Food & Medicine Homology

ISSN 3006-6867 (print); 3006-4252 (online)
https://doi.org/10.26599/FMH.2025.9420048

Research Article

Total Glycosides of *Cistanche deserticola* attenuates DSS-induced inflammatory bowel disease by regulating intestinal environmental homeostasis

Shao-shi Zhang[1, 2], Duo Feng[1, 2], Jian-zhang An[1, 2], Jian Zhao[1, 2], Jiang-yan Zhao[1, 2], Yu Guo[1, 2], Yong-jun Jiang[3], Wen-jie Yan[1, 2] (✉)

[1] *Beijing Key Laboratory of Bioactive Substances and Functional Food, College of Biochemical Engineering, Beijing Union University, Beijing 100191, China*
[2] *College ofBiochemical Engineering, Beijing Union University, Beijing 100023, China*
[3] *Inner Mongolia Sankou Biotechnology Co., LTD., Ordos 017000, Inner Mongolia, China*

Received: 2 May 2024 / **Revised:** 16 July 2024 / **Accepted:** 16 July 2024

Abstract: This study investigates the protective effects of total glycosides of *Cistanche* (TGs) on the intestinal tract of inflammatory bowel disease (IBD) mice induced by dextran sulfate sodium (DSS) from the perspectives of immune regulation and intestinal homeosta. We evaluated the IBD disease activity index (DAI) and histopathological changes of the colon tissue in mice, and validated the involvement of NF-κB and JAK2 in TGS-mediated IBD regulation by real-time quantitative PCR (qRT-PCR), serum enzyme-linked immunosorbent assay (ELISA), and TUNEL Cell Apoptosis Detection. Finally, we analyzed the changes in the intestinal microbiome. The results show that TGs can effectively improve the disease activity index of sick mice, alleviate the damage to the epithelial barrier, improve the morphology of the intestinal epithelial tissue, and reduce the infiltration of inflammatory cells in the damaged colon. TGs inhibit the activation of the two key signal targets of NF-κB and JAK2, reduce the expression of inflammatory factors such as IL-1 and TNF-α, and increase the expression of anti-inflammatory factor IL-10. In addition, TGs reduce the abundance of Proteobacteria, increase the proportion of *Bacteroidetes* and Thermotogae, and upregulate the numbers of *Bacteroides*, *Lachnospiraceae_NK4A136_group*, and *Oscillospiraceae* to alleviate intestinal microbiome dysbiosis and restore intestinal microenvironmental homeosta. In summary, these research results suggest that TGs can improve IBD by alleviating inflammation and restoring intestinal homeosta, providing experimental evidence for the mechanism of *Cistanche* intervention in IBD.

Keywords: Total Glycosides; intestinal disease; gut microbiota; echinoside; verminoside

Citation: Zhang S. S., Feng D., An J. Z., et al. Total Glycosides of *Cistanche deserticola* attenuates DSS-induced inflammatory bowel disease by regulating intestinal environmental homeostasis. *Food & Medicine Homology*, 2025, 2: 9420048. https://doi.org/10.26599/FMH.2025.9420048

1 Introduction

Inflammatory bowel disease (IBD) is a non-specific chronic intestinal inflammatory disease, encompassing ulcerative colitis (UC) and Crohn's disease (CD)[1]. Although its pathogenesis and etiology are still under investigation, it primarily affects the human digestive system. Several studies have reported that the predominant clinical manifestations of IBD include diarrhea, intestinal perforation, weight loss, and rectal bleeding. The disease is characterized by poor recovery rates and frequent relapses, with an increased risk of progression to colon cancer. Consequently, this condition exerts significant physiological and economic burdens on patients[2, 3].

Currently, it is widely accepted that the pathogenesis of IBD is associated with factors such as genetics, immune metabolism, and an imbalance in the structure of intestinal flora[4]. Numerous studies have demonstrated that maintaining environmental homeostasis in the human intestine plays a crucial role in the development of IBD[5]. Patients with IBD often experience disorders in their intestinal immune system and imbalances in their intestinal microflora[6]. One prominent manifestation is damage to the integrity of the intestinal epithelium, which is essential for maintaining intestinal homeostasis. It has been observed that patients with IBD exhibit abnormal metabolism in their intestinal epithelial cells, characterized by aberrant hyperplasia or apoptosis, exfoliation of the intestinal epithelial mucosa, and ulceration[7]. In our research group, we have identified nuclear factor kappa-B (NF-κB)and Janus Kinase 2 (JAK2) as potential key targets for regulating Total Glycosides of *Cistanche deserticola* (TGs) activity against early-stage IBD based

© National R&D Center for Edible Fungus Processing Technology 2024. Published by Tsinghua University Press. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Address correspondence to Wen-jie Yan, meyanwenjie@126.com

on network pharmacological predictions[8]. The NF-κB pathway is a fundamental pathway in the pathogenesis of IBD, playing a crucial role in regulating inflammation, apoptosis, immune response, and various other aspects[9]. The NF-κB pathway interacts with the JAK2/STAT3 pathway to form a complex signaling network, thereby increasing the complexity of IBD[10]. Normally, NF-κB resides in the cytoplasm and binds to its inhibitory protein IκB to form an inactive complex that prevents NF-κB activation. However, in IBD, damage to intestinal epithelial cells leads to increased permeability of the intestinal mucosal barrier. This allows harmful microorganisms or endotoxins to induce intracellular degradation of IκB through the mucosal barrier, resulting in release and nuclear migration of NF-κB for transcriptional activation of numerous inflammatory response genes. This includes upregulation of inflammatory factors and adhesion molecules such as Tumor Necrosis (TNF-α), Interleukin-1 beta (IL-1β), and Interleukin 10 (IL-10) which further exacerbate intestinal inflammation. Additionally, loss of normal inhibition on apoptosis aggravates excessive cell death and compromises integrity of the intestinal mucosal barrier. The NF-κB pathway plays a vital role in regulating apoptosis-related gene expression necessary for maintaining this integrity[11]. JAK2 is an enzyme belonging to Janus kinase family acting as downstream regulator for several inflammatory cytokine receptors; it represents a potential therapeutic target within JAK2/STAT3 pathway[12]. Interferons (IFNs), interleukins (ILs), etc, transmit signals by activating JAK2 pathway ultimately leading to initiation and maintenance of inflammatory responses. Studies have confirmed significant increase in mRNA expression levels of JAK2 among patients with IBD indicating its involvement[13].

Herba cistanches, also known as inch cloud and goblin, is a plant in deserticaceae with high medicinal value and has pharmacological effects such as anti-oxidation, anti-dementia, anti-fatigue, bowel laxative, immune regulation, liver protection and flora inhibition[14, 15]. It is mainly produced in Inner Mongolia, Xinjiang, Qinghai and other places, is a traditional Chinese medicine. According to "Compendium of Materia Medica": herba cistanches is sweet, mild and non-toxic, returning kidney and large intestine. Contemporary research has demonstrated that herba cistanches contains a diverse range of bioactive components, including phenylethanoid glycosides, polysaccharides, total flavonoids, and betaine. Among these constituents, the primary efficacy components are the TGs, which encompass acteoside, echinacoside, boschnaloside A, 2'-acetylacteoside, and tubuloside B[16]. The findings of various studies have demonstrated that TGs possess the ability to effectively mitigate the oxidative stress damage induced by inflammatory response and counteract the upregulation of inflammatory factor expression[17].

