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ORIGINAL RESEARCH

Validate the Treatment of Crohn's Disease with Chaihu Guizhi Decoction Through Network Pharmacology, Molecular Dynamics Simulation, and in vivo Experiments

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Objective: Using an integrated strategy combining network pharmacology, molecular dynamics simulations, and in vivo experiments, this study explores the stage-specific targets of Chaihu Guizhi Decoction (CGD) for the treatment of Crohn's disease (CD).

Methods: First, through the GEO database, the patients with Crohn's disease were divided into four groups: early patients (NCD), late patients (LCD), patients with postoperative recurrence (PCD) and patients without postoperative recurrence (UPCD), and the differential genes of each group were screened. Active ingredients in Chaihu Guizhi Decoction (CGD) with an oral bioavailability (OB) greater than 30% and drug-likeness (DL) exceeding 0.18 were screened using the TCMSP database, along with their corresponding potential targets. The target of CGD was intersected with four groups of differential genes. The common therapeutic targets of CGD for Crohn's disease in four groups and the specific therapeutic targets of each group were obtained. Core targets were identified through protein-protein interaction (PPI) network analysis. The potential mechanism of CGD in treating Crohn's disease was analyzed by enrichment of KEGG and GO. Finally, molecular docking and molecular dynamics simulation were used to verify the possibility of combining the effective ingredients of CGD with the therapeutic target of Crohn's disease. Construct a mouse model of inflammatory bowel disease and administer drug treatment. Use mouse intestinal tissue for Elisa, HE staining, and AB-PAS staining to validate the potential of drug therapy for inflammatory bowel disease. Meanwhile, Western blot experiments were conducted to verify the effect of quercetin on the expression of downstream target proteins.

Results: Through molecular docking verification, quercetin can bind to these six core genes. We analyzed quercetin and six gene protein products through molecular dynamics simulation. We found that the complex of quercetin and PTGS2 protein is relatively stable, which may be a therapeutic target. In animal studies, both CGD and quercetin significantly alleviated inflammation-induced intestinal shortening ($p < 0.05$). ELISA results demonstrated that CGD and quercetin markedly reduced the expression of pro-inflammatory cytokines, including IL-6, IL-1β, and TNF-α ($p < 0.05$). Furthermore, histopathological examinations via HE and PAS staining revealed a substantial mitigation of intestinal inflammation following treatment with CGD and quercetin.

Conclusion: This study found through animal experiments that CGD and quercetin can treat CD. Through network pharmacology, molecular dynamics simulations, and Western blot experiments, it was demonstrated that quercetin can treat inflammatory bowel disease by affecting the expression of PTGS2 and IL-1B proteins.

Keywords: Chaihu Guizhi decoction, Crohn's disease, network pharmacology, molecular dynamics simulation, traditional Chinese medicine, quercetin

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Introduction

Crohn's disease is a clinical subtype of inflammatory bowel disease (IBD). The etiology of CD is complex and unclear, which may be related to immune system imbalance, genetic susceptibility, intestinal flora changes and environmental factors.[1] It is generally believed that the pathogenesis of CD is the interaction of genetic susceptibility and environmental factors, which leads to the imbalance of immune response in the body, and then acts on the gastrointestinal tract, causing inflammation.[2] The chronic inflammation of CD can occur anywhere from the mouth to the anus, showing a discontinuous disease progression. If not intervened, the disease will continue to progress, causing irreversible damage to the intestine.[3–5] Common complications of Crohn's disease include intestinal obstruction caused by intestinal stenosis, fistula and gastrointestinal inflammation with severe intestinal diarrhea.[3] Among them, intestinal obstruction is one of the most serious and common complications. About 2/3 patients will have fibrotic intestinal obstruction 10 years after the diagnosis of Crohn's disease.[6] At present, the treatment plan for Crohn's disease is mainly to delay the progress of the disease and prevent complications through drug treatment and surgical treatment. After surgical treatment, the symptoms of CD patients can be relieved immediately, and the quality of life can be temporarily improved in the short term. Surgical intervention for CD carries a significant risk of complications, including short bowel syndrome, and is typically reserved for cases refractory to medical therapy.[7,8] Due to the gradual use of biological agents and other drugs, the risk of surgical intervention for CD patients has been reduced.[9] At present, drug therapy mainly depends on glucocorticoids, mesalazine preparations, immunosuppressants, biological agents and enteral nutrition.[10] Despite these options, long-term drug therapy often fails to fully control symptoms and is associated with considerable side effects and financial burden—especially given the high cost of biologic agents. Even with optimized treatment, around 50% of patients require surgery within ten years of diagnosis.[11] Traditional Chinese medicine herbs and prescriptions have unique effects on gastrointestinal diseases, and they may become a new choice for the treatment of Crohn's disease.[12] These limitations underscore the urgent need for more effective, affordable, and sustainable treatment alternatives.

In previous clinical observations, Chaihu Guizhi Decoction (CGD) has shown efficacy in improving immune function and alleviating symptoms in CD patients.[13] Research shows that, compared with western medicine alone, the combination of traditional Chinese medicine and western medicine can significantly reduce the clinical symptoms of Crohn's disease, reduce the level of inflammatory indicators in the body, promote the healing of intestinal mucosa, and reduce the recurrence rate and adverse reactions.[13,14] From the perspective of Traditional Chinese Medicine (TCM), the pathogenesis of Crohn's disease (CD) is primarily characterized by the accumulation of dampness-heat toxin in the intestines.[15] This TCM syndrome is often contemporarily interpreted as a manifestation of intestinal inflammation, dysbiosis (an imbalance in gut microbiota), and immune activation. Therefore, the corresponding TCM treatment strategy aims to clear heat and resolve toxin (which may correlate with exerting anti-inflammatory and antimicrobial effects), activate blood circulation to remove stasis (potentially corresponding to improving microcirculation and mitigating tissue fibrosis), and reinforce qi and fortify the spleen (which may be associated with regulating immune function and restoring intestinal barrier integrity).[15] In previous clinical studies, we found that CGD can improve the immune function of CD patients, and has a significant therapeutic effect on CD patients.[16] CGD comes from Zhang Zhongjing's Treatise on Febrile Diseases. The prescription is: *Paeonia lactiflora* 18 g, *Codonopsis pilosula* 15 g, *Cinnamomum cassia* 12 g, *Bupleurum chinense* 12 g, *Baikal Skullcap* 9 g, *Pinellia ternata* 9 g, *Licorice* 6 g, *Jujube* 5 pieces, *Ginger* 3 pieces. *Bupleurum chinense* and *Cinnamomum cassia* can soothe the liver and promote yang, reconcile the exterior and interior, warm and dredge the meridians, tonify yang, and invigorate qi and blood. *Paeonia lactiflora* can strengthen the spleen and stomach, complement deficiency, and warm yang and remove dampness. *Codonopsis pilosula* has the effects of strengthening the spleen and lung, supplementing the middle and qi. It can also enhance the hematopoietic function, improve microcirculation, lower blood pressure, expand blood vessels, and enhance immunity. *Jujube* can nourish yin and yang, *ginger* can benefit the spleen and stomach, and *licorice* can harmonize various drugs to play a synergistic role.[16] This study aims to systematically investigate the active components, core targets, and therapeutic mechanisms of CGD in the treatment of CD using an integrated strategy combining network pharmacology and molecular dynamics simulations. Our central hypothesis is that CGD alleviates intestinal inflammation through interactions with core targets such as PTGS2 and IL-1B.

Materials and Methods

Acquisition of Differential Genes in Crohn's Disease

In order to obtain the differential gene of Crohn's disease, download GSE102133 and GPL6244 files from Gene Expression Omnibus (GEO) database on June 26, 2022. 12 groups of normal transcriptional group data, 30 groups of postoperative recurrent transcriptional group data, 3 groups of postoperative non recurrent transcriptional group data, 18 groups of early diagnosis transcriptional group data, and 14 groups of late diagnosis transcriptional group data were obtained. The Crohn's disease patients in the data were divided into NCD, LCD, PCD, and patients without postoperative recurrence (UPCD). The difference genes of Fdr<0.05, | log2 (fold change) (FC) |>1 in each group were screened using the lima package of R-language Bioconductor. The four groups of differential genes were intersected to obtain four groups of common differential genes and each group's unique differential genes.

Acquiring the Effective Components and Pharmacological Targets of the Effective Components of CGD

The effective ingredients of *Paeonia lactiflora, Codonopsis pilosula, Cinnamomum cassia, Bupleurum chinense, Baikal Skullcap, Pinellia ternata, Licorice, Jujube* and *Ginger*. in CGD were obtained from Tcm Systems Pharmacy Database and Analysis Platform (TCMSP). Then set OB>30% and DL>0.18 to screen out the effective components and pharmacological targets that can be used as drugs.

