The “whole ingredients extract” of Astragali Radix improves the symptoms of dextran sulfate sodium (DSS)-induced ulcerative colitis in mice through systemic immunomodulation

Ke-Gang Linghu
University of Macau
Qiushuo Ma
University of Macau
Shi Hang Xiong
University of Macau
Mingming Zhao
University of Macau
Qiling Chen
University of Macau
Wen Xu
Fujian University of Traditional Chinese Medicine
Meiwan Chen
University of Macau
Jian-Ye Zhang
Guangzhou University of Chinese Medicine
Yuanjia Hu
University of Macau
Wei Xu
Fujian University of Traditional Chinese Medicine
Hua Yu (yuhuayu@vip.sina.com)
University of Macau https://orcid.org/0000-0003-0737-9925

Research Article

Keywords: Astragali Radix, whole ingredients extract, immunomodulation, ulcerative colitis

Posted Date: July 14th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1779435/v1

Abstract

Background

Ulcerative colitis (UC) is a common inflammatory intestinal disease. Astragali Radix (AR) is one of the traditional Chinese medicines used in clinic for UC treatment. In our previous study, the whole ingredient extract (WIE) from AR have been proved to possess better immunomodulatory effects on immunosuppressed mice compared with the traditional water extraction (WAE). At our present study, we further evaluated the systemic immune regulation and therapeutic effects of WIE compared with the WAE in the dextran sodium sulfate (DSS)-induced UC mice.

Methods

Gradient solvent extraction has been used to prepare the WIE of Astragalus. The HPLC-MS analysis approach has been employed to analyze and compare the chemical differences between WAE and WIE. UC model was reproduced in 3% DSS-induced C57BL/6 mice for 6 days. Flow cytometric analysis for splenic lymphocyte subset. Elisa kits were used to determine the cytokines in the serum and colon tissues. The histopathological characteristics of colon were evaluated by hematoxylin-eosin staining and immunohistochemistry

Results

The chemical compositions of WIE were more abundant than that in WAE, and amount of main active ingredients in WIE is higher than that in WAE. The WIE treatment altered a better action on reducing colitis disease activity index (DAI) and histological scores, as well as the recovered body weight and increased colon length in mice compared to the WAE group. Additionally, WIE showed a better effects than WAE in recovering the levels of white blood cells in peripheral blood and cytokines (IL-2, IL-6 and MCP-1) in serum or colon tissues, improving the percentage of CD3 + and the ratio of CD4+/CD8 + in the spleen, and inhibiting the spleen enlargement in DSS-induced UC mice.

Conclusions

WIE has a more complete chemical composition than WAE. Meanwhile, WIE possesses better therapeutic effect on UC and the resuming effect on dysfunctional immunity than WAE at the same dosage in mice.

Background

Ulcerative colitis (UC) is a chronic inflammatory bowel disease which happened in digestive system especially in the colon and rectum. Clinical symptoms are abdominal pain, diarrhea, bloody stool etc. The progression of UC is a long-term process that is easily to relapse. The UC patients often undertake the

high risk of colon cancer than ordinary people [1]. In the past decades, UC mainly happened in western countries, while at present the morbidity of UC is growing in Asian countries. The prevalence of UC has been becoming a noticeable problem in China [2].Most scholars believe that UC is caused by multiple factors, including infection, immunity, genetic and mentality. Although the exact pathological mechanisms of UC are still not well understood, immune system dysfunction and severe inflammatory response at colon and rectum are widely observed for most UC patients[3]. The influence of immune factors on the pathogenesis of UC are specifically manifested in the clinical symptoms of the disease, histopathology, and immunosuppressive therapy [4].

Currently, the main clinical use of drugs to treat UC are amino salicylic acid, glucocorticoid and immunosuppressant[5]. But these medicines could not cure UC completely, and the side effect is too severe. Extensive ongoing research efforts have been invested in developing the plant-based bioactive agents from traditional Chinese medicine for treating ulcerative colitis and made some achievements[6]. Astragali Radix (AR) is a traditional tonic herbal medicine and has been used to strengthen human immunity in China for more than two-thousand years[7]. In general, the water decoctions (water extracts, WAE) are conventionally used as main preparation for most Chinese medicines and health products. “Baizhu-Huangqi decoction”, a traditional Chinese medicine formula consisted with AR and another Chinese medicine has been used for UC treatment[8]. However, WAE mainly contains water-soluble components (polar macromolecules and small molecules), while alcohol-soluble components (weakly polar and non-polar small molecules) in the WAE are very limited. As a result, WAE contains incomplete chemical components compared to the herb itself, causing a loss of integrity in the corresponding pharmacological activity. Recently, astragalus polysaccharide (APS), as the main water-soluble component from RA, has attracted a lot of attention on UC treatment. Zhao et al reported that APS attenuated rat experimental colitis by inducing regulatory T cells in intestinal Peyer’s patches [9]. Tian et al revealed that APS alleviated murine colitis through inhibition of the NLRP3 inflammasome [10]. Lv et al reported that APS protected against dextran sulfate sodium (DSS)-induced colitis by inhibiting NF-κB activation [11]. However, according to the current reports, main components of AR include astragalosides Ⅰ-Ⅳ (saponins), polysaccharides, amino acids, flavonoids and trace elements, could the APS represent the whole function of AR on immune regulation and UC has not been investigated.

In our previous study, a “whole ingredients extract” (WIE) containing both polar and non-polar molecules of AR has been developed, and demonstrated to present better immunomodulatory effects than the WAE on cyclophosphamide (Cy)-induced immunosuppressive mice [12]. In this study, the therapeutic effects of WIE and WAE against UC through immunomodulation were further investigated and compared on DSS-induced mice model.

Methods

Chemicals and reagents

Astragalosides (Ⅰ, Ⅱ, Ⅲ and Ⅳ), calycosin-7-O-β-d-glucoside, ononin, calycosin and formononetin (the purities of all standards were higher than 98% by HPLC analysis) were purchased from Chengdu Pufeide Biotech Co., Ltd. (Chengdu, China). Acetonitrile (ACN) as HPLC grade was purchased from Merck (Darmstadt, Germany). All chemicals used were analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). Milli-Q water prepared from Sigma-Aldrich Co. (Millipore, MA, USA).

Mice anti-mouse CD3-FITC, anti-mouse CD4-APC, and anti-mouse CD8a-PE were provided by eBioscience (San Diego, CA, USA). Enzyme-linked immunosorbent assay (ELISA) kits IL-2, IL-6 and MCP-1 were supplied by Neobioscience Technology Co., Ltd. (Shenzhen, China). Primary antibodies against COX-2 and the secondary antibody were purchased from Cell Signaling Technology (Danvers, MA, United States), all antibodies were diluted with 1:1000. Astragaloside Ⅰ, Astragaloside Ⅱ, Astragaloside Ⅲ, Astragaloside Ⅳ(the purities of all standards were higher than 98% by HPLC analysis) were purchased from Chengdu Pefeide Biotech Co., Ltd. (Chengdu, China). Acetonitrile (ACN) purchased from Merck (Darmstadt Germany) is HPLC grade. And other chemicals used were analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

Herbs and herbal extracts

Astragalus Radix (Astragalus, *Astragalus membranaceus* (Fisch.) Bge. var. mongholicus (Bge.) Hsiao) was purchased from Guangjitang CSPC Pharm Group (Guizhou, China). The herb sample was identified by the Dr. YU Hua (Macao, China) and the voucher specimen (HQ-2017001) was deposited in Institute of Chinese Medical Sciences, University of Macau.

The WIE and WAE were prepared with the methods as reported previously. For WIE, 400 g of powered Astragalus (400g) was gradient-extracted with 10-fold volume of 95% ethanol, 50% ethanol and water at 60℃ for 1 h for each, the filtered extracts were concentrated under reduced pressure, and then lyophilized using a Virtis freeze dryer. On the other hand, the WAE was prepared with the same protocol but using water instead of the ethanol.

HPLC-MS analysis

The contents of total polysaccharides in WIE and WAE were determined using phenol-sulfuric acid method [12].

