scientific reports

Check for updates

OPEN

Exploring the mechanism of Suxin Hugan Fang in treating ulcerative colitis based on network pharmacology

Qiang Huang[1,7], Weijie Peng[2,7], Qing Luo[2,7], Wenchang Zhao[3,4,5], Weibo Dai[2]✉, Huifen Zeng[5]✉, Hoi Leong Xavier Wong[6] & Xianjing Hu[3,4]✉

As a traditional Chinese medicine formula used in clinical practice for an extended period, Suxin-Hugan-Fang (SXHGF) exhibits excellent anti-inflammatory properties. However, the efficacy of SXHGF in treating ulcerative colitis (UC) and its mechanism of action are still unclear. In this study, the therapeutic effects of SXHGF on UC were evaluated using network pharmacology and experimental validation, while also investigating its mechanism of action. By administering DSS to C57BL/6 mice to construct a mouse model of ulcerative colitis, the therapeutic effect of SXHGF on ulcerative colitis was evaluated based on weight loss percentage, disease activity index, colon length changes, and pathological conditions as indicators. The main chemical components of SXHGF were determined by LC-MS-QTOF method. The potential targets and mechanisms of action of SXHGF in the treatment of UC were inferred using bioinformatics methods, and further validated through ELISA, IHC, and Western blotting assays. The experimental results demonstrate that SXHGF can suppress oxidative stress and oxidative damage in the colon tissue of UC mice, and alleviate DSS-induced ulcerative colitis by inhibiting the JAK2/STAT3 and NFκB pathways.

Keywords Ulcerative colitis, Chinese herbal prescription, Suxin-Hugan Fang, Inflammation, Oxidative injury

Abbreviations

BP	biological process
CC	cellular component
CD	Crohn's disease
DSS	dextran sodium sulfate
DAI	disease activity index
GO	gene ontology
HO1	heme oxygenase 1
IBD	inflammatory bowel disease
JAK2	Janus kinase 2
KEGG	kyoto encyclopedia of genes and genomes
Keap1	Kelch-like ECH-associated protein 1
MF	molecular function
MDA	malondialdehyde
MUC2	mucin 2
MyD88	myeloid differentiation factor 88

[1]Department of Pharmacy, Xiaolan People's Hospital of Zhongshan, Zhongshan 528415, Guangdong, PR China. [2]Pharmacology Laboratory, Zhongshan Hospital, Guangzhou University of Chinese Medicine, Zhongshan 528401, Guangdong, PR China. [3]Dongguan Key Laboratory of Chronic Inflammatory Diseases, The First Dongguan Affiliated Hospital, Guangdong Medical University, Dongguan 523121, Guangdong, PR China. [4]Guangdong Provincial Key Laboratory of Research and Development of Natural Drugs, School of Pharmacy, Guangdong Medical University, Dongguan 523808, Guangdong, PR China. [5]Clinical Trial Institution, Xiaolan Hospital, Southern Medical University, Zhongshan 528415, Guangdong, PR China. [6]Centre for Cancer and Inflammation Research, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong 999077, PR China. [7]Qiang Huang, Weijie Peng and Qing Luo contributed equally. ✉email: daiweibo007@163.com; 13420342005@163.com; thuxj@gdmu.edu.cn

Nrf2	nuclear factor erythroid 2–related factor 2
NQO1	NA(D)H quinone oxidoreductase 1
NF-κB	nuclear factor kappa-B
NLRP3	nucleotide-binding oligomerization domain (NOD)-like receptor pyrin domain containing 3
OGG1	8-oxoguanine DNA glycosylase-1
SOD2	superoxide dismutase 2
SXHGF	Suxin-Hugan-Fang
STAT3	signal transducer and activator of transcription 3
TCM	traditional Chinese medicine
TLR4	toll-like receptor 4
UC	ulcerative colitis
8-OHDG	8-hydroxy-2-deoxyguanosine

Ulcerative colitis (UC), a kind of inflammatory bowel disease (IBD) that is caused by chronic inflammation of the digestive tract, is characterized by diarrhea, bleeding in the colon, and body weight loss[1]. The incidence of UC in Western countries is 12 ~ 26 per 100,000, while rapidly increasing in the newly industrialized countries of Asia and Latin America, and is likely to continue to rise as urbanization increases in the coastal cities[2]. Although the prevalence of UC has been leveling off in developed countries, the number of cases is still huge[3]. UC is reported to seriously affect the quality of a patient's life, and cause a series of complications, such as colorectal cancer, which would greatly shorten the patients' life span[4]. Hence, it is of great significance to develop new drugs to treat or prevent UC.

Oxidative stress is reported to be closely related to the pathogenesis of UC[5]. The accumulation of ROS and MDA may cause damage to the genes involved in cell growth or differentiation, leading to cell dysfunction[6]. Oxidative stress promotes the separation of nuclear factor erythroid 2-related factor 2 (Nrf2) and Keap1 complex, and reduces the expression of Nrf2, thereby down-regulating its downstream target genes related to anti-oxidant and -inflammation[7]. Long-term oxidative stress would lead to DNA damage by increasing the levels of 8-OHDG, and 8-oxoguanine DNA Glycosylase (OGG1) during the pathogenesis of UC[8].

Amino salicylic acids, sterols, immunological agents, and biological antibodies are the drugs that are commonly used for UC therapy in the clinic[9–11]. However, the side effects and incomplete treatment of these drugs are limiting their application[12–14]. Hence, other new drugs or alternative remedies are still largely in demand. Traditional ethnic medicine has been used for thousands of years to treat or prevent intestinal diseases, and it is a good resource to find new remedies for UC therapy. Chinese herbal medicine has shown good efficacy and few side effects in the prevention and treatment of UC, and is a good chance for developing new drugs to treat UC[15,16].

Network pharmacology, combining bioinformatics and pharmacology to construct complex regulatory networks and predict the possible pharmacological targets and signal pathways, is usually used to study traditional Chinese medicine (TCM), which harbors the characteristics of multi-components, -mechanisms, and -targets of action and is too complicated to uncover its mechanism using classical methodology[17]. Hence, it is of great significance to apply network pharmacology to study the complex pharmacological mechanism of TCM[18].

Suxin-Hugan-Fang (SXHGF) is a clinical prescription that has been used for more than 10 years. It consists of 10 kinds of herbs, including *Paeonia tacti lora Pall.* (Bai Shao, BS), *Plantago asiatica* L. (Che Qian Cao, CQC), *Lophatherum gracile* Brongn. (Dan Zhu Ye, DZY), *Glycyrrhiza uralensis* Fisch. (Gan Cao, GC), *Chrysanthemum morifolium* Ramat. (Ju Hua, JH), *Hypericum japonicum* Thunb. (Di Er Cao, DEC), *Prunella vulgaris* L. (Xia Ku Cao, XKC), *Artemisia capillaris* Thunb. (Yin Chen, YC), *Curcuma rcenyujin* Y, H. *Chenet* C. Ling (Yu Jin, YJ), *Jasminum grandiflorum*L. (Su Xin Hua, SXH). The therapeutic effects of BS, GC, XKC, and JH on UC have been scientifically validated[19]. At the same time, our previous studies have demonstrated that SXHGF effectively suppresses the secretion of proinflammatory cytokines IL-6 and TNF-α, while concurrently attenuating the expression of NFκB, a key mediator in the classical inflammatory pathway. Therefore, we hypothesize that SXHGF may exhibit promising anti UC effects; however, to date, no studies have investigated the therapeutic potential of SXHGF in UC treatment.

This study is designed to investigate the therapeutic function of SXHGF in treating colitis in mice and uncover its potential mechanism via integrating network pharmacology and experimental validation. In this study, a colitis mice model induced by DSS was used to evaluate the efficacy of SXHGF against UC. The bioinformatics methodology, including searching the oral bioavailability, drug-like, UniProt, GO, and KEGG database were used to speculate the underlying mechanism, which was further verified via ELISA, H&E, IHC, and Western Blot assays. The whole design of this study is shown in Fig. 1.