Target of CGD in Treating Crohn's Disease at Each Stage

The pharmacological targets of the effective ingredients of CGD were intersected with the differential genes of the four groups of Crohn's disease. Finally, the common target genes and specific targets of each group of CGD for Crohn's disease were obtained. The genes obtained from the intersection serve as potential therapeutic targets.

Enrichment Analysis

The r language package, including "ClusterProfiler", "org. Hs. eg Db" and "Enrichplot", is used to enrich and analyze the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of potential target genes of CGD for Crohn's disease treatment. Set P-value cutoff=0.05 and q-value cutoff=0.05, enrich the data of GO and KEGG through the language pack "org. Hs. eg Db", and use output to draw a Circos diagram.

The Core Target of CGD in Treating Crohn's Disease

Upload the common targets of CGD in each stage of Crohn's disease treatment to the STRING database (version 11.0) to obtain the target-target function-related protein interaction network, target interaction in protein-protein interaction (PPI) network diagram and tsv data. Import the data into the Cytoscape software, use its CytoNCA, select the scores of Betweenness, Closeness, Degree, Eigenvector, LAC, and Network, and screen out all the genes greater than the median value in the six scores, that is, core genes.

Molecular Docking

Molecular docking can predict the binding possibility and potential interaction between proteins and small molecules. First, download the SDF molecular structure of the active ingredient in the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Use the ChemBio3D Draw module in Chem3D software (14.0.0.117) to optimize the MM2 force field of the active ingredients of the drug, generate the minimum energy structure, and convert it to the mol2 format.The protein structure of Crohn's disease potential therapeutic target related protein comes from PDB database (https://www.rcsb.org/). Use PyMol (2.5.1) to perform the remove solvent, remove organic and remove inorganic command operations on the protein structure. Then AutoDock Tools is used to hydrogenate and charge the protein and convert it into the PDBQT format required for molecular docking. Use Autodock Vina software to perform molecular docking of proteins and ligands. Input the minimum binding energy structure in molecular docking to Discovery Studio 2021 Client to display the 2d structure of molecular docking, and then use PyMol

(2.5.1) software to conduct 3d visual expression of molecular docking results to display the binding site, binding mode, and interaction force between the effective components of drugs and proteins.

Molecular Dynamics Simulations

First, the small ligand molecules were parameterized by gaff2 force field and am1-bcc charge method through acpype software (v2023.3.1), and the protein macromolecules were processed by GMX pdb2gmx tool of GROMACS using charmm36m force field (version 2023). Subsequently, a cubic water box (TIP3P water model) with a margin of 1.2 nm was added, and Na^{+}/cl^{-} ions were added to physiological concentration. NVT equilibration (100 PS, v-rescale hot bath, 310 K) and NPT equilibration (100 PS, Parrinello Rahman pressure control, 1 bar) were performed. The whole process was quality controlled by energy convergence, temperature / pressure stability (fluctuation $< \pm 2$ k/ ± 5 bar) and structural convergence (C α -rmsd<0.2 nm).

Cell Culture

The NCM460 cells were obtained fromthe cell bank of the typical culture preservation committee, Chinese Academy of Sciences, Shanghai, China. The cells were cultured in 1640 medium containing 10% fetal bovine serum and 1% penicillin streptomycin. NCM460 cells were treated with 10ug/mL LPS for 24h to construct the enteritis cell model.

CCK8

NCM460 cells were seeded in 96 well plates with a density of 5000 cells/well. NCM460 cells were treated with different concentrations of quercetin (0, 0.2, 0.5, 1, 5, 10, 25, 50 μ mol/L) for 24h. CCK8 reagent was added and incubated in the cell incubator for 1h, and the absorbance at 450nm was measured by microplate reader.

Western Blotting

Protein extraction from colon tissues or cultured cells was performed using RIPA lysis buffer supplemented with protease and phosphatase inhibitors. The protein concentration was quantified with a BCA assay kit. Equal amounts of protein (20–30 μg) were separated by SDS-PAGE and subsequently transferred onto PVDF membranes. After blocking with 5% non-fat milk in TBST for 1 hour at room temperature, the membranes were incubated overnight at 4°C with primary antibodies against PTGS2, IL-1B, and β-actin. Following three washes with TBST, the membranes were incubated with HRP-conjugated secondary antibodies (dilution 1:5000) for 1 hour at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system. Band intensities were quantified with ImageJ software, and the expression levels of target proteins were normalized to β-actin.

Animal Model

This study has been approved by Anhui Medical University Experimental Animal Ethics Committee (LLSC20242418). Randomly divide 30 BALB/c mice aged 6–8 weeks into 6 groups: control group,TNBS model group, low-dose CGD group (5g/kg/d), medium-dose CGD group (10g/kg/d), high-dose CGD group (20g/kg/d), and quercetin group (30mg/kg/d), with 5 mice in each group. The experimental operators and result scorers were unaware of the grouping information. Using absolute ethanol, normal saline, and TNBS to prepare a 2% TNBS solution. To create a model of inflammatory bowel disease in mice, TNBS was first applied to the skin of the mice. Seven days later, TNBS was injected into the mouse intestine through the anus at a dose of 100mg/kg per mouse to establish an inflammatory bowel disease model. The drug treatment group was given medication by gavage for 7 days on the second day after the construction of the intestinal inflammation model. On the eighth day, the mice were euthanized and their intestinal tissues were collected. All programs and operations of the experiment were conducted in strict compliance with the National Laboratory Animal Welfare Standards: Laboratory Animal—Guideline for Ethical Review of Animal Welfare (GB/T35892–2018).

Histological Analysis

Intestinal tissue were preserved in a solution of 10% neutral buffered formalin for 24 h, processed by embedding in paraffin, and then sectioned for hematoxylin and eosin (H&E) staining and periodic acid- Schiff (PAS) staining.

ELISA

The ELISA assay kit was purchased from Quanzhou Ruixin Biological Technology Co, LTD Quanzhou, Chin. Use ELISA kit to detect IL-6, IL-1 β, TNF - α and IL-10 inflammatory cytokines. Grind the mouse intestinal tissue into a homogenate using a grinder. Set the centrifuge to 4 °C, 12000rpm, centrifuge for 15 minutes, and collect the supernatant. After diluting the standard and sample, incubate at 37 ° C for 2 hours. Add enzyme-linked immunosorbent assay antibody and incubate at room temperature for 1 hour. Finally, add TMB colorimetric solution and react in the dark for 30 minutes. After termination, use an enzyme-linked immunosorbent assay (ELISA) reader to detect the absorbance at 450nm.

Statistical Analysis

Use GraphPad Prism software (version 8.0) to process experimental data. Analysis of Variance (ANOVA) is used to evaluate differences between multiple groups, supplemented by Tukey's post hoc test. All experiments were repeated three times. Set the p-value to 0.05.

Result

Bioinformatics

Acquisition of the Pharmacological Target of CGD

The TCMSP database, 249 pharmacological targets were obtained by screening the pharmacological targets of each active ingredient (Figure 1A). In TCMSP, under the conditions of OB>30% and DL>0.18, 13 effective ingredients were screened from *Paeonia lactiflora*, 13 effective ingredients were screened from *Pinellia ternata*, 17 effective ingredients were screened from *Bupleurum chinense*, 21 effective ingredients were screened from *Codonopsis pilosula*, 29 effective ingredients were screened from *Jujube*, 92 effective ingredients were screened from *Licorice*, and 7 effective ingredients were screened from *Cinnamomum cassia*, 36 effective ingredients were screened from *Baikal Skullcap* and 5 effective ingredients were screened from *Ginger* (Figure 1B).

Identification of Crohn's Disease Differential Genes and Specific Differential Genes of Each Group

First, 530 LCD differential genes (Figure 2A–D); 264 PCD differential genes (Figure 2B–F); 547 NCD differential genes were screened from the GEO database (Figure 2C–E). The differential gene of UPCD is only WNT5A (Figure 2G). Through the intersection of NCD, LCD and PCD, 196 common genes, 152 NCD specific genes, 102 LCD specific genes and 25 PCD specific genes were obtained (Figure 3A).

Potential Pharmacological Targets of CGD in the Treatment of Crohn's Disease at Each Stage

By crossing the differential genes of each group of Crohn's disease with the pharmacological target of CGD, the overlapping genes are taken as the potential pharmacological target of CGD in treating Crohn's disease. Finally, 28 common potential pharmacological targets for LCD, NCD and PCD, 2 specific potential pharmacological genes for LCD and 5 specific potential pharmacological targets for NCD were obtained (Figure 3B–E).