The contents of Saponins (Astragaloside Ⅰ~Ⅳ) and flavonoids (calycosin-7-O-β-d-glucoside, ononin, calycosin and formononetin) were determined by a Waters Alliance HPLC system coupled with a Water ACQUITY QDa Mass Detector (Waters Corp., Milford, USA). Samples were eluted on a Waters Atlantis T3 column (150 mm×2.1 mm, 3.0 μm) maintained at 25 ºC. Elution was performed with a mobile phase of A (0.1% formic acid in water) and B (0.1% formic acid in ACN) under a gradient program: 0–2 min, 21% B; 2–12 min, 22–25% B; 12–20 min, 25–50% B; 20–30 min, 50–70% B. The flow rate was 0.4 mL/min, and the injection volume was 5 μL. The analytes were monitored by mass spectrometry with an electrospray ion source operating in the positive ion mode (ESI+) using single ion recording (SIR). The monitored ions were m/z 269.09 ($[M + H]^+$, formononetin), 285.05 ($[M + H]^+$, calycosin), 431.21 ($[M + H]^+$, ononin), 447.13

([M + H]$^+$, calycosin-7-O-β-d-glucoside), 891.39 ([M + Na]$^+$, astragaloside I), 849.44 ([M + Na]$^+$, astragaloside II) and 807.30 ([M + Na]$^+$, astragaloside III and IV), respectively. Between two injections, the column was washed with 100% B for 3 min and equilibrated with the initial mobile phase for 5 min.

Experimental animals and treatments

Male C57BL/6 mice (22–24 g) supplied by the Faculty of Healthy Science Animal Centre of University of Macau were fed on a standard laboratory diet with free access to water at a controlled temperature of 22 ± 1°C and relative humidity of 50% with a 12 h light/dark cycle. After one week of acclimatization, mice were randomly divided into six groups, control (CTRL), model (DSS, 3%), high dose of WIE (WIE-H, 3 g/kg), medium dose of WIE (WIE-M, 1.5 g/kg), low dose of WIE (WIE-L, 0.3 g/kg) and WAE group (WAE-H, 3 g/kg). Each group has six mice by random allocation. The control group was free to intake water for 14 days. Other groups were free to intake 3% dextran sulfate sodium (DSS) solution for 7 days, then changed to water for another 7 days. Extracts were administrated orally to the mice for 14 days in WIE and WAE groups. The body weight of each mouse was measured and recorded every day. All the experimental protocols were in accordance with the National Institutes of Health guidelines for the Care of Use of Laboratory Animals, and approved (reference No: UMARE-004-2020) by the Animal Research Ethics Committee, University of Macau, Macao SAR, China.

Colon index and spleen index

In the end of experiment, mice were sacrificed by CO_2 inhalation, the colon was dissected, and the length of the colon was measured using a ruler. Colon index (cm/g) = length of colon (cm)/body weight (g). The spleen was immediately excised and weighed. Spleen index (mg/g) = weight of spleen (mg)/body weight (g).

White blood cell counting

Wiping the mice tail with warm water, then disinfect the tail with alcohol. Use a small surgical scissors to cut off the end of tail and a pipette to accurately suck blood for 5 μL into 95 μL of 0.2% acetic acid, shake well immediately. According to the method of counting red blood cells, drip the white blood cell suspension into the counting cell plate, count the white cell after they sink down (about 1 min).

ELISA assay for serum and colon

In the end of the experiment, blood samples were collected from the orbits of mice. To obtain serum, each blood sample was centrifuged at 4,000 rpm for 10 min at 4°C. Additionally, the colon tissues in different groups were lysed by cell lysis buffer (Beyotime, Jiangsu, China), and the protein concentration was detected using a BCA protein assay kit (Thermo Fisher Scientific, MA, USA). Cytokines (IL-2, MCP-1, and IL-6) in the serum and lysate were measured using mouse ELISA kits according to the manufacturer's protocol.

Flow cytometry analysis

At the end of the experiment, spleens were isolated from mice. The spleen was then gently grinded and filtrated through a 40 μM strainer. For purpose of wiping out red blood cells, red cell lysis buffer was added to the single cell suspension for 5 min and centrifuged for 5 min at 200g. Then, $5–10\times10^5$ splenocytes was incubated with FITC-CD3, APC-CD4, PE-CD8a for 30 min at 4 ℃ in the darkness. Use PBS to wash the cells for two times and re-suspend into the 500 μL PBS. The numbers of $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes was measured by LSRFortessa™ Flow Cytometer (BD, USA) and signified as percentage of total number of lymphocytes.

Histopathology and immunohistochemistry

The colon tissue was fixed with 10% neutral phosphate buffered formalin for 7 days. After washing with tap water, the tissues were sequentially placed in gradient ethanol: 70% for 45 min, 80% for 45 min, 95% for 45 min, 100% I for 45 min, 100% II for 45 min. Place the tissue in xylene-ethanol mixture for 45 min; place the tissue in xylene 20–60 min; place the tissue in melted paraffin at 70 ° C temperature for 2 h. Then colon tissue was embedded in the paraffin wax by using the embedding tool. Cut the tissue into 6 μm sections on paraffin section machine (Thermo, UK).

Place the slices of colon tissue in the oven at 70 ° C for 30 min. Soak in xylene I, II each for 15 min, 100% ethanol I, II each for 5 min, 95% ethanol for 5 min, 80% ethanol for 3 min, 70% ethanol for 3 min, distilled water for 10 min. Hematoxylin staining was performed for 5–10 min, then slices were rinsed with tap water for 5 min, and differentiated with 0.5% hydrochloric acid ethanol for 30 s. After that, stain with 0.5% eosin for 10 s and rinse with tap water for 25 min. Then sink in 75% ethanol for 2 min, 80% ethanol for 2 min, 95% ethanol for 5 min, absolute ethanol for 5 min, and xylene I, II each for 5 min. Finally, the neutral gum was sealed and dried.

For immunohistochemistry assay, slices were deparaffinized and rehydrated by standard methods, then placed in 3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase. Following a rinse in deionized water, slices were transferred to a microwave-safe rack and immersed in 10 mM citrate buffer, pH 6.0, at 95℃ and microwaved on high power for 10 min. Cooling for 10 min, slices were soaked in phosphate buffered saline (PBS) for 5 min. Then the cyclooxygenase-2 (COX-2) was stained according to the commercial kit (Solarbio, Shanghai, China).

Statistical analysis

All data from a minimum three experiments were presented as mean ± SD. Data were analyzed on GraphPad Prism 6.0 software based on a one-way ANOVA with Dunnet's multiple comparisons test; $P < 0.05$ was considered difference significantly.

Results

Chemical characterization of two extracts

The data for chemical characterization of WIE and WAE were summarized in Table 1. The results indicated that the similar extraction efficiencies of total extract and total polysaccharides for WIE (35.78% and 43.6%) and WAE (34.65% and 45.2%). However, the extraction efficiencies for the small molecules (flavonoids, Astragaloside I and Astragaloside III) of WIE were significantly higher to those of WAE, indicating the more abundance of small molecules in WIE.

Table 1

Table 1 Chemical characterization of WIE and WAE. Data are presented as Mean ± SD (n = 3).

Content (%, g/100g extract)	WIE	WAE
Total polysaccharides	43.47 ± 0.35	44.63 ± 0.55
Calycosin-7-O-β-d-glucoside	0.0641 ± 0.0006	0.0191 ± 0.0002
Ononin	0.0151 ± 0.0001	0.0032 ± 0.0000
Calycosin	0.0311 ± 0.0001	0.0305 ± 0.0005
Formononetin	0.0478 ± 0.0007	0.0304 ± 0.0004
Astragaloside I	0.3676 ± 0.0063	N.D.
Astragaloside II	0.0285 ± 0.0005	0.0360 ± 0.0004
Astragaloside III	0.0109 ± 0.0005	0.0047 ± 0.0002
Astragaloside IV	0.0091 ± 0.0001	0.0318 ± 0.0004

The effects of WAE and WIE on food, liquid intake and animal growth

As shown in Fig. 2D, the mice in the control group keep the weight stable throughout the whole experiment. Compared with the control group, the body weight of the mice in the groups given DSS has been decreasing since the fourth day. The model group showed a dramatical decline (21% from the initial body weight) till the ninth day. While the WAE and WIE treatment significantly reversed the decline of body weight of mice after the DSS administration, and the weight loss in the WIE-H group is the smallest, followed by the WAE-H group and WIE-M group.

