American Journal of Biomedical and Life Sciences
2019; 7(6): 143-147
http://www.sciencepublishinggroup.com/j/ajbls
doi: 10.11648/j.ajbls.20190706.13
ISSN: 2330-8818 (Print); ISSN: 2330-880X (Online)

Empirical Study on the Therapeutic Effect of Astragalus Polysaccharides in a Mouse Model of Ulcerative Colitis

Xi Qinhua[1, †], Teng Yajie[1, †], Li Yueqin[1], Dai Juan[1], Zhang Guangbo[2, *], Chen Weichang[1, 2, *]

[1]Department of Gastroenterology, First Affiliated Hospital of Soochow University, Suzhou, China

[2]Institute of Clinical Immunology, First Affiliated Hospital of Soochow University, Suzhou, China

Email address:

zhanggbsuzhou@hotmail.com (Zhang Guangbo), weichangchen@126.com (Chen Weichang)

[*]Corresponding author

† Xi Qinhua and Teng Yajie are co-first authors.

To cite this article:

Xi Qinhua, Teng Yajie, Li Yueqin, Dai Juan, Zhang Guangbo, Chen Weichang. Empirical Study on the Therapeutic Effect of Astragalus Polysaccharides in a Mouse Model of Ulcerative Colitis. *American Journal of Biomedical and Life Sciences.* Special Issue: *Inflammatory Bowel Disease*. Vol. 7, No. 6, 2019, pp. 143-147. doi: 10.11648/j.ajbls.20190706.13

Received: September 14, 2019; **Accepted**: October 29, 2019; **Published**: November 13, 2019

Abstract: This study was performed to study the effects of astragalus polysaccharides (APS) in the treatment of ulcerative colitis (UC), and to explore whether myeloid-derived suppressor cells (MDSC) involve in this process. UC model was established by dextran sulfate sodium salt (DSS) inducement in mouse. Then the effects of APS on UC was evaluate at the levels of cytology and molecular biology: exploring the influence of APS on MDSC by analyzing the levels of MDSC before and after the treatments of APS; evaluating the therapeutic effects of APS on UC by pathologic histology. Data showed that the levels of MDSC in bone marrow, spleen, peripheral blood of UC mice were significantly decreased after intervention with APS, indicating APS inhibited the level of MDSC significantly. After depletion bone marrow cells in mice, we further found that the therapeutic effects of APS in $MDSC^-$ group was significantly reduced compared with $MDSC^+$ group. It was discovered through pathological analysis that, compared with UC model group, APS intervention group mainly manifested as reduced infiltrating acute and chronic inflammatory cells, necrotic epithelial cells and epithelial ulceration. Therefore, APS could reduce the inflammatory cell infiltration in colonic tissues of UC model mice, repair the damaged colonic mucosa, and promote ulcer healing. In conclusion, APS has a potential application in the treatment of ulcerative colitis, and is dependent or partially dependent on MDSC to achieve this effects.

Keywords: Astragalus Polysaccharides, Ulcerative Colitis, Mouse Model, MDSC

1. Introduction

Inflammatory bowel disease (IBD) includes Crohn's disease and ulcerative colitis (UC), which is associated with unclear etiology and pathogenesis. At present, most scholars believe that persistent intestinal infection, intestinal mucosal barrier defect, intestinal immunoregulatory abnormality, genetic and environmental factors have jointly participated in the disease occurrence process [1-3]. Among them, immune factor has been well recognized as a crucial factor in the IBD pathogenesis, which has always been the research hotspot.

Myeloid-derived suppressor cells (MDSCs) are a class of important immune regulatory cells, and mouse MDSCs are defined as cells with positive $Gr\text{-}1^+$ and $CD11b^+$, but no uniform definition standard is available for human MDSCs so far. The suppression of MDSCs is mainly related to the production of arginase and inducible nitric oxide synthase (iNOS). Besides, it can promote Treg differentiation, suppress T-cell response and weaken the NK function, thus exerting a key role in tumor immunity and inflammatory response. With the deepening of basic research, the expression profile and clinical significance of MDSCs in disease have attracted attention [4]. This paper had adopted DSS modeling to analyze the effect of APS on MDSCs and the therapeutic effect on UC.