Enrichment Analysis of Target Genes KEGG and GO in the Treatment of Crohn's Disease with CGD

The KEGG and GO enrichment analysis of the potential pharmacological targets of CGD for treating LCD, NCD and PCD showed that CGD can be used to treat patients through inflammatory response, inflammatory response, cellular response to chemical stimulus, cytokine-mediated signaling pathway, response to oxygen-containing compound, response to chemical, chemokine receptor binding, CXCR chemokine receptor binding, chemokine activity, G protein-coupled receptor binding, interleukin-1 receptor binding, HIF-1 signaling pathway, Inflammatory bowel disease, Chemokine

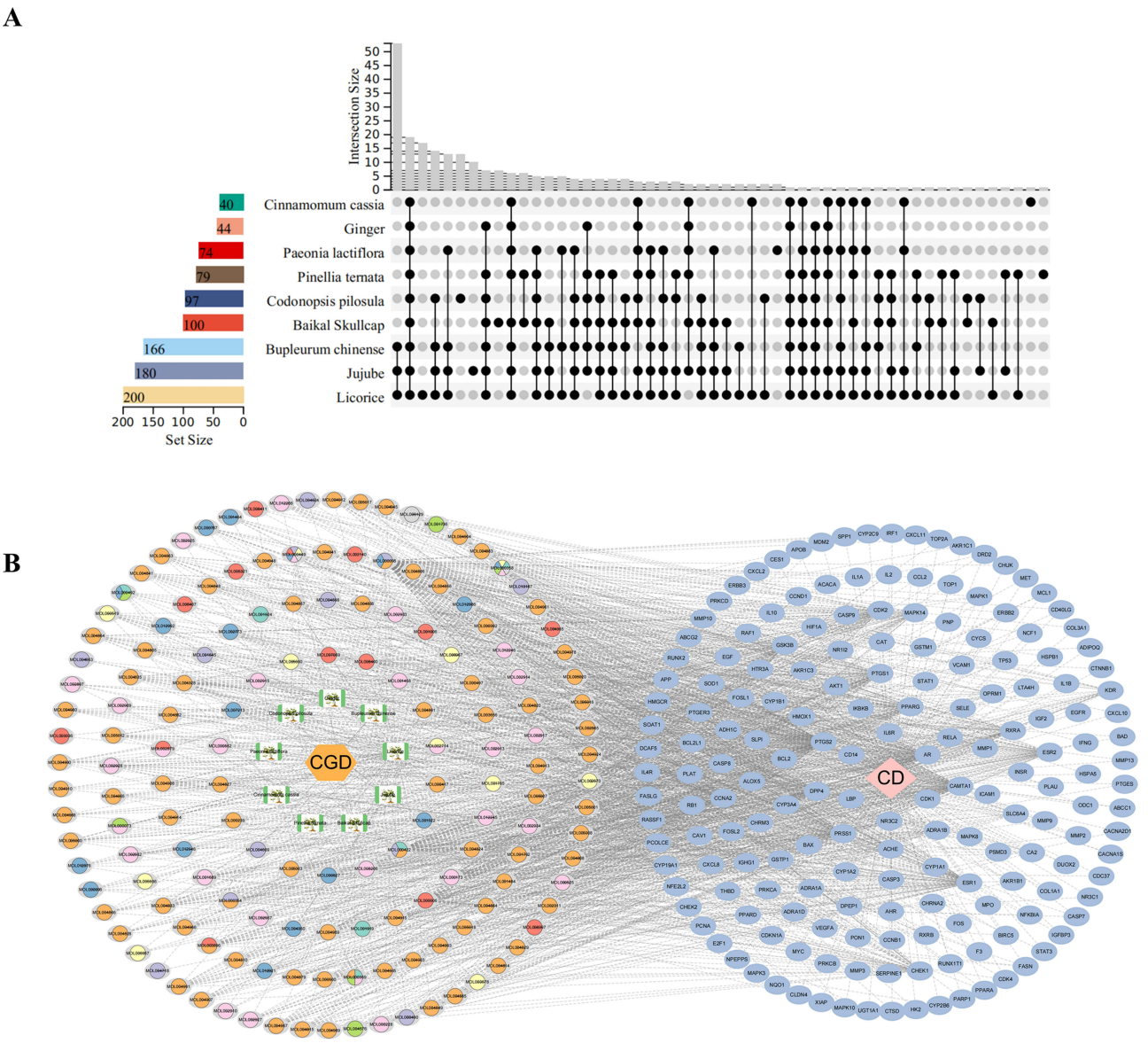

Figure 1 Acquisition of the pharmacological target of CGD (**A**) Pharmacological target of each drug component of CGD. (**B**) Drug-Target-Disease visualization chart (the color number of active ingredients indicates the types of drugs in the compound).

signaling pathway, Drug metabolism - cytochrome P450, TNF signaling pathway, NF-kappa B signaling pathway and other mechanisms to achieve the purpose of treating Crohn's disease (Figure 3F and G).

CGD for the Treatment of Crohn's Disease Core Gene

The potential 28 common pharmacological targets of CGD for the treatment of Crohn's disease in three groups were input into STRING, and the protein interaction network and tsv files were obtained. Import the data obtained by STRING into Cytoscape, and screen six core genes (HIF1A, CCL2, PTGS2, IL1A, IL1B, CXCL8) through CytoNCA (Figure 4A).

Molecular Docking

Effective Drug Components and Therapeutic Targets for Crohn's Disease

When analyzing the drugs corresponding to these six core genes, we found that PTGS2 can interact with 116 active ingredients in CGD (Figure 4B). We speculate that PTGS2 may be an important target gene of CGD in the treatment of Crohn's disease. According to the TCMSP database, we found that six core target genes were regulated by quercetin. We

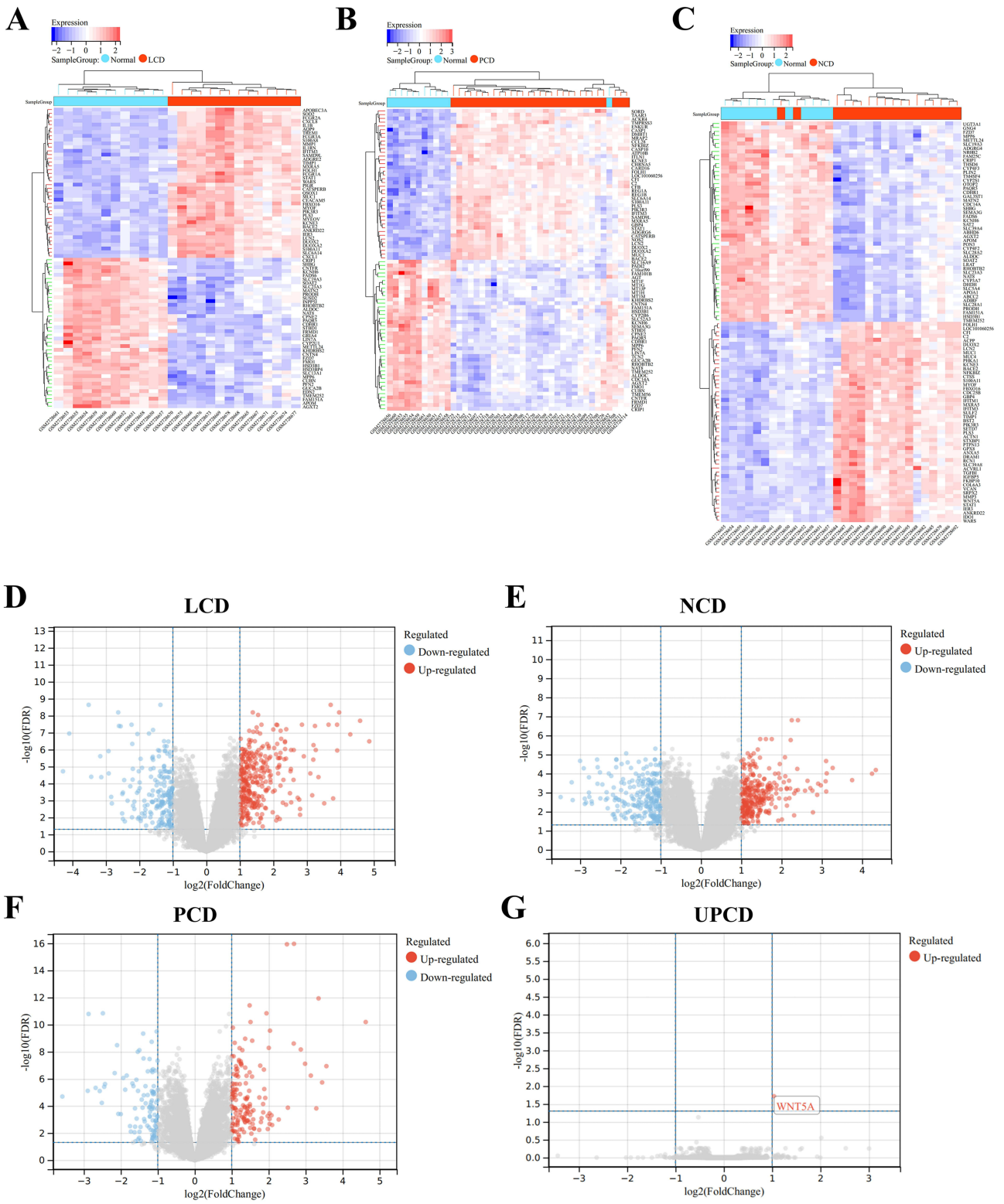

Figure 2 Differential genes in different stages of Crohn's disease. (**A**) Differential genes of late patients (LCD). (**B**) Differential genes of patients with postoperative recurrence (PCD). (**C**) Differential genes of early patients (NCD). (**D**) Differential genes of late patients (LCD). (**E**) Differential genes of early patients (NCD). (**F**) Differential genes of patients with postoperative recurrence (PCD). (**G**) Differential genes of patients without postoperative recurrence (UPCD).

