Drug Design, Development and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Combining Metagenomics, Network Pharmacology and RNA-Seq Strategies to Reveal the Therapeutic Effects and Mechanisms of Qingchang Wenzhong Decoction on Inflammatory Bowel Disease in Mice

Yali Yuan[1,2,*], Hairong Hu[1,*], Zhongmei Sun[3,*], Wenting Wang[4], Zhibin Wang[1], Mengyu Zheng[5], Yunqi Xing[1], Wenji Zhang[1], Muyuan Wang[1], Xinyu Lu[1], Yitong Li[1], Chengtao Liang[1], Zhengdao Lin[1], Chune Xie[6], Junxiang Li[1], Tangyou Mao[1]

[1]Dongfang Hospital, Beijing University of Chinese Medicine, Beijing, People's Republic of China; [2]Hebei North University, Zhangjiakou, Hebei, People's Republic of China; [3]Tianjin Nankai Hospital, Tianjin, People's Republic of China; [4]Beitaipingzhuang Community Health Service Center, Beijing, People's Republic of China; [5]King's College, London, UK; [6]Shenzhen Bao'an Traditional Chinese Medicine Hospital, Shenzhen, People's Republic of China

*These authors contributed equally to this work

Correspondence: Tangyou Mao; Junxiang Li, Email maotangyouqun@126.com; lijunxiang1226@163.com

Background: Inflammatory bowel disease (IBD) is a chronic and recurrent inflammatory disease that lacks effective treatments. Qingchang Wenzhong Decoction (QCWZD) is a clinically effective herbal prescription that has been proven to attenuate intestinal inflammation in IBD. However, its molecular mechanism of action has not been clearly elucidated.

Purpose: We aimed to probe the mechanism of QCWZD for the treatment of IBD.

Methods: The dextran sulfate sodium (DSS)-induced mouse model of IBD was used to identify the molecular targets involved in the mechanism of action of QCWZD. Metagenomics sequencing was utilized to analyze the differences in gut microbiota and the functional consequences of these changes. Network pharmacology combined with RNA sequencing (RNA-seq) were employed to predict the molecular targets and mechanism of action of QCWZD, and were validated through in vivo experiments.

Results: Our results demonstrated that QCWZD treatment alleviated intestinal inflammation and accelerated intestinal mucosal healing that involved restoration of microbial homeostasis. This hypothesis was supported by the results of bacterial metagenomics sequencing that showed attenuation of gut dysbiosis by QCWZD treatment, especially the depletion of the pathogenic bacterial genus *Bacteroides*, while increasing the beneficial microorganism *Akkermansia muciniphila* that led to altered bacterial gene functions, such as metabolic regulation. Network pharmacology and RNA-seq analyses showed that Th17 cell differentiation plays an important role in QCWZD-based treatment of IBD. This was confirmed by in vivo experiments showing a marked decrease in the percentage of $CD3^+CD4^+IL\text{-}17^+$ (Th17) cells. Furthermore, our results also showed that the key factors associated with Th17 cell differentiation (IL-17, NF-κB, TNF-α and IL-6) in the colon were significantly reduced in QCWZD-treated colitis mice.

Conclusion: QCWZD exerted beneficial effects in the treatment of IBD by modulating microbial homeostasis while inhibiting Th17 cell differentiation and its associated pathways, providing a novel and promising therapeutic strategy for the treatment of IBD.

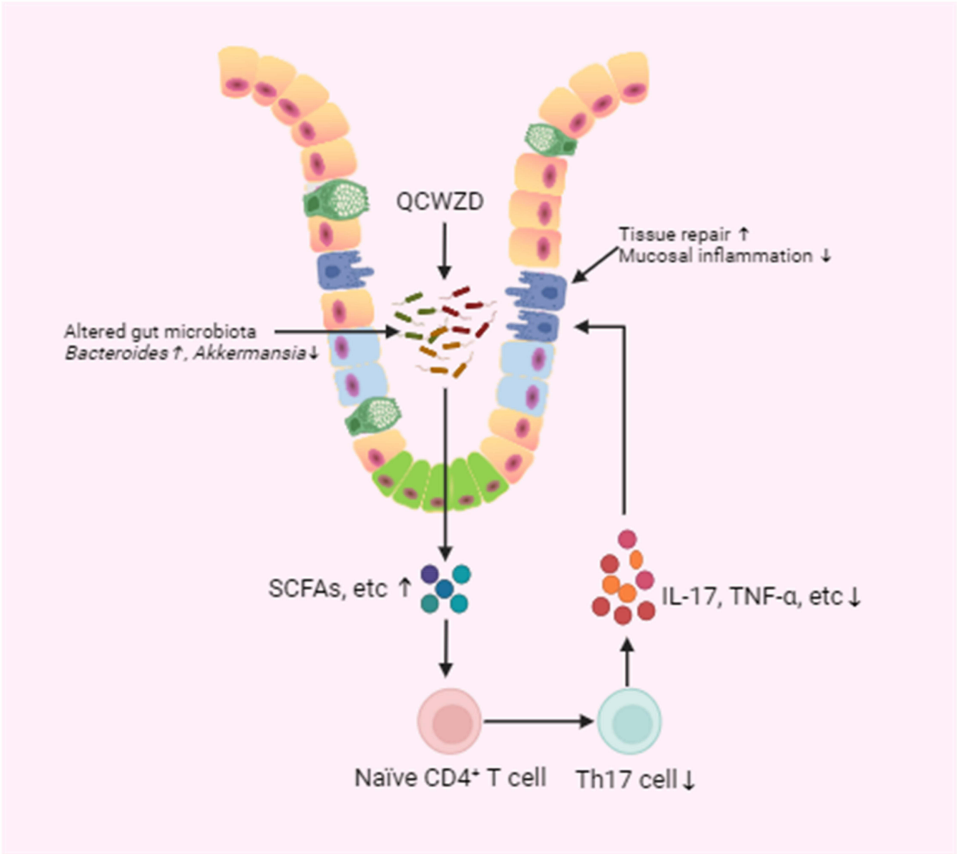
Keywords: intestinal bowel disease, Qingchang Wenzhong Decoction, microbial homeostasis, Th17 cells, mucosal immunity

Introduction

Inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), is characterized by chronic recurrent diffuse inflammatory changes in the colorectal mucosa.[1,2] In recent decades, the incidence and prevalence of IBD have consistently increased throughout the world, becoming a global public health challenge,

Received: 1 July 2024
Accepted: 10 September 2024
Published: 24 September 2024

Graphical Abstract



especially in North America and Western Europe.[3] The currently approved treatments for IBD do not produce satisfactory results due to several limitations and related side effects such as low responsiveness, opportunistic infections, and refractoriness.[4,5] Therefore, there is an urgent need to develop novel and safe therapies for IBD.

Traditional Chinese Medicine (TCM), an ancient system of medicine with thousands of years of clinical practice history in China to prevent and treat diseases, focuses on the integrity of the human body and Nature. TCM has been able to produce effective and comprehensive therapeutic effects on diseases through medicinal decoctions prepared by the combination of various natural products taken from Nature.[6,7] Driven by modern technology, TCM has received extensive attention and shown many advances in treating IBD due to its multi-component, multi-targeted and multi-pathway therapeutic approach.[8–10] Qingchang Wenzhong Decoction (QCWZD) is an effective traditional herbal prescription for the treatment of IBD that is composed of *Coptis chinensis* Franch, *Zingiber officinale* Roscoe, *Sophora flavescens* Aiton, *Strobilanthes cusia* (Nees) Kuntze, *Sanguisorba officinalis* L, *Dolomiaea costus* (Falc). Kasana & A. K. Pandey, *Panax notoginseng* (Burkill) F. H. Chen, and *Glycyrrhiza glabra* L (All plants names have been checked with http://mpns.kew.org on August 8, 2024). Our previous study[11] found that its main components include ginsenoside Rb1, gallic acid, berberine hydrochloride, ginsenoside Rg1, and liquiritin, and so on. Research has found that ginsenoside Rb1 exhibits anti-inflammatory, anti-oxidant, anti-apoptotic, and anti-autophagy properties,[12] gallic acid has great potential in maintaining intestinal health through its anti-inflammatory and antibacterial properties, as well as regulating immune responses,[13] berberine hydrochloride has the effect of reducing intestinal inflammation and regulating gut microbiota in mice,[14] ginsenoside Rg1 protects hippocampal neurons, improves synaptic plasticity, enhances cognitive function, and boosts immunity,[15] liquiritin can target Th17 cells differentiation.[16] In our

previous prospective, randomized, double-blind, and double-dummy clinical study, we showed that QCWZD significantly improved the clinical symptoms, promoted intestinal mucosal healing, and improved the quality of life of patients, suggesting that QCWZD may be a novel therapeutic option for IBD.[17] Moreover, in a mouse model of colitis, we found that QCWZD treatment alleviated intestinal inflammation and accelerated intestinal mucosal healing, whose mechanism was related to the modulation of intestinal dysbiosis.[11] However, due to the nature of bacterial 16S rRNA amplicon sequencing, the specific types and functions of regulating microbiota were still unclear. Metagenomics sequencing is a new microbial research method that uses high-throughput sequencing technology to sequence and analyze the genomes of all microbial genetic materials in environmental samples, including culturable and unculturable microorganisms. The purpose of this method is to study microbial diversity, population structure, evolutionary relationships, functional activity, interaction relationships and relationships with the environment.[18] Compared to 16S rRNA amplicon sequencing, metagenomics sequencing can isolate entire genes and allows much deeper characterization of the microbiome complexity, as well as the functional status of genes identified from the screening.[18] Therefore, it can better reveal the role of gut microbiota. In this study, metagenomics sequencing was utilized to further analyze the differences in gut microbiota at different phylogenetic levels, especially the species level, as well as analyzing the functional consequences of key genes in the altered microbiota of a dextran sulfate sodium (DSS)-induced mouse model of colitis.

Meanwhile, TCM has the characteristics of multi-component, multi-targeted, and multi-pathway therapy. Network pharmacology is based on a variety of emerging technologies and concepts, providing a new method to predict the mechanism of action of drugs and provide data on the occurrence and progression of diseases from multi-component, multi-targeted pathways. At present, network pharmacology is being extensively used in TCM because of its holistic and systematic characteristics.[19,20] In this study, network pharmacology was combined with an RNA-seq strategy to predict possible targets of QCWZD. Our data showed that T helper cell 17 (Th17) differentiation played an important role in QCWZD treatment of IBD. This finding was confirmed by in vivo experiments showing a marked decrease in the percentage of Th17 cells and associated key factors such as IL-17, TNF-α and IL-6 in the colon. Our results revealed that QCWZD exerts beneficial effects on intestinal inflammation in mice by modulating microbial homeostasis, regulating metabolic pathways including the biosynthesis of amino acids, phenylalanine, tyrosine, tryptophan, arginine, pantothenate and coenzyme A, glutathione, monobactam, and novobiocin, while elevating propanoate metabolism, streptomycin biosynthesis, valine, leucine, and isoleucine degradation, various types of N-glycan biosynthesis, and the glycosphingolipid biosynthesis-ganglio series pathway, and inhibiting Th17 cell differentiation and its associated pathway. These findings provided novel insights into the regulatory role of QCWZD in the treatment of IBD and support the clinical use of herb-based complementary and alternative therapies.