As for the change in liquid intake in Fig. 2B, the liquid intake in the groups given DSS were higher than that in the water group in the first four days of the experiment. From the fifth day, the liquid intake in the DSS groups began to decrease significantly, lower than that in water group. After the eighth day's treatment of Astragalus extract, the liquid intake of the treatment group increased.

In Fig. 2C, there was no obvious difference in food intake in the first five days of the experiment, while from the sixth day, the food intake in the DSS groups were significantly reduced. The model group showed a dramatical decline till the ninth day. The Astragalus extract treatment group showed a short-

term decline and began to recover from the seventh day. There is no obvious change of food intake from the beginning to the end of experiment in the control group.

The effects of WAE and WIE on disease activity index and colon index

A significant reduction in body weight and increased stool frequency were observed in mice with DDS-induced colitis, compared to their initial conditions. Stools of these mice were loose or watery containing some mucus and/or blood. DSS group mice with colitis display significant increase of DAI (Fig. 3A), while treatment with WIE in high dose significantly reversed the level of DAI, followed by WAE-H, WIE-M and WIE-L group.

To investigate the therapeutic effects of Astragalus extract on UC, the colon lengths in different groups were compared. A representative colonic appearance of mice in each group is shown in Fig. 3B. As shown in Fig. 3C, the colon of the DSS group was significantly shortened compared with the control group. Compared with the model group, each treatment showed a significant alleviation of colon shortening to varying degrees. Taken together, the effect of WIE-H is better than the WAE-H and WIE-M groups. While the low dose of WIE did not improve the length of colon obviously, compared with the DSS group.

WAE and WIE reduced the production of IL-6 and MCP-1 while increased the IL-2 in the serum and colon of DSS-induced mouse

As shown in Fig. 4, the expressions of MCP-1 and IL-6 were markedly increased in DSS-induced mice, while treatment with Astragalus extract significantly reduced MCP-1 and IL-6 expression in colon tissues and serum of mice with UC, especially in the WIE-H, WAE-H, and WIE-M groups. IL-2 level was reduced significantly in DSS-induced mice, but the production of this cytokine was markedly increased after treatment with Astragalus extract both in tissues and serum.

WAE and WIE decreased the white blood cell counting while increasing the spleen index

White blood cell and spleen index represent the systemic immune function in a mouse. As shown in Fig. 5A, the spleen index of mice in the DSS group was significantly higher than that of the control group, which means the enlargement of spleen after inducement with DSS in mice. However, after treatment with Astragalus extract, the spleen index in the WIE-H, WAE-H and WIE-M groups were decreased significantly. As shown in Fig. 5B, the number of peripheral white blood cell (PWBC) in DSS-induced mice were markedly reduced. After treatment with Astragalus extract, there is an obvious recovery of PWBC numbers, especially the WIE-H could significantly enhance the PWBC numbers in UC mice.

WAE and WIE upregulated the level of CD3 + and CD4+/CD8 + T lymphocytes in spleen

From the Fig. 6, immunophenotype of splenocytes was evaluated by counting $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes using flow cytometry analysis. The percentages of $CD3^+$ lymphocytes (Fig. 5A, C), and the ratio of $CD4^+/CD8^+$ were determined to be significantly decreased in DSS-treated mice (Fig. 5B, D). Treatment with WIE-H and WAE-H could increase the percentages of $CD3^+$ and the $CD4^+/CD8^+$ ratio significantly.

The effects of WAE and WIE on histopathological evaluation

The histopathological results of colonic mucosa in each group of mice are shown in Fig. 7A. It can be seen from the control group mice that the colon mucosa tissue is intact, the crypt structure is complete, and no edema were observed in the submucosa. In the DSS group, the mucosal epithelium was severely damaged and shed, the crypt structure was disappeared, ulcers were formed, and a large number of inflammatory cells were infiltrated into submucosa. Treatment with the WIE-H, WIE-M and WAE could alleviate the above pathological features, reduce epithelial tissue shedding and inflammatory cell infiltration.

COX-2 is a critical protein associated with the inflammatory condition of colon mucosa tissue. To further evaluate the protective effect of Astragalus extract on DSS-induced ulcerative colitis, we detected the expression of COX-2 in colon tissue with immunohistochemical analysis. As shown in Fig. 7B, a large number of COX-2 positive cells were observed in the colon mucosa of DSS-treated mice. In UC colon tissue treated with Astragalus extract, the COX-2 positive cells were significantly reduced, and WIE-H group showed the most significant inhibition effect, followed by the WIE-M and WAE-H.

Discussion

Ulcerative colitis (UC) is a chronic inflammatory intestinal disease, immune system dysfunction and severe inflammatory response at colon and rectum are the typical characteristics. In recent years, several traditional Chinese medicines (TCM) have been shown to protect against UC [6]. Astragali Radix (AR) is a popular Chinese medicine, which has been traditionally used to promote the immune function [13]. “Baizhu-Huangqi decoction”, a traditional Chinese medicine formula consisted with AR and another Chinese medicine has been used for UC treatment. Based on the traditional use of water decoction (water extracts, WAE), many modern researchers focused their study on the water extract of herb medicine, astragalus polysaccharide (APS) is one of the main bioactive components from water extracts of AR and has been revealed to show anti-colitis effects [14]. Astragaloside IV (ASI) is a monomeric compound identified from APS and also been confirmed to exert anti-UC effects in vivo and in vitro [15]. However, because of the different physical and chemical properties, the alcohol-soluble components (weak polar and nonpolar small molecules) in water extract are very limited. As a result, compared with the herbal medicine itself, WAE contains incomplete chemical components, leading to a decrease in the integrity of the corresponding pharmacological activity. Therefore, our research group developed the whole ingredient extract (WIE) method, trying to retain the small molecule compounds in AR to make its ingredients more

complete. Further study showed that WIE of AR is better immune recovery than WAE in cyclophosphamide (Cy)-induced immunosuppressive mice. In this study, we evaluated and compared the immune regulation effects between the WIE and WAE on DSS-induced UC model.

The spleen belongs to the peripheral immune organs and is the largest lymphoid organ in the human body, which plays important roles in regard to red blood cells (erythrocytes) and the immune system. Many studies have initially detected the state of the body's immune function by detecting changes in the spleen weight index. As shown in Fig. 4A, our results showed that the spleen index of the DSS group is much higher than that of the control group, it suggests that the dysfunction of the spleen in the DSS group. Compared with the DSS group, the spleen index of the WIE-H, WIE-M and WAE-H groups was declined ($P < 0.01$), indicating that Astragalus extract may improve the spleen function to enhance the immune function of UC mice. Similarly, results from Fig. 4B indicated that, compared with the control group, the white blood cells (WBCs) in the DSS group are reduced ($P < 0.01$), suggesting the suppressed immune function in the UC mice, treatment with AR extract could improve the level of WBCs, and the WIE-H showed a better effect on increase the level of WBCs than WAE-H.

There are two types of acquired immune systems including cellular immunity and humoral immunity, and cellular immunity is dominant. T lymphocytes are the most important regulatory components in cellular immunity. T lymphocytes are not only effector cells of cellular immunity, but also play an important role in regulating the immune response. T cells are grouped into a series of subsets based on their function, CD3 is present on all mature T cell membranes, CD8 is distributed on the surface of killer T cells, and CD4 is distributed on the helper cells. The proportion of $CD3^+$ T cell and the ratio of $CD4^+/CD8^+$ are critical parameters for immune system. As shown in Fig. 5, DSS treatment not only induced the decrease of proportion of $CD3^+$ T cell but also a significant decline of the ratio of $CD4^+/CD8^+$, suggesting the suppressed immune function in the UC mice. However, this immune-suppressed status was reversed after the treatment of the AR extract, and WIE-H showed a better modulatory effect than that of WAE-H (Fig. 5).

