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ORIGINAL RESEARCH

Gegen Qinlian Decoction Ameliorated DSS-Induced Colitis by Attenuating Inflammation, Restoring Intestinal Mucosal Barrier and Modulating Gut Microbiota

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Background: *Gegen Qinlian* Decoction (GQD), a traditional Chinese medicine formula, has shown significant therapeutic potential for colitis. However, the specific mechanisms by which GQD exerts its effects remain poorly understood, limiting its application in UC treatment.

Purpose: In this study, we utilized a dextran sodium sulfate-induced colitis model in mice to evaluate the efficacy of GQD and to elucidate its underlying mechanisms.

Methods: Network pharmacology was employed to explore the potential targets and signaling pathways of GQD, which were subsequently validated using Western blot. The effects of GQD on gut barrier integrity, inflammation, and oxidative damage were assessed through immunofluorescence, immunohistochemistry, and ELISA. Additionally, 16S rRNA high-throughput sequencing was conducted to examine alterations in the gut microbiota composition.

Results: The results demonstrated that GQD alleviated the colitis symptoms in mice, as evidenced by an increase in goblet cell numbers, upregulation of ZO-1, MUC-2, and Occludin expression, and a decrease in apoptosis in intestinal epithelial cells. GQD effectively suppressed the expression of pro-inflammatory and oxidative mediators, including interleukin-6, tumor necrosis factor-α, interleukin-1β, nitric oxide, malondialdehyde, and myeloperoxidase, with inhibition rates ranging from 47% to 66%. According to the results from Western blot, the phosphorylation levels of p53, Akt, STAT3, and MAPK inflammatory signaling pathway-related proteins (p38, ERK, and JNK) were significantly inhibited with GQD treatment. Furthermore, GQD re-regulated composition of gut microbiota, including inhibiting the harmful bacteria (*Proteobacteria, Bacteroides*, and *Romboutsia*) enrichment and restoring the probiotics (*Lactobacillus* and *Allobaculum*). The correlation analysis revealed that the abundances of gut microbiota were closely related to the key protein expression in colon.

Conclusion: This study revealed GQD can alleviate colitis by attenuating inflammation, restoring intestinal mucosal barrier and modulating gut microbiota.

Keywords: *Gegen Qinlian* decoction, anti-inflammation, intestinal barrier, inflammatory signaling pathway, gut microbiota

Introduction

Ulcerative colitis (UC) is a chronic, non-specific inflammatory condition of the colon, primarily manifested by pathological intestinal mucosal injury, abdominal pain, diarrhea, loose stools, and bloody stool. Due to its chronic and relapsing nature, UC has evolved into a highly prevalent global disease.[1] Due to its chronic and relapsing nature, UC has evolved into a highly prevalent global disease. Since the 20th century, the incidence rate in most of the countries is on the rise. In the past decade, at least 1.5 million and 2 million people suffer from the disease, posing a substantial threat to

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global public health.[2] The standard treatment for UC typically involves chemically synthesized drugs such as mesalazine, glucocorticoids, azathioprine, biologics, JAK inhibitors, and calcineurin inhibitors.[3] Although these conventional therapies have shown some success in managing UC, the treatment is often accompanied by significant side effects and the risk of complications. Consequently, there is an urgent need to explore alternative treatment strategies for alleviating UC.

UC is a multifactorial disease with a complex pathogenesis, primarily involving inflammation and gut microbiota dysbiosis.[4] External factors can activate specific inflammatory signaling pathways, such as the MAPK and PI3K-Akt pathways, leading to the overexpression of pro-inflammatory mediators. This overexpression compromises the integrity of the intestinal mucosal barrier, ultimately resulting in ulcer formation.[5] Additionally, elevated levels of pro-inflammatory cytokines disrupt the gut microbiota by altering the intestinal environment and mucosal layer, further exacerbating the progression of UC through an imbalance between beneficial and harmful bacteria.[6] Therefore, strategies aimed at reducing inflammation and regulating gut microbiota are crucial for effective UC treatment.

Gegen Qinlian Decoction (GQD), a classic formula originating from the ancient Chinese pharmacopeia "Treatise on Cold Damage", has been widely used for over a thousand years for the treatment of UC.[7] According to the pharmacopeia, GQD is composed of kudzu vine root (*Puerariae Lobatae Radix*), Huangqin (*Scutellariae Radix*), coptis chinensis (*Coptidis Rhizoma*), and Gancao (*Glycyrrhiza uralensis*). It was determined that GQD has an average overall effectiveness rate of 92.4% in treating UC, with a 47.2% average cure rate.[8] Besides, GQD is also used to treat other colon diseases, such as diarrhea, irritable bowel syndrome, and chemotherapy-induced gastrointestinal side effects.[9] In recent years, the pharmacological mechanism of GQD was regarded. The published studies revealed that GQD can ameliorate the colitis via the anti-inflammatory and antioxidative effects.[10,11] However, the specific signaling pathways and gene targets through which GQD exerts its effects on colitis remain unclear.

Furthermore, GQD is widely demonstrated with significant regulatory effects on gut microbiota.[12] After the main ingredients in GQD are digested in the small intestine by enzymes and microorganisms, short-chain fatty acids (SCFAs) are subsequently produced, leading to alterations in the gut microbiota.[13] This process is positively meaningful for the gut health.

Therefore, this study aims to investigate the therapeutic effects of GQD on dextran sulfate sodium (DSS)-induced colitis in mice, and further exploring its potential mechanisms. The findings of this research will provide a deeper theoretical basis and scientific guidance for the use of GQD in the treatment of colitis.

Materials and Methods

Reagents

Puerariae Lobatae Radix, Scutellariae Radix, Coptis chinensis, and *Glycyrrhiza uralensis* were purchased from Beijing Tong Ren Tang Co., Ltd. (Beijing, China). DSS (molecular weight 36,000–50,000 Da; Lot number: 0216011080) was bought from MP Biomedicals Inc (Santa Ana, CA, USA). IL-6, TNF-α, IL-1β, and IL-10 ELISA kits were purchased from NeoBioscience Technology Co. Ltd (Shenzhen, China). MPO, SOD, and MDA oxidase kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The rabbit monoclonal antibody of p-AKT (9271L), AKT (9272T), p-JNK (4668T), JNK (925ZS), p-ERK (4370), ERK (4695), p53 (9282), p-p53 (2521) was purchased from Cell Signaling Technology Co. Ltd (Danvers, Massachusetts, USA). ZO-1 (21,773), MUC2(27675), Occludin (27260), and F4/80 (28463) antibodies were bought from Proteintech Group Co. Ltd (Wuhan, China). The rabbit monoclonal antibody of β-actin (303975), p-STAT3 (334,465), STAT3 (T56566F) were bought from Abmart Shanghai Co., Ltd. (Shanghai, China). The one-step TUNEL in situ apoptosis kit was purchased from Elabscience Biotechnology Co. Ltd (Wuhan, China). The AB-PAS staining kit was obtained from Beijing Solarbio Science & Technology Co. Ltd (Beijing, China).

Network Pharmacology Analysis

Traditional Chinese Medicine System Pharmacology (TCMSP) database (https://old.tcmsp-e.com/tcmsp.php) was used to find active compounds in GQD. Then, active compounds were initially screened according to two important indicators in ADME of oral relative bioavailability ≥30% and drug-likeness ≥0.18. And the protein targets of related compounds were supplied by PubChem database (https://pubchem.ncbi.nlm.nih.gov/). By using "colitis" as the keyword, and related

targets were collected from the (OMIM) (http://www.omim.org/) and Gene Cards (https://www.genecards.org/). Protein–protein interaction (PPI) network constructed with related targets using String database (https://string-db.org/).

Molecular Docking and Visualization

The structure of the components and target proteins were downloaded from the TCMSP database and the PubChem database. The process of compound-target molecular docking and binding affinity was performed using Autodock Vina. The top 10 core target proteins and the top 5 core compounds from the network pharmacology results were selected for pairwise molecular docking. The top 7 pairs based on binding energy were used for visualization by PyMol software.

Preparation of Gegen Qinlian Decoction

GQD was extracted by soaking *Puerariae Lobatae Radix* (15 g), *Scutellariae Radix* (9 g), *Coptis chinensis* (9 g), and *Glycyrrhiza uralensis* (6 g) in sterile distilled water at five times their weight overnight. The mixture was then boiled for 30 min, filtered, and the process was repeated with the remaining residue. The two decoctions were combined and concentrated to 39 mL, yielding a final concentration of 1 g/mL.

Animal Experiment Design

Thirty male C57BL/6J mice (4 weeks of age, 18–22 g) were purchased from Guangdong Zhiyuan Biomedical Technology Co., Ltd (Guangdong, China). The mice were housed in plastic cages under controlled conditions: 50–60% humidity, 25±1°C temperature, and a 12-h light/dark cycle. All experiments were conducted following the guidelines approved by the Ethics Review Committee of Qingyuan People's Hospital Experimental Animal Center (Approval No. LAEC-2024-017). The animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council (NRC).

