Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.125522

Protective effect of five-flavor sophora flavescens enteric-coated capsules on inflammatory bowel disease and its molecular mechanism

Yanru XIANG[1], Bing JIANG[2], Shirong ZHONG[3], Yinglin WU[4], Jiaqi LIU[5], Zhizhi WANG[6*], Yan WU[1*]

Abstract

This study aims to investigate the effect of five-flavor sophora flavescens enteric-coated capsules (FSEC) on TNF-α-induced inflammatory bowel disease and its molecular mechanism. Wistar Rats were divided divided into 6 groups: Normal control group (group A): normal diet, drinking water; Model group (group B): 100 μg/L TNF-α; FSEC high-dose group (group C): 100μg/L TNF-α + FSEC (432 mg/kg); FSEC medium-dose group (group D): 100 μg/L TNF-α + FSEC (216 mg/kg); FSEC low-dose group (group E): 100 μg/L TNF-α + FSEC (108 mg/kg); Positive control group (group F): 100 μg/L TNF-α + 500 mg/kg sulfasalazine (SAZ). Animals in each group were intragastrically administered twice daily for 7 days. Animals were sacrificed 24 hours after the last treatment and colon tissues were collected for subsequent experiments. The results of HE staining showed that the colonic tissue of TNF-α-fed animals appeared damage, while the colonic tissue of animals treated with FSEC was improved to various degrees, and the histological characteristics of colon were basically recovered in the high-dose group, suggesting that FSEC could be used to treat TNF-α-induced colonic tissue damage. According to the results of ELISA and immunohistochemistry, the recovery of colonic tissue structure in rats treated with different doses of FSEC might be related to the decrease of TNF-α, IL-6, IL-17, TLR-4 and NF-κB proteins expression. According to the results of Western blotting, TNF-α-pretreated IEC-6 cells cultured with medicated serum decreased the expression of TRIF and IFN-γ proteins. These results suggest that FSEC has a protective effect on ulcerative colitis (UC), and the mechanism may be through inhibiting the activation of TLR-4/NF-κB signaling pathway and preventing the release of related inflammatory factors.

Keywords: five-flavor sophora flavescens enteric-coated capsules; ulcerative colitis; TLR-4/NF-κB signaling pathway; inflammatory factors.

Practical Application: This study not only provides the possibility to develop the value of Chinese herbal medicine, but also provides experimental basis and theoretical support for medical staff in the prevention and treatment of ulcerative colitis.

1 Introduction

Ulcerative colitis (UC), also known as chronic non-specific UC, is an unexplained rectal and colitis lesion, which has been classified as one of the modern refractory diseases by the world Health Organization (WHO) and seriously affects the normal life of patients (Regueiro et al., 2023). Clinically, it is mainly characterized by recurrent abdominal pain, diarrhea, mucopurulent bloody stools, tenesmus and so on. UC has no name of specific disease in ancient books of traditional Chinese medicine (TCM), and its clinical symptoms belong to the categories of "chronic dysentery", "intestinal dysentery", "diarrhea", "hematochezia" and "intestinal wind" in TCM. The course of UC is mainly chronic, alternating with attacks and remissions. A few symptoms can be persisted and gradually worsened, even developed to the possibility of carcinogenesis. In recent years, the incidence of inflammatory bowel disease has been increasing year by year (Jayasooriya et al., 2023; Abdel-Rahman & Morgan, 2023), especially the incidence of UC increased most significantly (Tian, 2021). Western medicine mainly uses medicines, such as aminosalicylic acid, hormone, immunosuppressive agent and biological agent, but it is difficult to be a long-term choice because of the expensive treatment cost and repeated illness after drug withdrawal (Song, 2018). Moreover, adverse reactions, drug dependence and other adverse situations are easy to occur after long-term use (Li et al., 2012). Therefore, it is particularly urgent to explore an efficient and safe therapeutic strategy.

TCM has unique advantages in regulating immunologic function, repairing intestinal mucosa, and relieving intestinal inflammation (Wang & Shao, 2022), and thus has received the attention from the majority of researchers. From the perspective of TCM, the etiology of UC can be divided into external and internal causes. Exogenous factors mainly include exogenous six evils, especially the evil of dampness and heat. And internal factors mainly include inadequate congenital endowment, emotional disorders, improper diet, visceral decay and other

Received 30 Nov., 2022
Accepted 19 Jan., 2023
[1]*Department of Integrative Medicine Anorectal, The First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, China*
[2]*Department of Integrated Chinese and Western medicine, Gansu University of Traditional Chinese Medicine, Gansu, Lanzhou, China*
[3]*Department of Rehabilitation Medicine, The Jiangxi Provincial People's Hospital, Nanchang, Jiangxi, China*
[4]*Department of Anesthesiology, The First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, China*
[5]*Department of Ophthalmology, The First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, China*
[6]*Department of Gastroenterology, The First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, China*
*Corresponding author: 15600787650@163.com; 495272212@qq.com

factors. As Zhao (2014) have concluded that deficiency of both spleen and kidney, imbalance of liver and spleen, and blocking the large intestine with damp-heat are mostly the syndromes of deficiency in origin and excess in superficiality. The deficiency in origin is deficiency of spleen and kidney, and the excess in superficiality is stasis of large intestine due to dampness, heat, blood stasis and toxin (Zhao, 2014). Chen J have believed that this disease is always a mixture of deficiency and excess, and the syndromes of deficiency in origin and excess in superficiality. Deficiency of spleen is the root of disease, and then the disease extends to kidney for a long time, which leads to deficiency of both spleen and kidney. In addition, the damp turbidity, heat toxin, blood stasis and other evils can be mutually affected, which is the superficiality of disease (Chen, 2005). Notably, Professor Xu Jingfan, a famous veteran teran doctors of TCM, also have believed that the pathogenesis of UC is the deficiency of spleen, which damages the liver and kidney. Deficiency of spleen and stagnation of dampness are its pathological basis. The critical pathogenesis is stagnation of intestines and organs due to damp-heat and blood stasis (Dai, 2019). According to the etiology and pathogenesis of UC, it is quite effective to treat this disease by taking TCM orally, enema with TCM, acupuncture and so on (Chen et al., 2022; Yu & Zhang, 2022; Xie & Lu, 2022). Among them, five-flavor sophora flavescens entered-coated capsules (FSEC) is a typical case of clinical application with TCM prescriptions. This drug is a new type of medicine developed by Professor Tong Zhanqi and his team for UC. This drug is an enteric-coated capsule, the content is grayish green powder, the smell is tiny, and the taste is slightly bitter. Meanwhile, FSEC can also be processed into functional food to promote the absorption capacity of the intestinal tract, thus contributing to the improvement of appetite (Wang et al., 2021). It is mainly composed of five medicines, consisting of sophora flavescens, bletilla striata, indigo naturalis, sanguisorba officinalis and raw licorice. Among them, according to the modern pharmacological studies, sophora flavescens, as the main component in FSEC, can not only relieve skin itching (He et al., 2015), but also process its related extracts (consisting of matrine, oxymatrine, Sophora flavescens tincture and so on) into skin care products (such as sophora flavescens gel) to balance oil secretion and remove skin endotoxin, so as to play a role in whitening and skin care (Shin et al., 2013). Notably, through a multicenter, randomized, double-blind, controlled study, it is found that the total effective rate of this drug can be as high as 92%, and it has almost no side effects (Tong et al., 2010), so as to be favored by clinicians. Although FSEC is effective in treating UC, its mechanism of action is not completely clear.

Thus, the aim of this study was to investigate the effect of FSEC on TNF-α-mediated UC and the underlying molecular mechanism through in vivo and in vitro experiments. We hope that these findings not only provide a new scientific basis for further supporting the development of TCM in the future, but also provide a new experimental basis for the application of FSEC in the prevention and treatment of inflammatory bowel disease. The experimental flow chart for this study is shown in Figure 1.

