Intervention mechanism of Traditional Chinese medicine on the expression of TGF - β 1, IL-10 and MAdCAM-1 in ulcerative colitis

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Abstract

Background: Ulcerative colitis (UC) is a chronic non-specific inflammatory bowel disease that invades the colonic mucosa and is characterized by ulceration. It has been listed as one of the modern refractory diseases by the World Health Organization, and has become a hot issue in the field of Gastroenterology of Traditional Chinese medicine. This study aimed to determine the intervention mechanism of Traditional Chinese medicine in the treatment of UC.

Methods: The pathological changes of intestines were observed. TGF - β1, IL-10 and MadCAM-1 in the intestinal tissue or in the serum of UC rats were detected in order to explore the intervention mechanism of the Chinese medicine (Sodium Houttuynia, Matrine and HQHLD) in an immune-TNBS-ethanol rat model.

Results: Sodium Houttuynia, Matrine and HQHLD could alleviate intestinal inflammation reaction in the UC by lessening the pathological changes and fibrosis of the intestine, regulating the expression of TGF - β1, IL-10 and MadCAM-1 in the intestine or in the serum, and by reducing lymphocyte homing to intestinal tissues.

Conclusions: This study revealed the intervention mechanism of Traditional Chinese medicine in the treatment of UC, which provided further theoretical and scientific basis for the treatment of clinical ulcerative colitis. Our results will likely help expand clinical practice using HQHLD, Matrine and Sodium Houttuynia as effective Chinese medicines to treat ulcerative colitis.

1. Introduction

Ulcerative colitis is a chronic non-specific inflammatory bowel disease that invades the colonic mucosa and is characterized by ulceration. In recent years, the incidence rate of UC has been increasing year by year. Research has demonstrated that ulcerative colitis has a complex etiology and pathogenesis, lingering course, great difficulty in cure, easy to relapse after recovery. It is a precancerous lesion of colon cancer, and has hundreds of extraintestinal manifestations[1, 2]. It has been listed as one of the modern refractory diseases by the World Health Organization and has become a hot issue in the field of digestive system of traditional Chinese medicine.

Although it is generally believed that salicylazosulfapyridine (SASP) and Azathioprine can control the progression of ulcerative colitis well, the treatment of UC can also cause lung lesions, such as pulmonary polycythemia, eosinophilic pneumonia, interstitial pneumonia and allergic pneumonia[3–5]. Therefore, alternative treatment options are needed. Traditional Chinese medicine has a history of thousands of years in disease treatment. At present, there are reports on the treatment of ulcerative colitis with traditional Chinese medicine[6–10]. In this study, we chose Sodium Houttuynia, Matrine and HQHLD to study the intervention mechanism of traditional Chinese medicine in the treatment of UC according to clinical experience.

2. Materials And Methods

2.1 The Information about Traditional Chinese Medicine

To mimic the traditional route of administration, the Traditional Chinese Medicine was suspended in distilled water and given to the animals intragastrically using a feeding tube. We administered Huangqi Huanglian Decoction (Table 1) which consist of 13 Chinese herbal medicines. This compound decoction free granule was purchased in Dongzhimen Hospital (Beijing) and was dissolved in water at 70°C for 30 min, stored at-20°C. Two Traditional Chinese Medicine monomer we used were as follows: Matrine (lot number: 110805–200508, China Institute of biological medicine), Sodium Houttuynia (lot number: 100247–199601, China Institute of biological medicine). Western medicine control is Sulfasalazine (lot number: H31020450, Shanghai Sanwei Pharmaceutical Co., Ltd.)

Table 1
Composition of Huangqi Huanglian Decoction

Chinese medicinal plant	The plant name in"The Plant List"	Batch number	Weight (g)
Astragalus membranaceus	*Astragalus membranaceus* (Fisch.) Bunge	19071411	30
Coptis Chinensis	*Coptis chinensis* Franch	19080711	10
P. veitchii Lynch	*Paeonia veitchii* Lynch	19040411	15
Paeonia Lactiflora Pall	*Paeonia lactiflora* Pall.	19070321	15
Aucklandia lappa Decne	*Aucklandia lappa* DC.	19093041	6
Galla chinensis	*Rhus chinensis* Mill.	19020211	6
Licorice	*Glycyrrhiza glabra* L.	19102121	6
Panax notoginseng	*Panax notoginseng* (Burkill) F.H.Chen	19021161	3
Atractylodes macrocephala	*Atractylodes macrocephala* Koidz	19082551	10
Areca nut	*Areca abdulrahmanii* J.Dransf.	19061031	10
Sanguisorba officinalis L	*Sanguisorba officinalis* L.	19051651	15
Dandelion	*Taraxacum borealisinense* Kitam.	19081521	20
Coix Seeds	*Coix aquatica* Roxb. Seeds	19090431	20