All mice were randomly assigned to five groups (n = 6): control group, DSS group, DSS+low-dose GQD group (DSS +GQD-L), DSS+medium-dose GQD group (DSS+GQD-M), and DSS+high-dose GQD group (DSS+GQD-H). Except for the control group, all other groups were given a 3% DSS solution for 7 days to induce colitis. During this period, the GQD groups received different concentrations of GQD via intragastric administration, while the control and DSS groups received the saline. During the period of experiment, body weight, fecal consistency, the presence of blood in stools, and general behavior were observed and recorded daily. On the 10th day, the mice were anesthetized and euthanized. Colon length was measured, and colon and cecal contents were collected for further analysis.

Colon Histopathologic Analysis

A 1 cm segment of the colon was fixed in 4% paraformaldehyde, dehydrated in a graded ethanol series, cleared with xylene, embedded in paraffin, and sectioned at 3 μm thickness for hematoxylin and eosin (H&E) staining. The sections were then examined under a microscope for histopathological analysis.

Biochemical Analysis

The colon tissue was homogenized in phosphate buffered solution at a ratio of 1:9 (m/v). After centrifugation, the supernatant was collected for biochemical analysis. MPO, SOD, MDA, NO, IL-6, TNF-α, IL-1β, and IL-10 in colon tissues were detected using ELISA kits.

Immunohistochemistry (IHC)

Paraffin-embedded colon tissue sections were deparaffinized in xylene and rehydrated through a graded alcohol series. Antigen retrieval was followed by treatment with a 3% endogenous peroxidase inhibitor and blocking with goat serum. The sections were then incubated overnight at 4°C with the primary antibody (F4/80). The following day, secondary antibody incubation was performed for 1 h at room temperature, followed by DBA staining, hematoxylin counterstaining, differentiation with alcohol hydrochloric acid, and a wash to restore the blue color. The sections were then dehydrated, cleared with xylene, and sealed with neutral gum. Images were captured randomly using an optical microscope.

Immunofluorescence (IF) Staining

Paraffin-embedded colon tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval. After permeabilization with 0.5% Triton X-100 and blocking with goat serum, the sections were incubated overnight at 4°C with primary antibodies (ZO-1, MUC2, Occludin). The next day, secondary antibodies were applied for 1 h at room temperature, followed by DAPI staining. Anti-fade mounting medium was used to preserve fluorescence, and images were randomly captured with a confocal microscope (LSM900) for analysis.

For TUNEL staining, deparaffinized and rehydrated colon tissue sections were processed according to the instructions of the One-step TUNEL In Situ Apoptosis Kit (Elabscience, China) to detect apoptotic cells. The images were captured using a confocal microscope (LSM900) for further analysis.

Alcian Blue-Periodic Acid Schiff (AB-PAS) Staining

Paraffin sections of colon tissue were deparaffinized in xylene and gradient alcohol until hydration was achieved. The paraffin sections were subjected to complete AB-PAS staining using Alcian Blue-Periodic Acid Schiff (AB-PAS) Stain Kit, and then observed and photographed under a microscope. Observe with an optical microscope and randomly capture images for further analysis.

Western Blotting

Total protein was extracted from colon tissue using RIPA lysis buffer containing protease and phosphatase inhibitors, and protein concentration was quantified using a BCA assay kit (Abbkine, Wuhan, China). Protein samples were separated by 10% SDS-PAGE and transferred onto a PVDF membrane. The membranes were then probed with primary antibodies against β-actin, p38, p-p38, JNK, p-JNK, ERK, p-ERK, AKT, p-AKT, STAT3, p-STAT3, p53, and p-p53, followed by HRP-conjugated secondary antibodies. Protein expression was visualized using ECL luminescent solution and analyzed using the Gel imaging system (Bio-Rad ChemiDoc). Quantitative analysis was performed with ImageJ software.

16S rRNA Sequencing and Bioinformatic Analysis

DNA was extracted from mouse stool using the DNA Stool Kit (Tiangen Biotech Co., Ltd, Beijing, China). The V3-V4 region of the 16S rRNA gene was amplified using the primers ACTCCTACGGGAGGCAGCAG-3′ and 5′-GGACTACHVGGGTWTCT AAT-3′. Following PCR amplification, the products were purified and sequenced on the Illumina MiSeq platform (Illumina, SD, USA).

Statistical Analysis

The statistical analysis used GraphPad Prism 8.0.2. All the experiment data are presented as the mean ± SD, analyzed by ANOVA. The significant difference is represented by different lowercase letters (a, b and c) between treatments. $P< 0.05$ was regarded as statistically significant.

Results

Network Pharmacology Analysis

Network pharmacology is an emerging subject combining the bioinformatics, systems biology, and polypharmacology, and has been widely used for studying on the mechanism and targets of natural herbal medicine. Therefore, network pharmacology was performed to predict the target of GQD on colitis in this study. A total of 242 acting targets of GQD were determined as searched related database. A total of 6018 potential targets for colitis were searched in the online Human Mendelian Genetic Database (OMIM) (http://www.omim.org/) and Gene Cards (https://www.genecards.org/). Therefore, Venn diagram was drawn according to this information (Figure 1A). The result indicated there were 176 intersection targets between GQD and colitis.

Figure 1B shows the protein–protein interaction (PPI) network constructed with related targets using String database (https://string-db.org/). The targets such as p53, Jun, STAT3, Akt, TNF-α, MAPK1, and IL-6 were located at the central network of PPI network, revealing that they were probably the key proteins. The "components-targets" network was

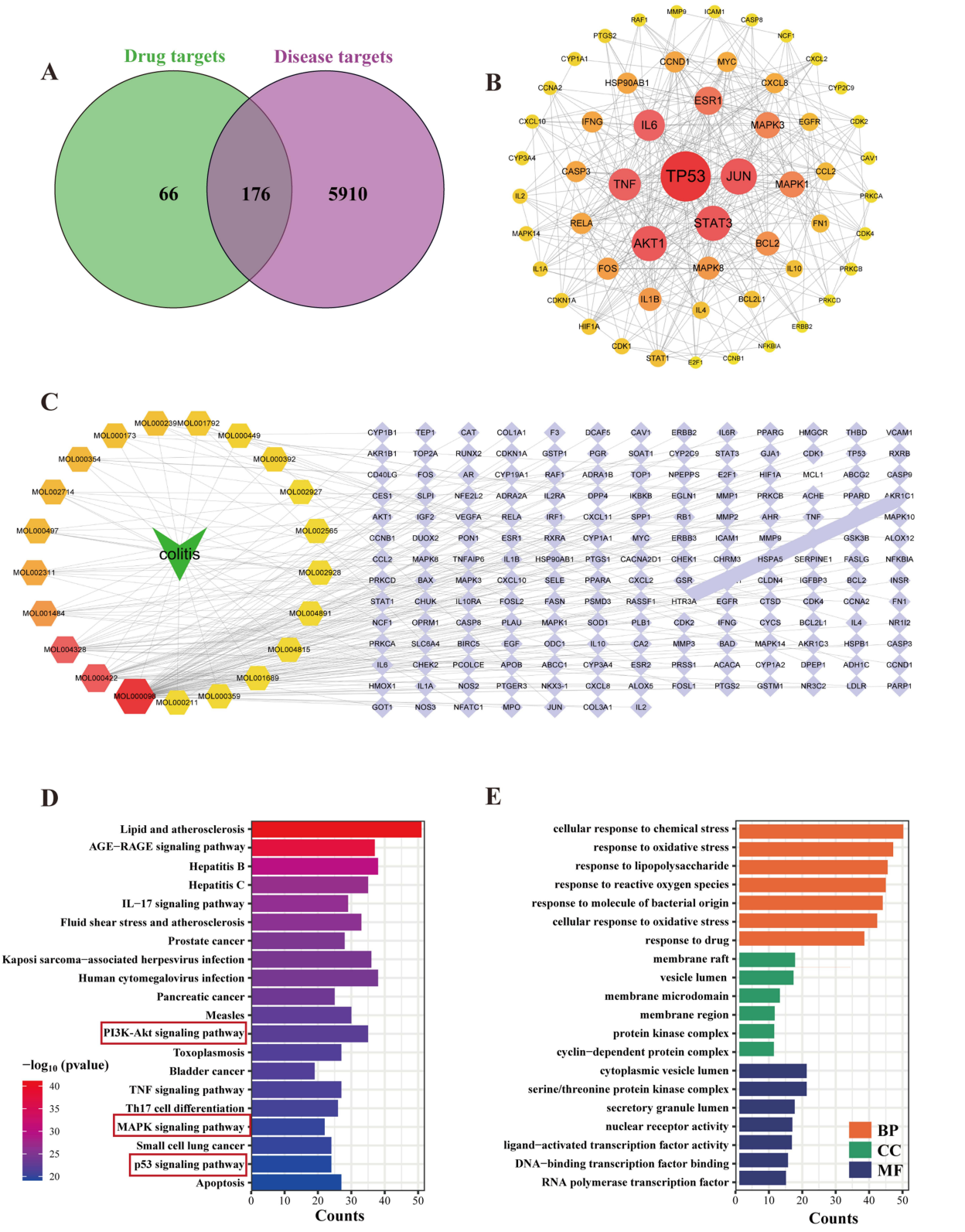

Figure 1 Network pharmacology analysis of *Gegen-Qinlian* Decoction (GQD) in treating colitis. (**A**) Venn diagram showing 176 overlapping targets between GQD (green) and colitis (purple). (**B**) PPI network of key hub genes. (**C**) Compound-target network, illustrating interactions between the active compounds in GQD and the common targets. (**D**) KEGG pathway enrichment analysis. (**E**) GO enrichment analysis.