2 Materials and methods

2.1 Animal maintenance

36 adult male Wistar rats (8 weeks old, 180-200 g) were purchased from Zhejiang Vital River Laboratory Animal Technology Co., Ltd. (SCXK, Zhejiang, China) and maintained in a temperature-controlled room (20-25 °C, 45-50% relative humidity) on a 12/12 h dark/light cycle with free access to water and food. The operating procedures of all animals were

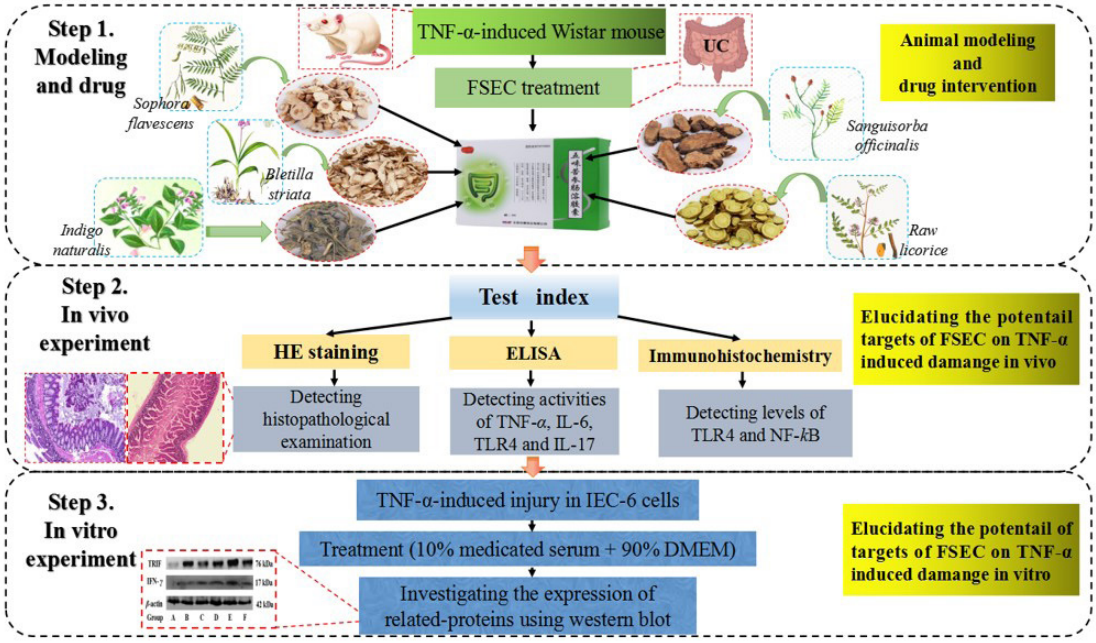

Figure 1. The flowchart of strategies for investigating the effect and mechanisms of FSEC on TNF-α-induced colonic injury. UC, ulcerative colitis; FSEC, five-flavor sophora flavescens enteric-coated capsules.

performed in according with the institutional guidelines for the care and use of laboratory animals and were approved by the ethics committee of the First Affiliated Hospital of Nanchang University (Jiangxi). After 1 weeks of acclimatization, these Wistar rats were used for subsequent experiments.

2.2 Drug preparation

FSEC was purchased from Beijing Zhonghui Pharmaceutical Co., Ltd. (Beijing, China), strength 0.4 g/capsule, cat. no. Z20150002, and the powder in FSEC was dissolved with sterile distilled water to prepare solutions with final concentrations of 108 mg/kg, 216 mg/kg and 432 mg/kg, respectively. Sulfasalazine (SAZ) was purchased from BioChemika Co., Ltd., purity ≥ 96.0%, cat. no. S838221, and the drug was refined and dissolved with sterile distilled water to prepare a solution with a final concentration of 500 mg/kg. TNF-α was purchased from Jiangsu Jingmei Biotechnology Co., Ltd. (Jiangsu, China), purity ≥ 98%, cat. no. kx30H-T, and the powder was dissolved with sterile distilled water to prepare a solution with a final concentration of 100 μg/L.

2.3 Main reagents

DMEM medium and phosphate buffered saline (PBS) were all purchased from Hyclone Co., Ltd (Logan, USA). Trypsin-EDTA solution, HE staining kit, radioimmunoprecipitation (RIPA), phenylmethylsulfonyl fluoride (PMSF), SDS-PAGE gel preparation kit, Tween-20, 10 x TBS, 10 x electrophoresis buffer, 10 x transfer buffer, BCA protein assay kit, and 4 x bromophenol blue buffer were all purchased from Solarbio Co., Ltd (Beijing, China). Penicillin-streptomycin was purchased from Basal Media Co., Ltd (Shanghai, China). Fetal bovine serum (FBS) was purchased from BI Co., Ltd (Grand Island, USA). 4% paraformaldehyde and additional enhanced chemiluminescent substrate kit were all purchased from Biosharp Co., Ltd (Beijing, China). Three-color prestained protein marker was purchased from YEASEM Co., Ltd (Shanghai, China). Xylene, 95% absolute ethanol and sodium citrate buffer were all purchased from DAMAO (Tianjin, China). Difco™ skimmed milk powder was purchased from Biotopped Co., Ltd (Beijing, China). The corresponding ELISA kits for TNF-α, IL-6, TLR4 and IL-17 were purchased from CoSinProtein Co., Ltd (Beijing, China; cat. no. KET6032, KET7009, KE1714 and YT5976, respectively). Mouse monoclonal TLR4 and mouse monoclonal NF-κB antibodies were all purchased from Protein Tech Group Co., Ltd (Chicago, USA; cat. no. 66350-1-Ig and KHC0634, respectively). Rabbit polyclonal TRIF, mouse monoclonal IFN-γ, mouse monoclonal β-actin and Horseradish peroxidase (HRP)-conjugated goat anti-mouse secondary antibodies were all purchased from Gen Tex Co., Ltd (Los Angeles, USA; cat. no. GTX65931, GTX34794, GTX629630 and GTX213111-01, respectively). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody was purchased from Zhongshan Golden Bridge Co., Ltd (Beijing, China; cat. no. ZB-2301)

2.4 Experimental instruments

Clean bench (SW-CJ-1F, Suzhou Purification Equipment Co., Ltd, China), incubator (BPN-RHP, Shanghai Yiheng Scientific Instrument Co., Ltd, China), 283-Frozen microtome (CM1850, Leica Instruments Co., Ltd, Germany), light microscope (Axio Imager Vario, Zeiss Co., Ltd, Germany), high flux frozen tissue grinder (YM-24LD, Shanghai Yuming Instrument Co., Ltd, China), microplate reader (KHB-ST-360, Shanghai Kehua Bioengineering Co., Ltd, China), thermostatic oven (BPG-9100BH, Shanghai Yiheng Scientific Instrument Co., Ltd, China), fluorescence microscope (Axio Vert. A1, Zeiss Co., Ltd, Germany), high speed frozen centrifuge (J2-21, Beckman Co., Ltd, USA), high speed desktop centrifuge (5415D, Eppendorf Co., Ltd, Germany), dry bath (TU10, Shanghai Yiheng Scientific Instrument Co., Ltd, China), thermostatic air shaker (4581, Forma Co., Ltd, USA), gel imaging system (CHEMIDOCTM-XRS+, Bio-rad Co., Ltd, USA).

2.5 Experimental groups

Wistar rats were randomly divided into 6 groups: (I) control group (n = 6); (II) model group (n = 6); (III) positive control group (n = 6); (IV) low-dose group (108 mg/kg, n = 6); (V) medium-dose group (216 mg/kg, n = 6); and (VI) high-dose group (432 mg/mL, n = 6). Rats were treated by intragastric administration of TNF-α (100 μg/L, twice a day) for 1 week. The positive control group (TNF-α + SAZ) was treated by intragastric administration of TNF-α (100 μg/L, twice a day), along with intragastric administration of SAZ (500 mg/kg, twice a day) for 1 week. The FSEC-treated group (TNF-α + FSEC) was treated by intragastric administration of TNF-α (100 μg/L, twice a day), along with intragastric administration of FSEC (108, 216, or 432 mg/kg, twice a day) for 1 week. Concentrations of TNF-α, SAZ, and FSEC were referenced to the relevant literatures (Yuan et al., 2018; Sawarkar et al., 2015; Bu et al., 2021). Rats in the control group were treated by intragastric administration of the same amount of sterile distilled water. Finally, all animals were sacrificed 24 h after the last treatment.