Methods

Experimental Animals

All female C57BL/6 mice involved in this study were purchased from SPF Biotechnology Co., Ltd. (Beijing, China) and were maintained in a specific pathogen-free facility at Dongfang Hospital, Beijing University of Chinese Medicine, China. All experimental protocols were approved by the Animal Ethics Committee of Dongfang Hospital, Beijing University of Chinese Medicine (Ethics Approval Number: DFYY-202104-M) and all methods were carried out in accordance with relevant guidelines and regulations. This study was conducted in accordance with the ARRIVE guidelines. All the animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of the National Institutes of Health.

Induction of Experimental Colitis

After one week of adaptive feeding, the model group mice were administered 2.5% (w/v) DSS (molecular weight, 36–50 kDa; MP Biomedicals, Santa Ana, CA, United States) in drinking water to induce colitis, while the other mice continued to freely drink water for a total of one week. Starting from day 8, all mice were switched to normal drinking water and began to be orally administered with different drugs.

Preparation of QCWZD

QCWZD formulation includes 1.2 g *Coptis chinensis* Franch. (NO. 20001081), 2 g *Zingiber officinale* Roscoe (NO. 20012041), 1.8 g *Sophora flavescens* Aiton (NO. 20023621), 0.6 g *Strobilanthes cusia* (Nees) Kuntze (NO. 20021141), 3 g *Sanguisorba officinalis* L (NO. 20020522), 1.2 g *Dolomiaea costus* (Falc). Kasana & A. K. Pandey (NO. 20002101), 1.2 g *Panax notoginseng* (Burkill) F. H. Chen (NO. 20021141), and 1.2 g *Glycyrrhiza glabra* L. (NO. 20001281), and were purchased from Dongfang Hospital, Beijing University of Chinese Medicine (Beijing, China). The processing of the formulation into granule form was carried out by Beijing Tcmages Pharmaceutical Co., Ltd. (Beijing, China): the formulation was first extracted into a stimulating family decoction form and then concentrated and dried to form granules. Our previous study[11] has quantified the main components of QCWZD via high-performance liquid chromatography (HPLC), showing it to be comprised of ginsenoside Rb1 (6.2884 mg/g), gallic acid (0.7178 mg/g), berberine hydrochloride (4.6455 mg/g), ginsenoside Rg1 (8.3506 mg/g), and liquiritin (0.9661 mg/g), as shown in Supplementary Table 1.

Animal Groups and QCWZD Administration

Mice in the Control group (n=5) were administered sterile water throughout the experiment. The remaining mice received DSS in drinking water to induce colitis for 1 week, followed by oral administration of QCWZD at a dose of 0.36 g/kg (n=4), 1.8 g/kg (n=5), 9 g/kg (n=4) or sterile water (n=5) and mesalazine (n=5) for 7 days.

Disease Activity Index (DAI) Analysis

Body weight, stool consistency, and rectal bleeding were recorded in all mice daily to calculate the DAI according to a standard scoring system.[21]

Histological Analysis

Colonic tissues were collected from all mice, fixed in 10% neutral buffered formalin, embedded in paraffin, and then sectioned and stained with hematoxylin and eosin (H&E). The histopathological scores were summed, based on our previous study.[17] For inflammatory cell infiltration, 0 points for no infiltration, when inflammatory cells infiltrated into the lamina propria, submucosa and transmural inflammatory cell infiltration, the scores were assigned as 1, 2, and 3 points, respectively. For epithelial damage, 0 points for no mucosal damage, 1 point for discrete epithelial lesions, 2 points for erosions or focal ulcerations and severe mucosal damage with extensive ulceration extending into the bowel wall were assigned as 3 points.

Metagenomics Sequencing

Genomic DNA was extracted from fecal samples and detected by 1% agarose gel electrophoresis. The Covaris M220 focused ultrasonicator was used for DNA shearing to produce 450 bp genomic DNA fragments. The fragmented DNA was purified and amplified through NEXTFLEX ™ Rapid DNA Seq Kit to build a library for paired-end sequencing. Finally, high-throughput sequencing was performed through NovaSeq Reagent Kits/HiSeq X Reagent Kits. Raw sequence reads were filtered using Trimmatic (version 0.33) software and then aligned to the host genome sequence using Bowtie 2 (version 2.4.4) to remove host contamination. MEGAHIT (Version 1.1.2) software was used to assemble sequences with different sequencing depths, and then gene prediction was conducted by MetaGene software. Clustering with CD-HIT software was used to remove redundancy, compare the high-quality reads of each sample with the non-redundant gene set using SOAPaligner (Version 2.21), and count the abundance information of genes in the corresponding samples. The identified genes were annotated in terms of functions and classified using the Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations at all levels.

Network Pharmacology Analysis and Molecular Docking

The active components and potential targets of QCWZD were retrieved from the Traditional Chinese Medicine Systems Pharmacology (TCMSP, http://lsp.nwsuaf.edu.cn/tcmsp.php) database.[22] Information on IBD-related target genes was

collected from GeneCards[23] (https://www.genecards.or) and the online Mendelian Inheritance of Man database[24] (https://omim.org/) using the keyword "inflammatory bowel disease". Genes that were common to the set of predicted QCWZD molecular targets and the IBD-associated set of gene targets were uploaded to the STRING database of known and predicted protein-protein interactions[25] (https://string-db.org/) for delineating protein-protein interactions (PPI). Metascape (http://metascape.org/gp/index.html) was utilized for Gene Ontology (GO) while KEGG was employed for pathway enrichment analysis of the screened core target genes from the QCWZD and IBD gene sets, followed by visualization on the bioinformatics platform (http://www.bioinformatics.com.cn/). Cytoscape 3.7.2 was used to construct the Herbs-Compounds-Targets network, PPI network and the targets-pathways network.[26] The candidate active compounds and core targets went through pretreatments including water removal, hydrogenation, and atom typesetting via AutoDockTools. The molecular docking and binding affinity calculation were performed by AutoDock Vina. PyMol software was used to visualize the docking results.

RNA Sequencing

Total RNA was extracted from colonic tissues of mice in the Control, DSS, and DSS+QCWZD groups using TRIzol® Reagent according to the instructions of the manufacturer. RNA quality was determined via the 5300 Bioanalyser (Agilent) and RNA concentration was measured by using the ND-2000 (NanoDrop Technologies). RNA purification, reverse transcription, library construction and high-throughput RNA-seq was performed at Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd. (Shanghai, China) according to the instructions of the manufacturer (Illumina, San Diego, CA). The expression level of each transcript was calculated according to the transcripts per million reads (TPM) method to identify differentially expressed genes (DEGs). Differential expression analysis was performed using the DESeq2 based on $|\log2(\text{FoldChange})| > 1$ and padj <0.05. Next, GO functional enrichment and KEGG pathway analyses were carried out by GOATOOLS and SciPy Python-based libraries, respectively.

Flow Cytometry

Mesenteric lymph nodes (MLNs) were collected and placed in complete Dulbecco's modified Eagle's medium (cDMEM). Subsequently, single lymphocyte suspensions were prepared by grinding and filtration with a 200μm cell strainer. The lymphocytes suspension was stimulated with a cell stimulation cocktail (plus protein transport inhibitors) at 37°C in a humidified atmosphere with 5% CO2 for 8 h. Surface staining was performed using APC/Cyanine7 anti-mouse CD3 antibody (BioLegend, dilution, 1:200, Cat. No. 100222) and FITC anti-mouse CD4 antibody (BioLegend, dilution, 1:500, Cat. No. 100509). For intracellular staining, cells were marked with Brilliant Violet 421™ anti-mouse IL-17A antibody (BioLegend, dilution, 1:200, Cat. No. 506944). The percentages of $CD3^+CD4^+$ cells and $CD3^+CD4^+IL\text{-}17^+$ (Th17) cells were measured by flow cytometry.

Immunohistochemistry (IHC)

Colonic tissues were collected, frozen in OCT compound (Tissue Tek), stored at −80°C, cut into 5 μm sections, and stained for immunohistochemistry (IHC). After washing in phosphate-buffered saline (PBS), the sections were placed in 3% H_2O_2 solution to quench endogenous peroxidase activity; non-specific binding sites were blocked by incubation in 3% bovine serum albumin. Primary antibodies against IL-17 (Servicebio, dilution, 1:200, Cat. No. GB11110-1), TNF-α (Servicebio, dilution, 1:200, Cat. No. GB11188), and IL-6 (Servicebio, dilution, 1:200, Cat. No. GB11117) were applied overnight at 4°C followed by incubation with a horse radish peroxidase-conjugated goat anti-rabbit IgG (H+L) secondary antibody (Servicebio, dilution, 1:200, Cat. No. GB23303) for 50 min at room temperature. Finally, the slides were stained by incubation with 3.3′-Diaminobenzidine, counterstained with hematoxylin, dehydrated with ethanol, and sealed with neutral glue. The FiJi/ImageJ software was used to analyze chromogenic intensity.[27]

Quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-qPCR)

Trizol reagent was used to extract total RNA from the colon according to the instructions of the manufacturer. The primer pairs used to amplify GAPDH, IL-6, IL-17, and TNF-α are shown below: 5'-TGGAATCCTGTGGCATCCATGAAAC-3', 5'-TAAAACGCAGCTCAGTAACAGTCCG-3' for GAPDH. 5'-TAGTCCTTCCTACCCCAATTTCC-3', 5'-

TTGGTCCTTAGCCACTCCTTC-3' for IL-6. 5'-CCACGTCACCCTGGACTCTC-3', 5'-CTCCGCATTGACACAGCG-3' for IL-17. 5'-CCCTCACACTCAGATCATCTTCT-3', 5'-GCTACGACGTGGGCTACAG-3' for TNF-α. The relative expression of each target mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method.

Enzyme-Linked Immunosorbent Assay (ELISA)

100 mg colon fragments from mice were cut up and homogenized with 900 uL PBS, centrifuged at 3000 rpm for 15 min at 4°C to obtain a homogenate. The BCA Protein Assay Kit (Solarbio, Cat. No. PC0020) was used to determine and normalize the concentration of total protein. The levels of TNF-α and IL-6 protein expression were detected through conventional double-antibody sandwich ELISA. The above antibodies were purchased from BD Biosciences. The levels of colon IL-17 protein expression were detected using ELISA kits (Mlbio, Cat. No. YJ037866) according to the instructions of the manufacturer.

Statistical Analysis

The statistical analyses were performed using GraphPad Prism 8.0 software, and all data were presented as means ± standard errors of the means. Data comparison among multiple groups was conducted by one-way Analysis of Variance (ANOVA). Results were considered to be statistically significant if the P value was less than 0.05.