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performed to investigate the key components and acting targets of GQD on colitis, as exhibited in Figure 1C. The components in GQD such as glycyrol, inermine, naringenin, kaempferol, and quercetin was determined to be the potential active molecules. The interaction between these key targets and components is important for GQD to exert its pharmacological action on colitis. Therefore, it still needs more in-depth study to further verify this interaction.

The key target proteins of GQD were inputted into Bioconductor (http://www.bioconductor.org/), subsequently KEGG and GO enrichment analysis were performed, as shown in Figure 1D and E. According to the results, KEGG pathways involved in 266 signaling pathways, including AGE-RAGE signaling pathway, IL-17 signaling pathway, PI3K-Akt signaling pathway, TNF signaling pathway, MAPK signaling pathway, p53 signaling pathways, etc. GO enrichment analysis revealed that 2516 entries for biological process (BP), 219 entries for molecular function (MF), and 91 entries for cell components (CC). These results provided valuable direction into the mechanism by which GQD impact colitis.

Molecular Docking Analysis and Validation

Molecular docking was performed to investigate the interaction between active components in GQD and key targets on colitis. Five components and seven key hub proteins were paired for molecular docking, resulting in a total of 35 docking tests. The seven compound-protein pairings with the lowest binding energy from the docking were visualized using PyMOL, and the results were shown in Figure 2. TNF-quercetin, p53-inermine, IL-6-quercentin, Akt-inermine, Jun-naringenin, MAPK-inermine, and STAT3-inermine were determined to be the potential action mode of GQD on colitis, and their binding energy were −8.8, −8.4, −8.1, −7.9, −7.5, −7.1, and −7 kJ/mol, respectively (Table 1). The results indicated inermine (MOL001484), quercentin (MOL000098), and naringenin (MOL004328) in GQD were probably the key active components in the treatment of colitis, as they exhibit strong binding affinity with key proteins associated with the condition. However, these findings still need to be validated through in vivo experiments in real-world settings.

Effects of GQD on Body Weight, Colon Length and DAI Index in DSS-Induced Mice

To evaluate the therapeutic effect of GQD on colitis in mice, a DSS-induced experimental model was established. The schematic diagram of the animal experiments was shown in Figure 3A. The in vivo results demonstrated that mice in the DSS group exhibited obvious weight loss, colon length shortening, and an increase in DAI score compared to the control group (Figure 3B–F). Following GQD treatment, these symptoms were significantly alleviated. Specifically, compared to the DSS group, body weight increased by 60%, colon length increased by 40%, and the DAI score decreased by 50% in the GQD treatment groups. As mentioned above, GQD demonstrated potential in treating colitis.

Effects of GQD on Colon Histology in DSS-Induced Mice

H&E staining was employed to assess the impact of GQD on colon tissue in DSS-induced colitis mice. Compared to the control group, the DSS-induced mice exhibited extensive infiltration of inflammatory cells, disruption of the mucosal architecture, tight clustering of intestinal glands, and significant inflammatory lesions (Figure 4A and D). However, following treatment with varying doses of GQD, there was a noticeable improvement in the structure of the colon mucosa. The arrangement of the intestinal glands became more orderly, and the number of inflammatory cells in the submucosal layer was reduced to varying degrees.

AB-PAS staining was used to observe goblet cells, as which play a crucial role in secreting mucus to protect the intestinal barrier.[14] The results revealed a significant reduction in the number of goblet cells in the DSS group compared to the control group (Figure 4B and E). However, GQD treatment, particularly at high doses, markedly restored goblet cell numbers, indicating a protective effect against DSS-induced tissue damage.

Macrophages are widely distributed in intestinal mucosal layer of colon tissue, playing a crucial role in inflammation-related diseases.[15] Immunohistochemical analysis revealed a significant increase in macrophages in the colon tissues of the DSS group. In contrast, GQD treatment effectively reduced macrophage infiltration in a dose-dependent manner (Figure 4C and F). These findings suggest that GQD can enhance colon tissue morphology and modulate immune cell distribution, further exhibiting a therapeutic potential for colitis.

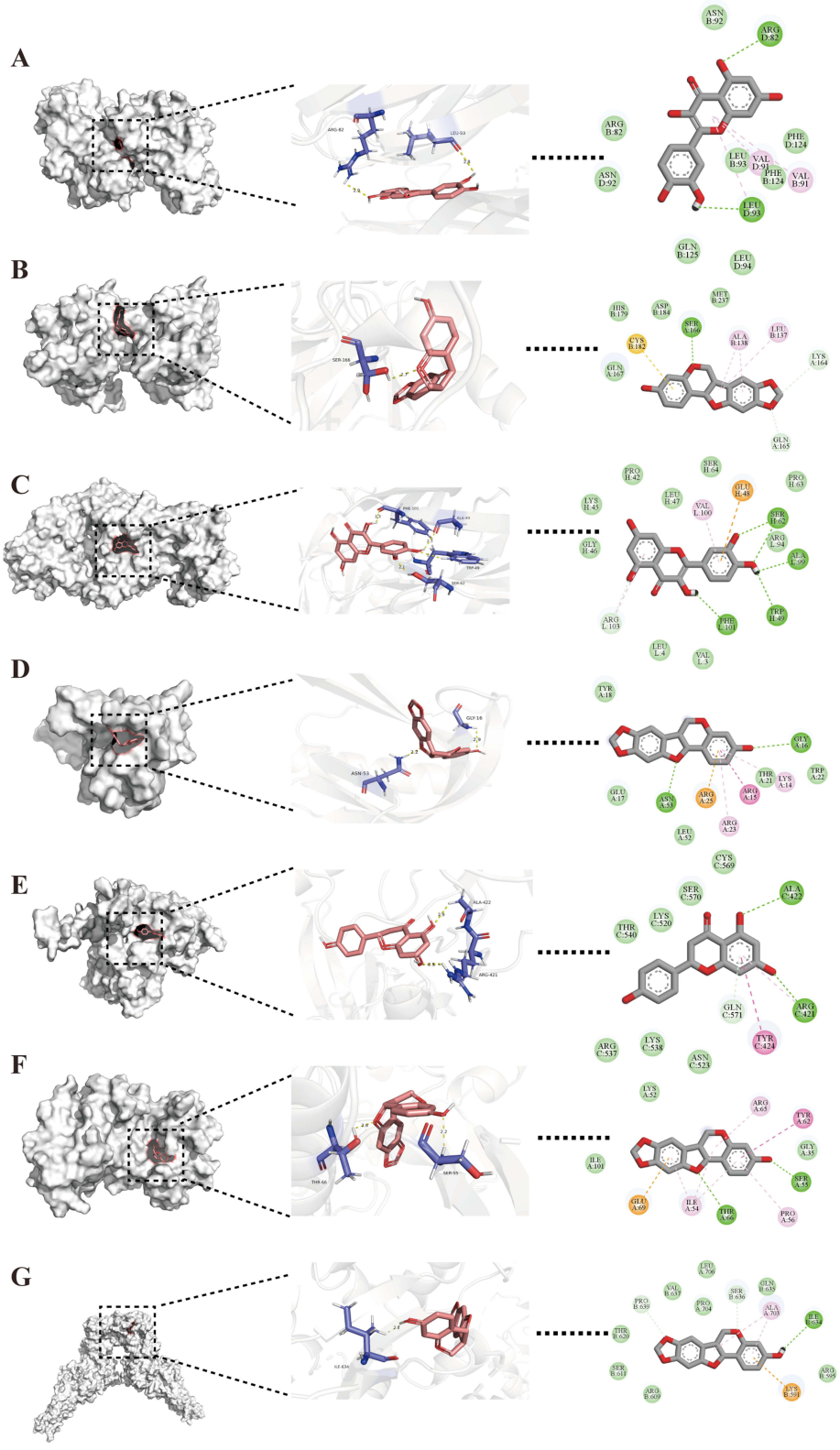

Figure 2 Molecular docking analysis of key compounds from GQD with target proteins related to colitis. (**A–G**) Each panel shows the docking pose (left), interaction details (middle), and 2D interaction diagram (right) for different compound-protein pairs.

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Table 1 The Binding Energy of Top 7 Compound-Protein Pairings

Compound-Protein Pairings	Binding Energy (kJ/mol)
TNF-quercetin	−8.8
MAPK-inermine	−8.4
Jun-naringenin	−8.1
IL-6-quercentin	−7.9
p53-inermine	−7.5
Akt-inermine	−7.1
STAT3-inermine	−7

Effects of GQD on Intestinal Mucosal Barrier in DSS-Induced Mice

The intestinal barrier is formed by a mucus layer composed of mucins, epithelial cells connected by tight junction proteins, and cell populations, playing an important factor in the occurrence and development of colitis.[16,17] To evaluate the impact of GQD on the intestinal barrier, the key markers (ZO-1, MUC2, and Occludin) were analyzed using immunofluorescence. Compared to the control group, the expression levels of ZO-1, MUC2, and Occludin in the DSS group were obviously downregulated (Figure 5A–E). However, the low-expression of these proteins in colon tissue was dose-dependently improved after treatment with three doses of GQD.