2. Materials and Methods

2.1. Experimental Animals

15 6-8-week-old balb/c clean male mice were purchased from Shanghai Slack Laboratory Animal Co., Ltd [SCXK

(Shanghai) 2012~0002], and raised in the clean standard cages. This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Soochow University.

2.2. Preparation of Major Reagents

5% DSS: DSS (MP Biomedicals, LLC, batch: M2709) was prepared into the 50 g/L DSS solution when it was to be used. The astragalus polysaccharides (APS) finished product was dissolved into the distilled water and prepared into the 80 g/L APS solution when it was to be used.

2.3. Construction of the Mouse Chronic UC Model and Intervention with APS

The 15 clean balb/c male mice were numbered and divided according to the random number table into group A (normal control group, n=5), group B (model control group, n=5) and group C (APS treatment group, n=5). The models were constructed following 1 week of adaptive feeding after being purchased when no abnormality was observed. Mice in model group and treatment group had free access to 5% DSS solution for 7 days continuously, and then to DSS-free distilled water for 14 days; while those in normal group could drink distilled water freely for 21 days to construct the chronic UC mouse model. At the same time of modeling, treatment group (group C) was given gavage of 100 mg/kg APS for once a day for 1 week; whereas normal control group (group A) and model control group (group B) were given gavage of equivalent amount of distilled water. Mouse spirit, fur, defecation, activity, diet and survival were observed every day. On the 22nd day of modeling, all mice were sacrificed to collect the peripheral blood, spleen, colon and bone specimens for subsequent use.

2.4. Sample Collection

On the 21st day of modeling, mice were fasting for food but not for water and drug for 24 h. On the 22nd day, peripheral blood stem cells were collected through the angular vein into the heparin sodium-containing test tube, so as to obtain the peripheral blood, spleen cells and skeleton cells of each mouse. In addition, colon from anus to the ileocecal junction was collected to observe inflammation and ulcer under visual inspection. 1 tissue specimen (2 mm x 10 mm) was collected from the distal colon (DC) of each mouse, fixed with 10% formalin, embedded with conventional paraffin, and sliced into continuous 4 μm pathological sections (Hemotoxylin and eosin staining, HE staining). Afterwards, the colonic mucosal injury was observed under the microscope.

2.5. Flow Cytometry

The detected specimens were divided into two groups, among which, one group was added with FITC anti-human CD14 monoclonal antibody (0.1 μg) and homotype control IgG-PE, while the other group was added with PE anti-human HLA-DR monoclonal antibody (0.1 μg) and FITC anti-human CD14 monoclonal antibody (0.1 μg) to react for 30 min in dark at 4°C. Subsequently, 1 ml red blood cell lysis buffer (10 × RCL buffer, Biolegend Cat. #420301) was added, respectively, and reacted in the 37°C incubator for about 10 min. Then, 2 ml PBS was added to terminate the reaction, and white blood cells were collected through centrifugation (1500 rpm, 5 min), and 0.5 ml PBS was loaded for detection. The proportion of mononuclear MDSCs in CD14+ cells was calculated as ($CD14^{+}HLA\text{-}DR^{-/low}$/CD14+) × 100%.

2.6. Statistical Analysis

The statistical softwares Graphpad prism 5 and SPSS. V13.0 were adopted for statistical analyses. Measurement data were expressed as mean± standard deviation, the means between two groups were compared through t-test, and means among at least three groups were compared by one-way analysis of variance (ANOVA), pair-wise comparison between groups was carried out using LSD-t test, and Spearman rank correlation analysis was adopted for correlation analysis. A difference of $P<0.05$ was deemed as statistically significant.