Results

Protective Effects of QCWZD Against DSS-Induced Colitis in Mice

A mouse model of DSS-induced colitis was used to determine the beneficial effects of QCWZD on intestinal inflammation (Figure 1A). Similar to our previous study, DSS administration in mice for 7 days induced severe intestinal inflammation, as demonstrated by body weight loss, diarrhea and hematochezia, colon shortening, and significantly increased disease activity index (DAI) scores compared to mice in the control group (Figure 1B–F). Colonic inflammation was gradually alleviated after cessation of DSS treatment in all groups. However, it is worth noting that administration of QCWZD led to faster remission of the changes caused by DSS treatment, especially in terms of body weight,

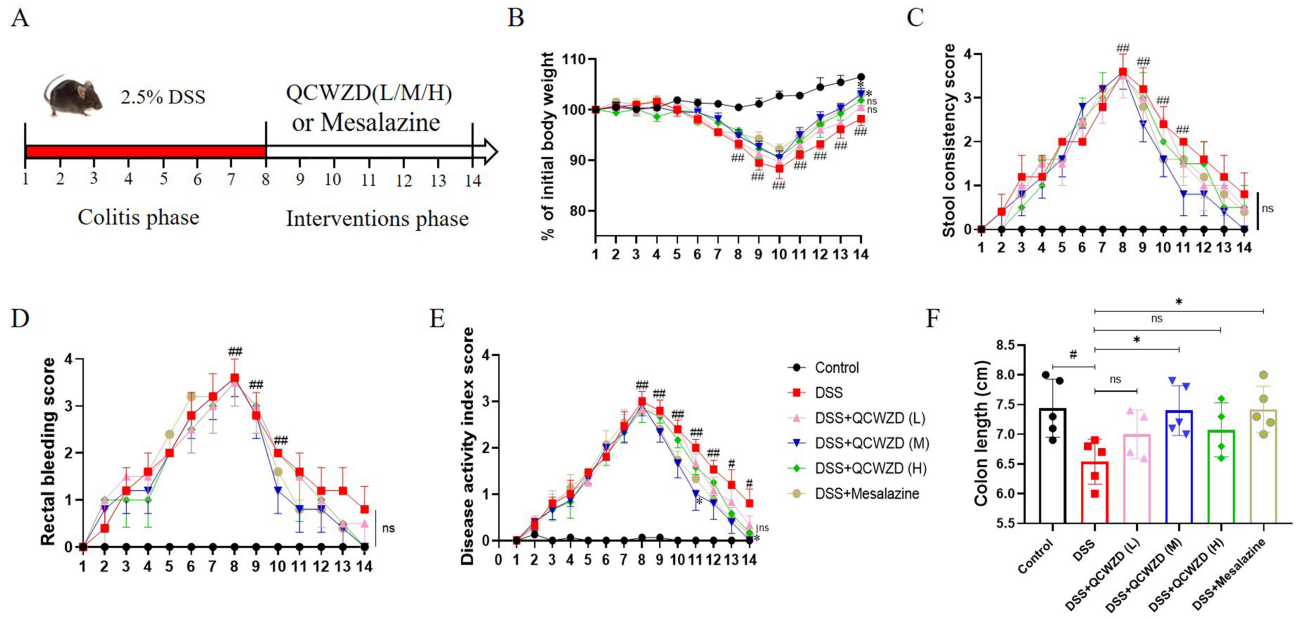


Figure 1 Protective Effects of Different Dosages of QCWZD Against DSS-Induced Colitis in Mice. (**A**) Experimental design. (**B**) Body weight. (**C**) Stool consistency. (**D**) Rectal bleeding. (**E**) Disease activity index (DAI) score. (**F**) Colonic length. The data shown are the mean ± the SEM (n=4-5 mice/group) from one of two experiments performed showing similar results. $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ versus the Control group; $^{*}P < 0.05$ versus the DSS group. Significance was determined by one-way ANOVA test (Tukey's multiple comparison test).

colon shortening, and DAI scores. These results indicate that QCWZD significantly improved colitis-related symptoms and ameliorated DSS-induced intestinal inflammation.

QCWZD Administration Alleviated DSS-Induced Pathological Damage

To further investigate the influence of QCWZD on DSS-induced colonic pathological changes in colitis mice, H&E staining was performed for histopathological analysis. As shown in Figure 2A, compared to the control mice, histological analysis of colon sections of DSS-induced colitis mice revealed distortions of crypts, severe mucosal necrosis, and inflammatory cell infiltration, whereas drugs treatment alleviated this tissue damage, especially in the DSS+QCWZD(M) and DSS+QCWZD(H) groups employing medium and high dosage of QCWZD, respectively (Figure 2B). These data suggest that QCWZD ameliorates pathological damage to the colon of DSS-treated mice, and the efficacy was comparable to mesalazine. Considering the results shown in Figures 1 and 2, administration of medium-dosage QCWZD was the most effective treatment for IBD. Therefore, the administration of QCWZD at a dose of 1.8 g/kg was selected for further mechanistic studies.

QCWZD Modulated Intestinal Microbial Homeostasis in Colitis Mice

Our previous and present findings showed that QCWZD treatment ameliorated intestinal inflammation and accelerated intestinal mucosal healing. Bacterial 16S rRNA amplicon sequencing revealed that the QCWZD mechanism of action was related to the modulation of intestinal dysbiosis.[17] In the present study, metagenomics sequencing was used to analyze the differences in the gut microbiota at different taxonomic levels, especially at the species level. The functional consequences of the QCWZD-based regulation of key genes in the altered microbiota of the DSS-induced colitis mouse model animals were also investigated. As shown in Figure 3A, principal component analysis (PCoA) revealed distinct colonic microbial communities among the three groups of mice, with a small difference in the microbial communities between the DSS+QCWZD-treated and control groups. Gut microbiota abundance at different taxonomic levels was quantified to further evaluate the microbiota composition in the different mouse groups. At the phylum level (Figure 3B), mice in the DSS-treated group showed a lower abundance of Verrucomicrobia and a higher abundance of phylum Bacteroidetes than mice in the control group. However, QCWZD administration reversed these changes. At the genus

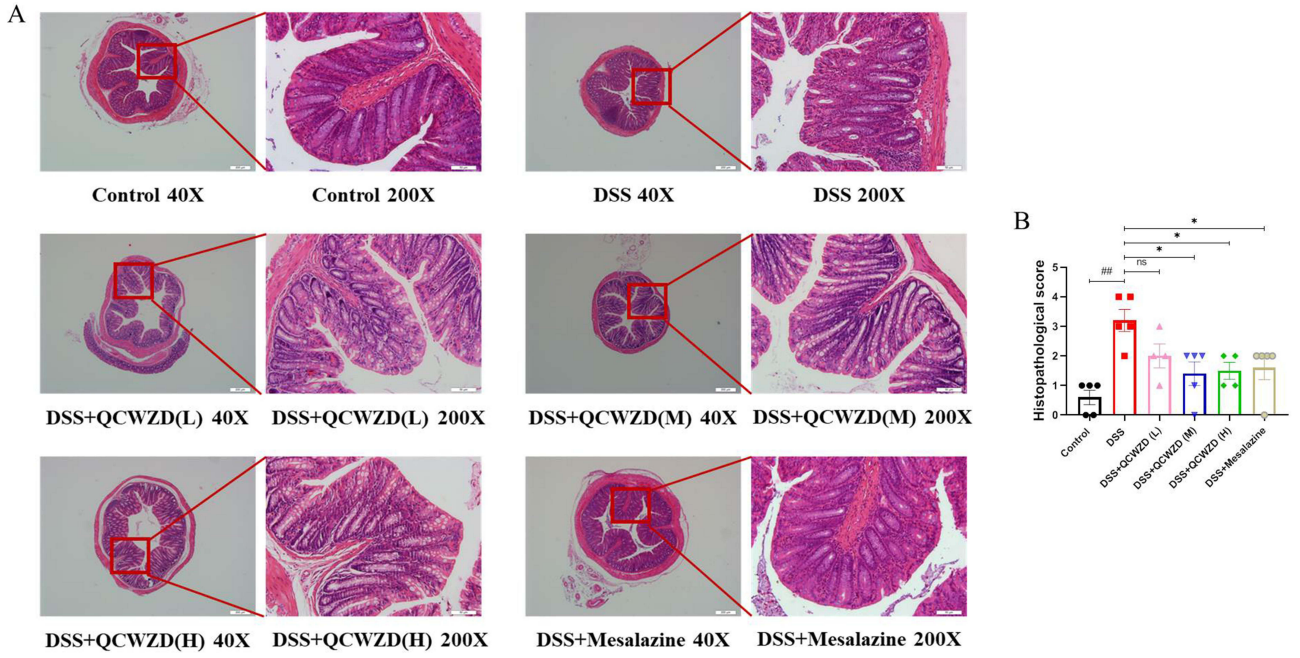


Figure 2 Administration of QCWZD Alleviated the DSS-Induced Pathological Damage. (**A**) H&E staining of the colon (200×). (**B**) Histological score. The data shown are the mean ± the SEM (n=4-5 mice/group) from one of two experiments performed showing similar results. $^{\#\#}P < 0.01$ versus the Control group; $^{*}P < 0.05$ versus the DSS group. Significance was determined by one-way ANOVA test (Tukey's multiple comparison test).

