eCAM Evidence-Based Complementary and Alternative Medicine

Herbal Medicines Useful to Treat Inflammatory and Ulcerative Gastrointestinal Disorders: Preclinical and Clinical Studies

Lead Guest Editor: Sérgio F. De Andrade
Guest Editors: Luísa M. Da Silva, Shahram Golbabapour, and Steve Harakeh

Herbal Medicines Useful to Treat Inflammatory and Ulcerative Gastrointestinal Disorders: Preclinical and Clinical Studies

Evidence-Based Complementary and Alternative Medicine

Herbal Medicines Useful to Treat Inflammatory and Ulcerative Gastrointestinal Disorders: Preclinical and Clinical Studies

Lead Guest Editor: Sérgio F. De Andrade
Guest Editors: Luísa M. Da Silva, Shahram Golbabapour, and Steve Harakeh

Editorial Board

Contents

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2018, Article ID 3605748, 2 pages
https://doi.org/10.1155/2018/3605748

Editorial

Herbal Medicines Useful to Treat Inflammatory and Ulcerative Gastrointestinal Disorders: Preclinical and Clinical Studies

Sérgio Faloni de Andrade[1], **Luisa Mota da Silva**[1], **Shahram Golbabapour**[2] and **Steve Harakeh**[3]

[1]*Núcleo de Investigações Químico-Farmacêuticas (NIQFAR), Universidade do Vale do Itajaí (UNIVALI), Rua Uruguai, No. 458, Centro, 88302-202 Itajaí, SC, Brazil*
[2]*Shiraz Transplant Research Centre, Shiraz University of Medical Sciences, Shiraz, Iran*
[3]*Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*

Correspondence should be addressed to Sérgio Faloni de Andrade; faloni@univali.br

Received 12 December 2017; Accepted 13 December 2017; Published 29 March 2018

Gastrointestinal disorders are among the most common illnesses that affect people nowadays. Their prevalence and incidence have been on the rise during the last decade. This high prevalence and incidence are due to the contemporary lifestyle we live. Such lifestyles include bad dietary habits, consumption of drugs, alcoholic drinks, and stress. It is very common that gastrointestinal disorders are characterized with inflammatory and ulcerative processes from the stomach or gut.

The main inflammatory and ulcerative disorders associated with the gastrointestinal tract include gastritis, ulcers, colitis, Crohn's disease, and mucositis. These disorders are difficult to treat. The recurrence and side effects are very common after treatment with available drugs. Based on that, there is an urgent need for the search for more effective and safe pharmacological options for the treatment of inflammatory and ulcerative gastrointestinal disorders. In the recent years, plant extracts and natural products have been sought for the important role in the treatment of the previously mentioned disorders. Thus, a great deal of effort and research has been undertaken to find suitable natural plants and compounds with proven potential. For this reason, hundreds of plant extracts and isolated active compounds have shown a promising potential. However, for the majority of them, it is necessary to conduct preclinical studies followed by the clinical studies in humans in order to be approved for human use. This special issue is focused on this topic. The edition consists of seven articles including a clinical study, three preclinical studies, and three reviews, which are briefly described below.

The clinical study was performed by B. Liu et al., who reported that the use of Chinese medicinal herbs mix composed of seven plants (CIF) and mesalazine in the treatment of ulcerative colitis (UC). In this study, 60 patients with chronic UC were treated only with either oral mesalazine or mesalazine in combination with CIF enema. The results showed that combination of mesalazine and CIF significantly improved the clinical symptoms, the colon mucosal conditions, the Mayo Clinic Disease Activity Index, and quality of life, when compared to mesalazine alone.

Considering the preclinical studies, one of the contributions is the work of T. Mao et al., who explored the mechanism of Qingchang Wenzhong Decoction (QCWZD), preparation derived from eight plants, in UC in rats models. The authors showed that QCWZD administration significantly alleviated colitis-associated inflammation, upregulating serum macrophage-stimulating protein (MSP) and receptor d'origine nantais (RON) expression in the colon, reduced the pAkt (protein kinase B [Akt], phosphorylated [p] Akt) levels, promoted zona occluden 1 (ZO-1) expression, and depressed claudin-2 expression. Of this mode, it was possible to conclude that QCWZD appears to attenuate DSS-induced UC in rats by upregulating the MSP/RON signaling pathway, contributing to understanding of QCWZD benefits in UC.

Still considering the preclinical studies, Y. Yang et al. described the anti-inflammatory effects and the underlying

mechanisms of GCZX-pill, Chinese herbal formula composed of six herbs, on trinitrobenzene sulfonic acid- (TNBS-) induced UC in rats. The results demonstrated that the GCZX-pill can attenuate colitis in rats and the anti-inflammatory effect of the GCZX-pill may be related to the reduction of enterochromaffin colonic cells hyperplasia and serotonin availability.

The third preclinical study was done by H. Zhang et al., who tested the effects of two Chinese herbal formulations, Erchen decoction (ECD) and Linguizhugan decoction (LGZGD), on insulin resistance in rats. The results indicated that ECD and LGZGD have protective effects against high-fat diet-induced liver insulin resistance and their underlying mechanisms involve the TNF-α and insulin pathway.

In the review articles, P. Ren et al. reported a comprehensive review concerning the efficacy and safety of Kangfuxinye enema (Chinese herbal medicine extracted from the *Periplaneta americana*) combined with mesalamine for the ulcerative colitis (UC) patients and in addition evaluated the grade of the quality of evidence by using the GRADE (grading of recommendations, assessment, development, and evaluation) approach. With this revision, the authors concluded that although Kangfuxinye enema seems to be effective and safe for treating UC patients in this systematic review, Kangfuxinye enema combined with mesalamine was weakly recommended due to very low to moderate quality of available evidence by the GRADE approach.

In another review, C. Wang et al. discussed another Chinese herbal formula known as Huangqin-Tang (HQT), composed of four ingredients: the roots of *Scutellaria baicalensis* Georgi, *Glycyrrhiza uralensis* Fisch, *Paeonia lactiflora* Pall, and the fruit of *Ziziphus jujuba* Mill. The authors concluded that available reports suggested that the efficacy of HQT or its components on UC is related to the intestinal environment improvement, immune modulation, and regulation of inflammatory pathways or cytokines. However, most of the data were based on animal studies or in vitro experiments; thus, the effects and mechanisms of HQT in UC patients remain to be explored or verified.

Finally, L. M. da Silva et al. did a review concerning the potential role of propolis in the treatment and prevention of gastrointestinal disorders. They reported and critiqued the studies showing the beneficial effects of propolis and its active compounds in the treatment of gastrointestinal diseases; however, they concluded that only few clinical trials have been conducted to prove their effectiveness and safety against human ulcers and their associated pathologies.

Thus, this special issue included different investigations related to the efficacy of herbal medicines in the treatment of inflammatory and ulcerative gastrointestinal disorders, based on clinical and preclinical studies as well as the revision of the literature related to the objective of this special issue.

Sérgio Faloni de Andrade
Luisa Mota da Silva
Shahram Golbabapour
Steve Harakeh

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2018, Article ID 2035820, 12 pages
https://doi.org/10.1155/2018/2035820

Review Article

Propolis and Its Potential to Treat Gastrointestinal Disorders

Luisa Mota da Silva[1], **Priscila de Souza**[1], **Soad K. Al Jaouni**[2,3], **Steve Harakeh**[3,4], **Shahram Golbabapour**[5] and **Sérgio Faloni de Andrade**[1]

[1]*Núcleo de Investigações Químico-Farmacêuticas (NIQFAR), Universidade do Vale do Itajaí (UNIVALI), Rua Uruguai 458, Centro, 88302-202 Itajaí, SC, Brazil*
[2]*Department of Pediatric Hematology/Oncology, Faculty of Medicine, King Abdulaziz University Hospital (KAUH), King Abdulaziz University (KAU), Jeddah, Saudi Arabia*
[3]*Yousef Abdullatif Jameel Research Chair for Prophetic Medicine Application, Faculty of Medicine, KAUH, KAU, Jeddah, Saudi Arabia*
[4]*Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*
[5]*Shiraz Transplant Research Centre, Shiraz University of Medical Sciences, Shiraz, Iran*

Correspondence should be addressed to Sérgio Faloni de Andrade; faloni@univali.br

Received 11 August 2017; Accepted 21 November 2017; Published 15 March 2018

Academic Editor: Salvatore Chirumbolo

There are a number of disorders that affect the gastrointestinal tract. Such disorders have become a global emerging disease with a high incidence and prevalence rates worldwide. Inflammatory and ulcerative processes of the stomach or intestines, such as gastritis, ulcers, colitis, and mucositis, afflict a significant proportion of people throughout the world. The role of herbal-derived medicines has been extensively explored in order to develop new effective and safe strategies to improve the available gastrointestinal therapies that are currently used in the clinical practice. Studies on the efficacy of propolis (a unique resinous aromatic substance produced by honeybees from different types of species of plants) are promising and propolis has been effective in the treatment of several pathological conditions. This review, therefore, summarizes and critiques the contents of some relevant published scientific papers (including those related to clinical trials) in order to demonstrate the therapeutic value of propolis and its active compounds in the treatment and prevention of gastrointestinal diseases.

1. Introduction

Propolis or "bee glue" is a resinous waxy-like substance. Honey bees produce it by mixing their saliva and beeswax with the exudates obtained from plants like tree buds, sap flows, leaves, branches, and barks of plants found in the vicinity of the beehive. The ultimate goal of propolis is for bees to protect their hives by utilizing it to seal cracks and protect bees from predators and microorganisms and provide thermal insulation [1–3]. The term propolis has originated from Greek word pro, for or in defense of, and polis, the city [1]. The color of propolis is variable and depends on the plants' type that the bees used to collect the resinous substances. Three main colors have been noted: green, red, brown, or black propolis [4]. For instance, the red propolis from Cuba or Venezuela has botanical origins identified as *Clusia nemorosa* Forsteronia G. Mey (Clusiaceae) and *Clusia scrobiculata* Benoist (Clusiaceae), respectively. Red propolis from Northeastern Brazil has *Dalbergia ecastaphyllum* (L.) Taub. (Leguminosae) as botanical source while Brazilian green propolis originates mainly from *Baccharis dracunculifolia* DC (Asteraceae). Therefore, the geographical location, plant sources, collection season, bee species, and solvents used in the extraction have an influence on the chemical composition and on the pharmacological activity of propolis preparation. Despite this wide range of its composition, the records indicate that propolis has been used in the folk medicine since 300 BC [5]. In the last decades, it has attracted the interest of researchers around the world because of its several biological and pharmacological properties, with over 2500 articles being published on Pubmed website (https://www.ncbi.nlm.nih.gov/pubmed/) about this substance over the last 30 years. Moreover, it has gained popularity as either an alternative medicine or as a dietary

supplement for health amelioration and disease prevention in various parts of the world, including the United States of America, European Union, Brazil, and Japan [6]. Nowadays, propolis has been widely used to treat several illnesses including those that affect the gastrointestinal tract, such as mucositis, colitis, gastritis, and peptic ulcer [7–10]. This is in addition to its potential to treat different forms of gastrointestinal cancer, as presented in this article. Thus, the aim of this review is to summarize and critique published articles related to studies on the use of propolis and its main active ingredients in the treatment of gastrointestinal disorders and other related disorders.

2. Methodology

Considering the main gastrointestinal disorders that propolis is normally used to treat, a search has been conducted on Pubmed (https://www.ncbi.nlm.nih.gov/pubmed/), Science Direct (http://www.sciencedirect.com/), and Medline (https://www.nlm.nih.gov/bsd/pmresources.html) databases using the terms "propolis and ulcer, propolis and gastroprotective, propolis and mucositis, propolis and colitis, propolis and gastrointestinal cancer." Relevant articles have been included in this review.

3. Propolis in the Treatment of Oral Mucositis

Oral mucositis (OM) is an inflammation of the oral mucosa of the mouth. OM is observed in cancer patients, especially those with squamous cell carcinoma located in the head and neck area, when treated by chemo and/or radiotherapy [11–14]. OM is one of the most serious complications that are facing cancer patients [15]. Many possible age and gender related complications result from OM. It has been reported that older patients have less ability to repair the damaged DNA associated with treatment and are, thus, more at risk of developing problems. On the other hand, younger patients have the ability to deal better with OM because they have a faster rate of proliferation of the epithelial cells and this will be an important factor in dealing with OM [16]. Females are more at risk of developing OM than males. Various risk factors have been reported including cigarette smoking, excessive alcohol intake, defective restorations, orthodontic appliances, ill-fitting prostheses, and other mucosal irritations [17]. The related risk factors are associated with the area of oral mucosal treated and the type, dose, and intensity of the chemotherapy used [18]. This is in addition to the frequent daily and repetitive radiation treatment [16].

The aggressive medical agents, such as cisplatin and 5-fluorouracil (5-FU), when used in the presence or absence of radiation therapy result in the development of OM in comparison to the use of "gentler" agents like gemcitabine [11]. The OM induced chemotherapy usually manifests itself during the first week after the beginning of therapy and peaks in the 2nd week. It appears first by thinning of oral tissues that leads to erythema. As these tissues become thinner, ulceration eventually occurs [14]. Potential complications include pain, increased risk of local and systemic infections, bleeding, and insufficient food intake, which may lead to breaks in treatment sessions [15].

The typical manifestations associated with OM include the following: atrophy, erythema, ulceration, and swelling of the mucosa [19]. Such manifestations are accompanied with pain, elevated risk of infection, and dysphasia and may lead to dehydration and malnutrition [13, 16, 17, 20]. Other medically related problems include xerostomia and sensational changes that may lead to reduced food intake and finally result in anorexia, malnutrition, and body mass loss and weakness [13].

The traditional way to manage OM is to educate the involved patient to comply with treatment and give the patient good nutritional support, hydration, use of saline rinses, topical and systemic pain relief, and infection surveillance [17]. So far, no therapy has been effective against OM. However, infections associated with OM are usually treated with antibiotics and antifungal agents. The short-term use of antibiotics will lead to the stabilization of resistant bacteria in the human gut for many years and may cause lots of complications associated with treatment [21, 22].

In this section, the role of the external use propolis in the treatment of OM and related mouth diseases will be discussed. The external use of propolis is defined by the application of pharmaceutical or natural products on the surface or point of illness [23]. External uses of propolis (EUP) include the use of pharmaceutical, cosmetic, and oral products such as ointment [24], gel [25], and mouthwash [26].

A recently published systematic review on propolis for oral health reported that it can reduce oral infection and dental plaque and treat stomatitis [27]. In another study, which evaluated the efficacy of ethanolic extract of propolis on radiation-induced mucositis in rats, propolis was found to effectively reduce and/or delay radiation-induced mucositis in an animal model. However, it is recommended that further studies need to be conducted to further confirm this effect [28].

Not all of the published studies have reported the geographical locations from where the propolis was collected [29–36]. In only one study the chemical composition of propolis has been mentioned which gives this study importance since it characterized the propolis tested and listed its chemical composition [30]. It has been reported that all the propolis from different areas had similar composition but its efficacy was concentration dependent [37]. In addition, different components were identified in propolis collected from different regions from the same country [38] and their adverse effects were identified from certain countries [39]. Based on that, the geographical location is a key factor in the safety and efficacy of propolis [40].

In studies where placebo was used, they used the same form in the control group without hinting to anything about taking into account the smell of the propolis [29, 30, 36, 41]. It would have been prudent to utilize indistinguishable placebo as compared to the experimental treatment. Considering the scent is crucial, since propolis has a distinct aromatic smell and subjects are usually familiar with its distinctive smell; this characteristic should be taken into consideration in future blind studies on propolis [42].

Propolis when used as an ingredient in mouthwashes showed protection against oral disease which is likely due to its antimicrobial efficacy [43]. There was no significant difference in the efficacy that is provided by propolis when used as a gel or as a mouthwash [44] or as a buccal paste [45].

We have published an open labeled randomized controlled recent study on the use of Saudi honey that is similar in so many ways to propolis on 40 pediatric cancer patients undergoing chemo/radiotherapy. The topical application of local Saudi honey resulted in a significant reduction of OM, associated with bacterial and fungal (candida) infections. The use of honey in the treatment of the patients has led to a reduction in the hospitalization time accompanied by a significant gain in body weight, delayed onset, reduced infections, and decreased severity of pain related to OM [46].

4. Propolis in the Therapeutic Management of Ulcerative Colitis

Ulcerative colitis (UC), a subtype of inflammatory bowel disease, is a chronic inflammatory condition that causes a constant inflammation of the colonic mucosa. It is characterized by significant morbidity and worsening in the quality of life of the affected patients [47, 48]. Although the exact etiology is unidentified, several authors suggest that an interaction between genetic and environmental factors, as well as a dysregulated immune system, can result in mucosal inflammation [49, 50]. The main clinical symptoms of UC are abdominal pain, diarrhea, and rectal bleeding, which are currently treated with mesalamine (5-aminosalicylic acid or 5-ASA), corticosteroids, immunosuppressants, antibiotics, and biologic therapies [such as the antitumor necrosis factor (TNF) agents]. However, the effectiveness of the available medical therapy and the list of a large number of important side effects are the two major concerns in clinical practice for the efficient and safe management of UC [48, 51–53]. Studies with novel therapies based on medicinal plants have been the focus of a pronounced number of investigations in the last years, which points out promising results in experimental trials (for review see [54]). In this sense, propolis and its active compounds have already been the target of several preclinical studies about its advantage in the treatment of UC.

The first evidence about the beneficial role of propolis on experimental UC was described in 1979 [55]. Since then, other studies have been conducted either with propolis or with its active components, in different animal models with induced UC. In 2007, Aslan et al. [56] showed the effectiveness of propolis in a model where acetic acid was used to induce colitis in rats. The intracolonic instillation of acetic acid is the simplest and most reproducible model of many characteristics of the human colitis [57]. In that study, propolis treatment was effective in attenuating UC, by mechanisms associated with decrease in oxidative stress and inflammation, which are key parameters in the pathogenesis of the disease [56]. Subsequently, the same group of researchers explored the effect of propolis on bacterial translocation using the same model of acetic acid induced experimental colitis. There are several evidences showing that luminal bacteria are involved in mucosal inflammatory responses in UC, whereby this bacteria causes the disruption of the intestinal mucosa barrier integrity [58]. Thus, the authors concluded that propolis was able to reduce bacterial translocation, due to its ability to restrict the damage caused by acetic acid induction, and results in the protection of the integrity of the intestinal wall [59]. More recently, another study using the same model of acetic acid-induced colitis in rats showed that the hydroalcoholic extract of Brazilian red propolis attenuated colitis, an effect associated with decreases in myeloperoxidase (MPO) activity, gross, and histological scores of tissue damage and the inducible isoform of nitric oxide synthase (iNOS) expression [60].

The properties of propolis were also evaluated in other experimental models, including the trinitrobenzene sulfonic acid- (TNBS-) induced colitis. TNBS intrarectal inoculation is capable of activating the immune response driven intestinal inflammation, which is characterized by infiltration of the lamina propria with CD4+ T cells, neutrophils, and macrophages [61–63]. Okamoto et al. showed the suppressive effect of Brazilian propolis on Th1 differentiation, an action related to a reduction on the severity of TNBS-induced UC in mice [64]. Additionally, the effects of propolis hydroalcoholic extract were also explored using TNBS-induced UC in rats, in which the decreases in the inflammatory infiltrate and the number of cysts and abscesses in the colonic mucosa established the anti-inflammatory action of the propolis extract [65]. Moreover, *Baccharis dracunculifolia* DC (Asteraceae), a medicinal plant that is the main botanical source of Brazilian green propolis, also demonstrated a positive action in attenuating the colonic damage induced by TNBS in rats [66].

Dextran sodium sulfate (DSS) is used as the principal chemical agent for induction of intestinal inflammation in experimental animals, which has the ability to disrupt the integrity of the mucosal barrier. The main macroscopic marks include loss of weight, diarrhea, and rectal bleeding, while ulcerations and granulocyte infiltrations are the basic microscopic findings [63, 67]. In addition to the ability to induce UC, DSS is also used as a chemical agent to cause colon tumorigenesis, after a prior administration of carcinogenic initiators [68, 69]. Thus, Doi et al. evaluated the effects of ethanolic and water-extracts produced from Brazilian green propolis in the model of inflammation-associated rat colon carcinogenesis (1,2-dimethylhydrazine plus DSS treatment). The authors showed that the ethanol based extract exerted its anticancer effects through the suppression of inflammatory factors, such as tumor necrosis factor (TNF-α) and iNOS [70].

A recently published study evaluated the effects of caffeic acid phenethyl ester (CAPE), one of the main compounds of propolis, in DDS-induced acute colitis in a mice model. CAPE-treated group exhibited a protection in the epithelial barrier from disruption accompanied by and a decrease in MPO activity and proinflammatory cytokines levels [71]. In addition, CAPE effects were also explored in a previous study using the model of peptidoglycan-polysaccharide- (PG-PS-) induced colitis in rats [72]. PG-PS causes chronic inflammation, granulomas, crypt abscesses, and fibrosis, being also described as one of the only models that closely resemble

Crohn's disease [73]. CAPE was able to attenuate the PGPS-induced colitis through its ability to inhibit the nuclear factor-κB (NF-κB) pathway, by reducing the production of proinflammatory cytokines and by induction of apoptosis in macrophages [72]. Notably, several authors have shown that NF-κB was upregulated in macrophages and epithelial cells of patients with inflammatory bowel disease [74–76]. Furthermore, other studies have also shown the ability of CAPES as an inhibitor of NF-κB activation [77–80]. Similarly, another compound found in propolis, the flavonoid quercetin, also demonstrated potential in inhibiting the NF-κB pathway in an experimental model of DSS induced rat colitis [81]. In addition, quercetin has been the subject of several studies about its property in attenuating colitic damage in different experimental models, such as acetic acid-induced UC in mice [82, 83] and on TNBS-induced colitis in rats [84, 85].

It is well established that flavonoids are the main active constituents of propolis [86, 87]. This is in addition to those that have, already, been mentioned above (CAPE and quercetin). For this reason, the potential role of flavonoids found in propolis against UC has also been investigated. Of this class of compounds, the studies developed with kaempferol, luteolin, and naringenin stood out. Park et al. [88] showed that the treatment with kaempferol was effective against the damage induced by DSS in the colonic mucosa of mice, an effect related to its anti-inflammatory properties. Luteolin was able to ameliorate DSS-induced UC, which was verified in different experimental trials [89, 90]. Finally, naringenin showed a protective effect against DSS-induced UC in mice [91, 92] and in an acetic acid model of colitis in rats [93]. On the other hand, although propolis and its main components have shown promising results in the treatment of experimental UC (mainly due to their antioxidative and anti-inflammatory properties), as verified by the several publications described here, these studies do not translate to human application, remaining to be explored its efficacy and safety in clinical trials.

5. Propolis and Its Potential to Treat Gastrointestinal Cancers

When it comes to the therapeutic effects of honey and bee products on various types of cancers, several outstanding original scientific works on propolis research can be highlighted. Apoptosis is one of the most important homeostatic features of a biological system that has a therapeutic critical role against cancer. Apoptosis is mainly mediated via caspase-independent and caspase-dependent pathways which can be stimulated through extrinsic signals (TNF family of cytokine receptors) and intrinsic signals (cytochrome c from mitochondria) [94]. Most of studies on herbal medicines and natural products have been conducted to find out bioactive components that possess significant therapeutic effects against different types of cancer and to assess the anticancerous effects of propolis and its extracts, including either its ethanolic or its aqueous extracts.

Among the various ingredients of propolis, caffeic acid phenethyl ester (CAPE) and artepillin C are two that are well-studied components showing anticancerous effects, which impose their bioactivity through apoptotic pathway. Moreover, essential oils of propolis are able to suppress human tumors through the reduction on the cell proliferation. Essential oils extracted from Xinjiang propolis were able to cause cell cycle arrest and apoptosis induction in HTC-116 (a human colorectal cancer cell line) [95].

CAPE, commonly present in propolis, has various biological activities including cytostatic and cytotoxic properties [96]. Cytotoxic effects of CAPE have been reported against oral squamous cell carcinoma and oral epidermoid carcinoma-Meng 1 [97]. These effects in addition to DNA degradation are attributed to apoptosis and altered redox state [98]. The authors showed that, while there is no cell cycle arrest against normal human oral fibroblast, treatments with 25 μM and 50 μM of CAPE for 24 h cause arrest at G2/M phase and sub-G0/G1 peak, respectively, in OEC-M1 cells which is an oral squamous cell carcinoma cell line. The apoptotic effect of CAPE is also associated with a selective scavenging ability for hydrogen peroxide as shown in human leukemic HL-60 cell line study [99]. CAPE is a strong suppressor of TNF activating ability for NFκB [100]. In fact, CAPE was able to suppress the activation of NF-κB, which is referred to as "a Critical Link between Inflammation and Cancer" [101]. Further studies showed that CAPE has cytotoxic effects against human leukemia [102], oral submucous fibroblast, neck metastasis of gingiva carcinoma, and tongue squamous cell carcinoma cells [103]. On the other hand, the inhibition of NF-κB implies apoptosis through Fas activation [104]. In BxPC-3 cells, a pancreas adenocarcinoma cell line, CAPE, was able to reduce mitochondrial transmembrane activity which leads to apoptosis through caspase activity of caspase-3/caspase-7 [105]. The therapeutic effect of CAPE against cholangiocarcinoma in extrahepatic biliary cancer cell line, human intrahepatic bile duct and human extrahepatic bile duct, intrahepatic bile ducts, and nonmalignant cholangiocyte cell line H69 showed that CAPE is able to inhibit NFκB and induces apoptosis [106].

CAPE is also capable of scavenging free radical through 5-lipoxygenase inhibition [107] and suppressing lipid peroxidation [108]. Inhibition of NFκB suppresses the level of inducible nitric oxide synthase and reduces its catalytic activity [109]. A study on CT26 colon adenocarcinoma showed that CAPE possesses angiogenesis effect that leads to the suppression of invasiveness on tumor cell and metastasis in murine [110]. Similarly, CAPE treatment of SKHep1 human hepatocellular carcinoma cells restricts invasion [111] and enhances glucose metabolism through adenosine monophosphate- (AMP-) activated protein kinase (AMPK) in skeletal muscle cells [112]. Self-renewal of breast cancer stem cells, isolated from MDA-231 cells which is a human triple-negative breast cancer model, demonstrated a dose-dependent inhibition by CAPE through the downregulation of the expression of CD44 (a marker of cancer-initiating cells in some malignancies [113]). The majority of these cells showed cell cycle arrest at G0/G1 level of the cell cycle [114].

In addition to CAPE, other ingredients of propolis such as artepillin C, galangin, kaempferol, and quercetin showed antiangiogenesis properties [115]. Artepillin C, found in Brazilian propolis, was able to suppress the formation of

membranous lipid peroxidation and 8-hydroxydeoxyguanosine [116]. In colorectal cancer, CAPE was able to suppress β-catenin/Tcell factor signalling, an important malignancy marker [117], and to affect crypt foci and colorectal tumor in rats [118]. Moreover, in an *in vitro* study on colon cancer cell lines showed a suppressive effect of artepillin C on Cip1/p21 protein, a quiescence state of G0/G1 phase arrest, which in turn is a conquest of cytostatic state in colon cancer [119]. Propolis also contains prenylflavanone compounds such as propolin G, which showed some therapeutic effects against glioma and glioblastoma, brain cancer cell lines through caspases-dependent pathway of apoptosis, and mitochondria pathways [120].

Galangin is a flavonoid that can be found in propolis and has antigenotoxic properties which makes propolis a valuable bioactive agent against cancerous proliferation through mechanisms involving NFκB, B-cell lymphoma-extra large [bcl-X(L)], and COX-2 (for a review see [121]). For instance, in human colon cancer cells (HCT-15 and HT-29), galangin is able to induce apoptosis and DNA condensation [122] and enhances "adenomatous polyposis coli gene product (APC)/Axin/glycogen synthase kinase-3 beta (GSK-3ß-) independent proteasomal degradation of ß-catenin" in adenomatous polyposis coli cancer cells and inhibits their proliferation [123]. Studies showed that galangin has suppressive effect against angiogenesis of ovarian cancer cells [124] and activates p38 MAPK and induces apoptosis through mitochondrial pathway in melanoma cells (B16F10) [125]. DNA fragmentation induced by galangin is another antiproliferative property of galangin as seen in HL-60 cells of a promyelocytic cell line [126].

Kaempferol is another flavonoid ingredient of propolis inducing apoptosis through activation of TNF-related apoptosis-inducing ligand (TRAIL) in SW480 cells, a human colon cancer [127], and inhibiting ribosomal protein S6 kinase (RSK2) and mitogen- and stress-activated kinase (MSK1), main regulators in tumor promoter induced cell transformation [128]. Moreover, this ingredient is able to suppress Src kinase activity and inhibits COX-2, through which kaempferol showed an effective preventive property against skin cancer [129]. Treatment with kaempferol causes a down-regulation of proliferation in human prostate cancer through suppression of proliferating-cell nuclear antigen (PCNA) and vascular cell adhesion molecule-1 (VCAM-1) [130]. Similar to the other flavonoids ingredients, quercetin exhibits anticancerous properties through stimulation of apoptotic pathways. For instance, 25 μM and 50 μM of quercetin suppress the proliferation of prostate cancer cell lines such as PC-3 and DU-145 and stimulate tumor suppressor genes [131].

Therapeutic properties of propolis seem to vary according to their geographical location. For instance, Chinese and Korean propolis suppress interleukin- (IL-) 6 [132], which is a critical mediator of solid malignancies [133]. In mice, ethanolic extract of Brazilian propolis regulates the level of Toll-like receptor- (TLR-) 4 [134], promoting gastric cancer through production of mitochondrial reactive oxygen species (ROS) [135], and the level of IL-4 [136], promoting tumor growth and invasion [137]. The extract also showed cytotoxic in HEp-2, human laryngeal epidermoid carcinoma [138]. Methanol extract of red propolis showed significant cytotoxicity against human pancreatic PANC-1 cancer cell line [139]. Korean propolis contains ethanol-soluble ingredients that inhibit NFκB [140] which is a potential anticancerous target [141]. Aqueous extracts of propolis inhibited proliferation of various cell lines such as McCoy, HeLa, SP2/0, HEp-2, and BHK21 [142]. Ethanolic extract of propolis contained more bioactive compounds than the water ones. Ethanolic extract of propolis increases TRAIL mediated apoptosis in malignant cells of human cervical cancer HeLa cell line [143], prostate cancer cells [144], and some human colon carcinoma cells such as CaCo2, HCT116, HT29, and SW480 [145]. Ethanolic extract of Polish to possesses chemopreventive effects against prostate cancer cells through apoptosis, which is activated by TRAIL receptor 2 [146].

6. Antiulcer Activity of Propolis

Gastric ulcer is defined as an injury to the gastric mucosa, which occurs due an imbalance between the luminal challenge exerted by the highly acidic and proteolytic properties of gastric juices and the ability of the mucosa to resist them [147]. This disease affects 10% of the world population, but its etiology is not completely understood [148]. There are various noxious agents to the stomach resulting in mucosal ulceration, such as *Helicobacter pylori* infections, prolonged ingestion of nonsteroidal anti-inflammatory drugs (NAIDs), alcoholic drinks, psychological stress, and cigarette smoking. On the other hand, the stomach protects itself through many defense mechanisms, mainly adequate blood flow and bicarbonate and mucus secretions [149].

The treatment of gastric ulcers is based on using antisecretory drugs, including type-2 histamine receptor antagonists (H2-RAs) and proton pump inhibitors (PPIs) [150], as well as antibiotics used to treat the *H. pylori* infections [151]. However, these therapeutic agents are typically associated with numerous adverse side effects, such as hypersensitivity, vitamin B12 and iron deficiency, arrhythmia, increased susceptibility to pneumonia, impotence, gynecomastia, bone fractures, hematopoietic changes, hypergastrinemia, and gastric cancer. In this context, natural products are considered as attractive sources for new antiulcer treatments. Among them, propolis has been used in folk medicine to treat gastric ulcer and this has boosted research in order to investigate and validate its use as an antiulcer agent as discussed below.

Investigations about the gastroprotective effects of ethanolic extract of propolis against ethanol-induced gastric ulcer in rats revealed that the administration of the extract prevented the occurrence of gastric ulcerations in a dose-dependent manner. Furthermore, propolis extract reduced the lipid peroxidation, based on both *in vivo* and *in vitro* experiments, and levels and scavenged the superoxide anion. So, the authors concluded that the gastric protective mechanism of ethanol propolis extract was due, at least in part, to its ability to protect the gastric mucosa from oxidative stress [152]. In another study, El-Ghazaly et al. [153] investigated the gastroprotective effect of an aqueous propolis extract which was evaluated using indomethacin-induced gastric ulcers in rats exposed or nonexposed to gamma radiation.

The results from this study confirmed that the pretreatment with the aqueous propolis extract to the irradiated or nonirradiated rats protected against gastric ulceration. Moreover, the extract increased the mucosal prostaglandin E2 (PGE2) levels and decreased the TNF-α and IL-1β amount in the plasma. Interestingly, the gastric acid antisecretory effect of the aqueous propolis extract was described by those authors and the beneficial effects measured were associated with a reduction in acid output and peptic acid activity, associated with increased mucin secretion. Given that the therapeutic properties of propolis may vary according to the geographical collection region, it is important to emphasize that the extract used by El-Ghazaly and collaborators [153] was obtained using raw propolis from many different countries and standardized in 13% of propolis containing not less than 0.05% organic aromatic acids, calculated as the total of caffeic acid, ferulic acid, and cinnamic acid, beyond traces of different flavonoids.

The propolis produced in the Southeastern region of Brazil is known as green propolis because of its color. The plant *Baccharis dracunculifolia* DC (Asteraceae) is the primary source for it, a common species found in the Brazilian Cerrado. Due to similarities among chemical constituents of green propolis with those present in *B. dracunculifolia*, this plant was identified as being the principal source of green propolis. The antiulcer activity of green propolis hydroalcoholic crude extract was evaluated by de Barros et al. [154] using models of acute gastric lesions induced by ethanol, indomethacin, or stress in rats. In this study, the green propolis extract (500 mg/kg, p.o.) reduced the indomethacin-induced gastric ulcers. Moreover, in the stress-induced ulcer a significant reduction in ulcer area in animals treated with green propolis extract (250 and 500 mg/kg) was observed. Regarding the antisecretory capability of green propolis extract, the authors described a reduction in the gastric juice volume, as well as in the total acidity after extract (250 and 500 mg/kg) administration in pylorus ligated rats. Therefore, in accordance with the findings on propolis from other countries, these data place the Brazilian green propolis as a promising antiulcer agent. Barros et al. [155] also described the gastroprotective properties of the main phenolic acids found in Brazilian green propolis. Similarly to the results obtained by Barros et al. [155], the oral treatment with caffeic, ferulic, and p-coumaric and cinnamic acids at doses of 50 and 250 mg/kg diminished the total area of the lesion induced by different harmful agents. In addition, the effects of these substances on gastric acid secretions were measured and the results revealed that the phenolic acids tested, except for p-coumaric, reduced the gastric acid secretion in rats at a dose of 50 mg/kg.

Up to this point, the reviewed studies have evaluated the antiulcer protective effects of propolis preparations. Such findings do not necessarily mean that they has a healing capacity against gastric ulcers [156]. In view of this, chronic gastric ulcers induced by acetic acid instillation at gastric serosa have been a widely used model for the evaluation of the gastric healing potential of natural products or herbal medicines. Indeed, Belostotskiĭ and collaborators [157] described the healing gastric effects after administration of honey, royal jelly, and propolis in rats exposed to acetic acid into the gastric serosa. Based on the above, it would be prudent to recommend conducting further studies to strengthen the the healing potential of propolis based preparations or its constituents against gastric ulcers. This is in addition to having a more comprehensive understanding of its antiulcer activities and a better understanding of the underlying mechanism(s) of action.

Despite many studies about the antiulcer potential of propolis, mainly its gastroprotective action, little is known regarding its activity against *H. pylori*. In this field, Villanueva et al. [158] evaluated the inhibitory activity of 22 propolis extracts obtained from nine of the 11 beekeeping Chilean regions on 10 strains of *H. pylori* isolated from the gastric mucosa. Interestingly, 100% of the tested extracts inhibited the *H. pylori* growth, but those authors also pointed out that the need for additional microbiological studies before a potential clinical trial of these natural products is warranted.

7. Conclusions

In conclusion, this review included a summary of the data published by many researchers related to the protective and/or treatment role that propolis and/or its active ingredients play against gastrointestinal associated disorders that affect humans. The focus was on the following: oral mucositis, ulcerative colitis, gastrointestinal cancers, and gastric ulcers. Analysis of the published work indicated that the efficacy of propolis in the treatment of gastrointestinal disorders could be attributed to its antioxidants and anti-inflammatory properties. The underling mechanism of action is mediated through the inhibition of some transcriptional factors and related proteins. Several experimental studies showed the beneficial effects of propolis and its related compounds in the treatment of gastrointestinal diseases. However, only few clinical trials have been developed to prove their effectiveness and safety against human ulcers and other involved pathologies. Future studies should focus on the potential role of propolis and its related ingredients either alone or as a complementary therapy to ongoing conventional therapy against gastrointestinal diseases in humans.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] V. S. Bankova, S. L. de Castro, and M. C. Marcucci, "Propolis: recent advances in chemistry and plant origin," *Apidologie*, vol. 31, no. 1, pp. 3–15, 2000.

[2] M. Simone-Finstrom and M. Spivak, "Propolis and bee health: the natural history and significance of resin use by honey bees," *Apidologie*, vol. 41, no. 3, pp. 295–311, 2010.

[3] S. H. Sung, G. H. Choi, N. W. Lee, and B. C. Shin, "External use of propolis for oral, skin, and genital diseases: a systematic review and meta-analysis," *Evidence Based Complementary and Alternative Medicine*, vol. 2017, Article ID 8025752, 10 pages, 2017.

[4] T. Ueda, M. Inden, K. Shirai et al., "The effects of Brazilian green propolis that contains flavonols against mutant copper-zinc superoxide dismutase-mediated toxicity," *Scientific Reports*, vol. 7, no. 1, pp. 1–11, 2017.

[5] E. L. Ghisalberti, "Propolis: a review," *Bee World*, vol. 60, no. 2, pp. 59–84, 1979.

[6] É. W. Teixeira, G. Negri, R. M. S. A. Meira, D. Message, and A. Salatino, "Plant origin of green propolis: bee behavior, plant anatomy and chemistry," *Evidence-Based Complementary and Alternative Medicine*, vol. 2, no. 1, pp. 85–92, 2005.

[7] J. M. Sforcin and V. Bankova, "Propolis: is there a potential for the development of new drugs?" *Journal of Ethnopharmacology*, vol. 133, no. 2, pp. 253–260, 2011.

[8] C. C. M. Gonçalves, L. Hernandes, C. A. Bersani-Amado, S. L. Franco, J. F. Silva, and M. R. Natali, "Use of propolis hydroalcoholic extract to treat colitis experimentally induced in rats by 2,4,6-trinitrobenzenesulfonic acid," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 853976, 11 pages, 2013.

[9] M. H. Akhavan-Karbassi, M. F. Yazdi, H. Ahadian, and M. J. Sadr-Abad, "Randomized double-blind placebo-controlled trial of propolis for oral mucositis in patients receiving chemotherapy for head and neck cancer," *Asian Pacific Journal of Cancer Prevention*, vol. 17, no. 7, pp. 3611–3614, 2016.

[10] J. M. Sforcin, "Biological Properties and Therapeutic Applications of Propolis," *Phytotherapy Research*, vol. 30, no. 6, pp. 894–905, 2016.

[11] M. Khan and N. Gupta, "Oral mucositis," *European Journal Dentistry*, vol. 3, pp. 405–411, 2013.

[12] C. Alvariño-Martín and M.-G. Sarrión-Pérez, "Prevention and treatment of oral mucositis in patients receiving chemotherapy," *Journal of Clinical and Experimental Dentistry*, vol. 6, no. 1, pp. e74–e80, 2014.

[13] B. Rohani, K. Pourfar, H. Pourshahidi, and S. H. Ebrahimi, "Oral manifestation of hematologic malignancies," *Jundishapur Science Medical Journal*, vol. 14, pp. 477–485, 2015.

[14] S. W. Redding, "Cancer therapy-related oral mucositis," *Journal of Dental Education*, vol. 69, no. 8, pp. 919–929, 2005.

[15] E. Jadaud and R. J. Bensadoun, "Low-level laser therapy: A standard of supportive care for cancer therapy-induced oral mucositis in head and neck cancer patients?" *Laser Therapy*, vol. 21, no. 4, pp. 297–303, 2012.

[16] W. J. Kostler, M. Hejna, C. Wenzel, and C. C. Zielinski, "Oral mucositis complicating chemotherapy and/or radiotherapy: options for prevention and treatment," *CA: A Cancer Journal for Clinicians*, vol. 51, no. 5, pp. 290–315, 2001.

[17] N. Cirillo, A. Vicidomini, M. Mccullough et al., "A hyaluronic acid-based compound inhibits fibroblast senescence induced by oxidative stress in vitro and prevents oral mucositis in vivo," *Journal of Cellular Physiology*, vol. 230, no. 7, pp. 1421–1429, 2015.

[18] A. Simões, F. P. Eduardo, A. C. Luiz et al., "Laser phototherapy as topical prophylaxis against head and neck cancer radiotherapy-induced oral mucositis: Comparison between low and high/low power lasers," *Lasers in Surgery and Medicine*, vol. 41, no. 4, pp. 264–270, 2009.

[19] S. Silverman Jr., "Diagnosis and management of oral mucositis," *The Journal of Supportive Oncology*, vol. 5, no. 2, pp. 13–21, 2007.

[20] C. D. M. V. Brasil, M. S. Serpa, T. R. T. de França, and J. F. L. de Castro, "Management of oral mucositis," *Archive of Oncology*, vol. 20, no. 3-4, pp. 57–61, 2012.

[21] H. E. Jakobsson, C. Jernberg, A. F. Andersson, M. Sjölund-Karlsson, J. K. Jansson, and L. Engstrand, "Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome," *PLoS ONE*, vol. 5, no. 3, Article ID e9836, 2010.

[22] C. Jernberg, S. Löfmark, C. Edlund, and J. K. Jansson, "Long-term impacts of antibiotic exposure on the human intestinal microbiota," *Microbiology*, vol. 156, no. 11, pp. 3216–3223, 2010.

[23] N. Li, Y. Q. Li, H. Y. Li, W. Guo, and Y. P. Bai, "Efficacy of externally applied chinese herbal drugs in treating psoriasis: a systematic review," *Chinese Journal of Integrative Medicine*, vol. 18, no. 3, pp. 222–229, 2012.

[24] S. Iyyam Pillai, P. Palsamy, S. Subramanian, and M. Kandaswamy, "Wound healing properties of Indian propolis studied on excision wound-induced rats," *Pharmaceutical Biology*, vol. 48, no. 11, pp. 1198–1206, 2010.

[25] J. M. Senedese, A. R. Rodrigues, M. A. Furtado et al., "Assessment of the mutagenic activity of extracts of Brazilian propolis in topical pharmaceutical formulations on mammalian cells in vitro and in vivo," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 315701, 7 pages, 2011.

[26] E. M. Pereira, J. L. da Silva, F. F. Silva et al., "Clinical evidence of the efficacy of a mouthwash containing propolis for the control of plaque and gingivitis: a phase II study," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 750249, 7 pages, 2011.

[27] Y. J. Hwu and F. Y. Lin, "Effectiveness of propolis on oral health: a meta-analysis," *Journal of Nursing Research*, vol. 22, no. 4, pp. 221–229, 2014.

[28] L. Ghassemi, E. Zabihi, R. Mahdavi, M. Seyedmajidi, S. Akram, and M. Motallebnejad, "The effect of ethanolic extract of propolis on radiation induced mucositis in rats," *Saudi Medical Journal*, vol. 31, no. 6, pp. 622–626, 2010.

[29] S. A. Atanasovska, M. Popovska, I. Muratovska, K. Mitic, E. Stefanovska, and V. Radojkova Nikolovska, "Therapeutic effect of proaftol in treatment of recurrent aphthous stomatitis," *Pril (Makedon Akad Nauk Umet Odd Med Nauki)*, vol. 35, no. 3, pp. 195–202, 2014.

[30] H. M. Capistrano, E. M. De Assis, R. M. Leal, M. E. Alvarez-Leite, S. Brener, and E. M. Bastos, "Brazilian green propolis compared to miconazole gel in the treatment of Candida-associated denture stomatitis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 947980, 6 pages, 2013.

[31] N. R. Ngatu, T. Saruta, R. Hirota et al., "Antifungal efficacy of Brazilian green propolis extracts and honey on Tinea capitis and Tinea versicolor," *European Journal of Integrative Medicine*, vol. 3, no. 4, pp. e275–e281, 2011.

[32] J. M. Park and C. J. Han, "A effects of pine pollen and propolis skin treatment on acne skin," *Journal of the Korean Society of Cosmetology*, vol. 19, no. 6, pp. 1103–1111, 2013.

[33] M. Q. Yin, "A clinical study of efficacy of propolis ointment combined with routine care for the diabetes mellitus with foot ulcer," *World Health Digest*, vol. 10, no. 9, pp. 248-249, 2013.

[34] M. Mousavi, R. Mannani, M. Mottaghi, B. Torkan, and H. Afrouzan, "Comparing the effect of propolis vaginal cream and metronidazol vaginal gel for treatment of bacterial vaginosis," *Journal of Zanjan University of Medical Sciences and Health Services*, vol. 24, no. 106, pp. 42–50, 2016.

[35] N. Vynograd, I. Vynograd, and Z. Sosnowski, "A comparative multi-centre study of the efficacy of propolis, acyclovir

and placebo in the treatment of genital herpes (HSV)," *Phytomedicine*, vol. 7, no. 1, pp. 1–6, 2000.

[36] World Health Organization (WHO), ICD-10 Version: 2016, 2016, http://apps.who.int/classifications/icd10/browse/2016/en.

[37] S. Huang, C.-P. Zhang, K. Wang, G. Li, and F.-L. Hu, "Recent advances in the chemical composition of propolis," *Molecules*, vol. 19, no. 12, pp. 19610–19632, 2014.

[38] M. A. Savka, L. Dailey, M. Popova et al., "Chemical composition and disruption of quorum sensing signaling in geographically diverse United States propolis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 472593, 10 pages, 2015.

[39] V. D. Wagh, "Propolis: a wonder bees product and its pharmacological potentials," *Advances in Pharmacological Sciences*, vol. 2013, Article ID 308249, 11 pages, 2013.

[40] M. G. Miguel and M. D. Antunes, "Is propolis safe as an alternative medicine?" *Journal of Pharmacy and Bioallied Sciences*, vol. 3, no. 4, pp. 479–495, 2011.

[41] M. Piredda, G. Facchinetti, I. Stan et al., "Propolis for prevention of chemo-induced oral mucositis in breast cancer patients: a randomized controlled trial," *Annals of Oncology*, vol. 26, supplement 6, 2015.

[42] S.-H. Sung, G.-H. Choi, N.-W. Lee, and B.-C. Shin, "External Use of Propolis for Oral, Skin, and Genital Diseases: A Systematic Review and Meta-Analysis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2017, Article ID 8025752, 2017.

[43] V. Dodwad and B. Kukreja, "Propolis mouthwash: a new beginning," *Journal of Indian Society of Periodontology*, vol. 15, no. 2, pp. 121–125, 2011.

[44] J. L. Chen, X. H. Fan, and L. L. Ma, "A clinical study of efficacy of propolis treatment for recurrent oral aphthae," *Capital Medicine*, vol. 16, no. 18, pp. 43-44, 2009.

[45] H. S. Ali and B. K. Abdul Rasool, "Propolis buccal paste in treatment of aphthous ulceration: formulation and clinical evaluation," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 4, no. 4, pp. 29–33, 2011.

[46] S. K. Al Jaouni, M. S. Al Muhayawi, A. Hussein et al., "Effects of Honey on Oral Mucositis among Pediatric Cancer Patients Undergoing Chemo/Radiotherapy Treatment at King Abdulaziz University Hospital in Jeddah, Kingdom of Saudi Arabia," *Evidence-Based Complementary and Alternative Medicine*, vol. 2017, Article ID 5861024, 2017.

[47] F. Magro, P. Gionchetti, R. Eliakim et al., "Third european evidence-based consensus on diagnosis and management of ulcerative colitis. part 1: definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders," *Journal of Crohn's and Colitis*, vol. 11, no. 6, pp. 649–670, 2017.

[48] J. Panés and I. Alfaro, "New treatment strategies for ulcerative colitis," *Expert Review of Clinical Immunology*, vol. 21, no. 1, pp. 1–11, 2017.

[49] D. C. Baumgart and S. R. Carding, "Inflammatory bowel disease: cause and immunobiology," *The Lancet*, vol. 369, no. 9573, pp. 1627–1640, 2007.

[50] C. Abraham and J. H. Cho, "Inflammatory bowel disease," *The New England Journal of Medicine*, vol. 361, no. 21, pp. 2066–2078, 2009.

[51] D. C. Baumgart and W. J. Sandborn, "Inflammatory bowel disease: clinical aspects and established and evolving therapies," *The Lancet*, vol. 369, no. 9573, pp. 1641–1657, 2007.

[52] S. Ben-Horin, M. Margalit, P. Bossuyt et al., "Combination Immunomodulator and Antibiotic Treatment in Patients With Inflammatory Bowel Disease and Clostridium difficile Infection," *Clinical Gastroenterology and Hepatology*, vol. 7, no. 9, pp. 981–987, 2009.

[53] M. Harbord, R. Eliakim, D. Bettenworth et al., "Third european evidence-based consensus on diagnosis and management of ulcerative colitis. part 2: current management," *Journal of Crohn's and Colitis*, vol. 11, no. 7, pp. 769–784, 2017.