2.2 Animals

Male Wistar rats weighing 200 ± 10 g were purchased from the Academy of Military Medical Laboratory Animal Centre (Beijing, China). Ten male New Zealand rabbits weighing about 3 kg were purchased from Haidian Thriving Animal Farm (Beijing, China). All animals were housed at SPF Animal Laboratory of Dongzhimen Hospital (Beijing, China) with free access to food and water and were kept in a regulated environment (23 ± 2℃) under a 12-hour light/dark cycle (with light turning on at 8:00 am). All animal procedures were performed strictly within national regulations and the guidelines of the National Institutes of Health (NIH) and approved by the Animal Experimentation Committee at Beijing University of Chinese Medicine. The rats were randomly divided into four groups as follows: normal 1 w (n = 10); model 1 w (n = 15): immune-2,4,6-trinitrobenzenesulfonic acid (TNBS)-Ethanol induced UC rats, (1 week after TNBS-ethanol enema); normal 5 w (n = 10); model 5 w (n = 15): immune-TNBS-ethanol induced UC rats, 5 weeks after TNBS-ethanol enema.

2.3 Induction of Ulcerative Colitis and Traditional Chinese Medicine

Antigen preparation: Ten rabbits were killed by CO_2 suffocation, then the colon was removed immediately and rinsed with sterile saline and the mucosa was scraped off. The mucosa was mixed with equal saline and homogenized 30 times with a tight Dounce homogenizer (Sigma, USA). Samples were further disrupted by intermittent sonication (six 30 s pulses with a 1 min cooling-down period in between) and then centrifuged at 3,000 rpm (Eppendorf, Germany) for 30 min at 4℃. The supernatant was then aliquoted and stored at -20℃. The protein concentration was measured by bicin choninic acid (BCA) assay (CoWin Bioscience, China). Antigen preimmunization and TNBS enema: Rabbit intestinal mucosal antigen solution was mixed with an equal volume of Freunds complete adjuvant in order to prepare the antigen emulsion. Wistar rats were immunized with the antigen emulsion in the paws and groin alternately at day 1, day 15, and day 22. Each immune volume contained 8 mg of antigen protein per rat. On day 29, the rats were anesthetized with 1% Pentobarbital Sodium, and then a medical-grade polyurethane cannula for enteroclysm (external diameter 2mm) was inserted into the anus, and the tip was advanced to 8 cm proximal to the anal verge. TNBS (Sigma, USA) dissolved in 50% ethanol was instilled into the colon rapidly with TNBS 100mg / Kg (0.5-0.7ml per rat). Then the rats were maintained in a head-down position for one minute to prevent leakage of the intra-intestinal instillation. The rats that did not die were randomly divided into seven groups after the TNBS-alcohol enema. Subsequently, the rats with ulcerative colitis received an intragastric administration with Traditional Chinese Medicine. All the experimental rats were killed by CO_2 asphyxiation after 1 week and 5 weeks later respectively.

2.4The histopathological and fibrotic changes of the Intestine were observed

The bowel tissue of the rats was taken and soaked in 4% paraformaldehyde solution. The 4% paraformaldehyde solution was changed every day. One week later, the fixed specimens were routinely dehydrated with alcohol gradient, transparent xylene, embedded with paraffin, and sectioned at 5 mm thick. Two sections were taken from each rat for HE staining and Masson staining. Then the

morphological changes and fibrotic changes of the bowel tissue in each group were observed and photographed under the light microscope.

2.5 Enzyme Linked Immunosorbent Assay

Each group of rats was not given anything to eat for 24–48 hours before killing. After anaesthetizing with 1% Pentobarbital Sodium by peritoneal injection, blood was drawn from the abdominal aorta, the blood serum was deep frozen in liquid nitrogen, and all rats were killed using CO_2 suffocation. The bowel tissue was also taken off, washed with normal saline, the water was absorbed using filter paper, and the tissue was deep frozen in liquid nitrogen. When the tissue was used, it was disrupted with an ultrasonic disintegrator using intermittent sonication (six 30 s pulses with a 1 min cooling-down period in between), and then the sample was centrifuged at 3,000 rpm for 20 mins at 4°C. The supernatant was collected, TGF-β1 was measured in the bowel and in the serum, MadCAM1 in the serum was detected with an ELISA kit (Beijing Kangyua Ruide biotechnical Co. Ltd.).

2.6 Real-time PCR Analysis

The total RNA from the bowel tissue was extracted by using a TRZol reagent extraction kit (Invitrogen Life Technologies,Inc). The mRNA expressions of MadCAM1 and GAPDH in the bowel of the rats in each group were detected by real-time PCR. The sequences of the PCR primers were depicted in Table 2. Ct of every sample was analyzed by MxPro-Mx3000p software. The relative amount of each gene was calculated utilizing the expression of GAPDH as internal control and using the Eq. $2^{-\triangle\triangle Ct}$ where △Ct = (Ct gene-Ct GAPDH). △△Ct=△Ct-average (△Ct normal), cDNA$2^{-\triangle\triangle Ct}$. All real-time PCR experiments were performed with Pwer SYBR Green PCR Master Mix (Applied Biosystems company,USA).