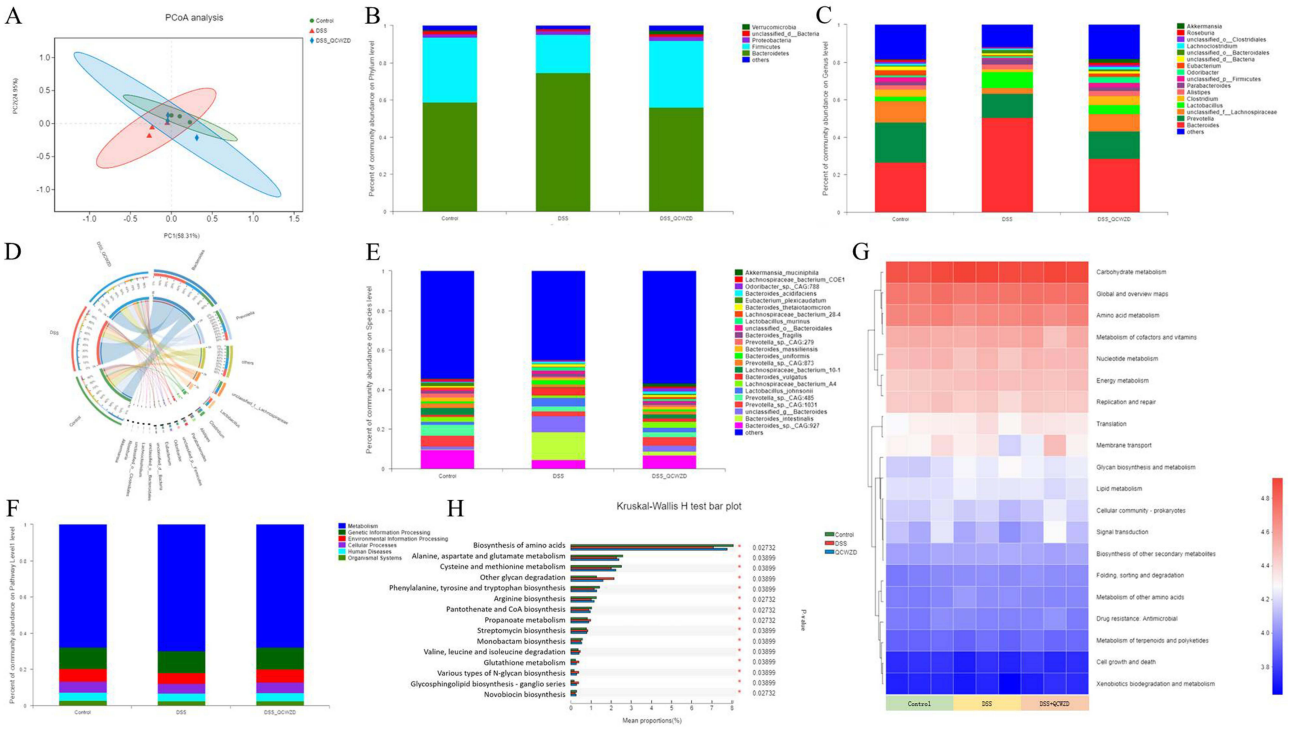


Figure 3 QCWZD Modulated Intestinal Microbial Homeostasis in colitis mice. Fresh feces were collected for bacterial metagenomics sequencing analysis (n=3 mice/group). (**A**) PCoA analysis based on Bray-Curtis distance among different samples. (**B**–**E**) Bacterial taxonomic profiling in the phylum, genus and species level of gut bacteria from different mouse groups. (**F**) Histogram of KEGG pathway composition and abundance at level 1. (**G**) Differential composition and abundance at level 2. (**H**) Heat map of metabolic pathways associated with metabolic regulation at level 3.

level, compared to the control group, DSS treatment enriched the levels of *Bacteroidales* and *Bacteroides* genera and decreased the levels of *Eubacterium* and *Lachnospiraceae* genera whereas QCWZD administration restored the original levels of the aforementioned species and increased the level of the probiotic genus *Akkermansia* (Figure 3C and D). Similar to our previous findings, analysis of the microbiota at the species level revealed a decreased abundance of the pathogenic species *Bacteroides vulgatus*. Moreover, our results showed that other species of the genus *Bacteroides*, such as *Bacteroides intestinalis, Bacteroides acidifaciens*, and *Bacteroides fragilis* were reduced after QCWZD treatment. In addition, QCWZD administration significantly elevated the abundance of beneficial microorganisms, including *Akkermansia muciniphila, Lachnospiraceae bacterium COE1, Lachnospiraceae bacterium 28–4* and *Lachnospiraceae bacterium 10–1* (Figure 3E).

To probe bacterial gene functions, we annotated the gut microbiota composition via KEGG pathway analysis. The results of this analysis are displayed in Figure 3F, depicting the level 1 pathway of each group of mice: the level of expression of Metabolism pathways was reduced and the level of expression of Environmental Information Processing pathways was increased in the DSS+QCWZD group compared to the DSS group. At level 2 of the KEGG pathway analysis, QCWZD intervention reduced the expression of the pathways for Carbohydrate metabolism, glycan biosynthesis and metabolism, and the metabolism of other amino acids in the DSS group (Figure 3G). We assessed the effect of QCWZD treatment on metabolic pathways associated with metabolic regulation in DSS-treated mice at level 3 in each group. Some metabolic processes, including the biosynthesis of amino acids, phenylalanine, tyrosine, tryptophan, arginine, pantothenate and coenzyme A, glutathione, monobactam, and novobiocin, were reduced. Conversely, other metabolic processes, including propanoate metabolism, streptomycin biosynthesis, valine, leucine, and isoleucine degradation, various types of N-glycan biosynthesis, and the glycosphingolipid biosynthesis-ganglio series pathway were elevated. QCWZD administration reversed these changes in the DSS group (Figure 3H). Therefore, our results provide strong support for the hypothesis that QCWZD administration influenced the composition and function of the gut

microbiota, especially the metabolic pathways associated with metabolic regulation in mice with colitis, thus contributing to the maintenance of intestinal microbial homeostasis.

Th17 Cell Differentiation Plays an Important Role in QCWZD Treatment of IBD Based on Network Pharmacology Analysis

Network pharmacology analysis was used to explore the possible targets of QCWZD treatment for IBD and we retrieved 146 active compounds and 263 targets of QCWZD from the TCMSP database. The Herbs-Compounds-Targets (H-C-T) network showed that QCWZD included a variety of active ingredients that may modulate multiple molecular targets to effectively treat IBD (Figure 4A). A total of 103 overlapping targets were obtained after 263 targets of QCWZD treatment were combined with 1223 IBD-related targets collected after merging and deleting duplicates (Figure 4B). These core gene targets of QCWZD treatment for IBD were screened and identified using the CytoHubba plug-in, where the top 30 genes generated by the Maximal Clique Centrality method were regarded as core genes (Figure 4C), and these included TNF-α, IL-6, IL-1B, JUN, and CCL2 that may play a crucial role in the anti-IBD effects of QCWZD.

The 30 core genes we identified were uploaded to Metascape for GO and KEGG pathway enrichment analyses. Finally, 949 GO entries and 127 KEGG signaling pathways were obtained. Based on the expression levels of the enriched genes and their associated *P* values, the top 20 significantly enriched pathways are presented in a bubble plot in Figure 4E. As shown in Figure 4E, Th17 cell differentiation, and the associated TNF-α and IL-17 signaling pathways were the key pathways involved in the effects of QCWZD against IBD. In addition, by constructing a target pathway network, we found that several targets were associated with multiple pathways (Figure 4D). Finally, three main active components (quercetin, kaempferol, beta-sitosterol) of QCWZD were selected to conduct in silico molecular dockings with four candidate target proteins in the key signaling pathways, including TNF-α, IL-6, and IL-17, respectively. The results showed strong binding affinity among them (Figure 4F and G; Supplementary Table 2). Collectively, these data illustrate that QCWZD may exert beneficial effects on intestinal inflammation in mice through a combination of multiple pathways and targets. Th17 cell differentiation plays an essential role in the QCWZD treatment for IBD.

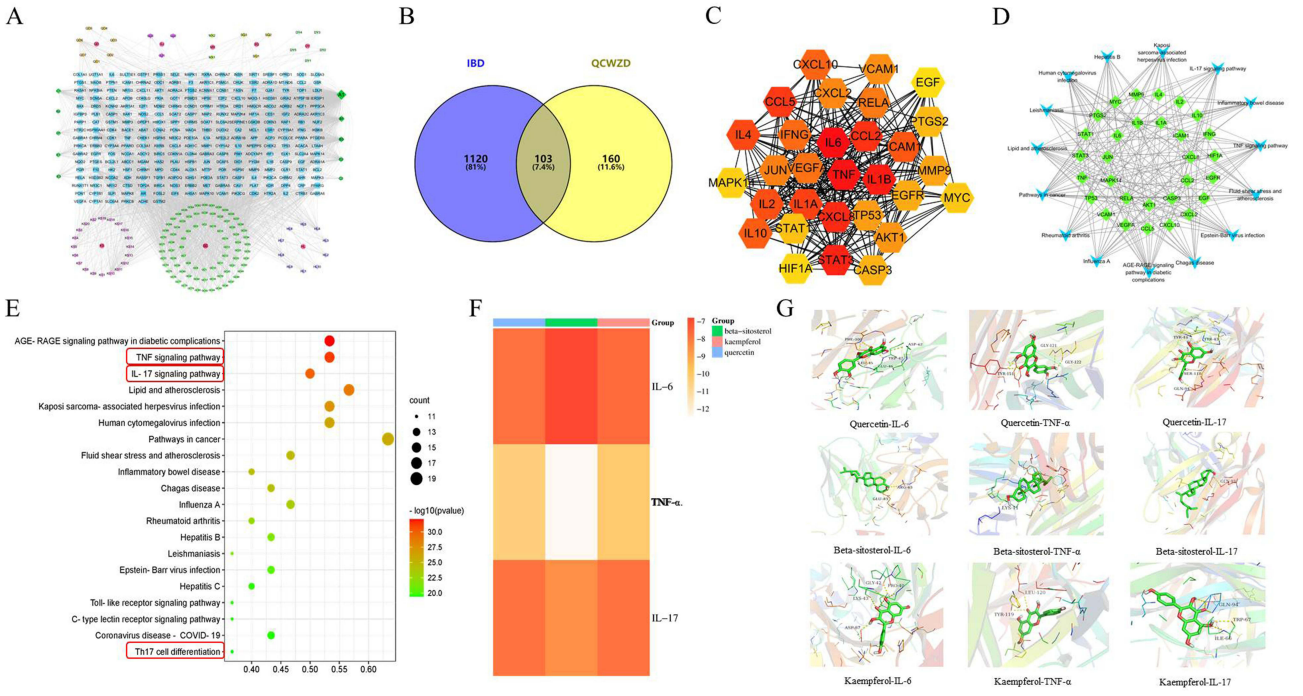


Figure 4 Th17 cell differentiation plays an important role in the treatment of QCWZD against IBD based on network pharmacology analysis. (**A**) Herbs-Compounds-Targets (H-C-T) network diagram. (**B**) Venn diagram of targets for QCWZD treating IBD. (**C**) PPI network of the core genes. (**D**) Target-pathway network. (**E**) KEGG enrichment analysis for 30 core targets. (**F**) Heat map for the binding energies of docked components within the active sites of tested targets. (**G**) Molecular docking results.

RNA-Seq Analysis Showed That QCWZD Regulated the Expression of Genes Related to IBD

To elucidate the underlying mechanisms of action of QCWZD treatment for IBD and validate our predicted results, colonic tissues dissected from the Control, DSS, and DSS + QCWZD mouse groups were subjected to RNA-seq analyses. The average number of raw reads obtained was 5.55×10^7 and the average number of clean reads obtained after filtering was 5.52×10^7. The average Q20 of the clean reads was > 98.41% (Supplementary Table 3). There were 2474 DEGs in the DSS group compared to the control group (1276 up-regulated and 1198 down-regulated DEGs) and 4006 DEGs expressed in the DSS+QCWZD group compared to the DSS group (1893 up-regulated and 2113 down-regulated DEGs) (Figure 5A–C). GO function enrichment analysis (Figure 5D) showed that, compared to the DSS group, the DEGs in the DSS+QCWZD group were mainly enriched for metabolic processes, catabolic processes, cell proliferation, cytokine secretion, T-cell receptor signaling pathway, and inflammatory responses. Regarding the cellular function composition, the DEGs were enriched for oxidant detoxification, spindle, and spindle assembly. KEGG analysis showed that the DEGs in a comparison of the DSS and DSS+QCWZD groups were enriched for 328 pathways, and included the TNF-α signaling pathway, chemokine signaling pathway, lipid metabolism, and glutathione metabolism. The top 20 most enriched pathways are shown in Figure 5E. Further enrichment analysis of the signaling pathways derived from the DEGs suggested that QCWZD treatment affected Th17 cell differentiation and the associated TNF-α and IL-17 signaling pathways (Supplementary Figure 1A and B).