[54] M. T. Santana, L. M. Cercato, J. P. Oliveira, and E. A. Camargo, "Medicinal Plants in the Treatment of Colitis: Evidence from Preclinical Studies," *Planta Medica*, vol. 83, no. 7, pp. 588–614, 2017.

[55] P. Dan, E. H. M, and S. Jarnum, "Effect of the natural product propolis on ulcerative colitis and Crohn's disease," *UgeskrLaeger*, vol. 141, no. 28, pp. 1888–1890, 1979.

[56] A. Aslan, M. Temiz, E. Atik et al., "Effectiveness of mesalamine and propolis in experimental colitis," *Advances in Therapy*, vol. 24, no. 5, pp. 1085–1097, 2007.

[57] B. R. MacPherson and C. J. Pfeiffer, "Experimental production of diffuse colitis in rats," *Digestion*, vol. 17, no. 2, pp. 135–150, 1978.

[58] F. Guarner and J.-R. Malagelada, "Role of bacteria in experimental colitis," *Best Practice & Research Clinical Gastroenterology*, vol. 17, no. 5, pp. 793–804, 2003.

[59] A. Aslan, M. Cetin, M. Temiz, N. Sahinler, E. Besirov, and N. Aban, "The effect of propolisis and mesalazine on bacterial translocation in an experimental colitis model," *Saudi Medical Journal*, vol. 28, no. 10, pp. 1605–1607, 2007.

[60] G. Barbosa Bezerra, L. de Menezes, A. S. dos Santos et al., "Hydroalcoholic extract of Brazilian red propolis exerts protective effects on acetic acid-induced ulcerative colitis in a rodent model," *Biomedicine Pharmacotherapy*, vol. 85, pp. 687-396, 2017.

[61] M. F. Neurath, I. Fuss, B. L. Kelsall, E. Stüber, and W. Strober, "Antibodies to interleukin 12 abrogate established experimental colitis in mice," *The Journal of Experimental Medicine*, vol. 182, no. 5, pp. 1281–1290, 1995.

[62] S. Wirtz, C. Neufert, B. Weigmann, and M. F. Neurath, "Chemically induced mouse models of intestinal inflammation," *Nature Protocols*, vol. 2, no. 3, pp. 541–546, 2007.

[63] R. M. Gadaleta, O. Garcia-Irigoyen, and A. Moschetta, "Exploration of Inflammatory Bowel Disease in Mice: Chemically Induced Murine Models of Inflammatory Bowel Disease (IBD)," *Current Protocols in Mouse Biology*, vol. 7, no. 1, pp. 13–28, 2017.

[64] Y. Okamoto, T. Hara, T. Ebato, T. Fukui, and T. Masuzawa, "Brazilian propolis ameliorates trinitrobenzene sulfonic acid-induced colitis in mice by inhibiting Th1 differentiation," *International Immunopharmacology*, vol. 16, no. 2, pp. 178–183, 2013.

[65] C. C. M. Gonçalves, L. Hernandes, C. A. Bersani-Amado, S. L. Franco, J. F. D. S. Silva, and M. R. M. Natali, "Use of propolis hydroalcoholic extract to treat colitis experimentally induced in rats by 2,4,6-trinitrobenzenesulfonic acid," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 853976, 11 pages, 2013.

[66] S. H. Cestari, J. K. Bastos, and L. C. Di Stasi, "Intestinal anti-inflammatory activity of baccharis dracunculifolia in the trinitrobenzenesulphonic acid model of rat colitis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 524349, 2011.

[67] I. Okayasu, S. Hatakeyama, M. Yamada, T. Ohkusa, Y. Inagaki, and R. Nakaya, "A novel method in the induction of reliable

experimental acute and chronic ulcerative colitis in mice," *Gastroenterology*, vol. 98, no. 3, pp. 694–702, 1990.

[68] N. Toyoda-Hokaiwado, Y. Yasui, M. Muramatsu et al., "Chemopreventive effects of silymarin against 1,2-dimethylhydrazine plus dextran sodium sulfate-induced inflammation-associated carcinogenicity and genotoxicity in the colon of gpt delta rats," *Carcinogenesis*, vol. 32, no. 10, pp. 1512–1517, 2011.

[69] K. Arigesavan and G. Sudhandiran, "Carvacrol exhibits antioxidant and anti-inflammatory effects against 1, 2-dimethyl hydrazine plus dextran sodium sulfate induced inflammation associated carcinogenicity in the colon of Fischer 344 rats," *Biochemical and Biophysical Research Communications*, vol. 461, no. 2, pp. 314–320, 2015.

[70] K. Doi, M. Fujioka, Y. Sokuza et al., "Chemopreventive action by ethanol-extracted brazilian green propolis on post-initiation phase of inflammation-Associated rat colon tumorigenesis," *In Vivo*, vol. 31, no. 2, pp. 187–197, 2017.

[71] M. N. Khan, M. E. Lane, P. A. McCarron, and M. M. Tambuwala, "Caffeic acid phenethyl ester is protective in experimental ulcerative colitis via reduction in levels of pro-inflammatory mediators and enhancement of epithelial barrier function," *Inflammopharmacology*. In press.

[72] L. R. Fitzpatrick, J. Wang, and T. Le, "Caffeic acid phenethyl ester, an inhibitor of nuclear factor-κB, attenuates bacterial peptidoglycan polysaccharide-induced Colitis in rats," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 299, no. 3, pp. 915–920, 2001.

[73] L. Reingold, K. Rahal, P. Schmiedlin-Ren et al., "Development of a peptidoglycan-polysaccharide murine model of Crohn's disease: Effect of genetic background," *Inflammatory Bowel Diseases*, vol. 19, no. 6, pp. 1238–1244, 2013.

[74] R. D. Ellis, J. R. Goodlad, G. A. Limb, J. J. Powell, R. P. H. Thompson, and N. A. Punchard, "Activation of nuclear factor kappa B in Crohn's disease," *Inflammation Research*, vol. 47, no. 11, pp. 440–445, 1998.

[75] G. Rogler, K. Brand, D. Vogl et al., "Nuclear factor κB is activated in macrophages and epithelial cells of inflamed intestinal mucosa," *Gastroenterology*, vol. 115, no. 2, pp. 357–369, 1998.

[76] H. Bantel, C. Berg, M. Vieth, M. Stolte, W. Kruis, and K. Schulze-Osthoff, "Mesalazine inhibits activation of transcription factor NF-κB in inflamed mucosa of patients with ulcerative colitis," *The American Journal of Gastroenterology*, vol. 95, no. 12, pp. 3452–3457, 2000.

[77] K. Natarajan, S. Singh, T. R. Burke Jr., D. Grunberger, and B. B. Aggarwal, "Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-κB," *Immunology*, vol. 93, no. 17, pp. 9090–9095, 1996.

[78] J. Shvarzbeyn and M. Huleihel, "Effect of propolis and caffeic acid phenethyl ester (CAPE) on NFκB activation by HTLV-1 Tax," *Antiviral Research*, vol. 90, no. 3, pp. 108–115, 2011.

[79] M. S. Cho, W. S. Park, W.-K. Jung et al., "Caffeic acid phenethyl ester promotes anti-inflammatory effects by inhibiting MAPK and NF-κB signaling in activated HMC-1 human mast cells," *Pharmaceutical Biology*, vol. 52, no. 7, pp. 926–932, 2014.

[80] L. Li, W. Sun, T. Wu, R. Lu, and B. Shi, "Caffeic acid phenethyl ester attenuates lipopolysaccharide-stimulated proinflammatory responses in human gingival fibroblasts via NF-κB and PI3K/Akt signaling pathway," *European Journal of Pharmacology*, vol. 794, pp. 61–68, 2017.

[81] M. Comalada, D. Camuesco, S. Sierra et al., "*In vivo* quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-κB pathway," *European Journal of Immunology*, vol. 35, no. 2, pp. 584–592, 2005.

[82] C. F. S. Guazelli, V. Fattori, B. B. Colombo et al., "Quercetin-loaded microcapsules ameliorate experimental colitis in mice by anti-inflammatory and antioxidant mechanisms," *Journal of Natural Products*, vol. 76, no. 2, pp. 200–208, 2013.

[83] D. Dodda, R. Chhajed, and J. Mishra, "Protective effect of quercetin against acetic acid induced inflammatory bowel disease (IBD) like symptoms in rats: Possible morphological and biochemical alterations," *Pharmacological Reports*, vol. 66, no. 1, pp. 169–173, 2014.

[84] D. Dodda, R. Chhajed, J. Mishra, and M. Padhy, "Targeting oxidative stress attenuates trinitrobenzene sulphonic acid induced inflammatory bowel disease like symptoms in rats: role of quercetin," *Indian Journal of Pharmacology*, vol. 46, no. 3, pp. 286–291, 2014.

[85] I. Castangia, A. Nácher, C. Caddeo et al., "Therapeutic efficacy of quercetin enzyme-responsive nanovesicles for the treatment of experimental colitis in rats," *Acta Biomaterialia*, vol. 13, pp. 216–227, 2015.

[86] V. Bankova, "Chemical diversity of propolis and the problem of standardization," *Journal of Ethnopharmacology*, vol. 100, no. 1-2, pp. 114–117, 2005.

[87] V. Bankova, "Recent trends and important developments in propolis research," *Evidence-Based Complementary and Alternative Medicine*, vol. 2, no. 1, pp. 29–32, 2005.

[88] M.-Y. Park, G. E. Ji, and M.-K. Sung, "Dietary Kaempferol suppresses inflammation of dextran sulfate sodium-induced colitis in mice," *Digestive Diseases and Sciences*, vol. 57, no. 2, pp. 355–363, 2012.

[89] Y. Nishitani, K. Yamamoto, M. Yoshida et al., "Intestinal anti-inflammatory activity of luteolin: role of the aglycone in NF-κB inactivation in macrophages co-cultured with intestinal epithelial cells," *BioFactors*, vol. 39, no. 5, pp. 522–533, 2013.

[90] Y. Li, L. Shen, and H. Luo, "Luteolin ameliorates dextran sulfate sodium-induced colitis in mice possibly through activation of the Nrf2 signaling pathway," *International Immunopharmacology*, vol. 40, pp. 24–31, 2016.

[91] T. Azuma, M. Shigeshiro, M. Kodama, S. Tanabe, and T. Suzuki, "Supplemental naringenin prevents intestinal barrier defects and inflammation in colitic mice," *Journal of Nutrition*, vol. 143, no. 6, pp. 827–834, 2013.

[92] W. Dou, J. Zhang, A. Sun et al., "Protective effect of naringenin against experimental colitis via suppression of Toll-like receptor 4/NF-κB signalling," *British Journal of Nutrition*, vol. 110, no. 4, pp. 599–608, 2013.

[93] S. S. Al-Rejaie, H. M. Abuohashish, M. M. Al-Enazi, A. H. Al-Assaf, M. Y. Parmar, and M. M. Ahmed, "Protective effect of naringenin on acetic acid-induced ulcerative colitis in rats," *World Journal of Gastroenterology*, vol. 19, no. 34, pp. 5633–5644, 2013.

[94] J. C. Reed, "Mechanisms of apoptosis," *The American Journal of Pathology*, vol. 157, no. 5, pp. 1415–1430, 2000.

[95] A. Ghupur, Y. Rahman, T. Abdureyim, A. Tuerxun, and M. Amet, "Effect of essential oils extracted from Xinjiang propolis on cell proliferation, cell cycle progression and apoptosis in human colorectal cancer cell line HCT-116," *World Chinese Journal of Digestology*, vol. 19, no. 14, pp. 1469–1475, 2011.

[96] D. Grunberger, R. Banerjee, K. Eisinger et al., "Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester

isolated from propolis," *Experientia*, vol. 44, no. 3, pp. 230–232, 1988.

[97] Y.-T. Lee, M.-J. Don, P.-S. Hung et al., "Cytotoxicity of phenolic acid phenethyl esters on oral cancer cells," *Cancer Letters*, vol. 223, no. 1, pp. 19–25, 2005.

[98] C. Chiao, A. M. Carothers, D. Grimberger, G. Solomon, G. A. Preston, and J. C. Barrett, "Apoptosis and altered redox state induced by caffeic acid phenethyl ester (cape) in transformed rat fibroblast cells," *Cancer Research*, vol. 55, no. 16, pp. 3576–3583, 1995.

[99] Y.-J. Chen, M.-S. Shiao, and S.-Y. Wang, "The antioxidant caffeic acid phenethyl ester induces apoptosis associated with selective scavenging of hydrogen peroxide in human leukemic HL-60 cells," *Anti-Cancer Drugs*, vol. 12, no. 2, pp. 143–149, 2001.

[100] K. Natarajan, S. Singh, T. R. Burke Jr., D. Grunberger, and B. B. Aggarwal, "Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-κB," *Proceedings of the National Acadamy of Sciences of the United States of America*, vol. 93, no. 17, pp. 9090–9095, 1996.

[101] M. Karin, "NF-kappaB as a critical link between inflammation and cancer," *Cold Spring Harbor Perspectives in Biology*, vol. 1, no. 5, Article ID a000141, 2009.

[102] J. H. Chen, Y. Shao, M. T. Huang, C. K. Chin, and C. T. Ho, "Inhibitory effect of caffeic acid phenethyl ester on human leukemia HL-60 cells," *Cancer Letters*, vol. 108, no. 2, pp. 211–214, 1996.

[103] Y.-J. Lee, P.-H. Liao, W.-K. Chen, and C.-C. Yang, "Preferential cytotoxicity of caffeic acid phenethyl ester analogues on oral cancer cells," *Cancer Letters*, vol. 153, no. 1-2, pp. 51–56, 2000.

[104] M. Watabe, K. Hishikawa, A. Takayanagi, N. Shimizu, and T. Nakaki, "Caffeic acid phenethyl ester induces apoptosis by inhibition of NFkappaB and activation of Fas in human breast cancer MCF-7 cells," *The Journal of Biological Chemistry*, vol. 279, no. 7, pp. 6017–6026, 2004.

[105] M. Chen, W. Chang, C. Lin et al., "Caffeic acid phenethyl ester induces apoptosis of human pancreatic cancer cells involving caspase and mitochondrial dysfunction," *Pancreatology*, vol. 8, no. 6, pp. 558–565, 2008.

[106] P. Onori, S. DeMorrow, E. Gaudio et al., "Caffeic acid phenethyl ester decreases cholangiocarcinoma growth by inhibition of NF-κB and induction of apoptosis," *International Journal of Cancer*, vol. 125, no. 3, pp. 565–576, 2009.

[107] M. Touaibia and M. Guay, "Natural product total synthesis in the organic laboratory: Total synthesis of caffeic acid phenethyl ester (CAPE), a potent 5-lipoxygenase inhibitor from honeybee hives," *Journal of Chemical Education*, vol. 88, no. 4, pp. 473–475, 2011.

[108] M. Hoşnuter, A. Gürel, O. Babucçu, F. Armutcu, E. Kargi, and A. Işikdemir, "The effect of CAPE on lipid peroxidation and nitric oxide levels in the plasma of rats following thermal injury," *Burns*, vol. 30, no. 2, pp. 121–125, 2004.

[109] Y. S. Song, E.-H. Park, G. M. Hur et al., "Caffeic acid phenethyl ester inhibits nitric oxide synthase gene expression and enzyme activity," *Cancer Letters*, vol. 175, no. 1, pp. 53–61, 2002.

[110] H.-F. Liao, Y.-Y. Chen, J.-J. Liu et al., "Inhibitory effect of caffeic acid phenethyl ester on angiogenesis, tumor invasion and metastasis," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 27, pp. 7907–7912, 2003.

[111] K. W. Lee, N. J. Kang, J. H. Kim et al., "Caffeic acid phenethyl ester inhibits invasion and expression of matrix metalloproteinase in SK-Hep1 human hepatocellular carcinoma cells by targeting nuclear factor kappa B," *Genes & Nutrition*, vol. 2, no. 4, pp. 319–322, 2008.

[112] E. S. Lee, K. Uhm, Y. M. Lee et al., "CAPE (caffeic acid phenethyl ester) stimulates glucose uptake through AMPK (AMP-activated protein kinase) activation in skeletal muscle cells," *Biochemical and Biophysical Research Communications*, vol. 361, no. 4, pp. 854–858, 2007.

[113] M. Zöller, "CD44: can a cancer-initiating cell profit from an abundantly expressed molecule?" *Nature Reviews Cancer*, vol. 11, no. 4, pp. 254–267, 2011.

[114] C. O. Omene, J. Wu, and K. Frenkel, "Caffeic Acid Phenethyl Ester (CAPE) derived from propolis, a honeybee product, inhibits growth of breast cancer stem cells," *Investigational New Drugs*, vol. 30, no. 4, pp. 1279–1288, 2012.

[115] M.-R. Ahn, K. Kunimasa, S. Kumazawa et al., "Correlation between antiangiogenic activity and antioxidant activity of various components from propolis," *Molecular Nutrition & Food Research*, vol. 53, no. 5, pp. 643–651, 2009.

[116] K. Shimizu, H. Ashida, Y. Matsuura, and K. Kanazawa, "Antioxidative bioavailability of artepillin C in Brazilian propolis," *Archives of Biochemistry and Biophysics*, vol. 424, no. 2, pp. 181–188, 2004.

[117] D. Xiang, D. Wang, Y. He et al., "Caffeic acid phenethyl ester induces growth arrest and apoptosis of colon cancer cells via the β-catenin/T-cell factor signaling," *Anti-Cancer Drugs*, vol. 17, no. 7, pp. 753–762, 2006.

[118] F. Borrelli, A. A. Izzo, G. Di Carlo et al., "Effect of a propolis extract and caffeic acid phenethyl ester on formation of aberrant crypt foci and tumors in the rat colon," *Fitoterapia*, vol. 73, no. 1, pp. S38–S43, 2002.

[119] K. Shimizu, S. K. Das, T. Hashimoto et al., "Artepillin C in Brazilian propolis induces G0/G1 arrest via stimulation of Cip1/p21 expression in human colon cancer cells," *Molecular Carcinogenesis*, vol. 44, no. 4, pp. 293–299, 2005.

[120] W.-J. Huang, C.-H. Huang, C.-L. Wu et al., "Propolin G, a prenylflavanone, isolated from Taiwanese propolis, induces caspase-dependent apoptosis in brain cancer cells," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 18, pp. 7366–7376, 2007.

[121] M. Y. Heo, S. J. Sohn, and W. W. Au, "Anti-genotoxicity of galangin as a cancer chemopreventive agent candidate," *Mutation Research - Reviews in Mutation Research*, vol. 488, no. 2, pp. 135–150, 2001.

[122] T. K. Ha, M. E. Kim, J. H. Yoon, S. J. Bae, J. Yeom, and J. S. Lee, "Galangin induces human colon cancer cell death via the mitochondrial dysfunction and caspase-dependent pathway," *Experimental Biology and Medicine*, vol. 238, no. 9, pp. 1047–1054, 2013.

[123] J. Gwak, J. Oh, M. Cho et al., "Galangin suppresses the proliferation of β-catenin response transcription-positive cancer cells by promoting adenomatous polyposis coli/axin/glycogen synthase kinase-3β-independent β-catenin degradation," *Molecular Pharmacology*, vol. 79, no. 6, pp. 1014–1022, 2011.

[124] H. Huang, A. Y. Chen, Y. Rojanasakul, X. Ye, G. O. Rankin, and Y. C. Chen, "Dietary compounds galangin and myricetin suppress ovarian cancer cell angiogenesis," *Journal of Functional Foods*, vol. 15, pp. 464–475, 2015.

[125] W. Zhang, Y. Lan, Q. Huang, and Z. Hua, "Galangin induces B16F10 melanoma cell apoptosis via mitochondrial pathway and sustained activation of p38 MAPK," *Cytotechnology*, vol. 65, no. 3, pp. 447–455, 2013.

[126] C. S. Bestwick and L. Milne, "Influence of galangin on HL-60 cell proliferation and survival," *Cancer Letters*, vol. 243, no. 1, pp. 80–89, 2006.

[127] T. Yoshida, M. Konishi, M. Horinaka et al., "Kaempferol sensitizes colon cancer cells to TRAIL-induced apoptosis," *Biochemical and Biophysical Research Communications*, vol. 375, no. 1, pp. 129–133, 2008.

[128] K. Yao, H. Chen, K. Liu et al., "Kaempferol targets RSK2 and MSK1 to suppress UV radiation-induced skin cancer," *Cancer Prevention Research*, vol. 7, no. 9, pp. 958–967, 2014.

[129] K. M. Lee, K. W. Lee, S. K. Jung et al., "Kaempferol inhibits UVB-induced COX-2 expression by suppressing Src kinase activity," *Biochemical Pharmacology*, vol. 80, no. 12, pp. 2042–2049, 2010.

[130] W. Qiu, Y.-h. Lei, M. Su, D.-j. Li, N. Zhang, and Y.-q. Shen, "Kaempferol inhibits proliferation of human prostate cancer PC-3 cells via down-regulation of PCNA and VCAM-1," *Chinese Pharmacological Bulletin*, vol. 4, pp. 553–557, 2011.

[131] H. K. Nair, K. V. K. Rao, R. Aalinkeel, S. Mahajan, R. Chawda, and S. A. Schwartz, "Inhibition of Prostate Cancer Cell Colony Formation by the Flavonoid Quercetin Correlates with Modulation of Specific Regulatory Genes," *Clinical and Diagnostic Laboratory Immunology*, vol. 11, no. 1, pp. 63–69, 2004.

[132] F. Hu, H. R. Hepburn, Y. Li, M. Chen, S. E. Radloff, and S. Daya, "Effects of ethanol and water extracts of propolis (bee glue) on acute inflammatory animal models," *Journal of Ethnopharmacology*, vol. 100, no. 3, pp. 276–283, 2005.

[133] K. Taniguchi and M. Karin, "IL-6 and related cytokines as the critical lynchpins between inflammation and cancer," *Seminars in Immunology*, vol. 26, no. 1, pp. 54–74, 2014.

[134] A. C. Pagliarone, C. L. Orsatti, and M. C. Búfalo, "Propolis effects on pro-inflammatory cytokine production and Toll-like receptor 2 and 4 expression in stressed mice," *International Immunopharmacology*, vol. 9, no. 11, pp. 1352–1356, 2009.

[135] X. Yuan, Y. Zhou, W. Wang et al., "Activation of TLR4 signaling promotes gastric cancer progression by inducing mitochondrial ROS production," *Cell Death & Disease*, vol. 4, no. 9, p. e794, 2013.

[136] A. C. Pagliarone, F. Missima, C. L. Orsatti, T. F. Bachiega, and J. M. Sforcin, "Propolis effect on Th1/Th2 cytokines production by acutely stressed mice," *Journal of Ethnopharmacology*, vol. 125, no. 2, pp. 230–233, 2009.

[137] V. Gocheva, H.-W. Wang, B. B. Gadea et al., "IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion," *Genes & Development*, vol. 24, no. 3, pp. 241–255, 2010.

[138] M. C. Bufalo, J. M. Candeias, and J. M. Sforcin, "In vitro cytotoxic effect of Brazilian green propolis on human laryngeal epidermoid carcinoma (HEp-2) cells," *Evidence Based Complementary and Alternative Medicine*, vol. 6, no. 4, pp. 483–487, 2009.

[139] S. Awale, F. Li, H. Onozuka, H. Esumi, Y. Tezuka, and S. Kadota, "Constituents of Brazilian red propolis and their preferential cytotoxic activity against human pancreatic PANC-1 cancer cell line in nutrient-deprived condition," *Bioorganic & Medicinal Chemistry*, vol. 16, no. 1, pp. 181–189, 2008.

[140] Y. S. Song, E.-H. Park, G. M. Hur, Y. S. Ryu, Y. M. Kim, and C. Jin, "Ethanol extract of propolis inhibits nitric oxide synthase gene expression and enzyme activity," *Journal of Ethnopharmacology*, vol. 80, no. 2-3, pp. 155–161, 2002.

[141] H.-M. Shen and V. Tergaonkar, "NFκB signaling in carcinogenesis and as a potential molecular target for cancer therapy," *Apoptosis*, vol. 14, no. 4, pp. 348–363, 2009.

[142] M. F. Najafi, F. Vahedy, M. Seyyedin, H. R. Jomehzadeh, and K. Bozary, "Effect of the water extracts of propolis on stimulation and inhibition of different cells," *Cytotechnology*, vol. 54, no. 1, pp. 49–56, 2007.

[143] E. Szliszka, Z. P. Czuba, M. Domino, B. Mazur, G. Zydowicz, and W. Krol, "Ethanolic extract of propolis (EEP) enhances the apoptosis-inducing potential of TRAIL in cancer cells," *Molecules*, vol. 14, no. 2, pp. 738–754, 2009.

[144] E. Szliszka, Z. P. Czuba, J. Bronikowska, A. Mertas, A. Paradysz, and W. Krol, "Ethanolic extract of propolis augments TRAIL-induced apoptotic death in prostate cancer cells," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 535172, 11 pages, 2011.

[145] M. Ishihara, K. Naoi, M. Hashita, Y. Itoh, and M. Suzui, "Growth inhibitory activity of ethanol extracts of Chinese and Brazilian propolis in four human colon carcinoma cell lines," *Oncology Reports*, vol. 22, no. 2, pp. 349–354, 2009.

[146] E. Szliszka, A. Sokół-Łętowska, A. Z. Kucharska, D. Jaworska, Z. P. Czuba, and W. Król, "Ethanolic extract of polish propolis: chemical composition and TRAIL-R2 death receptor targeting apoptotic activity against prostate cancer cells," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 757628, 12 pages, 2013.

[147] R. H. Hunt, M. Camilleri, S. E. Crowe et al., "The stomach in health and disease," *Gut*, vol. 64, no. 10, pp. 1650–1668, 2015.

[148] S. K. Sahoo, H. B. Sahoo, D. Priyadarshini, G. Soundarya, C. Kishore Kumar, and K. Usha Rani, "Antiulcer activity of ethanolic extract of Salvadora indica (W.) leaves on Albino rats," *Journal of Clinical and Diagnostic Research*, vol. 10, no. 9, pp. FF07–FF10, 2016.

[149] H. M. Ali Sidahmed, N. M. Hashim, S. Mohan et al., "Evidence of the gastroprotective and anti-helicobacter pylori activities of β-mangostin isolated from Cratoxylum arborescens (Vahl) blume," *Drug Design, Development and Therapy*, vol. 10, pp. 297–313, 2016.

[150] E. Sheen and G. Triadafilopoulos, "Adverse effects of long-term proton pump inhibitor therapy," *Digestive Diseases and Sciences*, vol. 56, no. 4, pp. 931–950, 2011.

[151] P. Malfertheiner, F. K. Chan, and K. E. McColl, "Peptic ulcer disease," *The Lancet*, vol. 374, no. 9699, pp. 1449–1461, 2009.

[152] C.-F. Liu, C.-C. Lin, M.-H. Lin, Y.-S. Lin, and S.-C. Lin, "Cytoprotection by propolis ethanol extract of acute absolute ethanol-induced gastric mucosal lesions," *American Journal of Chinese Medicine*, vol. 30, no. 2-3, pp. 245–254, 2002.

[153] M. A. El-Ghazaly, R. R. A. Rashed, and M. T. Khayyal, "Anti-ulcerogenic effect of aqueous propolis extract and the influence of radiation exposure," *International Journal of Radiation Biology*, vol. 87, no. 10, pp. 1045–1051, 2011.

[154] M. P. de Barros, J. P. B. Sousa, J. K. Bastos, and S. F. de Andrade, "Effect of Brazilian green propolis on experimental gastric ulcers in rats," *Journal of Ethnopharmacology*, vol. 110, no. 3, pp. 567–571, 2007.

[155] M. P. D. Barros, M. Lemos, E. L. Maistro et al., "Evaluation of antiulcer activity of the main phenolic acids found in Brazilian Green Propolis," *Journal of Ethnopharmacology*, vol. 120, no. 3, pp. 372–377, 2008.

[156] L. L. Périco, S. C. Heredia-Vieira, F. P. Beserra et al., "Does the gastroprotective action of a medicinal plant ensure healing effects? An integrative study of the biological effects of Serjania marginata Casar. (Sapindaceae) in rats," *Journal of Ethnopharmacology*, vol. 172, pp. 312–324, 2015.

[157] N. I. Belostotskiĭ, V. I. Kasianenko, E. A. Dubtsova, and L. B. Lazebnik, "Influence of honey, royal jelly and propolis on accelerating acetate healing of experimental gastric ulcers in rats," *Experimental and Clinical Gastroenterology*, vol. 6, pp. 46–50, 2009.

[158] M. Villanueva, M. González, H. Fernández et al., "*In vitro* antibacterial activity of Chilean propolis against *Helicobacter pylori*," *Revista chilena de infectología*, vol. 32, no. 5, pp. 530–535, 2015.

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2017, Article ID 7323129, 8 pages
https://doi.org/10.1155/2017/7323129

Research Article

A New Chinese Medicine Intestine Formula Greatly Improves the Effect of Aminosalicylate on Ulcerative Colitis

Baohai Liu,[1] Xuehua Piao,[2] Lianyi Guo,[1] Guijun Wang,[1] Weihua Sun,[1] Leming Gao,[3] Xuefeng Zheng,[1] and Yanli Fang[1]

[1]*Department of Gastroenterology, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou 121001, China*
[2]*Department of Traditional Chinese Medicine, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou 121001, China*
[3]*Administrative Logistics, The 2nd Dental Center, Peking University School of Stomatology, Beijing 100101, China*

Correspondence should be addressed to Xuehua Piao; piaoxuehua1982@126.com

Received 15 February 2017; Revised 13 August 2017; Accepted 29 August 2017; Published 20 November 2017

Academic Editor: Steve Harakeh

Ulcerative colitis (UC) is a chronic lifelong inflammatory disorder of the colon. Current medical treatment of UC relies predominantly on the use of traditional drugs, including aminosalicylates, corticosteroids, and immunosuppressants, which failed to effectively control this disease's progression and produced various side effects. Here, we report a new Chinese medicine intestine formula (CIF) which greatly improved the effect of mesalazine, an aminosalicylate, on UC. In the present study, 60 patients with chronic UC were treated with oral mesalazine alone or in combination with CIF enema. The combination of mesalazine and CIF greatly and significantly improved the clinical symptoms and colon mucosal condition and improved the Mayo Clinic Disease Activity Index and health-related quality of life, when compared to mesalazine alone. In particular, the addition of CIF further decreased serum levels of tumor necrosis factor-alpha and hypersensitivity C-reactive protein but in contrast increased interleukin-4. Thus, the results demonstrate the beneficial role of CIF in UC treatment, which may be mediated by the regulation of inflammation.

1. Introduction

Ulcerative colitis (UC) is a chronic and relapsing inflammatory disease caused by inflammation and sores in the lining of the large intestine and characterized clinically by recurrent episodes of bloody diarrhea, cramping, and abdominal pain and histologically by mucosal inflammation and injury [1, 2]. UC was reported to affect 120 to 200 per 100,000 people throughout the western world [3]. Recent reports also showed that the prevalence of UC in Asia was growing rapidly, including China [4, 5], Japan [6], and South Korea [7]. Although the pathogenesis mechanisms of UC are not completely understood, it is suggested that dysregulation of the pro/anti-inflammatory systems and antioxidant systems may be an important cause [2, 8].

Currently, the medical treatment of UC relies mainly on traditional drugs: aminosalicylates, corticosteroids, and immunosuppressants. These drugs reduce inflammatory injury and attenuate the expression of some proinflammatory molecules, but their side effects often result in reduced health-related quality of life and poor life satisfaction, particularly during long-term treatment [9, 10]. Therefore, there is an increasing interest in identifying alternative and more tolerable treatments for this disease.

Many traditional Chinese medicinal formulas have been proved to have beneficial effects on UC [11–15]. Here, we reported a new Chinese medicine intestine formula (CIF) for the treatment of UC. This CIF contains seven Chinese medicinal herbs: Radix Astragali Mongolici, Indigowood Root, Indigowood Leaf, Endoconcha Sepiae, *Bletilla striata*, *Cirsium japonicum*, and Common Cephalanoplos Herb. In the present study, the CIF greatly enhanced the effect of the traditional aminosalicylate mesalazine on UC patients including intestinal and extraintestinal symptoms.

2. Materials and Methods

2.1. Subjects. A total of 60 patients with left-sided UC were recruited from the Gastroenterology Department of the

Table 1: General patient information before mesalazine treatment alone (control group) or in combination with CIF enema (CIF group).

Parameters	Control	CIF	*P* value
Patients (males/females)	13/12	16/9	
Age (years)	45.07 ± 14.44	47.11 ± 12.08	0.576
Weight (kg)	60.88 ± 9.05	60.97 ± 9.99	0.974
Height (cm)	168.82 ± 5.49	163.62 ± 7.70	0.221
Disease duration (yr)	5.48 ± 2.12	5.16 ± 3.25	0.712
Heart rate/min	76.07 ± 4.81	76.42 ± 6.39	0.816
Systolic BP (mmHg)	117.00 ± 13.23	120.84 ± 14.97	0.285
Diastolic BP (mmHg)	72.53 ± 10.35	73.07 ± 11.65	0.846
Body temperature (°C)	36.48 ± 0.19	36.54 ± 0.22	0.313
Serum CRP (μg/mL)	5.86 ± 0.82	6.13 ± 1.27	0.261

First Affiliated Hospital of Jinzhou Medical University from August 2011 through July 2016. These patients had at least 6-month mild-to-moderate UC history based on Truelove and Witts criteria. The exclusion criteria include the following: (1) pregnant or breastfeeding patients; (2) patients with a history of alcohol or drug abuse; (3) recent malignancy, significant medical illness, or concurrent medication; (4) patients on an antidepressant drug; (5) patients with inability to complete the questionnaires and those with a psychiatric illness. These patients received treatment with mesalazine (control group) or mesalazine plus CIF enema (CIF group). Details of age, sex, weight, heart rate, body temperature, and others are shown in Table 1. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Jinzhou Medical University (Jinzhou, China). All participants provided written informed consent for this study.

2.2. Preparation of CIF. The CIF is composed of seven Chinese medicinal herbs: Radix Astragali Mongolici (50 g), Indigowood Leaf (50 g), Indigowood Root (50 g), Endoconcha Sepiae (30 g), *Bletilla striata* (30 g), *Cirsium japonicum* (10 g), and Common Cephalanoplos Herb (10 g). These herbs were immerged in 1000 ml of cool water and filtered. The filtrates were concentrated into a solution containing 1 g/ml of crude drugs and kept at 4°C until use. Patients in both control and CIF groups received oral mesalazine 1 g, 4 times daily for 8 weeks. Patients in CIF group were additionally treated with CIF enema (100 ml at bedtime) once daily during the 8 weeks of mesalazine treatment.

2.3. Colonoscopy Scores. Colonoscopy examination was performed before and after 8-week treatment. An Endoscopy Index was calculated by an experienced endoscopist. According to Mayo Endoscopic Score [16], the Endoscopic Index score (0–3 points) includes four categories: normal or inactive disease (0 points); erythema, decreased vascular pattern, and mild friability (1 point); marked erythema, absent vascular pattern, friability, and erosions (2 points); spontaneous bleeding and ulceration (3 points).

2.4. Score of Mayo Clinic Disease Activity Index. All patients received a medical evaluation with the Mayo Clinic Score System [17] before treatment and after 8-week treatment. Mayo Clinic Score System is one of the most commonly used activity indices for UC evaluation, in which Mayo Clinic Disease Activity Index (MCDAI) is counted based on the scores from four parameters (0–3 points each): stool frequency, rectal bleeding, endoscopic findings, and physician's global assessment. Thus, it can comprehensively evaluate the situation of UC patients. The MCDAI scores range from 0 to 12 points. Lower score refers to better health condition. According to the Mayo Clinic Score System, a clinical remission is defined as MCDAI 0–2 points with no individual subscore >1; an endoscopic remission is defined as endoscopic findings scored 0 or 1; and a clinical exacerbation is defined as 5 points together with an increase of endoscopic score of at least 1 point [14].

2.5. Assessment of Health-Related Quality of Life (HRQoL). We used the inflammatory bowel disease questionnaire (IBDQ) to assess HRQoL [18]. This disease-specific questionnaire comprises 32 questions which are divided into four health subscales: bowel symptoms (10 questions); systemic symptoms, including sleep disorders and fatigue (5 questions); emotional functions such as depression, aggression, and irritation (12 questions); and social function, meaning the ability to participate in social activities and to work (5 questions). The participants choose one from seven graded responses in each question (score: 1–7 points). Consequently, the total scores range from 32 to 224 points. Lower scores indicate worse HRQoL.

2.6. Scores of Clinical Symptoms. Clinical symptoms associated with UC were evaluated before and after 8-week treatment. The clinical symptoms include diarrhea, bloody stool, mucous stool, abdominal pain, abdominal distention, and tenesmus. Symptoms were scored by the following specific criteria: 0, no clinical symptoms; 3, minor symptoms with small effects on HRQoL; 6, moderate clinical symptoms with significant impairment in daily function; 9, severe clinical symptoms and severe debilitation of patients in terms of daily function.

2.7. Cure Standards. Cured. Clinical symptoms vanished and the mucosa shows normal tissue by colonoscopy. *Significant Improvement.* Clinical symptoms vanished and the colonoscopy result shows that the mucosal lesions are significantly improved. *Effective.* Clinical symptoms vanished and the colonoscopy result shows mild inflammation of the mucosa or false mucosal polyp formation. *Ineffective.* There is no improvement in both clinical symptoms and colonoscopy.

2.8. Measurement of Serum Tumor Necrosis Factor-Alpha (TNF-α), Interleukin-4 (IL-4), and Hypersensitivity C-Reactive Protein (hs-CRP). Serum levels of TNF-α, IL-4, and hs-CRP before and after 8-week treatment were measured with ELISA. Blood samples (5 ml each) were collected from all patients. Serum was acquired following centrifugation at

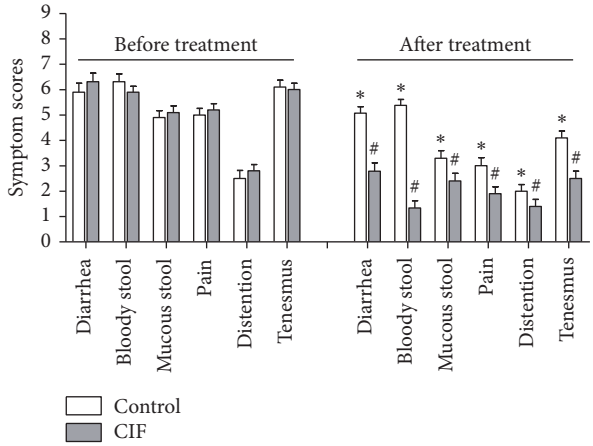


Figure 1: Effect of CIF on clinical symptoms of UC. Clinical symptoms associated with UC were evaluated in UC patients before and after 8-week mesalazine alone (control group) treatment or in combination with CIF enema (CIF group). Please note that CIF further and significantly reduced the symptom scores of all six symptoms ($n = 30$). $^{*}P < 0.05$ versus before treatment; $^{\#}P < 0.05$ versus control.

2,000g for 10 min at 4°C and aliquoted. The aliquots were stored at −20°C until use. The serum levels of TNF-α, IL-4, and CRP were measured using ELISA kits (cats. numbers EK0525, EK0404, and EK1316, resp.; Wuhan Boster Biological Engineering Co., Ltd., Wuhan, China), according to the manufacturer's protocols.

2.9. Erythrocyte Sedimentation Rate (ESR) Measurement. ESR was measured with an automated ESR analyzer.

2.10. Statistical Analysis. Data are presented as means ± SEM. Statistical analysis was performed with SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). One-way ANOVA and Tukey's test were used for group comparisons. $P < 0.05$ was considered statistically different.

3. Results

3.1. CIF Improves Clinical Symptoms. The main clinical symptoms of UC include diarrhea, bloody stool, mucous stool, abdominal pain, abdominal distention, and tenesmus. Thus, we first evaluated whether CIF improved these symptoms when added to mesalazine treatment (Figure 1). The scores of each symptom before treatment were not significantly different between mesalazine alone (control group) and mesalazine plus CIF enema (CIF group). After 8-week treatment, mesalazine alone moderately decreased the scores of all six symptoms ($P < 0.05$ versus before treatment). The addition of CIF significantly and markedly decreased the scores of all symptoms, when compared to the control group ($P < 0.05$). Thus, these results reveal an effective therapy of CIF on UC when in combination with mesalazine.

3.2. CIF Improves Mucosal Healing. We next examined whether the clinical effect of CIF was a consequence of mucosal healing. In the endoscopic examination, we observed a marked improvement of mucosal condition in both groups after 8-week treatment. As shown in Figure 2, mesalazine alone alleviated the mucous hyperemia. Mesalazine plus CIF caused a more marked improvement of mucous hyperemia, compared to mesalazine alone. The surface of the colon became smooth, and angiogenesis appeared in the impaired mucous. When endoscopic results from the 60 patients were scored, the data in all score grades (0–3) was not markedly different before treatment (Table 2). However, after 8-week treatment, there was a trend that more patients achieved mucosal healing in CIF plus mesalazine group than in mesalazine alone group. In CIF plus mesalazine group, 43.33% of the patients recovered completely (0 point), compared to 20% in mesalazine alone group. In score 1, the percentage was also bigger in CIF plus mesalazine group than in mesalazine alone group. In higher scores (2 and 3), which indicate worse mucosal conditions, the percentages were reversed. Thus, the results show that CIF greatly improves the mucosal condition of UC patients when added to mesalazine treatment.

3.3. CIF Improves Mayo Clinic Disease Activity Index. We evaluated the therapy effect of CIF on UC using Mayo Clinic Score System. Similar to the results in clinical symptoms and mucosal healing, there was no significant difference of MCDAI values before treatment between mesalazine alone and CIF plus mesalazine group (Figure 3(a)). Mesalazine alone moderately decreased the MCDAI values ($P < 0.05$). The addition of CIF significantly and markedly increased the effect of mesalazine in MCDAI scores. Thus, these results further support the notion that CIF is beneficial for mesalazine treatment in UC patients.

The ESR was also similar between the two groups before treatment. The two treatments decreased the ESR at an almost similar degree (Figure 3(b)), indicating that both treatments improved the disease condition.

3.4. CIF Improves HRQoL. We then evaluated the effect of CIF on HRQoL. Four parameters, that is, bowel symptoms, systemic symptoms, emotional function, and social function, were scored (Table 3). The four scores and total score were not significantly different before treatment between the two groups. Mesalazine alone moderately increased all scores including the total score. CIF further and significantly increased these scores ($P < 0.05$ versus control). Thus, the results, especially the improvements of extraintestinal characterizations, further support the beneficial effect of CIF on UC.

3.5. CIF Increases Total Efficacy. The effective rates were used to further evaluate the therapy effect of CIF after 8-week treatment. As shown in Table 4, the cure rate and significantly effective rate were markedly higher in CIF plus mesalazine group, compared to mesalazine alone group. Inversely, the ineffective rate was lower in CIF plus mesalazine group than

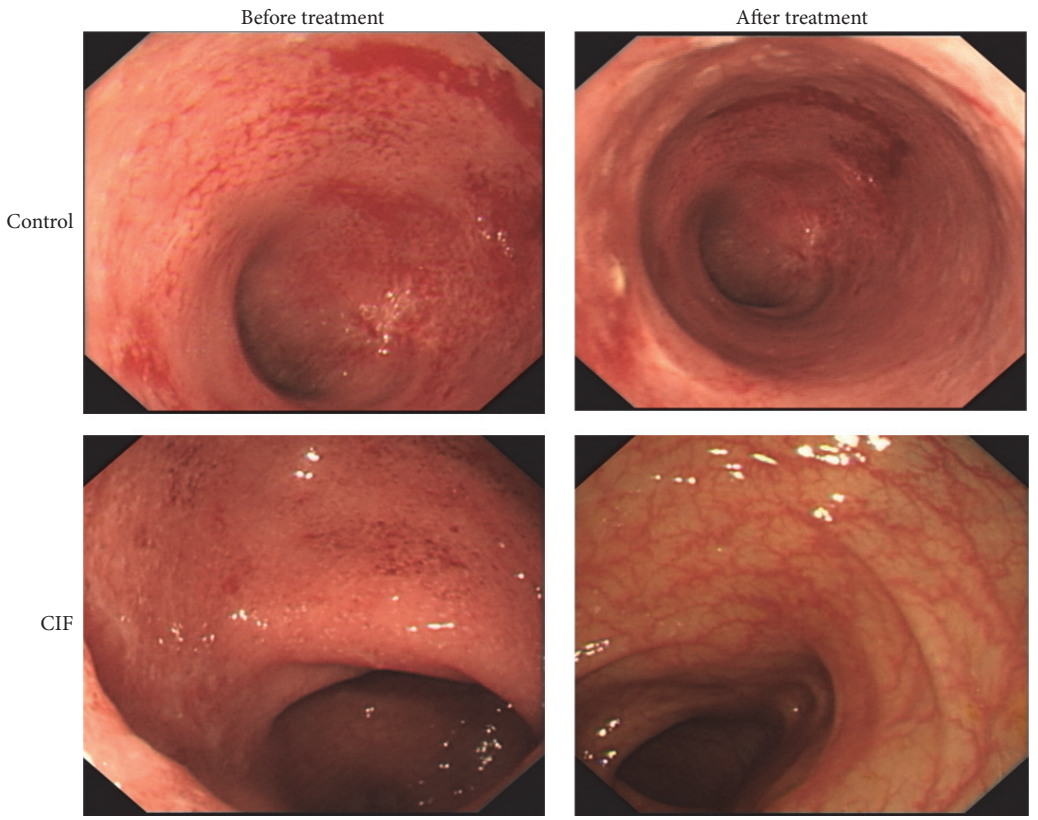


Figure 2: CIF improves mucosal healing. A representative endoscopic picture showing the mucosal condition before and after 8-week mesalazine treatment alone (control group) or in combination with CIF enema (CIF group).

Table 2: Mucosal scores before and after 8-week mesalazine treatment alone (control group) or in combination with CIF enema (CIF group).

	Mucosal score	Control (n, %)	CIF (n, %)
Before treatment	0	0/0%	0/0%
	1	7/23.3%	6/20%
	2	20/66.7%	18/60%
	3	3/10%	6/20%
After treatment	0	6/20.00%	13/43.33%
	1	8/26.67%	12/40%
	2	11/36.67%	5/16.67%
	3	5/16.67%	0/0.00%

in mesalazine alone group. The total effective rate was 93.3% and 73.3% in CIF plus mesalazine group and mesalazine alone group, respectively, and revealed a significant difference ($P < 0.05$). These results demonstrate that CIF increases the therapy effect of mesalazine on UC treatment.

3.6. Effect of CIF on Cytokines Levels and hs-CRP. These results above demonstrate the beneficial role of CIF in the treatment of UC. Next, we explored the possible mechanisms underlying it. UC is a type of inflammatory bowel disease. Proinflammatory cell infiltration and proinflammatory cytokines (like TNF-α and IL-1β) release are considered as important events in UC [2, 19]. In particular, some anti-TNF-α reagents showed a potential effect on UC treatment [20]. Thus, we examined whether CIF alleviates TNF-α release. As shown in Figure 4(a), mesalazine alone decreased serum level of TNF-α. CIF plus mesalazine further and significantly decreased the serum level of TNF-α, compared to mesalazine alone ($P < 0.05$). IL-4, an anti-inflammatory cytokine, was also reported to play an important role in the development of UC [21, 22]. Mesalazine treatment increased the serum level of IL-4. The addition of CIF further and significantly increased the serum level of IL-4, compared to mesalazine alone (Figure 4(b), $P < 0.05$). We next examined whether CIF may change another UC-related factor, hs-CRP level in serum. Like TNF-α results, mesalazine alone revealed a marked inhibitory effect on the serum level of hs-CRP, and CIF plus mesalazine further and significantly decreased its level compared to mesalazine alone (Figure 4(c), $P < 0.05$).

4. Discussion

The present study was set forth to evaluate the therapy effect of a new Chinese herb formula CIF on UC when in combination with mesalazine and the underlying mechanisms. In our results, although mesalazine alone exhibited a therapy effect on UC patients, the addition of CIF further and significantly improved intestinal symptoms, mucosal condition, and extraintestinal characterizations. In particular,

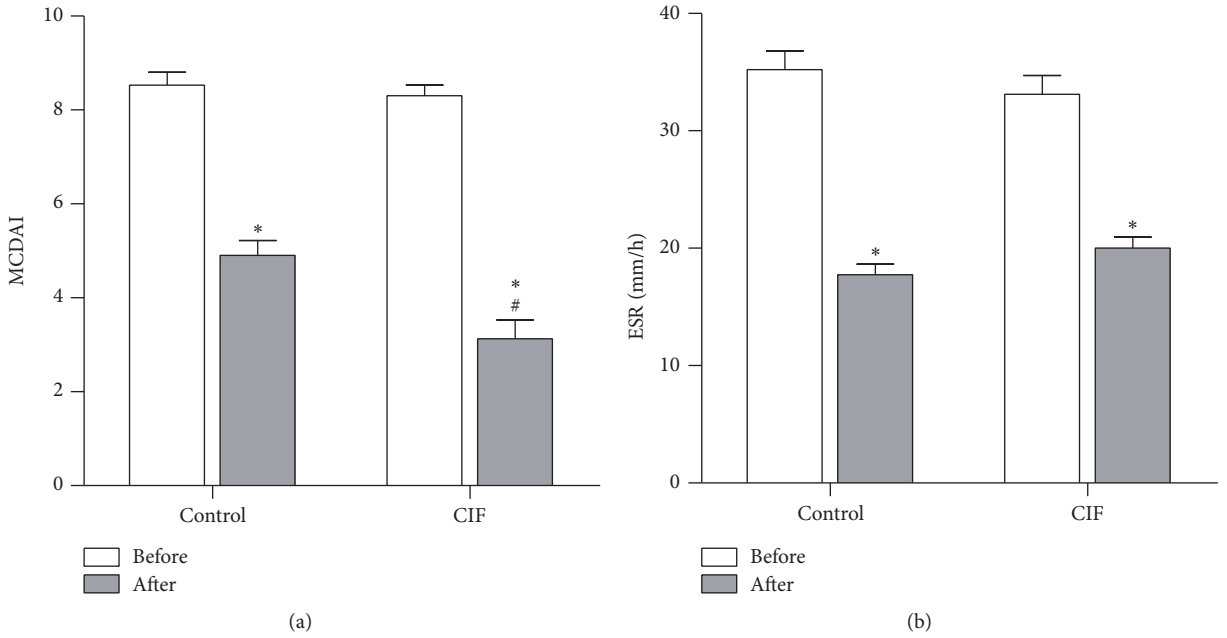


Figure 3: Effect of CIF on MCDAI and ESR. MCDAI associated with UC (a) and ESR (b) were evaluated in UC patients before and after 8-week mesalazine treatment alone (control group) or in combination with CIF enema (CIF group) ($n = 30$). $^{*}P < 0.05$ versus before treatment; $^{\#}P < 0.05$ versus control.