3. Results

3.1. Effect of APS on MDSC Expression in UC Mouse Model

3.1.1. APS Intervention Remarkably Down-regulated Mononuclear MDSC Cell Expression in Mouse Bone Marrow

The MDSCs in bone marrow of normal group accounted for 25.7%, while those in UC model group took up 38.0%, and the proportion in the bone marrow of UC model group was apparently higher than that in normal group ($P<0.001$), with the difference being statistically significant. The MDSCs in bone marrow of APS intervention group had accounted for 26.5%, which was markedly declined compared with that in model group, and the difference was significant ($P<0.001$) (Figure 1).

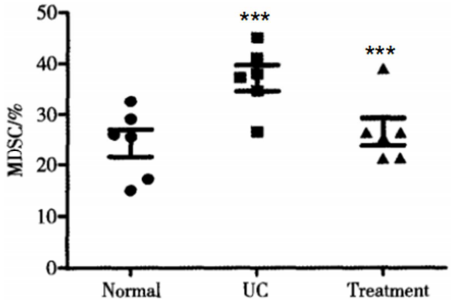


Figure 1. *Effects of astragalus polysaccharides on MDSC in bone marrow of UC mice.*

Note: The date is expressed as mean + SEM, with statistical method of one way ANOVA.
*** $P<0.001$.

3.1.2. APS Intervention Evidently Down-regulated Mononuclear MDSC Cell Expression in Mouse Spleen

After gavage in model group and APS group, the mononuclear MDSCs in mouse spleen of normal group took up 8.97%, while those in UC model group occupied 62.2%, and those in UC model group were markedly higher than those

in normal mice (P<0.001), with statistically significant difference. MDSCs in mouse spleen of APS intervention group had accounted for 42.7%, such a figure was markedly decreased compared with that in model group, and the difference was statistically significant (P<0.001) (Figure 2).

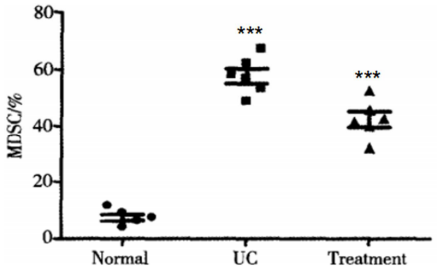


Figure 2. *Effects of astragalus polysaccharides on MDSC in spleen of UC mice.*

Note: The date is expressed as mean + SEM, with statistical method of one way ANOVA.
*** P<0.001.

3.1.3. APS Intervention Markedly Down-regulate Mononuclear MDSC Cell Expression in Mouse Peripheral Blood

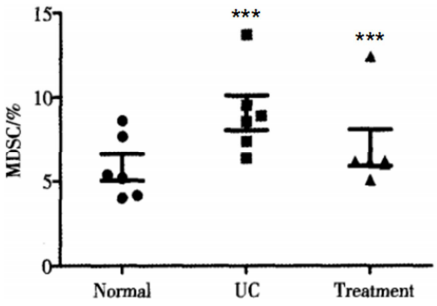


Figure 3. *Effects of astragalus polysaccharides on MDSC in blood of UC mice.*

Note: The date is expressed as mean + SEM, with statistical method of one way ANOVA.
*** P<0.001.

After gavage in model group and APS group, the mononuclear MDSCs in peripheral blood of each group were detected, as presented in Figure 3. The peripheral blood MDSCs in normal group accounted for 4.17%, while those in UC model group occupied 14.5%, and those in UC model group were apparently higher than those in normal mice (P<0.001), with the difference being statistically significant. The peripheral blood MDSCs of APS intervention group accounted for 8.58%, such a figure was dramatically decreased compared with that in model group, and the difference was statistically significant (P<0.001) (Figure 3).