2.6 Preparation of medicated serums

Blood samples were collected from the abdominal aorta of sacrificed Wistar rats in each group. Whole blood was allowed to stand at room temperature for 4 h, and centrifuged after the blood coagulation (3,000 rpm, 15 min). Subsequently, the upper serums were drawn with a 1 mL syringe, and the obtained serums were filtered and sterilized by attaching a 0.22 μm microporous filter membrane, and then the sterile serums were inactivated in a 56 °C water bath for 30 min. The collected sterile serums were placed into 10 mL centrifuge tubes, numbered according to experimental grouping, and frozen in a -80 °C refrigerator for future use.

2.7 Cell culture and modeling interventions

Murine intestinal epithelial cell line IEC-6 were obtained from the cell bank of Shanghai Institute of Biological Sciences (SIBS, Shanghai, China). Cells were cultured in DMEM complete medium (10% FBS + 90% DMEM) and incubated in an incubator (95% humidity, 5% CO_2 and 37 °C). To mimic UC, the cells were subcultured when they grew to 70%~80% fusion in the culture bottle. After subculture, they grew to about 80% fusion and then used serum free DMEM to culture for 24 h, and then

the cells were incubated with 100 μg/L TNF-α for 24h to induce cells damage.

2.8 HE staining

HE staining was used to pathologically analyze the colon tissue of Wistar rats. In conclusion, three colon tissues were randomly selected from each group, and the residual blood stains on the tissues were washed three times (5 min/time) with pre-cooled normal saline and fixed with 4% paraformaldehyde for 48 h. Each tissue was then processed by an automated tissue processor and embedded into paraffin. The paraffin blocks were made into routine pathological sections (5.0 μm thickness) and stained with hematoxylin-eosin. Morphological changes in the tissues were observed under a light microscope and photographed with a Zeiss camera.

2.9 ELISA assay

Three colon tissues were randomly selected from each group, and the residual blood stains on the tissues were washed three times (5 min/time) with pre-cooled normal saline. Water from tissue surface were absorbed with filter paper, tissues were cut into several pieces with scissors, then theses tissue blocks were wash three times with pre-cooled normal saline (5 min/time) and putted into a grinder to prepare 10% tissue homogenate. Subsequently, the homogenate was centrifuged (3,000 rpm, 15 min) to obtain the supernatants. The activities of TNF-α, IL-6, TLR4 and IL-17 in the supernatants of each group were detected by ELISA kits according to the manufacturer ‘s protocol with the help of a microplate reader.

2.10 Immunohistochemical detecting

After sampling, the fixed colon tissues were washed and dehydrated step by step with ethanol (2 h/step). The tissue blocks were placed in an equal volume mixture of xylene and pure ethanol for 2 h, placed in xylene for two times (2 h/time), placed in an equal volume mixture of xylene and paraffin for 2 h, and then placed in paraffin for two times (3 h/time). Subsequently, the processed tissue blocks were embedded into paraffin, and the paraffin blocks were placed in a – 20 °C freezer for 30 min before routine pathological sectioning (5.0 μm thickness). The slices were baked in a thermostatic oven for 30 min, soaked for two times with xylene (15 min/time), soaked with different concentrations of alcohol for 5 min, and rinsed with distilled water. The slices were repaired with heat in 0.1 mmol/L sodium citrate buffer for 15 min, allowed to cool naturally, washed for three times with PBS (5 min/time), soaked in 3% H_2O_2 for 10 min, and washed for three times with PBS (5 min/time). The slices were added corresponding primary antibodies (TLR4, NF-κB; both 1:50) and incubated at room temperature for 1 h, and washed with PBS for three times (5min/time). Then, the slices were added HRP labeled broad-spectrum secondary antibody (1:2,00) and incubated at room temperature for 30 min, and washed again with PBS for three times (5 min/time). When the color change of the slices is observed by DAB staining, the staining slices can be washed away with PBS and counterstained with hematoxylin. When the hydrochloric acid alcohol is differentiated, the light microscope can be used for observation. After washing for 10 min, the slices were placed in the oven for drying, placed in xylene for transparency twice (3 min/time), and placed in the oven for baking again for 15 min. Finally, the slices were observed under a fluorescence microscope and quantified with Image J software (Bio-rad Co., Ltd, USA).

2.11 Western blotting

Cells were lysed with RIPA containing 1% PMSF for 60 min at 4 °C, and the supernatants were collected after centrifugation (4 °C, 12,000 rpm, 10 min). The concentration of total protein in each group was determined using the BCA protein assay. Samples were mixed with 4 x bromophenol blue buffer and boiled at 100 °C for 10 min. Each sample suspension was separated 30 μg proteins with 10% SDS-PAGE and transferred them to PVDF membranes. The membranes were sealed with 5% skimmed milk at 37 °C for 2 h, and wash it with 1 x TBS containing 0.05% Tween-20 (1 x TBST) for three times (5 min/time). Then, membranes were incubated overnight at 4 °C with the corresponding primary antibodies: Anti-TRIF antibody (1:1,000), anti-IFN-γ antibody (1:1,000) and β-actin antibody (1:5,000). The membranes were washed with 1 x TBST for three times (5 min/time), and incubated with the secondary antibody (1:5,000) for 2 h at room temperature, and then the membranes were washed again with 1 x TBST for three times (5 min/time). Proteins were visualized using an additional enhanced chemiluminescent substrate kit and gel imaging system. Quantitative analysis of the results was performed using Image Lab software (Bio-rad Co., Ltd, USA).

2.12 Statistical analysis

Experimental data were analyzed using IBM SPSS 22.0 statistical software (SPSS Co., Ltd, Chicago, USA) and data were presented as mean ± standard deviation (SD). $P < 0.05$ indicates a statistically significant difference, and $P < 0.01$ indicates a significant difference between groups. Multiple groups were compared by one-way analysis of variance (ANOVA), and differences between the two groups were analyzed by LSD-t test.

3 Results

3.1 Effect of FSEC on colon tissue histopathology

As shown in Figure 2A, no ulcer surface was observed in the colon tissue of normal Wistar rats, and the glandular structure was clearly layered. In addition, the mucosa was neatly arranged, intact, and smooth. In contrast, the mucosa of Wistar rats in group B was severely destroyed (such as defects, necrosis and so on), ulcers of different depths were observed, the glands in the mucosa were disorganized and deformed, crypt abscesses in the lamina propria glands were formed, and some glands could also proliferate to form polypoid structures. As shown in Figure 2C-F, the FSEC-treated groups could effectively protect colon tissues from TNF-α-induced morphological damage in a dose-dependent manner. To further observe changes in relevant cells (suan as lymphocytes, plasma cells and so on) in each group, the magnification was adjusted to x400. The results showed (Figure 3A-F) that the control group appeared a few