$CD4^+$T cells secrete a variety of cytokines after activation. According to the different cytokines, T helper cells are divided into two functional subgroups, Th1 and Th2. Th1 mainly produces IL-2, IFN-γ, TNF etc. Th2 mainly produces IL-4, IL-5, IL-6, IL-10 etc. [16]. IL-2 is the most important and powerful T cell growth factor in the body, and it is a key factor to ensure the body's normal immune function [17]. IL-2 is produced by activated Th1 lymphocytes and stimulates macrophages, natural killer (NK) cells, and cytotoxic T cells in cell-mediated immune responses. As Fig. 3 showed that the IL-2 levels in serum and colon tissue of DSS mice were lower than those in the control group, but increased in WIE-H, WIE-M and WAE-H groups, indicating that AR extract may attenuate the immune function through balancing the level of IL-2.

IL-6 is a common pro-inflammatory cytokine. It has been showed that serum levels of this pro-inflammatory cytokine were significantly increased in IBD patients compared with healthy control [18]. Further research found a correlation between IL-6 expression and disease activity in patients with Crohn's disease (CD) and UC [19]. At the same time, Louis proposed that high serum levels of IL-6 could be used

as a prognostic marker for relapse in patients with resting CD [20]. Consistent with these reports, our results showed that, in the serum and colon tissue of DSS group, the expression of IL-6 was significantly higher than that of the control group, which could be decreased after the administration with WIE-H, WIE-M and WAE-H, indicating that IL-6 may participate in the regulatory process of Astragalus on UC.

Studies have confirmed that the expression of MCP-1 in UC lesions is higher than that in normal mucosa. Increased expression of MCP-1 was also observed in the lesions of CD patients, and the expression of MCP-1 was positively correlated with the severity of UC. In addition, recent studies have shown that mice with MCP-1 receptor deficiency are given DSS to induce experimental colitis animal models, and the number of ulcers in the intestine and the degree of inflammation both were reduced compared to normal mice. These illustrate the important role of MCP-1 in the pathogenesis of UC [21]. As shown in Fig. 3, the expression of MCP-1 in the intestinal tissues and serum of the DSS group was significantly increased compared to the control group. The expression of MCP-1 in the WIE-H, WIE-M and WAE-H were lower than that in the DSS group, which demonstrated that AR extract can reduce the inflammatory response in the UC model by down-regulating the MCP-1 expression.

Abovementioned content showed the immune-improvement effects of AR extract, and the WIE exhibited a better effect than WAE. Then, we made an assessment on therapeutic effects of UC through the body weight, disease activity index, colon length, and histopathological analysis. Clinical studies have shown that UC patients will develop diarrhea, bloody Stool [22]. Consistent with clinical studies, the mice in the model group gradually began to lose weight and accompany blood in the stool after DSS treatment. AR extract treatment could significantly inhibit the weight loss of mice and reduce the blood in the stool. Results showed that, with continuous treatment of DSS, the length of the colon is decreased, this is related to inflammation and edema of the colon. After colon edema, the fragility of the cells increases, and the cells are prone to rupture, forming intestinal ulcers. This leads to shrinking of the intestinal wall and shortening the length of the colon [23]. However, after treatment with AR extract, colon shortening was significantly reduced (Fig. 2). In UC model mice, colonic mucosal tissue often showed an obvious edema, hyperemia, shedding of epithelium, disappearance of crypt structure, and infiltration of inflammatory cells [24]. Consistent with these reports, in our result, the mice in the model group also exhibited the above pathological features. AR extract can alleviate the above symptoms and reduce the pathological score of mice in each group.

Conclusions

In summary, WIE-AR is more complete than WAE-AR in chemical integrity and the therapeutic effect of WIE-AR on UC and the resuming effect of WIE-AR on immune function are better than WAE-AR at the same dose in mice. This study suggests that WIE might be a better method than WAE to represent the whole function of herb medicine for some of traditional Chinese medicines.

Abbreviations

APS
Astragalus polysaccharide
AR
Astragali Radix
DAI
Disease activity index
DSS
Dextran sulfate sodium
IL-2
Interleukin-2
IL-6
Interleukin-6
MCP-1
Monocyte chemoattractant protein-1
PBS
Phosphate buffered saline
PWBC
Peripheral white blood cell
UC
Ulcerative colitis
WAE
Water extract
WIE
Whole ingredients extract

Declarations

Ethics approval and consent to participate

All the experimental protocols were in accordance with the National Institutes of Health guidelines for the Care of Use of Laboratory Animals, and approved (reference No: UMARE-004-2020) by the Animal Research Ethics Committee, University of Macau, Macao SAR, China.

Consent for publication

All of authors consent to publication of this study in Journal of Chinese Medicine.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interest.

Funding

This study was financially supported by the Science and Technology Development Fund, Macau SAR [FDCT No. 0159/2020/A3, No. 0096/2019/A2, and No. 0058/2020/AGJ], and the Research Committee of the University of Macau [MYRG2018-00043-ICMS].

Acknowledgements

Not applicable.

Authors' contributions

KL, WeiX and HY conceived and designed the study. KL, QM, SX, MZ and QC conducted the experiments. WenX, MC, JZ and YH provided the technical support and advices for the study. QM and KL wrote the manuscript. WeiX and HY revised the manuscript. All authors contributed to the review and the approval of the final manuscript.

Acknowledgments

Not applicable.

References

1. Guo W, Hu S, Elgehama A, Shao F, Ren R, Liu W, et al. Fumigaclavine C ameliorates dextran sulfate sodium-induced murine experimental colitis via NLRP3 inflammasome inhibition. J Pharmacol Sci [Internet]. Elsevier Ltd; 2015;129:101–6. Available from: http://dx.doi.org/10.1016/j.jphs.2015.05.003.
2. Lok KH, Hung HG, Ng CH, Kwong KC, Yip WM, Lau SF, et al. Epidemiology and clinical characteristics of ulcerative colitis in Chinese population: Experience from a single center in Hong Kong. J Gastroenterol Hepatol. 2008;23:406–10.
3. Neuman MG. Immune dysfunction in inflammatory bowel disease. Transl Res. 2007;149:173–86.
4. Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P, et al. Prophylaxis of pouchitis onset with probiotic therapy: A double-blind, placebo-controlled trial. Gastroenterology. 2003;124:1202–9.
5. Xu CT, Meng SY, Pan BR. Drug therapy for ulcerative colitis. World J Gastroenterol. 2004;10:2311–7.
6. Zheng K, Shen H, Jia J, Lu Y, Zhu L, Zhang L, et al. Traditional Chinese medicine combination therapy for patients with steroid-dependent ulcerative colitis: Study protocol for a randomized controlled trial. Trials [Internet]. Trials; 2017;18:4–9. Available from: http://dx.doi.org/10.1186/s13063-016-1763-9.