Materials and methods

Drugs and reagents

Dextran sulfate sodium (DSS, MW: 36000 ~ 50000 Da, S5036) was purchased from MP Biomedicals Inc. (California, USA) and Sulfasalazine (SASP, 33816) was obtained from MedChemExpress Co., Ltd (Shanghai, China). ELISA kits for IL-1β (370210818), IL-6 (385210818), TNF-α (569210610), LPS (261210607), MOP (411210519), MDA (417211010), ROS (653211010), IL-17 (374211202), 8-OHDG (108220104) were purchased from Tianjin Anoric Bio-technology CO., Ltd (Tianjin, China). Primary antibodies against ZO1 (AF5145), Occludin (DF7504), Claudin1 (AF0127), MUC2 (DF8390), MyD88 (AF5195), JAK2 (AF6022), HO1 (AF5393), NQO1 (DF6437), OGG1 (DF6401), SOD2 (AF5144), STAT3 (AF6294) were purchased from Affinity Biosciences LTD (OH, USA). The primary antibody of TLR4 (293072) was obtained from Santa Cruz (California, USA), Keap1 (D199574) was from Sangon Biotech Co., Ltd. (Shanghai, China) and Nrf2 (A0674), NLRP3 (A5652)

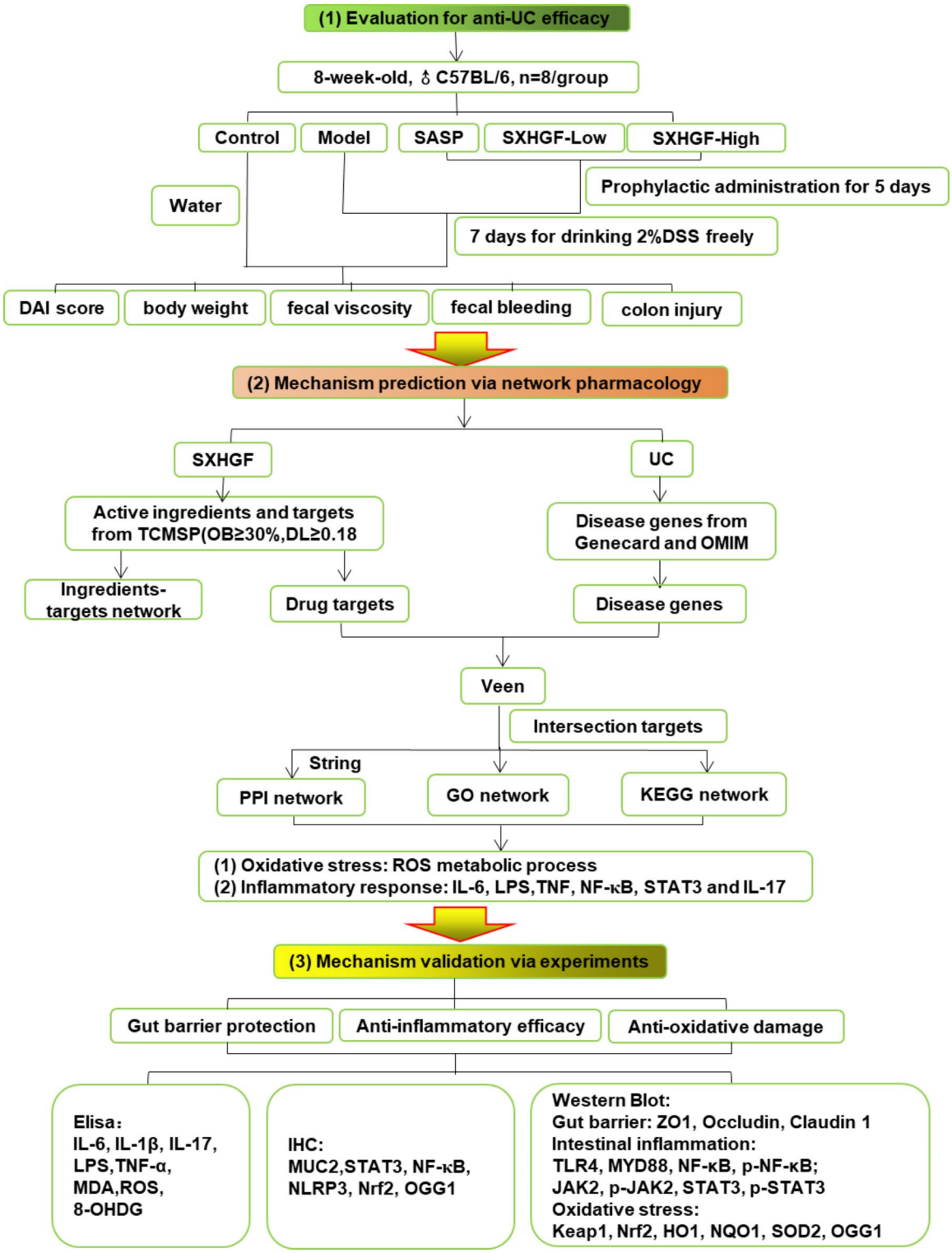

Fig. 1. Flow chart for the study of SXHGF against UC.

were from Abclonal Biotechnology Co., Ltd. (Wuhan, China). Primary antibodies of NF-κB (8242 S), p-NF-κB (3033T), p-JAK2 (3771 S), and p-STAT3 (9145 S) were obtained from Cell Signaling Technology, Inc. (Boston, USA). All materials of SXHGF were obtained from Sinopharm Group Feng Liao Xing Medicinal Material & Slices Co., Ltd (Foshan, China).

Preparation of SXHGF

The formula of SXHGF is including BS (25 g), CQC (15 g), DZY (15 g), GC (8 g), JH (15 g), DEC (5 g), XKC (15 g), YC (25 g), YJ (25 g), SXH (10 g). Briefly, the materials of SXHGF were soaked for 1 h and boiled twice with 10 times of distilled water (V/W) for 1 h each time. The decoction was then concentrated under a reduced pressure and frozen dried to obtain the powder. The powder was stored at -20℃ and redissolved with distilled water to prepare different concentrations of crude drugs before use. The percentage yield of SXHGF (extract/crude material) was 10.2%.

Network pharmacology analysis

Screening for the active components and targets of SXHGF

The active ingredients in SXHGF were searched out in the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP, https://www.tcmsp-e.com/) and screened based on the conditions of oral bioavailability (OB) ≥ 30% and drug-like (DL) ≥ 0.18. Some components that could not be searched out in TCMSP were obtained by referring to relevant literature. The potential targets of the active components were retrieved in the TCMSP and transformed into gene symbols of Homo sapiens species through the UniProt database (https://www.uniprot.org/).

Venn mapping for drug and disease targets

Searching with "ulcerative colitis" in the databases of GeneCards (https://www.genecards.org/) and Online Mendelian Inheritance in Man (OMIM, https://omim.org/) to obtain UC-associated targets. Merge the targets from the two databases and remove the repetitive targets. Subsequently, importing the SXHGF targets and UC targets into VENNY2.1 (https://bioinfogp.cnb.csic.es/tools/venny/index.html) to generate a Venn graph.

Construction of protein-protein-interaction (PPI) network

The intersected targets of SXHGF and UC were input into String (https://cn.string-db.org/) for retrieval and screened with the minimum interaction requirement score ≥ 0.4. The TSV data file was exported to make the PPI network using Cytoscape3.7.1. Each node represents a protein, and the higher the degree values, the bigger the shape is and the darker the color is.

Enrichment analysis of GO and KEGG pathway

Gene Ontology (GO) enrichment, including biological process (BP), cellular component (CC) and molecular function (MF), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were implemented by Bioconductor (R). GO term and KEGG pathway with *p*values < 0.05 were statistically significant[20].

Compounds analyzed by UHPLC-QTOF-MS

The chemical constituents of SXHGF were determined by LC-MS-QTOF (USA, Agilent 1290–6545). The chromatographic conditions of Ultra Performance Liquid Chromatography (UPLC) were as follows: chromatographic column (Zorbax Eclipse Plus C18, 3.0 × 150 mm, 1.8 μm); flow rate, 0.5 mL/min; column temperature, 30 ℃; sample concentration, 98.3 mg/mL (solvent: 20% methanol in water) of SXHGF; mobile phase, acetonitrile/water (0.1% FA); injection volume, 0.5 μL; gradient conditions, 5% acetonitrile (0 ~ 2 min) → 5% ~ 35% acetonitrile (2 ~ 20 min) → 35% ~ 50% acetonitrile (20 ~ 25 min) → 40% ~ 95% acetonitrile (24 ~ 30 min) and 95% acetonitrile (30 ~ 35 min). MS parameters were as follows: 300 ℃ of the drying temperature; 8 L/min of dry gas flow rate; 350 ℃ of sheath temperature; 11 L/min of sheath gas flow rate; 45 psi of atomizing gas pressure; 130 V of fragmentation voltage; 4000 V (+) / 3500 V (-) of capillary voltage; 0(+)/1000(-) of nozzle voltage; positive or negative auto MS/MS; 100 ~ 1000 of MS scan; 8 spectrum/s of scan rate; 50 ~ 1000 of MS/MS scan; 5 spectrum/s of scan rate with 3 precursor ion/cycle, threshold, 1000 counts, 0.01%; 10/20/40V of CE; narrow (1.3 DA) of quadrupole resolution; Agilent TCM database.

Animal treatment

The animal study was approved by the Animal Ethics and Welfare Committee of Zhongshan Hospital of Traditional Chinese Medicine (AEWC-2023004) and carried out in strict accordance with the guidelines for the management and use of laboratory animals. Tis study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org). Eight-week-old male C57BL/6 mice were purchased from Guangdong Medical Laboratory Animal Center (Foshan, China), and housed in a Specific Pathogen Free (SPF) room with a temperature of 22 ± 2℃, humidity of 70 ± 5% and accessed to food and water ad libitum. After a week of adaptive feeding, the mice were divided into normal, model (2% DSS), positive control (Sulfasalazine, SASP, 200 mg/kg, p.o.), SXHGF low-dose (SXHGF-L, 10.25 g/kg, p.o.) and high-dose (SXHGF-H, 20.50 g/kg, p.o.) groups with 8 mice for each group. The doses of SXHGF used in this study were converted from the human dose based on the body surface area and the dose of 10.25 g/kg equivalent to the human clinical dose.