Table 3: HRQoL scores before and after 8-week mesalazine treatment alone (control group) or in combination with CIF enema (CIF group).

	Before treatment		After treatment	
	Control	CIF	Control	CIF
Bowel symptoms	40.43 ± 3.34	40.77 ± 3.08	42.7 ± 4.25	46.97 ± 6.68
Systemic symptoms	19.2 ± 3.03	20.23 ± 2.12	21.67 ± 3.13	24.07 ± 5.32
Emotional function	48.7 ± 8.63	48.7 ± 9.08	57 ± 8.72	61.5 ± 10.06
Social function	20.57 ± 4.01	21.03 ± 3.37	22.30 ± 2.93	26.13 ± 6.23
Total	128.9 ± 11.86	130.73 ± 12.37	143.67 ± 11.23	158.67 ± 19.08

Table 4: Effective rate after 8-week mesalazine treatment alone (control group) or in combination with CIF enema (CIF group).

	Control (n/%)	CIF (n/%)
Cured	3/10%	10/33.3%
Significantly effective	6/20%	12/40%
Effective	13/43.3%	6/20%
Ineffective	8/26.7%	2/6.7%
Total effective rate	30/73.3%	30/93.3%

CIF decreased the serum level of proinflammatory factor TNF-α and increased the serum level of anti-inflammatory factor IL-4. These results demonstrated a therapy effect of CIF on UC, and anti-inflammatory activity may underlie the action mechanisms.

Chinese herbal medicine is widely used in the treatment of UC. In an analysis of 10,218 UC cases in China, 20.1% of the patients were treated with Chinese herbs, and 59.1% were treated with combined Chinese and western medicines (like mesalazine and/or corticosteroids) [23]. Thus, Chinese herbal medicine may offer an exciting potential for discovering new agents for UC treatment. Indeed, some pure and crude extractions from Chinese medicine herbs have been proved to have a therapy effect on UC in some experimental animal models [2, 15]. In the present study, we combined a Chinese herbal medicine formula CIF with classical mesalazine to treat UC patients. The 8-week treatment significantly improved the intestinal symptoms, mucosal condition, and extraintestinal characterizations in UC patients, compared to mesalazine alone, showing good therapy effect on UC. Among the seven components of this CIF, Radix Astragali Mongolici was used in another famous Chinese medicine formula to treat various gastrointestinal tract diseases, such as gastritis and stomach ulcer [24]. Indigowood Root has been used in traditional medicine for its potential anti-inflammatory effect. Indigowood Root protects against radiation-caused damage in the hematopoietic system with a significant reduction of serum TNF-α, IL-1beta, and IL-6 [25]. Endoconcha Sepiae is a classical herbal medicine used in gastrointestinal diseases. It was reported to have a

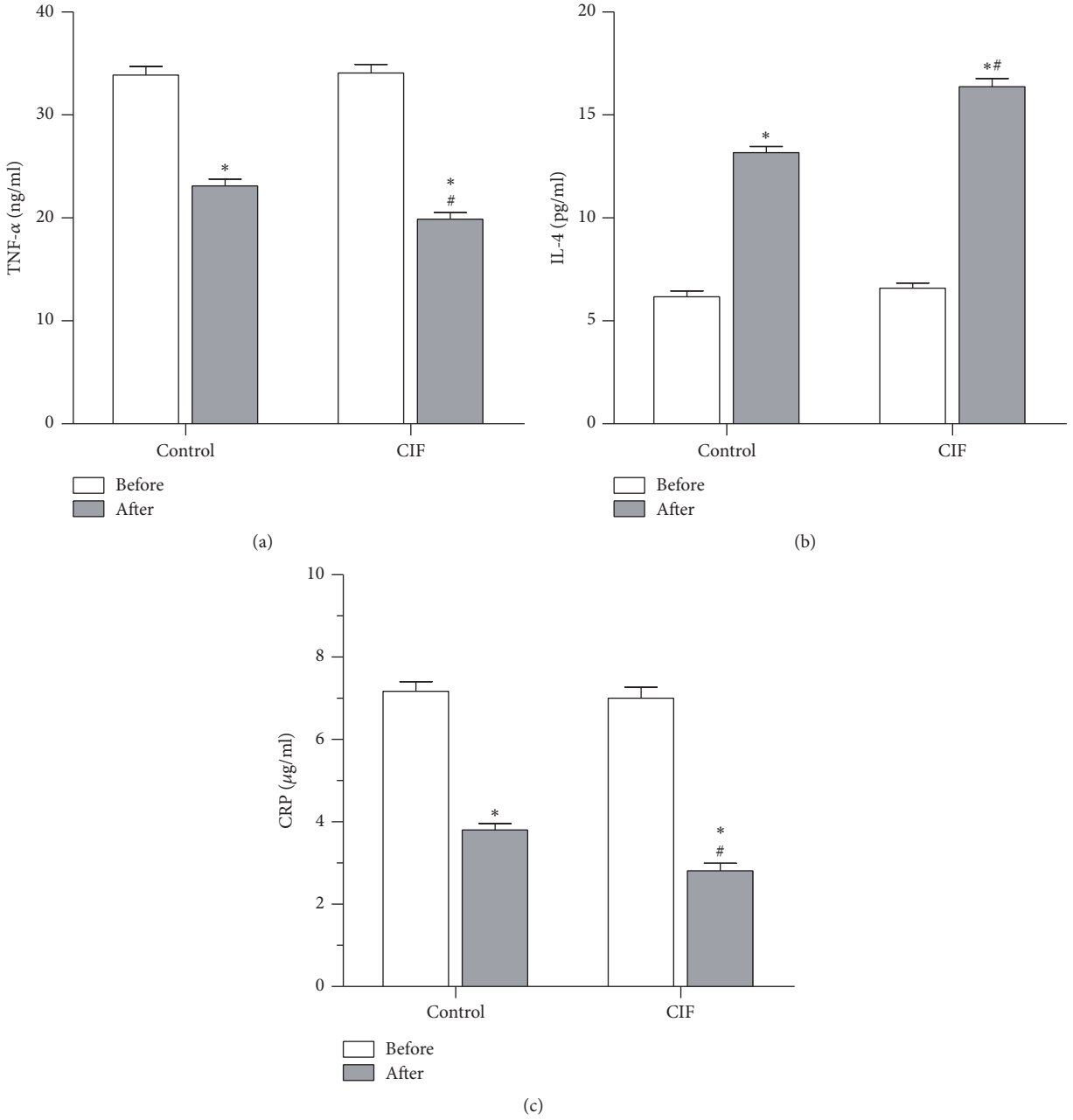


Figure 4: Effect of CIF on serum CRP and cytokines. Sera were obtained from patients before and after 8-week mesalazine treatment alone (control group) or in combination with CIF enema (CIF group) ($n = 30$). The levels of TNF-α (a), IL-4 (b), and hs-CRP (c) were measured with ELISA. $^{*}P < 0.05$ versus before treatment; $^{\#}P < 0.05$ versus control.

gastroprotective potential against indomethacin [26]. *Bletilla striata* has been not only widely used for the treatment of hematemesis, hemoptysis, and traumatic bleeding due to the efficacy of arresting bleeding with astringent action, but also topically applied to overcome ulcers, sores, swellings, and chapped skin due to the efficacy of dispersing swelling and promoting tissue regeneration [27]. An extract from *Bletilla striata* has been proved to have a good effect on wound healing [28]. *Cirsium japonicum* has been employed traditionally in the treatment of inflammatory symptoms. Its extracts and principal ingredient apigenin were reported to have a strong antioxidant activity [29, 30]. Luteolin, another major component of *Cirsium japonicum*, reduced D-galactosamine/lipopolysaccharide-stimulated high serum level of TNF-α and protein expression of TNF-α receptor-associated death domain [31]. These pharmacological effects of major components of CIF collectedly may contribute to the therapy results in UC patients in the present study.

Consistent with the anti-TNF-α effect of luteolin, one major component of *Cirsium japonicum* in D-galactosamine/lipopolysaccharide-induced liver damage model [31], this CIF also significantly reduced serum level of TNF-α in UC

patients. As proinflammatory cell infiltration and proinflammatory cytokine (like TNF-α) release are considered as important events in UC [2, 19], thus, the inhibition of TNF-α by CIF in the present study can be considered as an important action mechanism in UC treatment. Especially interesting is that our results showed that CIF in combination with mesalazine further increased the serum level of IL-4, compared to mesalazine alone. IL-4, as an anti-inflammatory factor, was increased in UC when effective treatments were given in some UC experimental models [32, 33]. Therefore, the increased anti-inflammatory factor IL-4 by CIF may become another important mechanism of CIF in UC treatment.

ESR and hs-CRP were used as a measure of the acute phase response in UC [34]. In particular, hs-CRP increased significantly when exacerbation of colitic symptoms occurred [35]. And effective treatment of Crohn's disease and UC significantly decreased the serum level of hs-CRP [36]. Consistent with the previous reports, our results showed that CIF further decreased the serum level of hs-CRP compared to mesalazine alone, indicating a good treatment effect of CIF on UC.

In conclusion, the present results demonstrate that CIF exerted a beneficial effect on UC patients in combination with classical mesalazine. The suppression of TNF-α and hs-CRP and increase of IL-4 may underlie the action mechanism of CIF.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This study was supported by the Research Startup Fund of Liaoning Medical University for Doctors and Teachers (Grant no. Y2012B014) and the Youth Science and Technology Startup Fund of the First Affiliated Hospital of Liaoning Medical University (Grant no. FY2012-17).

References

[1] E. Furrie, S. Macfarlane, J. H. Cummings, and G. T. Macfarlane, "Systemic antibodies towards mucosal bacteria in ulcerative colitis and Crohn's disease differentially activate the innate immune response," *Gut*, vol. 53, no. 1, pp. 91–98, 2004.

[2] B. Liu, X. Piao, L. Guo, S. Liu, F. Chai, and L. Gao, "Ursolic acid protects against ulcerative colitis via anti-inflammatory and antioxidant effects in mice," *Molecular Medicine Reports*, vol. 13, no. 6, pp. 4779–4785, 2016.

[3] J. Cosnes, C. Gowerrousseau, P. Seksik, and A. Cortot, "Epidemiology and natural history of inflammatory bowel diseases," *Gastroenterology*, vol. 140, no. 6, pp. 1785–1794, 2011.

[4] Q. Ouyang, R. Tandon, K. L. Goh et al., "The emergence of inflammatory bowel disease in the Asian Pacific region," *Current Opinion in Gastroenterology*, vol. 21, no. 4, pp. 4408–4413, 2005.

[5] K. Asakura, Y. Nishiwaki, N. Inoue, T. Hibi, M. Watanabe, and T. Takebayashi, "Prevalence of ulcerative colitis and Crohn's disease in Japan," *Journal of Gastroenterology*, vol. 44, no. 7, pp. 659–665, 2009.

[6] L. Prideaux, M. A. Kamm, P. P. De Cruz, F. K. L. Chan, and S. C. Ng, "Inflammatory bowel disease in Asia: a systematic review," *Journal of Gastroenterology and Hepatology*, vol. 27, no. 8, pp. 1266–1280, 2012.

[7] S.-K. Yang, S. Yun, J.-H. Kim et al., "Epidemiology of inflammatory bowel disease in the Songpa-Kangdong district, Seoul, Korea, 1986–2005: a KASID study," *Inflammatory Bowel Diseases*, vol. 14, no. 4, pp. 542–549, 2008.

[8] K. M. Sakthivel and C. Guruvayoorappan, "Protective effect of acacia ferruginea against ulcerative colitis via modulating inflammatory mediators, cytokine profile and NF-κB signal transduction pathways," *Journal of Environmental Pathology, Toxicology and Oncology*, vol. 33, no. 2, pp. 83–98, 2014.

[9] J. A. Katz, "Advances in the medical therapy of inflammatory bowel disease," *Current Opinion in Gastroenterology*, vol. 18, no. 4, pp. 435–440, 2002.

[10] C. J. Ooi and B. E. Sands, "Treatment of ulcerative colitis," *Current Opinion in Gastroenterology*, vol. 15, no. 4, pp. 298–301, 1999.

[11] G. Chen, Y. Yang, C. Hu et al., "Protective effects of Huangqin Decoction against ulcerative colitis and associated cancer in mice," *Oncotarget*, vol. 7, no. 38, pp. 61643–61655, 2016.

[12] P. Chen, X. Zhou, L. Zhang et al., "Anti-inflammatory effects of Huangqin tang extract in mice on ulcerative colitis," *Journal of Ethnopharmacology*, vol. 162, pp. 207–214, 2015.

[13] K. Kawashima, A. Nomura, T. Makino, K.-I. Saito, and Y. Kano, "Pharmacological properties of traditional medicine (XXIX): effect of hange-shashin-to and the combinations of its herbal constituents on rat experimental colitis," *Biological & Pharmaceutical Bulletin*, vol. 27, no. 10, pp. 1599–1603, 2004.

[14] F. Ke, P. K. Yadav, and L. Z. Ju, "Herbal medicine in the treatment of ulcerative colitis," *Saudi Journal of Gastroenterology*, vol. 18, no. 1, pp. 3–10, 2012.

[15] L. Zhao, H. Wu, A. Zhao et al., "The in vivo and in vitro study of polysaccharides from a two-herb formula on ulcerative colitis and potential mechanism of action," *Journal of Ethnopharmacology*, vol. 153, no. 1, pp. 151–159, 2014.

[16] P. Rutgeerts, W. J. Sandborn, B. G. Feagan et al., "Infliximab for induction and maintenance therapy for ulcerative colitis," *The New England Journal of Medicine*, vol. 353, no. 23, pp. 2462–2476, 2005.

[17] G. R. D'Haens, W. J. Sandborn, G. Zou et al., "Randomised non-inferiority trial: 1600 mg versus 400 mg tablets of mesalazine for the treatment of mild-to-moderate ulcerative colitis," *Alimentary Pharmacology & Therapeutics*, vol. 46, no. 3, pp. 292–302, 2017.

[18] W. H. Ren, M. Lai, Y. Chen, E. J. Irvine, and Y. X. Zhou, "Validation of the mainland Chinese version of the Inflammatory Bowel Disease Questionnaire (IBDQ) for ulcerative colitis and Crohn's disease," *Inflammatory Bowel Diseases*, vol. 13, no. 7, pp. 903–910, 2007.

[19] M. Oishi, K. Tokuhara, H. Miki et al., "Temporal and spatial dependence of inflammatory biomarkers and suppression by fluvastatin in dextran sodium sulfate-induced rat colitis model," *Digestive Diseases and Sciences*, vol. 59, no. 9, pp. 2126–2135, 2014.

[20] U. Billmeier, W. Dieterich, M. F. Neurath, and R. Atreya, "Molecular mechanism of action of anti-tumor necrosis factor antibodies in inflammatory bowel diseases," *World Journal of Gastroenterology*, vol. 22, no. 42, pp. 9300–9313, 2016.

[21] A. K. Bhan, E. Mizoguchi, R. N. Smith, and A. Mizoguchi, "Spontaneous chronic colitis in TCRα-mutant mice; an experimental model of human ulcerative colitis," *International Reviews of Immunology*, vol. 19, no. 1, pp. 123–138, 2000.

[22] I. Soufli, R. Toumi, H. Rafa, and C. Touil-Boukoffa, "Overview of cytokines and nitric oxide involvement in immunopathogenesis of inflammatory bowel diseases," *World Journal of Gastrointestinal Pharmacology and Therapeutics*, vol. 7, no. 3, pp. 353–360, 2016.

[23] X.-L. Jiang and H.-F. Cui, "An analysis of 10218 ulcerative colitis cases in China," *World Journal of Gastroenterology*, vol. 8, no. 1, pp. 158–161, 2002.

[24] D.-Y. Liu, C.-S. Pan, Y.-Y. Liu et al., "Huang Qi Jian Zhong pellet attenuates TNBS-induced colitis in rats via mechanisms involving improvement of energy metabolism," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 574629, 14 pages, 2013.

[25] W. C. You, W. C. Lin, J. T. Huang, and C. C. Hsieh, "Indigowood root extract protects hematopoietic cells, reduces tissue damage and modulates inflammatory cytokines after total-body irradiation: does Indirubin play a role in radioprotection?" *Phytomedicine*, vol. 16, no. 12, pp. 1105–1111, 2009.

[26] M. Chien, Y. Lin, F. Peng et al., "Gastroprotective potential against indomethacin and safety assessment of the homology of medicine and food formula cuttlebone complex," *Food Funct.*, vol. 6, no. 8, pp. 2803–2812, 2015.

[27] X. He, X. Wang, J. Fang et al., "Bletilla striata: medicinal uses, phytochemistry and pharmacological activities," *Journal of Ethnopharmacology*, vol. 195, pp. 20–38, 2017.

[28] L. Ding, X. Shan, X. Zhao et al., "Spongy bilayer dressing composed of chitosan–Ag nanoparticles and chitosan–Bletilla striata polysaccharide for wound healing applications," *Carbohydrate Polymers*, vol. 157, pp. 1538–1547, 2017.

[29] J. Yin, S. L. Heo, and M. H. Wang, "Antioxidant and antidiabetic activities of extracts from Cirsium japonicum roots," *Nutrition Research and Practice*, vol. 2, no. 4, pp. 247–251, 2008.

[30] J.-Y. Han, S.-Y. Ahn, C.-S. Kim et al., "Protection of apigenin against kainate-induced excitotoxicity by anti-oxidative effects," *Biological & Pharmaceutical Bulletin*, vol. 35, no. 9, pp. 1440–1446, 2012.

[31] W.-C. Lee, H. A. Jung, J. S. Choi, Y. S. Kim, and S.-M. Lee, "Protective effects of luteolin against apoptotic liver damage induced by D-galactosamine/lipopolysaccharide in mice," *Journal of Natural Products*, vol. 74, no. 9, pp. 1916–1921, 2011.

[32] W. Gao, Y. Guo, C. Wang et al., "Indirubin ameliorates dextran sulfate sodium-induced ulcerative colitis in mice through the inhibition of inflammation and the induction of Foxp3-expressing regulatory T cells," *Acta Histochemica*, vol. 118, no. 6, pp. 606–614, 2016.

[33] X. L. Huang, X. Zhang, X. Y. Fei et al., "Faecalibacterium prausnitzii supernatant ameliorates dextran sulfate sodium induced colitis by regulating Th17 cell differentiation," *World Journal of Gastroenterology*, vol. 22, no. 22, pp. 5201–5210, 2016.

[34] Y. Tolga, C. A. Mansur, and G. H. Savas, "Plasma viscosity: a potential predictor of both medical treatment response and clinical stage of ulcerative colitis," *Annali Italiani Di Chirurgia*, vol. 87, pp. 531–543, 2016.

[35] H. Shimizu, K. Arai, J. Tang, K. Hosoi, and R. Funayama, "5-Aminosalicylate intolerance causing exacerbation in pediatric ulcerative colitis," *Pediatrics International*, vol. 59, no. 5, pp. 583–587, 2017.

[36] R. Keil, M. Wasserbauer, Z. Zádorová et al., "Clinical monitoring: infliximab biosimilar CT-P13 in the treatment of Crohn's disease and ulcerative colitis," *Scandinavian Journal of Gastroenterology*, vol. 51, no. 9, pp. 1062–1068, 2016.

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2017, Article ID 4846876, 9 pages
https://doi.org/10.1155/2017/4846876

Research Article

Qingchang Wenzhong Decoction Attenuates DSS-Induced Colitis in Rats by Reducing Inflammation and Improving Intestinal Barrier Function via Upregulating the MSP/RON Signalling Pathway

Tangyou Mao,[1,2] Junxiang Li,[2] Lijuan Liu,[3] Weihan Zhao,[4] Yuyue Liu,[2] Kangli Gao,[5] Yi Guo,[1,2] Tianhong Xie,[1,2] Ningfei Li,[1] and Rui Shi[2]

[1]*Beijing University of Chinese Medicine, No. 11, North Third Ring East Road, Beijing 100029, China*
[2]*Gastroenterology Department, Dongfang Hospital, Beijing University of Chinese Medicine, No. 6, 1st Section, Fangxingyuan, Fangzhuang, Beijing 100078, China*
[3]*China-Japan Friendship Hospital, No. 2, Cherry East Road, Beijing 100029, China*
[4]*Gastroenterology Department, The First Affiliated Hospital, Shanxi University of Chinese Medicine, No. 2, Weiyang West Road, Xianyang 712000, China*
[5]*Gastroenterology Department, The First Affiliated Hospital, Anhui University of Chinese Medicine, No. 117, Mei Shan Road, Hefei 230031, China*

Correspondence should be addressed to Rui Shi; shiai588@163.com

Received 5 February 2017; Accepted 21 August 2017; Published 12 October 2017

Academic Editor: Steve Harakeh

Ulcerative colitis (UC) is a chronic, nonspecific, inflammatory disease for which an effective treatment is lacking. Our previous study found that Qingchang Wenzhong Decoction (QCWZD) can significantly improve the clinical symptoms of UC and ameliorate dextran sulphate sodium- (DSS-) induced ulcerative colitis in rats by downregulating the IP10/CXCR3 axis–mediated inflammatory response. The purpose of the present study was to further explore the mechanism of QCWZD for UC in rats models, which were established by 7-day administration of 4.5% dextran sulphate sodium solution. QCWZD was administered daily for 7 days; then we determined the serum macrophage-stimulating protein concentration (MSP) and recepteur d'origine nantais (RON) expression and its downstream proteins (protein kinase B [Akt], phosphorylated [p] Akt, occludin, zona occluden- [ZO-] 1, and claudin-2) in colon tissue using Western blotting and quantitative polymerase chain reaction. In DSS-induced UC, QCWZD significantly alleviated colitis-associated inflammation, upregulated serum MSP expression and RON expression in the colon, reduced the pAkt levels, promoted colonic occluding and ZO-1 expression, and depressed claudin-2 expression. In conclusion, the MSP/RON signalling pathway plays an important role in the pathogenesis of UC by involving the inflammatory response and improving intestinal barrier function. QCWZD appears to attenuate DSS-induced UC in rats by upregulating the MSP/RON signalling pathway.

1. Introduction

Ulcerative colitis (UC) is a nonspecific, chronic inflammation of the colon and rectum, primarily of the mucosal and submucosal layers [1, 2]. UC frequently occurs in young people and is characterized by abdominal pain, diarrhoea, and bloody mucopurulent stool [3]. UC-related complications including toxic giant colon, bleeding, perforation, and cancer seriously affect quality of life [4]. Thus, the World Health Organization lists UC as a miscellaneous problem [5].

The number of patients with UC is increasing annually in Asia, and the incidence of UC has increased more than 3 times in 10 years in China [6]. Because of delayed healing, the rate of UC recurrence is very high. In addition, the detection rate of UC-associated colorectal cancer (UC-CRC), which is one of the most serious complications, has

increased, and UC-CRC now accounts for 10–15% of deaths in patients with UC [7]. The disease process that leads to UC-CRC involves "continuous intestinal inflammation, suspicious atypical hyperplasia, a low degree of atypical hyperplasia, and highly atypical hyperplasia and cancer"; however, the later steps can be skipped [8]. Moreover, the progression through "inflammation to atypical hyperplasia to cancer" in patients with UC is more rapid than the progression of "adenoma to adenocarcinoma" in the general population [9, 10]. Thus, as an independent risk factor, intestinal inflammation is the first step in UC-CRC, and the risk of CRC increases with the severity of inflammation in patients with atypical hyperplasia or chronic UC. Furthermore, the length of the disease course is a key factor for cancer in patients with UC; with disease lasting 10 years and more, the average incidence rate of cancer increases exponentially [11]. Therefore, controlling inflammation in UC not only improves quality of life and work efficiency, but also prevents the development and reduces the incidence of UC-CRC.

The macrophage-stimulating protein (MSP)/recepteur d'origine nantais (RON) signalling pathway plays a critical role in the inflammatory process [12–14]. MSP is a hepatocyte-like cell growth factor and belongs to the plasminogen related growth factor family. Recent evidence indicates that MPS is deeply involved as an inhibitory factor in the endogenous inflammatory response. MSP is secreted by the hepatic cells into the blood as an inactive single strand (pro-MSP). In various pathological conditions, serum- and membrane-bound proteases can activate pro-MSP to become biologically active mature MSP, which is composed of alpha chains (60 kDa) and beta chains (30 kDa) at the RON binding site.

As the only specific binding protein of MSP, RON is activated when bound by MSP. Then, it not only exerts anti-inflammatory effects by inhibiting production of proinflammatory cytokines from peritoneal macrophages [15], but also maintains the homeostasis of the intestinal epithelium; regulates the proliferation, survival, and migration of intestinal epithelial cells; and promotes the repair of injured intestinal epithelial cells and mucosal barrier by regulating multiple downstream signalling cascades [16, 17]. Therefore, decreased MSP/RON pathway function, which decreases the anti-inflammatory processes and repair of intestinal mucosa, is one of the mechanisms of UC. The agents that activate the MSP/RON pathway could be drug targets for UC treatment.

Although research for UC has made great progress in recent years, effective treatment is still lacking. Aminosalicylates and glucocorticosteroids are currently the first-line drugs for mild-moderate and moderate-severe UC [18], respectively. The issues with these drugs include lack of tolerance to the drug and side effects such as the required treatment length and high recurrence rates. In addition, the efficacy and safety of immunomodulators and antibiotics, which are currently in the clinical trial stages, require further evaluation [19]. Thus, there is an increasing need for the development of more effective and less toxic agents for the treatment of UC.

We have already shown that QCWZD not only significantly improves the symptoms of active UC in patients with diarrhoea with mucous, pus, and blood [20], but also reduces damage to mucosal epithelial cells in rats models by downregulating the IP10/CXCR3 axis–mediated inflammatory response [21] and inhibiting proinflammatory cytokines (e.g., interleukin-6/tumour necrosis factor-alpha) secreted by macrophages [22]. Contrary to the destructive mechanism of IP10/CXCR3 axis, the MSP/RON signalling pathway plays a protective role in the pathogenesis of ulcerative colitis. So from the opposite perspective, we explored the possible protective mechanism of QCWZD in rats with DSS-induced UC, with the aim of providing a comprehensive experimental basis for the use of QCWZD as a potential therapeutic agent in the treatment of UC.

2. Materials and Methods

2.1. Qingchang Wenzhong Decoction Preparation. QCWZD formula granules, purchased from the Dong Fang Hospital pharmacy of the Beijing University of Chinese Medicine, were composed of eight commonly used herbs: 6 g Huanglian (coptis), 10 g Pao Jiang (ginger), 15 g Kushen (matrine), 6 g Qingdai (indigo), 30 g Diyutan (sanguisorba carbon), 6 g Mu Xiang (wood), 6 g Sanqi (pseudo-ginseng), and 6 g Gan Cao (licorice). Mesalazine was purchased from Losan Pharma GmbH, Germany.

2.2. Animals and Experimental Procedure. Sixty male Sprague-Dawley rats (weight, 180–220 g) were purchased from the Experimental Animal Science and Technology Co. Ltd. (Beijing, China; certificate no. SCXK-2011-0004) and raised in a specific pathogen-free animal room at the Research Institute of Chinese Medicine in the Chinese Academy of Traditional Chinese Medicine (Beijing, China), which is a temperature- (20–24°C), humidity- (50–60%), and light-controlled environment (12-h light/dark cycle), with ad libitum access to rodent feed and water. We used DSS solution (4.5%), which would simulate human pathogenesis and symptoms, to develop the rat colitis model.

After adaptive feeding for 1 week, the rats were randomly divided into five groups of 10 rats each for the 7-day intervention: blank control group, treated with 2 mL distilled water and free access to tap water; DSS-treated group, treated with 2 mL distilled water and free access to 4.5% DSS; low-dose QCWZD group, treated with 2 mL of 0.3 g/kg body weight (bw) QCWZD and free access to 4.5% DSS; medium-dose QCWZD group, treated with 2 mL of 0.6 g/kg bw QCWZD and free access to 4.5% DSS; high-dose QCWZD group, treated with 2 mL of 1.2 g/kg bw QCWZD; mesalazine group, treated with 2 mL of 0.03 g/kg bw mesalazine and free access to 4.5% DSS. We observed the general condition, weight, and faecal occult blood on a daily basis.

2.3. Reagents. DSS (MW36–50 KDa; MP Biomedical, Burlingame, CA, USA), an ultraviolet spectrophotometer (NANODROP 2000; Thermo Scientific, Wilmington, DE, USA), and enzyme-linked immunosorbent assay (ELISA) kits for MSP were purchased from Shanghai BlueGene Biotechnology Co., Ltd. (Shanghai, China) in addition to Anti-RON (ab125283), anti-pAkt (S473) (ab18206), anti-Akt (ab5893),

anti-occludin (Ab31721), anti-ZO-1 (SC-8146), and anti-claudin-2 (ab12593).

2.4. Detection of Serum Macrophage-Stimulating Protein Levels. The possible protective mechanism of QCWZD on UC was evaluated by measuring the MSP using ELISA kits, according to the manufacturer's instructions (Multiskan MK3; Thermo Scientific, Rockford, IL, USA).

2.5. Analyses of RON, Akt, p-Akt, Occludin, ZO-1, and Claudin-2 Expression. To investigate if QCWZD has a regulatory effect on RON expression, we conducted Western blot analysis, followed by quantitative polymerase chain reaction (qPCR). Then, to further clarify RON activity levels, we detected the levels of Akt and phosphorylated (p) Akt in the PI3K/Akt signalling pathway. Meanwhile, dysfunction of the MSP/RON pathway leads to disruption of the intestinal mucosal barrier; we determined the levels of occludin, zona occluden- [ZO-] 1, and claudin-2.

Protein was isolated from ice-cold colon tissues as described previously [23]; then, the protein was separated using 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. Membranes were immunoblotted with primary antibodies that recognized RON (1 : 1000), Akt (1 : 1000), p-Akt (1 : 500), occludin (1 : 1000), ZO-1 (1 : 500), claudin-2 (1 : 500), and GAPDH (1 : 1000) antibodies (TDY Biotech, Beijing, China). Peroxidase-conjugated secondary antibodies [goat polyclonal secondary antibody to rabbit (111-035-003), Jackson, USA] were also used. Densitometry was used to quantitate protein band intensities using the Gel Image System ver. 4.00 (Tanon, China).

Total RNA was extracted from the colon tissue samples after the 7 days of therapy, and the purity and concentration of the PCR were calculated. After reverse transcription, PCR amplification was performed using the TRIzol® method (TRIzol reagent; Invitrogen Life Technologies, Carlsbad, CA, USA). The primer sequences were 5′- TGCTTATTC-CCTCTCCCCGA -3′/5′- CCTCGGCTAGGAGCATCT-TG -3′ for RON, 5′- CAGACACCTTTGCACTTGGC -3′/5′- CTTGAGTAGGACCCCGAGGA -3′ for occludin, 5′- ATGACCGAGTCGCAATGGTT -3′/5′- TCTATCCCT-TGCCCAGCTCT -3′ for ZO-1, 5′- GTCAGCTTGCCA-GAGACACT -3′/5′- TTCGCTTGTCTTTTGGCTGC -3′ for claudin-2, and 5′- CCCATCTATGAGGGTTACGC -3′/5′- TTTAATGTCACGCACGATTTC -3′ for GAPDH (CWbio, Beijing, China). Changes in the target genes were determined using the 2−ΔΔCt method [24].

2.6. Statistical Analysis. SPSS v18.0 (IBM Corp, Armonk, NY, USA) was used for statistical analyses. Data are expressed as mean ± standard error. The data were compared between groups using one-way analysis of variance (ANOVA), followed by Student's t-tests. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of QCWZD on Serum MSP Level. All animals tolerated the entire experiment and no deaths occurred.

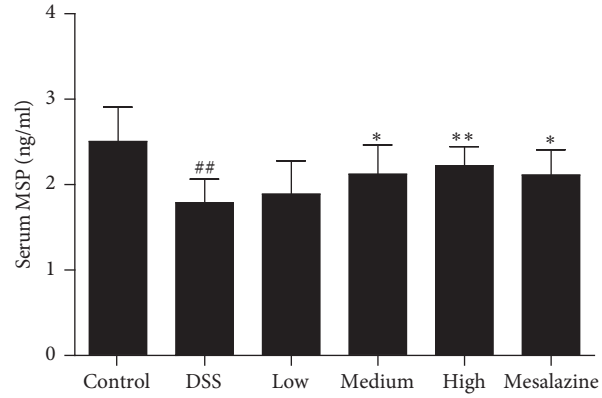


Figure 1: Effects of QCWZD on serum MSP level. Control, blank control group; DSS, DSS-treated group; low, low-dose QCWZD group; medium, medium-dose QCWZD group; high, high-dose QCWZD group; mesalazine, mesalazine group. $^{\#\#}P < 0.01$ versus the control group; $^{**}P < 0.01$, $^{*}P < 0.05$ versus the DSS group ($n = 10$ per group).

After intrarectal administration of QCWZD and mesalazine for 7 days, all rats were killed. Then we determined serum MSP level. As shown in Figure 1, serum MSP levels were significantly lower in the DSS group than in the control group (1.789 ± 0.2744 ng/ml in the DSS group versus 2.506 ± 0.4025 ng/ml, $P < 0.01$). Compared with the DSS group, the medium-dose QCWZD group (2.121 ± 0.3428 ng/ml), the high-dose QCWZD group (2.223 ± 0.2219 ng/ml), and the mesalazine group (2.111 ± 0.2958 ng/ml) showed significant activation ($P < 0.05$, $P < 0.01$, resp.) (Figure 1).

3.2. QCWZD Regulated Colonic RON and RON mRNA Expression in DSS-Induced UC Rats. As the only specific binding protein of MSP, RON is activated when bound by MSP; then we analysed RON level. Western blot analyses showed that the RON level was significantly lower in the DSS group than in the control group ($P < 0.01$, Figure 2(a)). Significantly higher RON levels were present in medium-dose QCWZD, high-dose QCWZD, and mesalazine groups than in the DSS group ($P < 0.05$, Figure 2(a)).

Next, we measured RON gene expression to confirm the effects of QCWZD on the colon. The qPCR analyses showed significantly lower RON mRNA levels in the DSS group than in the control group ($P < 0.01$, Figure 2(b)), and the medium-dose QCWZD, high-dose QCWZD, and mesalazine groups increased the RON gene expression compared to that in the control group ($P < 0.05$, $P < 0.01$, resp., Figure 2(b)).

3.3. QCWZD Regulated Colonic PI3K/Akt Signalling Pathway in DSS-Induced UC Rats. PI3K/Akt signalling pathway is an important regulatory pathway of RON activity; therefore, the level of PI3K/Akt expression can reflect RON activity [25], so we examined fold change of p-Akt level. As shown in Figure 3, the fold change of p-Akt level was significantly higher in the DSS group than in the control group ($P < 0.01$) and significantly lower in the medium-dose QCWZD, high-dose QCWZD, and mesalazine groups than in the DSS group ($P < 0.05$, $P < 0.01$, resp.).

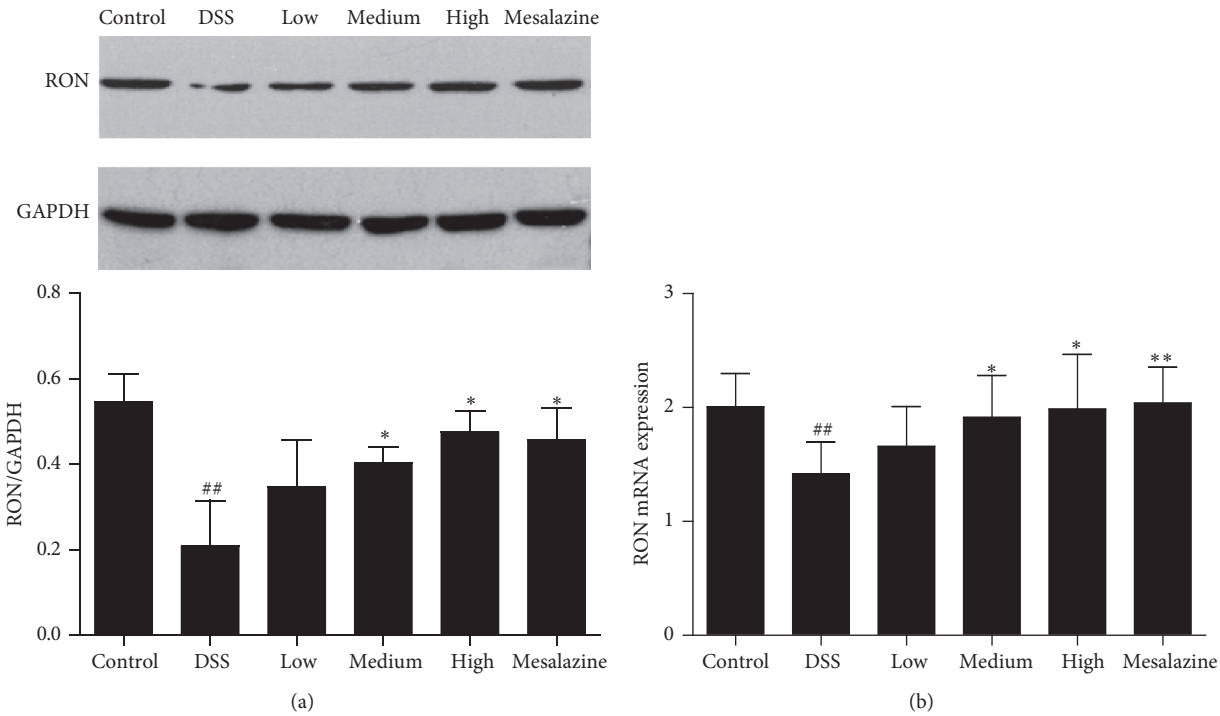


Figure 2: QCWZD regulated colonic RON (a) and RON mRNA (b) expression in DSS-induced UC rats. Control, blank control group; DSS, DSS-treated group; low, low-dose QCWZD group; medium, medium-dose QCWZD group; high, high-dose QCWZD group; mesalazine, mesalazine group. $^{\#\#}P < 0.01$ versus the control group; $^{**}P < 0.01$, $^{*}P < 0.05$ versus the DSS group ($n = 10$ per group).

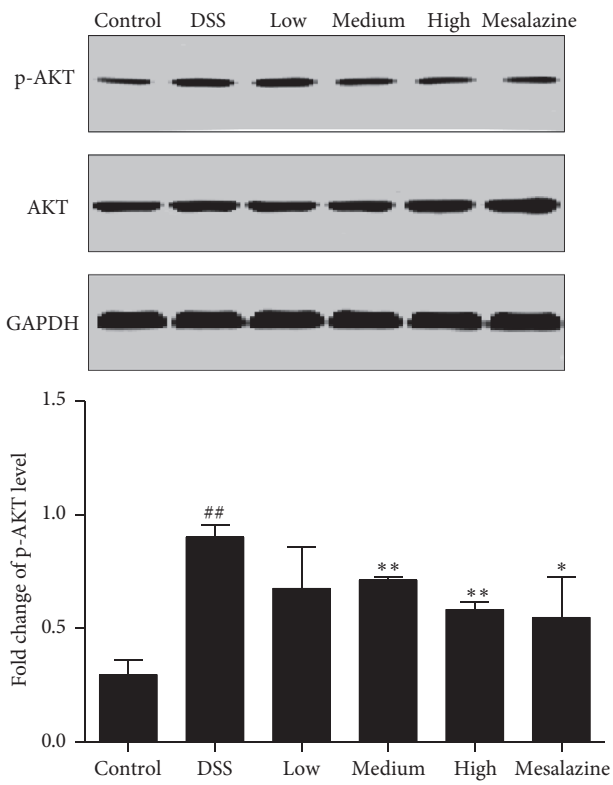


Figure 3: QCWZD regulated colonic PI3K/Akt signalling pathway in DSS-induced UC rats. Control, blank control group; DSS, DSS-treated group; low, low-dose QCWZD group; medium, medium-dose QCWZD group; high, high-dose QCWZD group; mesalazine, mesalazine group. $^{\#\#}P < 0.01$ versus the control group; $^{**}P < 0.01$, $^{*}P < 0.05$ versus the DSS group ($n = 10$ per group).

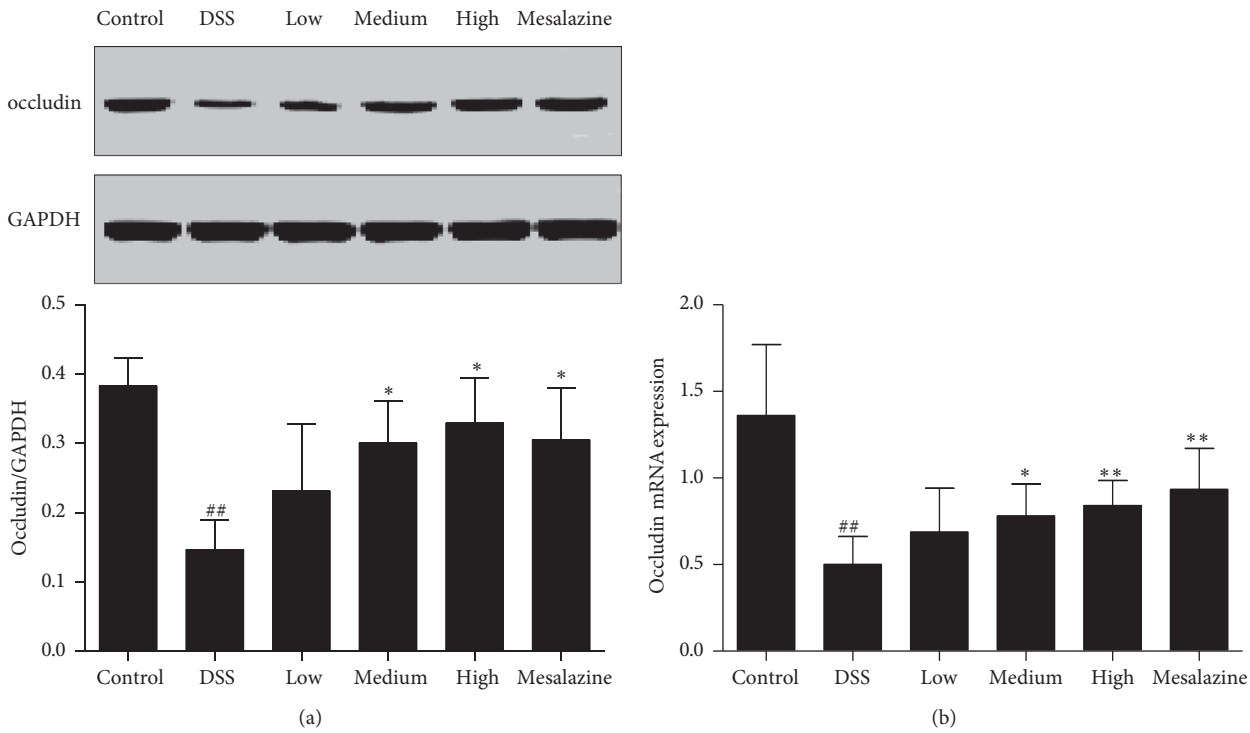


Figure 4: QCWZD increased colonic occludin (a) and occludin mRNA (b) expression in DSS-induced UC rats. Control, blank control group; DSS, DSS-treated group; low, low-dose QCWZD group; medium, medium-dose QCWZD group; high, high-dose QCWZD group; mesalazine, mesalazine group. $^{\#\#}P < 0.01$ versus the control group; $^{**}P < 0.01$, $^{*}P < 0.05$ versus the DSS group ($n = 10$ per group).

3.4. QCWZD Increased Colonic Occludin and Occludin mRNA Expression in DSS-Induced UC Rats. Disruption of tight junctions is an important basis for the pathogenesis of ulcerative colitis, and dysfunction of the MSP/RON pathway leads to disruption of the intestinal mucosal barrier, so occludin, a major protein in tight junctions, was examined. We found distinctly decreased occludin and occludin mRNA expression in the DSS group compared to that in the control group ($P < 0.01$, Figures 4(a) and 4(b)). Nevertheless, occludin and occludin mRNA expression were significantly induced in DSS-induced UC rats treated with medium-dose QCWZD, high-dose QCWZD, and mesalazine ($P < 0.05$, $P < 0.01$, resp., Figures 4(a) and 4(b)).

3.5. QCWZD Upregulated Colonic ZO-1 and ZO-1 mRNA Expression in DSS-Induced UC Rats. As a peripheral membrane protein in tight junctions, ZO-1 levels were analysed by Western blot. Compared to the control group, the DSS group showed significantly decreased ZO-1 expression ($P < 0.01$, Figure 5(a)), and the medium-dose QCWZD, high-dose QCWZD, and mesalazine showed promotion effects (versus the DSS group, $P < 0.05$, $P < 0.01$, resp., Figure 5(a)). Similarly, we found that the expression of the ZO-1 gene in the DSS group was decreased compared to that in the control group ($P < 0.01$, Figure 5(b)), and compared to the DSS group, medium-dose QCWZD, high-dose QCWZD, and mesalazine distinctly increased ZO-1 gene expression distinctly ($P < 0.05$, $P < 0.01$, resp., Figure 5(b)).

3.6. QCWZD Downregulated Colonic Claudin-2 and Claudin-2 mRNA Expression in DSS-Induced UC Rats. Upregulation of the tight junction protein claudin-2 is considered to contribute to the dysregulation of the epithelial barrier [26], so we analysed claudin-2 expression. Statistical analysis showed that, in rat with DSS-induced colitis, claudin-2 expression in the DSS group was higher than that in the control group ($P < 0.05$, Figure 6(a)) but was significantly lower than that in the QCWZD and mesalazine groups. And then we examined claudin-2 gene expression. We found that the claudin-2 gene expression was significantly higher than that in the control group. Treatment with medium-dose QCWZD, high-dose QCWZD, and mesalazine significantly reduced the claudin-2 gene expression ($P < 0.05$, $P < 0.01$, resp., Figure 6(b)).

4. Discussion

Our previous studies have shown that QCWZD had a prominent therapeutic effect on DSS-induced UC in rats, and we have made a preliminary study on the mechanism of QCWZD in treating ulcerative colitis from IP10/CXCR3 axis–mediated inflammatory response. Contrary to the destructive mechanism of IP10/CXCR3 axis, the MSP/RON signalling pathway plays a protective role in ulcerative colitis, so we continued to explore the possible protective mechanism of QCWZD in rats with DSS-induced UC from the opposite perspective, with the aim of providing a comprehensive experimental basis for the use of QCWZD.

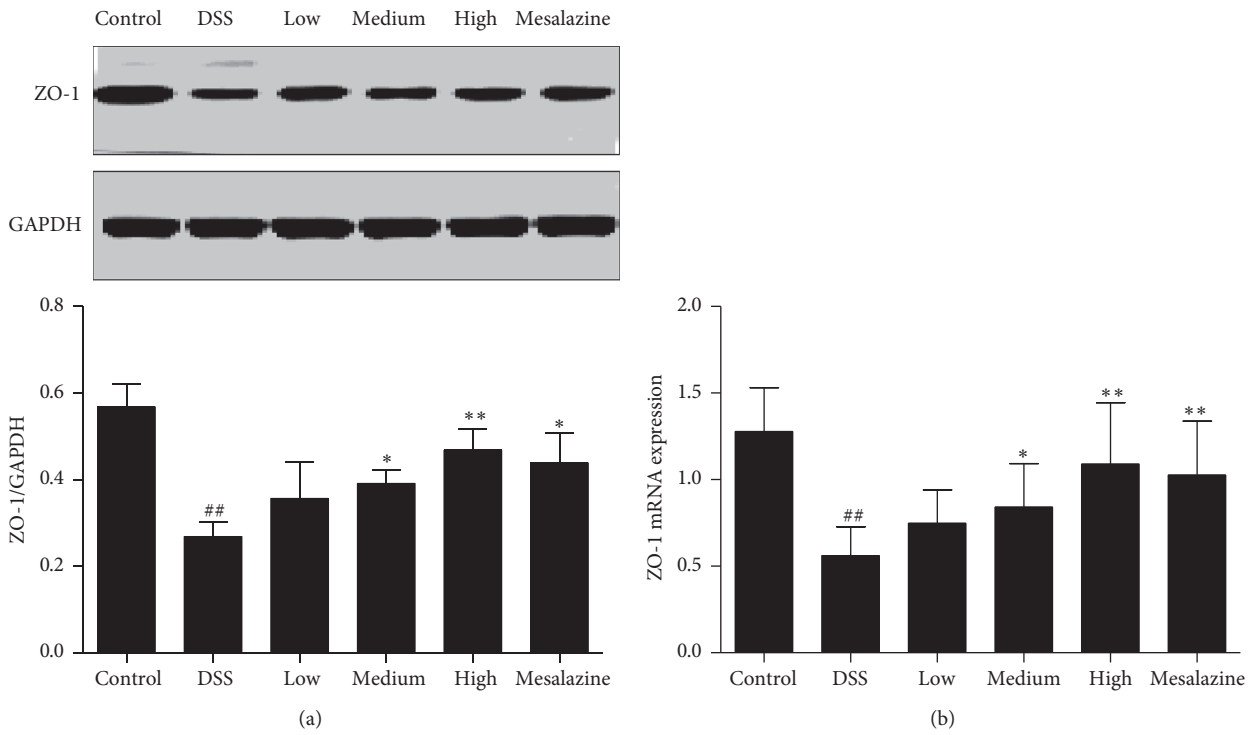


Figure 5: QCWZD upregulated colonic ZO-1 (a) and ZO-1 mRNA (b) expression in DSS-induced UC rats. Control, blank control group; DSS, DSS-treated group; low, low-dose QCWZD group; medium, medium-dose QCWZD group; high, high-dose QCWZD group; mesalazine, mesalazine group. $^{\#\#}P < 0.01$ versus the control group; $^{**}P < 0.01$, $^{*}P < 0.05$ versus the DSS group ($n = 10$ per group).