Table 2
PCR Primer(s)

Gene	Sequence of primers (5' -3')	Product Size	Cycling parameters
MadCAM-1	F:CCG AAA TCC ACC AGA ACC R: TCC AAT GCA CCG TCA CTC	81 bp	10min at 95°C, 30sec at 95°C, 30sec at 54°C, 20 sec at 72°C, for 40 cycles
GAPDH	F: CCA TGG AGA AGG CTG GG R: CAA AGT TGT CAT GGA TGA CC	195 bp	10min at 95°C, 30sec at 95°C, 30sec at 54°C, 20 sec at 72°C, for 40 cycles

2.7 Western blot Analysis

The bowel tissue was then lysed with 200 µl RIPA lysis buffer, the protein contents were quantitated using BCA protein reagent assay kit (CoWin Bioscience, China) and analysed by 10–12% of SDS-PAGE, followed by immunoblotting using enhanced chemiluminescence substrate (Merck Millipore) according to the manufacturer's instructions. The protein expressions of IL-10 in the bowel were detected by western blotting. Bands were visualised using a chemiluminescent detection system (ProteinSimple, San Jose, CA, USA).

2.8 Statistical Analysis

Quantitative data were expressed as mean ± standard deviation (S.D.). The significant difference between groups was assessed using the Student's t-test and one-way ANOVA, post hoc comparisons were made using the nonparametric Dunn multiple comparison test. In all tests, the criterion for statistical significance was $P < 0.05$. Statistical analysis was performed with SPSS Software 13.0.

3. Results

3.1 Toxicity evaluation of Traditional Chinese medicine on rats

After 4 weeks of drug administration, the weight of the model group rats significantly decreased, compared to that of the normal group rats ($P < 0.01$) (Fig. 1). The results showed that the weight of the rats decreased after modeling and increased after administration of the Traditional Chinese medicine. There was no significant difference in the serum ALT, AST, BUN and Cr ($P > 0.05$) after 4 weeks of administration (Fig. 2). The above results showed that there was no significant difference in body weight and hepatorenal function of all treatment groups. There was no hepatorenal damage after modeling and administration. Traditional Chinese medicine has no side effects on rats.

3.2 The protective effects of Traditional Chinese medicine on the intestine/ the colon tissue

After 4 weeks of administration, the colon tissue structure of the normal group was intact, the intestinal mucosa had no changed, and there was no inflammatory cell infiltration. In the model group, there were extensive lymphocytic inflammatory infiltration, necrosis of the colon wall, and a large number of ulcers (Fig. 3). In SASP group, the necrosis of the intestinal wall was significantly reduced, and there was still extensive inflammatory infiltration of lymphocytes in the intestinal mucosa, myometrium, submucosa and myometrium. Some of the mucosa surface had shallow ulcers, the surrounding mucosa structure was disordered, and the glands were atrophied. In the Sodium Houttuyniae group and Matrine group, mucosal ulcer formation and tissue necrosis were significantly reduced, but a large number of inflammatory cells still infiltrated the mucosal layer and submucosa, and the surrounding mucosal structure was disordered. In the HQHLD group, inflammatory cell infiltration and tissue necrosis were significantly reduced (Fig. 3). Massons staining visualized that substantial collagen tissue existed on the intestinal mucosal ulcer surface and the intestinal mucosal basement in the model group, even replace normal mucosa and glands. In the SASP group, the proliferation of collagen tissue on the surface of intestinal mucosa and the basal part was more than that in the normal group, but less than that of the model group. In the Sodium Houttuyniae group, the proliferation of collagen tissue in the intercellular and basal part of the intestinal mucosa was significantly lighter than that of the model group, but there was no significant difference compared to the normal group. In the Matrine group, the proliferation of collagen was more than in the normal group, but less than in the model group. In the HQHLD group, the proliferation of intercellular and basal collagen tissue was more than in the normal group, but less than that of the model group (Fig. 4).

3.3 Up-regulated expression levels of TGF-β1 and IL-10 in the Traditional Chinese Medicine groups

After 4 weeks of administration, the results showed that the expression of TGF - β1 in the bowel tissue of the model group was lower than that of the normal group, but there was no significant difference ($P > 0.05$). Moreover, there was no difference between the SASP group and the model group ($P > 0.05$). The expression of TGF - β1 in Sodium Houttuyniae group, matrine group and HQHLD group was significantly higher than that of the model group ($P < 0.01$), but there was no significant difference when compared with the normal group ($P > 0.05$) (Fig. 5A). After 4 weeks of administration, the level of TGF - β1 in the serum of the model group was higher than that of normal group ($P < 0.05$), the levels of TGF - β 1 in serum of Sodium Houttuyniae group and HQHLD group were also higher than that of the normal group ($P < 0.01$). In the SASP group and Matrine group, there was no significant difference compared with the normal group ($P > 0.05$) (Fig. 5B). WB results showed the IL − 10 level in the bowel tissue of the model group was significantly lower than that of the normal group ($P < 0.05$), the IL-10 level in the SASP group, Sodium Houttuyniae group, Matrine group and HQHLD group was not significantly different compared to the normal group ($P > 0.05$). The IL − 10 levels were significantly increased in all Chinese Medicine treatment groups, including Sodium Houttuyniae group, Matrine group and HQHLD group compared to the model group ($P < 0.05$) (Fig. 5C and 5D). The above results showed that the Sodium Houttuyniae group, Matrine group and HQHLD group could up-regulate the expression of TGF - β 1 and IL − 10, indicating that all Traditional Chinese medicine could also play an anti-inflammatory role by increasing TGF - β1 and IL − 10 in intestinal tissue.