From day 1 to 14, the mice of SASP- and SXHGF-treated groups received 5 days of prophylactic medication before the UC model was established and continued treatment with drugs for 9 days, while the normal and model groups were given distilled water. From day 5 to 12, mice except the normal control group were administered with 2% DSS for 7 days to induce the UC model and changed with distilled water from day 13 to 14. The general behaviors of mice were monitored every day and the parameters of body weight, rectal bleeding, fecal viscosity as well and DAI scores were measured regularly. After that, the mice were anesthetized with CO2, and blood samples were collected. The serum was isolated by centrifugation at 3000 rpm/min for 10 min and then stored at -80℃ before use. Mice were dissected to remove the colon tissue, part of which was immersed in 4% paraformaldehyde for H&E and IHC analysis, and the remaining was stored at -80℃ for biochemical analysis.

Disease activity index
The disease activity index (DAI) was assessed according to changes in body weight, fecal looseness, and fecal bleeding(Chi et al., 2021). The DAI scoring system is described in Table 1.

Histopathological staining
The dissected colon was fixed in 4% paraformaldehyde for 24 h, and then dehydrated, embedded in paraffin, and sliced (4 μm). The sections were stained with hematoxylin and eosin (H&E) and then taken pictures under an inverted microscope (Nikon ECLIPSE Ti2, 200×).

Immunohistochemistry analysis
For immunohistochemical (IHC) staining, the paraffin-embedded samples were sliced (4 μm) and sealed with 3% H_2O_2 at room temperature to inactivate the enzyme, and then boiled for 10 min in the sodium citrate buffer (10 mM, pH 6.0) followed by cooling at room temperature. The sections were blocked with normal goat serum and incubated overnight with primary antibodies (1:200) of MUC2, NF-κB, STAT3, Nrf2, OGG1, NLRP3, and corresponding secondary antibodies for 1 h at 4℃. The nuclei of slices were stained with DAPI and the expressions of target protein in the colon tissues were observed and photographed under an inverted microscope (Nikon ECLIPSE Ti2, 200×).

Western blot
Colon tissues were weighed and lysed with 10 times (V/W) of lysis buffer for 30 min on ice. The protein samples were collected after centrifugation at 15,000 rpm for 10 min, and the protein concentrations were determined by a bicinchoninic acid (BCA) kit (23227, Thermo Scientific, Massachusetts, USA) before denatured at 95℃ for 10 min. Protein samples were loaded into 8% ~ 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for running electrophoresis and then transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% non-fat milk for 2 h at room temperature, and incubated with corresponding primary antibodies of ZO1, Occludin, Claudin1, TLR4, NLRP3, NF-κB, p-NF-κB, MyD88, JAK2, STAT3, p-JAK2, p-STAT3, Nrf2, Keap1, HO1, SOD2, OGG1, NQO1 and beta-actin, for 18 h in 4 ℃, washed for 3 times and then incubated with corresponding secondary antibodies for 2 h at room temperature. An enhanced chemiluminescence (ECL) assay kit was used for developing the bands, and the gray values of target proteins were analyzed by Image J software.

ELISA assay
Colon samples were weighed, homogenized at 4℃ with 10 times of pre-cooled PBS containing phosphatase inhibitors and protease inhibitors, and then centrifuged at 15,000 rpm for 10 min to collect the supernatant. The levels of IL-6, IL-1β, IL-17, TNF-α, LPS, ROS, 8-OHDG, and MDA were determined by the commercially available kits following the manufacturer's instructions.

Statistical analysis
All data were expressed as mean ± SEM and statistical differences between the two groups were compared by Students' *t-test*, and $p < 0.05$ was considered to be statistically significant.

Results
Alleviation of SXHGF on DSS-induced UC of mice
UC is typically characterized by body weight loss, diarrhea, colonic injury, and bleeding. In this study, the body weight of model group strongly decreased from day 9 to 14, while those of the SXHGF-low dosage and -high dosage groups of mice significantly reversed, indicating the good alleviation of SXHGF against body weight loss of UC mice (Fig. 2A). The result of DAI scores also showed that DSS greatly increased the DAI score of UC mice (comparing with the control group, $p < 0.01$), while SXHGF significantly reduced it (comparing with the model group, $p < 0.01$) (Fig. 2B). Colon length reduction is also an important feature of UC mice. As shown in Fig. 2C - D, the colon length of DSS-induced UC mice was significantly reduced, while after SXHGF treatment, the colon lengths were remarkably increased (compared with the model group, $p < 0.01$). H&E staining was performed to systematically evaluate colonic injury. In the Control group, the colon tissue exhibited clear structure, intact mucosa epithelium, and no evident ulcer or inflammatory reaction. Mice in the Model group displayed severe pathological damage in colonic tissue, such as surface epithelial erosion, inflammatory cell infiltration, mucosal ulceration, crypt loss, and goblet cell reduction. These pathological lesions were significantly alleviated after SXHGF administration (Fig. 2E). Altogether, the results showed that SXHGF had a good alleviation against UC induced by DSS in mice. However, the detailed mechanism remained unclear.

Boday weight loss (%)	Fecal looseness	Fecal bleeding	Score
0	Normal	Normal	0
0 ~ 5%	Loose partial dry	Recessive bleeding	1
5%~10%	Loose partial wet		2
10%~15%	Diarrhea partial dry	bleeding	3
>15%	Diarrhea partial wet		4

Table 1. DAI scoring rules.

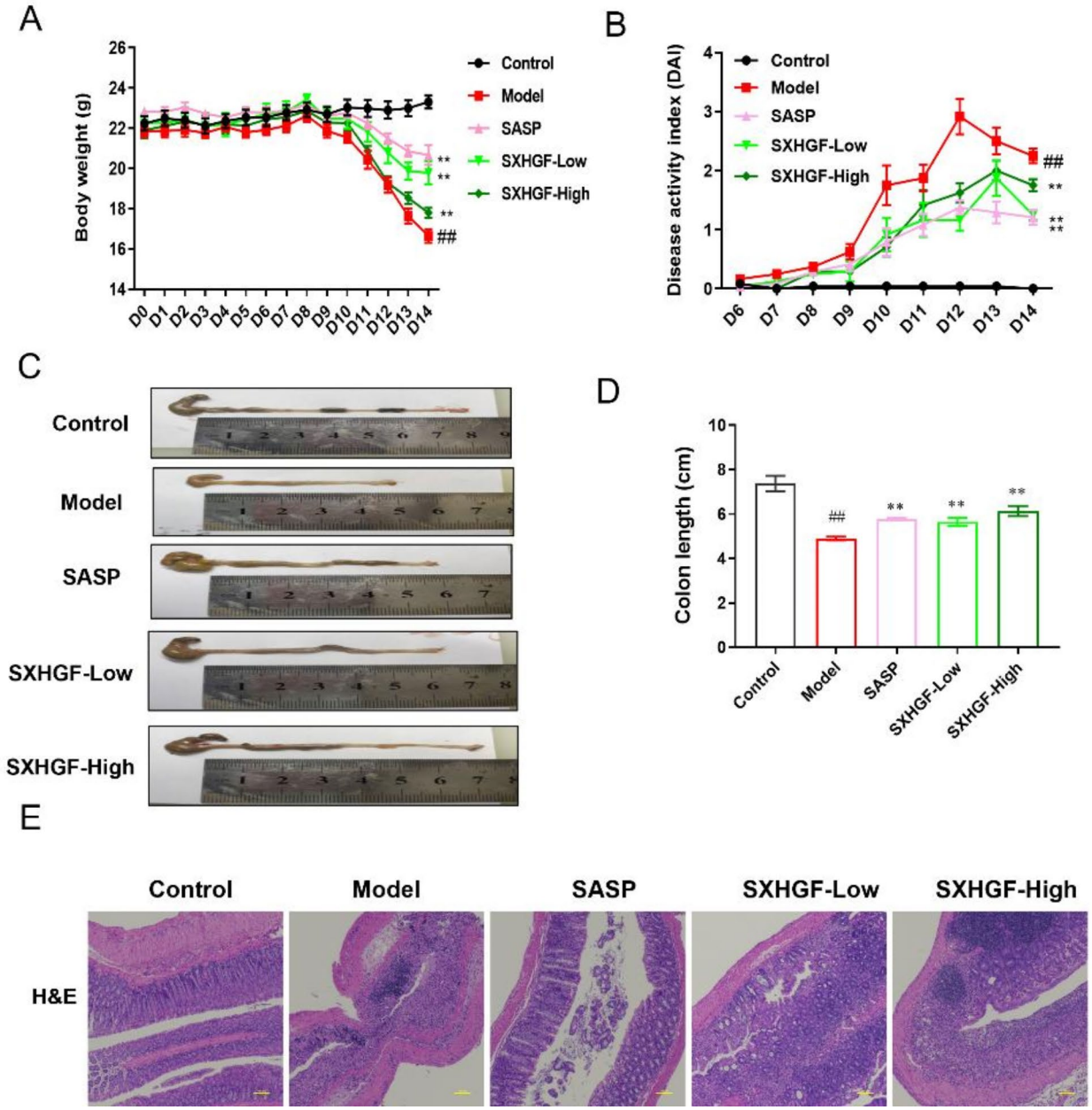

Fig. 2. SXHGF alleviated DSS-induced ulcerative colitis in mice. Effect of SXHGF on the body weight (A), DAI (B), and colon length (C) of colitis mice induced by DSS. (D) Representative images of colons of mice. (E) Histological analysis for colon tissues by H&E staining. Data are expressed as the mean ± SEM. Vs. the control group, $^{\#\#}P<0.01$; vs. the model group, $^{**}P<0.01$.