QCWZD Treatment Inhibited Th17 Cell Differentiation and Modulated IL-17 Signaling Pathway Activity in Mice

Based on preliminary network pharmacology predictions and RNA-Seq analyses, Th17 cell differentiation and the associated IL-17 signaling pathway are potential targets in the QCWZD treatment for IBD. To validate the effect of

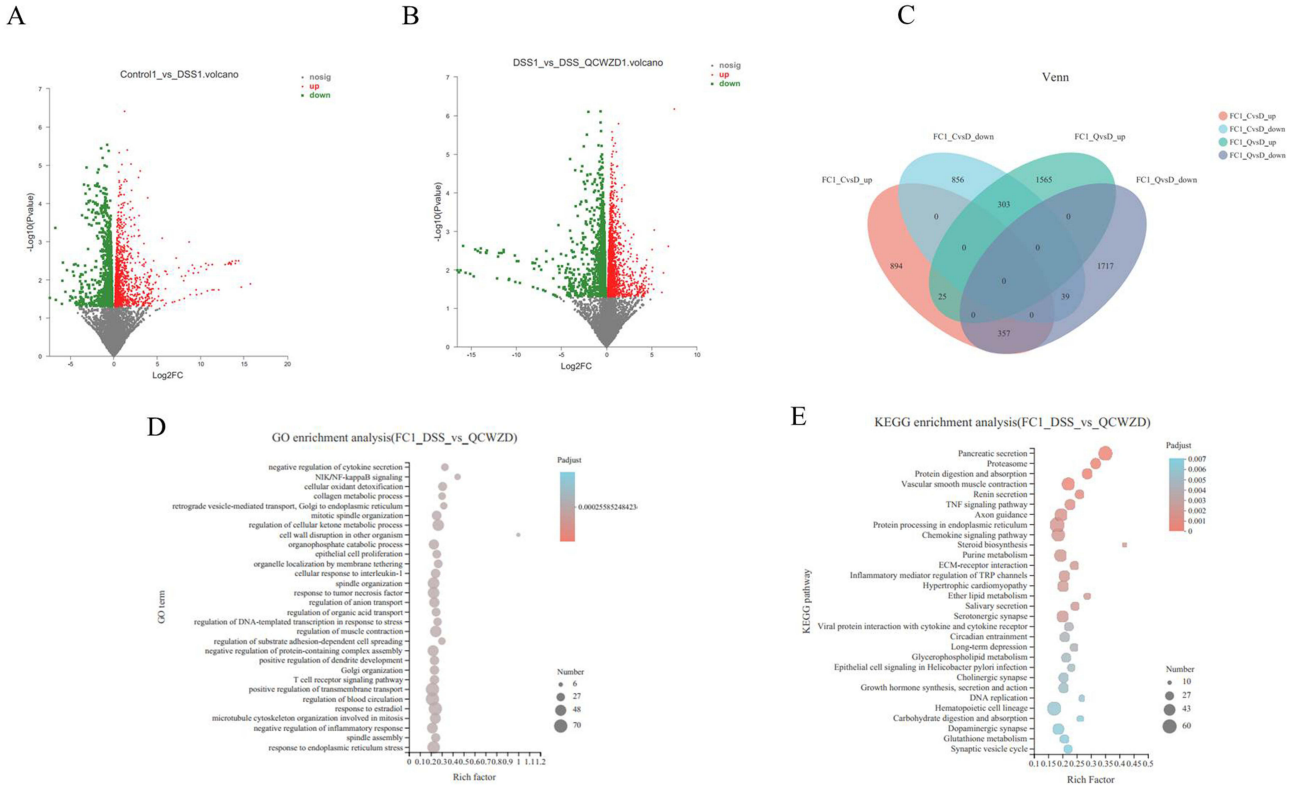


Figure 5 QCWZD regulated the expression of genes related to IBD via RNA-Seq analyses. Murine colonic tissues were collected for RNA-Seq analyses (n=5 mice/group). (**A**) Volcano plots of upregulated and downregulated differentially expressed genes (DEGs) between DSS-treated and Control groups of mice. (**B**) Volcano plots of upregulated and downregulated DEGs between DSS- and DSS+QCWZD-treated groups of mice. (**C**) Venn diagram illustration of RNA-seq analysis among the various experimental and control groups of mice. (**D**) The gene ontology (GO) and (**E**) Kyoto encyclopedia of genes and genomes (KEGG) analysis of DEGs between DSS- and DSS +QCWZD-treated groups of mice.

QCWZD on Th17 cell differentiation, we performed flow cytometric analyses of mesenteric lymph node contents obtained from all mice groups used in the in vivo experiments. As shown in Figure 6A, the percentage of CD3+CD4+ cells in the lymphocyte populations of mice with DSS-induced colitis was significantly higher than that in control mice, whereas this change was reversed by QCWZD treatment. In addition, analysis of IL-17 expression in the CD4+ T-cell compartment revealed a markedly elevated percentage of Th17 cells in the mesenteric lymph nodes isolated from DSS-induced mice. However, CD4+IL-17+ (Th17) cells decreased in the DSS+QCWZD group compared to the DSS group (Figure 6B). This finding validates our hypothesis that QCWZD administration inhibited Th17 cell differentiation in colitis mice.

The IL-17 signaling pathway, a downstream pathway of Th17 cell differentiation, was closely related to the underlying mechanism of action of QCWZD treatment for IBD, according to the results of network pharmacology analysis. Therefore, we quantified the IL-17 protein expression via IHC. Our results indicated that the positive expression of IL-17 in the colon of DSS-induced IBD mice had a larger range and darker brown staining compared to that in the control group. In contrast, QCWZD treatment significantly reduced IL-17 protein expression (Figure 6C). Furthermore, as shown in Figure 6D and E, the mRNA and protein expression levels of IL-17 were significantly increased in the DSS group compared to those in the Control group. Furthermore, QCWZD intervention markedly reduced IL-17 mRNA and protein expression levels, indicating that inhibition of IL-17 protein expression by QCWZD treatment inhibited IL-17 protein expression in the IL-17 signaling pathway and prevented DSS-induced colitis in mice.

QCWZD Administration Inhibited TNF-α Pathway-Related Protein Expression in Colitis Mice

KEGG pathway enrichment analysis from network pharmacology and RNA-Seq analysis indicated that the most important pathway in treating IBD with QCWZD was the TNF-α signaling pathway that was activated by Th17 cell

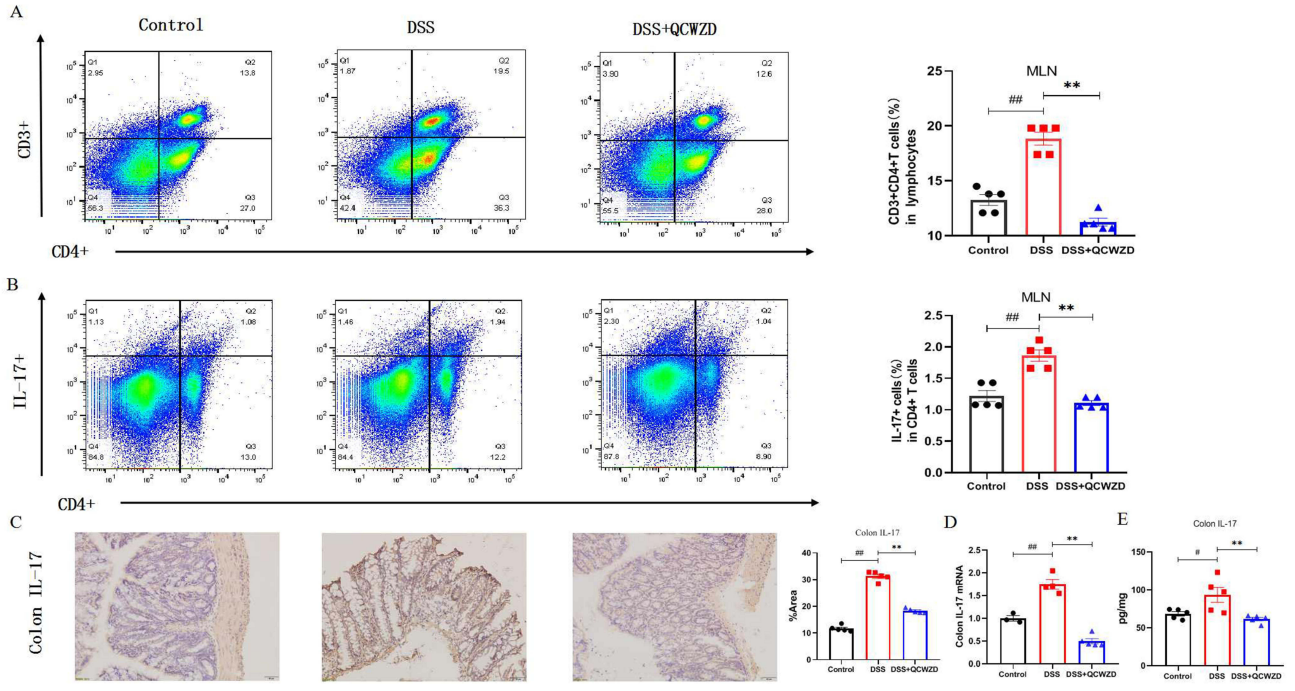


Figure 6 QCWZD Treatment Inhibited Th17 Cell Differentiation and Modulated IL-17 Signaling Pathway Activity in Colitis Mice. (**A**) The percentages of CD3+CD4+ T cells in lymphocytes by flow cytometry. (**B**) The percentages of CD3+CD4+IL-17+ (Th17) cells in lymphocytes by flow cytometry. (**C**) Representative immunohistochemistry staining and the quantification of IL-17 of different groups (×200). (**D**) RT-qPCR analysis for colonic IL-17 relative mRNA expression. (**E**) ELISA analysis for IL-17 in colon. The data shown are the mean ± the SEM (n=5 mice/group) from one of two experiments performed showing similar results. $^{\#\#}p < 0.01$ versus the Control group, $^{**}P < 0.01$ versus the DSS group. Significance was determined by one-way ANOVA test (Tukey's multiple comparison test).