Numerous studies have shown that the symptoms of acute intestinal mucosal injury induced by dextran sulfate sodium (DSS) are most similar to the clinical manifestations of human disease[18]. DSS mainly increases the intestinal mucosal permeability through various pathways, destroys the gastrointestinal immune system, causes the upregulation of inflammatory cytokines, and causes the imbalance of intestinal flora.Therefore, DSS was used to induce the IBD mouse model. To explore the effect of TGs on the IBD mouse model, which is most similar to the clinical manifestation of human disease. By inducing destruction of intestinal mucosal barrier, activation of T lymphocytes and single nucleus macrophages, caused upregulation of inflammatory cytokines, activated some pathways, increased intestinal permeability and led to intestinal mucosal damage, which could provide a good experimental basis for the prevention and control of inflammatory intestinal diseases in human and animals.

2 Materials and Methods

2.1 Chemical regents

TGs (Mass fraction ≥75%, to Echinoside and verminoside in total) was provided by Inner Mongolia Sankou Technology Co., Ltd. (Ordos, Inner Mongolia, China), Dextran sulfate sodium (MP USA has a molecular weight of 36000-50000), Urinary feces hidden blood test box (Nanjing Construction Institute of Biological Engineering, China), Ethanol absolute (Sinopharm Group Chemical Reagent Co., Ltd), Dimethylbenzene (Shanghai Lingfeng Chemical Reagent Co., Ltd., China), PBS buffer solution, Protease K, Broken membrane liquid, DAPI, Antifade mounting medium, and Tunel kit were purchansed from Servicebio (Wuhan Servicebio Technology Co., Ltd., China); Mouse Tumor necrosis factor α (TNF-α ELISA KIT), Mouse Interleukin 2 (IL-2 ELISA kit), Mouse Interleukin 10 (IL-10 ELISA kit), and Mouse Interleukin 1β (IL-1β ELISA kit) were obtained from CUSABIO (Cusabio Technology LLC, China).

2.2 DSS-induced experimental colitis

The SPF grade male BALB/c mice (5 – 8 weeks, 18 – 21 g) in this experiment, were provided by the Health Food Testing Center, School of Applied Arts and Sciences, Beijing Union University [Laboratory Animal Use License No: SYXK (Jing) 2017-0038]. In the feeding and SPF grade animal houses, the temperature was 23 ± 2 °C, the relative humidity was 50% to 70%, and the solar light was illuminated for 12 h. The animal experiment protocols for this study were approved by the Animal Laboratory of the Health Food Testing Center, School of Applied Arts and Sciences, Beijing Union University (Approval No: 20210720).

Three days after adaptive feeding, 40 mice were randomly divided into five groups by body weight, with 8 mice per group.

Control group (CG): Sterm-free water was consumed freely and gavaged with sterile water for 7 consecutive days. DSS model group (DG): Free drinking was supplemented with 3% DSS solution for 7 days. Low-dose group of TGs (LG): Free drink daily with 3% DSS solution, TGs concentration was given of 20 mg/kg through intragastric administration for 7 days. Medium-dosage group of TGs (MG): Free drink daily with 3% DSS solution, TGs concentration was given of 40 mg/kg through intragastric administration for 7 days. High-dose groups of TGs (HG): Free drink daily with 3% DSS solution, TGs concentration was given of 80 mg/kg through intragastric administration for 7 days.

Eating was stopped for 12 h after 7 days of continuous molding and killed by cervical dislocation. Immediately after death, removed the eyeball to collect blood, extractd the abdominal fluid and opened the abdominal cavity. And the colon length was measured, and organs such as mouse spleen, mesenteric lymph nodes, and pie nodes were collected.

2.3 Disease activity index (DAI)

Mice were weighed before gavage at a fixed time daily, their mental status and fecal traits were observed, and fecal hidden blood conditions were determined, and scored accordingly. As shown in Table 1.

2.4 Evaluation of the colon by histopathology

The mouse colons were harvested for 1cm and washed with 3%

Table 1 IBD code of points

score	Weight loss rate (%)	Fecal state	Fecal hidden blood
0	0	normal	achromatic colour
1	1-5		wathet blue
2	5-10	Paste stool	blue-green
3	10-15		navy blue
4	>15	Loose stools	Visible blood

Note: DAI score = (body quality score + fecal trait score + fecal cryptic blood score) / 3

normal saline. Samples were fixed in 4% paraformaldehyde solution for 24 h and prepared into HE sections by dehydration, transparency, wax embedding, slicing, dewaxing, hematoxylin-eosin staining, dehydration, sealing, etc. Stained sections were placed under a microscope to observe the inflammatory changes in the colonic tissue.

2.5 TUNEL Cell Apoptosis Detection

Already prepared colon paraffin sections were deparaxed to water, then repaired by protease K, membrane permeabilized, tunel reaction solution was incubated and stained with nuclear-anti-fluorescein quencher with DAPI, these five steps for TUNEL fluorescent sections. Finally, the slices were observed under a panoramic section scanner (3DHISTECH, PANNORAMIC DESK/MIDI/250/1000) and images were collected. DAPI stained nuclei are blue, UV stimulated, apoptotic nuclei are red.

2.6 Enzyme-linked immunosorbent assay (ELISA)

The collected mouse blood was centrifuged at 3,500 r/min for 10 min, and the supernatant was taken as the mouse serum. The expression of the inflammatory cytokines (IL-1β, TNF-α, IL-10) in the mouse serum was determined by using the kit according to the ELISA kit instructions.

2.7 Immunosignaling pathway was detected by Real-time quantitative PCR detection (qRT-PCR)

The mesenteric lymph nodes and Peyer's patches from mice were collected, sectioned into tissue blocks of approximately 0.5 cm, and immersed in a solution for preserving RNA stability. Following a designated soaking period, the tissue samples were extracted and total RNA was isolated through grinding and crushing in an RNA extraction solution. Subsequently, reverse transcription and PCR amplification techniques were employed to assess the activation of key immune signaling pathways including *GAPDH*, *NF-κB*, *mTOR*, and *JAK2* in both mouse mesenteric lymph nodes and Peyer's patches. The sequences of the primers used for qRT-PCR were shown in Table 2.

2.8 16S rRNA Amplification and Sequencing

After a 7-day period, fecal samples were collected within 24 h and subjected to genomic DNA extraction using the CTAB method. Subsequently, the purity and concentration of the extracted DNA were evaluated through agarose gel electrophoresis before undergoing dilution. Specific primers and barcodes were used to amplify the 16S rRNA genes in distinct regions (16S V3-V4). Each PCR mixture contained 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs, America), 0.2 μM of each primer, and 10 ng of target DNA. The cycling conditions consisted of a first denaturation step at 98 °C for 1 min, followed by 30 cycles at 98 °C (10 s), 50 °C (30 s), and 72 °C (30 s), with a final extension at 72 °C for 5 min. Mixed an equal volume of SYB green-containing loading buffer with PCR products and performed electrophoresis on 2% agarose gel for DNA detection. The PCR products were mixed in equal proportions, then purified using the Qiagen Gel Extraction Kit (Qiagen, Germany). Library construction was performed using the NEBNext® Ultra™ II DNA Library Prep Kit library-building kit, and the constructed libraries were prepared for Qubit and Q-PCR quantification. After qualifying the library, it was sequenced on an Illumina NovaSeq platform generating 250 bp paired-end reads[19, 20].

2.9 Gut microbiota analysis

The raw sequencing data contains some noise, so we splice and filter it to obtain clean data. Then, we use DADA2 in QIIME2 software (Version QIIME 2-202006) to denoise the effective tags and get initial ASVs (Amplicon Sequence Variants) (default: DADA2), followed by filtering out ASVs with an abundance less than 5. Each deweighted sequence generated after noise reduction using DADA2 is referred to as ASVs (Amplicon Sequence Variants), and the abundance table of these sequences in the sample is called a feature table, corresponding to the OTU table. For the resulting ASVs, species annotation was performed to obtain species information and abundance distributions. Perform abundance analysis, diversity calculations, and community structure comparisons on ASVs to evaluate species richness and evenness and explore differences between different samples or groups. At the same time, statistical analysis methods are used to test for significant differences in species composition and community structure, as well as to study the functional differences of communities in the samples through functional annotation analysis.