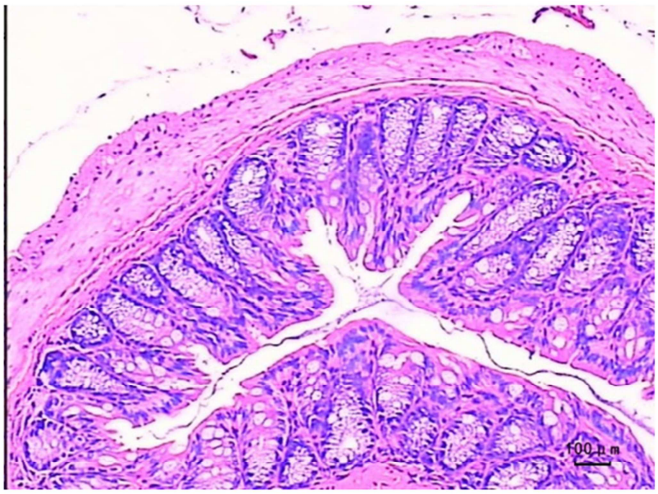
3.2. Colon Histopathological Changes in Mice of APS Intervention Group

3.2.1. Gross Observation

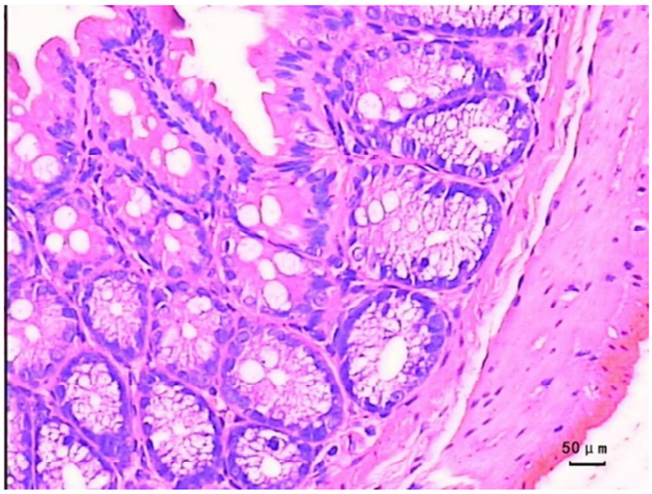
Normal group: clear intestinal duplicature texture was observed, with no erosion or ulcer; model group: multiple ulcers and punctate hemorrhage lesions were observed in intestinal mucosa, along with obvious congestion and edema in mucosa beside the ulcer, colonic wall thickening and stenosis; APS group: intestinal wall thickening and stenosis were seen, along with reduced intestinal mucosal ulcers and punctate hemorrhage lesions.

3.2.2. Optical Microscopy

Optical microscopic observation on UC experimental mouse model suggested that, the normal colon structure could be divided into mucous layer, submucous layer, muscular layer and serosal layer. The focal small ulcers were seen in model group, accompanying with adjacent epithelial cell regeneration and repair, as well as the prominent recess distortion, chronic inflammatory cell infiltration dominated by lymphocytes and mononuclear cells, while the inflammation mainly involved the mucous and submucous layers.



(× 100)



(× 200)