3.4 Traditional Chinese medicine application reduced the expression of MadCAM-1 mRNA and protein

After 4 weeks of drug administration, the model group had significantly higher level of MadCAM-1 mRNA expression in the bowel tissue than the normal group ($P < 0.01$), the expression level of MadCAM-1 mRNA in the bowel tissue of all treatment groups (SASP group, Sodium Houttuyniae group, matrine group and HQHLD group) was significantly lower than that of the model group ($P < 0.05$), but there was no significant difference in the SASP group and HQHLD group compared with the normal group ($P > 0.05$) (Fig. 6A). The above results showed, after 4 weeks of administration, all the administered types of Traditional Chinese Medicine could decrease the expression of MadCAM-1 mRNA in bowel tissue compared to the model group, which indicated that as seen in the SASP group, all Chinese Medicine groups could alleviate bowel inflammation by reducing lymphocyte homing to the bowel mucosa. ELISA showed that the serum level of MadCAM-1 in the model group did not increase significantly after 4 weeks of administration, but the transcription level of MadCAM-1 mRNA in the bowel tissue increased significantly, which may be caused by the homing of lymphocytes from peripheral to specific tissues. After 4 weeks of administration, the expression of MadCAM-1 protein of Matrine group and HQHLD group increased significantly in the serum, which was contrary to the expression level of MadCAM-1 mRNA in the bowel tissue (Fig. 6B). This may be due to the Matrine group

and HQHLD group being able to reduce the homing of lymphocytes to the bowel tissue and increasing the homing of lymphocytes to the peripheral blood or other tissues, so as to reduce the bowel inflammatory response.

4. Discussion

Traditional Chinese medicine has been used for treating diseases for thousands of years. It has not been widely used in the world because of its complex composition and obscure pharmacological mechanisms. With the development of science and network pharmacology, the mechanism of traditional Chinese medicine is now becoming more clear[11].On the basis of previous studies, the immune-TNBS-ethanol rat model was established. The intervention mechanism of Traditional Chinese medicine in UC rats was explored by observing the expression of related factors in rats. We found that Sodium Houttuyniae, Matrine and HQHLD could reduce the intestinal ulcer formation and tissue necrosis and decrease the intestinal inflammatory response of UC, so as to laterally verify the the effectiveness of traditional Chinese medicine against ulcerative colitis.

TGF - β and IL − 10 are both negative regulators of the inflammatory response[12]. TGF - β1 is not only a multi-effect cytokine, but also an important anti-inflammatory factor of UC. Normal expression can inhibit inflammatory reaction and cell proliferation, regulate cell growth, cell differentiation and immune function, while overexpression can promote the process of intestinal fibrosis[13, 14]. Rezaie et al. found that there was a lot of TGF - β1 in the saliva of UC patients, which was mainly expressed in the inflammatory cells of the lamina propria near the oral surface, suggesting that TGF - β1 may play an important role in promoting epithelial healing after mucosal injury. Babyatsky[15, 16] found that the expression of TGF - β was significantly increased in the mucosa of UC and CD patients. The expression of TGF - β1 mRNA was mainly located in the lamina propria cells on the surface of the intestinal mucosa with a large number of inflammatory cells in UC and CD patients and the TGF - β1 regulated the remodeling and functional characteristics of epithelial cells in the lamina propria of mucosa, which could be the key cytokine in the inflammatory phase. Monteleone[17] first revealed that the defect of the TGF - β1 cascade amplification signal was related to the pathogenesis of IBD. Therefore, TGF - β1 induction played an important role in the regulation of the intestinal immune system. The increase of TGF - β1 and its receptor TGF β RI in the active phase of UC could be the protective response of the body, which had the role of remodeling and maintaining the remission of UC pathological intestinal mucosa. IL − 10 was mainly produced by Th2 cells, monocytes, and macrophages. It can down-regulate the production of pro-inflammatory cytokines and Th1 cytokines, inhibit the activation of monocytes, macrophages, granulocytes and T cells, and play an important role in a stable mucosal environment. The anti-inflammatory cytokine IL − 10 dampens intestinal inflammation and is therefore a good candidate gene for IBD[18, 19]. In animal experiments, it was found that IL − 10 knockout mice could spontaneously lead to chronic non-infectious intestinal inflammation in the general environment without specific pathogenic bacteria[20]. Clinical studies had found that the expression of IL − 10 mRNA in the intestinal mucosa of UC patients was reduced, and so was the production of IL − 10 mRNA by monocytes in the lamina propria of the inflammatory colon mucosa. The over-expression of IL − 10 in different animal models of ulcerative colitis had proved beneficial[21]. It was found that IL − 10 modified by Lactococcus had a positive effect on the improvement of experimental colitis[22, 23]. Bifidobacterium modified IL − 10 expanded the bacterial species of IL-10 intestinal delivery[24].