2.10 Statistical analysis

Experimental data were statistically analyzed using SPSS (version 21.0), and the data were expressed as standard error of mean (SEM). Normal distributed data using Student's t test and non-normally distributed data were rank sum test and graphed using Graph Pad Prism 6.The significance level was expressed as $P < 0.05$ that was considered a statistically significant difference, with a confidence interval of 95% (95% CI).

3 Results

3.1 TGs effectively improved the DAI score induced by DSS

The DAI score is a crucial parameter for assessing IBD disease.

Table 2 The sequence of the primers

Pathway name	Upstream primers	Downstream primers
GAPDH	CCTCGTCCCGTAGACAAAATG	TGAGGTCAATGAAGGGGTCGT
NF-κB	CGAGTCTCCATGCAGCTACG	TTTCGGGTAGGCACAGCAATA
JAK2	GCTGAACCAAGGAGACGGAATA	GGCTGATCCCGTTGATTTCC

In this experiment, all the other four groups exhibited varying degrees of body weight loss and fecal occult blood, except for those in the normal group (Fig. 1). Among them, the DSS group demonstrated the highest rate of weight loss, most severe stool blood presence, and highest DAI score; visible fecal bleeding was observed after the sixth day of modulation. In contrast, in the TGs group, as the increase of dose concentration, there was a gradual decrease in DAI score and improvement in occult blood situation. Most notably, the TGs high-dose group displayed minimal weight loss and negligible fecal occult blood. These findings substantiate that TGs has potential to ameliorate physiological effects associated with colitis-induced diseases.

3.2 TGs improved the colon pathological histostasis in IBD mice

By HE staining, the colon tissue of normal mice was relatively intact, and the morphology of intestinal epithelial cells was intact. The DSS mice had severe colon structural damage, impaired epithelial barrier, significant inflammatory cell infiltration, and a severe degree of inflammation. The low and medium dose groups showed mucosal shedding and inflammatory cell infiltration, but the degree of inflammation was less severe than the model group, and the inflammation was moderate. And mice in the high dose group had essentially normal intestinal villus morphology with mild mononuclear infiltration and mild edema with a mild degree of inflammation (Fig. 2).

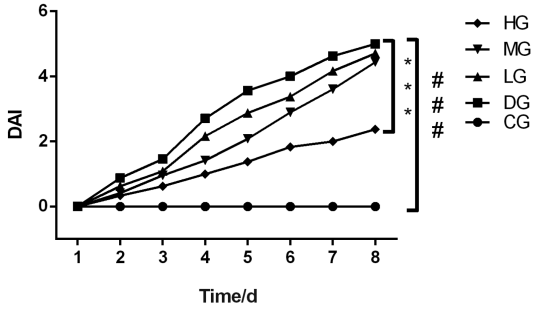


Figure 1 Effect of TGs on the IBD score in mice. Data were the mean ± SEM ($n = 8$). $^{###}P < 0.001$ versus the Control group; $^{***}P < 0.001$ versus the DSS model group.

3.3 TGs downregulated theNF-κB and JAK2 signaling pathways in IBD mice

NF-κB and JAK2 are two important targets for activating the inflammatory response, and the expression of NF-κB and JAK2 mRNA in mouse colon tissues can be detected.The results showed that: NF-κB and JAK2 mRNA expression were significantly increased in DSS control mice compared with normal mice, and NF-κB and JAK2 mRNA expression were significantly reduced in TGs administration mice compared with DSS control mice. Among them, the most significant decrease in the TGs high-dose group, and the expression levels were basically consistent with the normal group (Fig. 3). It showed that TGs effectively down-

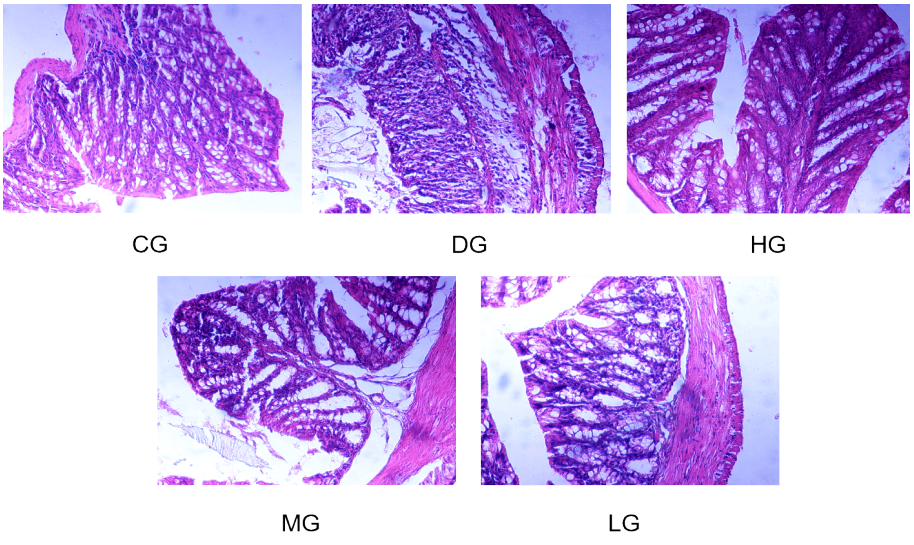


Figure 2 Effect of TGs on the colonic pathological tissue in mice (HE × 20).

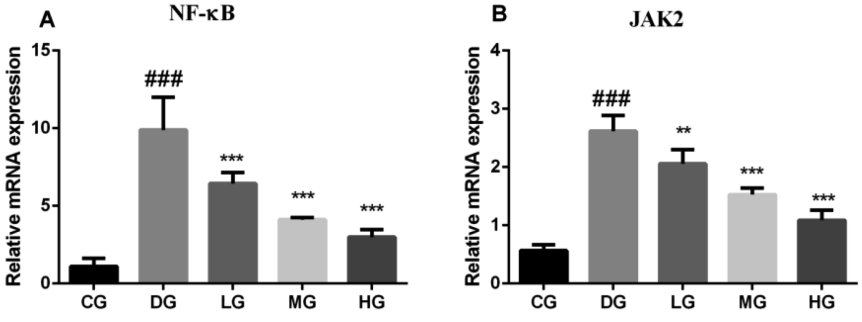


Figure 3 Effect of TGs on the expression levels of NF-κB and JAK2 mRNA in the mouse colon. (A) NF- κB mRNA expression level, (B) JAK2 mRNA expression level. Data were the mean ± SEM ($n = 8$). $^{###}P < 0.001$ versus the Control group; $^{**}P < 0.01$, $^{***}P < 0.001$ versus the DSS model group.

regulated the expression of the NF-κB and JAK2 signaling pathway.

3.4 TGs downregulated the serum inflammatory factor expression in IBD mice

The expression levels of inflammatory cytokines had an important role in IBD disease, and the expression levels of the serum inflammatory cytokines IL-1β, TNF-α, IL-10 in IBD mice were determined by the ELISA assay. The experimental results showed that the expression of pro-inflammatory factor IL-1β, TNF-α, and anti-inflammatory factor IL-10 was significantly increased in the serum of DSS control mice compared to the normal group (Fig. 4). After the intervention of TGs, there was an increase in the expression of serum inflammatory factor IL-10, while the expressions of IL-1β and TNF-α were significantly downregulated. Among them, the TGs group had the best intervention, and the inflammatory cytokine expression level was not significantly different from the normal group. It indicated that TGs was able to mitigate the inflammatory response by inhibiting proinflammatory factor expression.