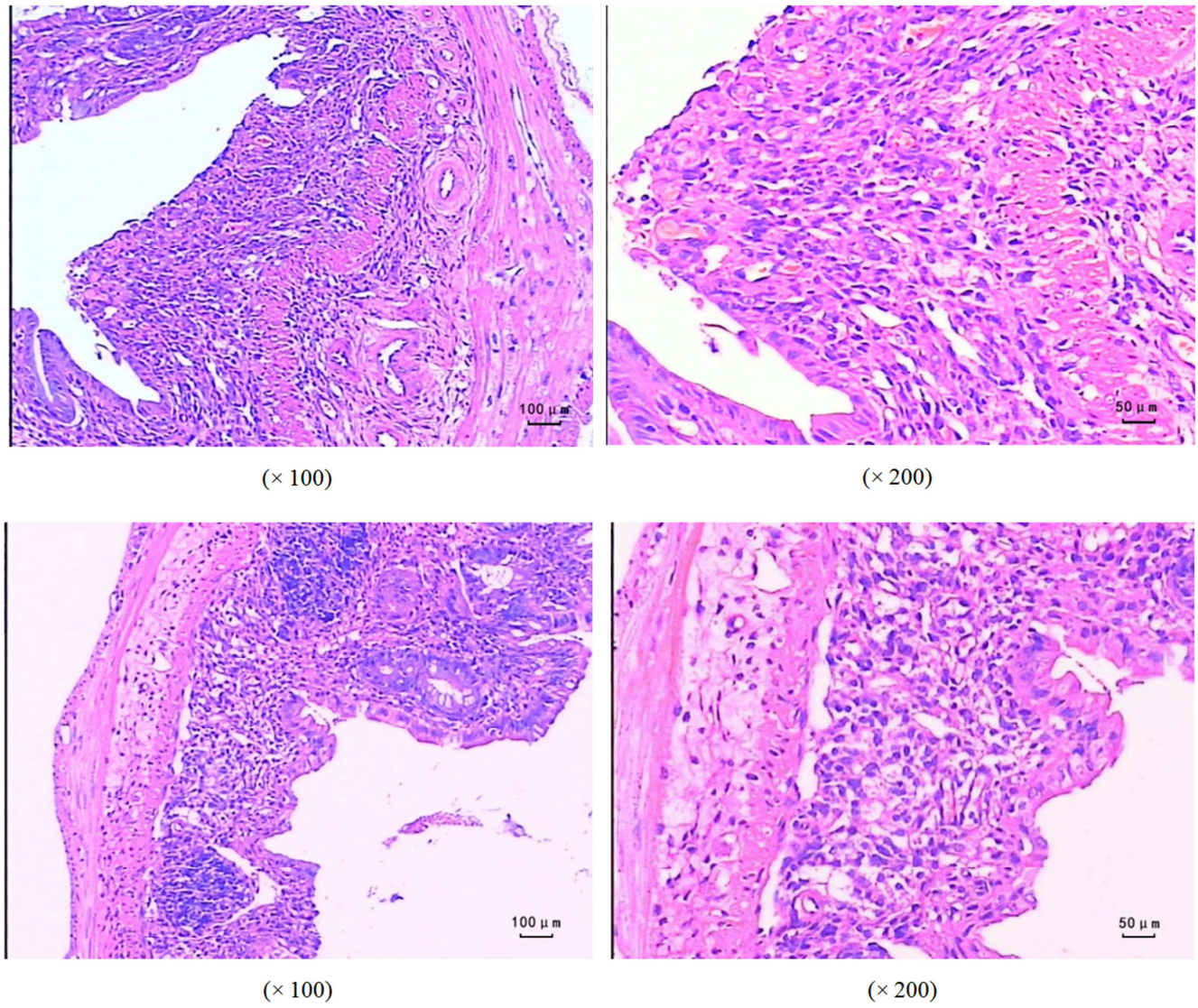


Figure 4. *Histopathological change in colon of UC mouse after the intervention of astragalus polysaccharides observed by optical microscopy.*

4. Discussion

Inflammatory bowel disease (IBD) is a group of non-specific chronic and recurrent gastrointestinal inflammatory disease with unknown cause, and chronic inflammatory response is the core pathological feature of IBD. How to relieve the inflammatory response and repair the gastrointestinal mucosa are the key problems urgently to be solved in the clinical treatment for IBD. The Qi and spleen tonifying prescription in traditional Chinese medicine (TCM) can enhance the gastrointestinal mucosal barrier function, promote the expression of mucosa-protecting factors and regulate the immunity. Typically, astragalus, the major component in the prescription, has the main active ingredient of astragalus polysaccharides (APS).