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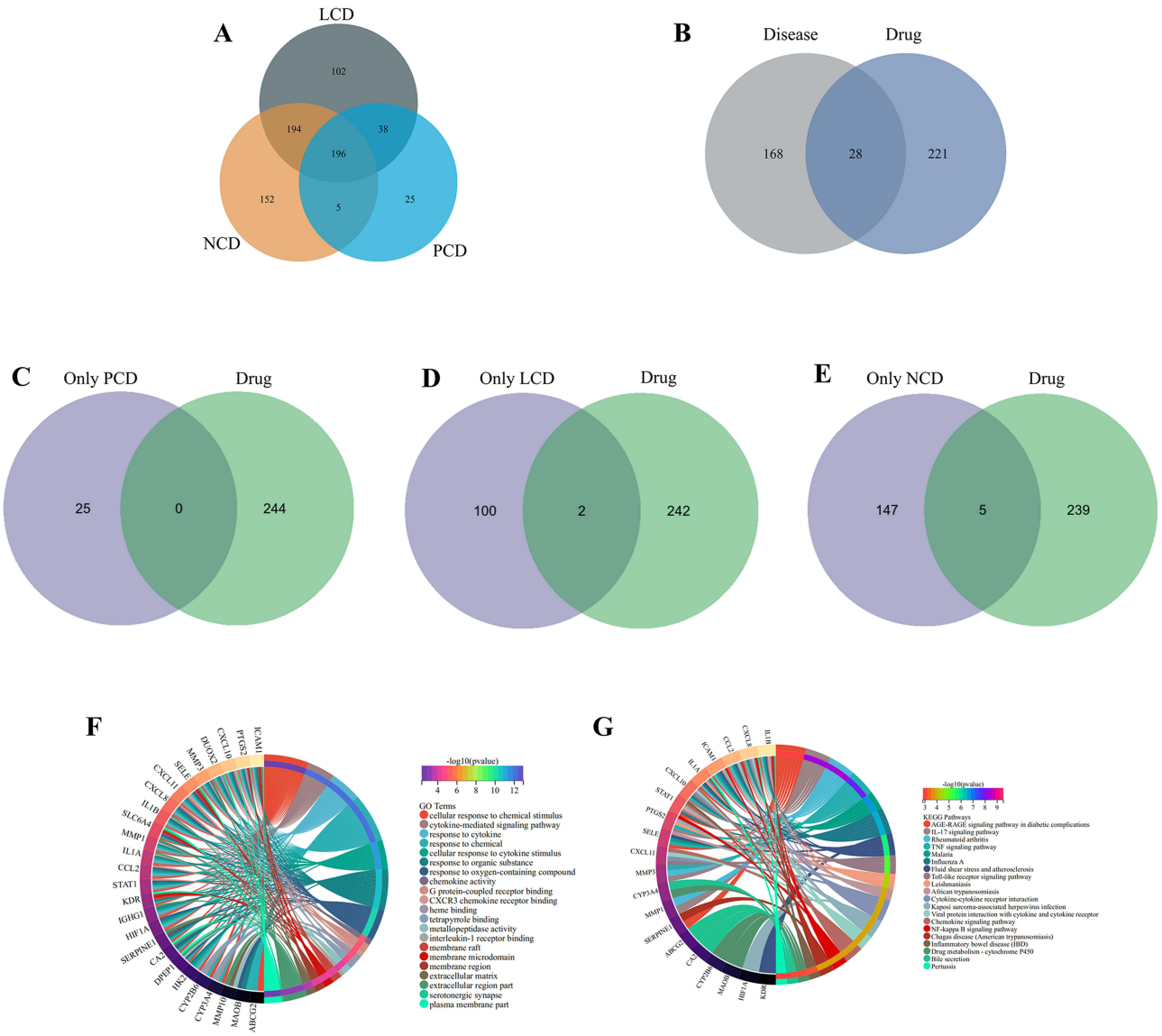

Figure 3 Pharmacological Target of Chaihu Guizhi Decoction (CGD) in Treating Crohn's Disease. (**A**) LCD, NCD and PCD differential genes. (**B**)Intersection of Pharmacological Target of CGD and Three Groups of Common Genes. (**C**) Intersection between PCD specific differential genes and CGD pharmacological targets. (**D**) Intersection between LCD specific differential genes and CGD pharmacological targets. (**E**) Intersection between NCD specific differential genes and CGD pharmacological targets. (**F**) GO enrichment analysis based on the intersection genes of the pharmacological target of Chaihu Guizhi Decoction and LCD, NCD, patients with PCD. (**G**) KEGG enrichment analysis based on the intersection genes of the pharmacological target of Chaihu Guizhi Decoction and LCD, NCD, PCD.

docked the target proteins of these six core genes with quercetin (Figure 5A and B, Supplementary Figure 1). The six core target genes can form hydrogen bonds with quercetin and bind stably. The molecular docking results showed that the binding energies of quercetin with IL-1A, IL1B, and PTGS2 were less than −7.5 kcal/mol (Table 1). This indicates that the complex formed by quercetin with IL-1A, IL-1B, and PTGS2 is stable Among these candidate targets, the binding energy of the complex formed by quercetin and PTGS2 is the lowest, at 9.181 kcal/mol. In the analysis of LCD, NCD specific differential genes and pharmacological targets of CGD, we found that formononetin can act on HSD3B2 and HSD3B3 in LCD, and Quercetin can act on NQO1. After molecular docking verification, we determined that Quercetin can combine with NQO1 through a hydrogen bond and other interactions (Table 1). In NCD, we found that Naringenin, Taxifolin, Paeoniflorin and Quercetin act on specific differential genes of NCD, which has the possibility to specifically treat NCD. Through molecular docking, it is verified that Paeoniflorin binds to CD14 through hydrogen bond, Naringenin and Taxifolin binds to MTTP through hydrogen bond, and Quercetin binds to PCOLCE through hydrogen bond (Tables 1 and 2). To sum