3.5 TGs slowed down apoptosis in colon epithelial cells in IBD mice

In the Fig. 5, We could observe the results of the TUNEL assay showed almost no TUNEL-labeled bright red fluorescence-positive cells in the colon epithelial tissue of mice in the blank group. In the blank group, the positive rate of the target region for quantitative analysis was 0.279%, indicating the basic absence of apoptotic features. However, bright red fluorescence positive cells marked with TUNEL in colonic epithelial tissues in DSS control mice, and the positive rate of the target area for quantitative analysis was 1.808%, indicating abnormal apoptosis of epithelial cell damage in mice. TUNEL fluorescent positive cells labeled with TUNEL were reduced in colonic epithelial tissues of TGs compared to DSS controls, and the positive rates of the quantitative target region were 0.942% and 0.888%, respectively. Of note, TUNEL decreased in the TGs, and the target region positive rate was 0.288% as that in the normal group, indicating the basic disappearance of apoptotic characteristics. So it was found thay TGs could maintain the integrity of intestinal barrier function by inhibiting the abnormal apoptosis of intestinal epithelial cells by HE analysis.

3.6 TGs improved the intestinal microbiota environment in IBD mice

To explore the effect of TGs on the mouse intestinal microbiota, gene amplification sequencing was performed at 16S rRNA on the illumina NovaSeq sequencing platform.Then, the noise reduction was performed using the DADA2 method, species annotation for each ASV using a pre-trained Naive Bayes classifier using QIIME2's classify-sklearn algorithm[21, 22]. According to the results of ASVs and research requirements of noise reduction, the common and unique ASVs between different groups were analyzed and plotted as Venn Graph (Fig. 6). A total of 1,862 ASVs were detected in the three groups, 1,358 ASVs were detected in CG, 1,070 ASVs were detected in DG, and 1,208 ASVs were detected in HG. Of these, CG and DG have a total of 775 ASVs and CG and HG have a total of 931 ASVs. Compared to the DG, the intestinal microbiota in the HG was more similar to that in the CG. To investigate the effects of TGs on the structural composition of mouse gut microbiota, we assessed microbial differentials at the intestinal portal and genus levels in mice.

The annotation results showed that at the phylum level, Bacteroidota, Firmicutes, and Proteobacteria were detected in the three groups of experimental mice, accounting for more than 94% of the total Bacteroidota, Firmicutes, and Proteobacteria. Compared with CG, the abundance of Proteobacteria in DG mice increased by 2.2%, that of Bacteroidetes increased by 17.78%, and that of Firmicutes decreased by 16.84%. Compared with DG, after TGs intervention, the abundance of Proteobacteria decreased by 3% and the ratio of Bacteroidota/Firmicutes returned to normal levels (Fig. 7A). The dominant bacterial genera in the analysis of experimental mice at the genus level were *Muribaculaceae*, *Bacteroides*, *Lachnospiraceae_NK4A136_group*, and *Kurthia*. Compared with CG mice, there was an increase in abundances of *Muribaculaceae*, *Bacteroides*, and *Kurthia* in DG mice while a decrease was observed in *Lachnospiraceae_NK4A136_group*. After TGs intervention, the abundances of *Muribaculaceae*, *Bacteroides* and *Kurthiadecreased* while the abundance of *Lachnospiraceae_NK4A136_groupincreased* (Fig. 7B). These results indicate that TGs has the ability to regulate and restore intestinal flora.

To further assess the differences and distance in fecal microbial structure in each group, we performed Alpha Diversity analysis index and Beta Diversity analysis index. Alpha Diversity was used to analyze the microbial community diversity[23-25] of Within-community, which reflects the diversity richness and diversity in a single sample. The Rarefaction Curve (Fig. 8A) was plotted by the Coverage-the Good's coverage index. Shannon index and Simpson index analyses indicated (Figs. 8B and C), DSS disrupted mouse gut microbiota diversity and decreased Shannon index and Simpson index. After the TGs treatment, the results showed the opposite trend. Although there was no significant difference after T-test ($P > 0.05$), the data trend still indicated that TGs protected the richness and diversity of microbial communities within the sample. Beta Diversity is a comparative analysis of the microbial community composition of different samples.Principal Co-ordinates Analysis (POCA). The analysis results show that (Fig. 8D), the distance between the DSS control group is the

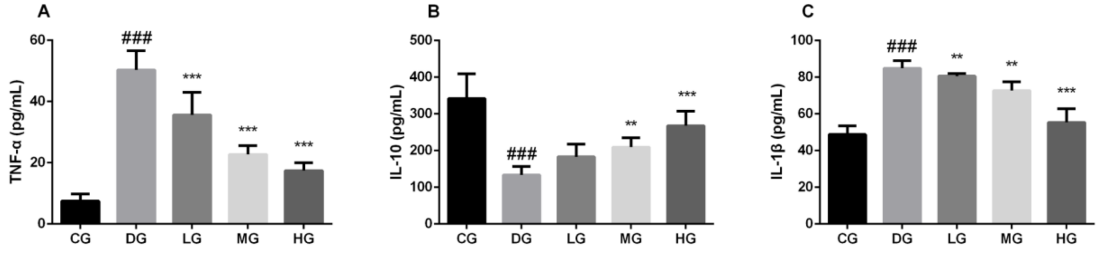


Figure 4 Effect of TGs on the expression level of inflammatory cytokines in mouse serum. (A) TNF-α expression level, (B) IL-10 expression level, (C) IL-1β expression level. Data were the mean ± SEM ($n = 8$). $^{###}P < 0.001$ versus the Control group; $^{**}P < 0.01$, $^{***}P < 0.001$ versus DSS model group.