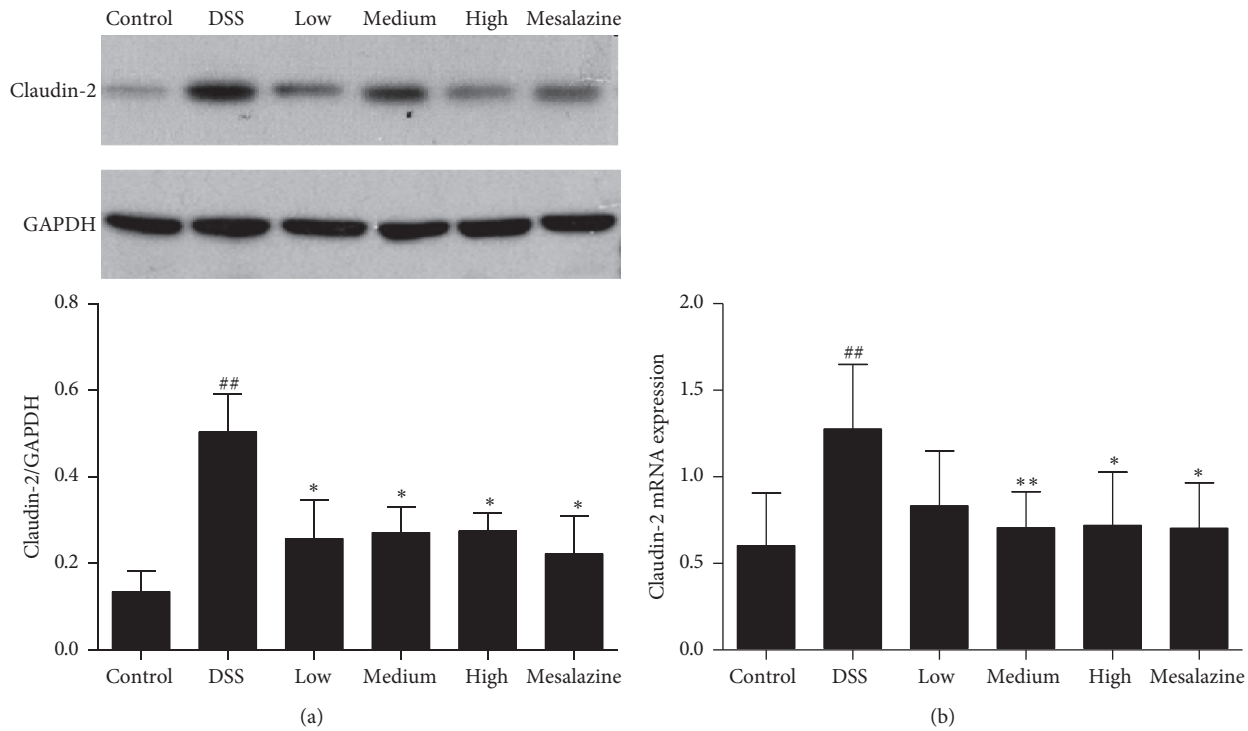


Figure 6: QCWZD upregulated colonic claudin-2 (a) and claudin-2 mRNA (b) expression in DSS-induced UC rats. Control, blank control group; DSS, DSS-treated group; low, low-dose QCWZD group; medium, medium-dose QCWZD group; high, high-dose QCWZD group; mesalazine, mesalazine group. $^{\#\#}P < 0.01$ versus the control group; $^{**}P < 0.01$, $^{*}P < 0.05$ versus the DSS group ($n = 10$ per group).

Although the aetiology and pathogenesis of UC have not been fully elucidated [27], it is commonly believed that immune inflammatory response and intestinal barrier function destruction play important roles in the pathogenesis of UC [28–30]. The MSP/RON signalling pathway is closely related to cytokine expression [31, 32], and the absence or inhibition of RON can reduce the migration and reproduction of epithelial cells and increase the production and release of inflammatory factors, which are more sensitive to external factors, thereby resulting in more severe colitis. In contrast, the activation of RON, via the binding of MSP to RON, regulates multiple downstream signalling cascades, which could inhibit peritoneal macrophages and exert anti-inflammatory effects, while regulating the proliferation, survival, and migration of and promoting the repair of injury to intestinal epithelial cells. In the present study, serum MSP levels were significantly lower in the DSS group than in the control group; similarly, the expression of RON mRNA and protein, which are MSP-specific receptors, in the colon tissue was significantly lower. In addition, our previous studies found that the DAI and MPO activity of the rats with UC were higher than that in the control group and with the 7-day QCWZD treatment; the DAI and MPO levels were significantly lower, which were negatively correlated with MSP and RON expression (Figures 1 and 2). This interesting phenomenon further supports the fact that the MSP/RON signalling pathway is closely related with the degree of inflammation and plays an important role in the pathogenesis of UC.

The PI3K/Akt signalling pathway is closely associated with the production and release of cytokines, which play an important role in abnormal immune responses [33–35]. Previous experiments have confirmed that the PI3K inhibitor Wortmannin inhibits the activation of PI3K and reduces the activity of Akt in the main downstream target in the colonic mucosa of patients with UC to achieve effective treatment [36]. Based on previous studies, we speculated that activated RON transduces a variety of signals that regulate different downstream pathways, including the PI3K/Akt signalling pathway, and has anti-inflammatory and epithelial repair effects. Thus, PI3K/Akt can be used as an indicator of the MSP/RON signalling pathway activity. In the present study, the fold changes of p-Akt level were significantly higher in the DSS group, indicating enhanced activity of the PI3K/Akt signalling pathway. With the 7-day QCWZD treatment, the activity was significantly lower (Figure 3).

The intestinal mucosal barrier is the first barrier of self-protection of the colon, so barrier dysfunction is an important material basis for the pathogenesis of ulcerative colitis [37, 38]. Recent studies have shown that altered expression of the tight junction protein is considered to contribute to the dysregulation of the epithelial barrier [2, 37, 39], and activation of the MSP/RON pathway differentially regulates tight junction function [40]. Therefore, we evaluated the expression of colonic tight junction protein. In this study, we chose occludin, ZO-1, and claudin-2 as the representative proteins of tight junctions. Occludin is a crucial tight junction protein in the pathogenesis of ulcerative colitis [41], which plays an important role in maintaining the integrity of intestinal mucosal barrier. As a peripheral membrane tight junction protein, ZO-1 can physically bind the distal C-terminus of occluding, so as to ensure the accurate connection of occludin protein [42]. With just the opposite, upregulation of claudin-2 is considered to contribute to the dysregulation of the epithelial barrier [26]. In the present study, compared with the control group, the expression of tight junction associated proteins including occludin and ZO-1 was decreased (Figures 4 and 5); claudin-2 was increased in DSS-induced UC rats (Figure 6), indicating that intestinal barrier function was damaged seriously. Treatment with QCWZD and mesalazine dependently increased occludin and ZO-1 expression (Figures 4 and 5) and decreased claudin-2 expression (Figure 6). These results suggest that QCWZD plays an important role in the protection of intestinal barrier function.

In the present study, we found that MSP/RON signalling pathway plays an important role in the pathogenesis of UC by involving the inflammatory response of macrophages and repairing epithelial cells. Intragastric administration of QCWZD significantly upregulated serum MSP level and RON expression in the colon, reduced the pAkt levels, increased colonic occluding and ZO-1 expression, and downregulated claudin-2 expression, so as to inhibit the intestinal inflammation, improve the intestinal mucosal barrier function, and finally achieve the purpose of repairing intestinal mucosa and treating ulcerative colitis.

5. Conclusions

Our results show that QCWZD ameliorates DSS-induced UC in rats mainly by reducing inflammation and improving intestinal barrier function via upregulating the MSP/RON signalling pathway.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Tangyou Mao and Junxiang Li contributed equally.

Acknowledgments

This study was supported by the National Science Foundation of China (Grant no. 81403369) and the self-selected topic of the Beijing University of Chinese Medicine (Grant no. 2015-JYB-XS172).

References

[1] B. Liu, T. Liu, X. Wang, X. Zheng, H. Wang, and L. Ma, "Effects of Guchang Capsule on Dextran Sulphate Sodium-Induced Experimental Ulcerative Colitis in Mice," *Evidence-based Complementary and Alternative Medicine*, vol. 2016, Article ID 3150651, 12 pages, 2016.

[2] X. Xu, C. Xu, S. M. Saud et al., "Effect of Kuijie Granule on the Expression of TGF-β/Smads Signaling Pathway in Patients with Ulcerative Colitis," *Evidence-based Complementary and*

Alternative Medicine, vol. 2016, Article ID 2601830, 9 pages, 2016.

[3] R. B. Sartor, "Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis," *Nature Clinical Practice Gastroenterology and Hepatology*, vol. 3, no. 7, pp. 390–407, 2006.

[4] R. J. Xavier and D. K. Podolsky, "Unravelling the pathogenesis of inflammatory bowel disease," *Nature*, vol. 448, no. 7152, pp. 427–434, 2007.

[5] G. Sclano, "Asthma, nasal polyposis and ulcerative colitis: A new perspective," *Clinical and Experimental Allergy*, vol. 32, no. 8, pp. 1144–1149, 2002.

[6] APDW 2004 Chinese IBD Working Group, "Retrospective analysis of 515 cases of Crohn's disease hospitalization in China: nationwide study from 1990 to 2003," *Journal of Gastroenterology and Hepatology*, vol. 21, no. 6, pp. 1009–1015, 2006.

[7] P. Munkholm, "Review article: the incidence and prevalence of colorectal cancer in inflammatory bowel disease," *Alimentary Pharmacology and Therapeutics*, vol. 18, no. s2, pp. 1–5, 2003.

[8] J. A. Eaden, K. R. Abrams, and J. F. Mayberry, "The risk of colorectal cancer in ulcerative colitis: a meta-analysis," *Gut*, vol. 48, no. 4, pp. 526–535, 2001.

[9] A. G. Ording, E. Horváth-Puhó, R. Erichsen et al., "Five-year mortality in colorectal cancer patients with ulcerative colitis or Crohn's disease: a nationwide population-based cohort study.," *Inflammatory bowel diseases*, vol. 19, no. 4, pp. 800–805, 2013.

[10] H. Matsuoka, H. Ikeuchi, M. Uchino et al., "Clinicopathological features of ulcerative colitis-associated colorectal cancer pointing to efficiency of surveillance colonoscopy in a large retrospective Japanese cohort," *International Journal of Colorectal Disease*, vol. 28, no. 6, pp. 829–834, 2013.

[11] T. Ullman, R. Odze, and F. A. Farraye, "Diagnosis and management of dysplasia in patients with ulcerative colitis and Crohn's disease of the colon," *Inflammatory Bowel Diseases*, vol. 15, no. 4, pp. 630–638, 2009.

[12] S. E. Kauder, L. Santell, E. Mai et al., "Functional consequences of the macrophage stimulating protein 689C inflammatory bowel disease risk allele," *PLoS ONE*, vol. 8, no. 12, Article ID e83958, 2013.

[13] R. M. Kulkarni, W. D. Stuart, D. Gurusamy, and S. E. Waltz, "Ron receptor signaling is protective against DSS-induced colitis in mice," *American Journal of Physiology - Gastrointestinal and Liver Physiology*, vol. 306, no. 12, pp. G1065–G1074, 2014.

[14] M. Witte, L. F. A. Huitema, E. E. S. Nieuwenhuis, and S. Brugman, "Deficiency in macrophage-stimulating protein results in spontaneous intestinal inflammation and increased susceptibility toward epithelial damage in zebrafish," *The American Journal of Physiology-Gastrointestinal and Liver Physiology*, vol. 11, no. 6, pp. 542–550, 2014.

[15] A. S. Bhatt, A. Welm, C. J. Farady, M. Vásquez, K. Wilson, and C. S. Craik, "Coordinate expression and functional profiling identify an extracellular proteolytic signaling pathway," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 14, pp. 5771–5776, 2007.

[16] P. K. Wagh, B. E. Peace, and S. E. Waltz, "Met-Related Receptor Tyrosine Kinase Ron in Tumor Growth and Metastasis," *Advances in Cancer Research*, vol. 100, pp. 1–33, 2008.

[17] N. Gorlatova, K. Chao, L. R. Pal et al., "Protein characterization of a candidate mechanism SNP for Crohn's disease: The macrophage stimulating protein R689C substitution," *PLoS ONE*, vol. 6, no. 11, Article ID e27269, 2011.

[18] M. Daperno, R. Sostegni, R. Rocca et al., "Medical treatment of severe ulcerative colitis," *Alimentary Pharmacology and Therapeutics*, vol. 16, supplement 4, pp. 7–12, 2002.

[19] J. Torres, R. K. Boyapati, N. A. Kennedy, E. Louis, J.-F. Colombel, and J. Satsangi, "Systematic review of effects of withdrawal of immunomodulators or biologic agents from patients with inflammatory bowel disease," *Gastroenterology*, vol. 149, no. 7, pp. 1716–1730, 2015.

[20] J. W. Chen, *Clinical effect of qingchang wenzhong on the treatment of ulcerative colitis*, Beijing University of Chinese Medicine, Beijing, China, 2015.

[21] L. J. Liu, Y. L. Wang, R. Shi et al., "Effect of Qingchang Wenzhong Decoction on colonic mucosa of TNBS-induced colitis rats," *Global Traditional Chinese Medicine*, vol. 7, no. 3, pp. 187–191, 2014 (Chinese).

[22] T.-Y. Mao, R. Shi, W.-H. Zhao et al., "Qingchang Wenzhong Decoction Ameliorates Dextran Sulphate Sodium-Induced Ulcerative Colitis in Rats by Downregulating the IP10/CXCR3 Axis-Mediated Inflammatory Response," *Evidence-based Complementary and Alternative Medicine*, vol. 2016, Article ID 4312538, 11 pages, 2016.

[23] W. D. Stuart, R. M. Kulkarni, J. K. Gray, J. Vasiliauskas, M. A. Leonis, and S. E. Waltz, "Ron receptor regulates Kupffer cell-dependent cytokine production and hepatocyte survival following endotoxin exposure in mice," *Hepatology*, vol. 53, no. 5, pp. 1618–1628, 2011.

[24] M. W. Pfaffl, "A new mathematical model for relative quantification in real-time RT-PCR.," *Nucleic Acids Research*, vol. 29, no. 9, article e45, 2001.

[25] W. Jing, A. Rajput, J. L. C. Kan et al., "Knockdown of Ron kinase inhibits mutant phosphatidylinositol 3-kinase and reduces metastasis in human colon carcinoma," *Journal of Biological Chemistry*, vol. 284, no. 16, pp. 10912–10922, 2009.

[26] K. Randall, N. Henderson, J. Reens et al., "Claudin-2 expression levels in ulcerative colitis: Development and validation of an in-situ hybridisation assay for therapeutic studies," *PLoS ONE*, vol. 11, no. 9, Article ID e0162076, 2016.

[27] A. Kaser, S. Zeissig, and R. S. Blumberg, "Inflammatory bowel disease," *Annual Review of Immunology*, vol. 28, pp. 573–621, 2010.

[28] S. R. Targan and L. C. Karp, "Defects in mucosal immunity leading to ulcerative colitis," *Immunological Reviews*, vol. 206, pp. 296–305, 2005.

[29] R. B. Sartor, "Pathogenesis and immune mechanisms of chronic inflammatory bowel diseases," *The American Journal of Gastroenterology*, vol. 92, no. 12, pp. 5–11, 1997.

[30] F. Casellas I Jordà, "TNF inhibitors in inflamatory bowel disease," *Medicina Clinica*, vol. 123, no. 16, pp. 627–634, 2004.

[31] A. Chaudhuri, "Regulation of macrophage polarization by RON receptor tyrosine kinase signaling," *Frontiers in Immunology*, vol. 5, article 546, 2014.

[32] M.-H. Wang, Y.-Q. Zhou, and Y.-Q. Chen, "Macrophage-stimulating protein and RON receptor tyrosine kinase: Potential regulators of macrophage inflammatory activities," *Scandinavian Journal of Immunology*, vol. 56, no. 6, pp. 545–553, 2002.

[33] S. K. Selvaraj, R. K. Giri, N. Perelman, C. Johnson, P. Malik, and V. K. Kalra, "Mechanism of monocyte activation and expression of proinflammatory cytochemokines by placenta growth factor," *Blood*, vol. 102, no. 4, pp. 1515–1524, 2003.

[34] A. L. Sabater, E. J. Andreu, M. García-Guzmán et al., "Combined PI3K/Akt and Smad2 activation promotes corneal endothelial

cell proliferation," *Investigative Ophthalmology and Visual Science*, vol. 58, no. 2, pp. 745–754, 2017.

[35] R. F. Shan, Y. F. Zhou, A. F. Peng, and Z. G. Jie, "Inhibition of aurora-B suppresses HepG2 cell invasion and migration via the PI3K/Akt/NF-κB signaling pathway in vitro," *Experimental and Therapeutic Medicine*, vol. 8, no. 3, pp. 1005–1009, 2014.

[36] X. L. Huang, J. Xu, X. H. Zhang et al., "PI3K/Akt signaling pathway is involved in the pathogenesis of ulcerative colitis," *Inflammation Research*, vol. 60, no. 8, pp. 727–734, 2011.

[37] M. Stio, L. Retico, V. Annese, and A. G. Bonanomi, "Vitamin D regulates the tight-junction protein expression in active ulcerative colitis," *Scandinavian Journal of Gastroenterology*, vol. 51, no. 10, pp. 1193–1199, 2016.

[38] L.-C. Tong, Y. Wang, Z.-B. Wang et al., "Propionate ameliorates dextran sodium sulfate-induced colitis by improving intestinal barrier function and reducing inflammation and oxidative stress," *Frontiers in Pharmacology*, vol. 7, article 253, 2016.

[39] P. Das, P. Goswami, T. K. Das et al., "Comparative tight junction protein expressions in colonic Crohn's disease, ulcerative colitis, and tuberculosis: a new perspective," *Virchows Archiv*, vol. 460, no. 3, pp. 261–270, 2012.

[40] K. Zhang, H.-P. Yao, and M.-H. Wang, "Activation of RON differentially regulates claudin expression and localization: Role of claudin-1 in RON-mediated epithelial cell motility," *Carcinogenesis*, vol. 29, no. 3, pp. 552–559, 2008.

[41] N. J. Ronaghan, J. Shang, V. Iablokov et al., "The serine protease-mediated increase in intestinal epithelial barrier function is dependent on occludin and requires an intact tight junction," *American Journal of Physiology - Gastrointestinal and Liver Physiology*, vol. 311, no. 3, pp. G466–G479, 2016.

[42] Y. Jiang, L. Liu, and J. J. Steinle, "Compound 49b regulates ZO-1 and occludin levels in human retinal endothelial cells and in mouse retinal vasculature," *Investigative Opthalmology & Visual Science*, vol. 58, no. 1, pp. 185–189, 2017.

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2017, Article ID 6019530, 15 pages
https://doi.org/10.1155/2017/6019530

Review Article

Kangfuxinye Enema Combined with Mesalamine for Ulcerative Colitis: A Systematic Review and GRADE Approach

Peng-wei Ren,[1] Wen-jie Yang,[1] Dan-dan Wang,[1] Jing-yan Shan,[2] De-ying Kang,[1] Qi Hong,[1] Shu Wen,[3] and Ru-wen Zhang[4]

[1]*Department of Evidence-Based Medicine and Clinical Epidemiology, West China Hospital, Sichuan University, Chengdu 610041, China*
[2]*West China School of Public Health, No. 4 West China Teaching Hospital, Sichuan University, Chengdu 610041, China*
[3]*West China School of Medicine and West China Hospital, Sichuan University, Chengdu 610041, China*
[4]*Department of Integrated Traditional Chinese and Western Medicine, West China Hospital, Sichuan University, Chengdu 610041, China*

Correspondence should be addressed to De-ying Kang; deyingkang@126.com

Received 16 December 2016; Accepted 21 June 2017; Published 7 August 2017

Academic Editor: Steve Harakeh

Objectives. To critically appraise the efficacy and safety of Kangfuxinye enema combined with mesalamine for the ulcerative colitis (UC) patients and in addition to grade the quality of evidence by using the GRADE (grading of recommendations, assessment, development, and evaluation) approach. *Methods*. A literature search was performed in the Cochrane Library, MEDLINE, EMBASE, CBM, CNKI, VIP, and WanFang Databases. The search restrictions were patients with UC and RCTs. Studies including other treatments except Kangfuxinye with mesalamine were excluded. *Results*. Nineteen studies met the inclusion criteria. We found significant benefits of Kangfuxinye combined with mesalamine against mesalamine alone in improving response rate as well as reducing the recurrence rate and inflammation rate; meanwhile, the increase of the adverse events rate was not observed. Furthermore, the symptoms remission rate and the cure time were insignificant statistically. Additionally, GRADE results indicated that the quality of evidence regarding the above 6 outcomes was rated from very low to moderate quality. *Conclusions*. Although Kangfuxinye enema seems effective and safe for treating UC patients in this systematic review, Kangfuxinye enema combined with mesalamine was weakly recommended due to very low to moderate quality of available evidence by the GRADE approach.

1. Introduction

Ulcerative colitis (UC) is one of the 2 major types of inflammatory bowel disease (IBD), along with Crohn disease but 3 times more common compared to it [1, 2]. The incidence of UC is 1.2–20.3 cases per 100,000 per year, and the developed countries, such as Northern Europe and North America, have the highest incidence of the disease [1, 3]. In Asia and the Middle East, the incidence is about 6.3 per 100,000 person-years. Universally, UC occurs mainly between the second and fourth decades of life [4]. In combination with the change of environment and other unknown reasons, UC has become a global emergence disease with increasing incidence and prevalence worldwide [5]. Typical symptoms of UC include abdominal pain, tenesmus, bloody diarrhea, passage of pus, mucus, or both, urgency, weight loss, and fever [6], which causes a miserable influence on the quality of life of the UC patients. Moreover, UC affects individuals in their most formidable and productive years of life, resulting in heavy burden on the patients' life, health care system, and society [7]. In addition, high relapse rates and protracted courses of disease also lead to the increasing risk of colorectal cancer [8, 9]. Therefore, UC often requires life-long maintenance therapy for relieving symptoms and/or to attenuate the inflammation while there is lack of curative treatment.

Mesalamine (USAN), also known as mesalazine (INN, BAN) or 5-aminosalicylic acid (5-ASA), is most commonly used as a first-line therapy for mild to moderate UC [10].

However, the majority of patients with UC exhibited low adherence and persistence to mesalamine, which has been an important barrier for successful management [11]. Indeed, the major consequences of nonadherence to 5-ASA for UC patients had a fivefold higher risk of relapse, an increased risk of colorectal cancer, and a reduced quality of life [8]. Once the first-line therapy fails, patients would turn to alternative medicine such as steroids [12], azathioprine [13], and the anti-tumour necrosis factor alpha (TNFa) agent infliximab [14]. Nevertheless, those alternative therapies always accompany increased risks of infection and malignancy.

At present, complementary and alternative medicine (CAM) is increasingly applied for treatment of IBD due to its potential efficacy [15, 16], and it accounts for about 21% of inflammatory bowel disease patients now [17]. Of those, Kangfuxinye, a pure Chinese herbal medicine extracted from the *Periplaneta americana*, has been widely used for treating ulcerative and inflammatory diseases [18, 19] due to its sound effects on anti-inflammatory and recovery of gastrointestinal mucosal, and animal studies have also suggested that the therapeutic effect of Kangfuxinye may be due, at least in part, to its stimulatory effect on nonspecific cellular defense mechanisms [20], making it one of the most addressed therapies for UC, especially in Chinese UC patients. Although previous studies had shown sound effects of Kangfuxinye for treating UC patients, the quality of the studies has become a common concern, thus further researches are needed before making recommendations for clinical practice. One previous systematic review (SR) [21] indicated Kangfuxinye having short-term benefits regarding the overall response and inflammation reduction, but its safety and long-term effect still remain unclear. In addition, the quality of evidence needs to be appraised and validated critically.

Therefore, the aims of this study were to systematically review the efficacy and safety of Kangfuxinye enema in combination with mesalamine according to the Cochrane Collaboration's guidance for SR and then to grade quality of the evidence and make recommendations for practice by using The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach [22] which is always used as an instrument for grading quality of evidence within systematic reviews and guidelines and for making evidence-based recommendations during guidelines development [23].

2. Methods

This study was conducted using the Cochrane Collaboration's approach [43] and this systematic review is consistent with the PRISMA (the Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist [44]. In addition, the GRADE approach [22] was also taken to grade the quality of evidence and make recommendation regarding the use of Kangfuxinye enema in the UC. Five methodological factors (risk of bias, inconsistency, indirectness, imprecision, and publication bias) were judged to downgrade or upgrade the quality of evidence [45]. Ethical approval and patient informed consent were waived because all data were extracted from previous studies.

Table 1: Rating scale for outcome ranking according to clinical importance.

Importance	Measure
Critical*	Recurrence rate
	Response rate
Important†	Inflammation reduction rate
	Symptom remission rate
	Adverse effects rate
	Time of cure
Not important‡	None

*Critical for making a decision and included in the evidence profile. †Important for making a decision and included in the evidence profile. ‡Not important for making a decision and not included in the evidence profile.

2.1. Criteria for Considering Studies for This Review

2.1.1. Type of Studies. Only RCTs, which were published or unpublished in English or Chinese, were identified for this review. Observational studies, quasi-randomized controlled trials (Q-RCTs), controlled clinical trials (CCTs) were excluded.

2.1.2. Types of Participants. Participants (male/female) diagnosed with UC and who met the indications for using Kangfuxinye as enema were included in this study.

2.1.3. Types of Interventions. Kangfuxinye enema combined with mesalamine served as the intervention and taking mesalamine alone served as the control. Any mode of the mesalamine was eligible for this review.

2.1.4. Types of Outcome Measures. We consulted with 5 clinicians specialized in UC from West China hospital, to identify possible outcomes relating to the UC's efficacy and safety as well as to rate clinical importance of each outcome with assigning a value of 1 (lowest importance) to 9 (highest importance). The results were then used to generate a mean score with standard deviation (SD) for each outcome. The importance of each outcome was classified according to the mean score. Three outcome categories were identified regarding the clinical importance: critical (mean score of 7–9), important but not critical (mean score of 4–6), and limited importance (mean score of 1–3) [22]. Critical and important outcomes in Table 1 were used to make recommendations (Table 1).

2.2. Search Strategies

2.2.1. Electronic Searches. The following databases were searched from the inception through March 31, 2016: Cochrane Central Register of Controlled Trials (CENTRAL, Ovid), MEDLINE (PubMed), EMBASE (Ovid), Chinese Biomedicine Database (CBM), China National Knowledge Infrastructure (CNKI), VIP Information Database (VIP), and WanFang Database. The search terms used were "Kangfuxinye"; "Mesalamine"; and "ulcerative colitis" in Chinese or English.

2.2.2. Search Other Sources. We also screened reference list of all obtained papers. Additionally, conference proceedings and dissertation abstracts were retrieved to identify unpublished studies.

2.3. Selection of Studies. Retrieved records including titles and abstracts were screened independently by 2 reviewers (P-W R and W-J Y) using EndNote 5.0 software after removal of duplications. The studies were included if they were Kangfuxinye enema combined with mesalamine against mesalamine alone. Observational studies, quasi-randomized controlled trials (Q-RCTs), controlled clinical trials (CCTs), and trials with paired interventions besides Kangfuxinye and mesalamine were excluded. Dissertations and abstracts were included when they contained sufficient details. All of the eligible studies were downloaded. Discrepancies were resolved via discussion or in consultation with the third reviewer (D-Y K).

2.4. Data Extraction and Management. All studies were reviewed by two reviewers (P-W R, W-J Y), who extracted data from the studies with the predeveloped forms including items such as the following: first author, publication year, sample size in each group, characteristics of participants (including age, sex, and degree of UC), diagnosis criteria of UC, details of Kangfuxinye enema and mesalamine, measured outcomes, follow-up (where available), and the number and reasons of missing participants.

Mean score changes from baseline to a particular endpoint were also abstracted. If unavailable, we extracted mean scores of baseline and endpoint as well as the SDs [43, 46]. Consensus was obtained by discussion or by consulting the third reviewer (D-Y K).

2.5. Assessment of Risk of Bias in Included Studies. Risk of bias for each eligible study was assessed by 2 reviewers (P-W R and W-J Y) using the Cochrane Collaboration's Risk of Bias Tool in 6 domains: random sequence generation, incomplete outcome measures, blinding of participants and personnel, and outcome assessors, and allocation concealment, and selective outcome reporting [43]. Disagreements were resolved by discussion between the two reviewers (P-W R, W-J Y), or with the arbitration of a third reviewer (D-Y K) being sought if necessary. There was no disagreement between the two reviewers on the risk of bias.

2.6. Data Synthesis and Statistical Analysis. A meta-analysis was performed by using the Review Manager (Version 5.3 for Windows; Cochrane Collaboration, Oxford, UK) if needed. For dichotomous data, pooled effect estimate was calculated using risk ratio (RR) with its 95% confidence interval (CI). For continuous data, overall treatment effect size was calculated using mean difference (MD) with its 95% CI when the same rating scale was used, or using standardized mean difference (SMD) if rating scales were different. A 2-sided $P \leq 0.05$ was considered as the threshold for statistical significance. Heterogeneity across study results was assessed using Cochrane's Q statistic with P value. I^2 statistic was used to quantify the degree of heterogeneity. If $P < 0.1$ or $I^2 > 50\%$, this indicates significant heterogeneity was present [43], and a random-effects model was applied to pool overall effect estimate; otherwise, a fixed-effects model was used. Subgroup analyses were carried out where available to investigate potential influence of clinical characteristics of participants or methodological quality on treatment effect size. Sensitivity analyses were performed where available to explore possible heterogeneity and its impact on the robustness of study results. If the number of included studies was sufficient ($n > 10$), a funnel plot or Egger's regression test was generated to detect potential publication bias [47, 48].

2.7. The GRADE Approach. Quality of evidence for each specific outcome among the included studies was evaluated by using the GRADE approach. Two authors (P-W R and W-J Y) received training on how to use GRADEro [49] in the 23nd Cochrane Colloquium (Vienna, Austria, from October 3 to 7, 2015), and separately assessed the quality in the estimate of each outcome. The evidence quality across each outcome was upgraded or downgraded determined by 5 primary domains (risk of bias, inconsistency, indirectness, imprecision, and publication bias) and was eventually categorized into 4 levels (high, moderate, low, and very low) [23].

3. Results

Our searches identified 202 potentially relevant studies, of which 193 references were all from electronic databases, 9 references from relevant reference lists, and no references were obtained from conference proceedings or dissertation abstracts. Finally, 19 studies [24–42] from electronic databases met our inclusion criteria. Further details were shown in Figure 1.

3.1. Characteristics of Included Studies. The characteristics of all included RCTs [24–42] were listed in Table 2. All RCTs were conducted in China and were published in Chinese. Males approximately account for half of the enrolled patients in each study. No dropouts were observed in these studies.

3.2. Assessment of Risk of Bias in Included Studies. The risk of bias of all included RCTs was assessed by using Cochrane Collaboration risk of bias tool. Because of inadequate reporting of randomization sequence generation and allocation concealment, all of the two items were judged as "unclear" which means that the potential risk of selection bias may exist. Of those, only two RCTs [24–42] used random number table to produce random sequence, whereas other trials just reported "randomly assigned" but failed to report on how sequence is produced. Details of allocation being concealed were unclear in all studies. Meanwhile, whether other important risks of bias existed could not be assessed due to paucity of data among the included trials. Overall, the included RCTs had moderate or high risks of bias in terms of 6 domains (Table 3).

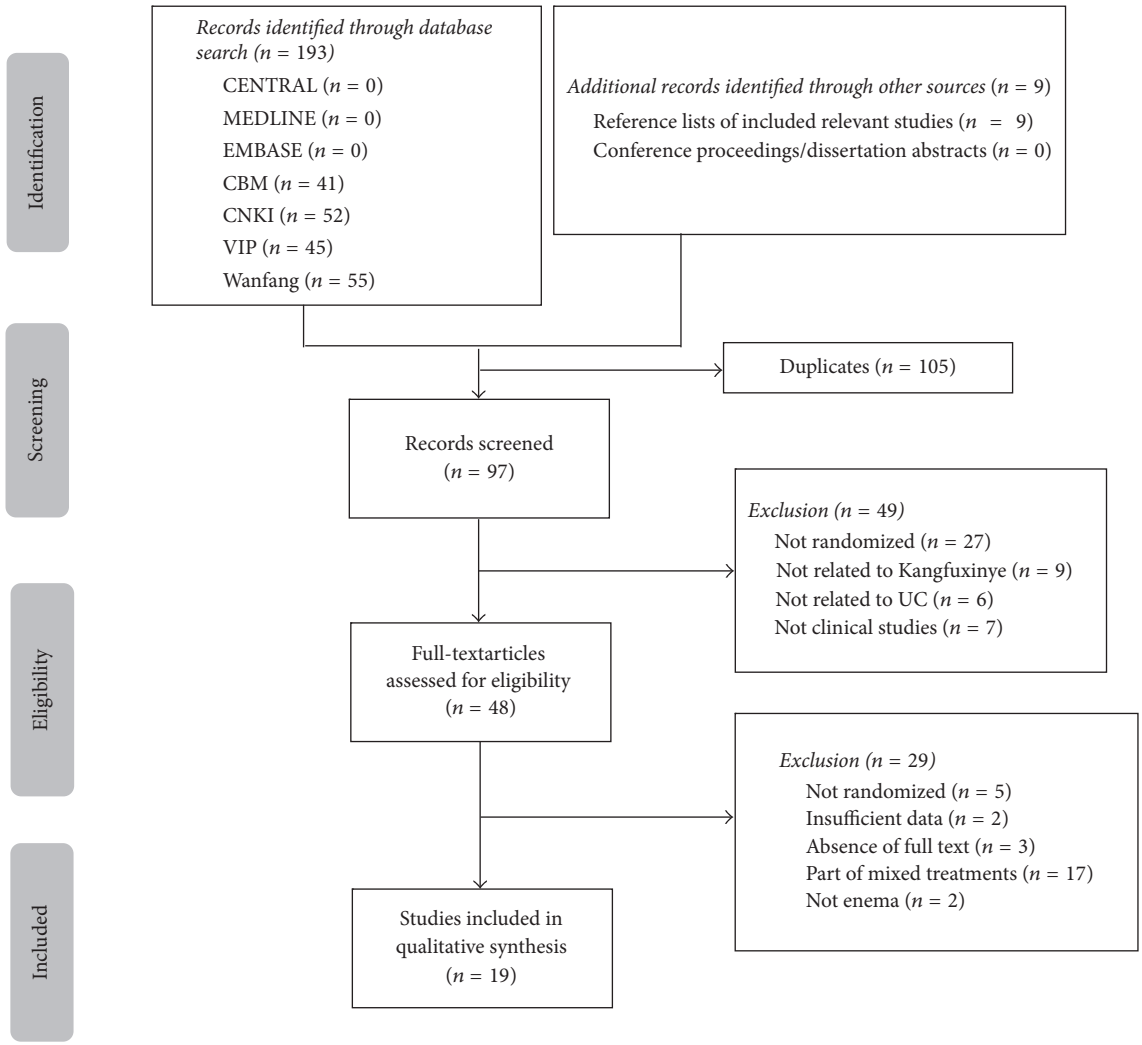


Figure 1: Flow diagram of the selection process. CENTRAL = Cochrane Central Register of Controlled Trials; CBM = Chinese Biomedicine Database; CNKI = China National Knowledge Infrastructure; VIP: Chinese Scientific Journals Database.

3.3. Critical Outcomes

3.3.1. Recurrence Rate. Five RCTs [24, 26, 29, 39, 41] including 360 patients reported recurrence rate. Recurrence was monitored after 3~12 months of follow-up among these trials. Compared with mesalamine, the meta-analysis indicated that Kangfuxinye combined with mesalamine enema reduced recurrence significantly (RR = 0.33, 95% CI: 0.20–0.53, $P < 0.001$) without heterogeneity ($I^2 = 0\%$, $P = 0.99$) (Figure 2). A GRADE analysis indicated that the quality of evidence supporting this outcome was moderate due to risk of bias (Table 4).

3.3.2. Response Rate. 16 RCTs [24–31, 33, 36–42] including 1236 patients reported response rate. The outcome measure was based on both physician's assessment and the results of endoscopy typically divided into four categories, including (1) recovery, (2) significant improvement, (3) mild improvement, and (4) no change. The meta-analysis suggested favourable effects of Kangfuxinye combined with mesalamine against mesalamine (RR = 1.19, 95% CI = 1.14 to 1.25, $P < 0.0001$; heterogeneity: $I^2 = 0\%$, $P = 0.89$) (Figure 3). A GRADE approach indicated that the quality of evidence supporting this outcome was low due to serious risk of bias (Table 4).

3.4. Important Outcomes

3.4.1. Inflammation Reduction Rate. Of those included 5 trials [25, 30, 33–35] providing examination of the inflammation reduction by endoscopy and endoscopy grading or scoring systems for inflammatory bowel diseases (IBD), a significant difference on the inflammation reduction rate was observed between two groups (fixed-effects model, RR = 1.30, 95% CI: 1.16–1.46, $P < 0.001$) without heterogeneity ($I^2 = 0\%$, $P = 0.44$) (Figure 4). A GRADE approach indicated that the quality of evidence supporting this outcome was low due to serious risk of bias (Table 4).

3.4.2. Symptom Remission Rate. Four studies [30, 32, 34, 35] including 269 patients reported symptom remission rate. The

Table 2: Characteristics of 19 included trials.

(a)

Studies	Number of participants		Age (mean ± SD/range, y)		Sex (male, %)		Degree of UC	Disease course (mean ± SD/range, y)		Diagnosis
	Experiment	Control	Experiment	Control	Experiment	Control		Experiment	Control	
Gong 2015 [24]	40	40	58.1 ± 8.2	58.4 ± 9.0	45.0	45.0	Mild to moderate	3.6 ± 1.6	3.3 ± 1.4	WGOPGDMIBD (revised 2010)
Han 2015 [25]	20	20	44 ± 13.1	43.5 ± 12.3	35.0	35.0	NR	4.8 ± 2.5	4.5 ± 2.3	NR
Huang 2013 [26]	40	40	67.1 ± 10.4	68.5 ± 8.6	42.5	50.0	Mild to severe	NR	NR	WGOPGDMIBD (revised 2010)
Jin 2015 [27]	90	90	45.2 ± 3.7	45.3 ± 3.5	54.4	54.4	NR	3.8 ± 1.4	3.5 ± 1.3	CTCMDTUC (revised 2010)
Kan 2013 [28]	46	46	20~74		NR	NR	NR	NR	NR	CCDTIBD (revised 2007)
Li 2015 [29]	50	50	34.9 ± 6.2	34.5 ± 6.5	66.0	66.0	NR	2.4 ± 0.7	2.5 ± 1.0	WGOPGDMIBD (revised 2010)
Liu 2015 [30]	30	30	24~62	27~63	40.0	40.0	NR	1~8	0.75~6	CCDTIBD (revised 2007)
Ma 2014 [31]	30	30	20~50	17~50	60.0	60.0	Mild to moderate	0.5~2	0.25~2	NR
Ouyang 2014 [32]	34	33	36.3 ± 5		NR	NR	Mild to moderate	NR	NR	CCDTIBD (revised 2007)
Ouyang 2011 [33]	42	42	20~78		NR	NR	Mild to moderate	NR	NR	NR
Tan 2014 [34]	35	35	43.5 ± 10.4	45.2 ± 11.3	54.3	54.3	NR	0.5~16	0.5~14	WGOPGDMIBD (revised 2010)
Wang 2013 [35]	36	36	18~70		NR	NR	Mild to moderate	NR	NR	CCDTIBD (revised 2007)
Xu 2014 [36]	20	20	23~55		NR	NR	Mild to moderate	NR	NR	NR
Yin 2014 [37]	27	27	51.02 ± 3.14	50.20 ± 3.03	48.1	48.1	Moderate to sever	5.49 ± 1.45	5.93 ± 1.57	NR
Yue 2013 [38]	55	55	20~74	19~74	41.8	41.8	Mild to moderate	8.5 ± 0.9	8.2 ± 0.8	CCDTIBD (revised 2012)
Zeng 2013 [39]	20	20	46.0 ± 3.5	45.0 ± 2.5	40.0	40.0	Mild to moderate	9.5 ± 0.5	8.5 ± 1.5	CCDTIBD (revised 2007)
Zhang 2012 [40]	28	28	NR	NR	NR	NR	NR	NR	NR	NR
Zhang 2014 [41]	30	30	45 ± 12	47 ± 11	46.7	46.7	Mild to moderate	NR	NR	NR
Zheng 2013 [42]	30	30	22~63	29~58	63.3	63.3	NR	0.7~14	0.9~9	CCDTIBD (revised 2007)

(b)

Studies	Intervention strategy	Control strategy	Course	Adverse events		Recurrence		Follow-up time (month)
				Experiment	Control	Experiment	Control	
Gong 2015 [24]	KFXY enema (50 ml of KFX in 150 ml of normal saline; 37°C) + mesalamine Enteric-coated Tablets 1.0 g tid	Mesalamine Enteric-coated Tablets 1.0 g tid	NR	5	3	3	11	6
Han 2015 [25]	KFXY enema (50 ml of KFX in 150 mL of normal saline; 37°C) + mesalamine 1.0 g qid	Mesalamine 1.0 g qid	NR	NR	NR	NR	NR	NR
Huang 2013 [26]	KFXY enema (30 ml of KFX in 150 ml of normal saline; 37°C; 20 min) + mesalamine 1.0 g qid	Mesalamine 1.0 g qid	4 w	3	0	7	19	12
Jin 2015 [27]	KFXY enema (50 ml of KFX in 150 mL of normal saline; 37°C) + mesalamine 1.5–4.0 g/day	Mesalamine 1.5–4.0 g/day	4 w	NR	NR	NR	NR	NR
Kan 2013 [28]	KFXY enema (50 ml of KFX in 50 mL of normal saline; 37°C) + mesalamine Slow Release Tablets 1.0 g qd	Mesalamine Slow Release Tablets 1.0 g qd	4 w	3	2	NR	NR	NR
Li 2015 [29]	KFXY enema (50 ml of KFX in 50 mL of normal saline; 37°C; 45 min) + mesalamine Slow Release Tablets 1.0 g qd	Mesalamine Slow Release Tablets 1.0 g qd	4 w	2	3	3	10	3

(b) Continued.

Studies	Intervention strategy	Control strategy	Course	Adverse events		Recurrence		Follow-up time (month)
				Experiment	Control	Experiment	Control	
Liu 2015 [30]	KFXY enema (100 ml; 20 min) + mesalamine 1.0 g tid	Mesalamine 1.0 g tid	8 w	NR	NR	NR	NR	NR
Ma 2014 [31]	KFXY enema (38–41°C; 45 min) + mesalamine Slow Release Tablets 1.0 g tid	Mesalamine Slow Release Tablets 1.0 g tid	4 w	1	0	NR	NR	NR
Ouyang 2014 [32]	KFXY enema (30 ml of KFX in 120 mL of normal saline; 37°C; 45 min) + mesalamine Slow Release Tablets 1.0 g qd	Mesalamine Slow Release Tablets 1.0 g tid	4 w	1	2	NR	NR	NR
Ouyang 2011 [33]	KFXY enema (50 ml of KFX in 50 mL of normal saline) + mesalamine 1.0 g tid	Mesalamine 1.0 g tid	4 w	0	0	NR	NR	NR
Tan 2014 [34]	KFXY enema (50 ml of KFX in 150 mL of normal saline; 37-38°C) + mesalamine 1.0 g tid	Mesalamine 1.0 g tid	4 w	0	0	NR	NR	NR
Wang 2013 [35]	KFXY enema (50 ml of KFX in 100 mL of normal saline) + mesalamine 1.0 g qid	Mesalamine 1.0 g tid	4 w	NR	NR	NR	NR	NR
Xu 2014 [36]	KFXY enema (50 ml of KFX in 150 mL of normal saline; 37°C) + mesalamine 1.0 g qid	Mesalamine 1.0 g tid	NR	NR	NR	NR	NR	NR
Yin 2014 [37]	KFXY enema (50 ml of KFX in 50 mL of normal saline) + mesalamine 1.0 g tid	Mesalamine 1.0 g tid	4 w	NR	NR	NR	NR	NR
Yue 2013 [38]	KFXY enema (50 ml of KFX in 50 mL of normal saline; 38°C; 45 min) + mesalamine 1.0 g tid	Mesalamine 1.0 g tid	4 w	0	0	NR	NR	NR
Zeng 2013 [39]	KFXY enema + mesalamine 1.0 g tid	Mesalamine 1.0 g tid	NR	0	0	0	2	6
Zhang 2012 [40]	KFXY enema + mesalamine 1.0 g tid	Mesalamine 1.0 g tid	2 w	NR	NR	NR	NR	NR
Zhang 2014 [41]	KFXY enema (50ml of KFX in 150 ml of normal saline; 37-38°C) + mesalamine 1.0 g tid	Mesalamine 1.0 g tid	4 w	2	0	5	14	12
Zheng 2013 [42]	KFXY enema (50 ml of KFX in 100 ml of normal saline; 37.5°C; >30 min) + mesalamine 1.0 g tid	Mesalamine 1.0 g tid	2 w	0	0	NR	NR	NR

WGOPGDMIBD = World Gastroenterology Organization Practice Guidelines for the Diagnosis and Management of IBD, CTCMDTUC = Consensus of Traditional Chinese Medicine Diagnosis and Treatment for Ulcerative Colitis. CCDTIBD = Chinese Consensus on the Diagnosis and Treatment of Inflammatory Bowel Disease (IBD). NR = not reported. KFXY = Kangfuxinye.

Table 3: Assessment of risk of bias in included studies.

Studies	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other sources of bias
Gong 2015 [24]	Random number table	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Han 2015 [25]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Huang 2013 [26]	Random number table	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Jin 2015 [27]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Kan 2013 [28]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Li 2015 [29]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Liu 2015 [30]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Ma 2014 [31]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Ouyang 2014 [32]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Ouyang 2011 [33]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Tan 2014 [34]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Wang 2013 [35]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Xu 2014 [36]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Yin 2014 [37]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Yue 2013 [38]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Zeng 2013 [39]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Zhang 2012 [40]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Zhang 2014 [41]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Zheng 2013 [42]	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear

Yes = low risk of bias; No = high risk of bias; unclear = uncertain risk of bias.

Table 4: Assessment of quality and summarizing the findings with the GRADE approach.

Quality assessment							Summary of findings				
							Study event rates (%)			Anticipated absolute effects	
Participants (studies) Follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall quality of evidence	With control	With Kangfuxinye + mesalazine	Relative effect (95% CI)	Risk with control	Risk difference with Kangfuxinye + mesalazine (95% CI)
Recurrence rate (critical outcome)											
360 (5 studies) 8 months	Serious[1]	No serious inconsistency	No serious indirectness	No serious imprecision[2]	Undetected[3]	⊕⊕⊕⊖ *MODERATE*[1,2,3] due to risk of bias	56/180 (31.1%)	18/180 (10%)	*RR 0.33* (0.2 to 0.53)	*Study population* *311 per 1000* *Moderate* *275 per 1000*	*208 fewer per 1000* (from 146 fewer to 249 fewer) *184 fewer per 1000* (from 129 fewer to 220 fewer)
Response rate (critical outcome)											
1236 (16 studies) 4 weeks	Very serious[4]	No serious inconsistency	No serious indirectness	No serious imprecision[5]	Undetected	⊕⊕⊖⊖ *LOW*[4,5] due to risk of bias	483/618 (78.2%)	575/618 (93%)	*RR 1.19* (1.14 to 1.25)	*Study population* *782 per 1000* *Moderate* *764 per 1000*	*148 more per 1000* (from 109 more to 195 more) *145 more per 1000* (from 107 more to 191 more)
Inflammation reduction rate (important outcome)											
326 (5 studies) 4 weeks	Very serious[6]	No serious inconsistency	No serious indirectness	No serious imprecision[7]	Undetected[3]	⊕⊕⊖⊖ *LOW*[3,6,7] due to risk of bias	113/163 (69.3%)	147/163 (90.2%)	*RR 1.3* (1.16 to 1.46)	*Study population* *693 per 1000* *Moderate* *700 per 1000*	*208 more per 1000* (from 111 more to 319 more) *210 more per 1000* (from 112 more to 322 more)
Symptom remission rate (important outcome)											
269 (4 studies) 4 weeks	Very serious[8]	Serious[9]	No serious indirectness	Serious[10]	Undetected[3]	⊕⊖⊖⊖ *VERY LOW*[3,8,9,10] due to risk of bias, inconsistency, imprecision	108/134 (80.6%)	124/135 (91.9%)	*RR 1.12* (0.96 to 1.3)	*Study population* *806 per 1000* *Moderate* *802 per 1000*	*97 more per 1000* (from 32 fewer to 242 more) *96 more per 1000* (from 32 fewer to 241 more)
Time of remission (important outcome; better indicated by lower values)											
19 (1 study) 4 weeks	Serious[11]	No serious inconsistency	No serious indirectness	Very serious[12]	Undetected[3]	⊕⊖⊖⊖ *VERY LOW*[3,11,12] due to risk of bias, imprecision	8	11	*MD-5.99* (−14.15 to 2.17)		The mean time of cure in the intervention groups was *5.99 lower* (14.15 lower to 2.17 higher)

Table 4: Continued.