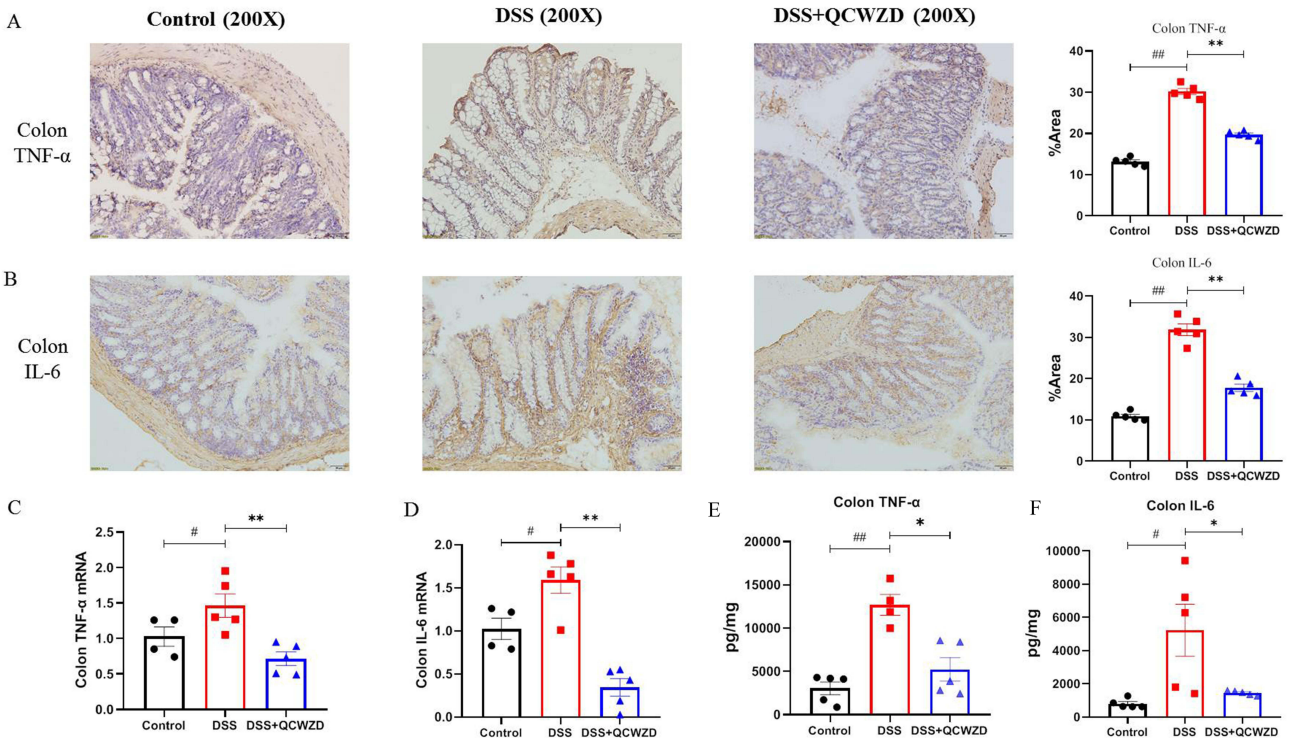


Figure 7 Supplementation of QCWZD inhibited TNF-α pathway-related protein expressions in colitis mice. (**A** and **B**) Representative immunohistochemistry staining and the quantification of TNF-α and IL-6 expression in different groups of mice (×200). (**C**) Inflammation-related cytokines such as colonic TNF-α expression was detected using quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR). (**D**) Inflammation-related cytokines such as colonic IL-6 expression was detected using RT-qPCR. (**E**) ELISA results for TNF-α protein expression in the mouse colon. (**F**) ELISA results for IL-6 protein expression in the mouse colon. Data are expressed as mean ± SEM (n=5 mice/group) from one of two experiments performed showing similar results. $^{\#\#}p < 0.01$ versus the Control group, $^{**}P < 0.01$ versus the DSS group. Significance was determined by the one-way ANOVA test (Tukey's multiple comparison test).

differentiation and the IL-17 signaling pathway. Therefore, we determined the expression levels of related proteins enriched in the TNF-α signaling pathway. As shown in Figure 7A and B, TNF-α and IL-6 protein expression was significantly increased in the DSS group, indicating severe colonic inflammation. In contrast, RT-qPCR and ELISA analysis indicated that the mRNA and protein expression levels of TNF-α and IL-6 in the colon were significantly reduced in the DSS+QCWZD group compared to the DSS group after QCWZD intervention (Figure 7C–F). These findings validated our hypothesis that QCWZD treatment modulated the expression of genes related to the TNF-α signaling pathway in mice with IBD. Taken together, these data demonstrate that QCWZD treatment ameliorated DSS-induced colonic damage and inflammatory responses in mice by regulating Th17 cell differentiation and the associated IL-17 and TNF-α pathways.

Discussion

IBD has evolved into a global disease with rising prevalence in every continent.[3] Current therapeutic approaches for IBD include corticosteroids, aminosalicylates, and biological therapies aim at controlling mucosal inflammation and inducing remission. However, these therapies are not curative and have various limitations.[4,5] In recent years, an increasing number of studies have aroused strong interest in TCM due to its better efficacy, and researchers have focused on its mechanism of action in treating IBD.[28,29] Our previous study found that QCWZD treatment attenuated intestinal inflammation and accelerated intestinal mucosal healing both in IBD patients and colitis mice, where the mechanism of action involved the modulation of gut dysbiosis. However, the specific types of regulating microbiota and their functions have not been clearly elucidated. Our present findings have revealed a regulatory effect of QCWZD on microbial composition, especially at the species level. Furthermore, this study has used metagenomics sequencing, network pharmacology, RNA-seq, and in vivo experiments to confirm that QCWZD regulates the function of key genes in altered microbiota as well as host Th17 cell differentiation. Our present findings provide novel insights into the

regulatory role of QCWZD in the treatment of IBD, and support the future use of herb-based complementary and alternative therapies.

Compelling evidence has shown that intestinal dysbiosis may cause intestinal mucosal inflammation leading to the onset of IBD.[30] Therefore, targeted regulation of gut microbiota plays a pivotal role in modulating intestinal homeostasis and has been considered an attractive therapeutic approach and a promising candidate in the treatment of IBD.[31] In this study, the results of metagenomics sequencing demonstrated that oral administration of QCWZD had a modulating effect on gut microbial homeostasis in DSS-induced colitis mice, especially elevating the relative level of the probiotic species *Akkermansia muciniphila. Akkermansia muciniphila* is a unique representative strain of the phylum Verrucomicrobia that plays a crucial role in regulating the host intestinal barrier and immune response. Thus, it is considered to be a microorganism with probiotic characteristics.[32,33] Previous research has shown that *Akkermansia muciniphila* participates in the immunomodulatory effects in mice by changing the B cell population and reducing the overall T cell and neutrophil population.[34] Our previous report also showed that colonization of *Akkermansia muciniphila* in mice promoted the growth of intestinal goblet cells and mucin production, thereby regulating the intestinal mucus barrier and improving the *C. rodentium* infection-induced colitis.[35] In addition, *Akkermansia muciniphila* administration upregulated IL-10 expression that promoted the production of probiotics and reduced the abundance of harmful species belonging to the bacterial phylum *Bacteroidetes*.[36] Numerous studies have reported the regulatory effect of TCM on the abundance of *Akkermansia muciniphila* in colitis mice and IBD patients,[37,38] and this has been confirmed in the results of our present study. It is worth noting that *Bacteroidetes* is considered a harmful genus that aggravates IBD.[39,40] Similarly, our study discovered that QCWZD reduced the abundance of various species belonging to the bacterial phylum *Bacteroidetes*, such as *Bacteroides intestinalis, B. acidifaciens, B. fragilis*, and *B. vulgatus. B. intestinalis* is a gram-negative bacterial species that can degrade complex arabinoxylans.[41] Existing studies have found that *B. intestinalis* is involved in the pathogenesis of some common diseases, including obesity and hyperlipidemia.[42] Our present data show a significant increase in the abundance of *B. intestinalis* that correlated positively with the occurrence of IBD, suggesting that *B. intestinalis* modulates intestinal inflammation. However, few reports have examined the relationship between *B. intestinalis* and colitis, and further research is needed on its specific role in colitis. *B. acidifaciens, B. fragilis*, and *B. vulgatus* increased the production of acetic acid and succinic acid, leading to the progression of colitis-related inflammation.[43] In addition, it has been shown that the pro-inflammatory toxin secreted by *B. fragilis* not only exacerbates the diarrhea symptoms of IBD patients, but also triggers a multi-step inflammatory cascade reaction through IL-17R, NF-κB, and Stat3 signaling in colonic epithelial cells.[44] Zamani et al found that it was associated with the development of ulcerative colitis and induced the development of diarrhea in these patients.[45] Pathogenic *B. vulgatus* was associated with IBD that activated NF-κB and led to a host inflammatory reaction.[46] However, there was an increase in certain bacterial species of the genus *Bacteroides*, such as *Bacteroides sp. CAG:927* and *B. massiliensis* that indicated a protective role of members of the bacterial phylum *Bacteroidetes* in IBD, as shown by Ryan et al.[47] The complex role of *Bacteroides* species in IBD may be related to a variety of factors, and further research is needed. Furthermore, KEGG pathway analysis also found that QCWZD regulated metabolic pathways associated with metabolic regulation, especially the biosynthesis of amino acids, such as tryptophan and arginine. Amino acids are vital for maintaining mucosal integrity and intestinal barrier function by reducing the levels of inflammation, oxidative stress and pro-inflammatory cytokines.[48] At present, dietary amino acids are known to have a therapeutic potential with respect to IBD treatment.[49] Tryptophan is an essential amino acid for intestinal mucosal cells, as well as an inflammatory inhibitor and an intestinal commensal bacteria regulator. It can ameliorate IBD by directly or indirectly acting on intestinal immunity and microbial homeostasis.[50] The lack of several tryptophan metabolism end-products leads to the progression or aggravation of IBD in patients and mice.[51] In addition, arginine biosynthesis plays an important role in IBD. Arginine metabolic pathways are differentially involved in IBD pathogenesis, l-arginine metabolism may serve as a target for clinical intervention in IBD patients.[52,53] Therefore, the results from this current study demonstrate that QCWZD protects against intestinal inflammation in mice by modulating host gut microbiota composition and functionality.