Mechanism prediction based on network pharmacology

Ingredients – targets network of SXHGF

The potential active ingredients of the 10 herbs in SXHGF were screened with the conditions of OB ≥ 30% and DL ≥ 0.18 through the TCMSP database. As a result, 192 active compounds in all were collected from the 10 kinds of herbal medicine of SXHGF, and 160 compounds were confirmed after consolidating the duplicated compounds. The corresponding disease targets of each compound were searched in the TCMSP database, and 239 targets were obtained after merging and removing the duplicates. As shown in Fig. 3A, the yellow diamonds in the middle represent the disease targets, the circles represent active compounds, and different colors represent different drugs.

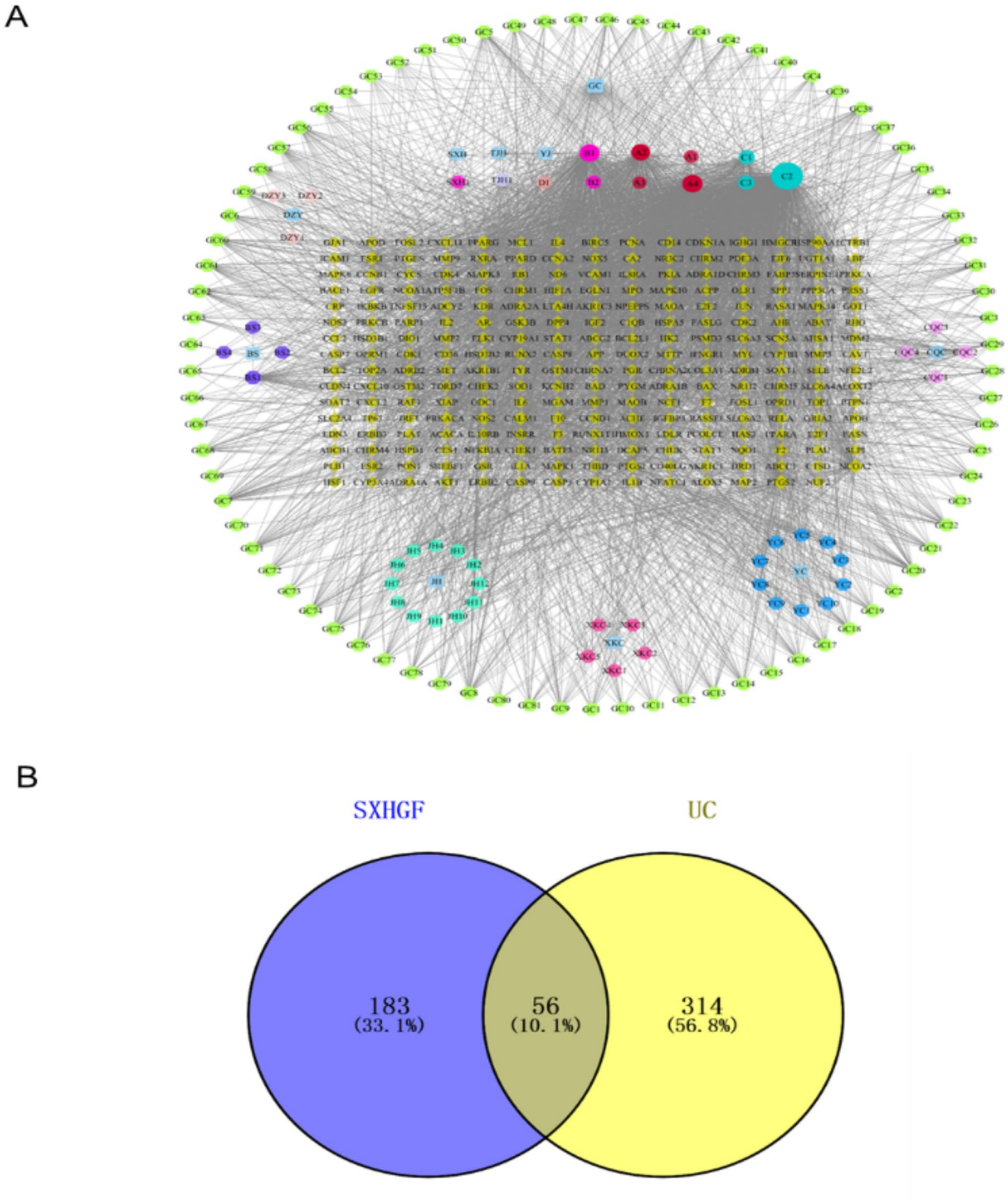

Fig. 3. Drug-component-disease-target network and Venn diagram. (A) Diagram of SXHGF ingredients-disease targets network. (B) The Venn diagram of the intersection of SXHGF and UC targets.

Veen diagram for the drug targets and disease targets

Through inputting "ulcerative colitis" into the Gene Card, 4825 disease genes were retrieved, with the highest score of 60.8385 and the lowest score of 0.1870. The average value was 2.4277, and 1215 genes were left after removing the genes with a score less than 2.4277, among which the average value of score was 6.4006[5], and 370 genes were left after removing the genes with a score less than 6.4006. At the same time, ulcerative colitis was searched in OMIM Genemap, and the genes were combined with the above genes after removing the

duplications, resulting in a total of 370 disease-related genes. Disease and drug-related genes were input into VEEN2.0 to obtain the Veen diagram for gene intersection, with a total of 56 genes in common (Fig. 3B).

PPI network construction

The 56 overlapping genes were input into String and a PPI core network diagram was made with Cytoscape3.7.1 (Fig. 4A). The thicknesses and colors of lines are representing the degree of interaction between different genes.

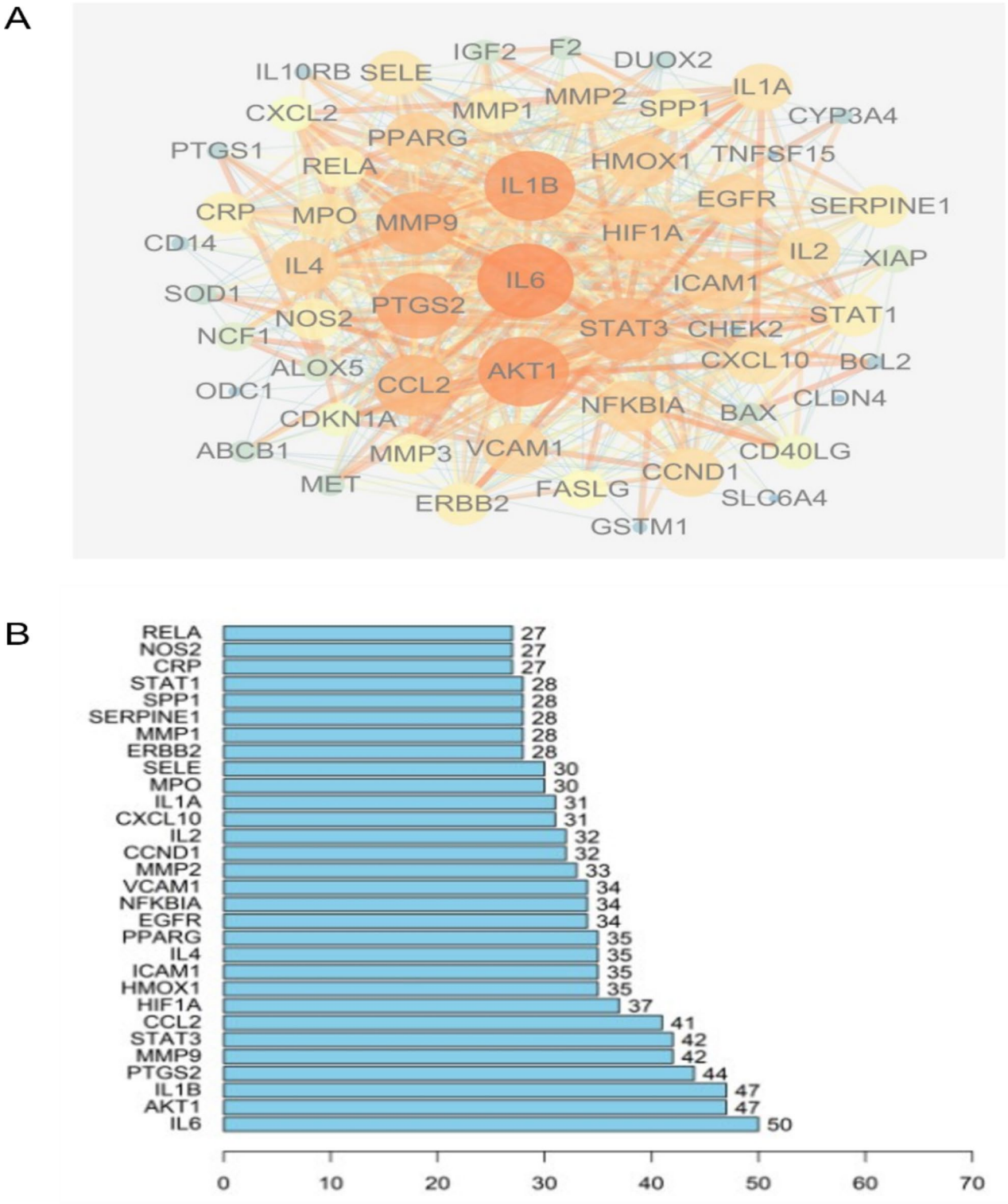

Fig. 4. PPI network construction. (A) Diagram of PPI network. (B) Bar plot of key nodes from the PPI network.