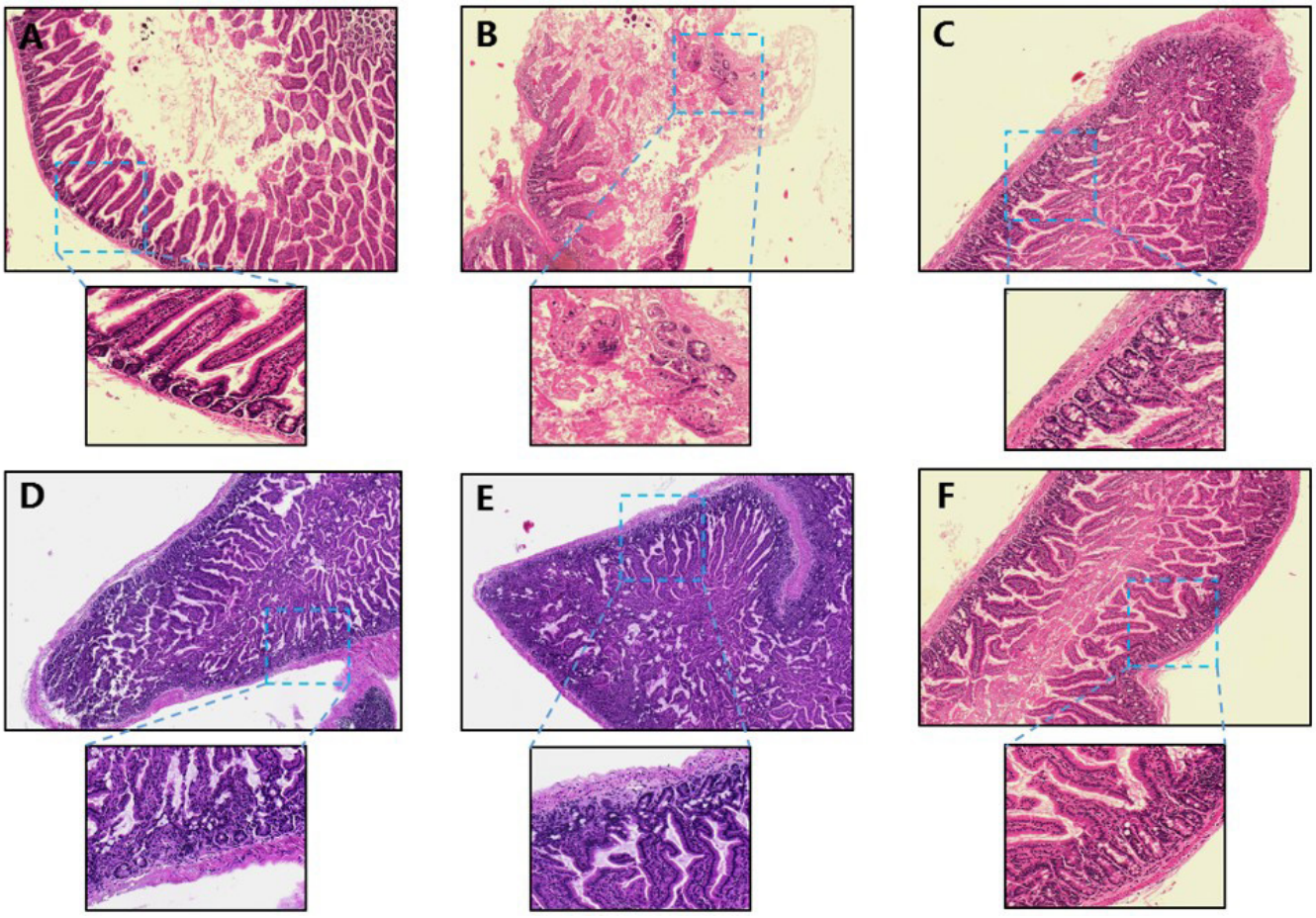

Figure 2. Histopathology of glandular structure in the colonic tissue. Magnifying glass, x200. Note: Blank control group (group A); Model group (group B); FSEC high-dose group (group C); FSEC medium-dose group (group D); FSEC low-dose group (group E); Positive control group (group F).

lymphocytes after HE staining, but no exudation of plasma cell, while the model group contained a large number of plasma cells in the lamina propria glands, and lymphocyte accumulation could occur at the base. In addition, compared with the model group, the drug treatment groups (FSEC or SAZ) improved the accumulation of lymphocytes and exudation of plasma cells with various degrees.

3.2 Effect of FSEC on TNF-α, IL-6, TLR4 and IL-17 protein activities

As shown in Table 1, compared with group A, the activities of TNF-α, IL-6, TLR4 and IL-17 proteins were significantly increased in the colon tissue of Wistar rats in group B (all $P < 0.01$). Compared with group B, the activities of TNF-α, IL-6, TLR4 and IL-17 proteins in the colon tissue of Wistar rats in the low-, medium-, and high-dose groups of FSEC (group E, D, and C, respectively) were dose-dependently decreased (all $P < 0.01$). In addition, there were no significant differences in the activities of TNF-α, IL-6, TLR4 and IL-17 proteins in colon tissue of Wistar rats between group C and group F (all $P > 0.05$).

3.3 Effect of FSEC on TLR4 and NF-κB proteins expression levels

As shown in Table 2, the expression levels of TLR4 and NF-κB proteins were significantly increased in group B compared with group A (all $P < 0.01$), while the expression levels of TLR4 and NF-κB proteins were significantly decreased in groups E, D, and C with a dose-dependent manner compared with group B (all $P < 0.01$). in addition, there were no significant differences in the expression levels of TLR4 and NF-κB proteins between group C and group F (all $P > 0.05$).

3.4 Effect of FSEC on TRIF and IFN-γ proteins expression levels

As shown in Figure 4 and Table 3, the relative expression levels of TRIF and IFN-γ protein in IEC-6 cells derived from murine small intestinal epithelial cells in group B showed an up-regulated trend compared with group A, while the relative expression levels of TRIF and IFN-γ proteins in IEC-6 cells treated with FSEC showed a dose-dependent decrease ($P < 0.05$). In addition, the relative expression levels of TRIF and IFN-γ proteins in IEC-6 cells