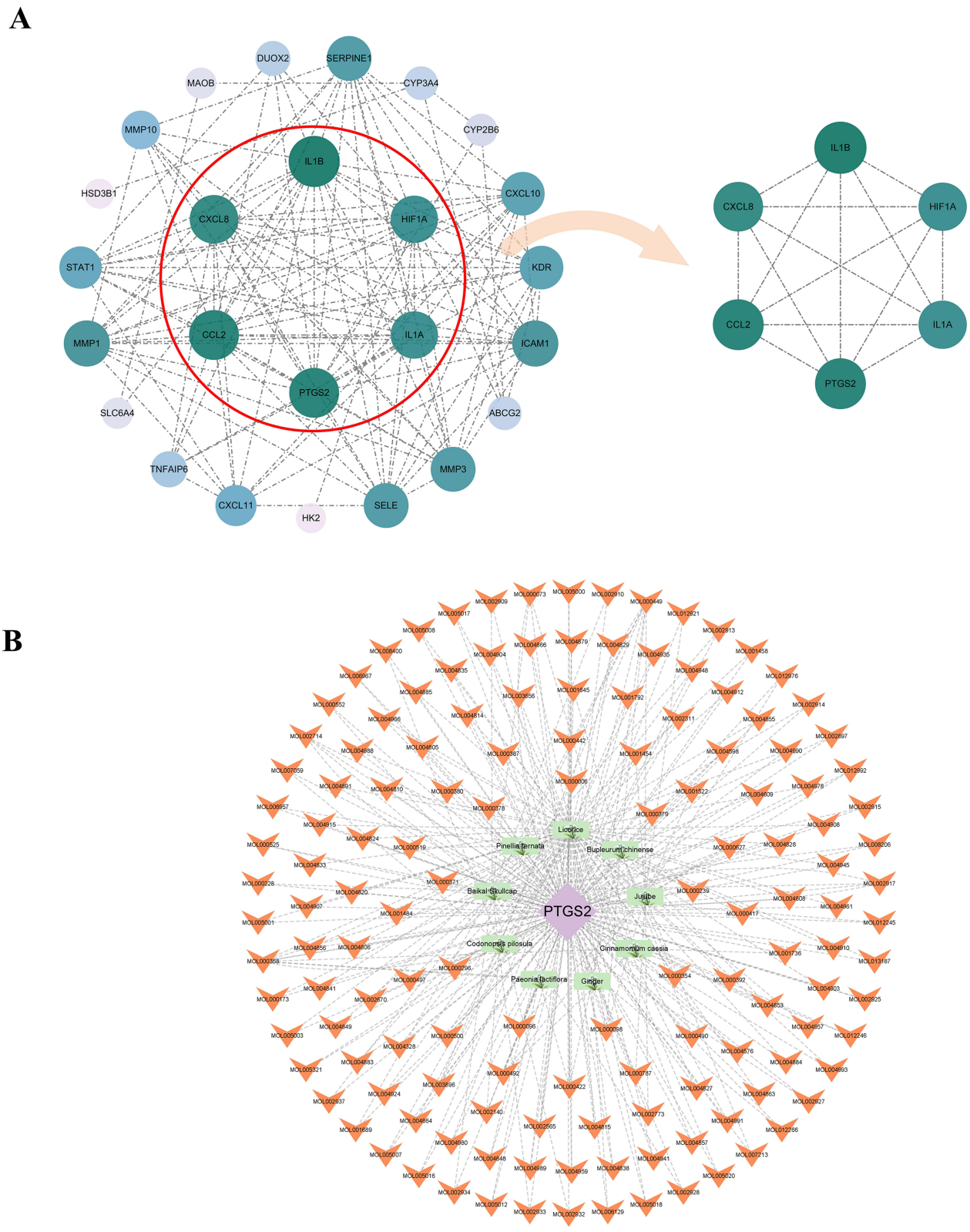

Figure 4 Visualization Chart of Chaihu Guizhi Decoction in Treating Crohn's Disease. (**A**) Core gene screening of common genes of LCD, NCD, PCD. (**B**) PTGS2 - Drug-Drug Composition Visualization Chart.

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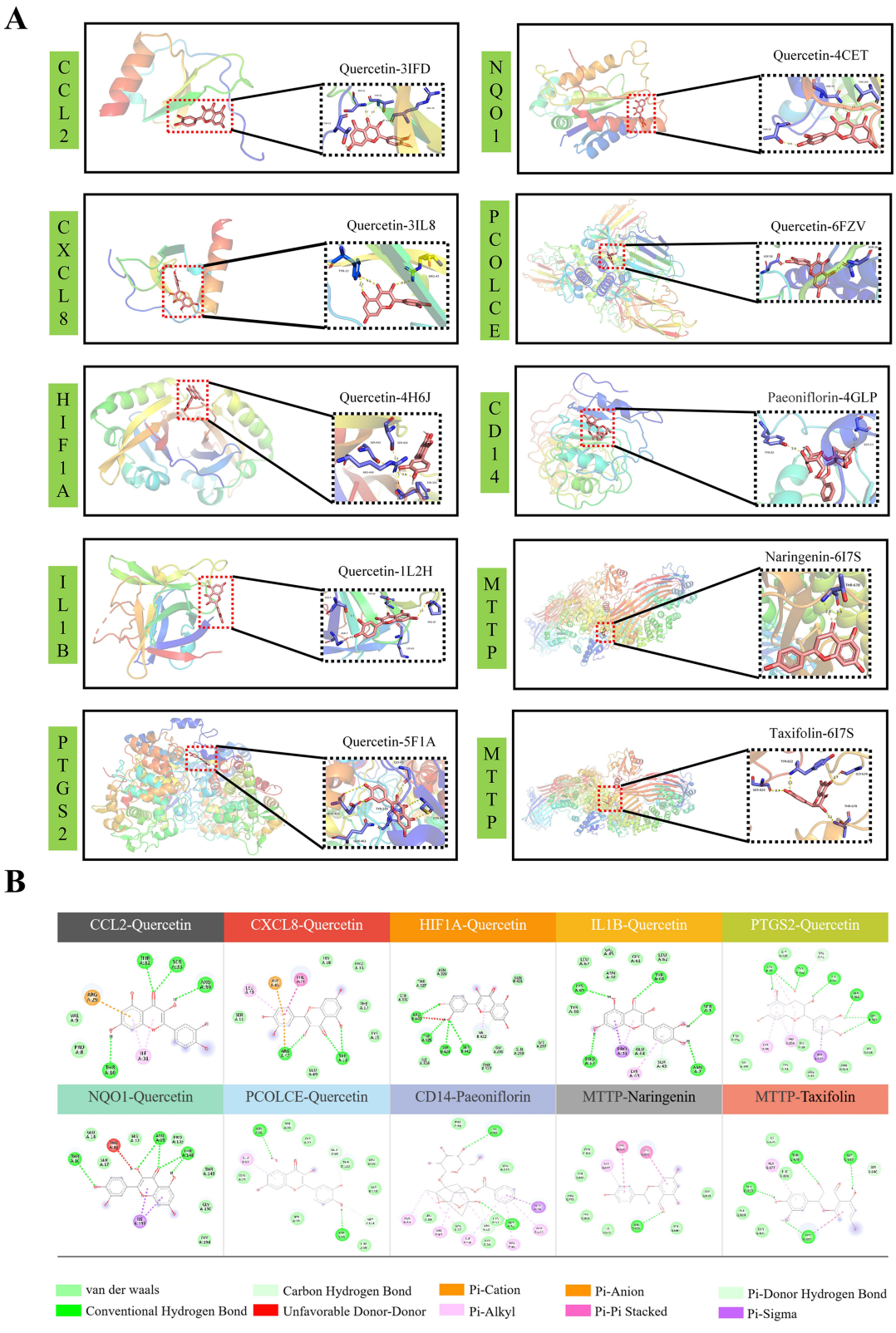
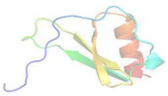
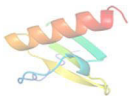
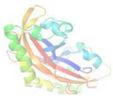
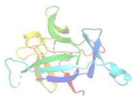
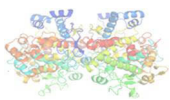
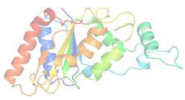
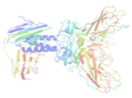


Figure 5 Molecular docking (**A**) 3D visualization of molecular docking. (**B**) 2D visualization of molecular docking.

Table 1 Visual Display of Molecular Docking Between Macromolecular Proteins and Small Molecular Ligands

Target	Target (PDB ID)	Target Structure	Compound	Affinity (kcal/mol)
CCL2	3IFD		Quercetin	−6.107
CXCL8	3IL8		Quercetin	−6.788
HIF1A	4H6J		Quercetin	−7.217
IL1A	5UC6		Quercetin	−7.618
IL1B	1L2H		Quercetin	−7.565
PTGS2	5F1A		Quercetin	−9.181
NQO1	4CET		Quercetin	−7.131
PCOLCE	6FZV		Quercetin	−7.279

(*Continued*)

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Table 1 (Continued).

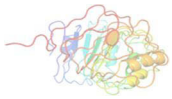
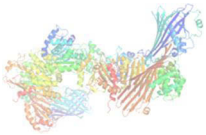
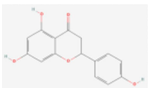
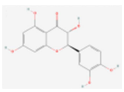
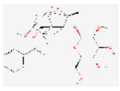
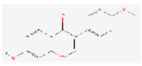
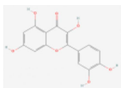
Target	Target (PDB ID)	Target Structure	Compound	Affinity (kcal/mol)
CD14	4GLP		Paeoniflorin	−7.41
MTTP	6I7S		Naringenin	−9.177
			Taxifolin	−8.085

Table 2 Effective Components of Chaihu Guizhi Decoction in the Precise Treatment of Crohn's Disease

Mol ID	Molecule Name	Molecule Structure	OB (%)	DL
MOL004328	Naringenin		59.29	0.21
MOL004576	Taxifolin		57.84	0.27
MOL001924	Paeoniflorin		53.87	0.79
MOL000392	Formononetin		69.67	0.21
MOL000098	Quercetin		46.43	0.28

up, we found that Quercetin may be used as a specific drug or long-term maintenance drug to treat Crohn's disease, and it is an important effective component of CGD in treating Crohn's disease. At the same time, because a large number of effective ingredients in CGD have the potential to combine with PTGS2, PTGS2 may be an important target of CGD in treating Crohn's disease.