7. Zhang C, Yang X, Wei J, ran, Chen N, mu han, Xu J ping, Bi Y qiong, et al. Ethnopharmacology, Phytochemistry, Pharmacology, Toxicology and Clinical Applications of Radix Astragali. Chin J Integr Med. 2021;27:229–40.
8. Li Ruliu C, Li G, Jing W, Wang Guixiang CW. Influence of Baizhu Huangqi Decoction single-drug extracts on ulcerative colitis in rats with different disease stages. China Jouunal Chineses Mater Medica. 2008;33:209–12.
9. Zhao HM, Wang Y, Huang XY, Huang MF, Xu R, Yue HY, et al. Astragalus polysaccharide attenuates rat experimental colitis by inducing regulatory T cells in intestinal Peyer's patches. World J Gastroenterol. 2016;22:3175–85.
10. Tian Z, Liu Y, Yang B, Zhang J, He H, Ge H, et al. Astagalus Polysaccharide Attenuates Murine Colitis through Inhibiton of the NLRP3 Inflammasome. Planta Med. 2017;83:70–7.
11. Lv Q, Qiao SM, Xia Y, Shi C, Xia YF, Chou GX, et al. Norisoboldine ameliorates DSS-induced ulcerative colitis in mice through induction of regulatory T cells in colons. Int Immunopharmacol. 2015;29:787–97.
12. Li ZX, Zhao GD, Xiong W, Linghu KG, Ma QS, Cheang WS, et al. Immunomodulatory effects of a new whole ingredients extract from Astragalus: A combined evaluation on chemistry and pharmacology. Chinese Med (United Kingdom) [Internet]. BioMed Central; 2019;14:1–10. Available from: https://doi.org/10.1186/s13020-019-0234-0.
13. Jin M, Zhao K, Huang Q, Shang P. Structural features and biological activities of the polysaccharides from Astragalus membranaceus. Int J Biol Macromol [Internet]. Elsevier B.V.; 2014;64:257–66. Available from: http://dx.doi.org/10.1016/j.ijbiomac.2013.12.002.
14. Lv J, Zhang Y, Tian Z, Liu F, Shi Y, Liu Y, et al. Astragalus polysaccharides protect against dextran sulfate sodium-induced colitis by inhibiting NF-κB activation. Int J Biol Macromol [Internet]. Elsevier B.V.; 2017;98:723–9. Available from: http://dx.doi.org/10.1016/j.ijbiomac.2017.02.024.
15. Wu S, Chen Z. Astragaloside IV alleviates the symptoms of experimental ulcerative colitis in vitro and in vivo. Exp Ther Med. 2019;2877–84.
16. Chen IH, Lai YL, Wu CL, Chang YF, Chu CC, Tsai IF, et al. Immune impairment in patients with terminal cancers: Influence of cancer treatments and cytomegalovirus infection. Cancer Immunol Immunother. 2010;59:323–34.
17. Neuner A, Schindel M, Wildenberg U, Muley T, Lahm H, Fischer JR. Prognostic significance of cytokine modulation in non-small cell lung cancer. Int J Cancer. 2002;101:287–92.
18. Takač B, Mihaljević S, Glavaš-Obrovac L, Kibel A, Suver-Stević M, Canecki-Varžić S, et al. Interactions Among Interleukin-6, C-Reactive Protein and Interleukin-6 (-174) G/C Polymorphism in the Pathogenesis of Crohn'S Disease and Ulcerative Colitis. Acta Clin Croat. 2020;59:67–80.
19. Xie T, Zhao C, Ding C, Zhang L, Cheng M, Chun C, et al. Postoperative Interleukin-6 Predicts Intra-abdominal Septic Complications at an Early Stage after Elective Intestinal Operation for Crohn's Disease Patients. Inflamm Bowel Dis. 2018;24:1992–2000.

20. Van Kemseke C, Belaiche J, Louis E. Frequently relapsing Crohn's disease is characterized by persistent elevation in interleukin-6 and soluble interleukin-2 receptor serum levels during remission. Int J Colorectal Dis. 2000;15:206–10.
21. Motomura Y, Khan WI, El-Sharkawy RT, Verma-Gandhu M, Verdu EF, Gauldie J, et al. Induction of a fibrogenic response in mouse colon by overexpression of monocyte chemoattractant protein 1. Gut. 2006;55:662–70.
22. Dai PC, Liu DL, Zhang L, Ye J, Wang Q, Zhang HW, et al. Astragaloside IV sensitizes non–small cell lung cancer cells to gefitinib potentially via regulation of SIRT6. Tumor Biol. 2017;39.
23. Geboes K. Lymphocytic, collagenous and other microscopic colitides: Pathology and the relationship with idiopathic inflammatory bowel diseases. Gastroenterol Clin Biol. 2008;32:689–94.
24. Triantafyllidis I, Poutahidis T, Taitzoglou I, Kesisoglou I, Lazaridis C, Botsios D. Treatment with Mesna and n-3 polyunsaturated fatty acids ameliorates experimental ulcerative colitis in rats. Int J Exp Pathol. 2015;96:433–43.

Figures

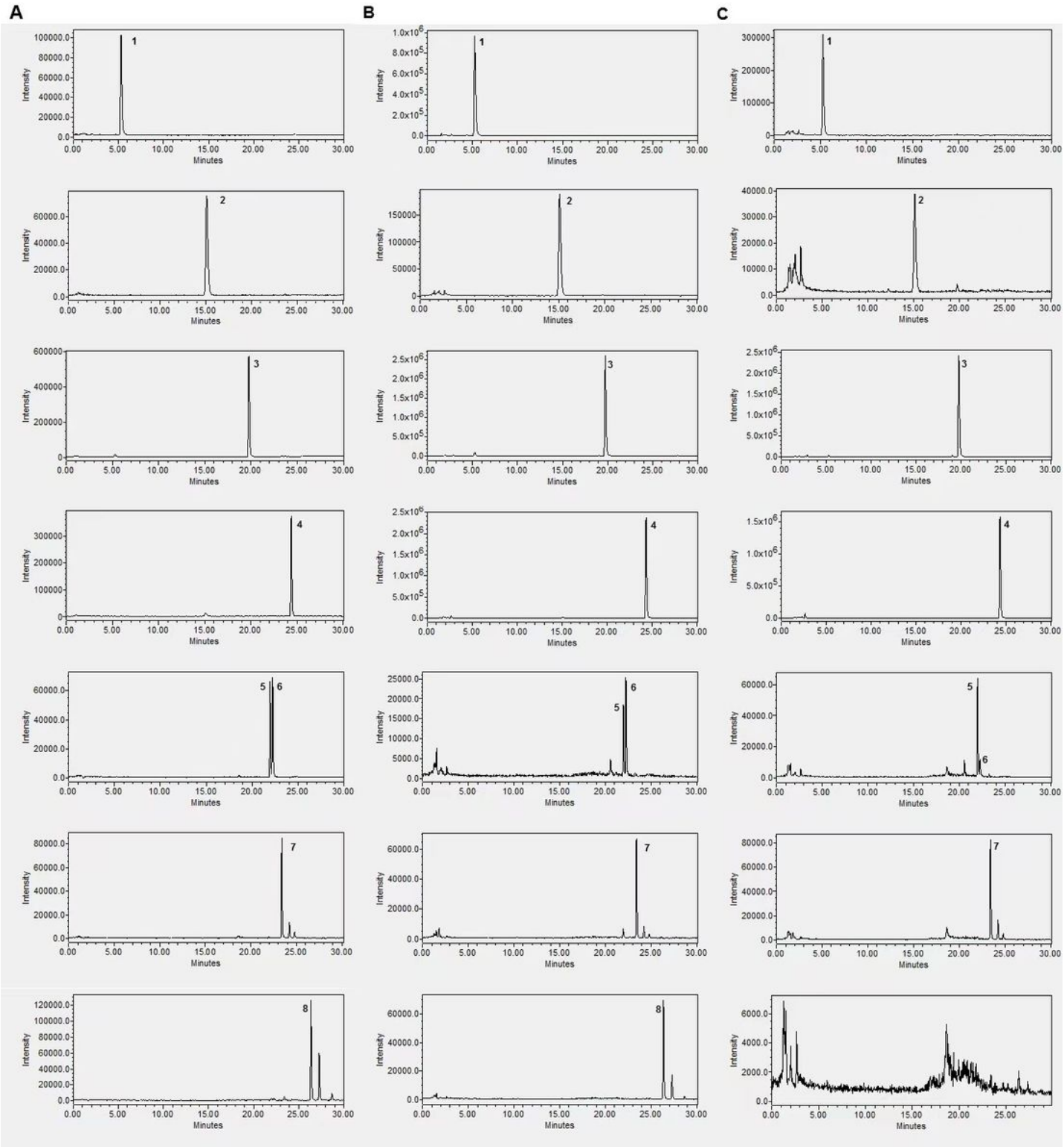

Figure 1

HPLC-MS chromatograms of (A) mixed standards, (B) WIE and (C) WAE. **WIE**: whole ingredient extract, **WAE**: water extract. **1**: calycosin-7-O-β-d-glucoside, **2**: ononin, **3**: calycosin, **4**: formononetin, **5**: astragaloside III, **6**: astragaloside III, **7**: astragaloside II, **8**: astragaloside I.