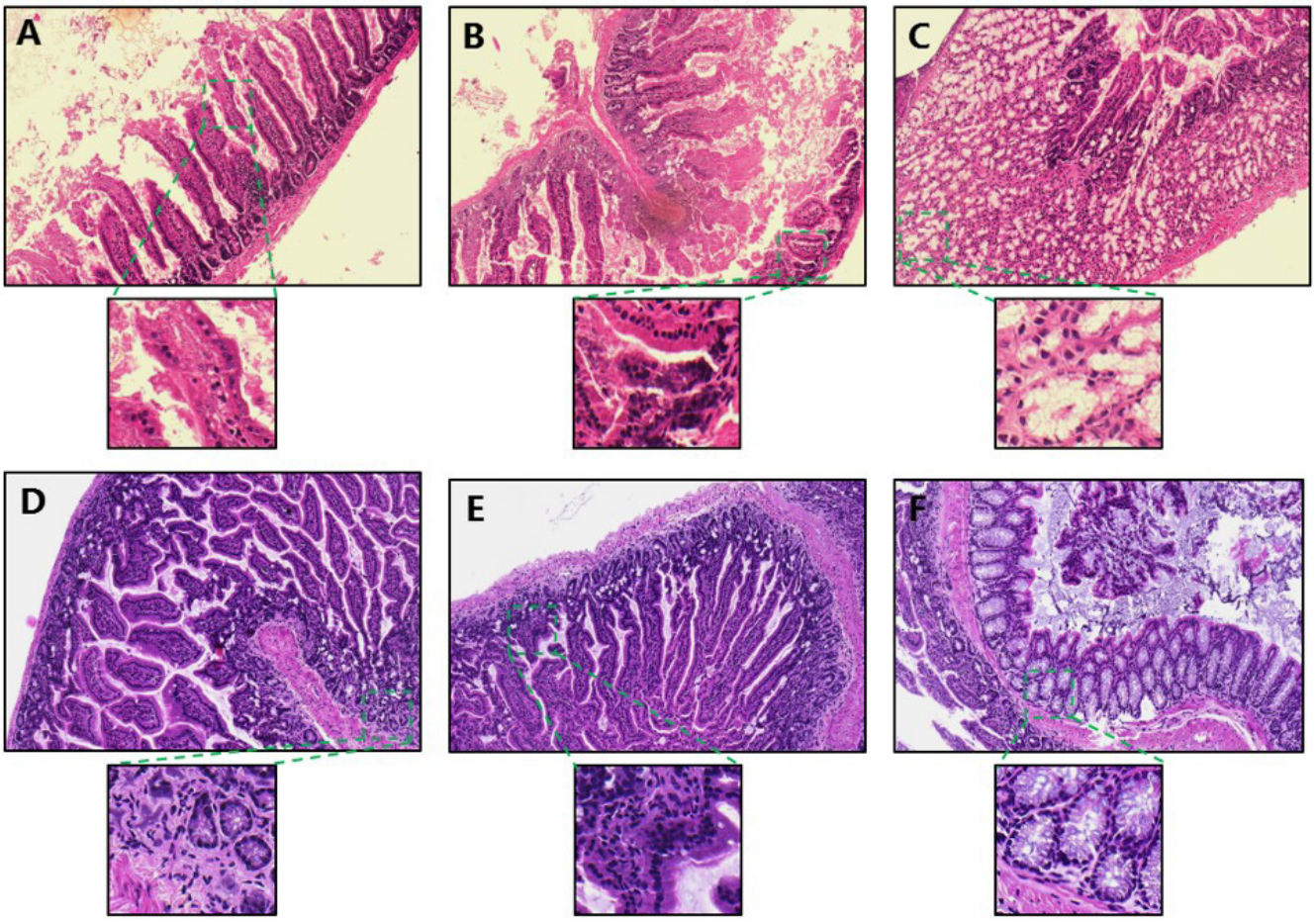

Figure 3. Distribution of lymphocyte and phlogocyte in the colonic tissue. Magnifying glass, x400. Note: Blank control group (group A); Model group (group B); FSEC high-dose group (group C); FSEC medium-dose group (group D); FSEC low-dose group (group E); Positive control group (group F).

Table 1. Comparison of the activities of TNF-α, IL-6, TLR4, and IL-17 proteins in each group ($\overline{x}$ ± S).

Group	TNF-α	IL-6	TLR4	IL-17
Group A	1.04 ± 0.01	1.05 ± 0.02	2.05 ± 0.01	1.03 ± 0.01
Group B	61.21 ± 0.13**	63.37 ± 0.13**	62.35 ± 0.11**	60.66 ± 0.11**
Group C	18.29 ± 7.11##	19.53 ± 6.2[1]##	15.01 ± 5.05##	11.76 ± 6.71##
Group D	30.39 ± 6.11##	30.11 ± 5.21##	30.25 ± 5.21##	31.65 ± 6.52##
Group E	41.29 ± 6.31##	42.35 ± 5.61##	42.62 ± 5.43##	40.21 ± 5.41##
Group F	19.21 ± 7.11##	17.81 ± 7.11##	16.01 ± 5.05##	12.11 ± 5.31##

Note: $^{**}P < 0.01$ vs. Group A; $^{\#\#}P < 0.01$ vs. Group B.

treated with TNF-α in the high-dose FSEC group were slightly lower than those in the positive control group, but the difference was not statistically significant ($P > 0.05$).

4 Discussion

UC is a chronic recurrent, non-specific, inflammatory bowel disease whose etiology and pathogenesis are not fully clarified. And abdominal pain, diarrhea, mucopurulent bloody stools and tenesmus are often used as the main clinical symptoms. Colonoscopic lesions are mainly found in the rectum and sigmoid colon, even

Table 2. Comparison of the expression levels of TLR4 and NF-κB proteins among groups (%, $\overline{x}$ ±S).

Group	TLR4	NF-k B
Group A	0.07 ± 0.01	0.05 ± 0.01
Group B	0.85 ± 0.01**	0.84 ± 0.01**
Group C	0.11 ± 0.02##	0.16 ± 0.04##
Group D	0.33 ± 0.01##	0.25 ± 0.12##
Group E	0.52 ± 0.02##	0.45 ± 0.04##
Group F	0.13 ± 0.02##	0.17 ± 0.04##

Note: $^{**}P < 0.01$ vs. Group A; $^{\#\#}P < 0.01$ vs. Group B.

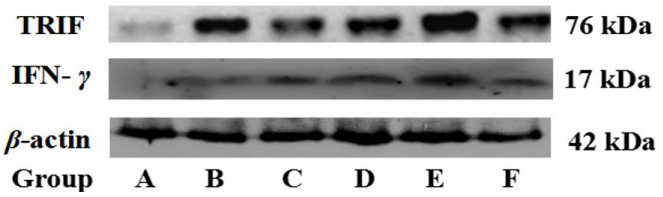

Figure 4. Expression of TRIF and IFN-γ proteins were detected by using western blot in each group. Note: Blank control group (group A); Model group (group B); FSEC high-dose group (group C); FSEC medium-dose group (group D); FSEC low-dose group (group E); Positive control group (group F).

Table 3. Comparison of the relative expression levels of TRIF and IFN- γ proteins in each group ($\bar{x}$ ±S).

Group	TRIF/β-actin	IFN- γ/β-actin
Group A	0.12 ± 0.12	0.15 ± 0.07
Group B	2.53 ± 3.15**	1.35 ± 0.11**
Group C	0.48 ± 3.11##	0.41 ± 3.02##
Group D	1.35 ± 3.21##	0.96 ± 3.42##
Group E	3.37 ± 0.13#	1.53 ± 3.21#
Group F	0.73 ± 3.21##	0.89 ± 3.51##

Note: **$P < 0.01$ vs. Group A; #$P < 0.05$; ##$P < 0.01$ vs. Group B.

up to the whole colon, and lesions are confined to the mucosa and submucosa (Zhao et al., 2021). Modern medicine generally believes that the factors of this disease mainly include immune disorders, infection, environment, genetics, psychology and so on (Lu et al., 2022; Zhu et al., 2022). Although the therapeutic strategies of western medicine often choose salicylic acid preparations, glucocorticoids, immunosuppressive agents and other means, there are still recurrent attacks after drug withdrawal, accompanied by many adverse reactions, and even appeared some problems (consisting of drug resistance, drug dependence and so on) (Ceng et al., 2021; Zhang et al., 2014). So far, the clinical treatment of UC remains difficult, and there is still a lack of specific and targeted therapeutic drugs. Therefore, exploring an effective and safe treatment remains a valuable research.

FSEC, a Chinese patent medicine developed from Chinese herbal medicine, its main components include five herbs: sophora flavescens, bletilla striata, indigo naturalis, ulmus officinalis and raw licorice root. Among them, sophora flavescens is the monarch drug, which enters the meridians of large intestine, heart, and bladder, can clear away heat and dampness, stop bleeding and stop dysentery. Ulmus officinalis enters the meridians of large intestine and liver, can cool blood, stop bleeding, detoxify, astringe sores. Moreover, the combination of ulmus officinalis and indigo naturalis can enhance its effects of clearing heat, detoxifying, cooling blood and stopping bleeding, which can become the ministerial drug. Bletilla bletilla has the effect of astringent, hemostasis and detumescence, which can assist the monarch drug and ministerial drug. Raw licorice can not only clear away heat, detoxify and relieve pain, but also harmonize various medicines. Modern pharmacology also finds that matrine, an alkaloid extracted from dried roots of sophora flavescens, has anti-inflammatory, antibacterial, immunomodulatory and antitumor effects. Notably, Zhang & Shen (2021) have found that the use of matrine has certain therapeutic effects on gastrointestinal diseases (such as UC and chronic atrophic gastritis), and its mechanism of action is just closely related to its antioxidant, anti-inflammatory and immunomodulatory effects. Bletilla polysaccharide, as the main active ingredient in Bletilla striata, has anti-tumor and immunoregulatory effects (Luo et al., 2019). The main components of indigo naturalis are indigo carmine, indirubin, indigo carmine, tryptamine, indigo naturalis and a large number of inorganic salts, which have anti-inflammatory effects (Zhang et al., 2022). Sanguisorbin, extracted from the root of sanguisorba officinalis, has antibacterial, antitumor, neuroprotective and hypoglycemic effects (Wu et al., 2022). Raw licorice is rich in glycyrrhizic acid, glycyrrhizin and other active components, which have antiviral, antioxidant and other pharmacological effects (Song et al., 2022). Although FSEC is a new national drug developed for UC, domestic and foreign scholars have conducted a large number of studies on the chemical composition and pharmacological effects of mono-drug in this Chinese patent medicine (Thang et al., 2022; Liu & Liu, 2022; Zhang et al., 2021), and they have achieved certain results, the compound mechanism of action in each component is still unclear. However, there is still a lack of in-depth understanding and research on its mechanism.