Quality assessment							Summary of findings				
							Study event rates (%)			Anticipated absolute effects	
Participants (studies) Follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall quality of evidence	With control	With Kangfuxinye + mesalazine	Relative effect (95% CI)	Risk with control	Risk difference with Kangfuxinye + mesalazine (95% CI)
Adverse effects rate (important outcome)											
531 (7 studies) 4 weeks	Serious[13]	No serious inconsistency	No serious indirectness	Serious[14]	Undetected[3]	⊕⊕⊖⊖ *LOW*[3,13,14] due to risk of bias, imprecision	10/262 (3.8%)	17/269 (6.3%)	*RR 1.58* (0.77 to 3.24)	*Study population* *38 per 1000*	*22 more per 1000* (from 9 fewer to 85 more)
										Moderate *44 per 1000*	*26 more per 1000* (from 10 fewer to 99 more)

[1]Only 2 studies used random number table to generate random sequence, whereas the 3 remaining trials just reported "randomly assigned" but no mention was made of sequence. Details on how allocation was concealed were unclear in these studies. [2]The 95% CI excluded a relative risk of 1.0 and the sample size ($n = 360$) met the optimal information size (OIS) criterion, which was calculated approximately as 114. [3]It was impossible to check publication bias because of limited number of trials for this outcome. [4]Only 2 studies used random number table to generate random sequence, whereas the 14 remaining trials just reported "randomly assigned" but no mention was made of sequence. Details on how allocation was concealed were unclear in these studies. [5]The 95% CI excluded a relative risk of 1.0 and the sample size ($n = 1236$) met the optimal information size (OIS) criterion, which was calculated as 176. [6]All of the 5 trials just reported "randomly assigned" but no mention was made of sequence. Details on how allocation was concealed were unclear in these studies. [7]The 95% CI excluded a relative risk of 1.0 and the sample size ($n = 326$) met the optimal information size (OIS) criterion, which was calculated approximately as 114. [8]All of the 4 trials just reported "randomly assigned" but no mention was made of sequence. Details on how allocation was concealed were unclear in these studies. [9]Inconsistencies were found among the 4 studies in the pooled results with a considerable heterogeneity ($I^2 = 68\%$, $P < 0.05$). [10]The 95% CI included a relative risk of 1.0 and the sample size ($n = 269$) failed to meet the optimal information size (OIS) criterion, which was calculated approximately as 290. [11]This study just reported "randomly assigned" but no mention was made of sequence. Details on how allocation was concealed were unclear in these studies. [12]Sample sizes and number of events ($n = 19$) were far less than the number of patients generated by a conventional sample size ($n = 300$) calculation for a single adequately powered trial, and the change of our confidence for this outcome was very serious, thus downgrading.[13]Only 2 studies used random number table to generate random sequence, whereas the 5 remaining trials just reported "randomly assigned" but no mention was made of sequence. Details on how allocation was concealed were unclear in these studies. [14]The 95% CI included a relative risk of 1.0 and the sample size ($n = 531$) failed to meet the optimal information size (OIS) criterion, which was calculated approximately as 1204.

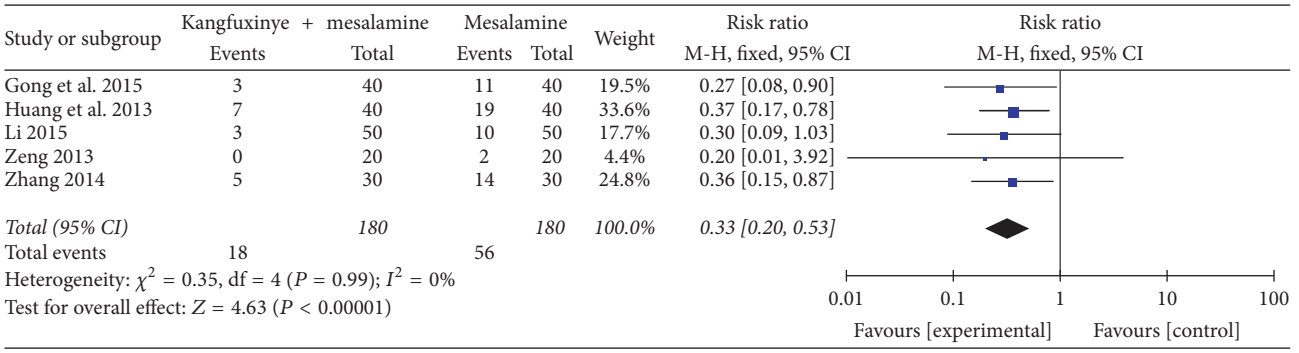


Figure 2: Efficacy of Kangfuxinye combined with mesalamine versus mesalamine on recurrence rate.

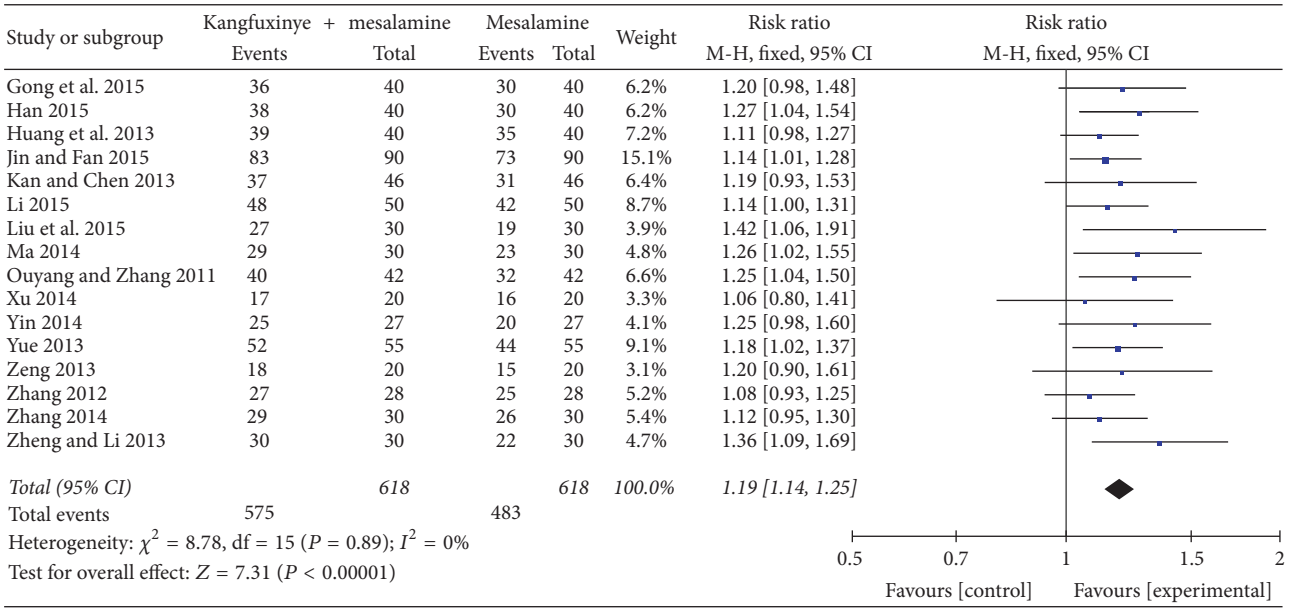


Figure 3: Efficacy of Kangfuxinye combined with mesalamine versus mesalamine on response rate.

outcome measure was based on both physician's assessment about the patients' general conditions and the patients' feeling. The meta-analysis indicated that no favourable effects of Kangfuxinye combined with mesalamine compared with mesalamine alone were observed (fixed-effect model, RR = 1.12, 95% CI = 0.96 to 1.30, P = 0.15) with moderate heterogeneity (I^2 = 68%, P = 0.02) (Figure 5). A GRADE approach indicated that the quality of evidence supporting this outcome was low due to the risk of bias, imprecision, and inconsistency (Table 4).

3.4.3. Time of Remission. One trial [37] involving 19 participants provided the time of remission, but it failed to present any benefit of Kangfuxinye in terms of shortening time of remission significantly (MD = −5.99, 95% CI: −14.15, 2.17, P = 0.15) (Figure 6). A GRADE analysis indicated that the quality of evidence supporting this outcome was very low due to high risk of bias and imprecision (Table 4).

3.5. Safety Evaluation. Of the 19 RCTs, 7 trials failed to report anything about adverse events, and the other 12 RCTs [24, 26, 28, 29, 31–34, 38, 39, 41, 42] reported adverse events rate. Five trials of those [33, 34, 38, 39, 42] reported no adverse events, while at least 1 adverse event was reported in the other 7 trials [24, 26, 28, 29, 31, 32, 41] which included 451 patients that were taken to explore the safety of Kangfuxinye combined with the mesalamine. The meta-analysis showed no difference in Kangfuxinye combined with mesalamine against mesalamine alone (fixed-effect model, RR = 1.58, 95% CI = 0.77 to 3.24, P = 0.21) without heterogeneity (I^2 = 0%, P = 0.74) (Figure 7). A GRADE approach indicated that the quality of evidence supporting this outcome was low due to risk of bias and imprecision (Table 4).

3.6. Publication Bias. Although an asymmetric funnel plot on the response rate was observed, the Egger et al. [48] test failed to identify any publication bias (P = 0.817) (Figure 8).

Study or subgroup	Kangfuxinye + mesalamine Events	Total	Mesalamine Events	Total	Weight	Risk ratio M-H, fixed, 95% CI
Han 2015	18	20	14	20	12.4%	1.29 [0.93, 1.77]
Liu et al. 2015	28	30	18	30	15.9%	1.56 [1.14, 2.12]
Ouyang and Zhang 2011	38	42	27	42	23.9%	1.41 [1.10, 1.80]
Tan et al. 2014	31	35	26	35	23.0%	1.19 [0.95, 1.50]
Wang 2013	32	36	28	36	24.8%	1.14 [0.93, 1.41]
Total (95% CI)		*163*		*163*	*100.0%*	*1.30 [1.16, 1.46]*
Total events	147		113			

Heterogeneity: $\chi^2 = 3.73$, df = 4 ($P = 0.44$); $I^2 = 0\%$

Test for overall effect: $Z = 4.51$ ($P < 0.00001$)

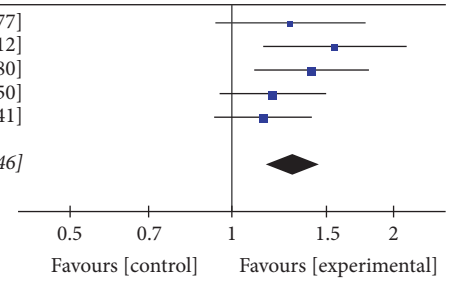


Figure 4: Efficacy of Kangfuxinye combined with mesalamine versus mesalamine on inflammation reduction rate.

Study or subgroup	Kangfuxinye + mesalamine Events	Total	Mesalamine Events	Total	Weight	Risk ratio M-H, random, 95% CI
Liu et al. 2015	27	30	19	30	16.0%	1.42 [1.06, 1.91]
Ouyang and Ran 2014	34	34	32	33	35.8%	1.03 [0.95, 1.12]
Tan et al. 2014	30	35	25	35	19.5%	1.20 [0.94, 1.54]
Wang 2013	33	36	32	36	28.7%	1.03 [0.89, 1.20]
Total (95% CI)		*135*		*134*	*100.0%*	*1.12 [0.96, 1.30]*
Total events	124		108			

Heterogeneity: $\tau^2 = 0.02$; $\chi^2 = 9.41$, df = 3 ($P = 0.02$); $I^2 = 68\%$

Test for overall effect: $Z = 1.42$ ($P = 0.15$)

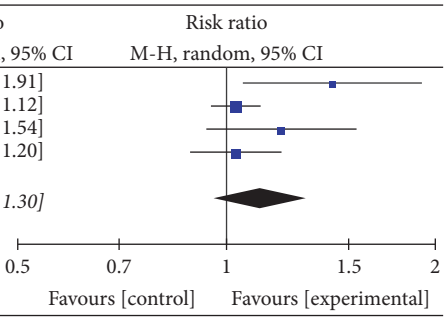


Figure 5: Efficacy of Kangfuxinye combined with mesalamine versus mesalamine on symptom remission rate.

Study or subgroup	Kangfuxinye + mesalamine Mean	SD	Total	Mesalamine Mean	SD	Total	Weight	Mean difference IV, fixed, 95% CI
Yin 2014	14.53	6.65	11	20.52	10.32	8	100.0%	−5.99 [−14.15, 2.17]
Total (95% CI)			*11*			*8*	*100.0%*	*−5.99 [−14.15, 2.17]*

Heterogeneity: not applicable

Test for overall effect: $Z = 1.44$ ($P = 0.15$)

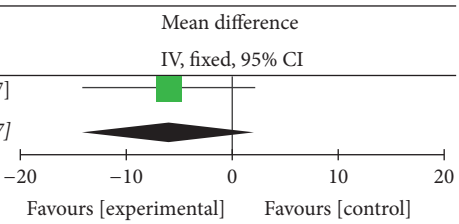


Figure 6: Efficacy of Kangfuxinye combined with mesalamine versus mesalamine on time of remission.

Study or subgroup	Kangfuxinye + mesalamine Events	Total	Mesalamine Events	Total	Weight	Risk ratio M-H, fixed, 95% CI
Gong et al. 2015	5	40	3	40	25.9%	1.67 [0.43, 6.51]
Huang et al. 2013	3	40	0	40	4.3%	7.00 [0.37, 131.28]
Kan and Chen 2013	3	46	2	46	17.3%	1.50 [0.26, 8.56]
Li 2015	2	50	3	50	25.9%	0.67 [0.12, 3.82]
Ma 2014	1	29	0	23	4.8%	2.40 [0.10, 56.30]
Ouyang and Ran 2014	1	34	2	33	17.5%	0.49 [0.05, 5.10]
Zhang 2014	2	30	0	30	4.3%	5.00 [0.25, 99.95]
Total (95% CI)		*269*		*262*	*100.0%*	*1.58 [0.77, 3.24]*
Total events	17		10			

Heterogeneity: $\chi^2 = 3.54$, df = 6 ($P = 0.74$); $I^2 = 0\%$

Test for overall effect: $Z = 1.25$ ($P = 0.21$)

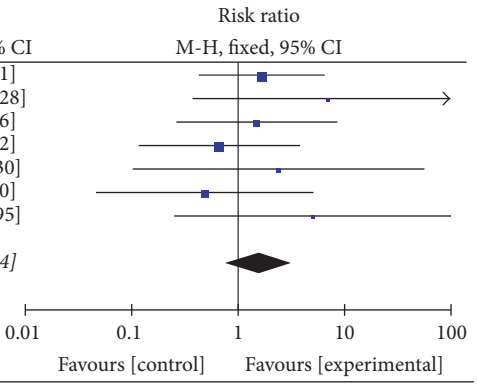


Figure 7: Efficacy of Kangfuxinye combined with mesalamine versus mesalamine on adverse events rates.

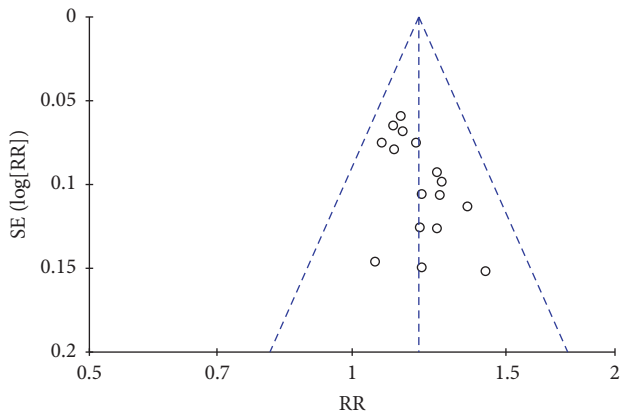


Figure 8: Funnel plot analysis on response rate of the 16 trials comparing Kangfuxinye combined with mesalamine versus mesalamine.

4. Discussion

19 RCTs involving 1685 patients were identified for this study. The results in our study showed that, compared to mesalamine alone, Kangfuxinye enema combined with mesalamine appeared to be more effective either in reducing recurrence rate or in improving response rate and the inflammation reduction rate. With regard to the symptom remission rate, time of remission, and adverse events rate, no significant benefits were observed. A GRADE approach indicated that most of evidences were rated as moderate, low, or very low quality. Compared with the outcome measured in previous systematic reviews, our review rated rank of relative outcomes according to clinical importance. What is more, the recurrence rate ranked as first of those outcomes due to high relapse rate of UC, and the following in descending order was response rate, inflammation reduction rate, symptom remission rate, and time of remission accordingly in the review.

Furthermore, the quality of evidence on the 6 preset outcomes was rated with the GRADE approach. The evidence of each outcome was downgraded one or two levels due to high risk of bias (poor reporting about randomization and allocation concealment), inconsistency, and imprecision. Five of the 6 outcomes were not downgraded in terms of inconsistency and the remission rate of symptoms was downgraded, which may be explained by heterogeneous patients' characteristics, disease cognition, and susceptibility to adverse events. Regarding imprecision, the OIS referred to the number of participants estimated by a sample size calculation for a single adequately powered trial [50]. If the total number of participants of a meta-analysis is lower than the OIS criterion, the quality of evidence should be downgraded because of imprecision [51]. In this study, the 95% CIs of the outcomes of adverse effects rate and symptom remission rate included a relative risk of 1.0 [51]; meanwhile the total number of participants for both outcomes (n = 561 and 269, resp.) of the meta-analysis did not exceed conventional sample size (n = 1204 and 290) calculation for a single adequately powered trial; therefore it was more likely to support downgrading the evidence quality due to imprecision. In addition, as the sample sizes (n = 19) of the time of remission were far less than the OIS (n = 300), our confidence in this outcome downgraded two levels. Because there were no significant differences either in baseline characteristics or in the outcomes measured in the included studies, the indirectness was considered as not serious; consequently none of these outcomes was downgraded. Potential publication bias was detected concerning the outcome of inflammation reduction rate through visual inspection. Therefore, the quality of evidence on this outcome was downgraded.

Overall, the quality of evidence with respect to the 6 critical or important outcomes was graded from moderate to very low, and limited data and insufficient follow-up time of long-term effects were more likely to warrant a weak recommendation of Kangfuxinye combined with mesalamine for treating UC patients. Kangfuxinye is crudely extracted by ethanol from dried *P. americana* whole body and has been approved by the China Food and Drug Administration (CFDA) (Z51021834). The main chemical compositions of Kangfuxinye are amino acids, small molecular peptides, and nucleotides. The present study indicated that *P. americana* extract can increase the levels of prostaglandin E2 (PGE2) [52]. And PGE2 can inhibit acid secretion and increase mucosal blood flow, both of which contribute to the repair of gastrointestinal mucosa [53]. Moreover, it also inhibits the release of inflammatory mediators in the gastric mucosa and inhibits neutrophils, monocytes, and macrophages at inflammatory sites [54]. Therefore, *P. americana* has a good effect on the gastrointestinal mucosal repairing and anti-inflammatory. A recent study [55] showed that the abstracts enema could accelerate the healing process in dinitrochlorobenzene (DNCB) and acetic acid- (AA-) induced ulcerative colitis rat, whose symptoms and histological features were similar to those of human UC. Moreover, the mechanism was also confirmed that the abstract of *P. americana* was able to encourage fibroblasts proliferation and collagen synthesis in in vitro fibroblast cell model, NIH 3T3 [55]. And a multitude of clinical researches have reported the positive effect of Kangfuxinye. One previous systematic review [21] concerning the clinical application of Kangfuxinye combined with mesalamine in treating UC patients has found that Kangfuxinye could significantly improve the response rate of the UC. However, with only 11 studies retrieved from the Chinese databases, only 2 outcomes (overall response rate and inflammation reduction rate) were taken to perform the pooled analysis, and the adverse events were not pooled due to unavailable data. Moreover, some of the included studies mixed with other interventions in the combination of mesalamine and Kangfuxinye enema.

In our study, a comprehensive literature search was conducted in 7 electronic databases, and, gray literature databases and references lists were taken to identify relevant studies. We also developed explicit eligibility criteria using PICOS (Participants, Intervention, Comparison, Outcome, Study design) format. Only those that compared Kangfuxinye enema combined with mesalamine against mesalamine alone were included. In addition, we graded 6 critical or important

outcomes according to their clinical importance to grade the quality of evidence by GRADE approach and the recurrence rate was taken as the most critical outcome used to explore the long-term effect of Kangfuxinye enema. Furthermore, we explored the safety of Kangfuxinye enema in terms of adverse events rate. By the way, as we searched relevant databases from the inception through March 31, 2016, the conclusion in our review may be recognized more and up to date, comprehensive, and robust. To the best of our knowledge, this is the first systematic review to grade the quality of evidence and then to generate recommendation regarding the use of Kangfuxinye in UC patients. Currently, rating an overall body of evidence by the GRADE approach is becoming an important and recommended explicit step in evidence synthesis initiatives [56]. With this approach, the details of potential limitations, including risk of bias, result inconsistency, indirectness imprecision, and publication bias are scrutinized for every outcome. And the approach provides us with a structured and transparent way to use this evidence for making a recommendation or decision, particularly for the low or unclear quality of evidence [56]. Therefore, it becomes one of the strengths in our study.

Nevertheless, several limitations should be specially addressed before the acceptance of the findings. Firstly, selection bias may occur in the methodological designs of included studies due to the inadequate reporting, although the review processes were appraised rigorously by 2 experienced and independent authors. Secondly, only two trials [24, 26] using a random method divided the groups, and the remaining 17 trials [25, 27–42] reported "randomly allocating" participants but the method of randomization was not described. Thirdly, none of the included trials reported allocation concealment, and whether a blinding method was used or not within 19 trials remains unclear, leading to the increase in risk of selection or performance bias. Last but not least, all included studies were conducted in China and were published in Chinese journals. Although the funnel plot and Egger's regression test failed to detect any publication biases, we could not rule out publication bias absolutely. As studies with statistically significant results are more likely to be published compared to those with null results [57], which seems more common in studies reported in Chinese and other Asian language [58, 59], the pooled RR reported in this study may be exaggerated compared to the true value. It is an important threat to the validity of systematic reviews and is difficult to combat except through the registration of all RCTs. In addition, in most studies, the effect of Kangfuxinye enema would be reduced without full contact with the ulcer on account of the fact that enema position of the patients did not vary according to the ulcer locations in the colon. We also noted that the participants of all the trials were all Chinese and whether it is still effective and could be applied to patients outside of China still needs to be further investigated.

5. Conclusions

Kangfuxinye enema addition to mesalamine may be effective and safe for UC patients. As the GRADE approach indicated very low to moderate quality of the evidence and lack of information about patients' preference, we suggest a weak recommendation for Kangfuxinye. Considering that all identified studies were of low quality and all were carried out in China, further rigorously designed and large-scale RCTs outside of China are warranted to improve the generalizability and applicability of this study results. And further GRADE approaches are also needed for grading quality of evidence regarding Kangfuxinye in combination with other additional or alternative medicine for UC patients.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Guarantor of the article is De-ying Kang. De-ying Kang conceived and designed the review. Peng-wei Ren, Wen-jie Yang, Jing-yan Shan, and Dan-dan Wang conducted literature searches, selected studies, assessed risk of bias, and extracted data. Peng-wei Ren, Wen-jie Yang, and Dan-dan Wang carried out analysis, applied GRADE, and interpreted results. Peng-wei Ren, De-ying Kang, and Qi Hong drafted the manuscript. All authors approved the final version of the manuscript.

Acknowledgments

The study was supported by Nonprofit Research Project from State Administration of Traditional Chinese Medicine of China, Grant no. 201207005.

References

[1] A. C. Ford, P. Moayyedi, S. B. Hanauer, and J. B. Kirsner, "Ulcerative colitis," *BMJ*, vol. 346, article f432, 2013.

[2] "Ulcerative Colitis," NIDDK, September 2014, https://www.niddk.nih.gov.

[3] S. Danese and C. Fiocchi, "Ulcerative colitis," *The New England Journal of Medicine*, vol. 365, no. 18, pp. 1713–1725, 2011.

[4] A. Ponder and M. D. Long, "A clinical review of recent findings in the epidemiology of inflammatory bowel disease," *Clinical Epidemiology*, vol. 5, no. 1, pp. 237–247, 2013.

[5] N. A. Molodecky, I. S. Soon, D. M. Rabi et al., "Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review," *Gastroenterology*, vol. 142, no. 1, pp. 46–54, e30, 2012.

[6] D. C. Baumgart and W. J. Sandborn, "Inflammatory bowel disease: clinical aspects and established and evolving therapies," *The Lancet*, vol. 369, no. 9573, pp. 1641–1657, 2007.

[7] C. B. Appleyard, G. Hernández, and C. F. Ríos-Bedoya, "Basic epidemiology of inflammatory bowel disease in Puerto Rico," *Inflammatory Bowel Diseases*, vol. 10, no. 2, pp. 106–111, 2004.

[8] S. V. Kane, "Systematic review: adherence issues in the treatment of ulcerative colitis," *Alimentary Pharmacology and Therapeutics*, vol. 23, no. 5, pp. 577–585, 2006.

[9] Z. Zhang and H. Kennedy, "Ulcerative colitis: Current medical therapy and strategies for improving medication adherence,"

European Journal of Gastroenterology and Hepatology, vol. 21, no. 1, pp. 1–8, 2009.

[10] T. M. Letter, *Treatment Guidelines*, vol. 7 of *Drugs for Inflammatory Bowel Disease*, The Medical Letter, New York, NY, USA, 2009.

[11] J. Lachaine, L. Yen, C. Beauchemin, and P. Hodgkins, "Medication adherence and persistence in the treatment of Canadian ulcerative colitis patients: analyses with the RAMQ database," *BMC Gastroenterology*, vol. 13, no. 1, article 23, 2013.

[12] S. C. Truelove and L. J. Witts, "Cortisone in ulcerative colitis; final report on a therapeutic trial," *British Medical Journal*, vol. 2, no. 4947, pp. 1041–1048, 1955.

[13] Y. Leung, R. Panaccione, B. Hemmelgarn, and J. Jones, "Exposing the weaknesses: a systematic review of azathioprine efficacy in ulcerative colitis," *Digestive Diseases and Sciences*, vol. 53, no. 6, pp. 1455–1461, 2008.

[14] P. Rutgeerts, W. J. Sandborn, B. G. Feagan et al., "Infliximab for induction and maintenance therapy for ulcerative colitis," *The New England Journal of Medicine*, vol. 353, no. 23, pp. 2462–2476, 2005.

[15] L. Langmead and D. S. Rampton, "Review article: complementary and alternative therapies for inflammatory bowel disease," *Alimentary Pharmacology and Therapeutics*, vol. 23, no. 3, pp. 341–349, 2006.

[16] J. Langhorst, H. Wulfert, R. Lauche et al., "Systematic review of complementary and alternative medicine treatments in inflammatory bowel diseases," *Journal of Crohn's & Colitis*, vol. 9, no. 1, pp. 86–106, 2015.

[17] M. Bensoussan, N. Jovenin, B. Garcia et al., "Complementary and alternative medicine use by patients with inflammatory bowel disease: results from a postal survey," *Gastroenterologie Clinique et Biologique*, vol. 30, no. 1, pp. 14–23, 2006.

[18] X. Xiao, C. Luo, S. Wang et al., "Experimental study on the analgesic and anti-inflammation of periplanetaamericana extract," in *The Insects Academic Conference the Fourth National Insects Resource Conference of the Three Provinces in central China (including Hunan, Hubei and Henan)*, pp. 238–241, Shaoshan Hunan, China, 2005.

[19] H. Chen, X. Lian, L. Jiang, and M. Zhong, "Clinical effect of Kangfuxinliqud in treatment of recurrent aphthous ulcer," *China Practical Medicine*, vol. 3, pp. 24-25, 2011.

[20] X. H. Liu, A. Bravo-Cuellar, I. Florentin, S. Orbach-Arbouys, G. Mathe, and J. Breard, "In vivo immunopharmacological properties of the traditional chinese medicine Kang Fu-Xin (KFX)," *International Journal of Immunotherapy*, vol. 5, no. 1, pp. 25–33, 1989.

[21] B. Niu, S. Ye, B. Chen, M. Zhang, D. He, and X. Wang, "Mesalamine combined with Kangfuxin liquid in the treatment of ulcerative colitis: a Meta-analysis," *Chinese Traditional Patent Medicine*, no. 11, pp. 2275–2279, 2014.

[22] H. J. Schünemann, J. Brożek, G. H. Guyatt et al., *GRADE Handbook for Grading Quality of Evidence and Strength of Recommendations*, The GRADE Working Group, 2016, http://www.guidelinedevelopment.org/handbook.

[23] G. Guyatt, A. D. Oxman, E. A. Akl et al., "GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings table," *Journal of Clinical Epidemiology*, vol. 64, no. 4, pp. 383–394, 2011.

[24] J. Gong, C. Yan, and L. Zhang, "Curative efficacy of mesalazine in combination with Kangfuxin solution enema in treatmentof ulcerative colitis and its effects on coagulation parameters," *Journal of Armed Police Medicine*, no. 5, pp. 440–443, 2015.

[25] J. Han, "A clinical study on treating ulcerative colitis by lavation of Kangfuxin liquid," *Journal of Medical Informatics*, vol. 28, no. 8, article 236, 2015.

[26] D. Huang, C. Lu, and Z. He, "The combination treatment of mesalamine and Kangfuxinye for the elders ulcerative colitis and its influence on inflammatory cytokines coagulation factors," *Chinese Journal of Gerontology*, no. 15, pp. 3769-3770, 2013.

[27] J. Jin and Y. Fan, "Clinical research on ulcerative colitis treated with enema of KangfuXinye combined with mesalamine granules," *Henan Traditional Chinese Medicine*, no. 11, pp. 2725–2727, 2015.

[28] C. Kan and J. Chen, "Clinical observation of Kangfuxin Liquid combined with gentamicin on treatment of ulcerative colitis," *Journal of Modern Drugs and Clinical*, no. 2, pp. 217–219, 2013.

[29] C. Li, "Observation on curative effect of ulcerative colitis treated with combination of Chinese and Western drugs," *Electronic Journal of Clinical Medicine Literature*, no. 27, pp. 5730-5731, 2015.

[30] Y. Liu, M. Chen, Z. Lu, and R. Liu, "Effect observation of Kangfuxin solution for retention enema combined with oral administration of mesalazine in the treatment of ulcerative colitis," *Journal of Chinese Contemporary Medicine*, vol. 22, no. 2, pp. 85–87, 2015.

[31] J. Ma, "Mesalazinecombinated with Kangfuxin for ulcerative colitis," *Chinese Journal of Geriatric Care*, vol. 12, no. 1, pp. 37-38, 2014.

[32] W. Ouyang and H. Ran, "Mesalazinecombinated with Kangfuxin for ulcerative colitis," *Chinese Journal of Integrated Traditional and Western Medicine on Digestion*, no. 7, pp. 400-401, 2014.

[33] Y. Ouyang and Y. Zhang, "Kangfuxinye enema combined with mesalamine treated for ulcerative colitis," *Jilin Medical Journal*, vol. 32, no. 16, pp. 3236-3237, 2011.

[34] Z. Tan, S. Zeng, and D. Wang, "Kangfuxinye enema combined with mesalamine treated for ulcerative colitis," *Guide of China Medicine*, vol. 12, no. 20, pp. 262-263, 2014.

[35] S. Wang, "Kangfuxinye enema combined with mesalamine treated for ulcerative colitis," *China Health Care Nutrition*, no. 11, article 463, 2013.

[36] N. Xu, "Kangfuxinye enema combined with mesalamine treated for 40 ulcerative colitis patients," *China Health Care Nutrition*, vol. 24, no. 7, article 4235, 2014.

[37] C. Yin, "The effect foKangfuxinye enema combined with mesalamine treated for ulcerative colitis," *Medical Information*, no. 28, article 266, 2014.

[38] J. Yue, "Observation the effect foKangfuxinye enema combined with mesalamine treated for ulcerative colitis," *Medical Information*, no. 16, article 184, 2013.

[39] Q. Zeng, "Observation the effect foKangfuxinye enema combined with mesalamine treated for ulcerative colitis," *Chinese and Foreign Medical Research*, vol. 11, no. 4, pp. 52-53, 2013.

[40] C. Zhang, "Observation the effect foKangfuxinye enema treated for ulcerative in short time," *Clinical Medical Engineering*, vol. 19, no. 3, pp. 404-405, 2012.

[41] L. Zhang, "The effect foKangfuxinye enema combined with mesalamine treated for ulcerative colitis," *Journal of Clinical Rational Use of Medicines*, vol. 7, no. 30, pp. 27-28, 2014.

[42] P. Zheng and C. Li, "A clinical trial of Kangfuxinye enema combined with mesalamine treated for ulcerative colitis," *Medical Information*, no. 29, pp. 193-194, 2013.

[43] J. Higgins and G. Sally, *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011)*, The Cochrane Collaboration, 2011.

[44] D. Moher, A. Liberati, and J. Tetzlaff, "Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement," *Journal of Clinical Epidemiology*, vol. 62, no. 10, pp. 1006–1012, 2009.

[45] G. H. Guyatt, A. D. Oxman, G. E. Vist et al., "GRADE: an emerging consensus on rating quality of evidence and strength of recommendations," *British Medical Journal*, vol. 336, no. 7650, pp. 924–926, 2008.

[46] G. R. Norman, "Issues in the use of change scores in randomized trials," *Journal of Clinical Epidemiology*, vol. 42, no. 11, pp. 1097–1105, 1989.

[47] M. Borenstein, LV. Hedges, J. P. T. Higgins, and H. R. Rothstein, *Meta-Regression*, John Wiley & Sons, Ltd, 2009.

[48] M. Egger, G. D. Smith, M. Schneider, and C. Minder, "Bias in meta-analysis detected by a simple, graphical test," *British Medical Journal*, vol. 315, no. 7109, pp. 629–634, 1997.

[49] J. Brożek, A. Nowak, P. Kunstman et al., "GRADEpro Guideline Development Tool (G2DT)," http://www.guideline-development.org.

[50] J. M. Pogue and S. Yusuf, "Cumulating evidence from randomized trials: Utilizing sequential monitoring boundaries for cumulative meta-analysis," *Controlled Clinical Trials*, vol. 18, no. 6, pp. 580–593, 1997.

[51] G. H. Guyatt, A. D. Oxman, R. Kunz et al., "GRADE guidelines: 6. Rating the quality of evidence-imprecision," *Journal of Clinical Epidemiology*, vol. 64, no. 12, pp. 1283–1293, 2011.

[52] H. W. Zhang, L. Y. Wei, Z. Y. Zhang, S. Z. Liu, and J. Zhang, "Preventive effect of periplaneta americana extract on stress ulcer bleeding in patients with acute lung injury or acute respiratory distress syndrome," *Chinese General Practice*, vol. 15, pp. 3878-3879, 2012.

[53] Y. Li, H. W. Wu, and X. J. Dong, "Mechanism of kangfuxin solution on gastric ulcer," *Chinese Remedies & Clinics*, vol. 8, pp. 495-496, 2008.

[54] X. Q. Xiao, S. P. Wang, C. Luo, S. R. Xu, S. J. Wu, and F. Zhong, "Preliminary study of periplaneta americana extract on gastric ulcer," *Journal of Tropical Medicine*, vol. 6, pp. 1274–1276, 2006.

[55] N. Li, R. Lu, Y. Yu et al., "Protective effect of Periplanetaamericana extract in ulcerative colitis rats induced by dinitrochlorobenzene and acetic acid," *Pharmaceutical Biology*, vol. 54, no. 11, pp. 2560–2567, 2016.

[56] P. Alonso-Coello, H. J. Schünemann, J. Moberg et al., "GRADE Evidence to Decision (EtD) frameworks: a systematic and transparent approach to making well informed healthcare choices. 1: introduction," *BMJ*, vol. 353, article i2016, 2016.

[57] K. Dickersin, S. Chan, T. C. Chalmersx, H. S. Sacks, and H. Smith Jr., "Publication bias and clinical trials," *Controlled Clinical Trials*, vol. 8, no. 4, pp. 343–353, 1987.

[58] J. Liu, E. Manheimer, Y. Shi, and C. Gluud, "Chinese herbal medicine for severe acute respiratory syndrome: a systematic review and meta-analysis," *Journal of Alternative and Complementary Medicine*, vol. 10, no. 6, pp. 1041–1051, 2004.

[59] P. Jüni, F. Holenstein, J. Sterne, C. Bartlett, and M. Egger, "Direction and impact of language bias in meta-analyses of controlled trials: empirical study," *International Journal of Epidemiology*, vol. 31, no. 1, pp. 115–123, 2002.

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2017, Article ID 8547257, 11 pages
https://doi.org/10.1155/2017/8547257

Research Article

The Anti-Inflammatory Effect of Guchangzhixie-Pill by Reducing Colonic EC Cell Hyperplasia and Serotonin Availability in an Ulcerative Colitis Rat Model

Yong Yang,[1] Xianwei Zhu,[1,2] Yifei Qin,[1] Guihai Chen,[1] Jing Zhou,[1] Lu Li,[2] Jianjun Guan,[1] Li Ma,[1] Yanyan Xue,[1] and Chenhui Li[1]

[1]*College of Basic Medicine, Shaanxi University of Chinese Medicine, Xi'an-Xianyang New Economic Zone, Xianyang, Shaanxi 712046, China*
[2]*Innovation Research Centre of Acupuncture Combined with Medicine, Shaanxi University of Chinese Medicine, Xi'an-Xianyang New Economic Zone, Shaanxi 712046, China*

Correspondence should be addressed to Xianwei Zhu; jater0228@outlook.com

Received 18 December 2016; Revised 5 April 2017; Accepted 5 June 2017; Published 6 August 2017

Academic Editor: Sérgio F. De Andrade

Ulcerative colitis (UC) is one of the major types of inflammatory bowel diseases (IBD). Abnormal colonic enterochromaffin (EC) cell hyperplasia and serotonin availability have been described in UC. Guchangzhixie-pill (GCZX-pill), a Chinese herbal formula composed of six herbs, is modified based on a traditional formula (Jiechangyan-pill) for inflammatory and ulcerative gastrointestinal disorders. This study aims to investigate the anti-inflammatory effect and the underlying mechanisms of GCZX-pill on trinitrobenzene sulfonic acid- (TNBS-) induced UC in rats. After orally administrating a GCZX-pill to UC rats for 14 days, the results of the inflammation evaluation, such as disease activity index (DAI), macroscopic score (MS), myeloperoxidase (MPO) activity, and other methods, suggested that the GCZX-pill showed remarkable anti-inflammatory results in UC rats. In addition, the abnormal EC cell numbers, colonic tryptophan hydroxylase (TPH) expression, and serotonin (5-HT) contents in TNBS-induced UC rats were significantly reduced by the GCZX-pill. This data demonstrates that the GCZX-pill can attenuate the inflammation in UC rats and the anti-inflammatory effect of the GCZX-pill may be medicated by reducing colonic EC cell hyperplasia and 5-HT availability.

1. Introduction

Ulcerative colitis (UC) is one of the major types of inflammatory bowel diseases (IBD) that arises as a result of the interaction between environmental and genetic factors leading to immunological responses and inflammation in the intestine. It is characterized by inflammation of the mucosa of the intestinal tract, causing ulceration, edema, bleeding, and fluid and electrolyte loss [1, 2]. In recent years, progress has been made in understanding the pathogenesis of UC and in developing improved therapeutic approaches which include treatment with immune-modifying agents (mercaptopurine or azathioprine) or one of the anti-TNF agents earlier in the course of UC. Nevertheless, many side effects, which were caused by these therapeutic approaches, have been reported [3–6]. Conversely, in clinical practice, many Chinese herbal formulas as an alternative treatment modality for the treatment of UC acquired an observably therapeutic effect [7, 8].

Colonic changes in UC are characterized by ulcerative lesions accompanied by a prominent infiltrate of immune cells as well as alteration in serotonin (5-hydroxytryptamine; 5-HT) [9, 10]. 5-HT, as neurotransmitter and paracrine signalling molecule in gastrointestinal tract (GIT), not only plays important roles in initiating peristaltic, secretory, vasodilatory, vagal, and nociceptive reflexes, but can also activate the immune cells and regulate the GIT inflammation and intestinal pathophysiology as has been reported in past studies [11, 12]. 5-HT is synthesized and released from enterochromaffin (EC) cells and the tryptophan hydroxylase

(TPH) which in EC cells is the rate-limiting enzyme in the 5-HT synthesis process [13]. Moreover, colonic EC cell hyperplasia has been reported recently. The underlying mechanisms of EC cell hyperplasia in UC are unknown, but they are considered to have close correlation with $CD4^+$ T lymphocytes, especially the T_{h1}/T_{h2} balance [14, 15]. These findings indicated that the targeting effect of EC cells and 5-HT is a therapeutic strategy for UC.

Guchangzhixie-pill (GCZX-pill) is modified based on a traditional formula (Jiechangyan-pill) for diarrhea, abdominal pain, UC, and irritable bowel syndrome (IBS). It contains *Prunus mume*, *Zingiber oj-jicinale* Rosc., *Coptis chinensis* Franch., *Pericarpium Papaveris*, *Radix Aucklandiae*, and *Corydalis yanhusuo* [16–18]. Past studies indicated that GCZX-pill relieved symptoms in UC or IBS patients. In recent years, more and more clinicians have successfully applied GCZX-pill in inflammatory and ulcerative gastrointestinal disorder prevention and treatment and have received a satisfactory clinical outcome [19–22]. Nevertheless, the effect of GCZX-pill in UC requires further clinical evidence and definitive mechanisms of action.

In this study, an experimental UC rat model induced by trinitrobenzene sulfonic acid (TNBS) was used. After treatment of the TNBS-induced UC rat model with GCZX-pill, the changes of colonic EC cells number, TPH expression, 5-HT, and some cytokine productions were investigated to gain an understanding of the pharmacological mechanism of the GCZX-pill.

2. Materials and Methods

2.1. Materials. All the herbs in GCZX-pill were provided by the Affiliated Hospital of Shaanxi University of Chinese Medicine (Xianyang, Shaanxi, China) and extracted according to the standard methods recommended by the Chinese Pharmacopoeia (2010). In this study, *Prunus mume* was collected from Dujiangyan, Sichuan, China; *Zingiber oj-jicinale* Rosc. was collected from Hancheng, Shaanxi, China; *Coptis chinensis* Franch. was collected from Shizhu, Chongqing, China; *Pericarpium Papaveris* was collected from Nongken, Gansu, China; *Radix Aucklandiae* was collected from Dali, Yunnan, China; and *Corydalis yanhusuo* was collected from Chenggu, Shaanxi, China. Currently, these dried powdered herbs are being stored in the Innovation Research Centre of Acupuncture Combined with Medicine of Shaanxi University of Chinese Medicine. A total of 100 g of the herbs was mixed and macerated in water for 30 min, subsequently decocted for 60 min three times, and rinsed 10 times (v/w) with distilled water. Then, the final residues were filtered using microfilter, concentrated, and then made into dried powder. Approximately 12.4 g of powder was obtained. The concentration of berberine hydrochloride in the product was measured (7.901 mg/g) for quality standard research according to the Drug Standard of Ministry of Public Health of the People Republic of China (WS3-B-3870-98) [16–18].

2.2. Animals. Male Sprague-Dawley rats (weighing approximately 220 g) were housed in polycarbonate cages in isolators under a 12 h light/12 h dark cycle, with controlled temperature $21 \pm 3^{\circ}$C and humidity 60%. In addition, rats were fed ad libitum with standard food and water. All animal care and experimental procedures complied with the regulations of Shaanxi Administration Rule of Laboratory Animal (authorization number: #109228/2014). All experiments in this study were approved by the Laboratory Animal Ethics Committee at the Shaanxi University of Chinese Medicine.

2.3. Induction of Experimental UC Rat Model. UC rats were induced by the intracolonic administration with TNBS [23, 24]. After being fasted for 24 hours, the rats were deeply anesthetized with chloral hydrate (350 mg/kg, i.p.). Rats were given 0.9% saline solution or TNBS-solution (30 mg/kg, soluble in 50% ethanol) via PE90 tubing inserted to 8 cm from the anus. Then, the rats given 0.9% saline solution were divided into the control group and treated with 0.9% saline solution. The UC rats were randomly divided into 5 groups ($n = 12$) and treated with 0.9% saline solution (i.p., TNBS group), or para-chlorophenylalanine (pCPA, TPH inhibitor, 150 mg/kg/d in 0.9% saline solution, i.p., pCPA group), or gavage administration of GCZX-pill (group 4–6) at a dose of 2.4, 4.8, and 9.6 g/kg/d (dissolved in water) once a day for 14 days.

2.4. Macroscopic Evaluation of Inflammation. The macroscopic colonic inflammations in the TNBS-induced UC rats before and after treatment with pCPA or GCZX-pill were evaluated by the methods of disease activity Index (DAI), macroscopic score (MS), colon thickness, and colonic edema [25–29]. The protocols are briefly described below.

2.4.1. DAI. The DAIs of the rats were based on body weight loss, stool consistency, and rectal bleeding and scored blindly by three investigators. The scores were quantified as follows: stool consistency: 0 (normal), 1 (soft), and 2 (liquid); body weight loss: 0 (<5%), 1 (5–10%), 2 (10–15%), 3 (15–20%), 4 (20–25%), and 5 (>25%); and rectal bleeding: 0 (absent) and 1 (present).

2.4.2. MS. After treatment of pCPA or GCZX-pill, the rats were passively euthanized with carbon dioxide. The colon was visualized and rapidly excised in its entirety and placed in a Petri dish containing sterile saline solution. The colons were opened along the mesenteric line and immediately evaluated for the presence of colonic inflammation. MS was quantified by a blinded investigator. The criteria were quantified as follows: the presence of points of stenosis and hypertrophic zones (0, absent; 1, 1 stenosis; 2, 2 stenoses; 3, more than 2 stenoses), mucus (0, absent; 1, present), adhesions (0, absent; 1, 1 adhesion between colon and other intra-abdominal organs; 2, 2 adhesions; 3, more than 2 adhesions), intraluminal hemorrhage (0, absent; 1, present), erythema (0, absent; 1, area $< 1\,cm^2$; 2, area $> 1\,cm^2$), and ulceration and necrosis zones (0, absent; 1, area $< 1\,cm^2$; 2, area $> 1\,cm^2$).

2.4.3. Colon Thickness. After the quantitation of MS, the length and weight of colon were measured, while weight/length ratio was calculated to estimate colon thickness.

2.4.4. Colonic Edema. After the estimation of colon thickness, 1 cm long colon segments were cut and weighed

immediately as the wet weight (WW) of colon. Then, the colon segments were placed in an oven set to 60°C for 3 days and weighed as the dry weight (DW) of colon. The (WW − DW)/DW ratio was calculated to estimate colonic edema.

2.5. Histological Evaluation. Three colon segments from each rat were used for hematoxylin-eosin staining by random choice. Then, the colons were fixed in 4% buffered formaldehyde and embedded in paraffin. Sections were cut to a thickness of 5 μm and stained with hematoxylin-eosin. The sections were examined under a light microscope (Olympus CKX41 31PHP) by a person unaware of the treatment.

2.6. Colonic MPO Activity Assay. The colonic samples were homogenized and centrifuged (40,000g for 15 min at 4°C) for obtaining the supernatants. The supernatants were diluted (1 : 10) with distilled water. The diluted supernatants (100 μl) were mixed with a buffer solution containing o-dianisidine (0.167 mg/ml, 900 μl) and H_2O_2. Each assay was performed in duplicate and the rate of change in absorbance was measured spectrophotometrically at 470 nm. One unit is defined as the amount of MPO enzyme that can metabolize 1 μmol of H_2O_2 per min at 25°C. Data were normalized with edema values and expressed as U/g of dry weight tissue [30, 31].

2.7. Enzyme-Linked Immunosorbent Assay (ELISA). The content of 5-HT (MBS725497, MyBioSource, CA) and the cytokine levels of TNF-α (MBS2507393, MyBioSource, CA), IFN-γ (ab46107, Abcam, UK), and IL-10 (ab100764, Abcam, UK) in the colon tissue were assayed by ELISA. The samples were measured according to the manufacturer's protocol. Absorbance at 450 nm in each well was measured using a spectrophotometer [32].

2.8. EC Cell Counting. An evolutionary Fontana-Masson staining was used for EC cell counting [33]. Briefly, the colonic sections were deparaffinized and rehydrated for Fontana-Masson staining (ab150669, Abcam, UK). The sections were incubated in ammoniacal silver solution (1 h, 60°C), gold chloride solution (0.2%, 30 seconds), sodium thiosulfate solution (5%, 2 min, at room temperature), and nuclear fast red solution successively. At last, the sections were dehydrated with alcohol and mounted in synthetic resin. Five random fields at 200x magnifications were counted in each section by a researcher blinded to the treatment; the number of EC cells per mm^2 of mucosa was quantified using Image J NIH software.