Molecular Dynamics Simulation

RMSD is the root mean square deviation in molecular dynamics simulation, which can be used to show the mobility of small molecules and the stability of proteins. The large value of RMSD and the large fluctuation of RMSD indicate that the structure has strong mobility and poor stability. The RMSD of the HIF1A protein complex is 0.3nm at 1–50ns, and the fluctuation amplitude is 0.2nm. At this time, the RMSD is stable, but after 50ns, the RMSD suddenly rises to 2nm, which shows that the protein complex of HIF1A and quercetin is unstable (Figure 6A). The RMSD of the protein complex of CCL2 was 1nm at 0–75ns, and suddenly increased to 5nm at 75ns, which showed that the protein complex of CCL2 was unstable The RMSD of CXCL8 protein complex has been fluctuating at 0.75nm, which shows that the protein complex of CXCL8 is unstable The RMSD of the protein complex of IL1B increased rapidly to 0.75nm at 0–15ns, and then fluctuated at 0.75nm. This shows that the protein complex of IL1B is unstable The protein complex structure of PTGS2 is stable, and its RMSD has been fluctuating at 0.4nm. The RMSD of the IL1A protein complex was 0.75nm at 0–55ns, but after 55ns, the RMSD suddenly rose to 1nm. This showed that the IL1A protein complex was unstable According to the results of RMSF, the RMSF value of HIF1A protein structure is high in the range of 200–500 (0.2–0.4 nm), indicating that this domain has significant conformational flexibility (Supplementary Figure 2). The N-terminus (residues 1–20) and C-terminus (residues 70–80) of CXCL8 showed bimodal flexible characteristics, and the RMSF peak reached 0.3 nm. The multidomain protein of PTGS2 showed a "wavy" RMSF distribution, and the membrane-bound domain (residues 100–150) had the highest RMSF (0.4–0.5 nm). The structure of IL1B is generally stable (rmsf<0.3 nm). However, a significant peak (0.6 nm) appeared in the functional loop region (residues 40–55). IL1A and IL1B are structurally homologous but more flexible. The C-terminal nuclear localization sequence (residues 160–200) RMSF is persistently >0.4 nm. CCL2 is a typical chemokine fold, and the N-terminal (residues 1–10) RMSF is significantly higher than the core. The number of hydrogen bonds between quercetin and PTGS2 protein structural complex is 2–3, that between quercetin and CCL2 protein structural complex is 1–2, that between quercetin and CXCL8 protein structural complex is 1–2, that between quercetin and HIF-1A protein structural complex is 1–2, that between quercetin and IL-1A protein structural complex is 1–2, and that between quercetin and IL-1B protein structural complex is 1–2, The number of hydrogen bonds between quercetin and CCL2 protein structural complex is 1–2 (Figure 6B). According to the results of molecular dynamics simulation, we concluded that the complex of quercetin and PTGS2 could exist stably, and quercetin could play a regulatory role by binding to PTGS2 protein.

In vitro and in vivo Experiments

CGD and Quercetin Relieve Intestinal Inflammation in the Enteritis Model Mice

In this study, a drug treated mouse model was constructed to validate the potential of CGD and quercetin in treating inflammatory bowel disease (Figure 7A). We observed that the body weight of mice in the TNBS group was lower than that in the drug treatment groups and the normal control group (Figure 7B). The Disease Activity Index (DAI) demonstrated a significant decrease in the CGD and quercetin treatment groups compared to the TNBS group (Figure 7C). When comparing the intestinal tracts of mice from each group, the colon length of the TNBS group was significantly shorter than that of the drug treatment groups and the NC group ($p<0.05$) (Figure 7D–F). There is a significant statistical difference in intestinal length between the CGD M and CGD H groups and the TNBS group in mice ($p<0.05$). However, there was no statistically significant difference in intestinal length between CGD L group and TNBS group mice, which may be due to the lower dosage of the drug, which cannot effectively alleviate intestinal inflammation. This means that CGD treatment for inflammatory bowel disease is dose-dependent. The ELISA results of mouse intestines showed that the inflammatory indicators IL-6, IL-1β and TNF-α in the TNBS group were significantly higher than those in the NC group ($p<0.05$), and IL-10 was lower than that in NC group ($p<0.05$). The IL-6, IL-1β and TNF-α levels in the CGD M, CGD H and Quercetin groups were lower than those in the TNBS group ($p<0.05$), and the IL-10 levels in the CGD M, CGD H and Quercetin groups were higher than those in the TNBS group ($p<0.05$) (Figure 7E). There was no statistically significant difference in IL-6, IL-1β and TNF-α levels between the CGD L group and the TNBS group. However, the IL-10 level in the CGD L group was higher than that in the TNBS group, with a statistically significant difference ($p<0.05$). This shows that CGD and quercetin can reduce inflammatory

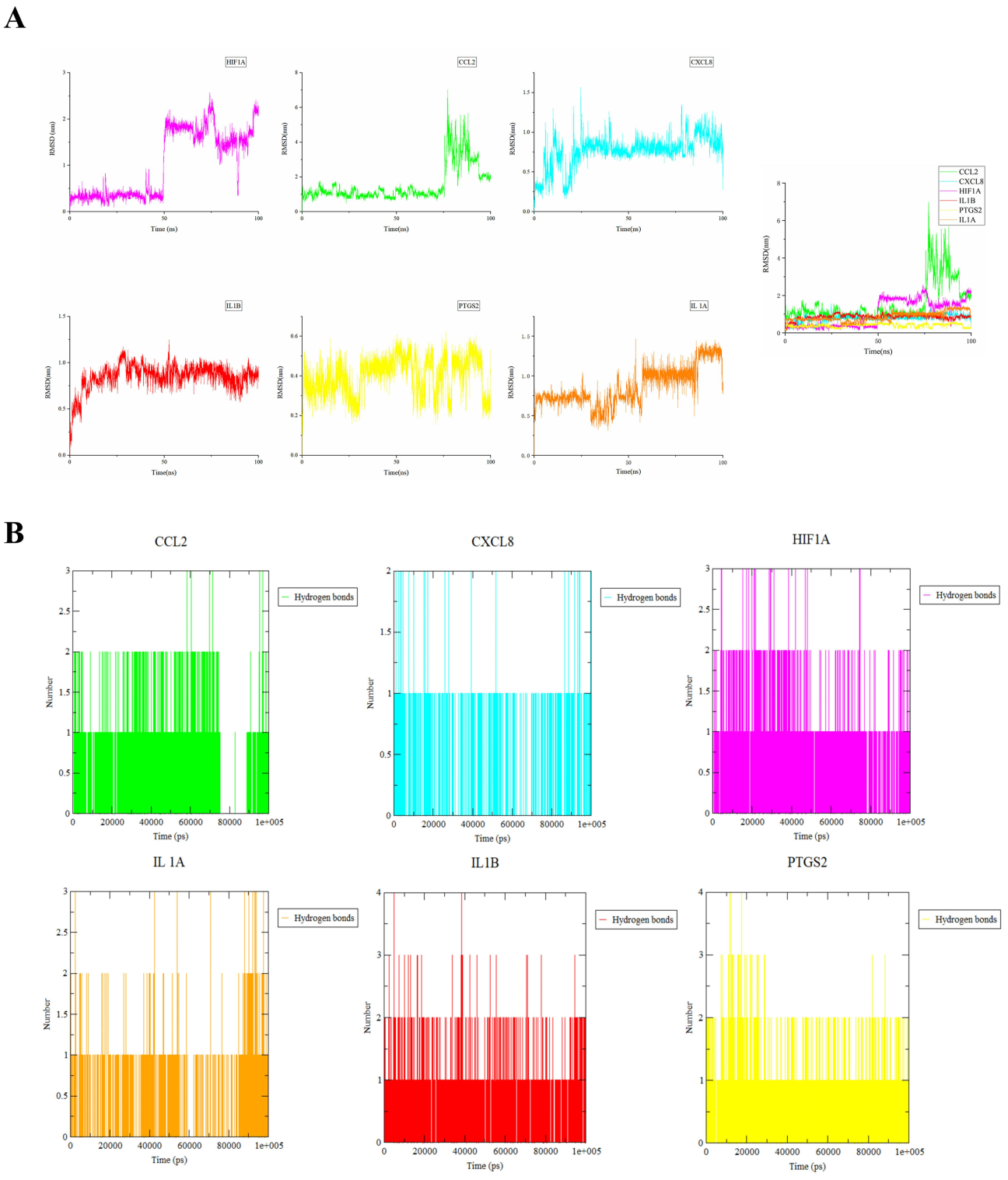


Figure 6 Molecular dynamic simulation (**A**) RMSD of six core pharmacological targets (HIF1A, CCL2, PTGS2, IL1A, IL1B, CXCL8) protein and quercetin complex. (**B**) Hydrogen bond between six core pharmacological targets (HIF1A, CCL2, PTGS2, IL1A, IL1B, CXCL8) and quercetin complex.

factors and alleviate intestinal inflammation in mice with inflammatory bowel disease. The HE results of mouse intestines showed that CGD and quercetin could significantly improve the inflammation of intestinal mucosa in mice with inflammatory bowel disease (Figure 7G). AB-PAS staining revealed a significant loss of goblet cells and thinning of