Increasing evidence suggests that tight junction (TJ) of intestinal epithelial cells plays an important role in the development and progression of UC (Zhang et al., 2017). MiR-224-3p, as a member of the miR-224 family, has been observed to have corresponding expression in tissues of colorectal cancer and UC (Olaru et al., 2013). In addition, Chen et al. (2015) have found that down-regulation of miR-224 can ameliorate pathological changes in colorectal cancer and UC, indicating that miR-224 can serve as a potential target for the treatment of colorectal cancer and UC. Previous studies have confirmed that miR-21 can play a cancer-promoting role by down-regulating the expression of tumor suppressor genes (Arias Sosa et al., 2017). Meanwhile, Sheedy (2015) have found that when the body was stimulated by external factors to produce an inflammatory response, miR-21 can show a tendency to be highly expressed, and is positively correlated with the degree of inflammatory response. In recent years, researchers have also found that miR-21 plays an important regulatory role in the progression of UC. As detected by Svrcek et al. (2013), miR-21 is highly expressed in intestinal mucosal tissues of UC patients and acts by inhibiting macrophage mannose receptors. Moreover, the expression level of miR-21 was positively correlated with the inflammatory response exhibited by UC, indicating that mirR-21 can be used as an important indicator to assess the degree of inflammatory response in UC (Naghdalipour et al., 2022). Another study report have showed that the expression level of miR-21 in colonic mucosal tissues of patients with active UC is significantly increased compared with that in inactive UC, which further suggests that this miRNA may have important value in the development of UC (Naghdalipour et al., 2022). During the development of UC, its persistently associated inflammatory response is the common feature and major factor in the disease and related complications (Tatiya-Aphiradee et al., 2018). Therefore, regulation of this molecular mechanism may also be a valuable pharmacological target. TNF-α, IL-6, TLR4, IL-17, NF-kB, TRIF, and IFN-γ are all important inflammatory factors and have some clinical significance for the diagnosis of UC (Du

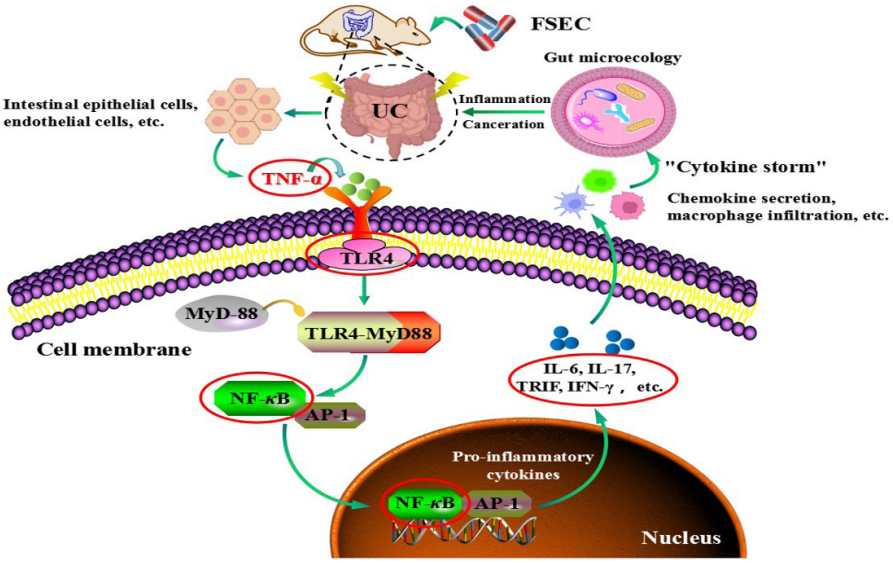

Figure 5. Schematic diagram of the molecular mechanism of FSEC on TNF-α-induced UC. UC, ulcerative colitis; FSEC, five-flavor sophora flavescens enteric-coated capsules. Note: the red circles represent the molecular targets involved in this study.

& Ha, 2020). Related studies have showed that disturbance of the internal environment (such as disturbed gut microbiota) can induce the increased expression of TLR4 on epithelial cells, endothelial cells and other membranes in intestinal mucosa, and combine with MyD88 to form TLR-MyD88 complex, so as to activate related signaling kinases, leading to the activation of transcription factor NF-κB, releasing NF-κB and translocating to the nucleus (Huang et al., 2023). When the number of NF-κB entering the nucleus increases, NF-κB entering the nucleus binds to DNA, thereby initiating a series of gene transcription, enhancing transcription, and promoting more inflammatory factors (such as TNF-α, IL-6, IL-17, TRIF, IFN-γ and so on), and then the phenomenon of chemokine secretion and macrophage infiltration occurs, ultimately forming a "cytokine storm" effect, aggravating the inflammatory response, mediating intestinal damage (such as ulcers, necrosis and), even causing carcinogenesis because of long-term accumulation (Xue et al., 2017). Thus, HE staining was firstly used to observe the surface phenomenon in this experiment, and the effect of FSEC on TNF-α-induced UC was assessed by pathological changes in colon tissue. The results showed that compared with the control group, the mucosa of colon tissue in the model group was severely damaged, some of them showed defects, necrosis, and formed ulcers of different depths. In addition, the glands in the mucosa were disorganized, and a large number of plasma cells exuded in the lamina propria, accompanied by glandular crypt abscess as well as glandular hyperplasia to form polypoid structures, while dense lymphocytes were observed at the base. Interestingly, compared with the model group, the above phenomena were improved in the drug treatment group to varying degrees, and the positive control group and FSEC high-dose group showed the lightest phenomenon, indicating that FSEC can effectively alleviate TNF-α-induced colonic tissue injury. In order to further explore the molecular mechanism, we measured the activities of TNF-α, IL-6, TLR4 and IL-17 proteins by using ELISA kits, and also detected the expression levels of TLR4 and NF-κB proteins by using immunohistochemistry. The results showed that the expression levels of TLR4 and NF-κB proteins were very weak or negative in colonic mucosal tissues without drug treatment, while the expression levels of these proteins were significantly enhanced in colonic mucosal tissues during the pathogenesis of UC, and TLR4 and NF-κB proteins showed a tendency to be down-regulated in the drug-treated group (FSEC or SAZ), indicating that the induction of UC was closely related to the activity of a large number of pro-inflammatory cytokines and genes (including TLR4 and NF-κB) in colonic mucosal tissues, while the treatment of FSEC could effectively inhibit the activity of these inflammatory factors and genes. In order to deeply investigate the cellular level, we also selected murine IEC-6 small intestinal epithelial cells as the research object, and determined the expression levels of TRIF and IFN-γ by using western blot technique. The results showed that TRIF and IFN-γ proteins were highly expressed in the model group compared with the control group, while the expression levels of TRIF and IFN-γ proteins in IEC-6 cells treated with FSEC were dose-dependently decreased, and this trend was consistent with the results of the positive control group, indicating that FSEC could improve TNF-α-mediated small intestinal epithelial cells IEC-6 injury by delaying the process of inflammatory response, and the molecular mechanism may be related to the decrease of TRIF and IFN-γ proteins expression.

5 Conclusion

In summary, we found that the development of UC is closely related to the "cytokine storm" effect, so as to aggravate the inflammatory response, leading to severe intestinal injury, even can develop to cancer for a long time. The results of histology showed that showed that FSEC could effectively ameliorate TNF-α-induced colonic tissue injury. Further studies have found that FSEC could inhibit the activation of TLR4/NF-κB signaling pathway, and then inhibit the activity of downstream related inflammatory factors (such as TNF-α, IL-6, IL-17, TRIF, IFN-γ), thereby inhibiting the inflammatory response and protecting colon tissues and cells from TNF-α-induced damage (Figure 5). However, more in vitro and

in vivo experiments as well as clinical studies are still needed to validate these results in the future, which not only provides the possibility to support the value of developing Chinese herbal medicines, but also provides more scientific basis for FSEC in the prevention and treatment of inflammatory diseases.