2.9. Western Blot Analysis. The expression of colonic TPH was analyzed by western blot analysis. Briefly, the total protein of the colon was extracted and quantified. Then the samples containing 30 μg of protein were boiled for 5 min and subjected to SDS-PAGE electrophoresis and then transferred to PVDF membranes. The PVDF membranes were incubated in a blocking buffer and then incubated with anti-tryptophan hydroxylase antibody (ab46757, Abcam, UK) for 1 night at 4°C. Subsequently, incubation with secondary antibodies labeled alkaline phosphatase (W3960, Promega, USA). The immunoblots were detected by western blue and quantified using the Image J program [34].

2.10. Immunohistochemistry Staining for SERT. The colonic sections were deparaffinized and rehydrated for immunohistochemistry staining for SERT. After the sections were quenched in methanol: 30% H_2O_2 (9 : 1) for 10 min and microwaved for 10 min in citrate buffer (10 mM, pH 6.0), the colonic sections were blocked in 3% BSA and incubated with anti-Serotonin Transporter (SERT) antibody (S1001-25B, USBiological, US) at 1 : 800 dilution overnight at 4°C, respectively. Followed by incubation with 2nd antibody conjugated with biotin (SA1022, Boster, PRC) and streptavidin conjugated with POD for 2 hours respectively. The immunoblots were detected by diaminobenzidine (DAB) (AR1022, Boster, PRC). The sections were examined under light microscope by a person unaware of the treatment and analyzed using Image J NIH software.

2.11. Statistics. All data are presented as mean ± standard error of the mean (SEM). Statistical analysis was conducted using SPSS 15.0 Software (SPSS Inc., Chicago, IL, USA). The differences between groups were considered significant using a one-way analysis of variance (ANOVA). After testing for homogeneity of variance, data of EC cell counting, TPH, cytokines levels, 5-HT, and 5-HIAA in colon were compared using Bonferroni's posttest. Nonparametric Kruskal–Wallis analysis followed by Dunn's posttest was applied for statistical comparison of DAI and MS. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Macroscopic Evaluation of Inflammation before and after Being Treated with GCZX-Pill. As shown in Figure 1(a), the DAIs in UC rats (TNBS group, $P < 0.01$, $n = 12$) were significantly increased when compared to that of the control rats. After pCPA treatment, the DAIs in the rats of pCPA group were significantly reduced ($P < 0.01$, $n = 12$). After GCZX-pill treatment, the DAIs in the rats of GCZX-pill groups were also significantly reduced when compared to that of the UC rats ($P < 0.05$, $n = 12$).

MS was based on the presence of adhesions, points of stenosis, mucus, erythema, and ulcers in colon, and the results were shown in Figure 1(b). Consistent with the results from DAI, the MS in UC rats (TNBS group, $P < 0.01$, $n = 12$) was significantly increased when compared to that of the control rats. After pCPA treatment, the MS in the rats of pCPA group was significantly reduced ($P < 0.01$, $n = 12$). After GCZX-pill treatment, the MS in the rats of GCZX-pill groups was also significantly reduced when compared to that of the UC rats ($P < 0.05$, $n = 12$).

The results of colon thickness were shown in Figure 1(c) and the results of colonic edema were shown in Figure 1(d). Consistent with the results from DAI and MS, both of the colon thickness and colonic edema in UC rats ($P < 0.01$ in colon thickness; $P < 0.05$ in colonic edema, $n = 12$, Dunn's test) were significantly increased when compared to that of the control rats. After pCPA or GCZX-pill treatment, the

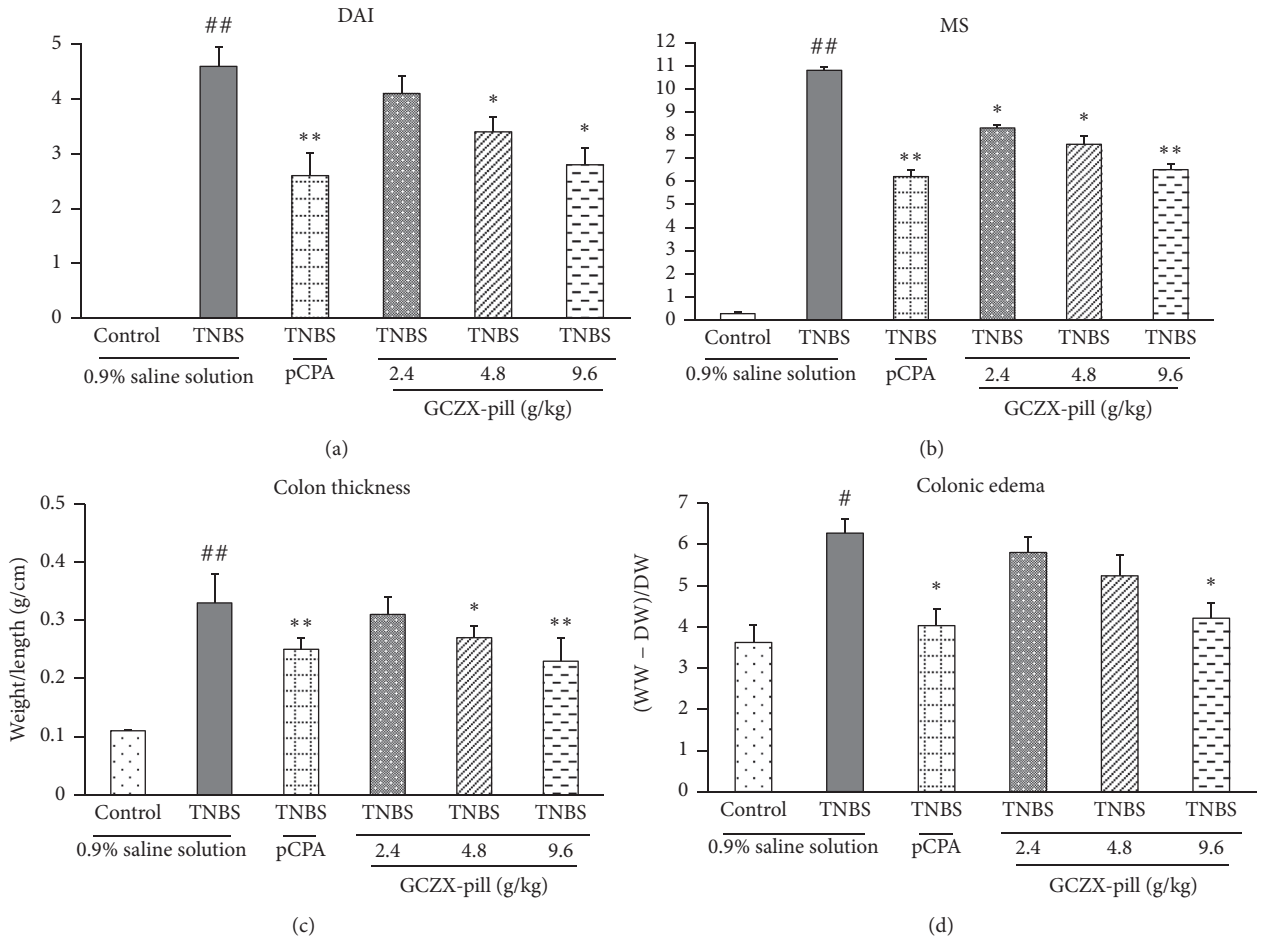


Figure 1: The macroscopic anti-inflammatory effects of GCZX-pill in TNBS-induced UC rats were evaluated by DAI (a), MS (b), colon thickness (c), and colonic edema (d). Data are shown as mean ± SEM ($n = 12$ per group). $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus the control rats; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus the UC rats; one-way ANOVA followed by Bonferroni's posttest; Kruskal–Wallis analysis followed by Dunn's posttest was applied for statistical comparison of DAI and MS.

colon thickness and colonic edema in the rats of pCPA and GCZX-pill groups were significantly reduced when compared to that of the UC rats ($P < 0.01$ in colon thickness; $P < 0.05$ in colonic edema, $n = 12$).

3.2. Histological Evaluation of Inflammation before and after Being Treated by GCZX-Pill. The results from the hematoxylin-eosin staining of colon sections were consistent with these alterations in macroscopic evaluation of inflammation changes (Figures 2(a)–2(d)). Relative to the colon section of the control rat (Figure 2(a)), the colon section of the UC rat (Figure 2(b)) showed that severe inflammation could be detected, including epithelial degeneration, inflammatory cell infiltration, and submucosal edema. After treatment with pCPA (Figure 2(c)) or GCZX-pill (Figure 2(d)), the colon sections from the rats of pCPA group or GCZX-pill group showed a marked reduction in the tissue disruption, mucosal ulcerations, and inflammatory cell infiltration.

3.3. Effects of GCZX-Pill on the MPO Activity in the Colon. MPO activity is an indicator of tissue neutrophil content (Figure 2(e)). The MPO activities in UC rats were significantly increased from 1.25 ± 0.31 U/mg to 3.18 ± 0.51 U/mg ($P < 0.01$, $n = 12$) when compared to that of the control rats. After pCPA treatment, the MPO activities in the rats of pCPA group were significantly reduced to 1.87 ± 0.43 U/mg ($P < 0.05$, $n = 12$). Also, the MPO activities were significantly reduced to 1.91 ± 0.44 U/mg ($P < 0.01$, $n = 12$) by treatment with GCZX-pill when compared to that of the UC rats.

3.4. Effects of GCZX-Pill on the Levels of Cytokines in the Colon. The results of the changes on the levels of cytokines indicated that the levels of cytokines in the colon were associated with the development of UC (Figure 3). The levels of TNF-α, IFN-γ, and IL-10 in the colon of UC rats (4.24 ± 0.37 pg/mg, 6.41 ± 0.27 pg/mg, and 2.21 ± 0.48 pg/mg for TNF-α, IFN-γ, and IL-10, $P < 0.01$, $n = 12$) were all significantly changed when compared to that of the control rats (1.15 ± 0.14 pg/mg, 3.92 ± 0.26 pg/mg, and 6.07 ± 0.56 pg/mg for TNF-α, IFN-γ, and IL-10). GCZX-pill treatment significantly reduced the levels of TNF-α and IFN-γ (2.06 ± 0.33 pg/mg, 3.47 ± 0.34 pg/mg for TNF-α and IFN-γ, $P < 0.01$, $n = 12$) and elevated the levels of IL-10 (3.87 ± 0.68 pg/mg, $P < 0.05$, $n = 12$) in the rats of high-dose GCZX-pill group. Similarly,

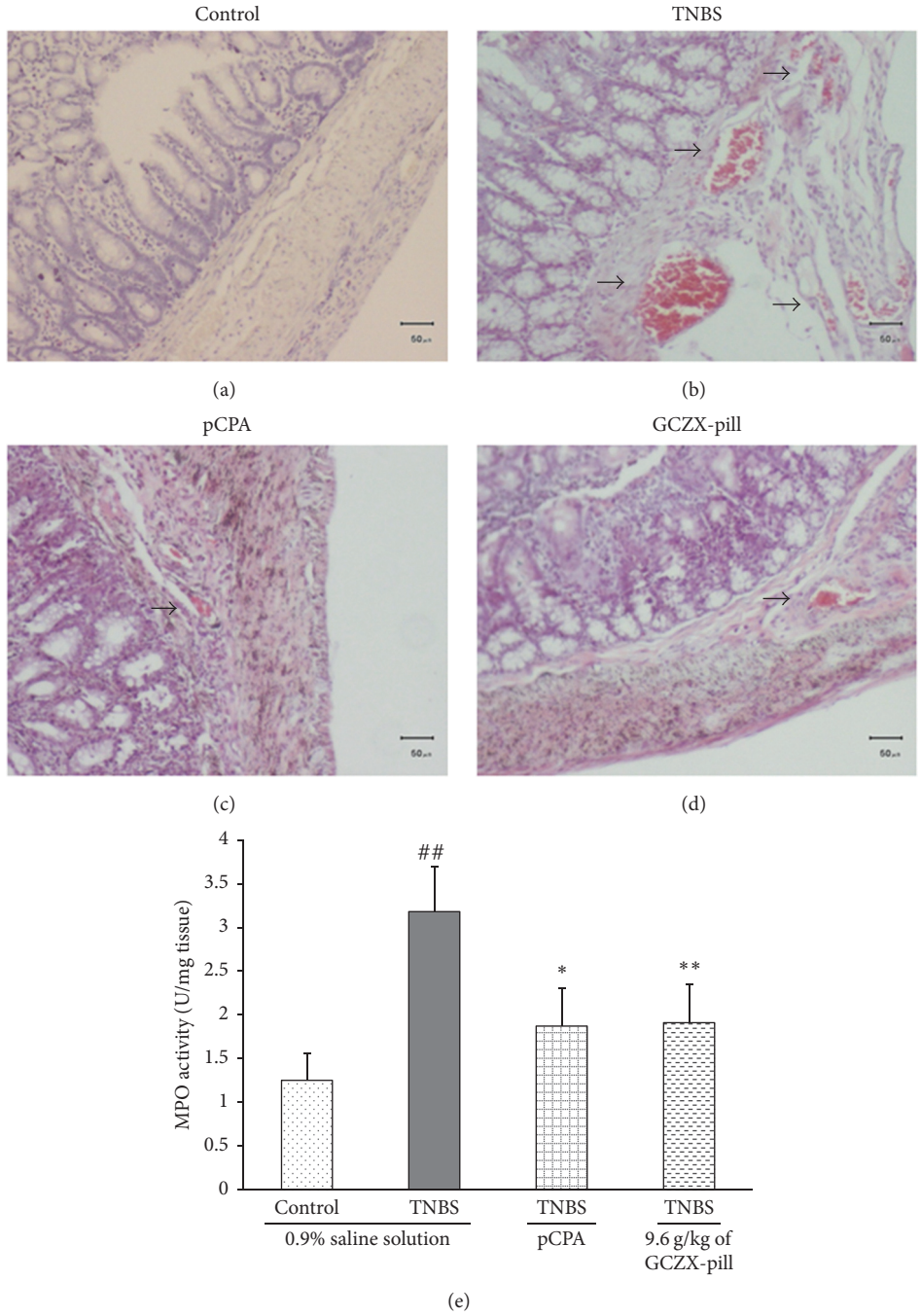


Figure 2: Representative hematoxylin-eosin stained sections of colonic specimens harvested from the control rats (a), from the TNBS-induced UC rats (b), from the TNBS-induced UC rats given pCPA (c), and from the TNBS-induced UC rats given GCZX-pill 9.6 g/kg (d), scale bar = 50 μm; the epithelial degeneration, neutrophilic infiltration, and submucosal edema are indicated by arrows. The effect of GCZX-pill on MPO activity in TNBS-induced UC rats is shown in (e). Data are shown as mean ± SEM ($n = 12$ per group). $^{\#\#}P < 0.01$ versus the control rats; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus the UC rats; one-way ANOVA followed by Bonferroni's posttest.

pCPA treatment also significantly reduced the levels of TNF-α and IFN-γ (2.15 ± 0.41 pg/mg and 4.02 ± 0.33 pg/mg for TNF-α and IFN-γ, $P < 0.01$, $n = 12$) but did not elevate the levels of IL-10 (2.19 ± 0.34 pg/mg).

3.5. Effects of GCZX-Pill on Colonic EC Cell Number and TPH Expression. The effect of GCZX-pill on colonic EC cells number was investigated (Figures 4(a)–4(e)) in this study. The colonic EC cell numbers in UC rats were significantly increased from 72.5 ± 6.8 per mm^2 to 182.4 ± 7.1 per mm^2 ($P < 0.01$, $n = 12$) when compared to that of the control rats. After pCPA treatment, the colonic EC cells numbers in the rats of pCPA group were significantly reduced to 162.3 ± 7.6 per mm^2 ($P < 0.05$, $n = 12$). Also, the colonic EC

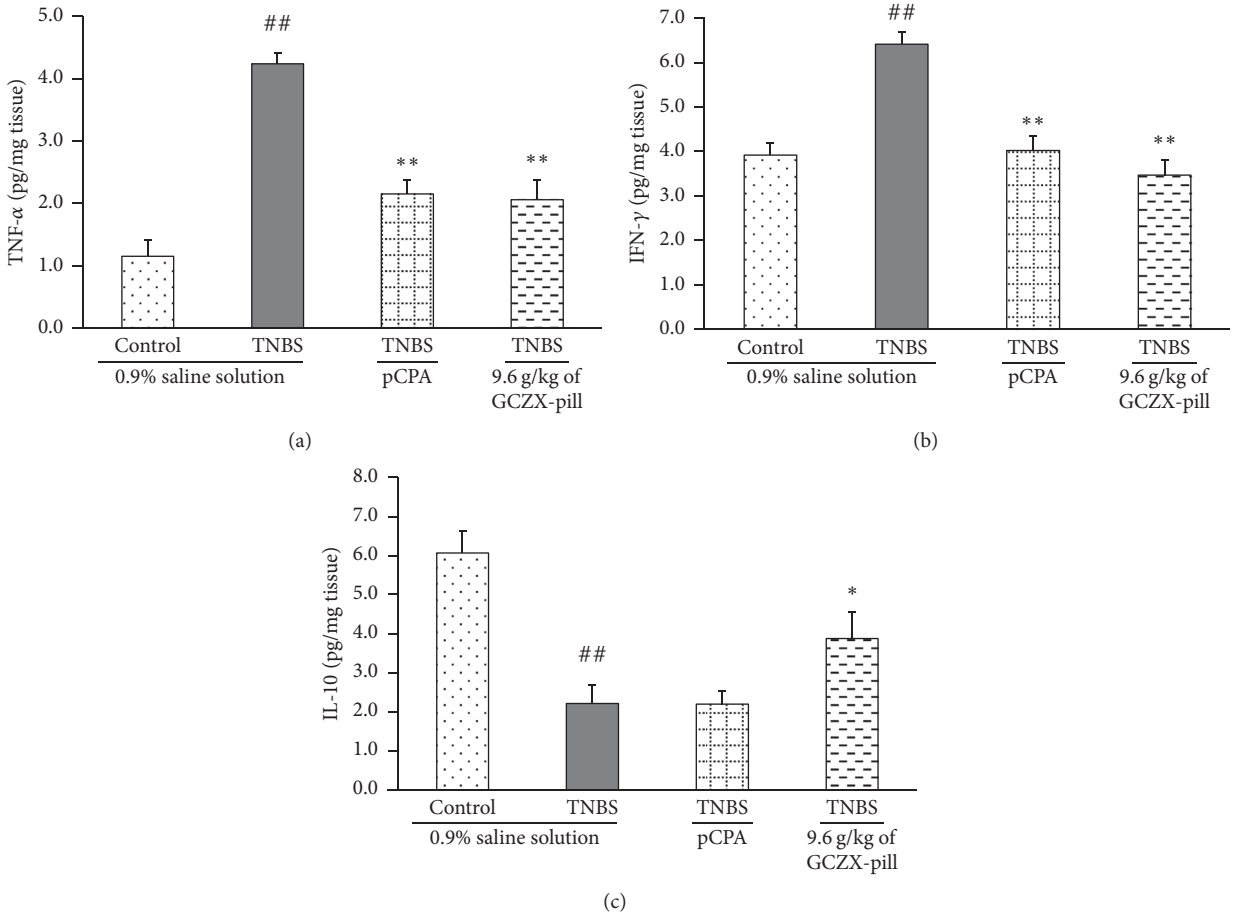


Figure 3: The effects of GCZX-pill on the levels of TNFα (a), IFNγ (b), and IL-10 (c) in TNBS-induced UC rats were investigated. Data are shown as mean ± SEM ($n = 12$ per group). $^{\#\#}P < 0.01$ versus the control rats; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus the UC rats; one-way ANOVA followed by Bonferroni's posttest.

cells numbers were significantly reduced to 101.5 ± 9.4 per mm^2 ($P < 0.01$, $n = 12$) by treatment with GCZX-pill when compared to that of the UC rats.

Consistent with the results from colonic EC cells number, the results (Figures 4(f) and 4(g)) also showed that colonic TPH expression in UC rats was significantly increased from 0.09 ± 0.052 to 0.28 ± 0.045 ($P < 0.01$, $n = 12$) when compared to that of the control rats. After pCPA or GCZX-pill treatment, the TPH expression in the rats of pCPA group or high-dose GCZX-pill group was significantly reduced to 0.24 ± 0.047 (pCPA group, $P < 0.05$, $n = 12$) and 0.19 ± 0.036 (high-dose GCZX-pill group, $P < 0.05$, $n = 12$) when compared to that of the UC rats.

3.6. Effects of GCZX-Pill on 5-HT Availability in the Colon. The effect of GCZX-pill on colonic 5-HT content was also investigated (Figure 5(a)) in this study. The colonic 5-HT content in UC rats was significantly increased from 1.26 ± 0.27 ng/mg to 3.11 ± 0.17 ng/mg ($P < 0.01$, $n = 12$) when compared to that of the control rats. After pCPA treatment, the colonic 5-HT content in the rats of pCPA group was significantly reduced to 1.62 ± 0.22 ng/mg ($P < 0.01$, $n = 12$). Also, the colonic 5-HT content was significantly reduced to 2.28 ± 0.31 ng/mg ($P < 0.05$, $n = 12$) by treatment with GCZX-pill when compared to that of the UC rats.

The colonic intensity of SERT immunoreactivity (Figures 5(b)–5(e)) in UC rats was significantly decreased when compared to that of the control rats ($P < 0.01$, $n = 12$). After treatment with GCZX-pill, there were no significant differences in the expression of SERT immunoreactive intensity between UC rats and GCZX-pill-treated rats, suggesting that GCZX-pill treatment has little effect on the SERT expression in UC rats.

4. Discussion

The results from this study indicated an important effect for GCZX-pill in inhibiting TNBS-induced UC. And the anti-inflammatory effect of GCZX-pill administration indicated that GCZX-pill significantly attenuated colitis. Meanwhile, the changes of EC cell numbers, colonic TPH expression, and 5-HT availability induced by GCZX-pill at colon were also indicated here.

To evaluate the anti-inflammatory effect of GCZX-pill, the well-established model of TNBS-induced colitis in rats which has resemblance to UC was used. In this study,

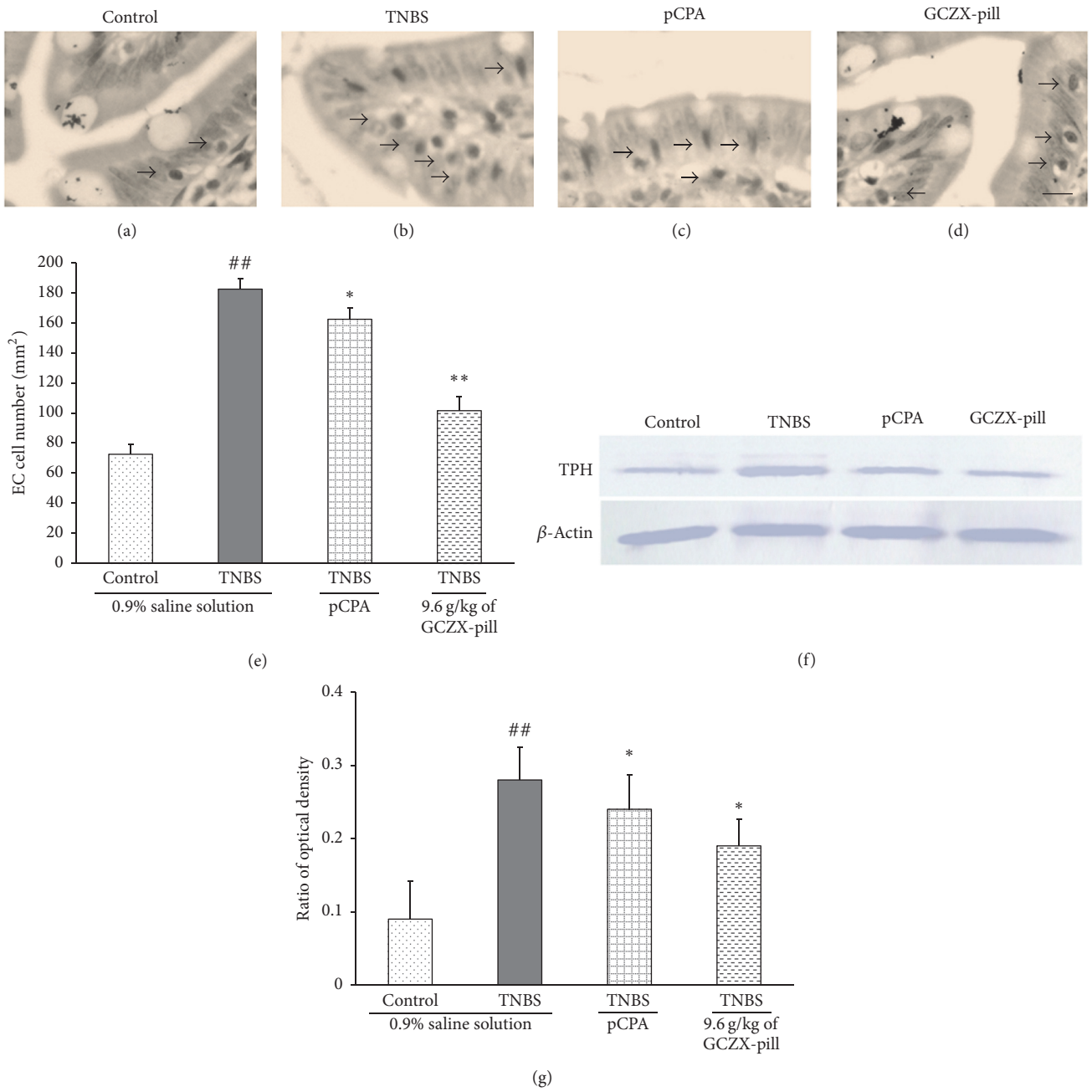


Figure 4: Representative Fontana-Masson stained sections of colonic EC cells specimens harvested from the control rats (a), from the TNBS-induced UC rats (b), from the TNBS-induced UC rats given pCPA (c), and from the TNBS-induced UC rats given GCZX-pill 9.6 g/kg (d), scale bar = 20 μm; the EC cells are indicated by arrows. Statistical graph of EC cell density is shown in (e). Representative western blot analysis figure of colonic TPH expression (relative to beta-actin) is shown in (f). Statistical graph of quantified optical density is shown in (g). Data are shown as mean ± SEM ($n = 12$ per group). $^{\#\#}P < 0.01$ versus the control rats; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus the UC rats; one-way ANOVA followed by Bonferroni's posttest.

GCZX-pill efficiently alleviated TNBS-induced UC. TNBS is widely used for studying ulcerative colitis because the TNBS-induced UC model is symptomatically, morphologically, and histopathologically very similar to that of human IBD [24]. When TNBS couples with high molecular weight proteins it can elicit significant immunologic responses by rendering those proteins immunogenic to the host immune system and induces diffuse colonic inflammation, which is characterized by increased leukocyte infiltration, edema, and ulceration [35, 36]. Experimentally, TNBS is dissolved in alcohol and is delivered intrarectally in rodents to induce colitis. Alcohol not only serves as a solvent or carrier, but also aids in inducing gut inflammation by breaking the mucosal barrier [37, 38]. However, TNBS also has inherent limitations that

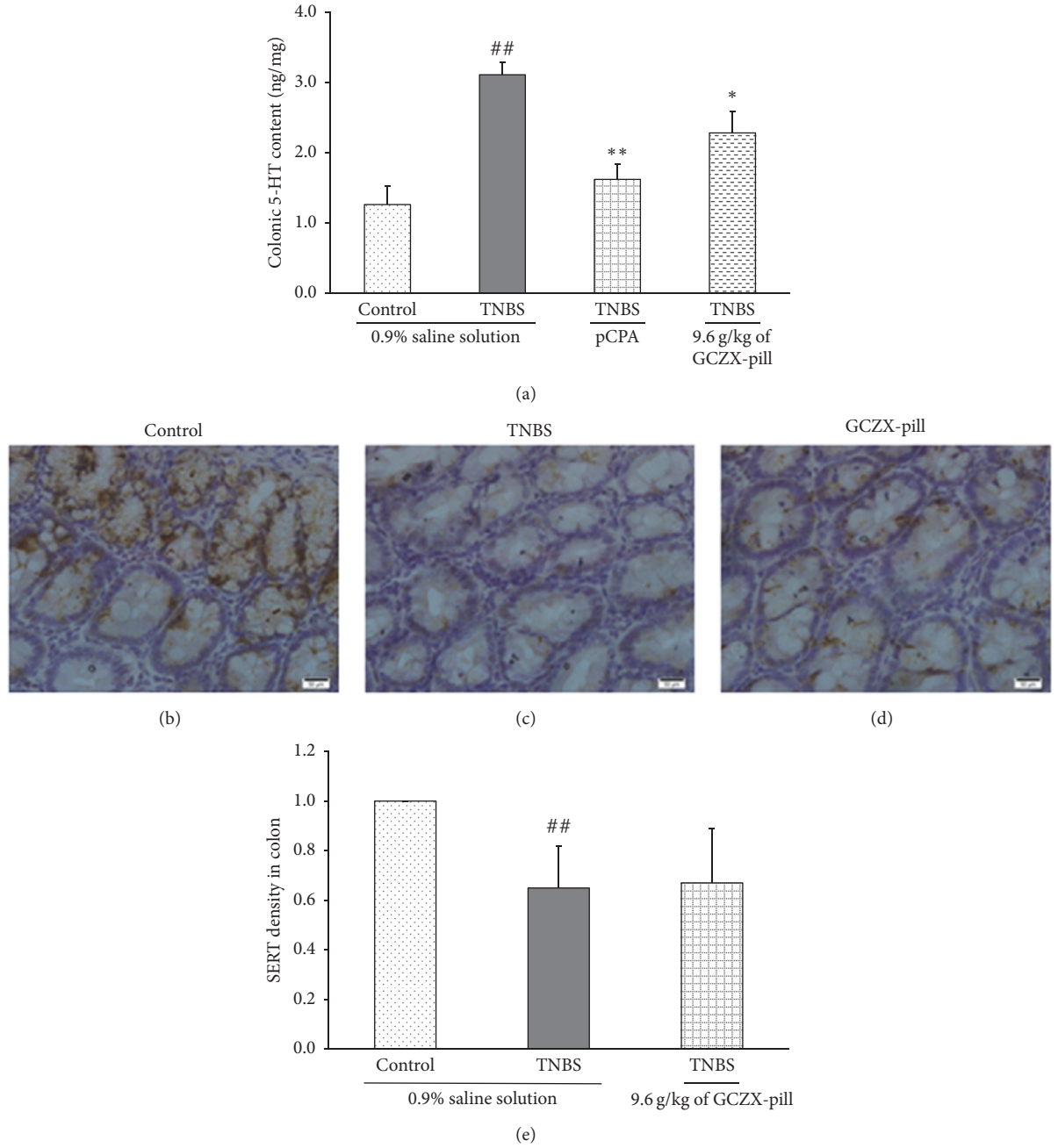


Figure 5: The effect of GCZX-pill on colonic 5-HT content in TNBS-induced UC rats is shown in (a). Representative immunohistochemical micrographs of colonic SERT expression specimens harvested from the control rats (b), from the TNBS-induced UC rats (c), and from the TNBS-induced UC rats given GCZX-pill 9.6 g/kg (d), scale bar = 50 μm. Statistical graph of SERT expression is shown in (e). Data are shown as mean ± SEM ($n = 12$ per group). $^{\#\#}P < 0.01$ versus the control rats; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus the UC rats; one-way ANOVA followed by Bonferroni's posttest.

it does not recapitulate the exact mechanisms that underlie its pathogenesis and specifically the role of gut microbiota [39]. The symptoms such as the attenuation of weight loss, abnormal defecation, rectal bleeding, adhesion, intraluminal hemorrhage, colonic thickness, and colonic edema were alleviated by GCZX-pill. Also, GCZX-pill reduced MPO activity, a marker of tissue neutrophil content.

As expected, EC cell hyperplasia and high-concentration of 5-HT were induced by TNBS which is consistent with the characteristic of UC. 5-HT was synthesized and released from enterochromaffin (EC) cells [13]. As a neurotransmitter, 5-HT plays an important role in the gastrointestinal tract. There are many serotonergic receptors that have been found on various immune cells such as B and T lymphocytes, monocytes,

macrophage, and dendritic cells [40]. In addition, 5-HT is also a chemotactic molecule for eosinophils, dendritic cells, and mast cells. Therefore, it is suggested that 5-HT plays an important role in influencing the immune system [41]. Results from this study showed that GCZX-pill treatment significantly alleviated inflammation in UC rats, and this effect was concomitant with the decreased colonic EC cell number and 5-HT concentration. TPH in EC cells is the rate-limiting enzyme in the 5-HT synthesis process, and the pCPA is one of the inhibitors of TPH [42, 43]. Results from this study show that pCPA depleted seriously the colonic 5-HT concentration and a well anti-inflammatory effect in TNBS-induced UC is shown. Therefore, all these results indicate that the decreased EC cell number and 5-HT concentration induced by GCZX-pill treatment may be responsible for attenuated the inflammation in UC rats.

The results from this study indicated that the levels of TNF-α, IFN-γ, and IL-10 in the colon were changed by treatment with GCZX-pill. Past study indicated that the levels of Th1 or Th2 inflammatory cytokines such as TNF-α, IFN-γ, and IL-10 play important roles in Th1/Th2 balance and expansion of $CD4^+$ T cells, and the classical Th1/Th2 pathways are thought to play a critical role in UC pathogenesis. In addition, the past reports indicated that EC cell hyperplasia is considered to have close correlation with $CD4^+$ T lymphocytes, especially the Th1/Th2 balance [14, 15, 44]. Therefore, the inflammation and hyperplastic colonic EC cells that were attenuated by GCZX-pill may be mediated by changing the levels of TNF-α, IFN-γ, and IL-10.

A similar phenomenon was observed where the hyperplastic colonic EC cells were reduced by pCPA. As an inhibitor of TPH, pCPA significantly reduced the colonic 5-HT content. Past studies indicated the reduced severity of colitis in $TPH1^{-/-}$ mice as compared with wild-type mice after dextran sodium sulfate- and dinitrobenzenesulfonic acid-colitis. Restoration of 5-HT in $TPH1^{-/-}$ mice by administration of a 5-HT precursor (5-hydroxy-l-tryptophan) enhanced the severity of colitis. Therefore, the inflammation could be attenuated via decreasing the colonic 5-HT content [40]. In addition, besides its well-characterized function as a neurotransmitter, 5-HT has been reported to be a potent immunoregulator. 5-HT has been reported to be a potent regulator of cytokine secretion in different kinds of cells [45]. Past studies have shown an important immunoendocrine axis in the gut in an enteric infection-induced model of gut inflammation, where secretory products from $CD4^+$ T cells interact with EC cells or their precursors to enhance 5-HT production in the gut [27, 46]. Therefore, it is not hard to understand that pCPA, as an inhibitor of TPH reducing the hyperplastic colonic EC cells, may be mediated by reducing colonic 5-HT content.

The serotonin reuptake transporter (SERT) plays important roles in 5-HT recycle in the colon tissue [12, 47]. The effect of GCZX-pill on the expression of SERT was also investigated in this study. Results from this study were consistent with that of the past studies which showed that SERT expression is downregulated in TNBS-induced colitis. Unfortunately, after treatment with GCZX-pill, there were no significant differences in the expression of SERT immunoreactive intensity between UC rats and GCZX-pill-treated rats. Therefore, this result indicated that GCZX-pill did not act on the recycling of 5-HT.

It is well known that most traditional Chinese medicine remedies are formulated by using individual herbs in combination. Under the guidance of traditional theory, the different herbs of certain formula are thought to increase therapeutic efficacy and reduce adverse effects simultaneously through multiple targets and biological pathways [48]. Results from this study indicated that the anti-inflammatory effect of GCZX-pill may be mediated by reducing colonic EC cell number, 5-HT content, and TPH expression, but not SERT expression, in UC rats. In conclusion, this study demonstrated that the GCZX-pill can attenuate the inflammation in UC rats. The anti-inflammatory effect of the GCZX-pill may be mediated by reducing colonic EC cell hyperplasia and 5-HT availability.

Conflicts of Interest

All authors in this research do not have any possible conflicts of interest.

Authors' Contributions

In this research, Dr. Xianwei Zhu and Yong Yang (M.M.) conceptualized and designed this research; Yifei Qin (M.M.), Jianjun Guan (M.M.), Li Ma (M.M.), Yanyan Xue (M.M.), and Chenhui Li (M.M.) participated in implementing various experiments of this study; Dr. Guihai Chen and Dr. Jing Zhou analyzed and interpreted the data of this study; Yifei Qin (M.M.) and Lu Li (M.M.) drafted the English manuscript. This study was supported by grants from the scientific and technological project in Shaanxi Province of China (Grant no. 2016SF-352).

Acknowledgments

The authors acknowledge the group of Innovation Research Centre of Acupuncture Combined with Medicine for their help in this study.

References

[1] J. Matricon, N. Barnich, and D. Ardid, "Immunopathogenesis of inflammatory bowel disease," *Self/Nonself—Immune Recognition and Signaling*, vol. 1, no. 4, pp. 299–309, 2010.

[2] Y.-Z. Zhang and Y.-Y. Li, "Inflammatory bowel disease: pathogenesis," *World Journal of Gastroenterology*, vol. 20, no. 1, pp. 91–99, 2014.

[3] U. Kopylov, S. Ben-Horin, and E. Seidman, "Therapeutic drug monitoring in inflammatory bowel disease," *Annals of Gastroenterology*, vol. 27, no. 4, pp. 304–312, 2014.

[4] S. Ardizzone, A. Cassinotti, G. Manes, and G. B. Porro, "Immunomodulators for all patients with inflammatory bowel disease?" *Therapeutic Advances in Gastroenterology*, vol. 3, no. 1, pp. 31–42, 2010.

[5] T. Bongartz, A. J. Sutton, M. J. Sweeting, I. Buchan, E. L. Matteson, and V. Montori, "Anti-TNF antibody therapy in

rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials," *Journal of the American Medical Association*, vol. 295, no. 19, pp. 2275–2285, 2006.

[6] L. E. Targownik and C. N. Bernstein, "Infectious and malignant complications of tnf inhibitor therapy in ibd," *American Journal of Gastroenterology*, vol. 108, no. 12, pp. 1835–1842, 2013.

[7] M. Sałaga, H. Zatorski, M. Sobczak, C. Chen, and J. Fichna, "Chinese herbal medicines in the treatment of IBD and colorectal cancer: a review," *Current Treatment Options in Oncology*, vol. 15, no. 3, pp. 405–420, 2014.

[8] P. Wan, H. Chen, Y. Guo, and A.-P. Bai, "Advances in treatment of ulcerative colitis with herbs: From bench to bedside," *World Journal of Gastroenterology*, vol. 20, no. 39, pp. 14099–14104, 2014.

[9] M. D. Coates, C. R. Mahoney, D. R. Linden et al., "Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome," *Gastroenterology*, vol. 126, no. 7, pp. 1657–1664, 2004.

[10] F. Magro, M. A. Vieira-Coelho, S. Fraga et al., "Impaired synthesis or cellular storage of norepinephrine, dopamine, and 5-hydroxytryptamine in human inflammatory bowel disease," *Digestive Diseases and Sciences*, vol. 47, no. 1, pp. 216–224, 2002.

[11] F. De Ponti, "Pharmacology of serotonin: what a clinician should know," *Gut*, vol. 53, no. 10, pp. 1520–1535, 2004.

[12] D. R. Linden, J.-X. Chen, M. D. Gershon, K. A. Sharkey, and G. M. Mawe, "Serotonin availability is increased in mucosa of guinea pigs with TNBS-induced colitis," *American Journal of Physiology—Gastrointestinal and Liver Physiology*, vol. 285, no. 1, pp. G207–G216, 2003.

[13] M. El-Salhy, Å. Danielsson, R. Stenling, and L. Grimelius, "Colonic endocrine cells in inflammatory bowel disease," *Journal of Internal Medicine*, vol. 242, no. 5, pp. 413–419, 1997.

[14] H. Wang, J. Steeds, Y. Motomura et al., "CD4+ T cell-mediated immunological control of enterochromaffin cell hyperplasia and 5-hydroxytryptamine production in enteric infection," *Gut*, vol. 56, no. 7, pp. 949–957, 2007.

[15] Y. Motomura, J.-E. Ghia, H. Wang et al., "Enterochromaffin cell and 5-hydroxytryptamine responses to the same infectious agent differ in Th1 and Th2 dominant environments," *Gut*, vol. 57, no. 4, pp. 475–481, 2008.

[16] X. Wang, L. Liu, J. Sun et al., "Application of superfine grinding technology in the production of Guchang Zhixie pills," *Modern Traditional Chinese Medicine*, vol. 37, pp. 79–81, 2014.

[17] Y. Liu, Z. Song, and Y. Zhang, "SQFM methods based on HPLC-DAD with five wavelengths for fingerprint determination of Guchang Zhixie pill and powders," *Chinese Journal of Experimental Traditional Medical Formulae*, vol. 21, pp. 65–70, 2015.

[18] Y. Liu, Z. Song, and Y. Zhang, "Application of Plackett-Burman-Central composite design for fingerprint analysis of Gu-Chang-Zhi-Xie pill by HPLC-DAD approach," *Modern Chinese Medicine*, vol. 17, pp. 590–595, 2015.

[19] W. Yuan, "Clinical research on treating diarrhea-predominant irritable bowel syndrome with the Guchang Zhixie pills," *Clinical Journal of Chinese medicine*, vol. 5, pp. 62-63, 2013.

[20] Y. Liu, "Clinical observation of Guchang Zhixie pills combined with compound glutamin entersoluble capsules in treatment of ulcerative colitis," *Drugs & Clinic*, vol. 31, pp. 182–185, 2016.

[21] H. Huang and S. Ji, "The therapeutic effect of combining Guchangzhixie Wan with Smecta in treating irritable bowel syndrome of diarrheal type," *Practical Clinical Journal of Integrated Traditional Chinese and Western Medicine*, vol. 6, pp. 11-12, 2006.

[22] H. Wu and Z. Chen, "Efficacy of Guchang Zhixie pill and sulfasalazine in treatment of paients with ulcerative colitis and influence of IL-2 and IL-8 in serum," *Liaoning Journal of Traditional Chinese Medicine*, vol. 41, pp. 462-463, 2014.

[23] H.-Y. Qin, J. C. Y. Wu, X.-D. Tong, J. J. Y. Sung, H.-X. Xu, and Z.-X. Bian, "Systematic review of animal models of post-infectious/post-inflammatory irritable bowel syndrome," *Journal of Gastroenterology*, vol. 46, no. 2, pp. 164–174, 2011.

[24] A. B. A. De Almeida, M. Sánchez-Hidalgo, A. R. Martín et al., "Anti-inflammatory intestinal activity of Arctium lappa L. (Asteraceae) in TNBS colitis model," *Journal of Ethnopharmacology*, vol. 146, no. 1, pp. 300–310, 2013.

[25] H. S. Cooper, S. N. Murthy, R. S. Shah, and D. J. Sedergran, "Clinicopathologic study of dextran sulfate sodium experimental murine colitis," *Laboratory Investigation*, vol. 69, no. 2, pp. 238–249, 1993.

[26] J. L. Wallace, W. K. MacNaughton, G. P. Morris, and P. L. Beck, "Inhibition of leukotriene synthesis markedly accelerates healing in a rat model of inflammatory bowel disease," *Gastroenterology*, vol. 96, no. 1, pp. 29–36, 1989.

[27] S. C. Bischoff, R. Mailer, O. Pabst et al., "Role of serotonin in intestinal inflammation: Knockout of serotonin reuptake transporter exacerbates 2,4,6-trinitrobenzene sulfonic acid colitis in mice," *American Journal of Physiology—Gastrointestinal and Liver Physiology*, vol. 296, no. 3, pp. G685–G695, 2009.

[28] S. D. Moore-Olufemi, R. A. Kozar, F. A. Moore et al., "Ischemic preconditioning protects against gut dysfunction and mucosal injury after ischemia/reperfusion injury," *Shock*, vol. 23, no. 3, pp. 258–263, 2005.

[29] W. I. Khan, P. A. Blennerhasset, A. K. Varghese et al., "Intestinal nematode infection ameliorates experimental colitis in mice," *Infection and Immunity*, vol. 70, no. 11, pp. 5931–5937, 2002.

[30] J. E. Krawisz, P. Sharon, and W. F. Stenson, "Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models," *Gastroenterology*, vol. 87, no. 6, pp. 1344–1350, 1984.

[31] M. B. Grisham, J. N. Benoit, and D. N. Granger, "Assessment of leukocyte involvement during ischemia and reperfusion of intestine," *Methods in Enzymology*, vol. 186, pp. 729–742, 1990.

[32] X. Zhu, Z. Liu, H. Qu et al., "The effect and mechanism of electroacupuncture at LI11 and ST37 on constipation in a rat model," *Acupuncture in Medicine*, vol. 34, no. 3, pp. 194–200, 2016.

[33] X. Zhu, Z. Liu, W. Niu et al., "Effects of electroacupuncture at ST25 and BL25 in a *Sennae*-induced rat model of diarrhoea-predominant irritable bowel syndrome," *Acupuncture in Medicine*, 2016.

[34] X. Zhu, H. Shinohara, R. Miyatake, and T. Hohsaka, "Novel biosensor system model based on fluorescence quenching by a fluorescent streptavidin and carbazole-labeled biotin," *Journal of Molecular Recognition*, pp. 485–491, 2016.

[35] C. O. Elson, R. B. Sartor, G. S. Tennyson, and R. H. Riddell, "Experimental models of inflammatory bowel disease," *Gastroenterology*, vol. 109, no. 4, pp. 1344–1367, 1995.

[36] F. Isik, T. Tunali Akbay, A. Yarat et al., "Protective effects of black cumin (*Nigella sativa*) oil on TNBS-induced experimental colitis in rats," *Digestive Diseases and Sciences*, vol. 56, no. 3, pp. 721–730, 2011.

[37] M. F. Neurath, I. Fuss, B. L. Kelsall, E. Stüber, and W. Strober, "Antibodies to interleukin 12 abrogate established experimental colitis in mice," *Journal of Experimental Medicine*, vol. 182, no. 5, pp. 1281–1290, 1995.

[38] M. Ikeda, F. Takeshima, H. Isomoto et al., "Simvastatin attenuates trinitrobenzene sulfonic acid-induced colitis, but not oxazalone-induced colitis," *Digestive Diseases and Sciences*, vol. 53, no. 7, pp. 1869–1875, 2008.

[39] E. Antoniou, G. A. Margonis, A. Angelou et al., "The TNBS-induced colitis animal model: an overview," *Annals of Medicine and Surgery*, vol. 11, pp. 9–15, 2016.

[40] J.-E. Ghia, N. Li, H. Wang et al., "Serotonin has a key role in pathogenesis of experimental colitis," *Gastroenterology*, vol. 137, no. 5, pp. 1649–1660, 2009.

[41] I. Cloëz-Tayarani and J.-P. Changeux, "Nicotine and serotonin in immune regulation and inflammatory processes: a perspective," *Journal of Leukocyte Biology*, vol. 81, no. 3, pp. 599–606, 2007.

[42] D. J. Walther, J.-U. Peter, S. Bashammakh et al., "Synthesis of serotonin by a second tryptophan hydroxylase isoform," *Science*, vol. 299, no. 5603, p. 76, 2003.

[43] D. J. Walther and M. Bader, "A unique central tryptophan hydroxylase isoform," *Biochemical Pharmacology*, vol. 66, no. 9, pp. 1673–1680, 2003.

[44] W. Strober and I. J. Fuss, "Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases," *Gastroenterology*, vol. 140, no. 6, pp. 1756–1767, 2011.

[45] T. Müller, T. Dürk, B. Blumenthal et al., "5-hydroxytryptamine modulates migration, cytokine and chemokine release and T-cell priming capacity of dendritic cells in vitro and in vivo," *PLoS ONE*, vol. 4, no. 7, Article ID e6453, 2009.

[46] S. Haub, Y. Ritze, I. Bergheim, O. Pabst, M. D. Gershon, and S. C. Bischoff, "Enhancement of intestinal inflammation in mice lacking interleukin 10 by deletion of the serotonin reuptake transporter," *Neurogastroenterology and Motility*, vol. 22, no. 7, pp. 826–e229, 2010.

[47] M. B. Hansen, "Neurohumoral control of gastrointestinal motility," *Physiological Research*, vol. 52, no. 1, pp. 1–30, 2003.

[48] L. Wang, G.-B. Zhou, P. Liu et al., "Dissection of mechanisms of Chinese medicinal formula Realgar-Indigo naturalis as an effective treatment for promyelocytic leukemia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 12, pp. 4826–4831, 2008.

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2017, Article ID 7016468, 7 pages
https://doi.org/10.1155/2017/7016468

Review Article

Huangqin-Tang and Ingredients in Modulating the Pathogenesis of Ulcerative Colitis

Chunyan Wang,[1] Xudong Tang,[2] and Li Zhang[1]

[1]*Institute of Digestive Diseases, China-Canada Center of Research for Digestive Diseases (ccCRDD), Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200032, China*
[2]*Xiyuan Hospital of China Academy of Chinese Medical Sciences, Beijing 100091, China*

Correspondence should be addressed to Li Zhang; zhangli.hl@163.com

Received 17 March 2017; Accepted 22 May 2017; Published 12 June 2017

Academic Editor: Luísa Mota da Silva

Ulcerative colitis (UC) is the most common inflammatory bowel disease worldwide. Current therapies in UC cause limitations, and herb medicine provides an important choice for UC treatment. Huangqin-Tang (HQT) is a well-known classical traditional Chinese herbal formula and has been used in China for thousands of years. A large number of pharmacological studies demonstrated HQT and its ingredients to be effective in treating UC. Though the therapeutic effect has been evaluated, comprehensive up-to-date reviews in this field are not yet available. Here we aim to review our current understanding of HQT and its ingredients in treating UC and how the agents modulate the main pathogenesis of the disease, including the intestinal environment, immune imbalance, inflammatory pathways, and oxidative stress. The summary on this issue may provide better understanding of HQT and its ingredients in treating UC and possibly help in promoting its clinical application.