Existing research suggests that, APS can regulate the immune function, enhance body metabolism, and exert a vital role in anti-inflammation, anti-ulcer, anti-allergic reaction in the body [5-7]. Moreover, it is recently discovered that the myeloid-derived suppressor cells (MDSCs) have great immune regulatory effect on IBD, but it has not been reported about whether APS is involved in regulating MDSCs.

It was discovered in this study from macroscopic morphology that, compared with UC model group, the colon morphology in APS treatment group mainly manifested as reduced epithelial tissue erosion and ulcers, as well as alleviated tissue edema. It was discovered through pathological analysis that, compared with UC model group, APS intervention group mainly manifested as reduced infiltrating acute and chronic inflammatory cells, necrotic epithelial cells and epithelial ulceration. Therefore, APS could reduce the inflammatory cell infiltration in colonic tissues of UC model mice, repair the damaged colonic mucosa, and promote ulcer healing.

Xia Ying et al. [8] suggested that APS possessed multiple immune enhancing effects, and the overall clinical response rate among the 30 UC patients treated with oral administration of APS combined with enema Xihuangbai mixture. Also, some research results indicate that, APS may have an important regulatory effect on the Th1/Th2 balance, and it can enhance

human immunity level [9-10]. Moreover, Han Wei et al. [11] proved that APS could boost the phagocytosis of macrophages. Xiang Jie et al. [12] discovered that APS could enhance the host humoral immunity to protect the host from intracellular bacterial infection. Han Jinchao et al. [13] found that APS could boost the expression of immunity-related co-stimulating molecules such as MHC-II, CD80, and CD86, on DC surface, so as to promote DC maturation. An Songlan et al. [14] discovered that the APS in astragalus could antagonize the cyclophosphamide-induced immunodeficiency in mice. Chen Wei et al. [15] verified that APS could correct the immune imbalance of cytokines. Zhao et al. [16] discovered that APS could induce macrophages to produce TNF-α and GM-CSF, increase NO production, and elevate the body immunity. Zang Kaihong et al. [17] found that APS can dose-dependently decrease MPO activity and the contents of TNF-α, TGF-β in colonic tissue, increase the content of EGF and expression level of Occludin and ZO-1 protein. Jun Lv et al. [18] discovered that APS could reduce NF-κB DNA phosphorylation activity and downregulate TNF-α,IL-1β,IL-6,IL-17 expressions and MPO activity in colitis.

In this study, we discovered that the Gr-1^{+}CD11b^{+}MDSCs were abnormally elevated in peripheral blood, spleen and bone marrow of UC model mice, therefore, it was speculated that MDSCs might be the important promoting factor of UC. Given the important role of APS in IBD, this study had analyzed whether APS affected the number of MDSCs, and whether its therapeutic effect was dependent (or at least partially) on MDSCs. Our results demonstrated that APS treatment in vivo could indeed reduce the MDSC levels in UC mice.

To sum up, APS exerts immune regulatory effects on multiple aspects, which has thereby attracted increasing attention and become the research hotspot. Most reports have focused on macrophages, dendritic cells, NK cells and Th cells, while the effect on MDSCs is rarely reported. According to our results, APS intervention on UC mouse model can apparently down-regulate the expression of MDSCs in mouse bone marrow, spleen and peripheral blood, suggesting that APS can markedly suppress MDSCs. Further experiment verifies that APS has outstanding suppression on MDSCs, and that the regulation of MDSCs by APS is the important mechanism of treatment. However, it remains to be further explored about how APS affects the number of MDSCs, and whether APS exerts an important regulatory effect on the MDSCs biological functions, especially for the release of inflammatory factors.

References

[1] Torres J, MD, Mehandru S, MD, Colombel J, Prof, Peyrin-Biroulet L, Prof. Crohn's disease. Lancet, The. 2017; 389 (10080): 1741-55.