Ethical approval

Experimental procedure was followed according with the institutional guidelines for the care and use of laboratory animals and were approved by the ethics committee of the First Affiliated Hospital of Nanchang University (Jiangxi).

Conflict of interest

The authors declare that they have no competing interests.

Availability of data and material

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

Funding

This study was supported by The Science and Technology Plan Project of Provincial Health Commission (grant no. 202310317) and The Science and Technology Plan Project of Provincial Health Commission (grant no. 202310195).

Acknowledgements

Authors are grateful to the teaching and non-teaching staffs of the First Affiliated Hospital of Nanchang University, Jiangxi, Nanchang for their kind help and support during this study.

References

Abdel-Rahman, L. I. H., & Morgan, X. C. (2023). Searching for a consensus among inflammatory bowel disease studies: a systematic meta-analysis. *Inflammatory Bowel Diseases*, 29(1), 125-139. http://dx.doi.org/10.1093/ibd/izac194. PMid:36112501.

Arias Sosa, L. A., Cuspoca Orduz, A. F., & Bernal Gómez, B. M. (2017). Deregulation of microRNAs in gastric cancer: up regulation by miR-21 and miR-106. *Revista de Gastroenterologia del Peru*, 37(1), 65-70. PMid:28489839.

Bu, Q., Han, Y. P., Miao, R., Huang, R., & Bao, X. Q. (2021). Effects of Wuwei Kushen enteric-coated capsules on inflammatory factors in mice with ulcerative colitis. *Guangdong Chemical Industry*, 48(2), 131-133. http://dx.chinadoi.cn/10.3969/j.issn.1007-1865.2021.02.055.

Ceng, J. W., Zheng, L. M., Chen, S. M., Chen, L. R., & Li, J. (2021). Multivariate analysis of adverse reactions in patients with severe ulcerative colitis treated with glucocorticoid. *Smart Healthcare*, 7(23), 102-104. http://dx.chinadoi.cn/10.19335/j.cnki.2096-1219.2021.23.033.

Chen, J. (2005). Analysis on the characteristics of the pathogenesis evolution of ulcerative colitis in traditional Chinese medicine. *Journal of Liaoning University of Traditional Chinese Medicine*, 3, 197. http://dx.chinadoi.cn/10.3969/j.issn.1673-842X.2005.03.002.

Chen, T., Xiao, H. R., Cai, H., & Xiao, Y. J. (2022). Research progress in the mechanism of anti apoptosis of intestinal epithelial cells in ulcerative colitis by traditional Chinese medicine and its extracts. *Clinical Journal of Practical Integrative Traditional Chinese and Western Medicine*, 22(12), 124-128. http://dx.chinadoi.cn/10.13638/j.issn.1671-4040.2022.12.036.

Chen, W., Fan, X. M., Mao, L., Zhang, J. Y., Li, J., Wu, J. Z., & Tang, J. H. (2015). MicroRNA-224: as a potential target for miR-based therapy of cancer. *Tumour Biology*, 36(9), 6645-6652. http://dx.doi.org/10.1007/s13277-015-3883-3. PMid:26254100.

Dai, L. M. (2019). TCM master Xu Jing-fan's experience of treating ulcerative colitis. *Henan Traditional Chinese Medicine*, 39(5), 677-681. http://dx.chinadoi.cn/10.16367/j.issn.1003-5028.2019.05.0167.

Du, L., & Ha, C. (2020). Epidemiology and pathogenesis of ulcerative colitis. *Gastroenterology Clinics of North America*, 49(4), 643-654. http://dx.doi.org/10.1016/j.gtc.2020.07.005. PMid:33121686.

He, X. R., Fang, J. C., Huang, L. H., Wang, J. H., & Huang, X. Q. (2015). *Sophora flavescens* Ait.: traditional usage, phytochemistry and pharmacology of an important traditional Chinese medicine. *Journal of Ethnopharmacology*, 172, 10-29. http://dx.doi.org/10.1016/j.jep.2015.06.010. PMid:26087234.

Huang, J., Wu, T. T., Zhong, Y. B., Huang, J. Q., Kang, Z. P., & Zhou, B. G. (2023). Effect of curcumin on regulatory B cells in chronic colitis mice involving TLR/MyD88 signaling pathway. *Phytotherapy Research*, 37(2), 731-742. PMid:36196887.

Jayasooriya, N., Baillie, S., Blackwell, J., Bottle, A., Petersen, I., Creese, H., Saxena, S., & Pollok, R. C. (2023). Systematic review with meta-analysis: time to diagnosis and the impact of delayed diagnosis on clinical outcomes in inflammatory bowel disease. *Alimentary Pharmacology & Therapeutics*, 10, apt.17370. http://dx.doi.org/10.1111/apt.17370.

Li, L., Chen, Y., & Yang, C. M. (2012). Correlation study of single nucleotide polymorphism of multiple drug resistance 1 and ulcerative colitis prednisone sensitivity. *Journal of Shandong University*, 50(5), 99-102.

Liu, C., & Liu, A. J. (2022). Structural characterization of an alcohol-soluble polysaccharide from Bletilla striata and antitumor activities in vivo and in vitro. *Chemistry & Biodiversity*, 19(12), e202200635. http://dx.doi.org/10.1002/cbdv.202200635. PMid:36282907.

Lu, X. F., Qin, W. N., & Geng, Y. L. (2022). Etiology, influencing factors and mechanism of ulcerative colitis. *China Journal of Traditional Chinese Medicine and Pharmacy*, 37(10), 6140-6144.

Luo, L., Liu, Y. Q., Cai, X., Wang, Y., Xue, J., Zhang, J., & Yang, F. (2019). Bletilla striata polysaccharides ameliorates lipopolysaccharide-induced injury in intestinal epithelial cells. *Saudi Journal of Gastroenterology*, 25(5), 302-308. http://dx.doi.org/10.4103/sjg.SJG_520_18. PMid:31044747.

Naghdalipour, M., Moradi, N., Fadaei, R., Rezghi Barez, S., Sayyahfar, S., Mokhtare, M., Fard, T. K., Fallah, S., & Esteghamati, A. (2022). Alteration of miR-21, miR-433 and miR-590 tissue expression related to the TGF-β signaling pathway in ulcerative colitis patients. *Archives of Physiology and Biochemistry*, 128(5), 1170-1174. http://dx.doi.org/10.1080/13813455.2020.1762656. PMid:32412349.

Olaru, A. V., Yamanaka, S., Vazquez, C., Mori, Y., Cheng, Y., Abraham, J. M., Bayless, T. M., Harpaz, N., Selaru, F. M., & Meltzer, S. J. (2013). MicroRNA-224 negatively regulates p21 expression during late neoplastic progression in inflammatory bowel disease. *Inflammatory Bowel Diseases*, 19(3), 471-480. http://dx.doi.org/10.1097/MIB.0b013e31827e78eb. PMid:23399735.

Regueiro, M., Hunter, T., Lukanova, R., Shan, M., Wild, R., Knight, H., Bannikoppa, P., & Naegeli, A. N. (2023). Burden of fatigue among patients with ulcerative colitis and crohn's disease: results from a global survey of patients and gastroenterologists. *Advances in Therapy*, 12, 123-125. PMid:36370288.

Sawarkar, S. P., Deshpande, S. G., Bajaj, A. N., & Nikam, V. S. (2015). In vivo evaluation of 5-ASA colon-specific tablets using experimental-

induced colitis rat animal model. *AAPS PharmSciTech*, 16(6), 1445-1454. http://dx.doi.org/10.1208/s12249-015-0331-z. PMid:26017284.

Sheedy, F. J. (2015). Turning 21: Induction of miR-21 as a key switch in the inflammatory response. *Frontiers in Immunology*, 6, 19. http://dx.doi.org/10.3389/fimmu.2015.00019. PMid:25688245.