The results of this study showed that the expression of anti-inflammatory factor TGF - β1 in intestinal tissue decreased after 4 weeks of administration, which indicated that the synthesis of TGF - β1 in the body decreased and could not play the role of inhibiting the of Traditional Chinese medicine inflammatory reaction. After treatment, TGF - β1 levels in each group had an upward trend, and the expression of TGF - β1 in all Traditional Chinese medicine group (Sodium Houttuyniae group, Matrine group and HQHLD group) increased significantly, and the most significant increase was found in the HQHLD group. The above results showed that the traditional Chinese medicine worked better in increasing the anti-inflammatory factors in the intestine, and it also showed that the anti-inflammatory effect of Traditional Chinese medicine compound was higher than that of a single monomer. After 4 weeks of administration, the level of TGF - β1 in the serum of the model group increased significantly, which could be owing to the activation of anti-inflammatory and repair mechanisms. The expression levels of TGF - β1 in all treatment groups were higher than in the normal group, especially in the Sodium Houttuyniae group and the HQHLD group, which indicated that Sodium Houttuyniae and HQHLD had stronger anti-inflammatory and repair functions, and also showed that the Sodium Houttuyniae and HQHLD were strengthened the body and removed pathogenic factors. The expression of IL − 10 in the model group was significantly lower than that of normal group. The expression of IL − 10 in all treatment groups was significantly higher than that in the model group. The increase of IL − 10 in Traditional Chinese medicine treatment groups were particularly significant, which indicated that the UC model inhibited the expression of anti-inflammatory factor IL − 10. The Sodium Houttuyniae, Matrine and HQHLD could increase IL − 10 and play an anti-inflammatory role.

Adhesion molecule is a kind of transmembrane glycoprotein which can mediate cell adhesion, chemotaxis and homing of lymphocytes. It is a kind of adhesion molecule selectively expressed on the surface of vascular endothelial cells of the intestinal mucosa and its related

lymphoid tissues, which mainly mediates the selective homing of lymphocytes to normal mucosa and participates in the immune response and inflammatory response in the gastrointestinal tract. The integrin family member α4β7 is the receptor of MadCAM-1. After CD45RB high CD4 + T cell reconstruction, the colitis model of SCID mice were treated with Anti-MadCAM-1 or α4β7 specific antibody, the number of lymphocytes in the colon was significantly reduced, suggesting that MadCAM-1 /α4β7 participated in the infiltration of lymphocytes into the inflammatory bowel[25]. Battat found that the inhibition of α4β7-mediated lymphocyte trafficking was effective in ulcerative colitis[26]. Clinical and animal experimental studies had confirmed that UC was related to the abnormal expression of MadCAM-1, and the expression of MadCAM-1 in vascular endothelial cells in the inflammatory colon of UC patients was up-regulated. The expression level of MadCAM-1 in the venules of the lamina propria of colon in mice with hapten induced colitis[27] or IL-2 gene knockout mice[28] was significantly increased. The expressions of MadCAM-1 in the lamina propria and submucosa of the mouse colitis model induced by Oxazolidone, TNBS and DSS were also increased. Shigematsu[29] applied anti-MadCAM-1 monoclonal antibodies to pretreat colitis in mice, which significantly reduced the adhesion of lymphocytes on endothelial cells of the intestinal mucosa. Inhibition of MAdCAM-1 and leukocyte recruitment can improve DSS induced colitis.

The results of the PCR experiments showed that the expression of MadCAM-1 mRNA in the intestinal tissue increased significantly after 4 weeks of drug administration, suggesting that the pathogenesis of intestinal injuries in this UC model was related to the directional homing of lymphocytes to the intestinal mucosa mediated by MadCAM-1. Sodium Houttuyniae, Matrine and HQHLD could reduce the expression of MadCAM-1 mRNA in intestinal tissues, the effect of HQHLD was particularly significant. After 4 weeks of drug administration, the expressions of MadCAM-1 in the serum of all treatment group were up-regulated, while the expressions of MadCAM-1 in intestinal tissue were down-regulated, which could be due to the increase of lymphocyte homing to the peripheral blood and other tissues, and the decrease of lymphocyte homing to the intestinal tissue in treatment groups, so as to reduce intestinal inflammatory reactions. The changes after administering HQHLD were the most significant, which indicates that the effect of Chinese medicine compound HQHLD on UC was the best.

5. Conclusion

This study revealed the intervention mechanism of traditional Chinese medicine in the treatment of UC, which provided further theoretical and scientific basis for the clinical treatment of ulcerative colitis. HQHLD, the representative prescription, had the most significant positive effect. The above results provided further theoretical and scientific basis for the clinic treatment of ulcerative colitis. Our results will likely help expand clinical practice using HQHLD as an effective Traditional Chinese medicine compound to treat ulcerative colitis.