Apoptosis of intestinal epithelial cells is also a key factor contributing to the damage of intestinal barrier.[18] To assess the apoptosis of intestinal epithelial cells in colon tissue, TUNEL staining was performed. The results showed a significant increase in epithelial cell death in the DSS group, while GQD treatment significantly reduced the rate of apoptosis (Figure 4C). Overall, GQD protects the intestinal barrier by promoting the production of tight junction proteins and mucins while also reducing the apoptosis of intestinal epithelial cells.

Effects of GQD on Inflammatory and Oxidative Stress Markers in DSS-Induced Colitis Mice

Inflammation and oxidative damage are the hallmark symptoms of colitis. During colitis, pro-inflammatory cytokines rapidly increase, triggering a cascade of reactions, such as oxidative stress, which exacerbate the condition.[19,20] In this study, both inflammatory factors and oxidative enzymes were investigated. As shown in Figure 6, the levels of pro-inflammatory cytokines (IL-6, TNF-α, IL-1β) and NO were significantly elevated in the DSS group compared to the control group. However, in the GQD treatment groups, a significant decreasing trend in these cytokines was observed, with the maximum inhibitory rate reaching 66% ($p < 0.05$). In contrast, the levels of anti-inflammatory cytokines IL-10 and SOD showed an opposite pattern, with maximum increases of 39% (Figure 6D and H). Additionally, the activity levels of MDA and MPO were consistent with the results of the pro-inflammatory cytokines (Figure 6E–G). In summary, GQD alleviated DSS-induced colitis in mice by suppressing the inflammatory response and mitigating oxidative stress.

Effects of GQD on Inflammatory Signaling Pathways and Target Proteins in Colon of DSS-Induced Colitis Mice

To explore the therapeutic mechanism of GQD on colitis in mice, the up-stream signaling pathway and target proteins excavated from network pharmacology were investigated, as shown in Figure 7. Figure 7A–D showed the alteration of key proteins in MAPK signaling pathway. Compared to the control group, the phosphorylation levels of p38, JNK, and ERK in the DSS group were significantly upregulated. However, the phosphorylation of these proteins was significantly inhibited with GQD treatment ($p < 0.05$). In particular, p-ERK expression in GQD group was nearly equivalent to that of the control group, all reduced to approximately 0.3.

STAT3, Akt, and p53 were determined to be the key hub proteins, therefore, they were further analyzed with Western blot, as shown in Figure 7E–H. The relative expression of p-STAT3, p-Akt, and p-p53 in the DSS group were significantly increased, compared to control. However, in the colon of GQD-treated mice, the relative expression of

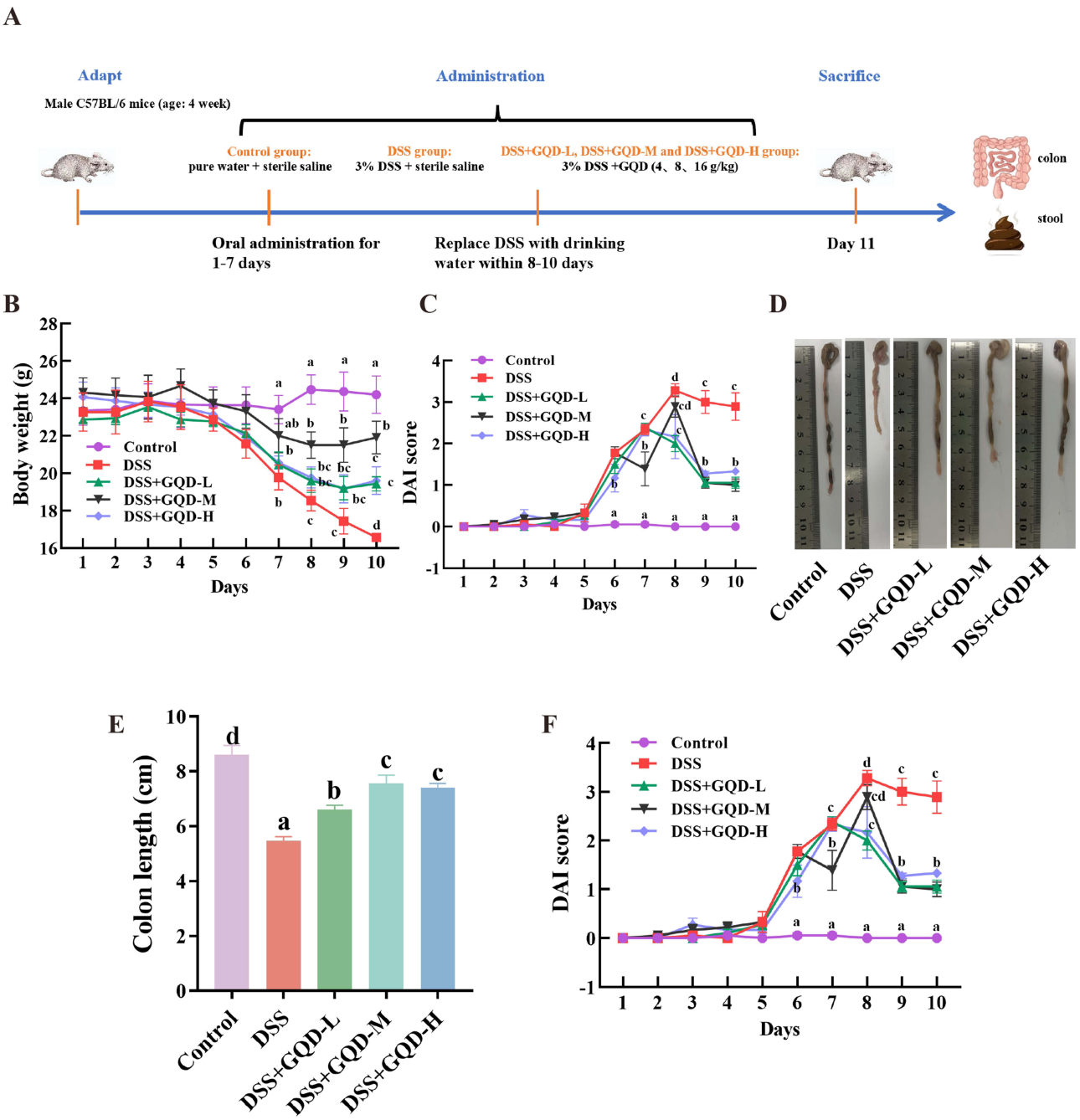

Figure 3 Effects of GQD on DSS-induced colitis in mice. (**A**) Experimental design. (**B**) Body weight changes over 10 days across different treatment groups. (**C**) Total body weight growth during the experiment. (**D**) Representative images of colon length from each group. (**E**) Quantification of colon length. (**F**) Disease Activity Index (DAI) scores across the experimental period. The significant difference is represented by different lowercase letters (a, b, and c) between treatments at $p < 0.05$.

these phosphorylated proteins was, respectively, reduced by 40%, 28%, and 74% in DSS+GQD-H group, as compared to DSS group ($p < 0.05$).

Effects of GQD on the Gut Microbiota of DSS-Induced Colitis in Mice

Gut microbiota plays a crucial role in the development and progression of colitis, its translocation can damage the integrity of the intestinal barrier and impact immune responses.[21] Therefore, the effects of GQD on gut microbiota of mice were investigated. According to the Venn diagram (Figure 8A), mice in control group exhibited the highest number of Operational Taxonomic Units (OTUs), while the DSS group was the lowest group. However, with the administration