Our results also provide strong evidence to support the hypothesis that QCWZD may ameliorate intestinal inflammation by reprogramming Th17 cell differentiation in mice. Th17 cells are a subset of T cells that arise by differentiation from naive T helper cells (Th0) after being stimulated by certain cytokines, including IL-6, IL-23, IL-21, and

transforming growth factor-β.[54] Th17 cells are believed to promote intestinal barrier function by stimulating epithelial cells to produce antimicrobial peptides.[55] Currently, an increasing number of reports show that Th17 cells are at the core of IBD-triggered pathogenesis and constitute a target of IBD therapy.[56–59] Alrafas et al found that microbial dysbiosis in colitis mice, especially *Akkermansia muciniphilia* and *Bacteroides acidifaciens*, may cause alterations in the production of short chain fatty acids and promote an inflammatory response via Th17 cells.[60] Changes in the colonization of the gut microbiome are associated with Th17 cell differentiation, leading to the increased expression of IL-17, TNF-α, IL-6, and other genes associated with the IL-17 and TNF-α signaling pathway involved in the intestinal immune disorder.[61] Regulation of the intestinal commensal bacteria, via oral probiotics, is considered a novel therapeutic approach for IBD that may also regulate Th17 cell differentiation. Th17 cells secrete factors such as IL-17 that are not strongly pro-inflammatory themselves but can recruit other immune cells expressing markedly pro-inflammatory factors, such as TNF-α and IL-6, and synergistically lead to a robust pro-inflammatory effect, participating in the mucosal host defence mechanism.[62,63] Interestingly, Li et al discovered that IL-17A secreted by γδT cells can upregulate the Act1-Occludin regulatory pathway to enhance the mucosal barrier function of mice with DSS-induced colitis, thereby exerting a protective effect.[64,65] However, recently emerging evidence has documented a pro-inflammatory effect of IL-17 during the active phase of intestinal inflammation[66] that is consistent with our present findings. It is worth mentioning that the activation of TNF-α and IL-17 signaling pathways not only damage the intestinal barrier function and play a central role in the pathogenesis of IBD,[67] but are also associated with Th17 cell differentiation.[68] TNF-α is activated by the IL-17 signaling pathway and induces Th17 cell differentiation like the downstream cytokine IL-6, thereby promoting IL-17 expression that further aggravates inflammation.[69] At the same time, TNF-α secretion is increased through a positive feedback loop, sustaining and amplifying the inflammatory process.[70] Therefore, we hypothesize that Th17 cell differentiation mediated by gut microbiota plays a crucial role in the QCWZD treatment of IBD.

In summary, our results revealed that QCWZD exerted beneficial effects on intestinal mucosal inflammation in mice through modulating microbial homeostasis, and inhibiting Th17 cell differentiation and its associated signaling pathways. These findings provide novel insights into the regulatory role of QCWZD in treating IBD and hold promise for herb-based complementary and alternative therapy.

Abbreviations

CD, Crohn's disease; cDMEM, complete Dulbecco's modified Eagle's medium; DAI, disease activity index; DSS, dextran sulfate sodium; GO, Gene Ontology; H&E, hematoxylin and eosin; IBD, inflammatory bowel disease; IHC, immunohistochemistry; IL-1β, Interleukin 1β; IL-6, Interleukin 6; IL-17, Interleukin 17; KEGG, Kyoto Encyclopedia of Genes and Genomes; MLNs, Mesenteric lymph nodes; NF-κB, nuclear factor kappa-B; PPI, protein-protein interactions; QCWZD, Qingchang Wenzhong Decoction; TCM, Traditional Chinese Medicine; TCMSP, Traditional Chinese Medicine Systems Pharmacology; Th17, T helper cell 17; TNF-α, transforming growth factor-α; UC, ulcerative colitis.

Data Sharing Statement

The data analyzed in this study can be obtained from the corresponding author upon reasonable request.

Acknowledgments

This work was supported by National Science Foundation of China (No. 82374411), Capital's Funds for Health Improvement and Research (shoufa 2022-4-4205), Unveiling and Leading Projects of Beijing University of Traditional Chinese Medicine (2023-JYB-JBQN-014), and China Association of Chinese Medicine Young Talent Support Project (NO. CACM-2022-QNRC2-A02).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

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

References

1. Alavinejad P, Hashemi SJ, Behl N, et al. Inflammatory bowel disease evolution in the past two decades: A chronological multinational study. *E Clinic Med*. 2024;70:102542. doi:10.1016/j.eclinm.2024.102542. PMID: 38525407; PMCID: PMC10959644.
2. Feuerstein JD, Moss AC, Farraye FA. Ulcerative Colitis. *Mayo Clin Proc*. 2019;94:1357–1373. doi:10.1016/j.mayocp.2019.01.018
3. Agrawal M, Jess T. Implications of the changing epidemiology of inflammatory bowel disease in a changing world. *United Eur Gastroenterol J*. 2022;10(10):1113–1120. doi:10.1002/ueg2.12317. PMID: 36251359; PMCID: PMC9752308.
4. Turner D, Ricciuto A, Lewis A, et al. STRIDE-II: An update on the selecting therapeutic targets in inflammatory bowel disease (STRIDE) initiative of the international organization for the study of IBD (IOIBD): Determining therapeutic goals for treat-to-target strategies in IBD. *Gastroenterol*. 2021;160:1570–1583. doi:10.1053/j.gastro.2020.12.031
5. Hazel K, O'Connor A. Emerging treatments for inflammatory bowel disease. *Ther Adv Chronic Dis*. 2020;11:2040622319899297. doi:10.1177/2040622319899297
6. Zhang S, Zhao L, Shen H, et al. International clinical practice guideline on the use of traditional Chinese medicine for ulcerative colitis by board of specialty committee of digestive system disease of world federation of Chinese medicine societies (2023). *Phytother Res*. 2024;38(2):970–999. doi:10.1002/ptr.8087. PMID: 38112572.
7. Yuan S, Li Y, Li J, et al. Traditional Chinese medicine and natural products: Potential approaches for inflammatory bowel disease. *Front Pharmacol*. 2022;13:892790. doi:10.3389/fphar.2022.892790
8. Chen F, Yin YT, Zhao HM, et al. Sishen pill treatment of DSS-induced colitis via regulating interaction with inflammatory dendritic cells and gut microbiota. *Front Physiol*. 2020;11:801. doi:10.3389/fphys.2020.00801
9. Ge W, Wang HY, Zhao HM, et al. Effect of Sishen pill on memory t cells from experimental colitis induced by dextran sulfate sodium. *Front Pharmacol*. 2020;11:908. doi:10.3389/fphar.2020.00908
10. Chen YL, Zheng YY, Dai YC, Zhang YL, Tang ZP. Systems pharmacology approach reveals protective mechanisms of Jian-Pi Qing-Chang decoction on ulcerative colitis. *World J Gastroenterol*. 2019;25:2603–2622. doi:10.3748/wjg.v25.i21.2603
11. Sun Z, Li J, Wang W, et al. Qingchang Wenzhong Decoction accelerates intestinal mucosal healing through modulation of dysregulated gut microbiome, intestinal barrier and immune responses in mice. *Front Pharmacol*. 2021;12:738152. doi:10.3389/fphar.2021.738152
12. Ling G, Zhang M, Chen C, et al. Progress of Ginsenoside Rb1 in neurological disorders. *Front Pharmacol*. 2024;15:1280792. doi:10.3389/fphar.2024.1280792. PMID: 38327982; PMCID: PMC10847293.
13. Yang K, Zhang L, Liao P, et al. Impact of gallic acid on gut health: Focus on the gut microbiome, immune response, and mechanisms of action. *Front Immunol*. 2020;11:580208. doi:10.3389/fimmu.2020.580208. PMID: 33042163; PMCID: PMC7525003.
14. Huang J, Yue M, Yang Y, et al. Protopine-type alkaloids alleviate lipopolysaccharide-induced intestinal inflammation and modulate the gut microbiota in mice. *Animals*. 2024;14(15):2273. doi:10.3390/ani14152273. PMID: 39123799; PMCID: PMC11311078.
15. Kong L, Liu Y, Li J, et al. Ginsenoside Rg1 alleviates chronic inflammation-induced neuronal ferroptosis and cognitive impairments via regulation of AIM2 - Nrf2 signaling pathway. *J Ethnopharmacol*. 2024;330:118205. doi:10.1016/j.jep.2024.118205. PMID: 38641079.
16. Guo D, Wang Q, Li A, et al. Liquiritin targeting Th17 cells differentiation and abnormal proliferation of keratinocytes alleviates psoriasis via NF-κB and AP-1 pathway. *Phytother Res*. 2024;38(1):174–186. doi:10.1002/ptr.8038. PMID: 37849425.
17. Wang Z, Chen C, Guo Y, et al. Therapeutic analysis of Qingchang Wenzhong formula for treatment of mild-moderate ulcerative colitis patients. *Chinese J Integ Trad West Med*. 2018;38:15–19.
18. Mirete S, Morgante V, González-Pastor JE. Functional metagenomics of extreme environments. *Curr Opin Biotechnol*. 2016;38:143–149. doi:10.1016/j.copbio.2016.01.017
19. Wang X, Wang ZY, Zheng JH, Li S. TCM network pharmacology: A new trend towards combining computational, experimental and clinical approaches. *Chin J Nat Med*. 2021;19:1–11. doi:10.1016/S1875-5364(21)60001-8
20. Ding P, Liu J, Li Q, et al. Investigation of the active ingredients and mechanism of hudi enteric-coated capsules in DSS-induced ulcerative colitis mice based on network pharmacology and experimental verification. *Drug Des Devel Ther*. 2021;15:4259–4273. doi:10.2147/DDDT.S326029
21. Zhou R, Huang K, Chen S, et al. Zhilining Formula alleviates DSS-induced colitis through suppressing inflammation and gut barrier dysfunction via the AHR/NF-κBp65 axis. *Phytomed*. 2024;129:155571. doi:10.1016/j.phymed.2024.155571. PMID: 38677270.
22. Ru J, Li P, Wang J, et al. TCMSP: A database of systems pharmacology for drug discovery from herbal medicines. *J Cheminform*. 2014;6:13. doi:10.1186/1758-2946-6-13
23. Stelzer G, Rosen N, Plaschkes I, et al. The genecards suite: From gene data mining to disease genome sequence analyses. *Curr Protoc Bioinfo*. 2016;54(1):1–30. doi:10.1002/cpbi.5.
24. Amberger JS, Bocchini CA, Schiettecatte F, et al. OMIM.org: Online Mendelian inheritance in man (OMIM®), an online catalog of human genes and genetic disorders. *Nucleic Acids Res*. 2015;43:D789–798. doi:10.1093/nar/gku1205
25. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2019;47:D607–D613. doi:10.1093/nar/gky1131
26. Doncheva NT, Morris JH, Gorodkin J, Jensen LJ. Cytoscape Stringapp: Network analysis and visualization of proteomics data. *J Proteome Res*. 2019;18:623–632. doi:10.1021/acs.jproteome.8b00702
27. Wu F, Shao Q, Cheng Z, et al. Traditional herbal formula Wu-Mei-Wan alleviates TNBS-induced colitis in mice by inhibiting necroptosis through increasing RIPK3 O-GlcNAcylation. *Chin Med*. 2021;16:78. doi:10.1186/s13020-021-00493-4
28. Yang L, Luo H, Tan D, et al. A recent update on the use of Chinese medicine in the treatment of inflammatory bowel disease. *Phytomed*. 2021;92:153709. doi:10.1016/j.phymed.2021.153709
29. Hu J, Huang H, Che Y, et al. Qingchang Huashi formula attenuates DSS-induced colitis in mice by restoring gut microbiota-metabolism homeostasis and goblet cell function. *J Ethnopharmacol*. 2021;266:113394. doi:10.1016/j.jep.2020.113394