Abbreviations

UC	Ulcerative colitis
IBD	Inflammatory bowel disease
TNBS	2,4,6-trinitrobenzenesulfonic acid
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
Cr	Serum creatinine
SASP	Sulphasalazine
HQHLD	Huangqi Huanglian Decoction
TGF-β1	Transforming growth factor-β 1
IL-10	Interleukin-10
MadCAM-1	Mucosal addressin cell adhesion molecule-1

Declarations

Ethics approval and consent to participate

The study was approved by the Animal Experimentation Committee at Beijing University of Chinese Medicine.

Authors Contributions

SJ and LN conceived and designed the study; SJ, LZ and XY performed the experiments and collected the data; LC, LN and MQ contributed to analyzing the data; SJ wrote the manuscript; XW and LZ made manuscript revisions. All authors read and approved the manuscript and all data were generated in-house and that no paper mill was used.

Conflict of interest

The authors declare that they have no competing interests.

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Figures

Figure 1

Toxicity evaluation of traditional Chinese medicine on rats. UC models were established with a mucous membrane of colon allergize combine with TNBS-alcohol enteroclysis. The model rats intervened with SASP, Sodium Houttuynia, Matrine, HQHLD. The changes of body weight were observed. Compared with Normal group, * P<0.05; ** P<0.01; compared with Model group, # P<0.05.

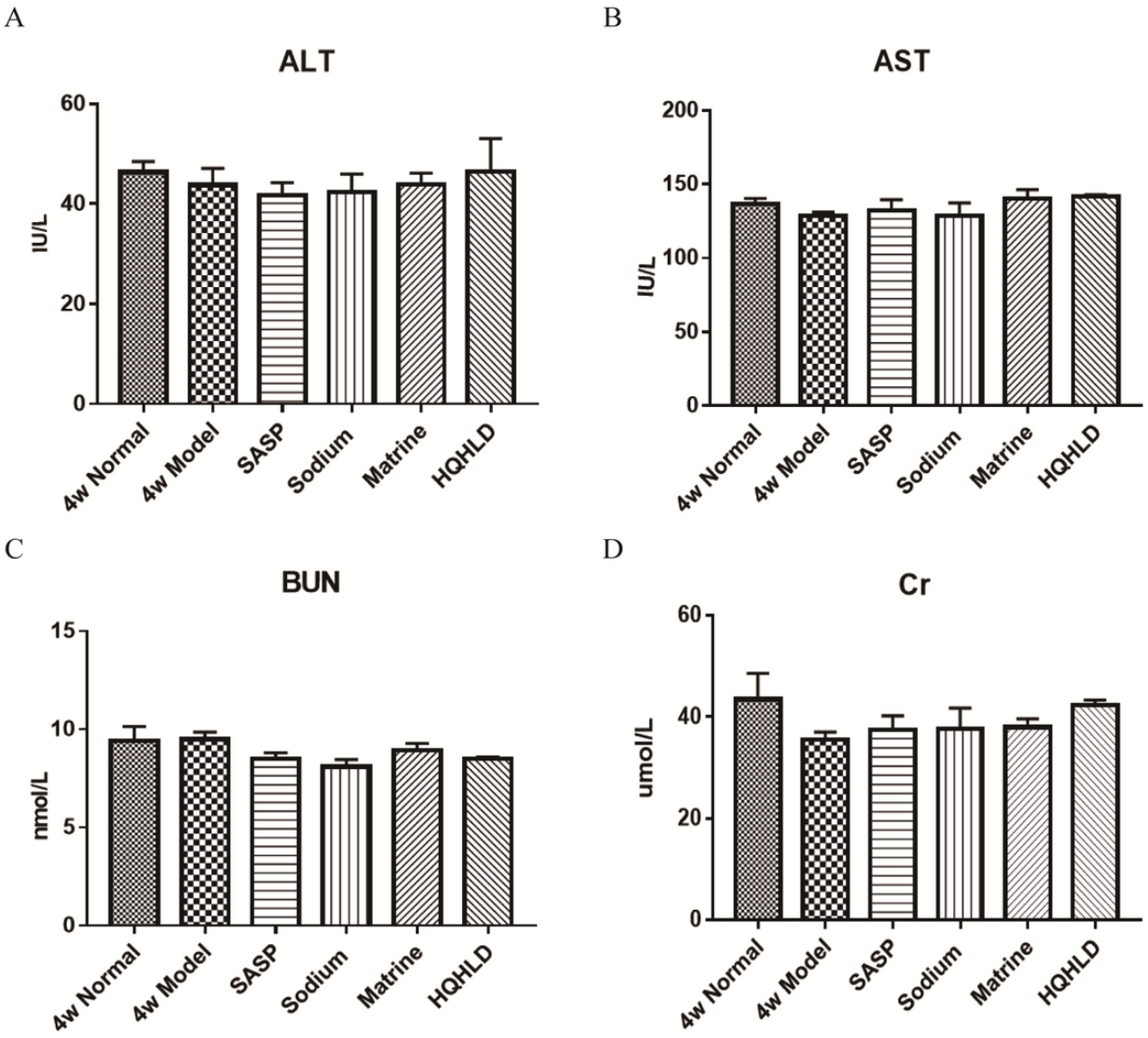

Figure 2

Toxicity evaluation of traditional Chinese medicine on rats regarding the hepatorenal function. UC models were established with a mucous membrane of colon allergize combined with TNBS-alcohol enteroclysis. The model rats intervened with SASP, Sodium Houttuynia, Matrine, HQHLD. ALT, AST, BUN and Cr were detected. Compared with Normal group, Compared with Normal group, * $P<0.05$; compared with Model group, # $P<0.05$; ## $P<0.01$.