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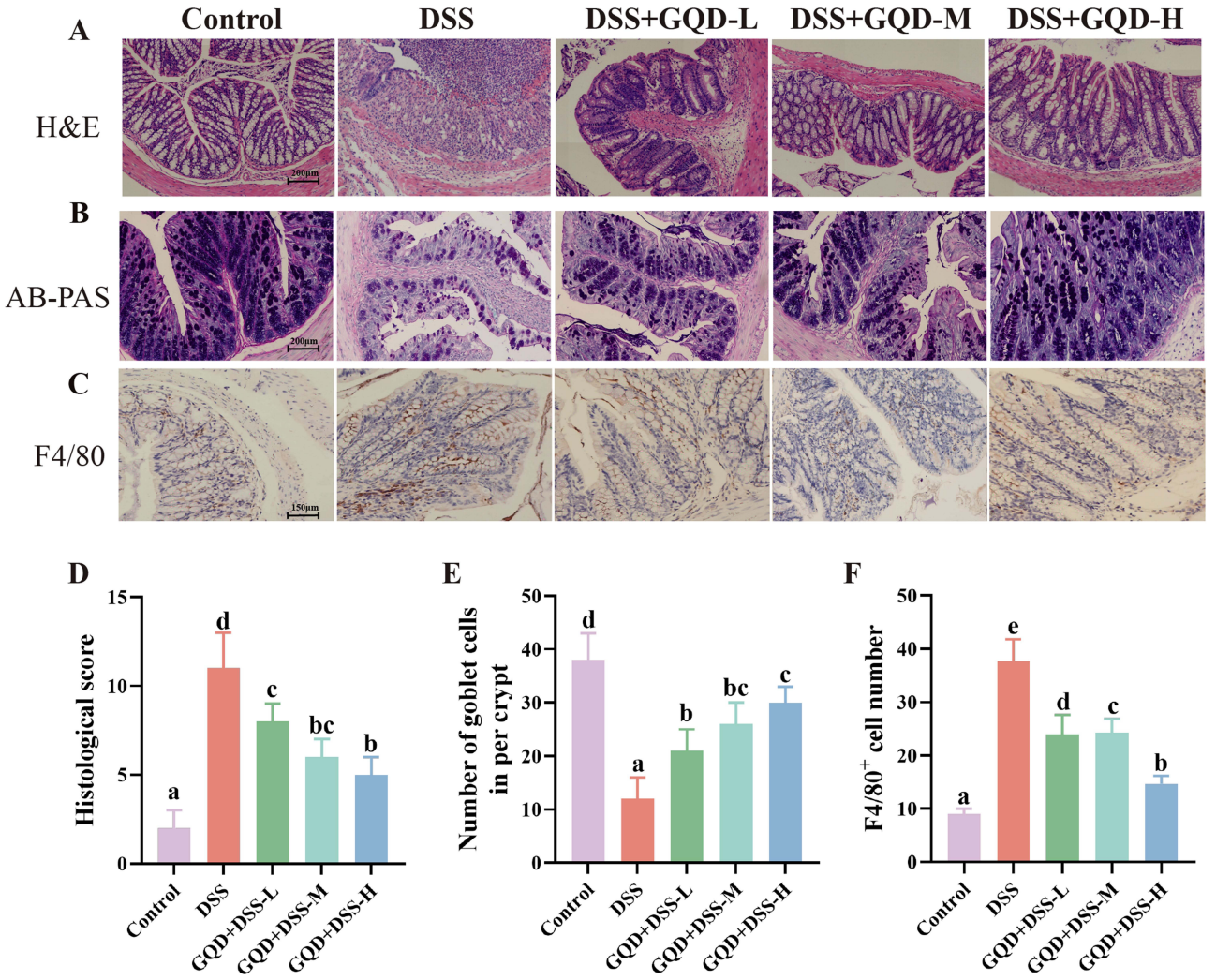

Figure 4 Histological analysis of colon tissues from DSS-induced colitis mice treated with different doses of GQD, (**A**) H&E (**B**) AB-PAS, (**C**) F4/80 staining images, (**D**–**F**) were the quantification for H&E, AB-PAS, and F4/80, respectively. The significant difference is represented by different lowercase letters (a, b, c, d, and e) between treatments at $p < 0.05$.

of GQD at three different doses, the number of OTUs significantly increased. Moreover, compared to the DSS group, the GQD-treated mice displayed a more similar OTU composition to that of the control group, as evidenced by the greater overlap in OTU numbers with the control. The Non-metric Multidimensional Scaling (NMDS) plot showed the similarity of gut microbiota composition among the samples in each group (Figure 8B). The clusters in control group are distinct from the DSS group, indicating a significant shift in microbial composition due to DSS treatment. The groups treated with GQD at low, medium, and high doses showed a trend toward the control group, with the medium and high-dose groups exhibiting closer clustering to the control.

Chao index, Shannon index, and ACE index were used to reflect the microbial diversity in mice gut, and the results as shown in Figure 8C and D. As compared to control, three diversity indices were all deceased in DSS group, indicating a loss of microbial diversity due to DSS treatment. However, low, medium, and high-doses of GQD treatment (DSS +GQD-L, DSS+GQD-M, and DSS+GQD-H) significantly restored these indices. Figure 8F exhibits the microbial dysbiosis index of mice in five groups. This index was significantly elevated in DSS group, reflecting a severe imbalance in the gut microbiota. GQD treatment dose-dependently reduced the dysbiosis index, with the high dose (DSS+GQD-H) showing the most significant reduction in dysbiosis.

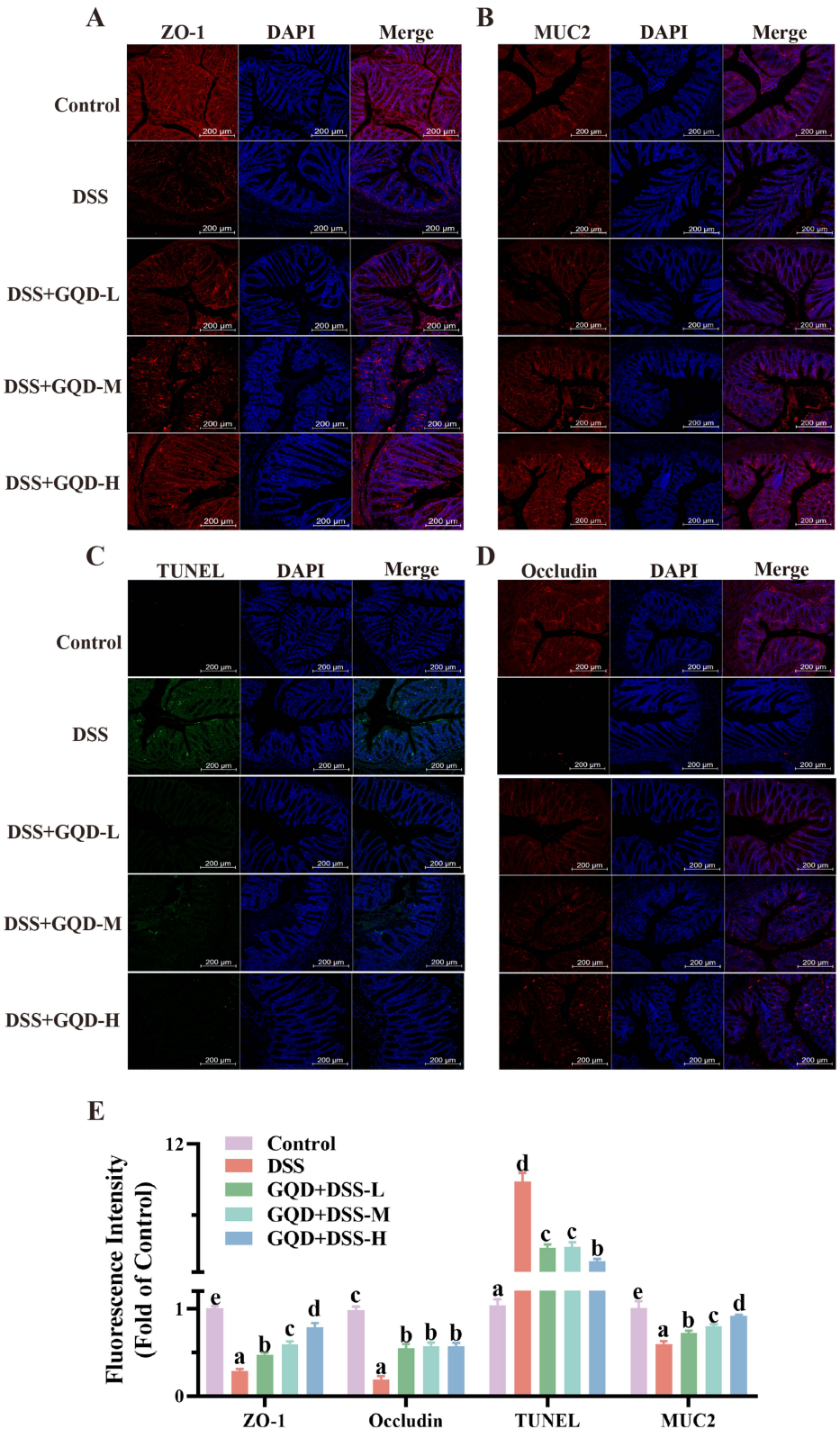

Figure 5 Immunofluorescence analysis of colon tissues from DSS-induced colitis mice treated with different doses of GQD. (**A**) ZO-1 staining, (**B**) MUC2 staining, (**C**) TUNEL staining, (**D**) Occludin staining, (**E**) the quantification for the four staining images. DAPI (blue) is used for nuclear counterstaining. The significant difference is represented by different lowercase letters (a, b, c, d, and e) between treatments at $p < 0.05$.