The larger and darker the lines are, the intensity of interaction between two targets is. According to the PPI bar chart shown in Fig. 4B, interleukin-6 (IL-6), AKT serine/threonine kinase 1 (AKT1), interleukin-1 beta (IL-1β), prostaglandin-endoperoxide synthase 2 (PTGS2), matrix metalloprotein (MMP9), signal transducer and activator of transcription 3 (STAT3), chemokine (C-C motif) ligand (CCL2), hypoxia-inducible factor 1 (HIF1A), heme oxygenase 1 (HMOX1) and intercellular adhesion molecule 1 (ICAM1) were the top ten targets with response values, which would probably be the potential target genes or critical target protein for SXHGF in treating UC.

GO and KEGG enrichment analysis

Based on the GO enrichment analysis, 1352 BP-related items in all were obtained and the top 20 were listed in Fig. 5A, including reactive oxygen species metabolic process, response to lipopolysaccharide, response to molecule of bacterial origin, response to oxidative stress, response to nutrient levels, response to reactive oxygen species, regulation of reactive oxygen species metabolic process, regulation of inflammatory response, response to nutrient, negative regulation of apoptotic signaling pathway, cellular response to oxidative stress, cellular response to lipopolysaccharide, cellular response to molecule of bacterial origin, cellular response to reactive oxygen species, response to the metal ion, cellular response to biotic stimulus, positive regulation of cytokine production, T cell activation, regulation of apoptotic signaling pathway and response to antibiotic. Fifteen items related to obtaining CC were collected and analyzed, including basal plasma membrane, membrane raft, membrane microdomain, membrane region, basal part of cell, caveola, external side of plasma membrane, plasma membrane raft, vesicle lumen, apical part of cell, NADPH oxidase complex, pore complex, apical plasma membrane, cytoplasmic vesicle lumen, basolateral plasma membrane (Fig. 5B). Sixty MF related items were collected and the top 20 were selected for analysis, including cytokine activity, cytokine receptor binding, receptor ligand activity, protein phosphatase binding, phosphatase binding, heme binding, tetrapyrrole binding, chemokine receptor binding, tumor necrosis factor receptor binding, integrin binding, ubiquitin-like protein ligase binding, antioxidant activity, tumor necrosis factor receptor superfamily binding, peroxidase activity, oxidoreductase activity, acting on peroxide as acceptor, ubiquitin protein ligase binding, growth factor receptor binding, repressing transcription factor binding, protein phosphatase 2 A binding, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen (Fig. 5C).

KEGG analysis showed that 123 pathways were associated with the efficiency of SXHGF treating UC, and the top 20 were selected to plot a bar chart, which included Lipid and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, TNF signaling pathway, Fluid shear stress and atherosclerosis, IL-17 signaling pathway, Measles, HIF-1 signaling pathway, NF-kappa B signaling pathway, Toxoplasmosis, Human cytomegalovirus infection, Kaposi sarcoma-associated herpesvirus infection, Hepatitis C, Small cell lung cancer, Prostate cancer, Influenza A, Malaria, Chagas disease, Pancreatic cancer, Leishmaniasis, Coronavirus disease - COVID-19 (Fig. 5D).

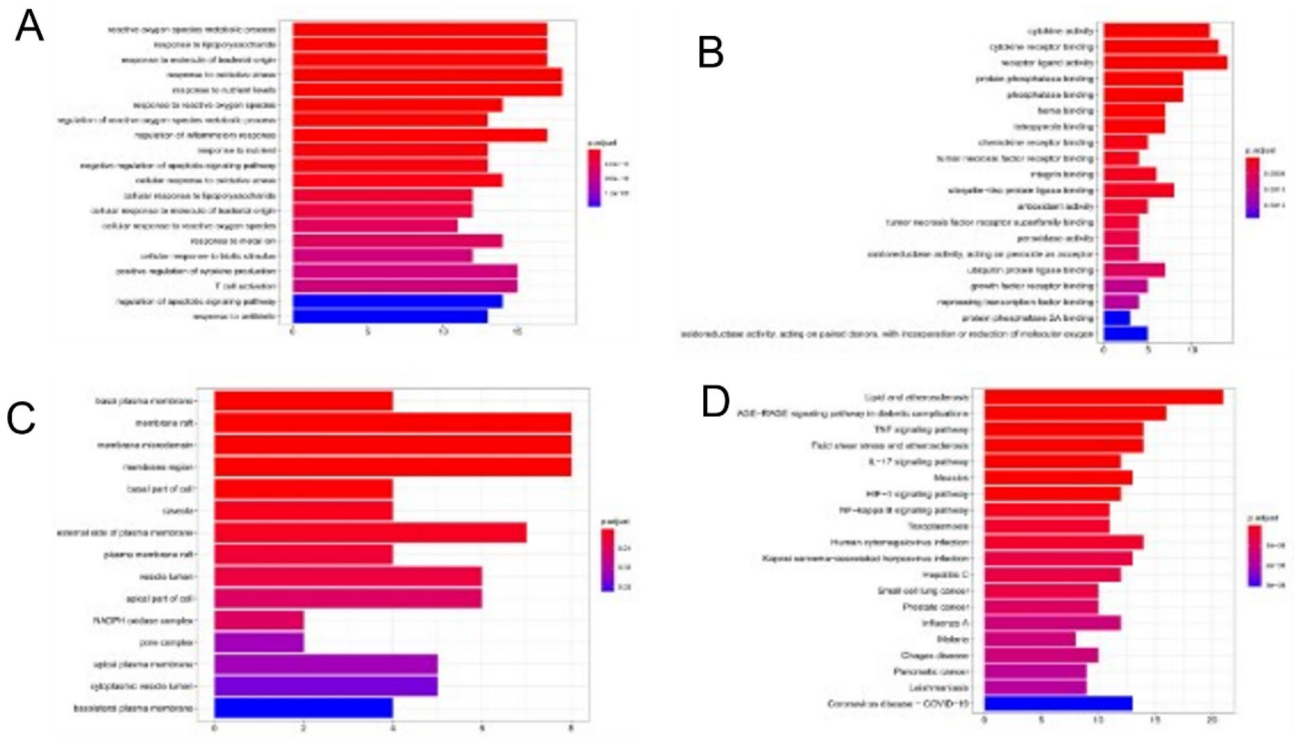

Fig. 5. GO and KEGG enrichment analysis. Bar plot of GO enrichment analysis of SXHGF in UC treatment: (A) Biological process, (B) Cellular Component, (C) Molecular Function. (D) Bar chart of KEGG enrichment analysis of SXHGF in UC treatment.

Altogether, GO and KEGG results showed that the effect of SXHGF in treating UC probably was mainly related to the regulations of the anti-inflammatory response and anti-oxidative stress signaling pathways.

Mechanism validation

Protection of SXHGF on the intestinal barrier of UC mice

Intestinal barrier dysfunction, such as intestinal permeability and a series of tight junction proteins lost, is a common clinical symptom of UC[21]. Hence, it is important to detect the expressions of intestinal barrier proteins for evaluating the therapeutic function of UC. In this study, the expressions of colonic barrier protein in UC mice, including ZO1, Occludin, and Claudin 1, were evaluated by Western Blot. As a result, the expressions of ZO1, Occludin, and Claudin 1 were decreased in the model group (compared with the control group, $p < 0.05$), while those of the SXHGF-treated groups were significantly increased (compared with the model group, $p < 0.05$) (Fig. 6A-B). The level of MUC2, another important protein harboring the functions of maintaining the intestinal flora, regulating the host innate immunity, and promoting intestinal homeostasis[22], was also evaluated via IHC staining, and the result indicated that SXHGF strongly inhibited the reduction of MUC2 expression induced by DSS (Fig. 6C), suggesting the well-protected effect of SXHGF on colon from injury. These data concluded that SXHGF alleviated the UC of mice by protecting intestinal barrier function.

Inhibition of SXHGF on the inflammatory response in UC mice

Sustained inflammatory response is the critical pathological mechanism of UC, and reducing NLRP3 inflammasome can diminish the acute inflammation as well as the tissue injury[23]. In this study, we evaluated the effect of SXHGF on the levels of pro-inflammatory cytokines and the expression of NLRP3 in the colons of UC mice. As a result, DSS significantly increased the levels of IL-1β, TNF-α, and IL-17, while SXHGF strongly reversed the effect, suggesting that SXHGF could inhibit the inflammatory response in UC mice via suppressing the signaling pathways associated with IL-1β (Fig. 7A), TNF-α (Fig. 7B), and IL-17 (Fig. 7C), which was consistent with the speculation of the network pharmacology. Additionally, the IHC staining assay showed that SXHGF remarkably reduced the high level of NLRP3 in the colon, suggesting the good, suppressed effect of SXHGF against inflammatory injury of UC mice induced by DSS (Fig. 7D). These results suggested that SXHGF had the potency of suppressing the inflammatory response as well as inflammatory tissue injury of UC induced by DSS.