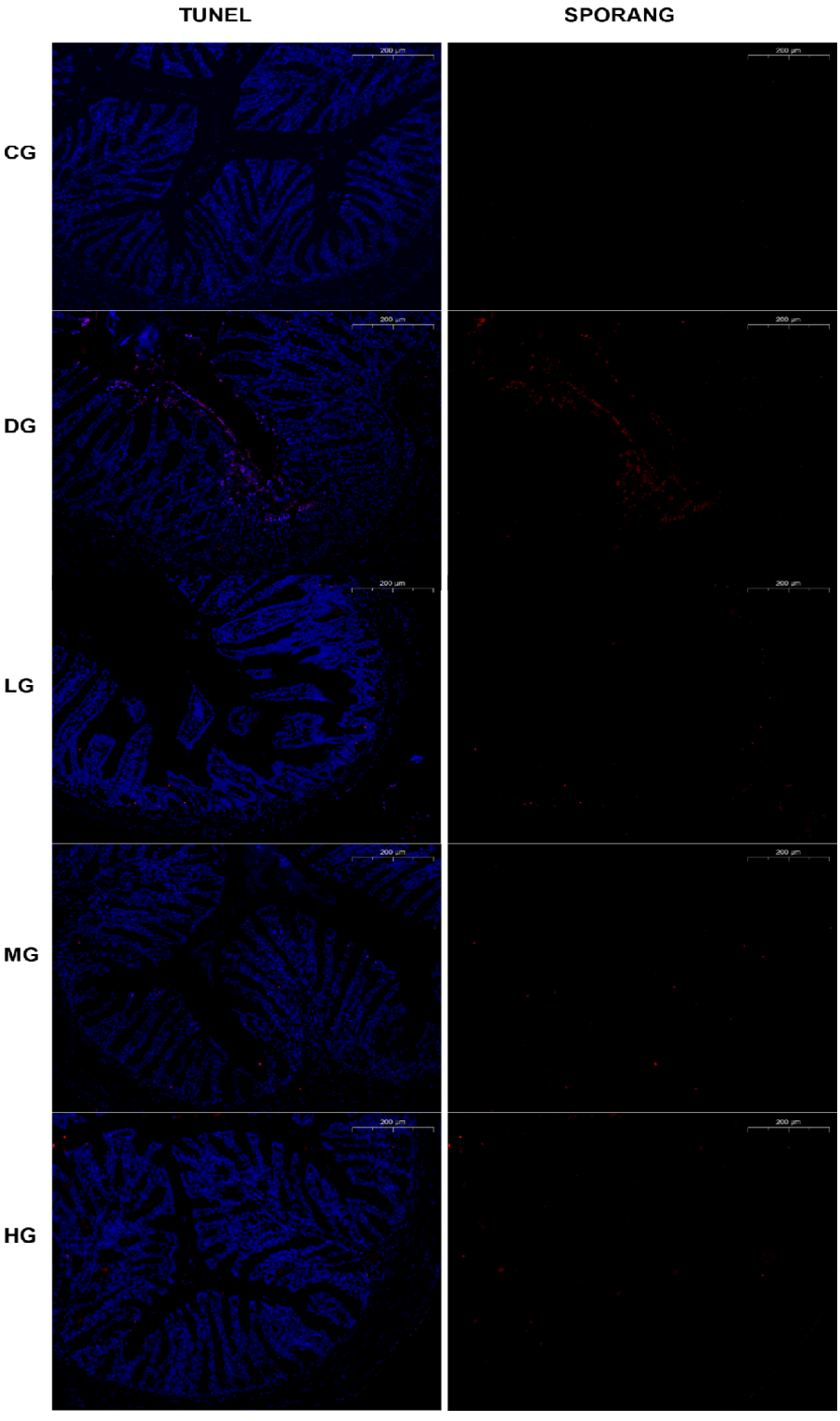


Figure 5 Effect of TGs on cell apoptosis in mouse epithelial tissues .

longest, and between the TGs high-dose treatment group and each sample of the normal group. This indicates that the mouse intestinal microbiota structure was somewhat restored after TGs treatment.

Further LefSe analysis was used to compare the differences in gut flora abundance at the phylum, class, order, family, and genus levels among the three groups of mice (CG, DG, and HG) Fig. 9, with a LDA value greater than 3.5. The LDA effect size[26] is an analytical tool utilized for identifying and interpreting high-dimensional biomarkers (such as genes, pathways, and taxa). It enables comparison of multiple populations by highlighting statistical significance and biological correlation while facilitating discovery of distinct bacterial genera between samples. The analysis revealed that the predominant bacterial groups in DG mice were *Bacteroidota phylum* and *Bacteroides Kurthia*. In mice overall, *Firmicutes* and *Eubacterium_nodatum_group Family_XIII_UCG_001L* were found to be dominant bacteria. These results indicate significant changes in intestinal flora among

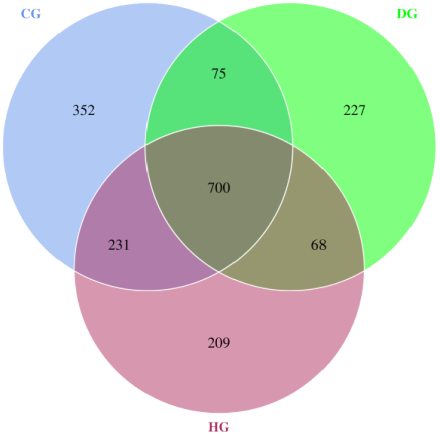


Figure 6 Venn analysis of ASVs ($n = 6$) .

DG mice. The dominant bacteria in HG mice were identified as *Lachnospiraceae_NK4A136_group* which significantly differed from those found in DG mice.

To further explore the effects of TGs on the gut microbiota, we used PICRUSt2 to make functional predictions of microbial communities in ecological samples. PICRUSt2 is a bioinformatic software for metagenomic function prediction based on the Marker gene. Functional prediction based on the KEGG Pathway database was performed based on the 16S sequencing data. According to the functional annotation and abundance information of the samples in the database, the top 15 abundance functions and their abundance information in each sample are selected to draw the heat map, and they are clustered from different functional levels (Fig. 10). The analysis revealed that, in comparison to CG mice, DG mice exhibited a significant

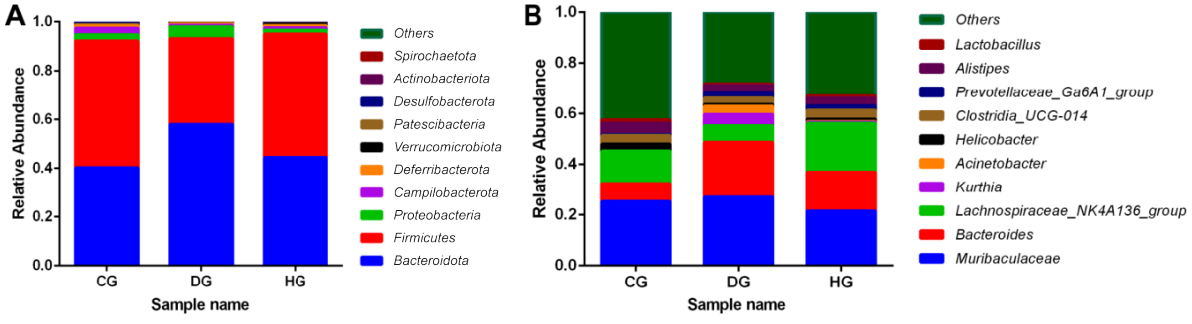


Figure 7 Effect of TGs on the structural composition of the mouse intestinal microbiota. (A) Horizontal structure composition of the phylum, (B) Horizontal structure composition of the genus. ($n = 6$).