30. Amoroso C, Perillo F, Strati F, Fantini MC, Caprioli F, Facciotti F. The role of gut microbiota biomodulators on mucosal immunity and intestinal inflammation. *Cells*. 2020;9:1234. doi:10.3390/cells9051234
31. Oka A, Sartor RB. Microbial-based and microbial-targeted therapies for inflammatory bowel diseases. *Dig Dis Sci*. 2020;65:757–788. doi:10.1007/s10620-020-06090-z
32. Cani PD, Depommier C, Derrien M, et al. Akkermansia muciniphila: Paradigm for next-generation beneficial microorganisms. *Nat Rev Gastroenterol Hepatol*. 2022;19(10):625–637. doi:10.1038/s41575-022-00631-9. PMID: 35641786.
33. Zheng M, Han R, Yuan Y, et al. The role of Akkermansia muciniphila in inflammatory bowel disease: Current knowledge and perspectives. *Front. Immunol*. 2023;13:1089600. doi:10.3389/fimmu.2022.1089600
34. Katiraei S, de Vries MR, Costain AH, et al. Akkermansia muciniphila exerts lipid-lowering and immunomodulatory effects without affecting neointima formation in hyperlipidemic APOE*3-Leiden. CETP mice. *Mol Nutr Food Res*. 2020;64(e1900732). doi:10.1002/mnfr.201900732
35. Mao T, Su CW, Ji Q, et al. Hyaluronan-induced alterations of the gut microbiome protects mice against citrobacter rodentium infection and intestinal inflammation. *Gut Microbes*. 2021;13:1972757. doi:10.1080/19490976.2021.1972757
36. Bian X, Wu W, Yang L, et al. Administration of akkermansia muciniphila ameliorates dextran sulfate sodium-induced ulcerative colitis in mice. *Front Microbiol*. 2019;10:2259. doi:10.3389/fmicb.2019.02259
37. Li Q, Cui Y, Xu B, et al. Main active components of Jiawei Gegen Qinlian decoction protects against ulcerative colitis under different dietary environments in a gut microbiota-dependent manner. *Pharmacol Res*. 2021;170:105694. doi:10.1016/j.phrs.2021.105694
38. Cai Y, Li S, Zhang X, et al. Integrated microbiome-metabolomics analysis reveals the potential therapeutic mechanism of Zuo-Jin-Wan in ulcerative colitis. *Phytomed*. 2022;98(153914). doi:10.1016/j.phymed.2021.153914
39. Hu L, Jin L, Xia D, et al. Nitrate ameliorates dextran sodium sulfate-induced colitis by regulating the homeostasis of the intestinal microbiota. *Free Radic Biol Med*. 2020;152:609–621. doi:10.1016/j.freeradbiomed.2019.12.002
40. Chen X, Lou L, Tang H, et al. Adsorptive granulomonocytapheresis alters the gut bacterial microbiota in patients with active ulcerative colitis. *J Clin Apher*. 2021;36:454–464. doi:10.1002/jca.21887
41. Pereira GV, Abdel-Hamid AM, Dutta S, et al. Degradation of complex arabinoxylans by human colonic bacteroidetes. *Nat Commun*. 2021;12:459. doi:10.1038/s41467-020-20737-5
42. Wang T, Han J, Dai H, et al. Polysaccharides from lyophyllum decastes reduce obesity by altering gut microbiota and increasing energy expenditure. *Carbohydr Polym*. 2022;295:119862. doi:10.1016/j.carbpol.2022.119862
43. Miyamoto Y, Itoh K. Bacteroides acidifaciens sp. nov. isolated from the caecum of mice. *Int J Syst Evol Microbiol*. 2000;50 Pt 1:145–148. doi:10.1099/00207713-50-1-145
44. Yang J, Wang X, Hu T, et al. Entero-toxigenic bacteroides fragilis contributes to intestinal barrier injury and colorectal cancer progression by mediating the BFT/STAT3/ZEB2 pathway. *Cell Cycle*. 2024;23(1):70–82. doi:10.1080/15384101.2024.2309005. PMID: 38273425; PMCID: PMC11005799.
45. Zamani S, Hesam Shariati S, Zali MR, et al. Detection of enterotoxigenic Bacteroides fragilis in patients with ulcerative colitis. *Gut Pathog*. 2017;9:53. doi:10.1186/s13099-017-0202-0
46. Cuív P Ó, de Wouters T, Giri R, et al. The gut bacterium and pathobiont Bacteroides vulgatus activates NF-κB in a human gut epithelial cell line in a strain and growth phase dependent manner. *Anaerobe*. 2017;47:209–217. doi:10.1016/j.anaerobe.2017.06.002
47. Ryan FJ, Ahern AM, Fitzgerald RS, et al. Colonic microbiota is associated with inflammation and host epigenomic alterations in inflammatory bowel disease. *Nat Commun*. 2020;11:1512. doi:10.1038/s41467-020-15342-5
48. Hissen KL, He W, Wu G, et al. Immunonutrition: facilitating mucosal immune response in teleost intestine with amino acids through oxidant-antioxidant balance. *Front Immunol*. 2023;14:1241615. doi:10.3389/fimmu.2023.1241615. PMID: 37841275; PMCID: PMC10570457.
49. Umeda S, Sujino T, Miyamoto K, et al. D-amino acids ameliorate experimental colitis and cholangitis by inhibiting growth of proteobacteria: Potential therapeutic role in inflammatory bowel disease. *Cell Mol Gastroenterol Hepatol*. 2023;16(6):1011–1031. doi:10.1016/j.jcmgh.2023.08.002. PMID: 37567385; PMCID: PMC10632532.
50. Li X, Zhang ZH, Zabed HM, Yun J, Zhang G, Qi X. An insight into the roles of dietary tryptophan and its metabolites in intestinal inflammation and inflammatory bowel disease. *Mol Nutr Food Res*. 2021;65:e2000461. doi:10.1002/mnfr.202000461
51. Michaudel C, Danne C, Agus A, et al. Rewiring the altered tryptophan metabolism as a novel therapeutic strategy in inflammatory bowel diseases. *Gut*. 2023;72(7):1296–1307. doi:10.1136/gutjnl-2022-327337. PMID: 36270778; PMCID: PMC10314090.
52. Baier J, Gänsbauer M, Giessler C, et al. Arginase impedes the resolution of colitis by altering the microbiome and metabolome. *J Clin Invest*. 2020;130(11):5703–5720. doi:10.1172/JCI126923. PMID: 32721946; PMCID: PMC7598089.
53. Li JY, Guo YC, Zhou HF, et al. Arginine metabolism regulates the pathogenesis of inflammatory bowel disease. *Nutr Rev*. 2023;81(5):578–586. doi:10.1093/nutrit/nuac070. PMID: 36040377; PMCID: PMC10086623.
54. Khantakova JN, Mutovina A, Ayriyants KA, et al. Th17 cells, glucocorticoid resistance, and depression. *Cells*. 2023;12(23):2749. doi:10.3390/cells12232749. PMID: 38067176; PMCID: PMC10706111.
55. Bonetti L, Horkova V, Grusdat M, et al. A Th17 cell-intrinsic glutathione/mitochondrial-IL-22 axis protects against intestinal inflammation. *Cell Metab*. 2024;36(8):1726–1744. doi:10.1016/j.cmet.2024.06.010. PMID: 38986617.
56. Jiang P, Zheng C, Xiang Y, et al. The involvement of TH17 cells in the pathogenesis of IBD. *Cytokine Growth Factor Rev*. 2023;69:28–42. doi:10.1016/j.cytogfr.2022.07.005
57. Liu YJ, Tang B, Wang FC, et al. Parthenolide ameliorates colon inflammation through regulating Treg/Th17 balance in a gut microbiota-dependent manner. *Theranostics*. 2020;10:5225–5241. doi:10.7150/thno.43716
58. Zhao Y, Luan H, Jiang H, et al. Gegen Qinlian decoction relieved DSS-induced ulcerative colitis in mice by modulating Th17/Treg cell homeostasis via suppressing IL-6/JAK2/STAT3 signaling. *Phytomed*. 2021;84:153519. doi:10.1016/j.phymed.2021.153519
59. Zhao J, Lu Q, Liu Y, et al. Th17 Cells in inflammatory bowel disease: Cytokines, plasticity, and therapies. *J Immunol Res*. 2021:8816041. doi:10.1155/2021/8816041.
60. Alrafas HR, Busbee PB, Nagarkatti M, Nagarkatti PS. Resveratrol modulates the gut microbiota to prevent murine colitis development through induction of Tregs and suppression of Th17 cells. *J Leukoc Biol*. 2019;106:467–480. doi:10.1002/JLB.3A1218-476RR
61. Wang Y, Yin Y, Chen X, et al. Induction of intestinal Th17 cells by flagellins from segmented filamentous bacteria. *Front Immunol*. 2019;10:2750. doi:10.3389/fimmu.2019.02750

62. Song M, Liang J, Wang L, et al. IL-17A functions and the therapeutic use of IL-17A and IL-17RA targeted antibodies for cancer treatment. *Int Immunopharmacol*. 2023;123:110757. doi:10.1016/j.intimp.2023.110757. PMID: 37579542.
63. Brackman LC, Dixon BREA, Bernard M, et al. IL-17 receptor A functions to help maintain barrier integrity and limit activation of immunopathogenic response to H. pylori infection. *Infect Immun*. 2024;92(1):e0029223. doi:10.1128/iai.00292-23. PMID: 38014948; PMCID: PMC10790819.
64. Li M, Wang B, Sun X, et al. Upregulation of intestinal barrier function in mice with dss-induced colitis by a defined bacterial consortium is associated with expansion of IL-17A producing gamma delta T cells. *Front Immunol*. 2017;8:824. doi:10.3389/fimmu.2017.00824
65. Li M, Gao J, Tang Y, et al. Traditional herbal medicine-derived sulforaphene LFS-01 reverses colitis in mice by selectively altering the gut microbiota and promoting intestinal gamma-delta T cells. *Front Pharmacol*. 2018;8:959. doi:10.3389/fphar.2017.00959
66. Zhou G, Kong WS, Li ZC, Xie RF, Yu TY, Zhou X. Effects of Qing Chang suppository powder and its ingredients on IL-17 signal pathway in HT-29 cells and DSS-induced mice. *Phytomed*. 2021;87:153573. doi:10.1016/j.phymed.2021.153573
67. Wei W, Mu S, Han Y, et al. Gpr174 knockout alleviates DSS-induced colitis via regulating the immune function of dendritic cells. *Front Immunol*. 2022;13:841254. doi:10.3389/fimmu.2022.841254
68. Hirata T, Osuga Y, Takamura M, et al. Recruitment of CCR6-expressing Th17 cells by CCL 20 secreted from IL-1β-, TNF-α-, and IL-17A-stimulated endometriotic stromal cells. *Endocrinol*. 2010;151:5468–5476. doi:10.1210/en.2010-0398
69. Alam MS, Otsuka S, Wong N, et al. TNF plays a crucial role in inflammation by signaling via T cell TNFR2. *Proc Natl Acad Sci U S A*. 2021;118:e2109972118. doi:10.1073/pnas.2109972118
70. Yao D, Dong M, Dai C, Wu S. Inflammation and inflammatory cytokine contribute to the initiation and development of ulcerative colitis and its associated cancer. *Inflamm Bowel Dis*. 2019;25:1595–1602. doi:10.1093/ibd/izz149