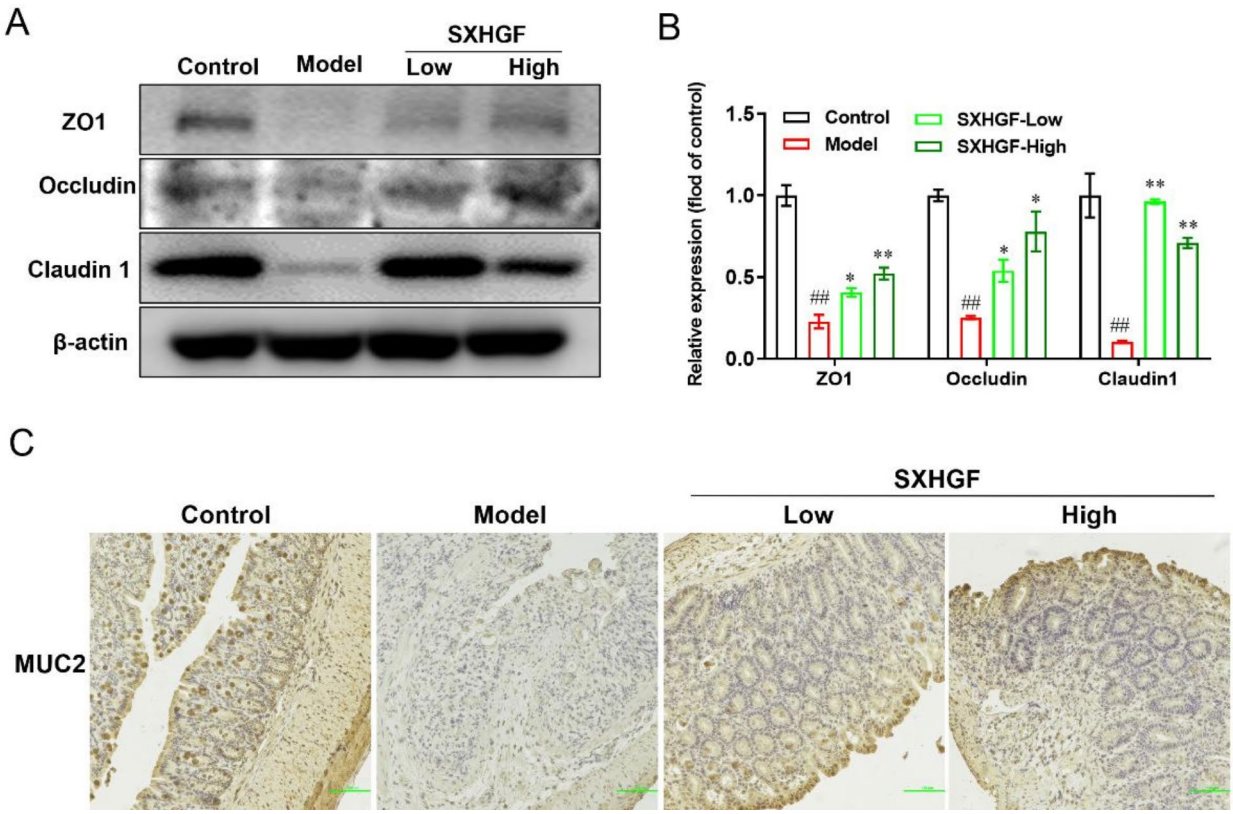

Fig. 6. SXHGF protected the intestinal barrier by increasing the tight junction protein expressions in DSS-induced colitis mice. (A) The expressions of ZO1, Occludin, and Claudin1 in the colon tissues of mice were analyzed by Western Blot. (B) Quantitative analysis for the tight junction proteins. (C) The expression of MUC2 in the colon tissue of UC mice was analyzed by IHC. Data are expressed as the mean ± SEM. Vs. the control group, $^{\#\#}P<0.01$; vs. the model group, $^{*}P<0.05$, $^{**}P<0.01$.

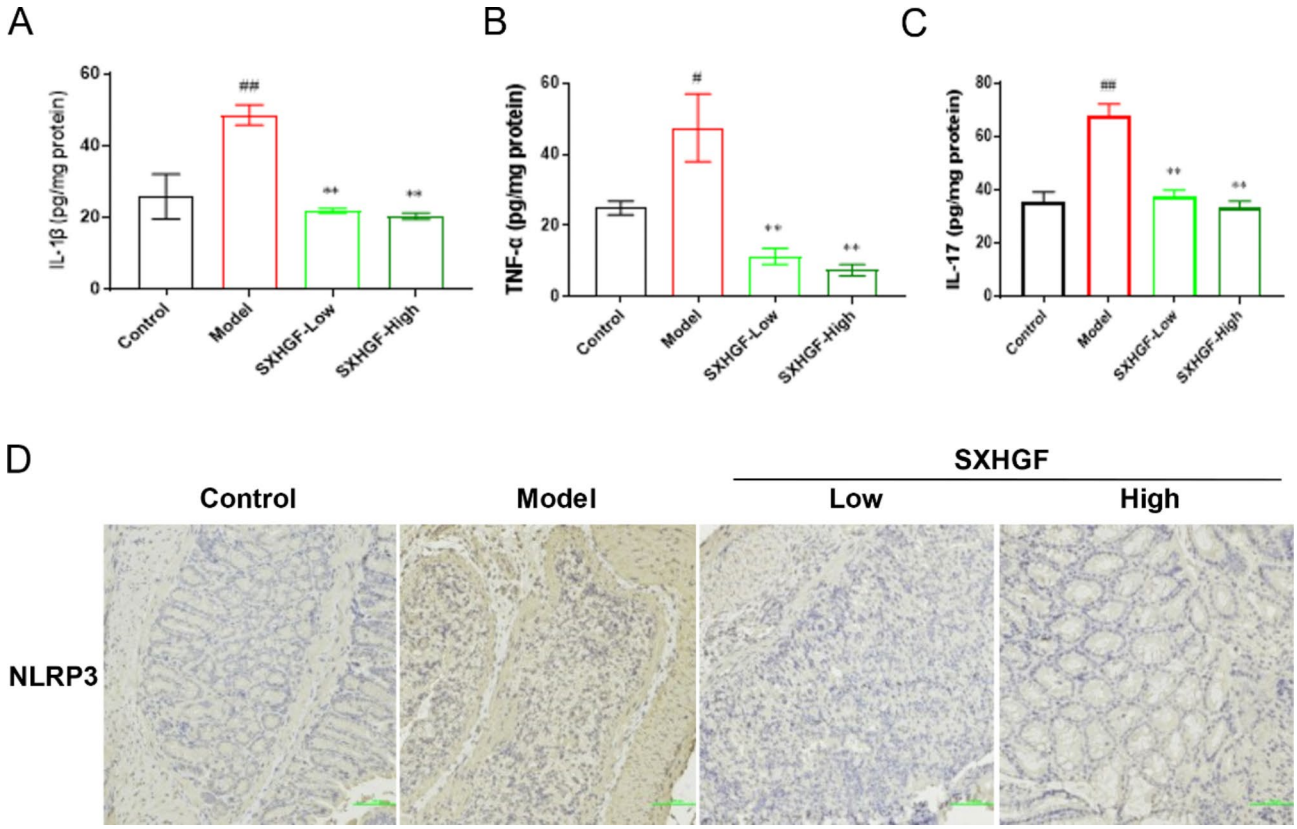

Fig. 7. SXHGF reduced the levels of pro-inflammatory cytokines and inflammasome in the colon of UC mice. The levels of pro-inflammatory cytokines IL-1β (A), TNF-α (B), and IL-17 (C) in the colon tissues were detected by ELISA assay. (D) The expression of NLRP3 in the colon tissue of UC mice was analyzed by IHC assay. Data are expressed as the mean ± SEM. Vs. the control group, $^{\#}P<0.05$, $^{\#\#}P<0.01$; vs. the model group, $^{**}P<0.01$.

Suppression of SXHGF on IL-6/JAK2/STAT3 and LPS/TLR4/ MyD88/ NF-κB pathways in UC mice

High levels of IL-6 and LPS in UC can induce a series of downstream inflammatory reactions, and reducing the production of IL-6 and LPS can suppress the inflammatory signaling pathways such as JAK2/STAT3 and TLR4/NF-κB/MyD88, thereby alleviating the intestinal inflammation[24,25]. By using the ELISA method, the level of LPS in the colon tissue of UC mice was detected, and the results showed that DSS significantly increased the level of LPS in the colon tissue of mice, while SXHGF reversed this effect. (Fig. 8A, compared with model group, $p<0.05$). Western Blot showed that the protein expressions of TLR4, NF-κB, p-NF-κB, and MyD88 in the model group were up-regulated (Fig. 8B-C), while those in the SXHGF-treated groups were significantly down-regulated (compared with the model group, $p<0.05$). Additionally, IHC results showed that SXHGF reduced the high levels of NF-κB in colon tissues induced by DSS, being consistent with the Western Blot results (Fig. 8D). On the other hand, SXHGF inhibits the increase of IL-6 in the colon tissue of UC mice (Fig. 8E, compared with the model group, $p<0.05$). Western blotting showed that SXHGF could also inhibit the expression of JAK2, P-JAK2, SATA3, and p-STAT3 in the colon tissue of UC mice (Fig. 8F-G). The IHC results also confirmed that SXHGF can suppress the expression of STAT3 in the colon tissue of UC mice (Fig. 8H). These results indicated that SXHGF alleviated DSS-induced UC by inhibiting the signaling pathways of IL-6/JAK2/STAT3 and LPS/TLR4/ MyD88/ NF-κB.