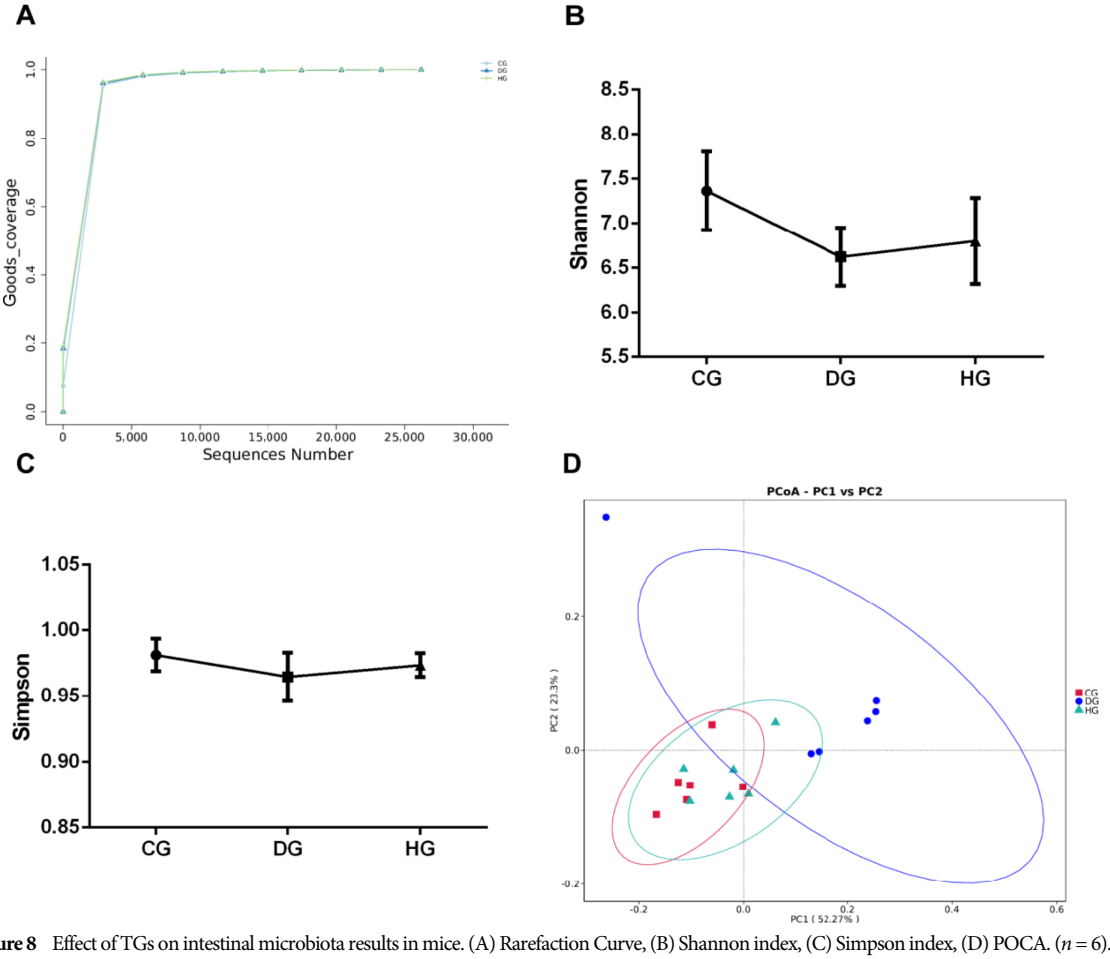


Figure 8 Effect of TGs on intestinal microbiota results in mice. (A) Rarefaction Curve, (B) Shannon index, (C) Simpson index, (D) POCA. ($n = 6$).

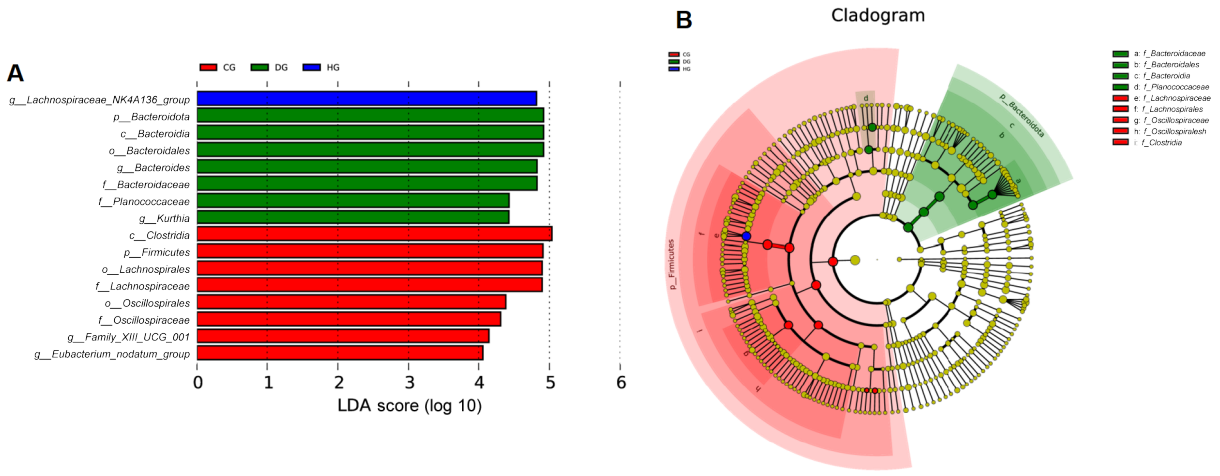


Figure 9 Analysis of the predominant microbes in mouse intestinal flora. (A) Bar graph of LDA value distribution. (B) Evolutionary branch diagram.

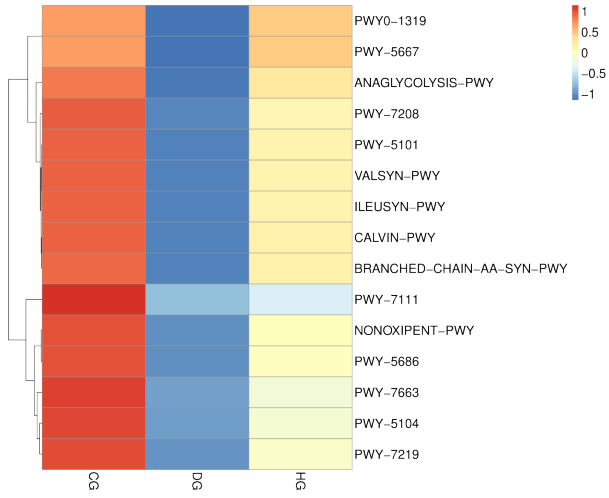


Figure 10 The PICRUSt2 functional annotation cluster heatma.

downregulation of the biological metabolic pathway NONOXIPENT-PWY (pentose phosphate pathway (non-oxidative branch)), as well as PWY-5101 (L-isoleucine biosynthesis II), PWY-5104 (L-isoleucine biosynthesis IV), PWY-7111 (pyruvate fermentation to isobutanol (engineered)), VALSYN-PWY (L-valine biosynthesis), ILEUSYN-PWY (L-isoleucine biosynthesis I (from threonine)), CALVIN-PWY (Calvin-Benson-Bassham cycle), PWY-7663 (gondoate biosynthesis (anaerobic)), PWY-7219 (adenosine ribonucleotides de novo biosynthesis), PWY0-1319 (CDP-diacylglycerol biosynthesis II), and BRANCHED-CHAIN-AA-SYN-PWY (superpathway of branched amino acid biosynthesis). However, following TGs intervention, the most notable pathways showing recovery were WYO0-1319 (CDP-diacylglycerol biosynthesis II), PWYE5667 (CDP-diacylglycerolbiosynthesisisI) and ANAGLYCOLYSIS-PWY (GlycolysisIII (fromglucose)).

4 Discussion

The present study aimed to investigate the protective effect of TGs on the intestinal tract of DSS-induced IBD mice, focusing on its role in regulating inflammation and maintaining intestinal homeostasis. Firstly, we assessed the homeostasis of the intestinal immune system in mice. The results from IBD score evaluation and HE staining demonstrated that TGs effectively ameliorated weight loss and fecal occult blood in infected mice, while preserving colonic epithelial morphology and mitigating inflammatory cell infiltration. In order to further investigate the regulatory mechanism of TGs on inflammatory response, we assessed the mRNA expression levels of two crucial targets involved in *NF-κB* and *JAK2* signaling pathways in mouse mesenteric lymph nodes. Additionally, we measured the serum levels of inflammatory factors (IL-1β, TNF-α, IL-10) in mice. The apoptosis of intestinal epithelial cells was evaluated using TUNEL fluorescence staining. NF-κB is a pivotal transcription factor that plays a significant role in regulating gene expression related to immunity, inflammation, and cell survival[27, 28]. As a key component of the JAK2/STAT3 pathway, JAK2 can collaborate with NF-κB to modulate the expression of inflammatory factors (TNF-α, IL-1β, IL-10) in IBD diseases[29-31]. This interaction ultimately affects the balance between survival and death of intestinal epithelial cells by influencing apoptosis-related gene expression and maintaining the integrity of the intestinal mucosal barrier. The results demonstrated that TGs effectively attenuated the expression of NF-κB and JAK2 mRNA, downregulated the production of pro-inflammatory cytokines TNF-α and IL-1β, and upregulated the secretion of anti-inflammatory cytokine IL-10. Additionally, TUNEL analysis revealed that TGs significantly ameliorated aberrant apoptosis in colonic epithelial cells in mice. These findings suggest that TGs may exert a synergistic regulatory effect on NF-κB and JAK2 to mitigate inflammation in IBD.