1. Introduction

Ulcerative colitis (UC) is the most common form of inflammatory bowel disease, it is characterized by chronic inflammatory disorders of the colonic mucosa, which starts in the rectum and generally extends proximally in a continuous manner through part of or the entire colon [1]. The disease is bifurcated into remitting and relapsing courses [2] and may have significant impact on the quality of life and personal burden through reduction in the ability to work [3, 4]. UC patients may require life-long treatment and have an increased risk of developing colorectal cancer [5, 6].

The global prevalence of UC is about 8 million, and the incidence and prevalence are increasing worldwide [7]. While the precise cause of UC is still unknown, it has been hypothesized that various factors such as geography, age, sex, genetic, environmental, gastrointestinal infection, and appendicectomy are responsible for the development of UC [1, 8–11]. The pathophysiology is related to epithelial barrier impairment, commensal microflora disorders, antigen recognition, dysregulation of immunological responses, leucocyte recruitment, and so forth. However, since the exact mechanisms of UC are still under investigation, treatment strategies are limited. The principle for UC treatment is divided into two categories according to the clinical activity and the extent of diseases, induction of remission, and maintenance of remission. The main agents consist of topical or oral mesalazine, oral or intravenous corticosteroids, immunosuppressive drugs, monoclonal antibodies of TNF-α, and colectomy [1]. However, the side effects and high cost limit the long-term application [12].

Recently, natural products have attracted lots of interest in preventing and treating UC. Traditional Chinese Medicines (TCM) and extracts have shown various beneficial treatment effects including bacteriostasis, anti-inflammation, and anticancer abilities [13]. Among them, Huangqin-Tang (HQT) is a well-known classical formula which derived from Shang Han Lun, consisting of four ingredients: the roots of *Scutellaria baicalensis* Georgi (scute), *Glycyrrhiza uralensis* Fisch (licorice), *Paeonia lactiflora* Pall. (peony), and the fruit of *Ziziphus jujuba* Mill (Chinese date). Using high-quality herbs picked by experienced herbalists and manufactured according to cGMP (current Good Manufacturing Practice),

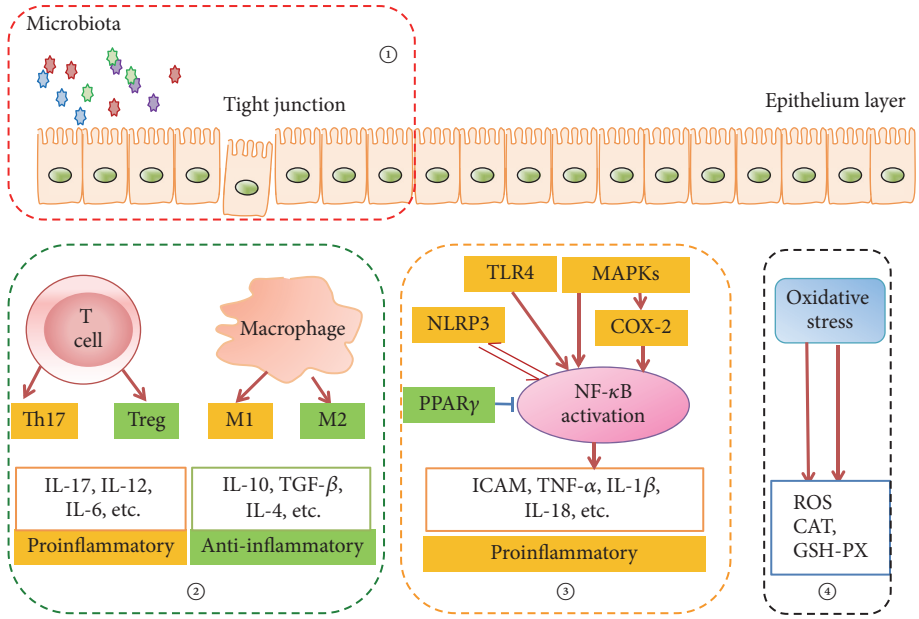


Figure 1: *HQT regulates the main pathogenesis of UC.* ① HQT and its ingredients modulate intestinal microbiota and the integrity of epithelium; ② HQT and its ingredients maintain the immune balance (Th17 versus Treg; M1 versus M2 type macrophages); ③ HQT and its ingredients target the inflammatory pathways, which could result in decrease of proinflammatory cytokines release; ④ HQT and its ingredients improve oxidative stress via enhancing antioxidants production. *Note.* ↑ indicates activated action and T indicates inhibited action.

HQT was extracted and named as PHY906 [14]. Using standardized chemical and biological fingerprints, the consistent preparations of PHY906 have been made and developed as an adjuvant for chemotherapy in various cancers.

Though HQT has been widely used in treating UC in China, the underlying mechanisms are still not clear. Pharmacokinetic studies on multiconstituents in HQT by the validated HPLC method showed that the main ingredients of HQT included baicalin, wogonoside, oroxylin-A-glucuronide, baicalein, wogonin, oroxylin-A, paeoniflorin, paeonimetabolin-I, liquiritin, liquiritigenin, glycyrrhizic acid, glycyrrhetinic acid, and visidulin I [15]. These constituents can have anti-inflammatory effect through different or similar mechanisms in UC treatment. Application of HQT or its ingredients in UC has been widely investigated; however, the systematic effect evaluation and mechanisms exploration are spotted, and we aim to summarize the current studies about the therapeutic effects or mechanisms of HQT or its main ingredients in UC (Figure 1), thus to better support its clinical use.

2. HQT and Ingredients in Targeting Intestinal Environment on UC

Imbalanced interactions with intestinal microbes lead to development of UC, and intestinal microbiota and epithelium provide environment for the pathogens.

There are approximately 10^{11}~10^{14} enteric commensal microorganisms in the gut. Under normal conditions, these commensal bacteria maintain the balance and help in regulating crucial nutrient provision, immune response, and energy metabolism. However, commensal microorganisms can be noxious for intestinal inflammation under certain circumstances, and the diversity and amount of microbiota are reduced in patients with UC compared to that in healthy humans [14]. Though the specific bacteria related to the incidence of UC have not been found, lots of studies have showed that the change of intestinal environment was closely related to UC, and there was significantly different bacterial colonies between UC patients and healthy people [16–18]. PHY906, the extracted HQT, has been reported to alter the profile of major intestinal bacteria species, and treatment of mice with PHY906 could significantly decrease *Bacteroides* and *E. rectale/C. coccoides* and increase *Clostridium leptum* specially in the colon [19]. In addition, *Paeonia lactiflora* root, the main constituent of HQT, has been demonstrated to inhibit the growth of harmful intestinal bacteria in human [20].

The intestinal epithelial cells structurally constitute crypts and villi in the intestine, with a single columnar cell lining with a tight junction. The epithelial integrity is maintained by tight junctions between epithelial cells, and external pathogens easily pass through the injured or incomplete intestinal epithelium, which is known to be involved in the pathogenesis of UC. Several lines of evidence showed that the ingredients of HQT can protect the epithelium from pathogen introduction. Wogonin is a flavonoid isolated from *Scutellaria baicalensis* Georgi, the predominant ingredient of HQT. Pretreatment of Caco-2 cells with wogonin protects intestinal barrier function in lipopolysaccharide (LPS) stressed condition. Both 10 μM and 50 μM wogonin attenuate the LPS-induced transepithelial electrical resistance and transport of fluorescent markers and upregulates claudin-1 and ZO-1, the representative tight junction proteins in

intestine [21]. Paeoniflorin, the main active ingredient of *Paeonia lactiflora* Pall., is confirmed to attenuate LPS-induced permeability in endothelial cells, and the ingredient (10, 30, and 100 μM) could inhibit dextran extravasation and leukocyte migration in a concentration-dependent manner [22]. Furthermore, the ingredient glycyrrhetinic acid also exerted protective effects on LPS stressed intestinal epithelial cells injury and expression of the epithelial tight junction molecules [23].

3. HQT and Ingredients in Regulating Immune Imbalance

The gut possesses an abundant and highly active immune system that is tightly regulated to prevent overreaction of immune responses [24]. Studies have provided the evidences that the dysfunction of gut immune system, mainly involving the abnormal percentage and disturbed differentiation of immune cells, can result in the increase of proinflammatory cytokines, epithelial permeability, and subsequent intestinal mucosa damage [25–27]. Both Th17 and regulatory T (Treg) cells are originated from $CD4^+$ T cells. Th17 cells, which secrete IL-17 and IL-22, promote inflammation process. On the contrary, Treg cells, which produces IL-10 and transforming growth factor-β (TGFβ), inhibit the inflammatory response [28, 29]. As one important subtype of T cells, $CD4^+CD25^+$ forkhead box $p3^+$ ($Foxp3^+$) Treg cells inhibit autoimmunity and protect the tissue against injury and their development is controlled by Foxp3.

Growing evidences suggest that the imbalance between Th17 cells and Treg cells may contribute to the development of UC [30–32]. The ingredients of HQT are reported to regulate immune imbalance in a series studies. In trinitrobenzenesulfonic acid- (TNBS-) induced UC mice, baicalin could ameliorate the severity of the disease by downregulating the number of Th17 cells and the levels of Th17-related cytokines (IL-17 and IL-6) and upregulating Treg cells and related TGFβ, IL-10, and Foxp3 levels [33]. Similar effect of baicalin was also observed in cultured T cells; it proved that baicalin induced Foxp3 protein expression and inhibited T cells proliferation [34]. $CD4^+CD29^+$ cells are helper T cells that can result in high activation of B cells and abnormal immune response. Previous study showed the percentage of $CD4^+CD29^+$ T cells significantly increased in UC patients, indicating $CD4^+CD29^+$ T cells might be an immunology index for monitoring UC [35, 36]. Baicalin can reduce the percentages of $CD4^+CD29^+$ T cells in cultured peripheral blood mononuclear cells from the UC patients, thus regulate immune balance, and relieve the UC-induced inflammation [37]. These studies inferred that baicalin may serve as a promising natural immunosuppressive compound for treating UC and related inflammatory diseases.

Macrophages play critical roles in both innate and adaptive immune responses and are classified into M1 and M2 phenotypes. M1 phenotype cells are stimulated by microbial products or other pathogens and produce many proinflammatory cytokines. In contrast, M2 type responses are the "resting" phenotype and are observed in healing-type circumstances without infections and generate anti-inflammatory cytokine. Macrophage M1 and M2 types execute opposite activities in tissues [38]. Once the M1/M2 balance is broken, immune-mediated inflammation occurs. Baicalin is reported to polarize macrophages to an M2 phenotype in murine peritoneum and ameliorate experimental inflammatory bowel disease in mice [39]. In mouse bone marrow precursors generated M1 and M2 cells, paeoniflorin inhibited LPS-induced M1 activity by reducing iNOS and NO production, whereas enhanced IL-4 provoked M2 function by upregulating Arg-1 production and activity [40], suggesting paeoniflorin can suppress M1 cells activity and enhance M2 cells function simultaneously. In addition, paeoniflorin is confirmed to significantly ameliorate the immune complex induced vascular damage, leucocyte infiltrates, and adhesion molecules expression [41]. The pharmaceutical effect of baicalin is reported to be associated with macrophage migration inhibitory factor downregulation, the quantity of macrophages, and the amount of M macrophage-related cytokines and macrophage inflammatory protein-3α [42].

B cells are important in the development of autoimmune disorders. Autoreactive B cells can present self-antigens to autoreactive T cells, produce autoantibodies and proinflammatory cytokines, and amplify inflammatory responses [25, 43, 44]. Inhibiting the abnormal activation of B cells is thought to be an effective strategy for UC treatment. It is reported that the B cell activation can be inhibited by paeoniflorin; in LPS-stimulated murine spleen B cells, paeoniflorin inhibits CD69/CD86 expression and B cell proliferation [45].

4. HQT and Ingredients in Modulating Inflammatory Pathways

Interactions between proinflammatory cytokines and their receptors lead to activation of intracellular signal transduction. And NF-κB has been recognized as a critical target in inflammatory process. The extracellular stimulus, like Ag, LPS, growth factors and inflammatory cytokines, signals, and so forth, could trigger NF-κB activation and induce inflammation in tissues. Therefore, inhibited NF-κB activation is considered to be an effective strategy for UC treatment [46].

Toll-like receptor 4 (TLR4), a key receptor for commensal recognition in innate immunity, is overexpressed in inflamed colonocytes [47, 48], and TLR4-mediated signal transduction can evenly lead to NF-κB activation [49]. In LPS stressed RAW264.7 cells, baicalin can block the TLR4/NF-κB pathway and inhibit IL-6 release and cell proliferation; in Sprague-Dawley rats, TNBS-induced UC status can be ameliorated by baicalin [50, 51]. In DSS-induced C57BL/6 mice, paeoniflorin could improve UC, and the beneficial effects are considered to be related to the downregulation of TLR4 expression and the blockage of NF-κB activation [52]. In addition, wogonin could attenuate the TLR4-mediated inflammatory response and maintain the single-layer membrane structure under LPS stressed Caco2 cells, and the positive effects might be achieved via TLR4-MyD88-TAK1-mediated NF-κB pathway [21].

Mitogen-activated protein kinases (MAPKs) are conserved among all eukaryotes and participate in multiple

cellular processes including cell growth, proliferation, differentiation, migration, inflammation, and survival. Numerous studies have described an increased expression of MAPKs in IBD patients [53]. There are several studies focusing on the therapeutic effects for UC by regulating MAPKs. Glycyrrhetinic acid had the inhibitory effects on IL-8 production in intestinal epithelial cells through blocking MAPKs phosphorylation, followed by I-KBα degradation and NF-κB activation [54]. Similarly, isoLQ, a chalcone found in licorice, could attenuate the DSS-induced colitis via suppressing MAPKs phosphorylation and NF-κB activation in inflamed colon tissue [55]. COX-2, one isoform of cyclooxygenase (COX), can be inducted by activating MAPK activity [56, 57]. And previous research has shown that the level of COX-2 increased in the intestinal tissues of UC, and COX-2 inhibitor could relieve intestinal inflammation in rats with UC [58–60]. PF2405, a standardized fraction of *S. baicalensis*, can significantly inhibit TNF-α induced COX-2 expression through JNK1/2 dephosphorylation and p38 MAPK in HT-29 cells and reduced the expression of proinflammatory cytokines and COX-2 in TNBS-induced colitis in female C57BL/6 mice [61].

Intercellular adhesion molecule 1 (ICAM-1) and TNF-α are the representative cytokines in inflammatory injuries. ICAM-1 induces the migration and infiltration of inflammatory cells into the lesion, whereas TNF-α can alter vascular and intestinal permeability [62]. It is reported that diammonium glycyrrhizinate could reduce TNF-α and ICAM-1 by inhibiting the NF-κB activation and improve intestinal inflammatory injury in a rat model [63]. NLRP3 inflammasome is a key component of inflammatory process and its dysregulation contributes to UC. The synthesis and accumulation of NLRP3 inflammasome can be activated by NF-κB and lead to the secretion of IL-1β and IL-18. It is reported that WG (the glucuronide metabolite of wogonin) could ameliorate DSS-induced colitis via inhibiting NF-κB and NLRP3 inflammasome activation [64]. In addition, there are studies that focus on the inflammatory cytokines. Paeoniflorin can play anti-inflammatory action by increasing the level of IL-17 and decreasing the level of IL-10 in recombinant human IL-1β-stimulated human peripheral blood mononuclear cells in vitro [65]. The glycyrrhizic acid could significantly reduce TNF-α and IL-1β levels in TNBS-induced colitis rats, and in vitro experiments indicated that glycyrrhizic acid could inhibit IL-6 and elevate IL-10 production in LPS-activated macrophages and significantly inhibit lymphocytes proliferation [66]. And similar effects are obtained in DSS-induced inflamed mucosa in rats [67].

Peroxisome proliferator-activated receptor γ (PPARγ), a member of the nuclear receptor family, has been recognized as an endogenous regulator of intestinal inflammation. It is reported that activating PPARγ can inhibit NF-κB activation. Oroxyloside, one of the ingredients of HQT, has been reported to activate PPARγ and prevent DSS-induced colitis through inhibiting NF-κB pathway [68]. Moreover, thioredoxin system is implicated in the regulation of NF-κB transactivation potential; there is one study that focuses on the inhibition of baicalin on the thioredoxin system and finds that baicalein can suppress mitogen induced thioredoxin activity in the unclear compartment of lymphocytes, thus limiting NF-κB dependent inflammatory responses [69]. In addition, it has been reported that estrogen can exert anti-inflammatory effects. Based on this background, one study shows that baicalein has estrogen-like activity and inhibits LPS-induced inflammatory cytokine production via regulating NF-κB pathway, suggesting the potential efficacy in preventing inflammation related diseases [70]. Scutellariae Radix extract was effective in treating acute DSS-induced UC with improving macroscopic and histological damage scores and enhancing recovery of normal colonic secretory function [71].

5. HQT Ingredients in Inhibiting Oxidative Stress

The available evidence suggests that oxidative stress may be involved in the pathogenesis of UC [72, 73]. The reactive oxygen species (ROS) are produced in excess by the inflamed mucosa and overwhelm the endogenous defenses in inflammatory intestinal diseases [74]. Among the masses of related products of oxidative stress, catalase (CAT) and phospholipid hydroperoxide glutathione peroxidase (GSH-Px) can reduce the intestinal damage by strengthening the oxidation resistance or reducing lipid hydroperoxides to their corresponding alcohols [75, 76]. One study showed that the possible mechanisms of baicalin in protecting UC were associated with the inhibition of oxidative stress; baicalin could increase the activities of CAT and GSH-Px in LPS-stimulated RAW264.7 cells and TNBS-induced UC rats [77].

6. Conclusions and Perspectives

In summary, available reports suggested that the mechanisms under the efficacy of HQT or its components on UC are related to intestinal environment improvement, immune modulation, and regulation of inflammatory pathways or cytokines (Figure 1). Since most data are interpreted from animal studies or in vitro experiments, the effects and mechanisms of HQT in UC patients remain to be explored or verified. In addition, most of the current studies are observational studies and the targets of the components remain unclear.

Though, along with the upgrade of the Chinese drugs ingredient detection tools, the compounds of Chinese herbal prescription have made some progress, its standardization is still the important obstacle for traditional Chinese medicine. And these studies are influenced by the quality, origin, and different processing method of single herb. Future studies should focus on the standardization of the compounds and the best compatibility in accordance with the disease database and traditional Chinese medicine database in order to achieve the best effect. In addition, different prescriptions for the acute phase or remission phase of UC patients should also be considered.

Conflicts of Interest

The authors declared no conflicts of interest.

Acknowledgments

This work was supported by the Shanghai Rising-Star Program (no. 17QA1404000).

References

[1] I. Ordás, L. Eckmann, M. Talamini, D. C. Baumgart, and W. J. Sandborn, "Ulcerative colitis," *The Lancet*, vol. 380, no. 9853, pp. 1606–1619, 2012.

[2] Y. Yokoyama, K. Matsuoka, T. Kobayashi et al., "A large-scale, prospective, observational study of leukocytapheresis for ulcerative colitis: Treatment outcomes of 847 patients in clinical practice," *Journal of Crohn's and Colitis*, vol. 8, no. 9, pp. 981–991, 2014.

[3] P. Kawalec, "Indirect costs of inflammatory bowel diseases: Crohn's disease and ulcerative colitis. A systematic review," *Archives of Medical Science*, vol. 12, no. 2, pp. 295–302, 2016.

[4] J. Sammut, J. Scerri, and R. B. Xuereb, "The lived experience of adults with ulcerative colitis," *Journal of Clinical Nursing*, vol. 24, no. 17-18, pp. 2659–2667, 2015.

[5] G. Rogler, "Chronic ulcerative colitis and colorectal cancer," *Cancer Letters*, vol. 345, no. 2, pp. 235–241, 2014.

[6] J. Gordillo, E. Cabré, E. Garcia-Planella et al., "Thiopurine Therapy Reduces the Incidence of Colorectal Neoplasia in Patients with Ulcerative Colitis. Data from the ENEIDA Registry," *Journal of Crohn's & colitis*, vol. 9, no. 12, pp. 1063–1070, 2015.

[7] F. Carbonnel, J. F. Colombel, and J. Filippi, "Methotrexate is not superior to placebo for inducing steroid-free remission, but induces steroid-free clinical remission in a larger proportion of patients with ulcerative colitis," *Gastroenterology*, vol. 150, no. 2, pp. 380–388.e4, 2016.

[8] H. Khalili, E. S. Huang, A. N. Ananthakrishnan et al., "Geographical variation and incidence of inflammatory bowel disease among US women," *Gut*, vol. 61, no. 12, pp. 1686–1692, 2012.

[9] S. M. Quezada and R. K. Cross, "Association of age at diagnosis and ulcerative colitis phenotype," *Digestive Diseases and Sciences*, vol. 57, no. 9, pp. 2402–2407, 2012.

[10] H.-S. Lee, S. H. Park, S.-K. Yang et al., "Appendectomy and the clinical course of ulcerative colitis: A retrospective cohort study and a nested case-control study from Korea," *Journal of Gastroenterology and Hepatology (Australia)*, vol. 30, no. 3, pp. 470–477, 2015.

[11] R. E. Childers, S. Eluri, C. Vazquez, R. M. Weise, T. M. Bayless, and S. Hutfless, "Family history of inflammatory bowel disease among patients with ulcerative colitis: A systematic review and meta-analysis," *Journal of Crohn's and Colitis*, vol. 8, no. 11, pp. 1480–1497, 2014.

[12] V. Ung, N. X. Thanh, K. Wong et al., "Real-life treatment paradigms show infliximab is cost-effective for management of ulcerative colitis," *Clinical Gastroenterology and Hepatology*, vol. 12, no. 11, pp. 1871–1878, 2014.

[13] G. Chen, Y. Yang, M. Liu et al., "Banxia xiexin decoction protects against dextran sulfate sodium-induced chronic ulcerative colitis in mice," *Journal of Ethnopharmacology*, vol. 166, pp. 149–156, 2015.

[14] R. Tilton, A. A. Paiva, J.-Q. Guan et al., "A comprehensive platform for quality control of botanical drugs (PhytomicsQC): a case study of Huangqin Tang (HQT) and PHY906," *Chinese Medicine*, vol. 5, article 30, 2010.

[15] F. Zuo et al., "Pharmacokinetic study on the multi-constituents of Huangqin-Tang decoction in rats," *Biological and Pharmaceutical Bulletin*, vol. 26, no. 7, pp. 911–919, 2003.

[16] T. Yukawa, T. Ohkusa, T. Shibuya et al., "Nested culture method improves detection of fusobacterium from stool in patients with ulcerative colitis," *Japanese Journal of Infectious Diseases*, vol. 66, no. 2, pp. 109–114, 2013.

[17] T. Nomura, T. Ohkusa, I. Okayasu et al., "Mucosa-associated bacteria in ulcerative colitis before and after antibiotic combination therapy," *Alimentary Pharmacology and Therapeutics*, vol. 21, no. 8, pp. 1017–1027, 2005.

[18] T. Ohkusa, N. Sato, T. Ogihara, K. Morita, M. Ogawa, and I. Okayasu, "Fusobacterium varium localized in the colonic mucosa of patients with ulcerative colitis stimulates species-specific antibody," *Journal of Gastroenterology and Hepatology (Australia)*, vol. 17, no. 8, pp. 849–853, 2002.

[19] W. Lam et al., "The number of intestinal bacteria is not critical for the enhancement of antitumor activity and reduction of intestinal toxicity of irinotecan by the Chinese herbal medicine PHY906 (KD018)," *BMC Complementary and Alternative Medicine*, vol. 14, 490 pages, 2014.

[20] L. T. M. Ngan, J.-K. Moon, J.-H. Kim, T. Shibamoto, and Y.-J. Ahn, "Growth-inhibiting effects of Paeonia lactiflora root steam distillate constituents and structurally related compounds on human intestinal bacteria," *World Journal of Microbiology and Biotechnology*, vol. 28, no. 4, pp. 1575–1583, 2012.

[21] W. Wang, T. Xia, and X. Yu, "Wogonin suppresses inflammatory response and maintains intestinal barrier function via TLR4-MyD88-TAK1-mediated NF-κB pathway in vitro," *Inflammation Research*, vol. 64, no. 6, pp. 423–431, 2015.

[22] H. Xu, J. Song, X. Gao et al., "Paeoniflorin attenuates lipopolysaccharide-induced permeability of endothelial cells: Involvements of F-Actin expression and phosphorylations of PI3K/AKT and PKC," *Inflammation*, vol. 36, no. 1, pp. 216–225, 2013.

[23] L.-N. Hu, X.-Y. Fang, H.-L. Liu et al., "Protective effects of 18β-glycyrrhetinic acid on LPS-induced injury in intestinal epithelial cells," *Chinese Journal of Natural Medicines*, vol. 11, no. 1, pp. 24–29, 2013.

[24] T. T. MacDonald and G. Monteleone, "Immunity, inflammation, and allergy in the gut," *Science*, vol. 307, no. 5717, pp. 1920–1925, 2005.

[25] E. G. W. Schmidt, H. L. Larsen, N. N. Kristensen, S. S. Poulsen, M. H. Claesson, and A. E. Pedersen, "B cells exposed to enterobacterial components suppress development of experimental colitis," *Inflammatory Bowel Diseases*, vol. 18, no. 2, pp. 284–293, 2012.

[26] J. C. Hoving, F. Kirstein, N. E. Nieuwenhuizen et al., "B cells that produce immunoglobulin e mediate colitis in BALB/c mice," *Gastroenterology*, vol. 142, no. 1, pp. 96–108, 2012.

[27] D. Haribhai, J. Ziegelbauer, S. Jia et al., "Alternatively activated macrophages boost induced regulatory T and Th17 cell responses during immunotherapy for colitis," *Journal of Immunology*, vol. 196, no. 8, pp. 3305–3317, 2016.

[28] W. Jiang, J. Su, X. Zhang et al., "Elevated levels of Th17 cells and Th17-related cytokines are associated with disease activity in patients with inflammatory bowel disease," *Inflammation Research*, vol. 63, no. 11, pp. 943–950, 2014.

[29] E. Owaga, R.-H. Hsieh, B. Mugendi, S. Masuku, C.-K. Shih, and J.-S. Chang, "Th17 cells as potential probiotic therapeutic targets in inflammatory bowel diseases," *International Journal of Molecular Sciences*, vol. 16, no. 9, pp. 20841–20858, 2015.

[30] S. Lim, S. Lee, J. Jeong, H. S. Choi, H. B. Chang, and D. Kim, "DW2007 ameliorates colitis and rheumatoid arthritis in mice by correcting Th17/Treg imbalance and inhibiting NF-κB activation," *Biomolecules & Therapeutics*, vol. 24, no. 6, pp. 638–649, 2016.

[31] Y. Gong, Y. Lin, N. Zhao et al., "The Th17/Treg Immune Imbalance in Ulcerative Colitis Disease in a Chinese Han Population," *Mediators of Inflammation*, vol. 2016, Article ID 7089137, 2016.

[32] J. Yao, C. Wei, and J. Y. Wang, "Effect of resveratrol on Treg/Th17 signaling and ulcerative colitis treatment in mice," *World Journal of Gastroenterology*, vol. 21, no. 21, pp. 6572–6581, 2015.

[33] Y. Zou, S.-X. Dai, H.-G. Chi et al., "Baicalin attenuates TNBS-induced colitis in rats by modulating the Th17/Treg paradigm," *Archives of Pharmacal Research*, vol. 38, no. 10, pp. 1873–1887, 2015.

[34] J. Yang, X. Yang, and M. Li, "Baicalin, a natural compound, promotes regulatory T cell differentiation," *BMC Complementary and Alternative Medicine*, vol. 12, article 64, 2012.

[35] Y. Yokoyama, K. Fukunaga, Y. Fukuda et al., "Demonstration of low-regulatory CD25High+CD4+ and high-pro-inflammatory CD28-CD4+ T-cell subsets in patients with ulcerative colitis: Modified by selective granulocyte and monocyte adsorption apheresis," *Digestive Diseases and Sciences*, vol. 52, no. 10, pp. 2725–2731, 2007.

[36] Y. Zhu et al., "$CD4^+CD29^+$T cells are blamed for the persistent inflammatory response in ulcerative colitis," *International Journal of Clinical and Experimental Pathology*, vol. 8, no. 3, pp. 2627–2637, 2015.

[37] F.-Y. Yu, S.-G. Huang, H.-Y. Zhang et al., "Effects of baicalin in CD4 + CD29 + T cell subsets of ulcerative colitis patients," *World Journal of Gastroenterology*, vol. 20, no. 41, pp. 15299–15309, 2014.

[38] N. Wang, H. Liang, and K. Zen, "Molecular mechanisms that influence the macrophage M1-M2 polarization balance," *Frontiers in Immunology*, vol. 5, article 614, 2014.

[39] W. Zhu, Z. Jin, J. Yu et al., "Baicalin ameliorates experimental inflammatory bowel disease through polarization of macrophages to an M2 phenotype," *International Immunopharmacology*, vol. 35, pp. 119–126, 2016.

[40] T. Zhai, Y. Sun, H. Li et al., "Unique immunomodulatory effect of paeoniflorin on type i and II macrophages activities," *Journal of Pharmacological Sciences*, vol. 130, no. 3, pp. 143–150, 2016.

[41] T. Chen, Z.-P. Guo, L. Wang et al., "Paeoniflorin suppresses vascular damage and the expression of E-selectin and ICAM-1 in a mouse model of cutaneous Arthus reaction," *Experimental Dermatology*, vol. 22, no. 7, pp. 453–457, 2013.

[42] S.-X. Dai, Y. Zou, Y.-L. Feng, H.-B. Liu, and X.-B. Zheng, "Baicalin down-regulates the expression of macrophage migration inhibitory factor (MIF) effectively for rats with ulcerative colitis," *Phytotherapy Research*, vol. 26, no. 4, pp. 498–504, 2012.

[43] J. L. Reyes, A. Wang, M. R. Fernando et al., "Splenic B cells from hymenolepis diminuta-infected mice ameliorate colitis independent of T cells and via cooperation with macrophages," *The Journal of Immunology*, vol. 194, no. 1, pp. 364–378, 2015.

[44] L. Wang, A. Ray, X. Jiang et al., "T regulatory cells and B cells cooperate to form a regulatory loop that maintains gut homeostasis and suppresses dextran sulfate sodium-induced colitis," *Mucosal Immunology*, vol. 8, no. 6, pp. 1297–1312, 2015.

[45] J. Zhang, H. Li, R. Huo et al., "Paeoniflorin selectively inhibits LPS-provoked B-cell function," *Journal of Pharmacological Sciences*, vol. 128, no. 1, pp. 8–16, 2015.

[46] A. K. Pandurangan, S. Ismail, Z. Saadatdoust, and N. M. Esa, "Allicin alleviates dextran sodium sulfate- (DSS-) induced ulcerative colitis in BALB/c mice," *Oxidative Medicine and Cellular Longevity*, vol. 2015, Article ID 605208, 13 pages, 2015.

[47] Y. Tan, K.-F. Zou, W. Qian, S. Chen, and X.-H. Hou, "Expression and implication of toll-like receptors TLR2, TLR4 and TLR9 in colonic mucosa of patients with ulcerative colitis," *Journal of Huazhong University of Science and Technology - Medical Science*, vol. 34, no. 5, pp. 785–790, 2014.

[48] M. Fukata et al., "Constitutive activation of epithelial TLR4 augments inflammatory responses to mucosal injury and drives colitis-associated tumorigenesis," *Inflammatory Bowel Diseases*, vol. 17, no. 7, pp. 1464–1473, 2011.

[49] M. A. Khan, C. Ma, L. A. Knodler et al., "Toll-like receptor 4 contributes to colitis development but not to host defense during Citrobacter rodentium infection in mice," *Infection and Immunity*, vol. 74, no. 5, pp. 2522–2536, 2006.

[50] L. Cui, L. Feng, Z. H. Zhang, and X. B. Jia, "The anti-inflammation effect of baicalin on experimental colitis through inhibiting TLR4/NF-κB pathway activation," *International Immunopharmacology*, vol. 23, no. 1, pp. 294–303, 2014.

[51] J. Feng et al., "Baicalin down regulates the expression of TLR4 and NFkB-p65 in colon tissue in mice with colitis induced by dextran sulfate sodium," *International Journal of Clinical and Experimental Medicine*, vol. 7, no. 11, pp. 4063–4072, 2014.

[52] J. Zhang, W. Dou, E. Zhang et al., "Paeoniflorin abrogates DSS-induced colitis via a TLR4-dependent pathway," *American Journal of Physiology - Gastrointestinal and Liver Physiology*, vol. 306, no. 1, pp. G27–G36, 2014.

[53] M. Coskun, J. Olsen, J. B. Seidelin, and O. H. Nielsen, "MAP kinases in inflammatory bowel disease," *Clinica Chimica Acta*, vol. 412, no. 7-8, pp. 513–520, 2011.

[54] O. H. Kang et al., "Inhibition of interleukin-8 production in the human colonic epithelial cell line HT-29 by 18 beta-glycyrrhetinic acid," *International Journal of Molecular Medicine*, vol. 15, no. 6, pp. 981–985, 2005.

[55] Y. H. Choi, J.-K. Bae, H.-S. Chae et al., "Isoliquiritigenin ameliorates dextran sulfate sodium-induced colitis through the inhibition of MAPK pathway," *International Immunopharmacology*, vol. 31, pp. 223–232, 2016.

[56] C. C. Chen et al., "Tumor necrosis factor-alpha-induced cyclooxygenase-2 expression via sequential activation of ceramide-dependent mitogen-activated protein kinases, and IkappaB kinase 1/2 in human alveolar epithelial cells," *Molecular Pharmacology*, vol. 59, no. 3, pp. 493–500, 2001.

[57] K. Subbaramaiah, W. J. Chung, and A. J. Dannenberg, "Ceramide regulates the transcription of cyclooxygenase-2: Evidence for involvement of extracellular signal-regulated kinase/c-Jun N-terminal kinase and p38 mitogen-activated protein kinase pathways," *Journal of Biological Chemistry*, vol. 273, no. 49, pp. 32943–32949, 1998.

[58] C. Jobin, O. Morteau, D. S. Han, and R. B. Sartor, "Specific NF-κB blockade selectively inhibits tumour necrosis factor- α-induced COX-2 but not constitutive COX-1 gene expression in HT-29 cells," *Immunology*, vol. 95, no. 4, pp. 537–543, 1998.

[59] I. I. Singer, D. W. Kawka, S. Schloemann, T. Tessner, T. Riehl, and W. F. Stenson, "Cyclooxygenase 2 is induced in colonic epithelial cells in inflammatory bowel disease," *Gastroenterology*, vol. 115, no. 2, pp. 297–306, 1998.

[60] K. M. Sakthivel and C. Guruvayoorappan, "Amentoflavone inhibits iNOS, COX-2 expression and modulates cytokine profile, NF-κB signal transduction pathways in rats with ulcerative colitis," *International Immunopharmacology*, vol. 17, no. 3, pp. 907–916, 2013.

[61] W.-Y. Jiang, G. S. Seo, Y.-C. Kim, D. H. Sohn, and S. H. Lee, "PF2405, standardized fraction of Scutellaria baicalensis, ameliorates colitis in vitro and in vivo," *Archives of Pharmacal Research*, vol. 38, no. 6, pp. 1127–1137, 2015.

[62] A. F. Hagel et al., "Plasma histamine and tumour necrosis factor-alpha levels in Crohn's disease and ulcerative colitis at various stages of disease," *Journal of physiology and pharmacology*, vol. 66, no. 4, pp. 549–556, 2015.

[63] H. Yuan, W.-S. Ji, K.-X. Wu, J.-X. Jiao, L.-H. Sun, and Y.-T. Feng, "Anti-inflammatory effect of Diammonium Glycyrrhizinate in a rat model of ulcerative colitis," *World Journal of Gastroenterology*, vol. 12, no. 28, pp. 4578–4581, 2006.

[64] Y. Sun, Y. Zhao, J. Yao et al., "Wogonoside protects against dextran sulfate sodium-induced experimental colitis in mice by inhibiting NF-κB and NLRP3 inflammasome activation," *Biochemical Pharmacology*, vol. 94, no. 2, pp. 142–154, 2015.

[65] X. Dai, L.-W. Wang, X.-Y. Jia et al., "Paeoniflorin regulates the function of human peripheral blood mononuclear cells stimulated by rhIL-1β by up-regulating Treg expression," *Immunopharmacology and Immunotoxicology*, vol. 37, no. 3, pp. 252–257, 2015.

[66] Y. Liu, J. Xiang, M. Liu, S. Wang, R. J. Lee, and H. Ding, "Protective effects of glycyrrhizic acid by rectal treatment on a TNBS-induced rat colitis model," *Journal of Pharmacy and Pharmacology*, vol. 63, no. 3, pp. 439–446, 2011.

[67] T. Kudo, S. Okamura, Y. Zhang, T. Masuo, and M. Mori, "Topical application of glycyrrhizin preparation ameliorates experimentally induced colitis in rats," *World Journal of Gastroenterology*, vol. 17, no. 17, pp. 2223–2228, 2011.

[68] X. Wang, Y. Sun, Y. Zhao et al., "Oroxyloside prevents dextran sulfate sodium-induced experimental colitis in mice by inhibiting NF-κB pathway through PPARγ activation," *Biochemical Pharmacology*, vol. 106, pp. 70–81, 2016.

[69] R. S. Patwardhan, D. Sharma, M. Thoh, R. Checker, and S. K. Sandur, "Baicalein exhibits anti-inflammatory effects via inhibition of NF-κB transactivation," *Biochemical Pharmacology*, vol. 108, pp. 75–89, 2016.

[70] G.-W. Fan, Y. Zhang, X. Jiang et al., "Anti-inflammatory activity of baicalein in LPS-stimulated RAW264.7 macrophages via estrogen receptor and NF-κB-dependent pathways," *Inflammation*, vol. 36, no. 6, pp. 1584–1591, 2013.

[71] H.-L. Chung, G. G.-L. Yue, K.-F. To, Y.-L. Su, Y. Huang, and W.-H. Ko, "Effect of Scutellariae Radix extract on experimental dextran-sulfate sodium-induced colitis in rats," *World Journal of Gastroenterology*, vol. 13, no. 42, pp. 5605–5611, 2007.

[72] A. Tuzun et al., "Oxidative stress and antioxidant capacity in patients with inflammatory bowel disease," *Clinical Biochemistry*, vol. 35, no. 7, pp. 569–572, 2002.

[73] A. Rezaie, R. D. Parker, and M. Abdollahi, "Oxidative stress and pathogenesis of inflammatory bowel disease: an epiphenomenon or the cause?" *Digestive Diseases and Sciences*, vol. 52, no. 9, pp. 2015–2021, 2007.

[74] A. D. Millar, D. S. Rampton, C. L. Chander et al., "Evaluating the antioxidant potential of new treatments for inflammatory bowel disease using a rat model of colitis," *Gut*, vol. 39, no. 3, pp. 407–415, 1996.

[75] S.-K. Ku, B.-I. Seo, and J.-H. Park, "Effect of Lonicerae Flos extracts on reflux esophagitis with antioxidant activity," *World Journal of Gastroenterology*, vol. 15, no. 38, pp. 4799–4805, 2009.

[76] P. A. Olsvik, T. Kristensen, R. Waagbø et al., "MRNA expression of antioxidant enzymes (SOD, CAT and GSH-Px) and lipid peroxidative stress in liver of Atlantic salmon (Salmo salar) exposed to hyperoxic water during smoltification," *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, vol. 141, no. 3, pp. 314–323, 2005.

[77] J. Yao, X. Cao, R. Zhang et al., "Protective effect of baicalin against experimental colitis via suppression of oxidant stress and apoptosis," *Pharmacognosy Magazine*, vol. 12, no. 47, pp. 225–234, 2016.

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2017, Article ID 1589871, 11 pages
https://doi.org/10.1155/2017/1589871

Research Article

Erchen Decoction and Linguizhugan Decoction Ameliorate Hepatic Insulin Resistance by Inhibiting IRS-1Ser307 Phosphorylation In Vivo and In Vitro

Huicun Zhang,[1,2] Na Ta,[3] Pengmin Chen,[4] and Hongbing Wang[1]

[1] *Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Beijing 100010, China*
[2] *Beijing Institute of Traditional Chinese Medicine, Beijing 100010, China*
[3] *China National Health Development Research Center, Beijing 100019, China*
[4] *China-Japan Friendship Hospital, Beijing 100029, China*

Correspondence should be addressed to Huicun Zhang; zhanghuicun728@126.com

Received 8 November 2016; Revised 6 January 2017; Accepted 27 February 2017; Published 29 May 2017

Academic Editor: Sérgio F. De Andrade

Erchen decoction (ECD) and Linguizhugan decoction (LGZGD), both are Chinese herbal formula, have been used clinically for the treatment of nonalcoholic fatty liver disease (NAFLD). However, their therapeutic mechanisms are still unclear. Because insulin resistance (IR) is a key etiological factor in the pathology of high-fat diet- (HFD-) induced NAFLD, in this study, the protective effects of ECD and LGZGD on HFD-induced insulin resistance in rats were evaluated and their mechanisms were investigated by OGTT and Western blot. The results showed that treatment with ECD and LGZGD significantly improved insulin resistance and liver damage in rats, evidenced by supported serum aminotransferase levels and the histopathological examination. ECD and LGZGD also showed significant protective effects against HFD-induced hyperlipidemia and the inhibition of the hepatocyte proliferation by palmitate. Furthermore, supplementation of ECD and LGZGD decreased TNF-α, NF-κB, and IRS-1Ser307 phosphorylation expressions in vivo and in vitro. These results indicated that ECD and LGZGD have protective effects against HFD-induced liver IR and their underlying mechanisms involve the TNF-α and insulin pathway. These findings would be beneficial for understanding of the therapeutic effects of ECD and LGZGD in treatment of NAFLD.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a disease located in hepatic lobule, and its pathological characteristics are the liver cells diffuse fatty degeneration and fat accumulation without excessive drinking history of clinical syndrome. NAFLD is composed of four pathological processes including simple fatty liver, fatty liver, fatty liver fibrosis, and fatty liver cirrhosis. At present, the disease may belong to a genetic environment-metabolic stress related diseases [1–4].

NAFLD is often associated with overweight (obesity), type 2 diabetes, and hyperlipidemia and other metabolic disorders which are related to insulin resistance (IR). The "two strikes" theory considers that insulin resistance is the first hit in the development of NAFLD and degeneration of hepatocytes is very sensitive to damage factors; the second attack comes from oxidative stress and lipid peroxidation and inflammation, which result in the second hit. At present, the research concerning NAFLD pathogenesis is mainly focused on IR, because IR is the potential abnormal factor in most patients. IR is initiating and the central part in the pathogenesis of NAFLD [5–8].

The theory of traditional Chinese medicine believes that NAFLD is due to eating too much fat and generates the disorder of spleen and stomach's transportation. Liver fails to disperse phlegm turbidity and phlegm turbidity is formed. Phlegm turbidity is obstructed in the liver and formed NAFLD at last. ECD's function is removing dampness and phlegm. ECD is partial to dryness; LGZGD's function is warming drink and strengthening spleen to eliminate dampness. LGZGD is partial to warm. They are both widely used in treating phlegm production of spleen. ECD and

LGZGD are widely applied for the treatment of the digestive, cardiovascular, and respiratory system diseases. At present, there are few researches concerning ECD and LGZGD in the treatment of insulin resistance of NAFLD, so we intend to study the similarities and differences of molecular mechanisms of ECD and LGZGD in treatment of insulin resistance of NAFLD; and it may provide theoretical guidance to clinical application for both decoctions.

2. Materials and Methods

2.1. Laboratory Animals and Cells. Experimental SD rats (Beijing Weitong Lihua Research Center for Experimental Animals)and hepatocytes of rats (BRL) were purchased from cell resource center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

2.2. Main Reagents and Drugs. DMEM medium and Fetal calf serum were obtained from GE Healthcare; anti-TNF-α antibody (sc-1350) and anti-NF-κB antibody (sc-372) were obtained from Santa Cruz Biotechnology; anti-IRS-1Ser307 antibody (AI623) was obtained from Beyotime Biotechnology, China, and anti-GAPDH (TA-08) and ECL display color liquid were purchased from Beijing Zhongshan Jinqiao Biological Technology Co., Ltd., China.

2.3. Preparation of ECD and LGZGD. ECD consists of four dried crude herbs listed as follows: *Pinellia ternata*, Pericarpium Citri Reticulatae, *Poria cocos*, and licorice. The ratio of herbs *Pinellia ternata*, Pericarpium Citri Reticulatae, *Poria cocos*, and licorice is 5 : 5 : 3 : 2. LGZGD is composed of *Poria cocos*, cassia twig, Rhizoma Atractylodis Macrocephalae, and licorice, and their ratio is 4 : 3 : 2 : 2. On the basis of standards specified in the Chinese Pharmacopoeia (2010 edition), all the herbs were provided by Beijing Tong Ren Tang Medicinal Materials company. ECD and LGZGD were prepared in our laboratory from a mixture of chopped crude herbs, extracted in distilled water at 100°C for 2 h. ECD solution was condensed to the density of 0.9 g/crude herb/ml, while the extract LGZGD had liquid density of 0.66 g/crude herb/ml and was stored at −20°C until further use.

2.4. In Vivo Experimental Design. Forty male Sprague-Dawley (SD) rats, weighing 120 ± 20 g, were obtained from Beijing Weitong Lihua Research Center for Experimental Animals; they were maintained in a temperature-controlled room (25 ± 1°C on a 12 h : 12 h light-dark cycle) in the animal center (Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Beijing, China). The study was carried out under the guidelines for animal experimentation set by them, and the protocol was approved by the animal studies ethics committee of Beijing Hospital of Traditional Chinese Medicine, Capital Medical University.

After acclimation for a week, forty rats were randomly divided into five groups of 8 rats each. One group (normal diet, ND, $n = 8$) of rats were fed with 11.4% kcal fat diet (Beijing science and cooperation Feed Technology Limited Company, Beijing, China; protein: 27.5%, carbohydrate: 65.8%, and fat: 11.4% kcal/g), and the other four groups (high-fat diet, HFD, $n = 8$; high-fat diet along with ROG and HFD + ROG, $n = 8$; high-fat diet along with ECD and HFD + ECD, $n = 8$; and high-fat diet along with LGZGD and HFD + LGZGD, $n = 8$) were fed with 33.1% kcal fat diet (Beijing science and cooperation Feed Technology Limited Company, Beijing, China; protein: 19.6%, carbohydrate: 47.1%, and fat: 33.1% kcal/g). High-fat diet lasted for 8 weeks to establish NAFLD rat model [9, 10]. From the ninth week, rats were dosed by oral gavage once per day for 4 weeks with ROG, ECD, and LGZGD of 5 ml/kg·d. After 4 weeks of treatment, blood samples were taken from the rats after anesthesia. The rats were then sacrificed and the liver was removed and stored at −80°C for subsequent analysis.

2.5. Serum Biochemical Parameter Analysis. The analysis of serum including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and levels of blood glucose (GLU), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL) were measured by 7160 automatic biochemical analyzer (Hitachi, Japan) according to the manufacturer's instructions.

2.6. Histological Examination and Assessment. Sections of the liver samples (4 μm thick) or the frozen liver tissues (5 μm thick) were stained with hematoxylin-eosin (H&E) or Oil Red O and were examined under light microscope (Olympus Medical Systems Corp., Tokyo, Japan). Nonalcoholic fatty liver disease activity score was used to evaluate seriousness of NAFLD. Steatosis (on a scale from 0 to 3), lobular inflammation (on a scale from 0 to 3), and hepatocellular ballooning (on a scale from 0 to 2) are the foundation of assessment system. Higher score demonstrates increasing severity of the disease [11].

2.7. Liver Index Detection. Liver index was calculated as a ratio (%) of the organ weight (g) to body weight (g) [12].

2.8. Oral Glucose Tolerance Test (OGTT). At the 12th weekend, rats were fasted for 6 hours and 2 g/kg of glucose was orally administered. Then, instant blood sugar apparatus was used to measure blood samples collected from tail veins at 0 min (without glucose load), 60 min, and 120 min (after glucose load) [13].

2.9. Insulin Resistance Index. Insulin resistance was calculated by means of the homeostatic model assessment index (HOMAIR). HOMA-IR = [fasting blood glucose (FBG) $*$ fasting insulin (FINS)]/22.5 [14].

2.10. Preparation of ROG-, ECD-, and LGZGD-Containing Serum. ROG-containing serum, ECD-containing serum, and Linguizhugan-containing serum groups rats were given ROG, ECD, and LGZGD by oral gavage individually twice a day for 3 consecutive days (0.5 ml/100 g body weight/time); blood was collected 1 h after the last administration via abdominal aorta and then centrifuged. Serum of the same

group was pooled, filtered through 0.22 um filter, inactivated at 56°C for 30 minutes, split, and stored at −80°C [15].

2.11. Cell Culturing. BRL cells were cultured in DMEM containing 10% fetal bovine serum with 5% CO_2 in a cell culture incubator at 37°C.