Shin, D. H., Cha, Y. J., Joe, G. J., Yang, K. E., Jang, I. S., Kim, B. H., & Kim, J. M. (2013). Whitening effect of Sophora flavescens extract. *Pharmaceutical Biology*, 51(11), 1467-1476. http://dx.doi.org/10.3109/13880209.2013.799708. PMid:24106757.

Song, X. M. (2018). Analysis of side effects of mesalazine combined with bifidobacteria in the treatment of severe ulcerative colitis. *Chinese Journal of Metallurgical Industry Medicine*, 35(1), 62-63. http://dx.chinadoi.cn/10.13586/j.cnki.yjyx1984.2018.01.059.

Song, X., Liu, S. J., Fan, J., Shen, Y., He, L. W., & Meng, X. C. (2022). Discussion on clinical application based on the differences of chemical composition and pharmacological action between radix glycyrrhizae preparata and raw radix glycyrrhizae. *China Medicine and Pharmacy*, 12(19), 114-117. http://dx.chinadoi.cn/10.3969/j.issn.2095-0616.2022.19.029.

Svrcek, M., El-Murr, N., Wanherdrick, K., Dumont, S., Beaugerie, L., Cosnes, J., Colombel, J.-F., Tiret, E., Flejou, J.-F., Lesuffleur, T., & Duval, A. (2013). Overexpression of microRNAs-155 and 21 targeting mismatch repair proteins in inflammatory bowel diseases. *Carcinogenesis*, 34(4), 828-834. http://dx.doi.org/10.1093/carcin/bgs408. PMid:23288924.

Tatiya-Aphiradee, N., Chatuphonprasert, W., & Jarukamjorn, K. (2018). Immune response and inflammatory pathway of ulcerative colitis. *Journal of Basic and Clinical Physiology and Pharmacology*, 30(1), 1-10. http://dx.doi.org/10.1515/jbcpp-2018-0036. PMid:30063466.

Thang, P. N. T., Tran, V. H., Vu, T. A., Vinh, N. N., Huynh, D. T., & Pham, D. T. (2022). Determination of antioxidant, cytotoxicity, and acetylcholinesterase inhibitory activities of alkaloids isolated from sophora flavescens ait: grown in dak nong, vietnam. *Pharmaceuticals*, 15(11), 1384. http://dx.doi.org/10.3390/ph15111384. PMid:36355556.

Tian, M. (2021). Research progress in pathogenesis of ulcerative colitis. *Chinese Journal of Urban and Rural Enterprise Hygiene*, 36(2), 92-94. http://dx.chinadoi.cn/10.16286/j.1003-5052.2021.02.034.

Tong, Z. Q., Yang, B., Chen, B. Y., & Zhao, M. L. (2010). A multi-center, randomized, single-blind, controlled clinical study on the efficacy of composite sophora colon-soluble capsules in treating ulcerative colitis. *Chinese Journal of Integrative Medicine*, 16(6), 486-492. http://dx.doi.org/10.1007/s11655-010-0562-5. PMid:21110172.

Wang, L. C., Wang, D. G., Wu, W. Y., Chen, S. Y., Xing, Y. T., Lin, X. Y., & Lin, T. (2021). Discussion on medication rules of anorexia treated by "dictionary of traditional Chinese medicine prescriptions" based on literature mining. *Chinese Journal of Ethnomedicine Ethnopharmacology*, 30(17), 17-22. http://dx.chinadoi.cn/10.0017/j.issn.1007-8517.2021.17.006.

Wang, Q. R., & Shao, Q. (2022). Recent progress of traditional Chinese medicine in treating inflammatory bowel disease by maintaining intestinal flora homeostasis. *China Journal of Chinese Materia Medica*, 47(22), 5997-6004.

Wu, L. L., Xu, H. Y., Zhang, L. Q., & Li, Y. M. (2022). Research progress on chemical constituents and pharmacological activities of sanguisorba officinalis. *Modern Trad China Med Mater Med-World Sci Tech*, 24(1), 360-378. http://dx.chinadoi.cn/10.11842/wst.20210302005.

Xie, L. Y., & Lu, J. Q. (2022). Clinical observation on treating chronic ulcerative colitis by acupuncture plus Chinese herbs orally. *Chinese Journal of Chinese Medicine*, 14(16), 39-41. http://dx.chinadoi.cn/10.3969/j.issn.1674-7860.2022.16.010.

Xue, B., Liu, X. L., Dong, W. W., Liang, L. L., & Chen, K. Y. (2017). EGCG maintains Th1/Th2 balance and mitigates ulcerative colitis induced by dextran sulfate sodium through TLR4/MyD88/NF-κB signaling pathway in rats. *Canadian Journal of Gastroenterology & Hepatology*, 30, 57-68.

Yu, S. J., & Zhang, X. Y. (2022). Clinical research progress of Chinese medicine retention enema in the treatment of ulcerative colitis. *Guangming Journal of Chinese Medicine*, 37(20), 3830-3832. http://dx.chinadoi.cn/10.3969/j.issn.1003-8914.2022.20.060.

Yuan, Y. H., Ding, D. K., Zhang, N., Xia, Z. Q., Wang, J. X., Yang, H. Y., Guo, F., & Li, B. (2018). TNF-α induces autophagy through ERK1/2 pathway to regulate apoptosis in neonatal necrotizing enterocolitis model cells IEC-6. *Cell Cycle*, 17(11), 1390-1402. http://dx.doi.org/10.1080/15384101.2018.1482150. PMid:29950141.

Zhang, K. R., Liu, Z. Y., Wang, L. L., Liang, Y., He, T. T., & Fan, Y. P. (2021). Antibacterial activity and mechanism study of sanguisorba officinalis L. Extracts against multidrug-resistant escherichia coli. *Problems in Veterinary Medicine*, 42(10), 61-65. http://dx.chinadoi.cn/10.3969/j.issn.1007-5038.2021.10.011.

Zhang, M. F., & Shen, Y. Q. (2021). Research progress on pharmacologic effect of matrine against gastrointestinal tract diseases. *Anti-Infection Pharmacy*, 18(4), 467-471. http://dx.chinadoi.cn/10.13493/j.issn.1672-7878.2021.04-002.

Zhang, S. S., Shen, H., Zheng, K., & Ye, B. (2017). Consensus of TCM diagnosis and treatment experts on ulcerative colitis (2017). *China Journal of Traditional Chinese Medicine and Pharmacy*, 32(8), 3585-3589.

Zhang, X., Xu, X. T., Mohammed, I., Xiao, L., Sun, L. X., & Zhang, L. Y. (2022). Research progress of pharmacologic effects and mechanism on ulcerative colitis of Indigo Naturalis and its active ingredients. *Drug Eva Research*, 45(5), 997-1002. http://dx.chinadoi.cn/10.7501/j.issn.1674-6376.2022.05.025.

Zhang, Y. J., Xu, J. J., Wang, P., & Wang, J. L. (2014). Multidrug resistance gene and its relationship to ulcerative colitis and immune status of ulcerative colitis. *Genetics and Molecular Research*, 13(4), 10837-10851. http://dx.doi.org/10.4238/2014.December.19.5. PMid:25526204.

Zhao, J. (2014). Research on the literature of ulcerative colitis treated by TCM syndrome differentiation. *Asia-Pacific Traditional Medicine*, 10(2), 35-38.

Zhao, J., Li, J. P., & Liu, X. L. (2021). Clinical characteristics and colonoscopic manifestations of ulcerative colitis. *Chinese Journal of Practical Medicine*, 48, 40-43.

Zhu, X. H., Deng, X., Wang, S. Q., & Zhao, L. Q. (2022). Analysis of recurrence factors of ulcerative colitis and prevention and treatment strategies of traditional Chinese medicine. *Beijing Journal of Traditional Chinese Medicine*, 41(9), 968-972. http://dx.chinadoi.cn/10.16025/j.1674-1307.2022.09.006.