Suppression of SXHGF on oxidative stress in UC mice

Oxidative stress can induce ROS production and long-term oxidation would cause sustained damage to colon tissues[26]. In this study, the effect of SXHGF on the oxidative stress of UC mice was detected by Western Blot, ELISA, and IHC assays. The results showed that the levels of ROS (Fig. 9A) and MDA (Fig. 9B) were significantly increased in the model group, while those in the SXHGF-treated groups were significantly reduced (compared with the model group). Furthermore, the expressions of the critical anti-oxidative stress proteins, including Nrf2, HO1, and NQO1 were down-regulated after DSS induction, while significantly up-regulated after SXHGF treatment (Fig. 9C-D). The IHC result also indicated that SXHGF strongly enhanced the expression of Nrf2 in the colon tissues of UC mice (Fig. 9E). In all, these data suggested that SXHGF alleviated UC of mice via enhancing the efficiency of anti-oxidative stress, which was consistent with our prediction of the network pharmacology.

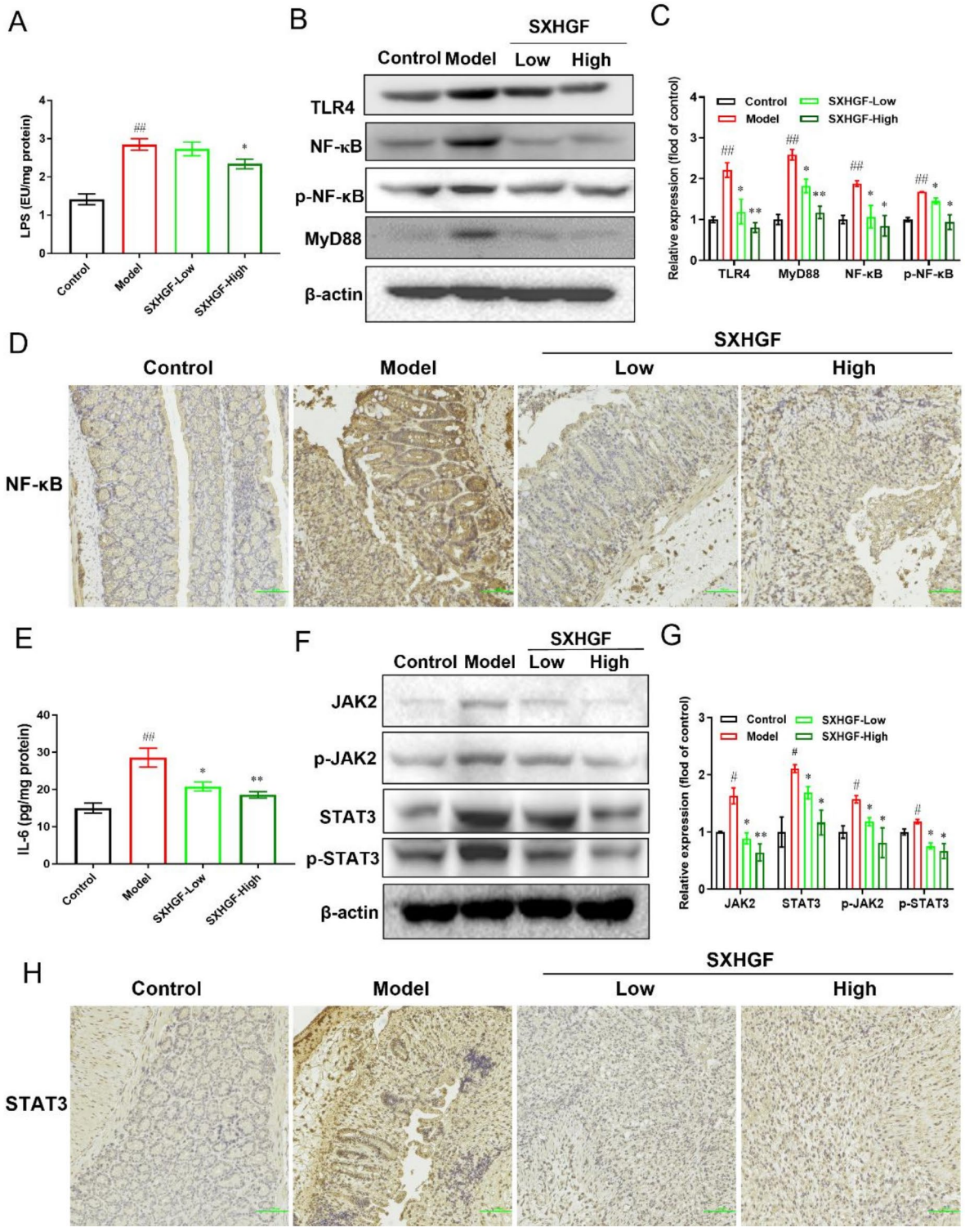

Fig. 8. SXHGF alleviated UC by inhibiting IL-6/JAK2/STAT3 and LPS/TLR4/NF-κB/MyD88 signal pathways. (A) The level of LPS in the colon tissues of UC mice was analyzed by ELISA assay. (B) Effect of SXHGF on the protein expressions of LPS/TLR4/NF-κB/MyD88 signaling pathway determined by Western Blot. (C) Quantitative analysis for TLR4, NF-κB, p-NF-κB, and MyD88 protein expressions. (D) Effect of SXHGF on the expression of NF-κB determined by IHC assay. (E) The level of IL-6 in the colon tissues of UC mice was analyzed by ELISA assay. (F) Effect of SXHGF on the protein expressions of IL-6/JAK2/STAT3 signaling pathway determined by Western Blot. (G) Quantitative analysis for JAK2, p-JAK2, STAT3, p-STAT3 protein expressions. (H) Effect of SXHGF on the expression of STAT3 determined by IHC assay. Data are expressed as the mean ± SEM. Vs. the control group, $^{\#}P<0.05$, $^{\#\#}P<0.01$; vs. the model group, $^{*}P<0.05$, $^{**}P<0.01$.