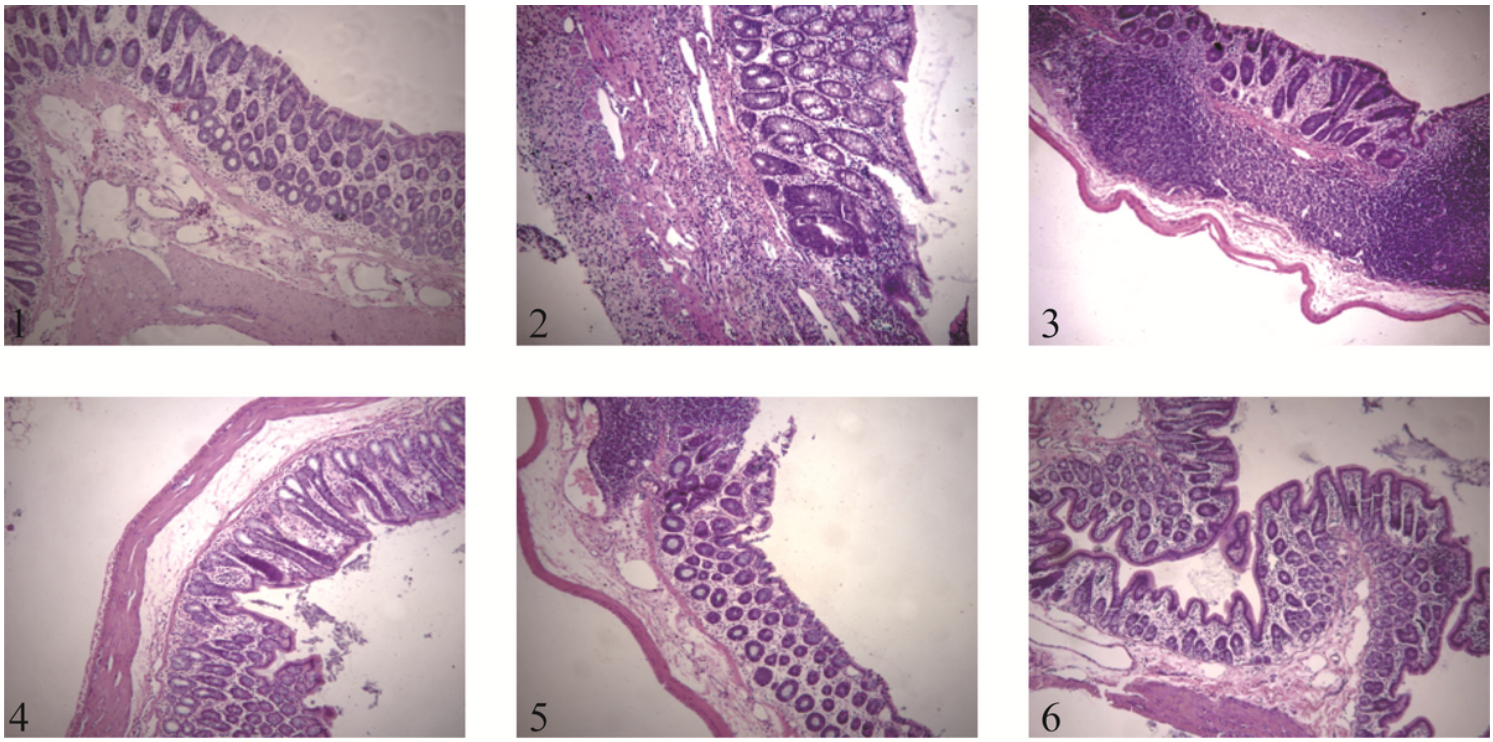

Figure 3

HE staining was performed to observe the histopathological changes of the intestine/ the bowel tissue. UC models were established with a mucous membrane of colon allergize combine with TNBS-alcohol enteroclysis. Changes of Bowel in 4w, and the model rats intervened with SASP, Sodium Houttuynia, Matrine, HQHLD. 1(4w normal), 2(4w model), 3(SASP group), 4(Sodium Houttuynia), 5(Matrine), 6(HQHLD group).

Figure 4

Masson staining was used to observe tissue collagen . UC models were established with mucous membrane of colon allergize combine with TNBS-alcohol enteroclysis. Changes of Bowel in 4w, and the model rats intervene with SASP, Sodium Houttuynia, Matrine, HQHLD. 1(4w normal), 2(4w model), 3(SASP), 4(Sodium Houttuynia), 5(Matrine), 6(HQHLD group).