[2] Ungaro R, MD, Mehandru S, MD, Allen PB, MD, Peyrin-Biroulet L, Prof, Colombel J, Professor. Ulcerative colitis. Lancet, The. 2017; 389 (10080): 1756-70.

[3] Ni J, Wu G, Albenberg L, Tomov V. Gut microbiota and IBD: causation or correlation? NATURE REVIEWS GASTROENTEROLOGY & HEPATOLOGY. 2017; 14 (10): 573-84.

[4] Hao WB, Xiang FF, Liu QL, et al. Research progress of myeloid-derived suppressor cells in tumor microenvironment [J]. Journal of Immunology, 2017, 33 (8): 729-732.

[5] Yang M, Qian XH, Zhao DH, et al. Effects of Astragalus polysaccharides to the erythroid lineage and microarray analysis in K562 cells [J]. Ethnopharmacol, 2010, 127 (2): 242-251.

[6] Zhang Y. Traditional Chinese medicine combined with western medicine in treating ulcerative colitis [J]. Chinese Journal of Practical Chinese and Western Medicine, 2005, 11 (18): 512-514.

[7] Yang WB, Chen QL, Ma LY, et al. Effect of astragalus polysaccharides on rabbit atherosclerotic endothelial cell function [J]. Shaanxi Medical Journal, 2005, 34 (8): 914-918.

[8] Xia Y, Qin ZR, Shi YM. Therapeutic effect observation on 30 cases of ulcerative colitis treated with internal and external treatment [J]. Shanghai Journal of Traditional Chinese Medicine, 2000, 46 (10): 24.

[9] Weng L, Liu Y, Liu XY, et al. Effect of astragalus polysaccharides injection on mouse immune function [J]. Journal of Immunology, 2003, 19 (3): 243-244.

[10] Jiang H, Zhang QY. Effect of astragalus polysaccharides on umbilical blood Th1/Th2 functional balance in newborns [J]. Chinese Journal of Perinatal Medicine, 2003, 6 (4): 225-228.

[11] Han W, Liu ZQ, Sun L. Clinical research of using Salvia miltiorrhiza and astragalus injection in treating ulcerative colitis [J]. Journal of Changchun College of Traditional Chinese Medicine, 2005, 21 (4): 7.

[12] Xiang J, Wang YB, Xu T, et al. Effect of astragalus polysaccharides on the host resistance to Listeria bacteria [J]. Journal of Wuhan University: Medical edition, 2007, 28 (6): 741-743.

[13] Han JC, Yu YS, Tang ZH, et al. Effect of astragalus polysaccharides on the differentiation and maturation of mouse dendritic cells [J]. Shanghai Medicine, 2006, 29 (12): 873-876.

[14] An SL, Zhang SY, Pu HS. Effect of astragalus polysaccharides in annual astragalus on mouse immune organ index [J]. Medical Journal of Yanbian University, 2007, 30 (1): 20-22.

[15] Chen W, Li YM, Yu MH, et al. Immune regulatory effect of astragalus polysaccharides on the T-cell subset in diabetic mice [J]. China Journal of Modern Medicine, 2007, 17 (1): 28-31.

[16] Zhao LH, Ma ZX, Zhu J, et al. Characterization of polysaccharides from astragalus radix as the macrophage stimulator [J]. Cellular Immunol, 2011, 271 (2): 329-334.

[17] Zang KH, LI YD, ZHU LJ, et al. Study on the repair effect on colon mucosa and mechanism of astragalus polysaccharides in rats with ulcerative colitis [J]. GANSU UNIVERSITY OF CHINESE MEDICINE, 2018, 035 (003): P. 5-10.

[18] Lv J, Zhang Y, Tian Z, Liu Y, Liu F, Shi Y, et al. Astragalus polysaccharides protect against dextran sulfate sodium-induced colitis by inhibiting NF-κB activation. International Journal of Biological Macromolecules. 2017; 98: 723-9.