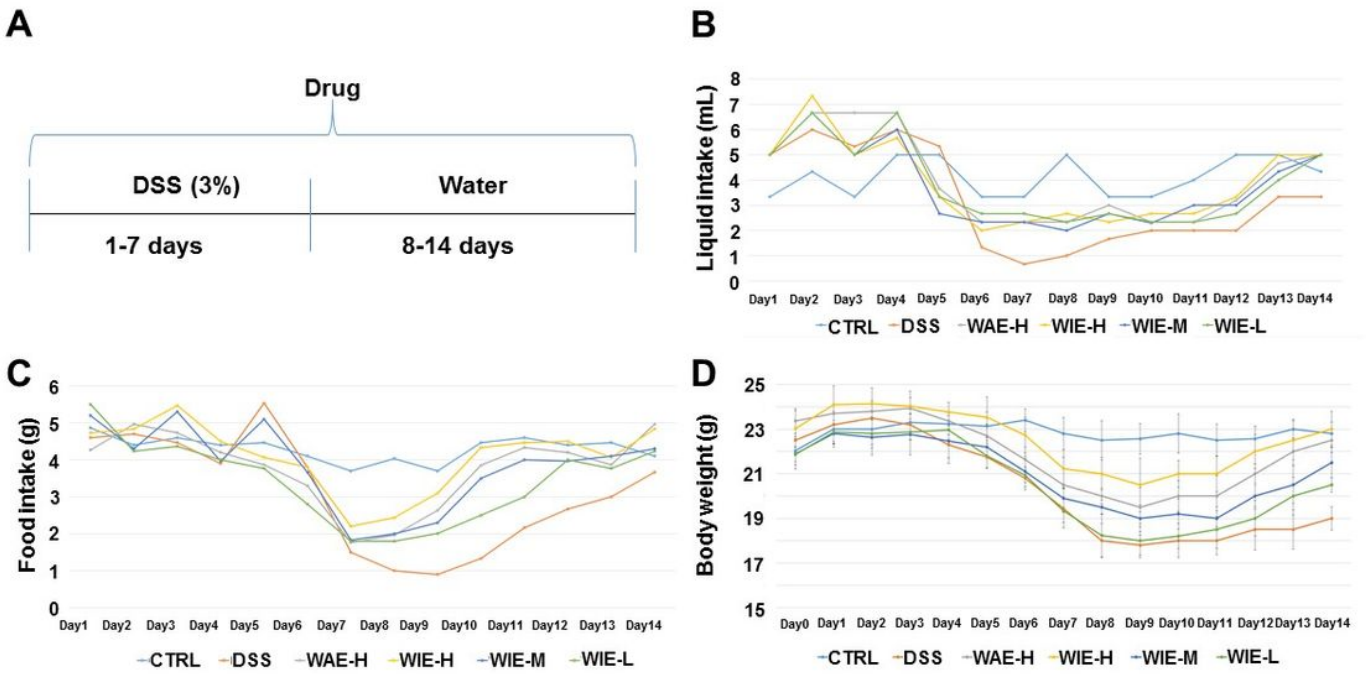

Figure 2

Food, liquid intake and body weight of experimental animals. (A) The route diagram of the whole experiment. (B) The changes of the liquid intake during the experiment period. (C) The changes of the food intake during the experiment period. (D) The changes of the body weight during the experiment period. Control (CTRL), model (DSS, 3%), high dosage of WIE (WIE-H, 3 g/kg), medium dosage of WIE (WIE-M, 1.5 g/kg), low dosage of WIE (WIE-L, 0.3 g/kg) and WAE group (WAE-H, 3 g/kg).

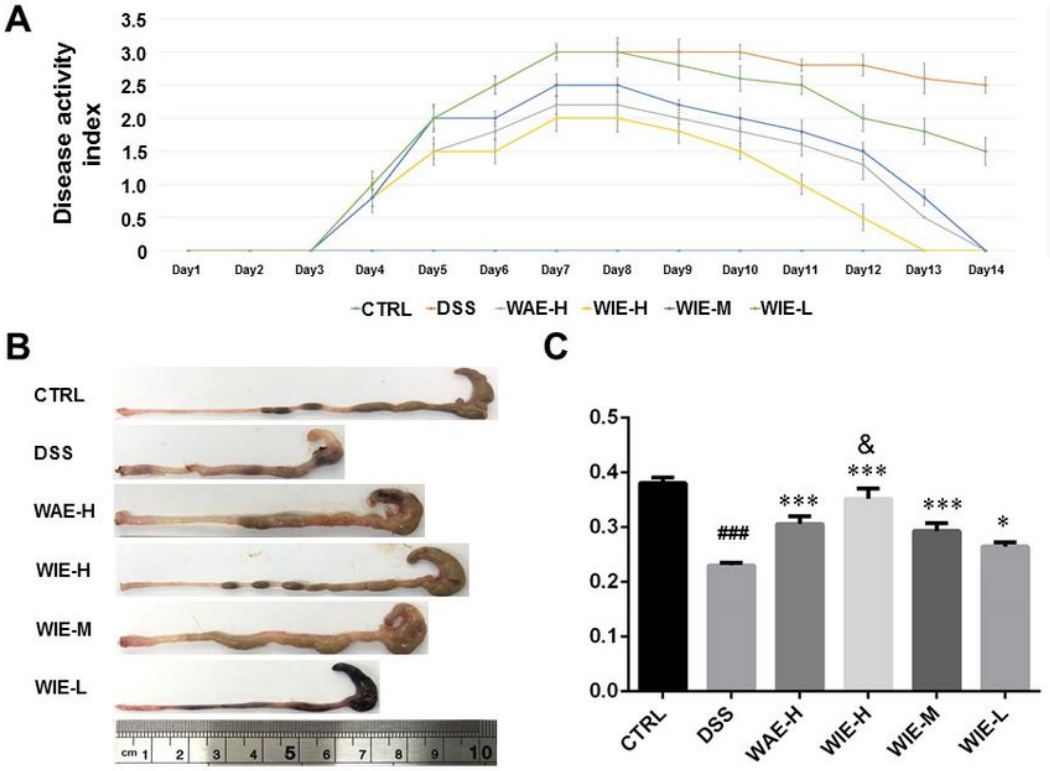

Figure 3

Effects of Astragalus extracts on disease activity index and colon index in DSS-induced UC. (A) The changes of the disease activity index during the experiment period. (B) Mice were sacrificed at day 15, the colon was dissected, measured, and imaged. (C) Colon index of different groups mice (Colon length/Body weight). Data are presented as Mean±SD (n=6). Control (CTRL), model (DSS, 3%), high dosage of WIE (WIE-H, 3 g/kg), medium dosage of WIE (WIE-M, 1.5 g/kg), low dosage of WIE (WIE-L, 0.3 g/kg) and WAE group (WAE-H, 3 g/kg). ### P<0.001 vs CTRL; *** P <0.001, * P <0.05 vs DSS; & P <0.05 vs WAE-H.