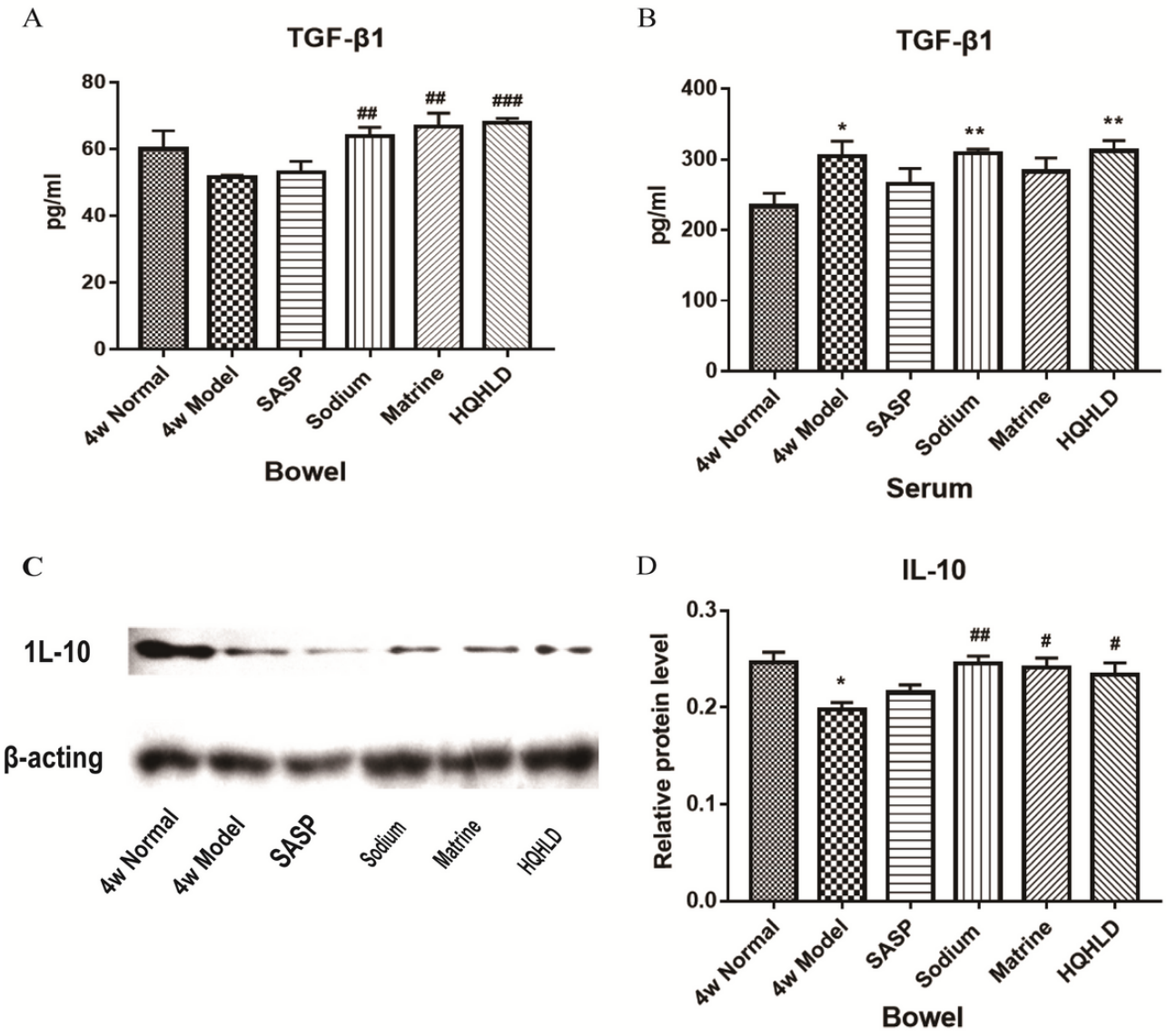

Figure 5

Traditional Chinese medicine groups up-regulated the expression levels of TGF-β1 and IL-10. UC models were established with mucous membrane of colon allergize combine with TNBS-alcohol enteroclysis. The model rats intervene with SASP, Sodium Houttuynia, Matrine, HQHLD. (A) TGF - β1 in bowel. (B) TGF - β1 in serum. (C) IL-10 in bowel (WB). (D) IL-10 level in bowel (three independent experiments). Compared with Normal group, Compared with Normal group, *P<0.05; **P<0.01;***P<0.001. Compared with Model group, # P<0.05; ## P<0.01; ### P<0.001.

Figure 6

Traditional Chinese medicine application reduced the expression of MadCAM-1 mRNA and protein. UC models were established with mucous membrane of colon allergize combine with TNBS-alcohol enteroclysis. The model rats intervene with SASP, Sodium Houttuynia, Matrine, HQHLD. (A) MadCAM-1 in bowel. (B) MadCAM-1 in serum. Compared with Normal group, Compared with Normal group, ***P<0.001. Compared with Model group, ## P<0.01; ### P<0.001.