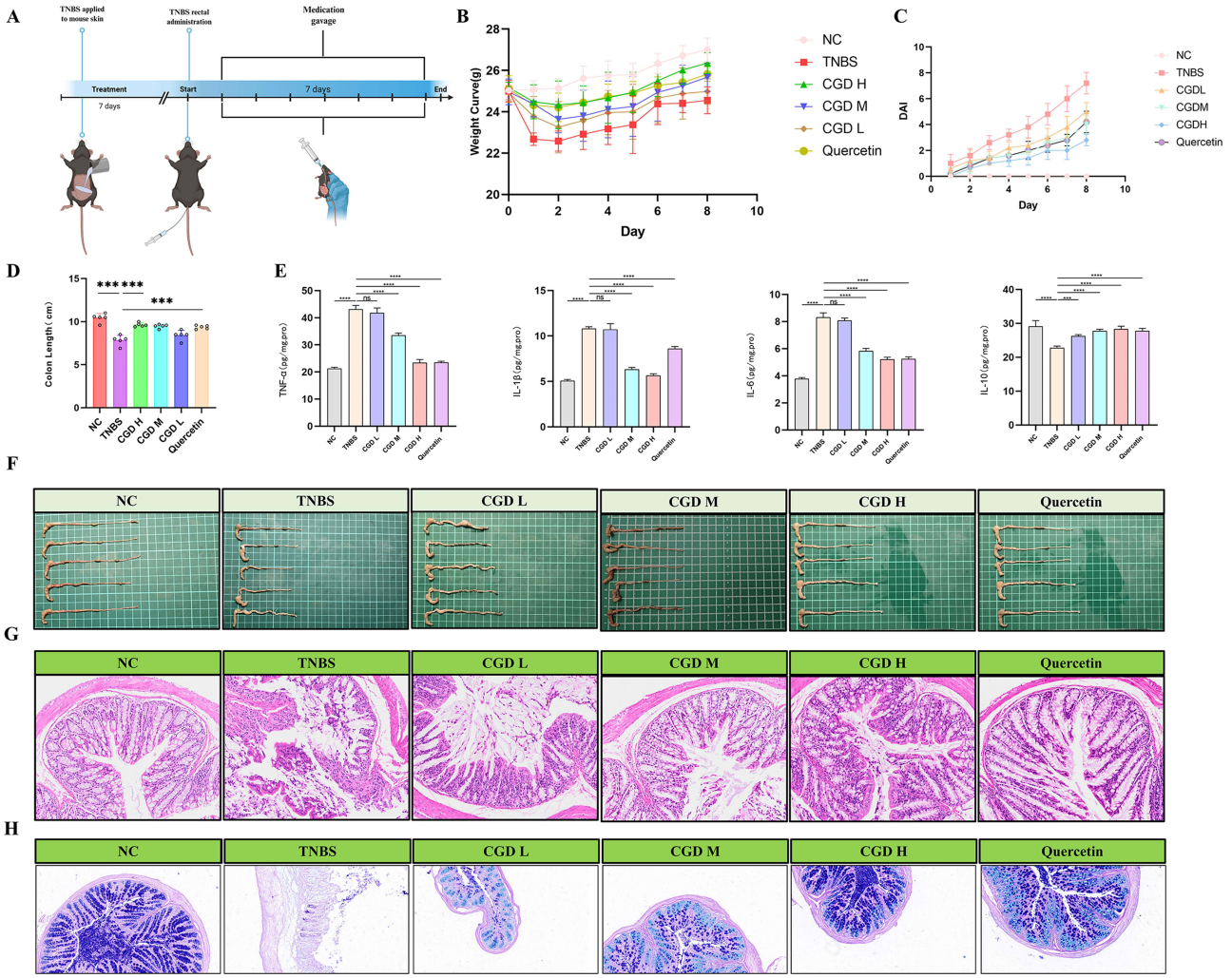


Figure 7 Animal experiments (**A**) Mouse modeling process diagram. (**B**)Mouse body weight. (**C**) Mouse Disease Activity Index (**D**) Mouse intestinal length. (**E**) Elisa results of mouse intestinal inflammation indicators. (**F**) Mouse intestinal. (**G**) Mouse intestinal HE results. (**H**) AB-PAS staining of mouse intestine ****p<0.0001; ***p<0.001; ns = not significant, p>0.05.

the mucus layer in the TNBS model group, both of which were effectively reversed after administration of CGD and quercetin (Figure 7H). In summary, we can conclude that CGD can improve intestinal inflammation in colitis model mice and has a dose-dependent effect. Quercetin is an important active ingredient in CGD for treating enteritis.

Quercetin Inhibits PTGS2 Protein Expression

The CCK8 results of quercetin on NCM460 cells showed that when the maximum concentration of quercetin was 10umol/L, it could play a pharmacological role without damaging normal intestinal cells (Figure 8A). We extracted the protein of NCM460 cells treated with 10 umol/L quercetin for 24 hours. Western blot results showed that quercetin could inhibit the expression of PTGS2 and IL-1B proteins in the inflammatory cell model ($p<0.05$) (Figure 8B and C). We also extracted proteins from mouse intestinal tissues. Western blot results showed that quercetin could also inhibit the protein expression of PTGS2 and IL-1B in mice with inflammatory bowel disease ($p<0.05$) (Figure 8B and C). In conclusion, quercetin can alleviate the inflammation of inflammatory bowel disease mice by acting on the protein expression of PTGS2 and IL-1B.

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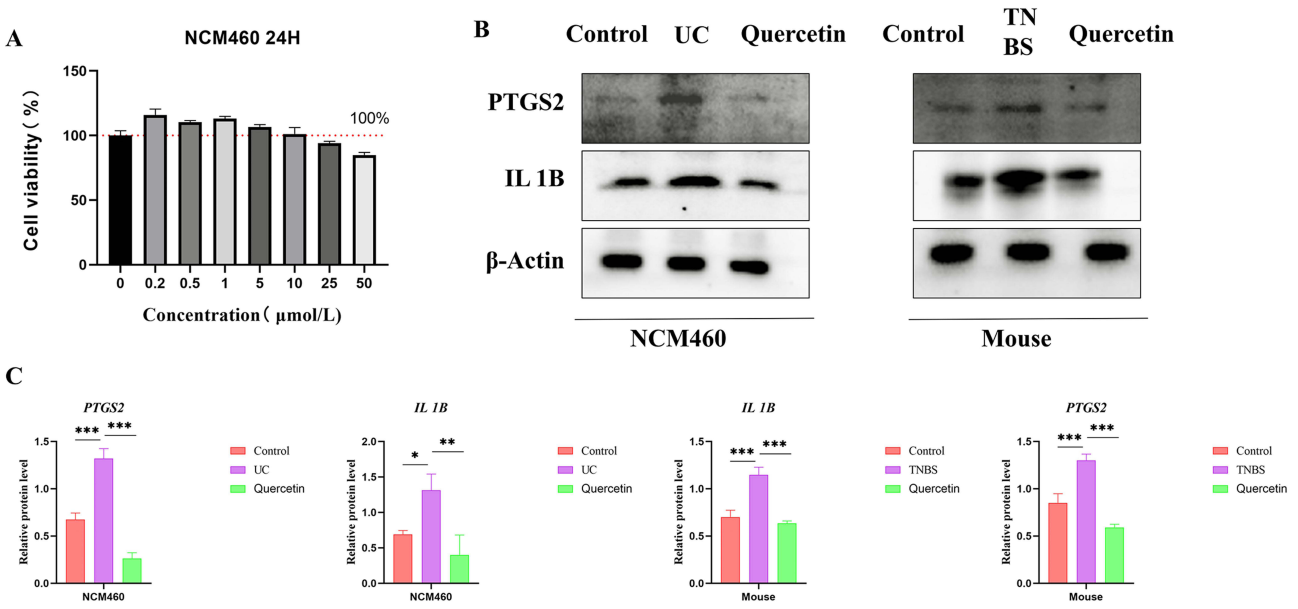

Figure 8 Regulation of gene expression by drugs (**A**) CCK8 assay of quercetin on NCM460 cells (**B**)Quercetin regulates the expression of PTGS2 and IL-1B proteins (**C**) WB result visualization ***p<0.001; **p<0.01; *p<0.05.