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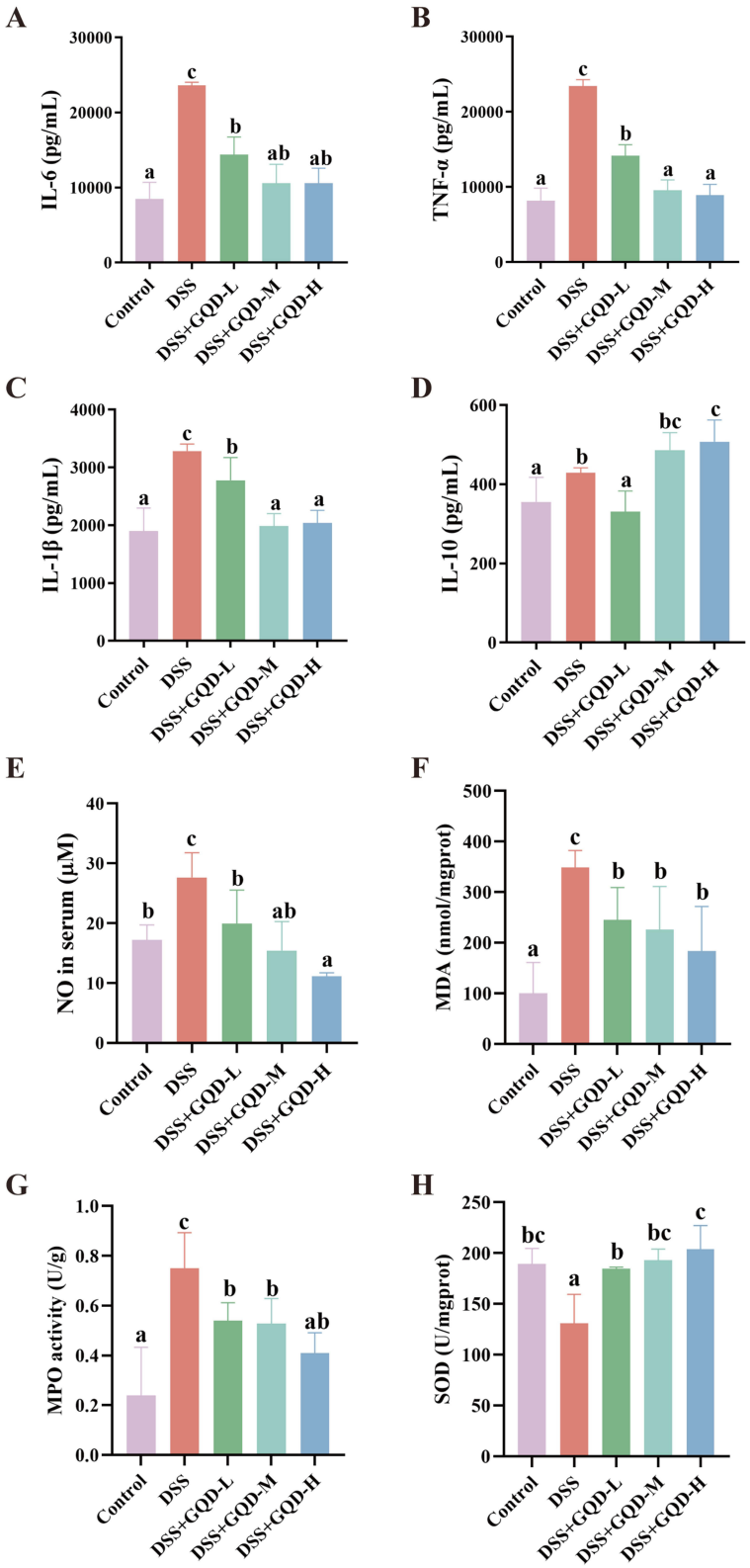

Figure 6 Analysis of inflammatory and oxidative stress markers in colon tissues from DSS-induced colitis mice treated with GQD. (**A**) IL-6. (**B**) TNF-α. (**C**) IL-1β. (**D**) IL-10. (**E**) NO. (**F**) MDA. (**G**) MPO. (**H**) SOD. Data are presented as mean±SEM with different letters indicating significant differences between groups. The significant difference is represented by different lowercase letters (a, b, and c) between treatments at $p < 0.05$.

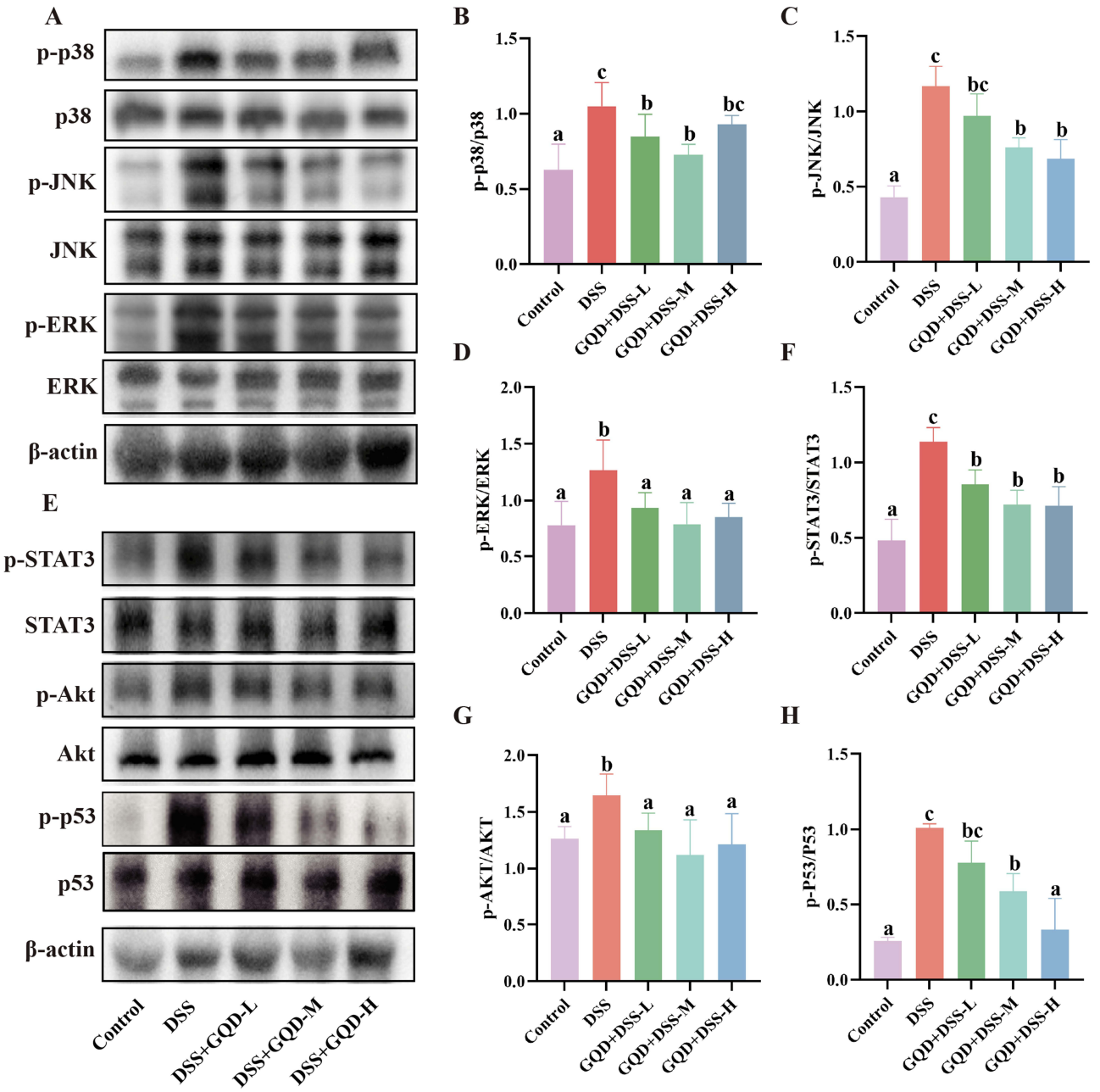

Figure 7 Western blot analysis of signaling pathways in colon tissues from DSS-induced colitis mice treated with GQD. (**A**) Representative blots for the levels of key proteins (p38, JNK, and ERK) on MAPK signaling pathway. (**B–D**) Quantification of the phosphorylation ratios for p38, JNK, and ERK. (**E**) Representative blots for the verification of hub proteins from network pharmacology. (**F–H**) Quantification of the phosphorylation ratios for STAT3, Akt, and p53. The significant difference is represented by different lowercase letters (a, b, and c) between treatments at $p < 0.05$.

To further elucidate the regulatory effects of GQD on colitis, the composition of gut microbiota was analyzed, with the relative abundance at the phylum level presented in Figure 9. In DSS group, the relative abundance of *Proteobacteria* significantly increased compared to the control group (Figure 9A and C). GQD treatment at low (DSS+GQD-L), medium (DSS+GQD-M), and high (DSS+GQD-H) doses effectively reduced the relative abundance of *Proteobacteria*, with reduction rates of 1.5%, 84.4%, and 47.9%, respectively, compared to the control. *Firmicutes*/*Bacteroidetes* (F/B) ratio is a common index for reflecting the microbial structure in mice gut (Figure 9D). However, the results indicated there was no significant difference among the five groups.