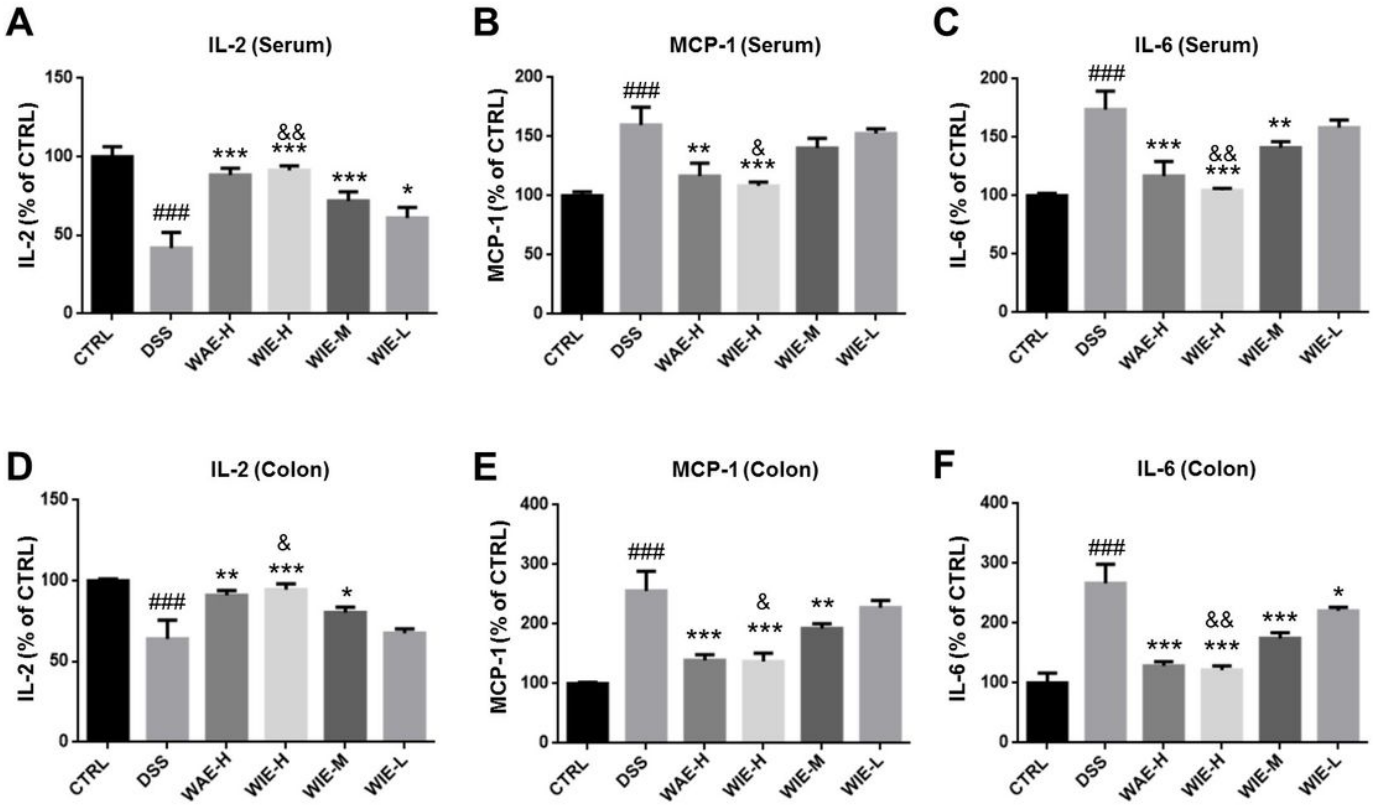

Figure 4

Effects of Astragalus extracts on cytokines in serum and colon in DSS-induced mice. (A) IL-2 level in serum of DSS-induced UC mice. (B) MCP-1 level in serum of DSS-induced UC mice. (C) IL-6 level in serum of DSS-induced UC mice. (D) IL-2 expression in colon of DSS-induced UC mice. (E) MCP-1 expression in colon of DSS-induced UC mice. (F) IL-6 expression in colon of DSS-induced UC mice. Data are presented as Mean ± SD (*n*=6). Control (CTRL), model (DSS, 3%), high dosage of WIE (WIE-H, 3 g/kg), medium dosage of WIE (WIE-M, 1.5 g/kg), low dosage of WIE (WIE-L, 0.3 g/kg) and WAE group (WAE-H, 3 g/kg). ### P<0.001 vs CTRL; *** P <0.001, ** P <0.01, * P <0.05 vs DSS; && P <0.01, & P <0.05 vs WAE-H.

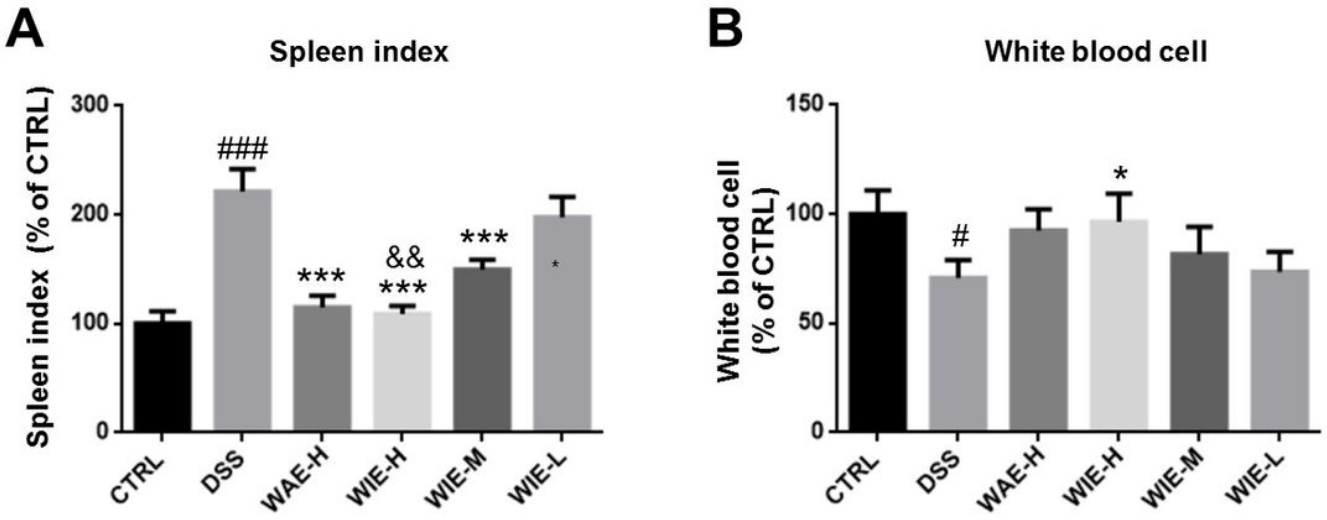

Figure 5

Effects of Astragalus extracts on spleen index and peripheral blood white cells in DSS-induced mice. (A) Effects of Astragalus extracts on spleen index of DSS-induced UC mice. (B) The counts of white blood cells in DSS-induced UC mice. Data are presented as Mean±SD (*n*=6). Control (CTRL), model (DSS, 3%), high dosage of WIE (WIE-H, 3 g/kg), medium dosage of WIE (WIE-M, 1.5 g/kg), low dosage of WIE (WIE-L, 0.3 g/kg) and WAE group (WAE-H, 3 g/kg). ### P<0.001, # P<0.05 vs CTRL; *** P<0.001, * P<0.05 vs DSS; && P<0.01 vs WAE-H.