2.12. Palmitate on the Proliferation of BRL Cells. BRL cells were plated into 96-well plate at an initial concentration of 5000 cells/hole. When BRL cell reached 60% confluence, BRL cells were followed by the addition of different final concentrations of palmitate (PA) (0, 0.05, 0.1, 0.2, 0.25, and 0.5 mM) culture medium. BRL cells that were incubated with normal culture medium for 24 h and 48 h were used as negative controls in the in vitro experiments. After 24 hours and 48 hours of coculture with PA, the BRL cells' proliferation was measured by MTT assay.

2.13. ROG-, ECD-, and LGZGD-Containing Serum on the BRL Cells Viability Assay. BRL cells were plated into 96-well plate at an initial concentration of 5000 cells/hole. When BRL cell reached 60% confluence, BRL cells were divided into 5 groups: ND group, HFD group, ROG group, ECD group, and LGZGD group: the ND group, DMEM with 20% rat serum; HFD group, containing 20% rat serum and 0.25 mM palmitate; ROG group, 20% ROG containing serum and 0.25 mM palmitate; ECD group, 20% ECD containing serum and 0.25 mM palmitate; LGZGD group, 20% LGZGD containing serum and 0.25 mM palmitate. After 24 hours and 48 hours of the coculture, BRL cells' proliferation was measured by MTT assay. The OD values were compared to determine whether the ECD- and LGZGD-containing serums have an effect on cell viability.

2.14. In Vitro Experimental Design. After culturing for 24–48 hours in 6-well plates, BRL cells were divided into 5 groups: ND group, HFD group, ROG group, ECD group, and LGZGD group (just as mentioned above). Each group of cells was incubated at 37°C with 5% CO_2 for 48 hours and then total protein was extracted.

2.15. Western Blot Analysis

2.15.1. Protein Extract. The liver tissue and BRL cells treated as above were collected and lysed in ice-cold RIPA lysis buffer (Beyotime, Shanghai, China) for 30 minutes. Protein extracted by lysis buffer was transferred into a precooled EP tube and centrifuged at 20000 r/min × 10 min at 4°C. The supernatant was immediately transferred into a precooled EP tube and mixed with an equal volume of buffer solution. The sample was subsequently boiled at 100°C for 10 min and stored at −20°C. A portion was used to determine the lysate protein concentration via the BCA (Beyotime Biotechnology, China) method, and another portion was stored at −80°C.

2.15.2. Protein Analysis. 80 μg liver and 30 μg BRL cells denatured protein liquid were separated by 10% SDS gel electrophoresis (SDS-PAGE). The proteins from the gel were then transferred to nitrocellulose membrane. The membrane was blocked with 8% nonfat milk in TBST at room temperature for 1 h and then anti-TNF-α antibody (sc-1350), anti-NF-κB antibody (sc-372), anti-IRS-1Ser307 phosphorylation antibody (AI623), and anti-GAPDH antibody (TA-08) were added and incubated at 4°C overnight. After the membrane was washed three times for 10 min each in TBST, secondary antibody was added, and the membrane was incubated at 37°C for 1 h. The membrane was washed again, stained with ECL, and exposed to X-ray film. Image analysis was then performed.

2.16. Statistical Analysis. ImageJ analysis software was used to conduct the image analysis. Each image analysis result was represented as means ± standard deviation ($\overline{x} \pm s$), and a one-way analysis of variance was used to analyze data in the statistical software package SPSS 17.0. The results were considered significantly different when $P < 0.05$.

3. Results

3.1. Effects of ECD and LGZGD on Body Weight and Liver Index of HFD-Fed Rats. At the end of the 12th week, there was a significant difference in the body weight between the treatment and HFD groups (Figure 1). Compared with the ND group, the liver index of the rats in the HFD group was elevated ($P < 0.05$). ROG, ECD, and LGZGD significantly decreased liver index, and no significant difference existed in the liver index of the rats in the treatment group (Figure 1).

3.2. Effects of ECD and LGZGD on Liver Morphology and Histopathology. H&E and oil red O stained sections were used to obverse the livers' histological changes. HFD group rats' liver tissue showed abundant fat deposition and mononuclear inflammatory cells infiltration, and a large number of fat vacuoles and ballooning existed in cytoplasm (Figure 2). Histological examination showed that fatty degeneration, inflammation, and hepatocyte ballooning in ECD group, LGZGD group, and ROG group were alleviated compared to HFD group (Table 1).

3.3. Effects of ECD and LGZGD on Serum Lipids, ALT, and AST Activities of HFD-Fed Rats. At the end of administration, the serum TC, TG, LDL, ALT and AST levels of the rats in the HFD group were significantly increased compared to those of ND group ($P < 0.05$), while serum HDL level was reduced in the HFD group rats ($P < 0.05$). ROG, ECD, and LGZGD lowered TG, TC, and LDL levels when compared with the HFD group ($P < 0.05$). ROG, ECD, and LGZGD failed to effectively increase HDL ($P > 0.05$). Compared with the HFD group, except ECD, ROG and LGZGD significantly decreased the serum ALT and AST levels ($P < 0.05$), and there was no significant difference between ROG and LGZGD groups. ECD significantly decreased the serum ALT without AST ($P < 0.05$), suggesting that ECD and LGZGD could protect the liver injury induced by the HFD feeding (Table 2).

3.4. Effect of ECD and LGZGD on Oral Glucose Tolerance Test in HFD-Fed Rats. After overnight fasting for 12 h, there existed no difference in blood glucose of each group. One

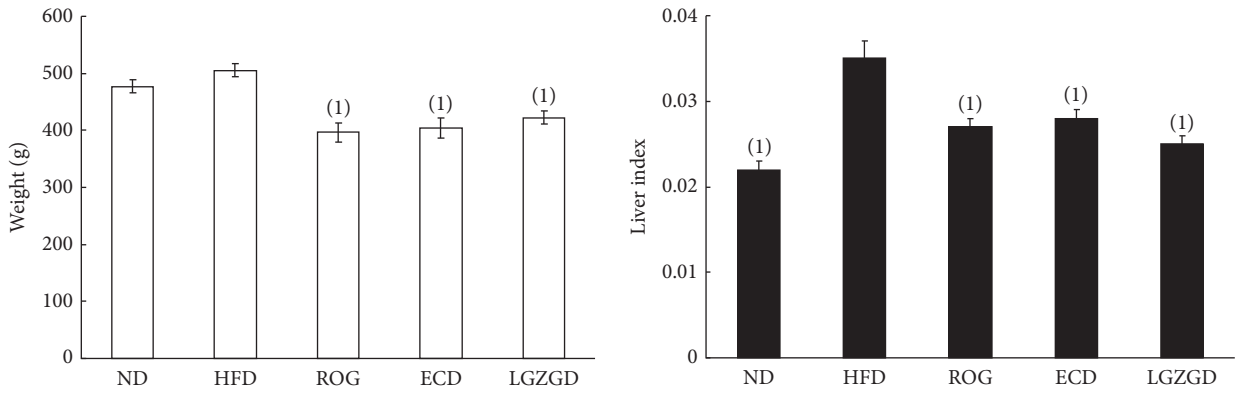


Figure 1: Rat's body weight and liver index. Compared with the HFD group, $^{(1)}P < 0.05$.

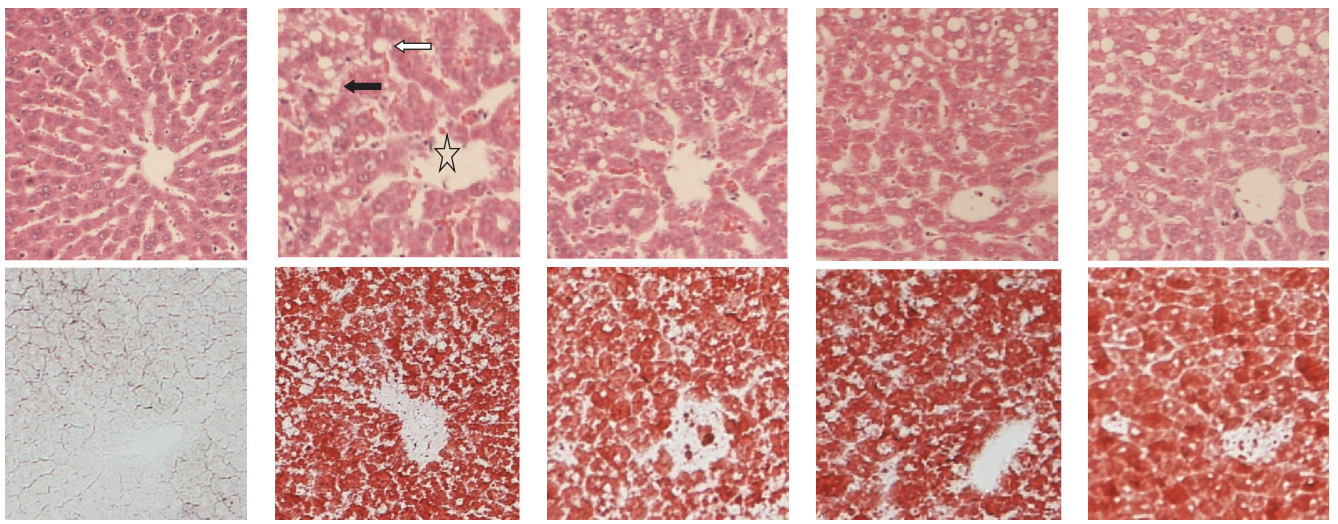


Figure 2: Effect of ECD and LGZGD on hepatic morphology and pathological changes. Histological micrograph of liver specimen from ND, HFD, HFD + ROG, HFD + ECD, and HFD + LGZGD rats (H&E staining and Oil Red O staining, magnification ×100). (a) ND group; (b) the HFD group; (c) the HFD + ROG group; (d) the HFD + ECD group; (e) the HFD + LGZGD group. The major histopathological change induced by HFD in rat's liver was hepatocyte steatosis (filled arrow) with inflammation (open pentagram) and ballooning (white arrow).

Table 1: Average score of histopathological findings in livers. ($\overline{x} \pm s$, $n = 5$).

Group	Steatosis	Inflammation	Ballooning
ND	0	0	0
HFD	2.00 ± 0.00^{a}	2.33 ± 0.52^{a}	2.00 ± 0.00^{a}
ROG	1.00 ± 0.00^{b}	1.17 ± 0.41^{b}	1.17 ± 0.75^{b}
ECD	1.00 ± 0.72^{b}	1.33 ± 0.52^{b}	1.16 ± 0.41^{b}
LGZGD	1.00 ± 0.56^{b}	1.35 ± 0.46^{b}	1.18 ± 0.65^{b}

Quantitative data are expressed as mean ± SD. Statistical analysis of the data for multiple comparisons was performed by one-way ANOVA.
$^{a}P < 0.05$ versus the ND group and $^{b}P < 0.05$ versus the HFD group.

hour after intragastric administration of glucose, ROG group, ECD group, and LGZGD group's blood glucose was significantly reduced compared to HFD group ($P < 0.05$), and there was no significant difference among the three groups ($P > 0.05$); after 120 min, HFD group's blood glucose was still significantly higher than other groups ($P < 0.05$) and blood glucose of ECD group was significantly higher than LGZGD group ($P < 0.05$).

3.5. Effect of ECD and LGZGD on Insulin Resistance Index in Rats with Nonalcoholic Fatty Liver Disease. Compared with the ND group, insulin resistance index of HFD group was obviously higher ($P < 0.05$). Compared with HFD group, insulin resistance index of ROG, ECD, and LGZGD groups was decreased ($P < 0.05$) (Figure 4).

3.6. The Inhibition of Palmitate on Proliferation of Hepatocytes. After being incubated with palmitate for 24 h, the cells proliferation was decreased significantly starting from 0.2 mM

Table 2: Rats serum lipid and ALT/AST ($\overline{x} \pm s$, $n = 8$).

Group	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	ALT (U/L)	AST (U/L)
ND	1.43 ± 0.10^{a}	0.58 ± 0.10^{a}	1.15 ± 0.085	0.21 ± 0.01^{a}	80.2 ± 4.36^{a}	175.2 ± 17.82^{a}
HFD	2.32 ± 0.47^{b}	0.80 ± 0.11^{b}	0.78 ± 0.08^{b}	0.74 ± 0.19^{b}	115.6 ± 16.76^{b}	227.0 ± 9.80^{b}
ROG	1.27 ± 0.11^{a}	0.42 ± 0.01^{a}	0.87 ± 0.06^{b}	0.29 ± 0.02^{a}	98.4 ± 2.27^{a}	190.40 ± 8.14^{a}
ECD	1.31 ± 0.09^{a}	0.40 ± 0.03^{a}	0.83 ± 0.05^{b}	0.33 ± 0.03^{a}	99.8 ± 9.88^{a}	207.80 ± 14.14
LGZGD	1.35 ± 0.08^{a}	0.34 ± 0.03^{a}	0.88 ± 0.09^{b}	0.28 ± 0.02^{a}	68.2 ± 3.21^{a}	142.00 ± 7.416^{a}

Note. Quantitative data are expressed as mean ± SD. Statistical analysis of the data for multiple comparisons was performed by ANOVA. $^{a}P < 0.05$ versus HFD group; $^{b}P < 0.05$ versus ND group.

Table 3: Inhibition of palmitate on hepatocytes' proliferation ($\overline{x} \pm s$, $n = 6$).

Palmitate (mM)	Exposure time of palmitate	
	24 hours	48 hours
0	0.40 ± 0.01	0.51 ± 0.01
0.05	0.40 ± 0.01	0.49 ± 0.01
0.10	0.41 ± 0.01	$0.43 \pm 0.02^{\blacklozenge}$
0.20	$0.31 \pm 0.01^{\diamond}$	$0.41 \pm 0.01^{\blacklozenge}$
0.25	$0.30 \pm 0.01^{\diamond}$	$0.39 \pm 0.01^{\blacklozenge}$
0.50	$0.17 \pm 0.01^{\diamond}$	$0.25 \pm 0.01^{\blacklozenge}$

Note. Different concentrations of palmitate group compared with the control group (palmitate concentration is 0 mM): $P < 0.05$. $^{\diamond,\blacklozenge}$Different concentrations of palmitate group compared with the control group (palmitate concentration is 0 mM): $P < 0.05$.

Table 4: ECD and LGZGD's effect on hepatocytes proliferation ($\overline{x} \pm s$, $n = 6$).

Cell grouping	Serum containing effect time	
	24 hours	48 hours
ND group	$0.42 \pm 0.02^{\triangle}$	$0.53 \pm 0.01^{\blacktriangle}$
HFD group	0.29 ± 0.01	0.37 ± 0.01
ROG group	0.34 ± 0.01	$0.43 \pm 0.01^{\blacktriangle}$
ECD group	$0.36 \pm 0.03^{\triangle}$	0.37 ± 0.02
LGZGD Group	$0.35 \pm 0.01^{\triangle}$	$0.45 \pm 0.02^{\blacktriangle}$

Note. $^{\triangle;\blacktriangle}$ND group and drug-containing serum group compared with the HFD group: $P < 0.05$.

palmitate. Meanwhile after 48 hours of being stimulated with palmitate, 0.1–0.5 mM palmitate significantly inhibited the liver cells proliferation (Table 3). The results showed inhibition of palmitate on liver cell proliferation with time- and dose-dependence.

3.7. ECD- and LGZGD-Containing Serum Resists Inhibition of Palmitate on Hepatocytes Viability. After being stimulated with palmitate and incubated with it for 24 h, the cells proliferation was decreased significantly. ECD and LGZGD groups had significant ($P < 0.05$) effect on the cellular proliferation when compared with HFD group. That means ECD and LGZGD can significantly antagonize 0.25 mM palmitate's inhibition on proliferation of BRL. While being stimulated with palmitate lasted 48 hours, only ROG- and LGZGD-containing serum can effectively antagonize inhibition of palmitate when compared with HFD group (Table 4).

3.8. Effects of ECD and LGZGD on TNF-α, NF-κB, and IRS-1Ser307 Phosphorylation Expressions in Hepatic Tissues of HFD-Fed Rats. Hepatic total TNF-α, NF-κB, and IRS-1Ser307 phosphorylation expressions of HFD group's rats were significantly higher than those in ND group's rats ($P < 0.05$), but they were decreased in rats in ROG, ECD, and LGZGD group. NF-κB and IRS-1Ser307 phosphorylation expressions of rats in ROG group were more than those of rats in ECD and LGZGD groups and there was no difference between rats of ECD group and rats of LGZGD group ($P > 0.05$, Figure 5).

3.9. Effects of ECD- and LGZGD-Containing Serum on TNF-α, NF-κB, and IRS-1Ser307 Phosphorylation Expressions in BRL Cells Stimulated with Palmitate. As shown in Figure 7, in BRL cells being stimulated with palmitate for 48 h, TNF-α, NF-κB, and IRS-1Ser307 phosphorylations in HFD group were overexpressed; ROG-, ECD-, and LGZGD-containing serum could decrease TNF-α, NF-κB, and IRS-1Ser307 phosphorylation expressions. Except IRS-1Ser307 phosphorylation, there was significant difference ($P < 0.05$) in TNF-α and NF-κB expressions between ECD- and LGZGD-containing serums (Figure 6).

4. Discussion

Our present study showed that the administration of ECD and LGZGD has preventive effect against hepatic insulin resistance in vivo and in vitro by decreasing of IRS-1Ser307 and TNF-α.

In China, ECD and LGZGD have been used in clinical practice to alleviate NAFLD. Previous studies showed that ECD and LGZGD could ameliorate dyslipidemia and hepatic steatosis [16, 17]. However, the underlying molecular mechanism needs to be further investigated. IRS-1Ser307 phosphorylation protein activity is recognized as a major regulator of insulin resistance. So, in this study, we observed the effect of ECD and LGZGD on IRS-1Ser307 phosphorylation protein expression and the related pathway in insulin resistance in

vitro and in vivo. Rosiglitazone improves insulin resistance by reducing the livers' free fatty acid utilization, TNF-α release, and IRS-1Ser307 phosphorylation expression [18–22], so we take rosiglitazone as a positive control.

To evaluate the protective effect of ECD and LGZGD on NAFLD IR injury in vivo, we used HFD for 8 weeks to duplicate fatty liver model in rats. In vivo animal experiments showed that ROG, ECD, and LGZGD could decrease abnormal HOMA-IR caused by HFD. At the same time, ROG, ECD, and LGZGD could improve abnormal blood lipid as follows. For the rats in HFD groups, serum TG, LDL, and serum ALT and AST levels were higher compared to normal control group, while for HFD groups, HDL was lower than normal control group. These results indicated that the fatty liver model with triglycerides accumulation in the liver was induced successfully by the high-fat diet. Using these model rats, we demonstrated that ECD and LGZGD can reduce serum TG, TC, and LDL and there was no obvious difference among them. ROG, ECD, and LGZGD failed to significantly increase HDL (Table 2). ROG and LGZGD can significantly decrease the level of serum ALT and AST levels and LGZGD is better than ROG, while ECD remarkably decreases the level of serum ALT (Table 2).

In this study, compared with the ND group, although the weight of the rats in the HFD group did not increase significantly ($P > 0.05$), the liver index was elevated ($P < 0.05$). The difference of liver index in HFD group and ND group was due to the liver weight of rats in HFD group which was heavier than that of ND group's rats. Some reports found that, when compared with the ND group, the weight of the rats in the HFD group increases significantly. But there was no significant difference between the weight of the rats in the HFD group and that of the rats in the ND group in our study. We ascribed it to the difference of composition of high-fat diet between our study and others. In our study, HFD rats were fed with 33.1% kcal fat diet, while rats in HFD group were fed with 45–60% kcal fat diet in previous studies [23–25].

Insulin signal transduction change is the main factor for the fatty liver. Studies have shown that IRS-1Ser307 phosphorylation plays an important role in mediating negative feedback regulation of insulin transduction. It not only interferes in interaction between IRS-1 and insulin receptor but also hinders the role of IRS-1 tyrosine's phosphorylation. IRS-1 tyrosine's phosphorylation is downstream of insulin signaling. ECD and LGZGD have been used in clinical practice to alleviate NAFLD in China. Previous studies showed that ECD and LGZGD could ameliorate dyslipidemia and hepatic steatosis [16, 17]. However, the underlying molecular mechanism needs to be further investigated.

Considering the key role of IR activation in regulating lipid metabolism, we hypothesized that IR may play a key role in the effects of ECD and LGZGD against hepatic lipid accumulation. So, in this study, we observed the effect of ECD and LGZGD on IR both in vitro and in vivo. This research showed that ECD and LGZGD could decrease insulin resistance index (Figure 5) and improve NAFLD rats' abnormal glucose tolerance caused by high-fat diet. As for oral glucose tolerance test (OGTT), compared with HFD group, ROG, ECD, and LGZGD can effectively reduce 1-hour and 2-hour postprandial blood glucose ($P < 0.05$). 2-Hour postprandial blood sugar of LGZGD group was lower than that of ECD group (Figure 3). These findings indicated that ECD and LGZGD could ameliorate IR induced by HFD.

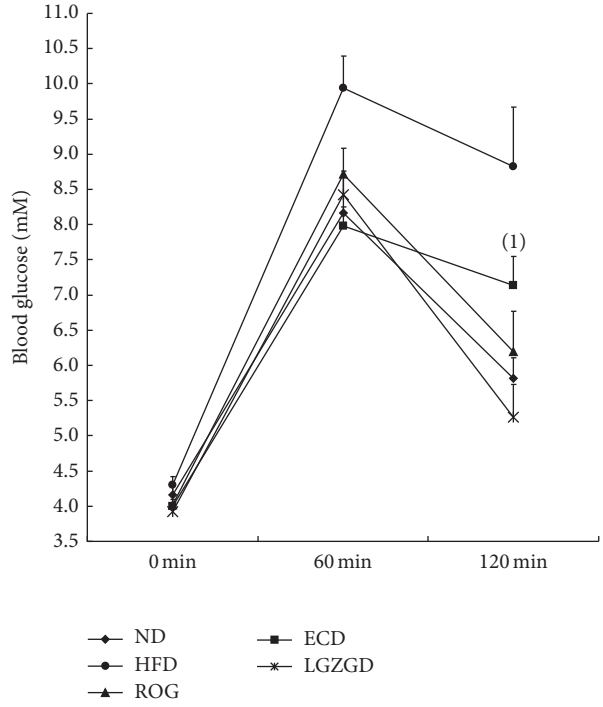


Figure 3: ECD and LGZGD on OGTT. $^{(1)}P < 0.05$ versus the LGZGD group.

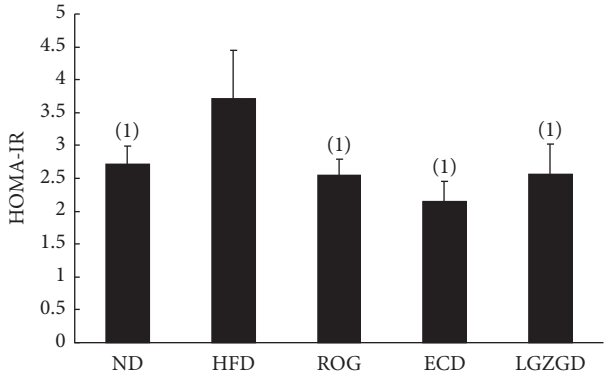


Figure 4: ECD and LGZGD on insulin resistance index. $^{(1)}P < 0.05$ versus the HFD group.

Therefore, in order to elucidate some of the underlying mechanisms involved in the protective effects of ECD and LGZGD on HFD-induced IR, the expression of IRS-1Ser307 phosphorylation associated with IR was detected by Western blot. Although ROG can effectively inhibit IRS-1Ser307 phosphorylation caused by HFD, the inhibition ability was weaker than ECD and LGZGD ($P < 0.05$). ECD and LGZGD could significantly inhibit IRS-1Ser307 phosphorylation but

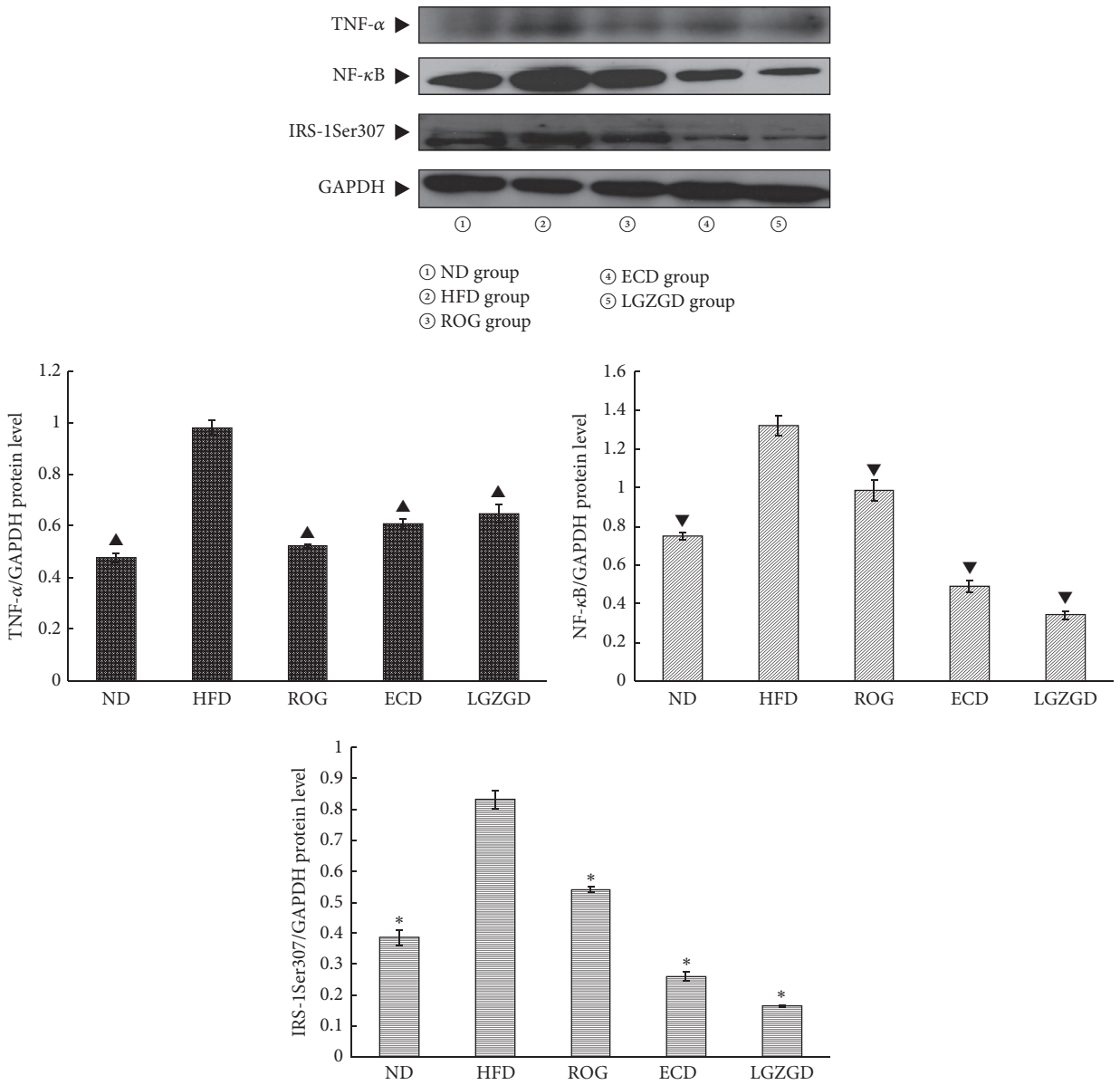


Figure 5: ECD and LGZGD on TNF-α, NF-κB, and IRS-1Ser307 phosphorylation expressions in hepatic tissues of HFD-fed rats. $^{\blacktriangle;\blacktriangledown;*}P < 0.05$ versus the HFD group.

inhibition of ECD was weaker than that of LGZGD (Figures 5 and 6).

TNF-α and NF-κB are involved in the pathogenesis of NAFLD. TNF-α makes IRS-1 serine phosphorylation and reduces the activity of insulin signaling pathway; moreover, TNF-α strengthens the lipolysis of adipose tissue and then more free fatty acids promote the secretion of TNF-α by TLR4/NF-κB pathway. Excessive TNF-α leads to abnormal mitochondria, oxidative stress, fatty acid beta oxidation overload, NF-κB overexpression, and fat deposition in the liver. TNF-α and NF-κB interact with each other, and they became vicious circle [26–28]. Therefore, inhibiting NF-κB activation and reducing the expression of TNF-α and IRS-1Ser307 phosphorylation were of great significance for the prevention and treatment of NAFLD insulin resistance. ECD and LGZGD seem to reverse the regulation of HFD on these genes.

In order to study further mechanism of ECD and LGZGD improving NAFLD IR in vitro, BRL cells, a kind of rat hepatocytes, were incubated with palmitate. We used 0.25 mM palmitate to stimulate BRL cells for 48 h to establish cellular NAFLD model. Free fatty acids (FFAs) have direct toxic effects on cell function and apoptosis. FFAs are composed of saturated fatty acid and unsaturated fatty acid. Damage of saturated fatty acid is much more than that of unsaturated fatty acids. Palmitate is the main component of saturated fatty acids [29, 30]. Palmitate inhibits on liver cell proliferation with time and dose dependence. The FFA from adipose tissue

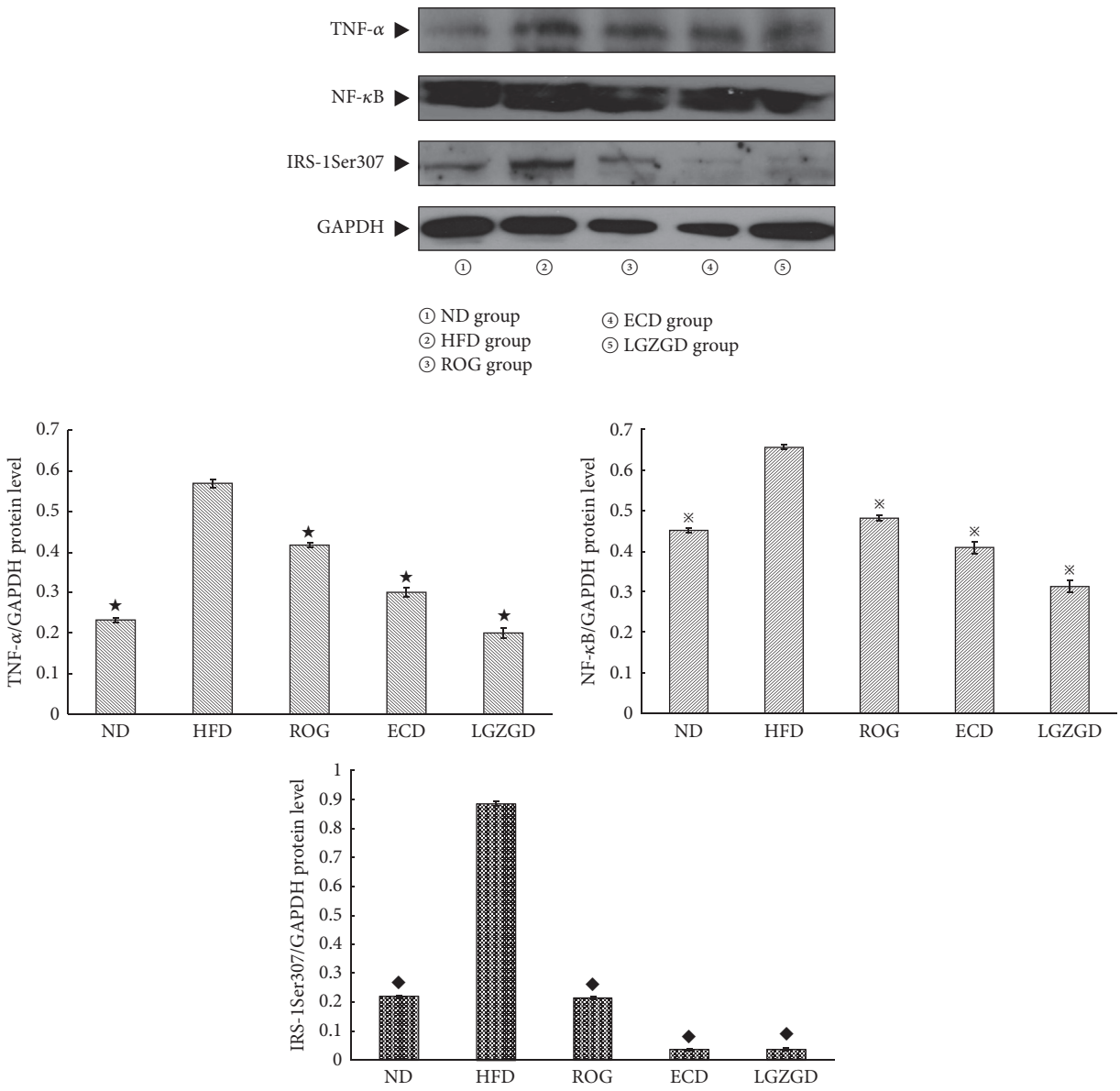
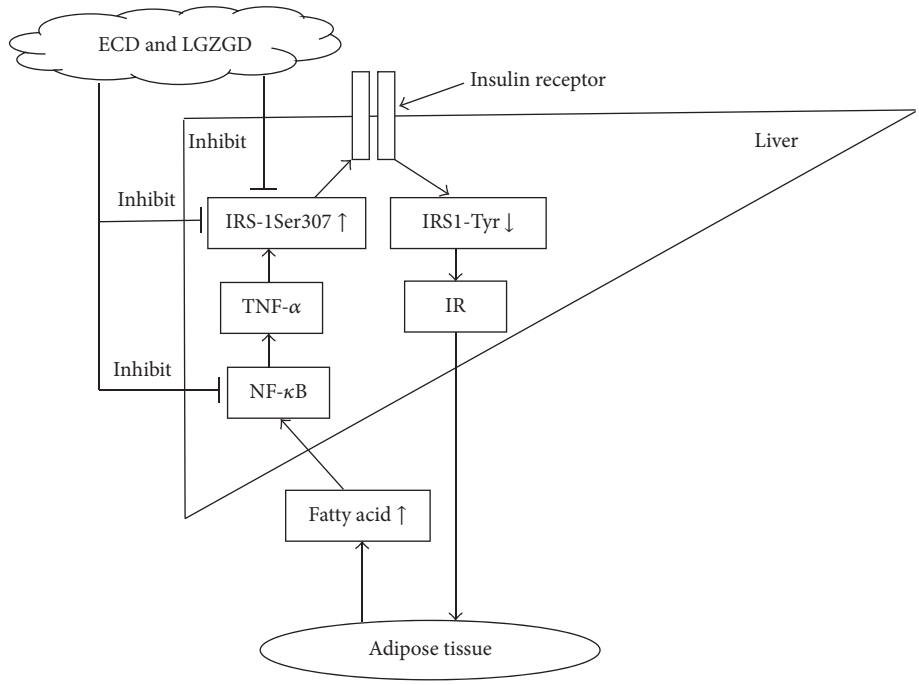


Figure 6: ECD and LGZGD serum containing on TNF-α, NF-κB, and IRS-1Ser307 phosphorylation expressions in BRL cells stimulated with palmitate. $^{\bigstar;※;\blacklozenge}P < 0.05$ versus the HFD group.

is the main source of liver fat, which accounts for 62%–82% of TG in liver [31]. Chinese medicines have numerous chemical compositions and possess multiple targets in human body. When traditional Chinese medicines are through stomach, intestine, and liver digestion and absorption, major medicinal effective ingredients go into the blood plasma and the effect of Chinese composite recipe will play a role [15]. Therefore, to reproduce the features of ECD and LGZGD after metabolism in digestive system, we prepared ECD- and LGZGD-containing serum. The same as the results in the animal study, supplementation of ECD and LGZGD decreased the phosphorylation levels of IRS-1Ser307 and TNF-α and hepatic nuclear protein expression of NF-κB in BRL cells induced by palmitate.

From the preceding discussion, we presume that ECD and LGZGD significantly not only inhibit TNF-α, NF-κB, and IRS-1Ser307 phosphorylation expression and improve insulin resistance but also decrease free fatty acids released by adipose tissue lipolysis and then decrease hepatic de novo lipogenesis and fat accumulation (Figure 7).

ECD and LGZGD are prepared from aqueous extracts of 4 medicinal herbs and two of them are the same. *Pinellia ternata*, Pericarpium Citri Reticulatae, *Poria cocos*, licorice, cassia twig, and Rhizoma Atractylodis Macrocephalae have been reported to possess anti-inflammatory effect [32–41]. In addition, cassia twig and Rhizoma Atractylodis Macrocephalae, two main constituents of LGZD, also showed antioxidant, antiadipogenic, and antiobesity activities and modulation of



↑: Increase; ↓: decrease; IRS-1Ser307; insulin receptor substrate-1 307 serine phosphorylation; IRS1-Tyr: insulin receptor substrate-1 tyrosine phosphorylation

Figure 7: ECD and LGZGD improve hepatic insulin resistance by inhibiting TNF-α, NF-κB, and IRS-1Ser307 phosphorylation expressions.

the gut microbial distribution [40]. Therefore, although ECD and LGZGD may contain hundreds of different chemical compounds, their active ingredients responsible for improving IR and liver protective activities are still unclear. Those researches mentioned above may explain ECD and LGZGD's activities partly.

Although ECD and LGZGD did not have any obviously antagonistic effect on the treatment of NAFLD IR in this study, further systematically experimental study is needed to identify whether they have some side effects.

5. Conclusion

Based on the above results indicating that the administration of ECD and LGZGD can suppress the development of HFD-induced fatty liver, we presume that inhibition of TNF-α, NF-κB, and IRS-1Ser307 phosphorylation expressions might be major contributor to the beneficial effects of ECD and LGZGD on decreasing hepatic lipid accumulation caused by IR. From these, the theoretical basis will be provided for the future clinical drug clinical application.

Abbreviations

IRS-1Ser307 phosphorylation: Insulin receptor substrate-1 Ser307 phosphorylation
TNF-α: Tumor necrosis factor-α
NF-κB: Nuclear transcription factor-κB
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
PA: Palmitate
FFA: Free fatty acid
HDL-C: High-density lipoprotein cholesterol
LDL-C: Low-density lipoprotein cholesterol
NAFLD: Nonalcoholic fatty liver disease
NASH: Nonalcoholic steatohepatitis
TC: Total cholesterol
TG: Triglyceride.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The Fund of Beijing Municipal Administration of Hospitals Incubating Program (Grant no. PZ, 2016013), the Cooperation Fund of Basic and Clinical Research of Capital Medical University (Grant no. 16JL24), the Fund of Beijing Hospital of Traditional Chinese Medicine (Grant no. YJ-201722), and the Fund for Beijing Science & Technology Development of TCM (QN2016-24) are acknowledged.

References

[1] A. C. Guidorizzi de Siqueira, H. P. Cotrim, R. Rocha et al., "Non-alcoholic fatty liver disease and insulin resistance: importance of risk factors and histological spectrum," *European Journal of Gastroenterology and Hepatology*, vol. 17, no. 8, pp. 837–841, 2005.

[2] L. M. Grant and M. Lisker-Melman, "Nonalcoholic fatty liver disease," *Annals of Hepatology*, vol. 3, no. 3, pp. 93–99, 2004.

[3] D. M. S. Ferreira, R. E. Castro, M. V. MacHado et al., "Apoptosis and insulin resistance in liver and peripheral tissues of morbidly obese patients is associated with different stages of non-alcoholic fatty liver disease," *Diabetologia*, vol. 54, no. 7, pp. 1788–1798, 2011.

[4] G. Luef, M. Rauchenzauner, M. Waldmann et al., "Non-alcoholic fatty liver disease (NAFLD), insulin resistance and lipid profile in antiepileptic drug treatment," *Epilepsy Research*, vol. 86, no. 1, pp. 42–47, 2009.

[5] G. Marchesini, E. Bugianesi, G. Forlani et al., "Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome," *Hepatology*, vol. 37, no. 4, pp. 917–923, 2003.

[6] A. J. G. Hanley, K. Williams, A. Festa, L. E. Wagenknecht, R. B. D'Agostino Jr., and S. M. Haffner, "Liver markers and development of the metabolic syndrome: The Insulin Resistance Atherosclerosis Study," *Diabetes*, vol. 54, no. 11, pp. 3140–3147, 2005.

[7] S. Xanthakos, L. Miles, J. Bucuvalas, S. Daniels, V. Garcia, and T. Inge, "Histologic spectrum of nonalcoholic fatty liver disease in morbidly obese adolescents," *Clinical Gastroenterology and Hepatology*, vol. 4, no. 2, pp. 226–232, 2006.

[8] J. K. Hae, J. K. Hyeong, E. L. Kwang et al., "Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults," *Archives of Internal Medicine*, vol. 164, no. 19, pp. 2169–2175, 2004.

[9] S. N. K. Al-Shaaibi, M. I. Waly, L. Al-Subhi, M. H. Tageldin, N. M. Al-Balushi, and M. S. Rahman, "Ameliorative effects of pomegranate peel extract against dietary-induced nonalcoholic fatty liver in rats," *Preventive Nutrition and Food Science*, vol. 21, no. 1, pp. 14–23, 2016.

[10] J. Liu, L. Han, L. Zhu, and Y. Yu, "Free fatty acids, not triglycerides, are associated with non-alcoholic liver injury progression in high fat diet induced obese rats," *Lipids in Health and Disease*, vol. 15, no. 1, article 27, 2016.

[11] D. E. Kleiner, E. M. Brunt, M. Van Natta et al., "Design and validation of a histological scoring system for nonalcoholic fatty liver disease," *Hepatology*, vol. 41, no. 6, pp. 1313–1321, 2005.

[12] Y.-X. Chen, L.-N. Lai, H.-Y. Zhang et al., "Effect of artesunate supplementation on bacterial translocation and dysbiosis of gut microbiota in rats with liver cirrhosis," *World Journal of Gastroenterology*, vol. 22, no. 10, pp. 2949–2959, 2016.

[13] Q. Zhang, X. Xiao, M. Li et al., "Berberine moderates glucose metabolism through the GnRH-GLP-1 and MAPK pathways in the intestine," *BMC Complementary and Alternative Medicine*, vol. 14, article 188, 2014.

[14] G. Mattace Raso, R. Simeoli, R. Russo et al., "Effects of sodium butyrate and its synthetic amide derivative on liver inflammation and glucose tolerance in an animal model of steatosis induced by high fat diet," *PLoS ONE*, vol. 8, no. 7, Article ID e68626, 2013.

[15] Z.-Z. Meng, J.-H. Hu, J.-X. Chen, and G.-X. Yue, "Xiaoyaosan decoction, a traditional Chinese medicine, inhibits oxidative-stress-induced hippocampus neuron apoptosis in vitro," *Evidence-based Complementary and Alternative Medicine*, vol. 2012, Article ID 489254, 2012.

[16] T. liu, L. Yang, L. Zhang, H. Song, D. Li, and G. Ji, "The discrepancies of different traditional chinese medicine prescription against nonalcoholic fatty liver," *Zhing Xi Yi Jie He Xue Bao*, no. 10, pp. 1120–1126, 2009.

[17] P. Zhang, "Modified er chen decoction for treatment of 137 cases of nonalcoholic fatty liver disease," *Jiang Shu Zhong Yi Yao*, no. 2, pp. 33–34, 2013.

[18] P. Aramwit, P. Bunmee, and O. Supasyndh, "Effectiveness and tolerability of rosiglitazone on insulin resistance and body composition in nondiabetic Thai patients undergoing continuous ambulatory peritoneal dialysis: a 12-week pilot study," *Current Therapeutic Research—Clinical and Experimental*, vol. 70, no. 5, pp. 377–389, 2009.

[19] R.-T. Lin, H.-C. Pai, Y.-C. Lee et al., "Electroacupuncture and rosiglitazone combined therapy as a means of treating insulin resistance and type 2 diabetes mellitus: a randomized controlled trial," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 969824, 9 pages, 2013.

[20] T. Basturk, A. Unsal, T. Ulas et al., "Effects of rosiglitazone treatment on insulin resistance and TNF-alpha levels in patients with chronic kidney disease: a prospective study," *European Review for Medical and Pharmacological Sciences*, vol. 16, no. 11, pp. 1519–1524, 2012.

[21] X. Zhou and S. You, "Rosiglitazone inhibits hepatic insulin resistance induced by chronic pancreatitis and IKK-β/NF-κB expression in liver," *Pancreas*, vol. 43, no. 8, pp. 1291–1298, 2014.

[22] G. Jiang, Q. Dallas-Yang, S. Biswas, Z. Li, and B. B. Zhang, "Rosiglitazone, an agonist of peroxisome-proliferator-activated receptor γ (PPARγ), decreases inhibitory serine phosphorylation of IRS1 in vitro and in vivo," *Biochemical Journal*, vol. 377, no. 2, pp. 339–346, 2004.

[23] S. Ezquerro, L. Mendez-Gimenez, S. Becerril et al., "Acylated and desacyl ghrelin are associated with hepatic lipogenesis, beta-oxidation and autophagy: role in NAFLD amelioration after sleeve gastrectomy in obese rats," *Scientific Reports*, vol. 6, Article ID 39942, 2016.

[24] C. Park, J. Kim, E. B. Lee et al., "Aronia melanocarpa extract ameliorates hepatic lipid metabolism through PPARγ2 down-regulation," *PLoS ONE*, vol. 12, no. 1, Article ID e0169685, 2017.

[25] W. Chiang, C. Y. Huang, C. R. Paul, Z. Lee, and W. Lin, "Lipolysis stimulating peptides of potato protein hydrolysate effectively suppresses high-fat-diet-induced hepatocyte apoptosis and fibrosis in aging rats," *Food & Nutrition Research*, vol. 60, no. 1, Article ID 31417, 2017.

[26] S. E. Shoelson, L. Herrero, and A. Naaz, "Obesity, inflammation, and insulin resistance," *Gastroenterology*, vol. 132, no. 6, pp. 2169–2180, 2007.

[27] G. N. Chaldakov, I. S. Stankulov, M. Hristova, and P. I. Ghenev, "Adipobiology of disease: adipokines and adipokine-targeted pharmacology," *Current Pharmaceutical Design*, vol. 9, no. 12, pp. 1023–1031, 2003.

[28] M. Saghizadeh, J. M. Ong, W. T. Garvey, R. R. Henry, and P. A. Kern, "The expression of TNF alpha by human muscle. Relationship to insulin resistance," *The Journal of Clinical Investigation*, vol. 97, no. 4, pp. 1111–1116, 1996.

[29] G. Musso, R. Gambino, and M. Cassader, "Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD)," *Progress in Lipid Research*, vol. 48, no. 1, pp. 1–26, 2009.

[30] S. H. Ibrahim, R. Kohli, and G. J. Gores, "Mechanisms of lipotoxicity in NAFLD and clinical implications," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 53, no. 2, pp. 131–140, 2011.

[31] A. E. Feldstein, N. W. Werneburg, A. Canbay et al., "Free fatty acids promote hepatic lipotoxicity by stimulating TNF-α expression via a lysosomal pathway," *Hepatology*, vol. 40, no. 1, pp. 185–194, 2004.

[32] F.-H. Zheng, P. Wei, H.-L. Huo et al., "Neuroprotective effect of gui zhi (ramulus cinnamomi) on ma huang- (herb ephedra-) induced toxicity in rats treated with a ma huang-gui zhi herb pair," *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 913461, 2015.

[33] H.-L. Yu, T.-F. Zhao, H. Wu et al., "Pinellia ternata lectin exerts a pro-inflammatory effect on macrophages by inducing the release of pro-inflammatory cytokines, the activation of the nuclear factor-κB signaling pathway and the overproduction of reactive oxygen species," *International Journal of Molecular Medicine*, vol. 36, no. 4, pp. 1127–1135, 2015.

[34] J.-H. Wang, S. Bose, H.-G. Kim, K.-S. Han, and H. Kim, "Fermented Rhizoma Atractylodis Macrocephalae alleviates high fat diet-induced obesity in association with regulation of intestinal permeability and microbiota in rats," *Scientific Reports*, vol. 5, article 8391, 2015.

[35] J.-L. Ríos, "Chemical constituents and pharmacological properties of poria cocos," *Planta Medica*, vol. 77, no. 7, pp. 681–691, 2011.

[36] X. Li, P. Lu, W. Zhang, B. Li, R. Yang, and K. Luo, "Study on anti-Ehrlich ascites tumour effect of Pinellia ternata polysaccharide in vivo," *African Journal of Traditional, Complementary, and Alternative Medicines*, vol. 10, no. 5, pp. 380–385, 2013.

[37] L. Wang, R. Yang, B. Yuan, Y. Liu, and C. Liu, "The antiviral and antimicrobial activities of licorice, a widely-used Chinese herb," *Acta Pharmaceutica Sinica B*, vol. 5, no. 4, pp. 310–315, 2015.

[38] T.-C. Kao, C.-H. Wu, and G.-C. Yen, "Bioactivity and potential health benefits of licorice," *Journal of Agricultural and Food Chemistry*, vol. 62, no. 3, pp. 542–553, 2014.

[39] Q.-C. Huang, M.-J. Wang, X.-M. Chen et al., "Can active components of licorice, glycyrrhizin and glycyrrhetinic acid, lick rheumatoid arthritis?" *Oncotarget*, vol. 7, no. 2, pp. 1193–1202, 2015.

[40] B. Han, Y. Gao, Y. Wang et al., "Protective effect of a polysaccharide from Rhizoma Atractylodis Macrocephalae on acute liver injury in mice," *International Journal of Biological Macromolecules*, vol. 87, pp. 85–91, 2016.

[41] S. Bose and H. Kim, "Evaluation of in vitro anti-inflammatory activities and protective effect of fermented preparations of rhizoma atractylodis macrocephalae on intestinal barrier function against lipopolysaccharide insult," *Evidence-based Complementary and Alternative Medicine*, vol. 2013, Article ID 363076, 2013.