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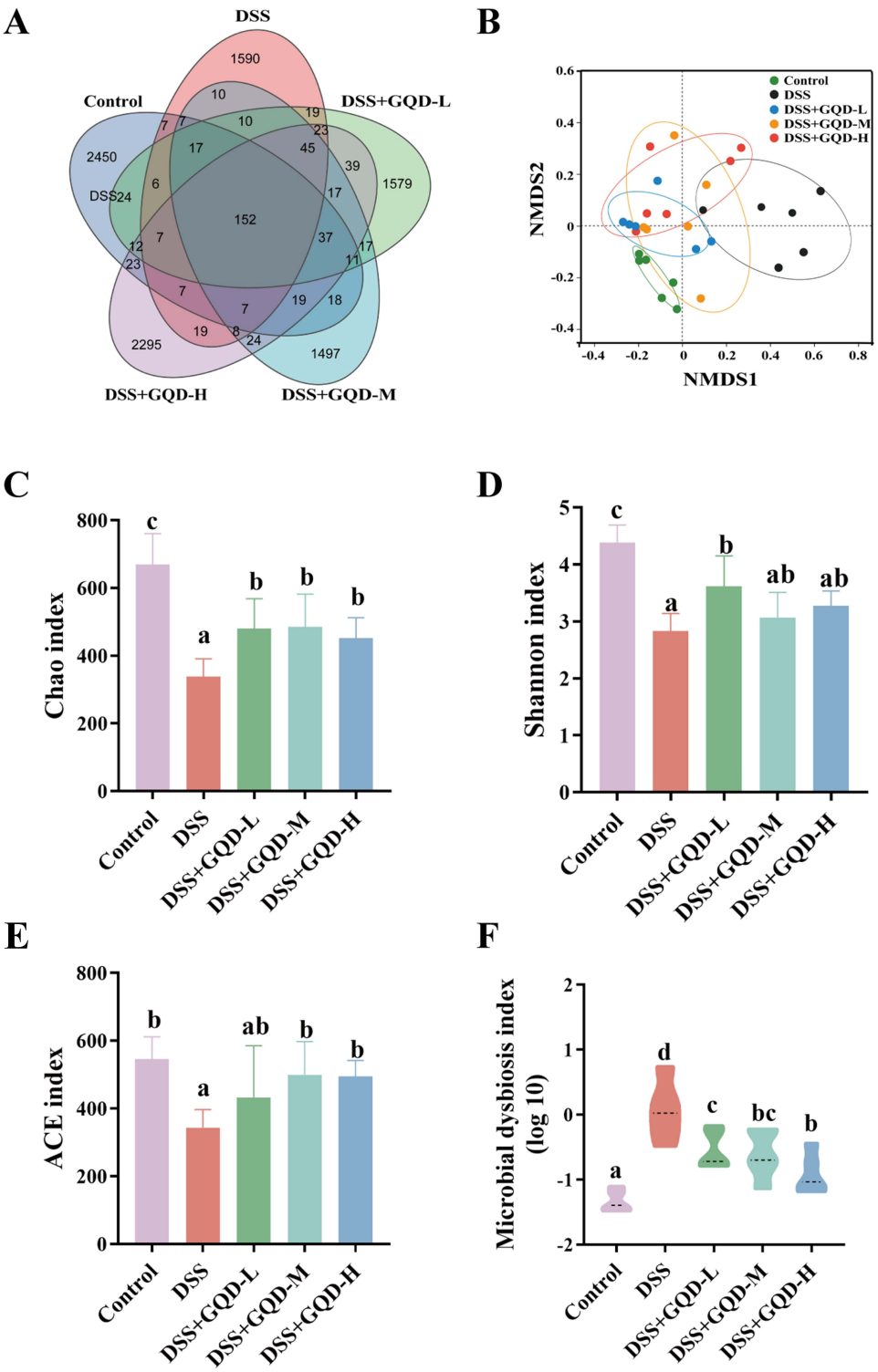

Figure 8 Analysis of gut microbiota diversity in DSS-induced colitis mice treated with GQD. (**A**) Venn diagram showing the shared and unique Operational Taxonomic Units (OTUs) among different treatment groups. (**B**) Non-metric multidimensional scaling (NMDS) plot illustrating the microbial community structure across groups. (**C–E**) Alpha diversity indices (Chao, Shannon, and ACE). (**F**) Microbial dysbiosis index across different groups. The significant difference is represented by different lowercase letters (a, b, and c) between treatments at $p < 0.05$.

At the genus level, significant decreases were observed in *Lactobacillus* and *Allobaculum* following DSS treatment, as shown in Figure 9B, E and H. The relative abundance of *Lactobacillus* dropped from 50.2% in the control group to 20.8%, while *Allobaculum* decreased from 13.2% to just 1.1%. In the three GQD treatment groups, mice in the DSS+GQD-H group

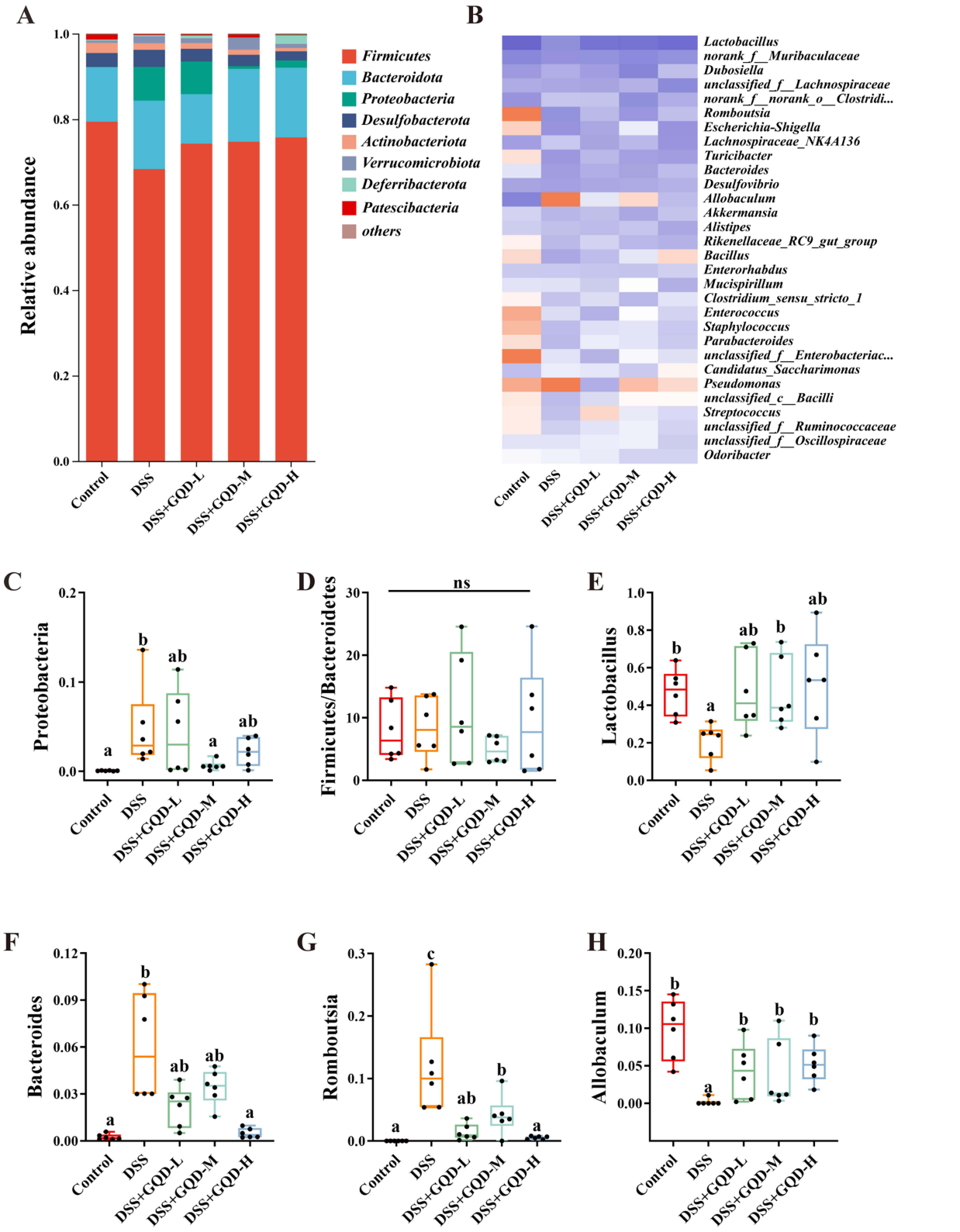

Figure 9 Analysis of gut microbiota composition in DSS-induced colitis mice treated with GQD. (**A**) Heatmap at genus level, showing the relative abundances of key bacterial genera in each group. (**B**) Bar chart of the relative abundance of major bacterial at phylum level. (**C–H**) Boxplots of selected bacterial taxa, including *Proteobacteria, Firmicutes/Bacteroidetes* ratio, *Lactobacillus, Bacteroides, Romboutsia*, and *Allobaculum*. The significant difference is represented by different lowercase letters (a, b, c, d, and e) between treatments at $p < 0.05$.

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exhibited an optimal gut microbial composition, with the relative abundance of these two genera significantly restored to 51% and 5.1%, respectively ($p<0.05$). Regarding *Bacteroides* and *Romboutsia* (Figure 9F and G), these genera were maintained at extremely low levels in the control group (close to 0). However, in the DSS group, both showed significant increases, with relative abundances reaching 5.3% and 10.4%, respectively. Following GQD treatment, particularly in the high-dose group, the overgrowth of *Bacteroides* and *Romboutsia* was significantly inhibited ($p<0.05$), reducing their levels close to those observed in the control group.

Spearman Correlation Analysis Between Gut Microbiota and Key Target Protein in Colon

To further clarify the relationship between gut microbiota and signal protein in colon, we conducted Spearman correlation analysis between phosphorylated proteins (p-p53, p-Akt, p-p38, p-JNK, and p-ERK) and the top 20 most abundant bacterial genera (Figure 10). Among them, *Lactobacillus* and *Allobaculum* exhibited strong negative correlations with p-p53 and p-JNK ($p < 0.01$). By contrast, *Romboutsia, Bacteroides*, and *Staphylococcus* demonstrated significant positive correlations with p-p53, p-Akt, or p-JNK. These results revealed that the abundances of gut microbiota were closely related to the key protein expression in colon.