We subsequently assessed the homeostasis of the gut microbiota in mice, and observed that treatment based on the gut microbiota also exhibited amelioration in the colitis model. The findings demonstrated that DSS perturbed both the abundance and community distribution of intestinal microflora in mice compared to normal mice, while TGs significantly mitigated this phenomenon. In terms of intestinal microflora structure, Firmicutes and Bacteroidetes were predominantly found in normal mice, followed by Proteobacteria. However, DSS induction led to a decrease in relative abundance of Firmicutes, Bacteroidetes, and Proteobacteria. After intervention with TGs, there was a decrease in relative abundance of Firmicutes and Proteobacteria as well. These findings are consistent with previous relevant studies[32, 33]. This result indicated that TGs could restore the homeostasis of the gut microbiota. Alpha Diversity and Beta Diversity analysis showed that it upregulated Shannon index and Simpson index of IBD diseased mice after TGs treatment, making the intestinal flora structure of diseased mice closer to that of

normal mice. This indicated that TGs restored its gut microflora structure while protecting the richness and diversity of mouse gut microbial community. The LefSe analysis revealed that the dominant bacterial genera in DG mice were *Bacteroides* and *Kurthia*, both of which are known to be pathogenic bacteria. *Bacteroides* is a gram-negative, obligate anaerobic genus commonly found in clinical specimens of enteritis and septicemia[34]. *Kurthia* is an irregularly spheroidal or short rod-shaped genus that can cause *Kutteriasis*[35]. The predominant strain observed in DG mice was *Lachnospiraceae_NK4A136_group*, belonging to the family *Trichospirillaceae*, which consists of anaerobic sporogenic bacteria[36]. This strain is considered potentially beneficial as it produces short-chain fatty acids (SCFAs) through the fermentation of dietary polysaccharides within the mouse gut[37]. SCFAs serve as an energy source and promote intestinal cell growth and differentiation. Furthermore, SCFAs possess anti-inflammatory properties and aid in repairing damaged intestinal mucosal barriers. Therefore, we hypothesized that the restoration of intestinal flora in IBD mice by TGs was achieved by regulating *Lachnospiraceae_NK4A136_group*. By predicting the biological function of intestinal microbiota, we observed abnormal expression of multiple biological metabolic pathways in the feces of DG mice compared to normal mice. Following TGs intervention, significant recovery was observed in three pathways: PWY0-1319 (CDP-diacylglycerol biosynthesis II), PWY-5667 (CDP-diacylglycerol biosynthesis I), and ANAGLYCOLYSIS-PWY. Among these, glycolysis III (from glucose) exhibited the most pronounced restoration. Both CDP-diacylglycerol I and II are crucial processes in biofilm synthesis[38]. CDP-diacylglycerol biosynthesis I involves the synthesis of CDP-diacylglycerol from CMP and diacylglycerol, catalyzed by the enzyme CDP-diacylglycerol synthase. In contrast, CDP-diacylglycerol II biosynthesis utilizes CTP and DAG as substrates. The products of these pathways, namely CDP-diacylglycerols, serve as direct precursors for phospholipid synthesis - a major component of cell membranes that plays a vital role in maintaining cellular junction stability and function[39]. Glycolysis III (from glucose) is a biochemical process that produces pyruvate from glucose; it is also known as the hexose phosphate pathway or glucose colysis's third stage[40]. Pyruvate serves as both a carbon source and energy source for some gut microorganisms while playing an essential role in maintaining environmental homeostasis within the gut by promoting metabolic activities.

5 Conclusion

In summary, this study investigated the mechanism by which TGs improve DSS-induced inflammatory bowel disease from two perspectives: regulation of intestinal inflammation and alterations in intestinal flora. The findings demonstrated that the high-dose group of TGs (80 mg/kg) exhibited the most effective therapeutic outcome, effectively alleviating pathological manifestations of IBD, reducing inflammation, mitigating disturbances in intestinal flora, regulating metabolic pathways, and restoring intestinal environmental homeostasis. The *in vivo* studies have demonstrated the direct inhibitory effects of TGs on two crucial targets, NF-κB and JAK2, leading to a reduction in inflammation. Furthermore, metagenomic function prediction of bacterial flora has revealed that TGs can also restore CDP-diacylglycerol biosynthesis II as well as Cdp-diacylglycerol biosynthesis I and Glycolysis III (from glucose). These three interconnected metabolic pathways work together to reduce inflammation and restore environmental homeostasis in the gut. As there were no previous studies on the impact of *Cistanche deserticola* on the intestinal flora of IBD mice, this study only conducted a preliminary exploration into its mechanism effects while providing an experimental foundation for further investigation into the mechanisms underlying *Cistanche deserticola*'s effects on IBD disease.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

Acknowledgements

This study was supported by the National Key Research and Development Program of China (No. 2023YFF1103802).

Ethics statement

The animal experimental protocol was authorized by the Animal Care and Use Ethics Committee of Beijing Union University (Approval No: 20210720), and all experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

References

[1] Cheng, C., Hua, J., Tan, J., et al. Identification of differentially expressed genes, associated functional terms pathways, and candidate diagnostic biomarkers in inflammatory bowel diseases by bioinformatics analysis. *Experimental and Therapeutic Medicine*, **2019**, 18: 278–288. https://doi.org/10.3892/etm.2019.7541

[2] Podolsky, D. K. Inflammatory bowel disease (2). *New England Journal of Medicine*, **1991**, 325: 1008–1016. https://doi.org/10.1056/NEJM199110033251406

[3] Han, Y., Ma, T. M., Lu, M. L., et al. Role of moxibustion in inflammatory responses during treatment of rat ulcerative colitis. *World Journal of Gastroenterology*, **2014**, 20: 11297–11304. https://doi.org/10.3748/wjg.v20.i32.11297

[4] Zhang, S. L., Wang, S. N., Miao, C. Y**.** Influence of Microbiota on Intestinal Immune System in Ulcerative Colitis and Its Intervention. *Frontiers in Immunology*, **2017**, 8: 1674. https://doi.org/10.3389/fimmu.2017.01674

[5] Asquith, M., Powrie, F. An innately dangerous balancing act: intestinal homeostasis, inflammation, and colitis-associated cancer. *Journal of Experimental Medicine*, **2010**, 207: 1573–1577. https://doi.org/10.1084/jem.20101330

[6] Kim, J. Y., Kajino-Sakamoto, R., Omori, E., et al. Intestinal epithelial-derived TAK1 signaling is essential for cytoprotection against chemical-induced colitis. *Plos One*, **2009**, 4: e4561. https://doi.org/10.1371/journal.pone.0004561

[7] Ou, J., Guan, X., Wang, J., et al. Epithelial NELF guards intestinal barrier function to ameliorate colitis by maintaining junctional integrity. *Mucosal Immunology*, **2022**, 15: 279–288. https://doi.org/10.1038/s41385-021-00465-9

[8] Wenjie, Y., Duo, F., Shaoshi, Z., et al. The Mechanism of Total Glycosides of Cistanche deserticola Y. Ma on Inflammatory Bowel Disease Based on Network Pharmacology and Animal Experiments. *Journal of Chinese Institute of Food Science and Technology*, **2023**, 23: 1–11. https://doi.org/10.16429/j.1009-7848.2023.10.001

[9] Morrison, H. A., Trusiano, B., Rowe, A. J., et al. Negative regulatory NLRs mitigate inflammation via NF-kappaB pathway signaling in inflammatory bowel disease. *Biomedical Journal*, **2023**, 46: 100616. https://doi.org/10.1016/j.bj.2023.100616

[10] Tao, J. H., Duan, J. A., Zhang, W., et al. Polysaccharides From Chrysanthemum morifolium Ramat Ameliorate Colitis Rats via