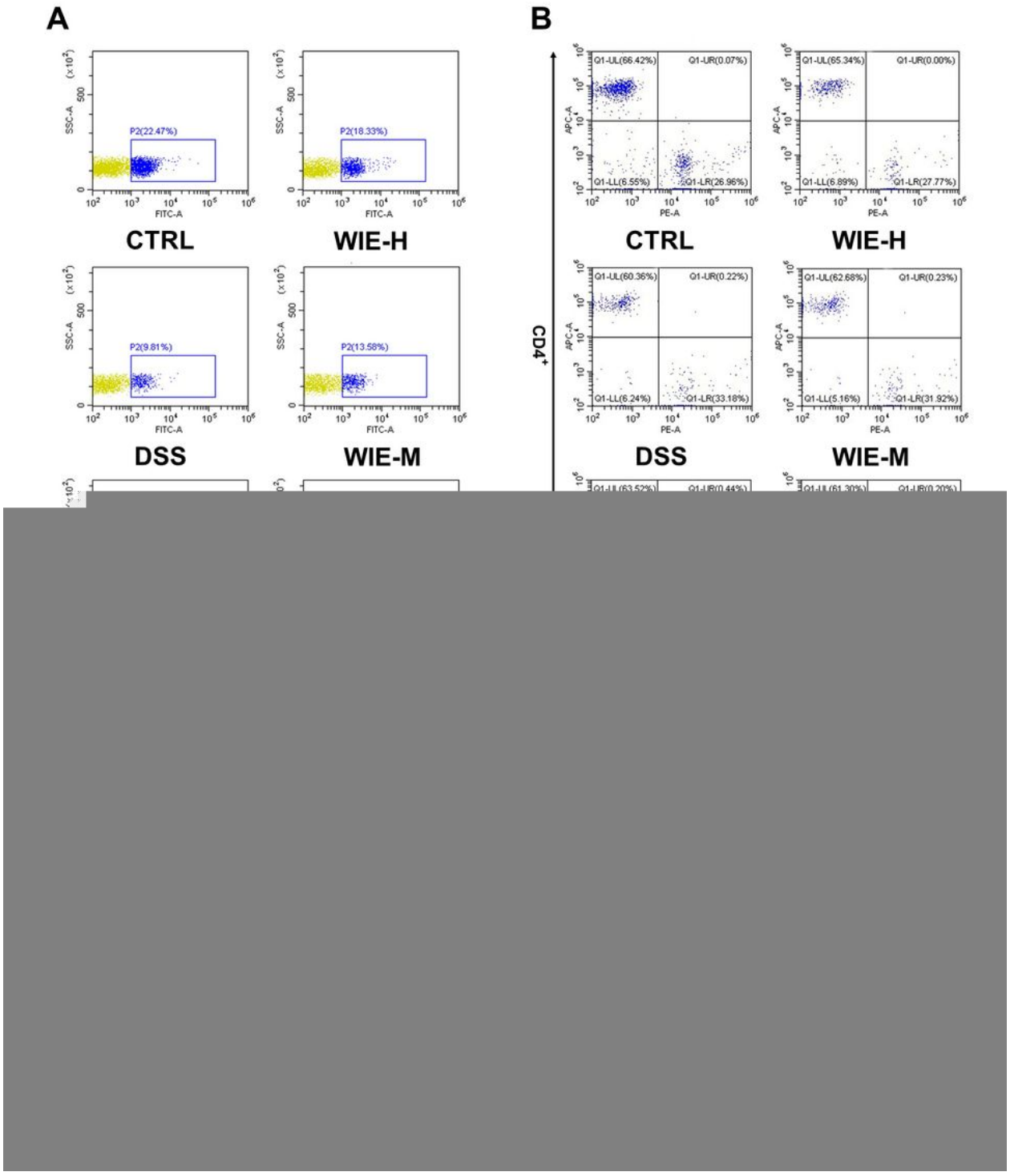

Figure 6

Counts of T lymphocytes determined by flow cytometry. (A) Representative images of $CD3^+$ counting determined by flow cytometry for mice treated by water, DSS, WAE and WIE. (B) Representative images of $CD4^+$ and $CD8^+$ counting determined by flow cytometry for mice given by water, DSS, WAE and WIE. (C) Proportion of $CD3^+$. (D) The ratio of $CD4^+/CD8^+$. Data are presented as Mean±SD (*n*=5). Control (CTRL), model (DSS, 3%), high dosage of WIE (WIE-H, 3 g/kg), medium dosage of WIE (WIE-M, 1.5 g/kg), low

dosage of WIE (WIE-L, 0.3 g/kg) and WAE group (WAE-H, 3 g/kg). ### P<0.001, # P<0.06 vs CTRL; *** P<0.001, ** P<0.01 vs DSS; && P<0.01, & P<0.05 vs WAE-H.

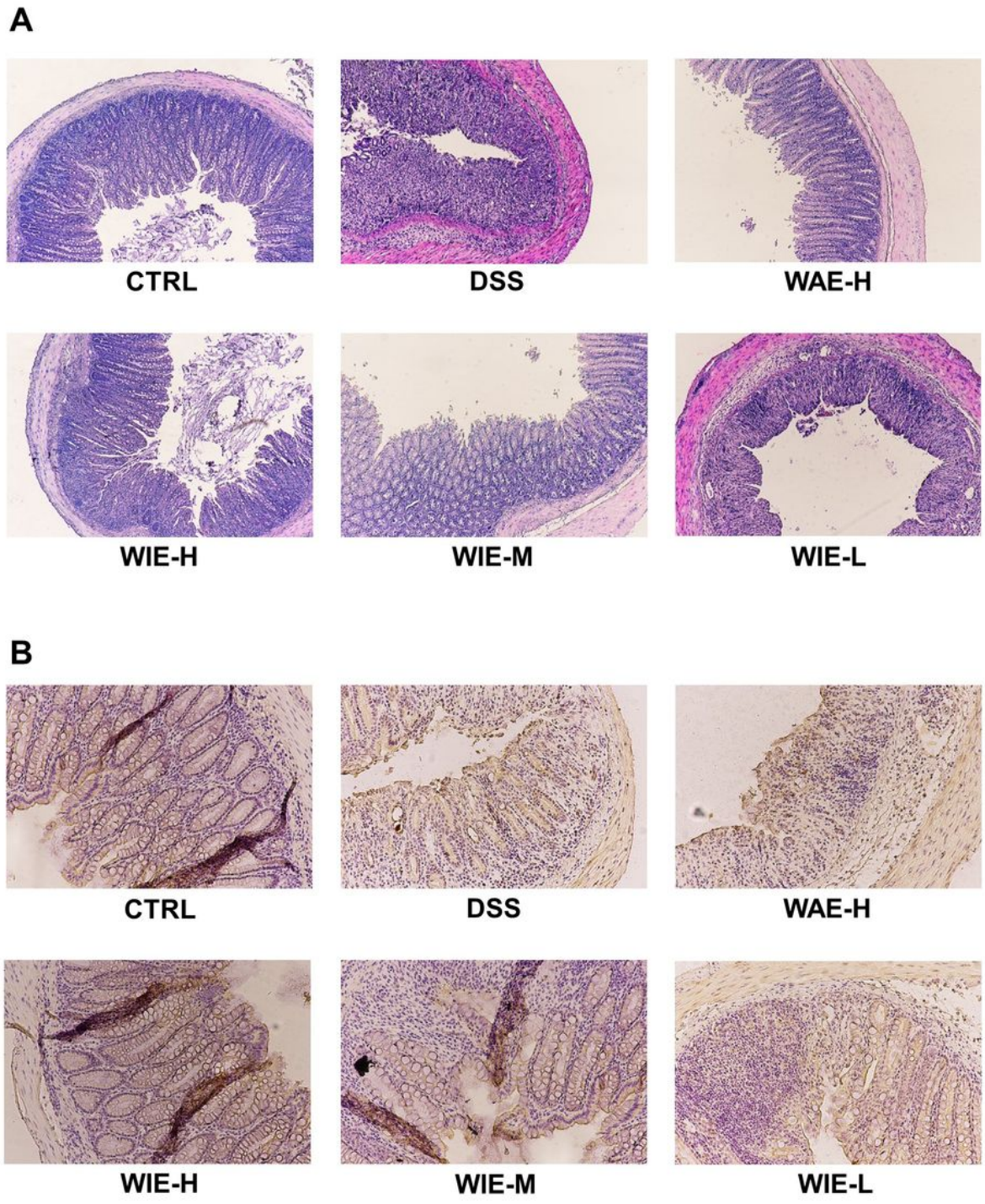

Figure 7

Effects of Astragalus extract on histopathological characterization of colon in DSS-induced mice. (A) Representative H&E staining images from the six different groups (×100). (B) Representative

immunohistochemistry images from the six different groups (×100). Control (CTRL), model (DSS, 3%), high dosage of WIE (WIE-H, 3 g/kg), medium dosage of WIE (WIE-M, 1.5 g/kg), low dosage of WIE (WIE-L, 0.3 g/kg) and WAE group (WAE-H, 3 g/kg).