Discussion

GQD is a traditional Chinese medicine (TCM) formula that has been used for thousands of years to treat colitis. Although a few studies have confirmed its efficacy, the precise mechanisms underlying its anti-colitis effects remain unclear. In this study, we integrated network pharmacology predictions with in vivo validation to first demonstrate that p53 might be involved in the therapeutic effects of GQD in ameliorating colitis. Furthermore, this study innovatively connected gut microbiota and key protein quantification such as p53 through Spearman correlation analysis, thereby providing an in-depth exploration of the mechanism by which GQD ameliorates colitis.

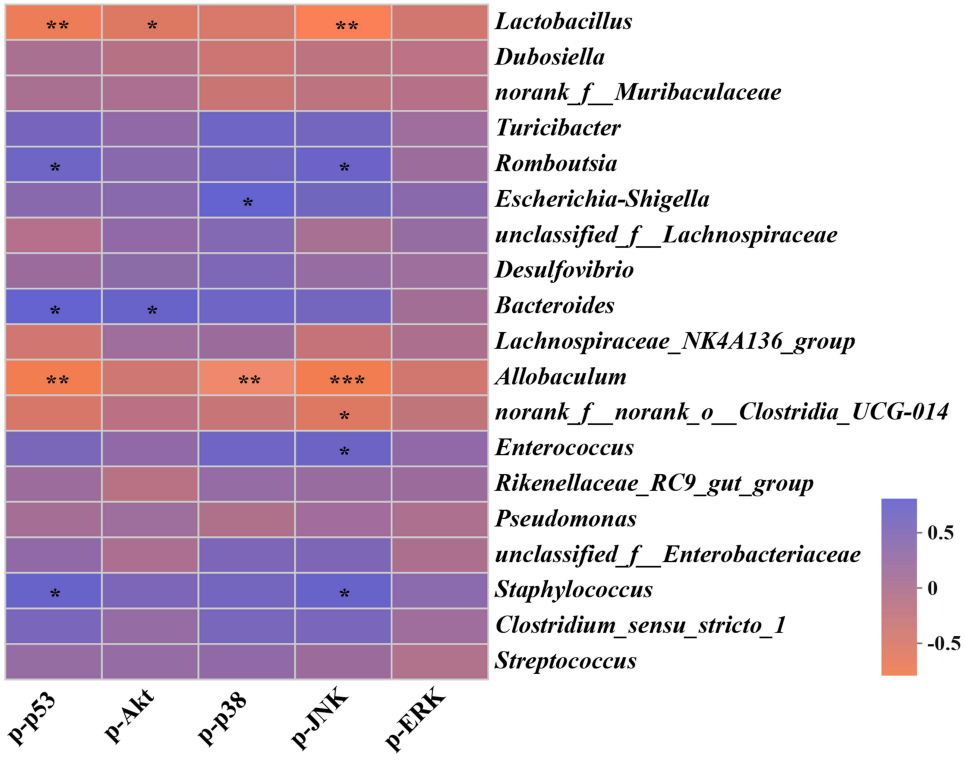

Figure 10 Spearman correlation analysis between gut microbiota and key target protein in colon; blue and red represent the positive and negative correlation, respectively; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

GQD is composed of various traditional Chinese medicines and contains multiple active ingredients. The synergistic effect between each ingredient can enhance the anti-colitis effect of GQD. Compound-target network illustrates that several key active ingredients contribute significantly to GQD alleviate colitis. The potential active ingredients with anti-colitis effects include glycerol, inermine, naringenin, kaempferol, and quercetin, with quercetin showing the highest binding affinity to the target. Previous study has shown that quercetin significantly improves intestinal barrier function and reduces inflammation in colitis models,[22] indicating it may play a key role in the therapeutic action of GQD. Furthermore, kaempferol and naringenin are recognized for their natural antioxidant and anti-inflammatory properties.[23] However, the key ingredient in GQD responsible for its therapeutic efficacy remains to be further elucidated.

The intestinal barrier plays a crucial role in preventing bacterial pathogens from penetrating intestinal tissue.[24] It has been proved that the increased permeability of this barrier is a key pathogenic mechanism in colitis; this is closely linked to the integrity of tight junction proteins and the presence of mucins.[25] ZO-1 and Occludin are the most extensively studied tight junction proteins, and they are crucial for regulating intestinal epithelial cell proliferation, promoting mucosal repair, and strengthening the intestinal barrier.[26] Furthermore, MUC2 is another crucial protein in protecting the intestinal barrier, as it regulates the formation of the mucus layer to exert its protective effects.[16] Therefore, ZO-1, Occludin, and MUC2 are deeply involved in maintaining the homeostatic of gut environment. This study revealed that GQD could significantly improve the down-regulation of these three proteins caused by DSS, showing a good protective effect on gut barrier. In summary, GQD alleviated pathological damage to colonic tissues by decreasing the permeability of intestinal barrier function.

The imbalance of inflammatory cytokines is the key factor resulting in colitis.[27] Inflammatory cytokines, including anti- and pro-inflammatory cytokines involve in the occurrence and development of colitis, their balance is crucial for observing inflammation degree in colon.[28] When pro-inflammatory cytokines dominate, the inflammation leads to tissue damage, ulcer formation, and colitis.[20] In this study, the pro-inflammatory cytokines including IL-6, TNF-α, and IL-1β in DSS-induced mice showed significant increase, while GQD reverse these trends. Furthermore, the up-stream inflammatory signaling proteins was also determined. According to the results from network pharmacology, GQD exerts its effects primarily by modulating the p53, Akt, STAT3, and MAPK signaling pathways (p38, JNK, and ERK). Western blot analysis revealed significant alterations in these proteins in colitis mice, which were notably improved with GQD treatment. The MAPK pathway, involving a cascade of kinase activations through ERK, JNK, and p38, plays a crucial role in regulating inflammation.[29] Both Akt and p53 are key regulators of apoptosis, oxidative stress, and inflammation. p53 promotes the phosphorylation of Akt by inducing the expression of phosphatase and tensin homolog (PTEN).[30] The activated Akt negatively regulates the MAPK pathway by inhibiting RAF-1, thereby influencing downstream signaling.[31] Therefore, it was suggested that GQD ameliorates colitis through inhibiting the overexpression of inflammatory cytokines.

The gut microbiota is the key factor in the development and progression of colitis. A healthy microbiota contributes to maintaining the intestinal barrier function and modulating immune responses, while dysbiosis can trigger the intestinal inflammation.[32] Therefore, the effects of GQD on gut microbiota were further evaluated. The results indicated that in the DSS group, *Proteobacteria, Bacteroides, Romboutsia* were enriched, while *Lactobacillus, Allobaculum* were decreased. However, GQD treatment reversed these trends. *Proteobacteria* is closely associated with the development and exacerbation of colitis, its overgrowth can disrupt the gut microbiota balance, damage the intestinal barrier, and exacerbate inflammation.[33] *Bacteroides* is a potential role in breaking down complex carbohydrates and producing short-chain fatty acids, while its translocation can impact the intestinal barrier integrity and accelerate the development of colitis.[34] Therefore, this revealed that GQD may protect the intestinal tract barrier via regulating the ratio of gut microbiota, which potentially improved the inflammation in colon. This aligns with and confirms the results of Figure 5. *Lactobacillus* is an acknowledged probiotic in digestive tract, it plays a crucial role in maintaining gut health and overall immune function. It has been proved that *Lactobacillus* can resist UC through inhibiting the growth of pathogenic bacteria and regulating the imbalance of inflammatory cytokines in gut.[35] *Allobaculum* plays a protective role in modulating metabolism and anti-inflammation in colon.[36] Therefore, the ameliorative effects of GQD on colitis may be related to the increase of these probiotic bacteria.

This study examined the therapeutic effects of GQD on colitis by focusing on gut barrier integrity, inflammation, and gut microbiota composition. In fact, there is close connection between these three aspects. When the barrier is compromised, pathogen translocation triggers immune dysregulation, which simultaneously induces inflammation.[37,38] Furthermore, the intestinal barrier can block harmful bacterial invasion and supports microbial diversity by providing a stable microenvironment.[39] In turn, the gut microbiota contributes to barrier maintenance through mechanisms like preserving the mucus layer. Concurrently, microbiota imbalance increases pro-inflammatory bacteria while suppressing anti-inflammatory strains, creating a vicious cycle of worsening inflammation and barrier damage.[40,41] In this study, GQD restored the integrity of the intestinal barrier, reduced inflammatory response, and increased gut microbiota diversity. This combined action may play a key role in its colitis-relieving mechanism.

Conclusion

In summary, the results demonstrated that GQD administration effectively alleviates DSS-induced colitis, as evidenced by improvements in clinical and histopathological parameters, protection of the intestinal mucosal barrier, regulation of pro-inflammatory mediators and oxidative enzymes in the colon, inhibition of inflammation-related protein activation, and enhancement of gut microbiota. These findings suggest that GQD mitigates colitis by ameliorating inflammation and restoring the balance between beneficial and harmful gut bacteria. This study offers crucial experimental insights and lays a strong foundation for future research into the therapeutic potential of GQD for colitis, ultimately advancing its prospects for clinical application.

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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 have no conflicts of interest to declare regarding the publication of this paper.

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