Regulation of the Metabolic Profiling and NF-kappa B/TLR4 and IL-6/JAK2/STAT3 Signaling Pathways. *Frontiers in Pharmacology*, **2018**, 9: 746. https://doi.org/10.3389/fphar.2018.00746
[11] Ibrahim, S., Zhu, X., Luo, X., et al. PIK3R3 regulates ZO-1 expression through the NF-kB pathway in inflammatory bowel disease. *International Immunopharmacology*, **2020**, 85: 106610. https://doi.org/10.1016/j.intimp.2020.106610
[12] Sopjani, M., Morina, R., Uka, V., et al. JAK2-mediated Intracellular Signaling. *Current Molecular Medicine*, **2021**, 21: 417–425. https://doi.org/10.2174/1566524020666201015144702
[13] Wang, L., Feng, Y., Wang, J., et al. Arbutin Ameliorates Murine Colitis by Inhibiting JAK2 Signaling Pathway. *Frontiers in Pharmacology*, **2021**, 12: 683818. https://doi.org/10.3389/fphar.2021.683818
[14] Wang, T., Zhang, X., Xie, W. Cistanche deserticola Y. C. Ma, "Desert ginseng": a review. *American Journal of Chinese Medicine*, **2012**, 40: 1123–1141. https://doi.org/10.1142/S0192415X12500838
[15] Shen, Z. The location of deficiency syndrome of kidney Yang. *Chinese Medical Journal*, **1999**, 112: 973–975.
[16] Dong, Y., Guo, Q., Liu, J., et al. Simultaneous determination of seven phenylethanoid glycosides in Cistanches Herba by a single marker using a new calculation of relative correction factor. *Journal of Separation Science*, **2018**, 41: 1913–1922. https://doi.org/10.1002/jssc.201701219
[17] Dong-Feng, X.U., Dong-Ming, Y., Yun-Zhi, F., et al. Effects of Cistanoside A on oxidative stress and inflammatory cytokines in radiation-induced pulmonary injury mice via TGF-β1/VEGF pathway. *Chinese Journal of Immunology*, **2019**, 35: 429–434. https://doi.org/10.3969/j.issn.1000-484X.2019.04.009
[18] Kim, J. J., Shajib, M. S., Manocha, M. M., et al. Investigating intestinal inflammation in DSS-induced model of IBD. *Jove-Journal of Visualized Experiments*, **2012**. https://doi.org/10.3791/3678
[19] Magoc, T., Salzberg, S. L. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, **2011**, 27: 2957–2963. https://doi.org/10.1093/bioinformatics/btr507
[20] Li, M., Shao, D., Zhou, J., et al. Signatures within esophageal microbiota with progression of esophageal squamous cell carcinoma. *Chinese Journal of Cancer Research*, **2020**, 32: 755–767. https://doi.org/10.21147/j.issn.1000-9604.2020.06.09
[21] Amir, A., McDonald, D., Navas-Molina, J. A., et al. Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. *Msystems*, **2017**, 2. https://doi.org/10.1128/mSystems.00191-16
[22] Bokulich, N. A., Kaehler, B. D., Rideout, J. R., et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome*, **2018**, 6: 90. https://doi.org/10.1186/s40168-018-0470-z
[23] Li, B., Zhang, X., Guo, F., et al. Characterization of tetracycline resistant bacterial community in saline activated sludge using batch stress incubation with high-throughput sequencing analysis. *Water Research*, **2013**, 47: 4207–4216. https://doi.org/10.1016/j.watres.2013.04.021
[24] Lozupone, C., Knight, R. UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, **2005**, 71: 8228–8235. https://doi.org/10.1128/AEM.71.12.8228-8235.2005
[25] Lozupone, C., Lladser, M.E., Knights, D., et al. UniFrac: an effective distance metric for microbial community comparison. *Isme Journal*, **2011**, 5: 169–172. https://doi.org/10.1038/ismej.2010.133
[26] Segata, N., Izard, J., Waldron, L., et al. Metagenomic biomarker discovery and explanation. *Genome Biology*, **2011**, 12: R60. https://doi.org/10.1186/gb-2011-12-6-r60
[27] Hotamisligil, G. S., Erbay, E. Nutrient sensing and inflammation in metabolic diseases. *Nature Reviews Immunology*, **2008**, 8: 923–934. https://doi.org/10.1038/nri2449
[28] Hu, L. H., Liu, J. Y., Yin, J. B. Eriodictyol attenuates TNBS-induced ulcerative colitis through repressing TLR4/NF-kB signaling pathway in rats. *Kaohsiung Journal of Medical Sciences*, **2021**, 37: 812–818. https://doi.org/10.1002/kjm2.12400
[29] Pistol, G. C., Marin, D. E., Rotar, M. C., et al. Bioactive compounds from dietary whole grape seed meal improved colonic inflammation via inhibition of MAPKs and NF-kB signaling in pigs with DSS induced colitis. *Journal of Functional Foods*, **2020**, 66: 103708. https://doi.org/10.1016/j.jff.2019.103708
[30] 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. *Phytomedicine*, **2021**, 84: 153519. https://doi.org/10.1016/j.phymed.2021.153519
[31] Ge, H., Zhang, B., Li, T., et al. Potential targets and the action mechanism of food-derived dipeptides on colitis: network pharmacology and bioinformatics analysis. *Food & Function*, **2021**, 12: 5989–6000. https://doi.org/10.1039/d1fo00469g
[32] Liang, Y. N., Yu, J. G., Zhang, D. B., et al. Indigo Naturalis Ameliorates Dextran Sulfate Sodium-Induced Colitis in Mice by Modulating the Intestinal Microbiota Community. *Molecules*, **2019**, 24. https://doi.org/10.3390/molecules24224086
[33] Tao, J. H., Duan, J. A., Jiang, S., et al. Polysaccharides from Chrysanthemum morifolium Ramat ameliorate colitis rats by modulating the intestinal microbiota community. *Oncotarget*, **2017**, 8: 80790–80803. https://doi.org/10.18632/oncotarget.20477
[34] Quaglio, A., Grillo, T. G., De Oliveira, E., et al. Gut microbiota, inflammatory bowel disease and colorectal cancer. *World Journal of Gastroenterology*, **2022**, 28: 4053–4060. https://doi.org/10.3748/wjg.v28.i30.4053
[35] Kovesdi, V., Stercz, B., Ongradi, J. Kurthia gibsonii as a sexually transmitted zoonosis: From a neglected condition during World War II to a recent warning for sexually transmitted disease units. *Indian Journal of Sexually Transmitted Diseases and Aids*, **2016**, 37: 68–71. https://doi.org/10.4103/0253-7184.180296
[36] Wu, Y., Ran, L., Yang, Y., et al. Deferasirox alleviates DSS-induced ulcerative colitis in mice by inhibiting ferroptosis and improving intestinal microbiota. *Life Sciences*, **2023**, 314: 121312. https://doi.org/10.1016/j.lfs.2022.121312
[37] Parada, V. D., De la Fuente, M. K., Landskron, G., et al. Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Frontiers in Immunology*, **2019**, 10: 277. https://doi.org/10.3389/fimmu.2019.00277
[38] Blunsom, N. J., Cockcroft, S. CDP-Diacylglycerol Synthases (CDS): Gateway to Phosphatidylinositol and Cardiolipin Synthesis. *Frontiers in Cell and Developmental Biology*, **2020**, 8: 63. https://doi.org/10.3389/fcell.2020.00063
[39] Jennings, W., Epand, R. M. CDP-diacylglycerol, a critical intermediate in lipid metabolism. *Chemistry and Physics of Lipids*, **2020**, 230: 104914. https://doi.org/10.1016/j.chemphyslip.2020.104914
[40] Liu, S., Zhao, W., Liu, X., et al. Metagenomic analysis of the gut microbiome in atherosclerosis patients identify cross-cohort microbial signatures and potential therapeutic target. *Faseb Journal*, **2020**, 34: 14166–14181. https://doi.org/10.1096/fj.202000622R