Discussion

CD is a chronic disease characterized by intestinal inflammation, fistula, abscess and other symptoms. The pathogenesis of CD is not yet clear, which may be caused by immune response disorder, intestinal flora change, genetic susceptibility and environmental factors.[17] The prevalence of CD is increasing year by year, and the patients are mainly young people.[18,19] However, there is no cure plan for CD at present, which makes CD patients suffer from long-term disease and seriously affects their quality of life. At present, CD patients mainly use aminosalicylic acid preparations, glucocorticoids, immunosuppressants, biological agents and other drugs. The therapeutic effect is limited, and biological agents are expensive.[10] This forces us to develop new drugs with better efficacy, cheaper price and fewer side effects as soon as possible. At present, Chinese medicine is widely used in the supplementary and alternative treatment of diseases, and has achieved obvious effects on respiratory, digestive, nervous, motor and other system diseases. However, traditional Chinese medicine has the characteristics of multi - component, multi - target, multi - pathway and so on. In order to screen out the target genes of traditional Chinese medicine for disease treatment and carry out precise treatment of diseases. Through network pharmacology, we screen the effective components of drugs and their target genes for precise treatment of diseases. In previous clinical studies, CGD has shown that it can promote the healing of colonic ulcer in CD patients, alleviate intestinal mucosal inflammation, and reduce the recurrence rate.[20]

In this study, we divided CD patients into NCD, LCD, PCD and UPCD according to different disease stages through GEO database. Then we discussed the common mechanism of CGD in treating NCD, LCD and PCD. The three groups of common genes were crossed with the pharmacological target of CGD, and 28 crossing genes were obtained. These 28 overlapping genes are regarded as common therapeutic targets of CGD in treating three groups of diseases. Through Cytoscape screening, six core genes (HIF1A, CCL2, PTGS2, IL1A, IL1B, CXCL8) were finally screened out, and quercetin can regulate these six core genes. Karhausen et al showed that the low expression of HIF-1 was related to the severe clinical symptoms of colitis. The increased expression of HIF-1 was associated with the remission of clinical symptoms.[21] Cosin-Roger et al found that the up-regulated expression of HIF-1A in CD patients can improve intestinal inflammatory symptoms by affecting autophagy or mTOR/NLRP3 pathway.[22] CCL2 and CXCL8 are chemokines. Chemokines are closely related to the occurrence and development of inflammation. They can recruit immune cells to the inflammatory site during the immune response and promote the development of inflammation. Ustekinumab can reduce the expression of CCL2, alleviate intestinal inflammation, and achieve the effect of treating CD.[23] IL-1 is a major mediator in the inflammatory environment and participates in the progression of various inflammatory diseases.[24,25]

Paola Menghini et al showed that the reduced expression of IL-1A can alleviate intestinal inflammation in Crohn's disease mouse model.[26] In CD, IL-1A can treat CD patients by influencing intestinal microorganisms and inducing the activation of JNK, p38-MAPK and NF-κB pathways.[27,28] Prostaglandin G/H synthase 2 (PTGS2 or COX2) is one of the key factors of cell response to inflammation. In IBD patients, NSAIDs may activate and aggravate the symptoms of patients, possibly because they reduce the production and activity of PTGS2. Quercetin is a natural polyphenol and a kind of flavonoid compound. It widely exists in vegetables, fruits and other plants, and has antioxidant, anti-inflammatory, anti-tumor and other effects.[29,30] These mechanisms suggest that these six core genes may be effective targets of CGD in treating CD.

The above therapeutic targets were verified by molecular docking. Quercetin can form hydrogen bond interactions with six core genes (HIF1A, CCL2, PTGS2, IL1A, IL1B and CXCL8). We compared the binding ability of quercetin with six core genes, and found that PTGS2 had the lowest binding energy (9.181 kcal/mol) and the strongest binding ability with quercetin. Then, we used molecular dynamics simulation to verify the structural stability and mobility of quercetin binding to six core gene proteins. We found that the RMSD of quercetin PTGS2 protein complex was small and fluctuated slightly. Their structures are relatively stable and small molecules have less mobility. At the same time, the results in vivo and in vitro showed that quercetin could reduce the protein expression of PTGS2 in NCM460 cells and the intestinal tract of inflammatory bowel disease mice ($p<0.05$). In conclusion, we can conclude that quercetin can reduce the expression of PTGS2 protein by binding to PTGS2 protein. However, PTGS2 has a dual effect in the treatment of intestinal inflammation. PTGS2 promotes intestinal mucosal healing in acute intestinal injury, but overexpression of PTGS2 during chronic inflammation can promote the progression of inflammation.[31] Inhibition of PTGS2 expression exacerbates inflammation related colon injury.[32] However, the mucosal repair of PTGS2 is mainly derived from the secretion of myeloid/endothelial cells, while the secretion of epithelial cells does not have this function.[33] Clinical studies have demonstrated the effectiveness and safety of COX-2 inhibitors in the treatment of IBD, indicating their potential for clinical application.[34] Etoricoxib is a novel anti-inflammatory drug with strong COX-2 selectivity. Clinical studies have shown that etoricoxib is effective and safe for most IBD patients, and does not exacerbate potential gastrointestinal and IBD related issues.[34] Meanwhile, various studies have shown that high expression of PTGS2 promotes the transformation of enteritis into tumors.[35] Therefore, targeting PTGS2 for the treatment of inflammatory bowel disease is not only safe and reliable, but also reduces the risk of tumor occurrence. Quercetin is a natural flavonol, which is widely used as food additive. Quercetin has long been considered to have antioxidant, anti-tumor, anti ulcer, anti diabetes, anti hypertension and anti depression properties, and has proved its therapeutic effect in inflammatory diseases such as asthma, arthritis, lung injury and diabetic vascular disease.[36–39] In animal experiments, quercetin has a protective effect on colitis induced by acetic acid, trinitrobenzene sulfonic acid (TNBS) or dextran sulfate sodium (DSS).[36,37,40] Quercetin can reduce the infiltration of macrophages, neutrophils, Th17 and other inflammatory cells, increase the proportion of Treg cells, and inhibit proinflammatory cytokines (such as IL-17, TNF- α And IL-6), and promote the secretion of IL-10 in colon tissue.[40,41] At the same time, quercetin itself has antioxidant activity and is an effective scavenger of reactive oxygen species (ROS).[42] It can significantly reduce the intestinal oxidative stress reaction, thereby reducing the symptoms of IBD and hindering the progression of the disease. In this study, we treated colitis model mice with drugs and found that CGD and quercetin can significantly reduce inflammatory factors (IL-6, IL-1β and TNF-α) in the intestines of colitis mice. After drug treatment, the intestinal shortening of colitis mice was improved. HE analysis of the intestine shows that CGD and quercetin can improve inflammation of the intestinal mucosa. However, low-dose CGD has limited improvement on enteritis, and there is no statistically significant improvement on intestinal inflammatory factors. However, moderate and high doses of CGD can significantly improve the inflammation in mice with enteritis. Quercetin is an important active ingredient in CGD. In this experiment, quercetin can significantly improve the inflammatory status of colitis mice. Thus, we conclude that quercetin may be an important effective ingredient in the treatment of inflammatory bowel disease with CGD.

However, this study only proved the feasibility of CGD in the treatment of inflammatory bowel disease in mice and cells. This is far from the clinical promotion and treatment of CD. We need to constantly improve relevant research, provide more evidence-based medicine, and prove the feasibility of CGD in the treatment of CD. This study relies on animal models to simulate human Crohn's disease (CD); however, these are essentially acute or semi-acute

chemically-induced models that cannot fully replicate the complex disease course and heterogeneity of human CD. Secondly, although quercetin is a promising natural bioactive compound, its pharmacokinetic properties pose major challenges for clinical translation. Its low absorption and high metabolic rate may result in prototype drug concentrations reaching the affected intestinal tract that are far lower than the effective concentrations observed in in vitro experiments, thereby potentially compromising its efficacy in vivo. Additionally, variations in the chemical composition of Chaihu Guizhi Decoction (CGD) may occur due to differences in the source of herbal materials and preparation processes.

Conclusions

This study verified the feasibility of CGD in the treatment of CD through network pharmacology, molecular dynamics simulation, in vivo and in vitro experiments. Quercetin in CGD can alleviate intestinal inflammatory injury by inhibiting the expression of PTGS2 and IL-1B protein. This provides us with a new scheme for the treatment of Crohn's disease.

Abbreviations

Crohn's disease, CD; inflammatory bowel disease, IBD; Chaihu Guizhi Decoction, CGD; early patients, NCD; late patients, LCD; patients with postoperative recurrence, PCD; patients without postoperative recurrence, UPCD; Gene Expression Omnibus, GEO; Tcm Systems Pharmacy Database and Analysis Platform,TCMSP; Bioavailability, OB; Drug-likeness, DL; Hematoxylin-eosin staining, HE.

Data Sharing Statement

The data are available to academic researchers upon request.

Ethics Approval and Consent to Participate

This study has been approved by Anhui Medical University Experimental Animal Ethics Committee (LLSC20242418). This study has been approved by The Clinical Research Ethics Committee of The First Affiliated Hospital of Anhui Medical University (LLSC20242418).

Consent to Publish

All authors gave their consent for publication.

Acknowledgments

All authors read and approved the manuscript.

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.

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Disclosure

The authors declare that no competing or conflicting interests exist for this work.

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