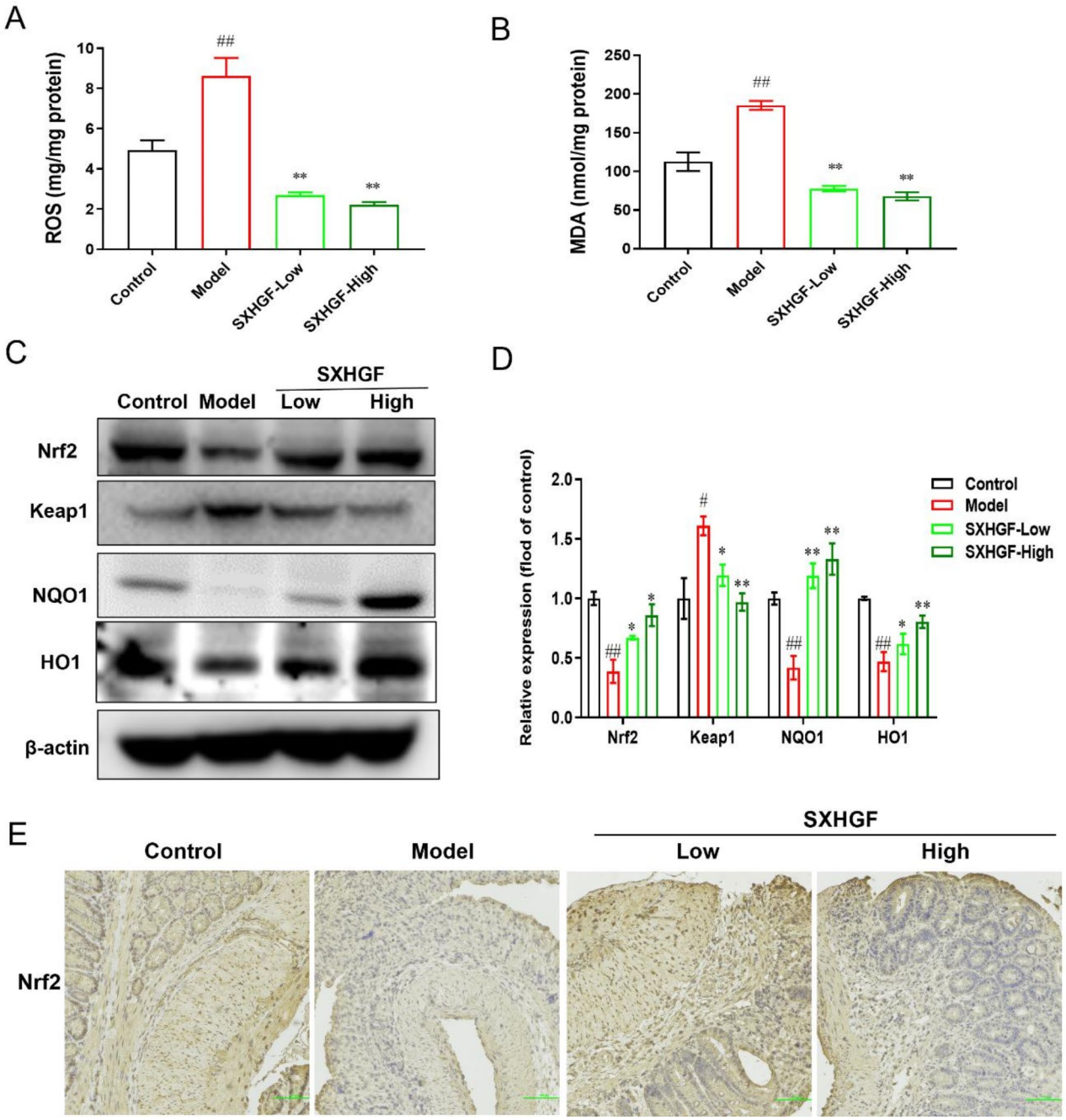

Fig. 9. SXHGF strengthened the colonic anti-oxidative stress in UC mice induced by DSS. The levels of ROS (A) and MDA (B) were evaluated by ELISA assay. Effect of SXHGF on the expressions of Keap1/Nrf2 pathway evaluated by Western Blot (C) and quantitative analysis (D). (E) Effect of SXHGF on the expression of Nrf2 in the colon tissue of UC mice determined by IHC assay. Data are expressed as the mean ± SEM. Vs. the control group, $^{\#}P<0.05$, $^{\#\#}P<0.01$; vs. the model group, $^{*}P<0.05$, $^{**}P<0.01$.

Inhibition of SXHGF on oxidative injury in UC mice

It was reported that a long course of oxidative stress would induce oxidative injury, during which the levels of 8-hydroxy-deoxyguanosine (8-OHDG) and DNA repair enzyme 8-oxyguanine DNA glycosylase (OGG1) would be strongly promoted, while SOD2 expression would be decreased[27]. Hence, the levels of OGG1, SOD2, and 8-OHDG can reflect the occurrence of oxidative injury. In our study, the levels of 8-OHDG and OGG1 were abnormally increased, while SOD2 was significantly decreased in the UC model group. However, after SXHGF treatment, the levels of OGG1, SOD2, and 8-OHDG were significantly reversed (compared with the model group, $p<0.05$), showing the excellent suppression of SXHGF against oxidative injury of the UC mice (Fig. 10A-C). Furthermore, IHC staining showed that SXHGF reduced the high expression of OGG1 in the colon tissues of UC mice, which was consistent with the Western Blot result (Fig. 10D). Altogether, these data showed

that SXHGF alleviated UC by suppressing the oxidative injury of the colon tissues in mice, as predicted by the network pharmacological analysis.

Identification of SXHGF by UHPLC-QTOF-MS

The main components in SXHGF were identified by UPLC-Q-TOF/MS analysis, and the results were shown in Fig. 11. A total of 21 components were identified in anionic mode (Fig. 11A, Table S2), including isoscopoletin, Dihydroxyacetone, narcissoside and hellicoside, etc. Amount to 41 compounds were identified in the positive ion mode (Fig. 11A, Table S3), including (+)-Catechin, coumarin, paeoniflorin and quercetin. The detailed information of each compound in SXHGF is shown in Table S2 and Table S3.

Discussion

UC, a gastrointestinal disease of unknown etiology seriously affecting the quality of life of patients, has developed into a global digestive disease[28,29]. The prevalence of UC exceeds 0.3% in North America, Europe, and Oceania and rapidly increases year by year in newly industrialized countries such as Asia and Africa[30]. Patients with UC would be suffering from a series of complications, such as secondary liver injury, neurological disease, and depression[31,32]. In addition, patients with UC are prone to develop colorectal cancer[33,34]. Therefore, it is urgent to accelerate the drug development for the prevention and treatment of UC.

In clinical practice, salicylic acid and immunosuppressant drugs have a high frequency of incomplete treatment and side effects[35,36]. Anti-TNF regimens were reported to induce psoriasis and lupus-like syndrome, as well as rare skin cancers[37]. In the long-term maintaining therapy using immune modulator thiopurines, 33% (33/100) of patients with CD and 46% (22/48) of patients with UC relapsed when they are taking, and most patients choose to stop taking the drugs due to recurrence or side effects[28]. At present, taking Western medicine alone is not satisfactory enough in the treatment of UC, and people are looking for other complementary and alternative therapies. TCM has a unique advantage in the prevention and treatment of digestive diseases[38]. Wu-mei-wan, a TCM used in the folk treating digestive diseases, was reported to inhibit the progression of colon inflammation and fibrosis, reduce the cytokines of IL-6 and IFN-γ, and down-regulate the expressions of P65 and STAT3[39]. In this study, it was proved that SXHGF, a TCM used for treating hepatitis in the clinic, had an excellent effect against UC with few side effects.

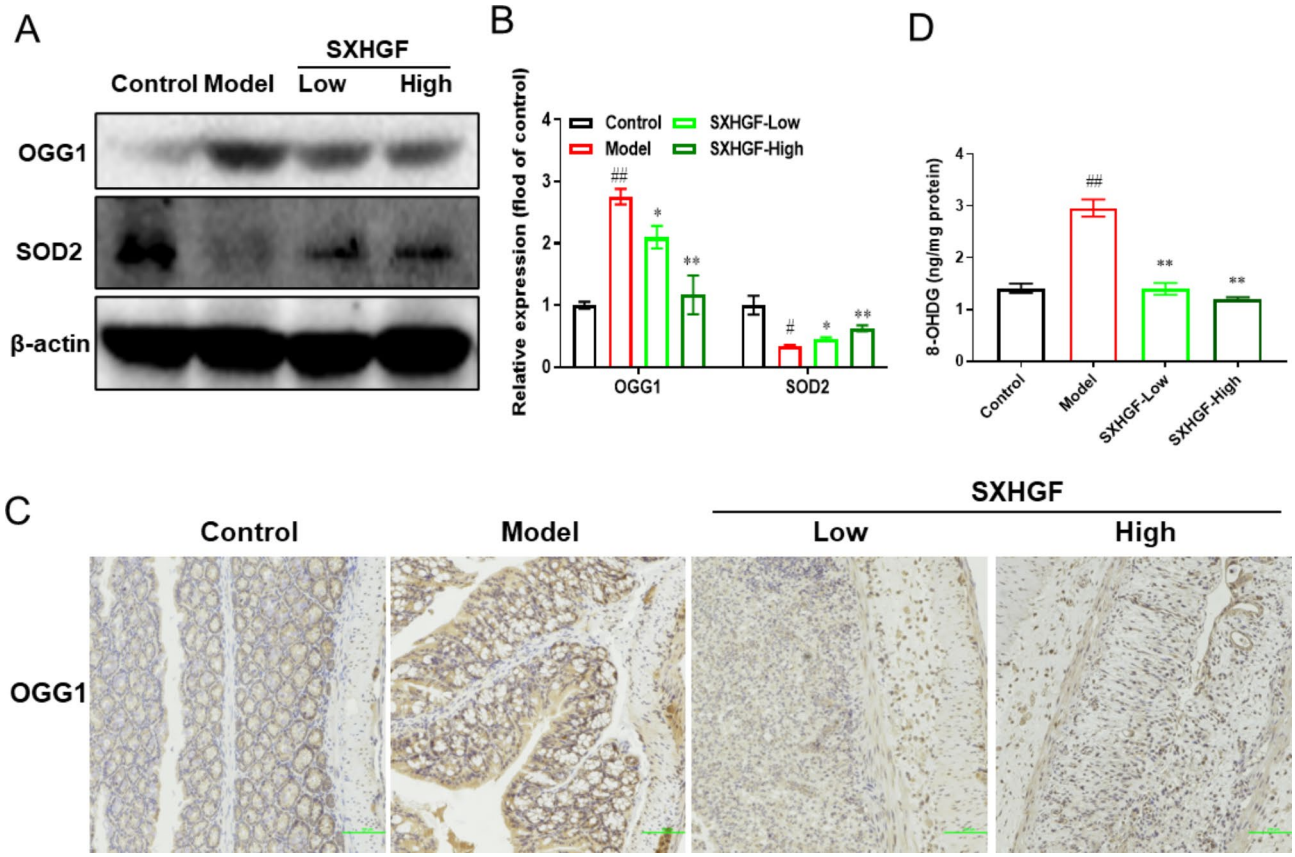

Fig. 10. SXHGF suppressed the oxidative damage of the colon tissues in UC mice. The effect of SXHGF on the expressions of SOD2 and OGG1 was determined by Western Blot assay (A) and quantitative analysis (B). (C) The effect of SXHGF on the level of 8-OHDG in the colon tissues of UC mice was analyzed by ELISA assay. (D) The effect of SXHGF on OGG1 expression was analyzed by IHC assay. Data are expressed as the mean ± SEM. Vs. the control group, $^{\#}P<0.05$, $^{\#\#}P<0.01$; vs. the model group, $^{*}P<0.05$, $^{**}P<0.01$.

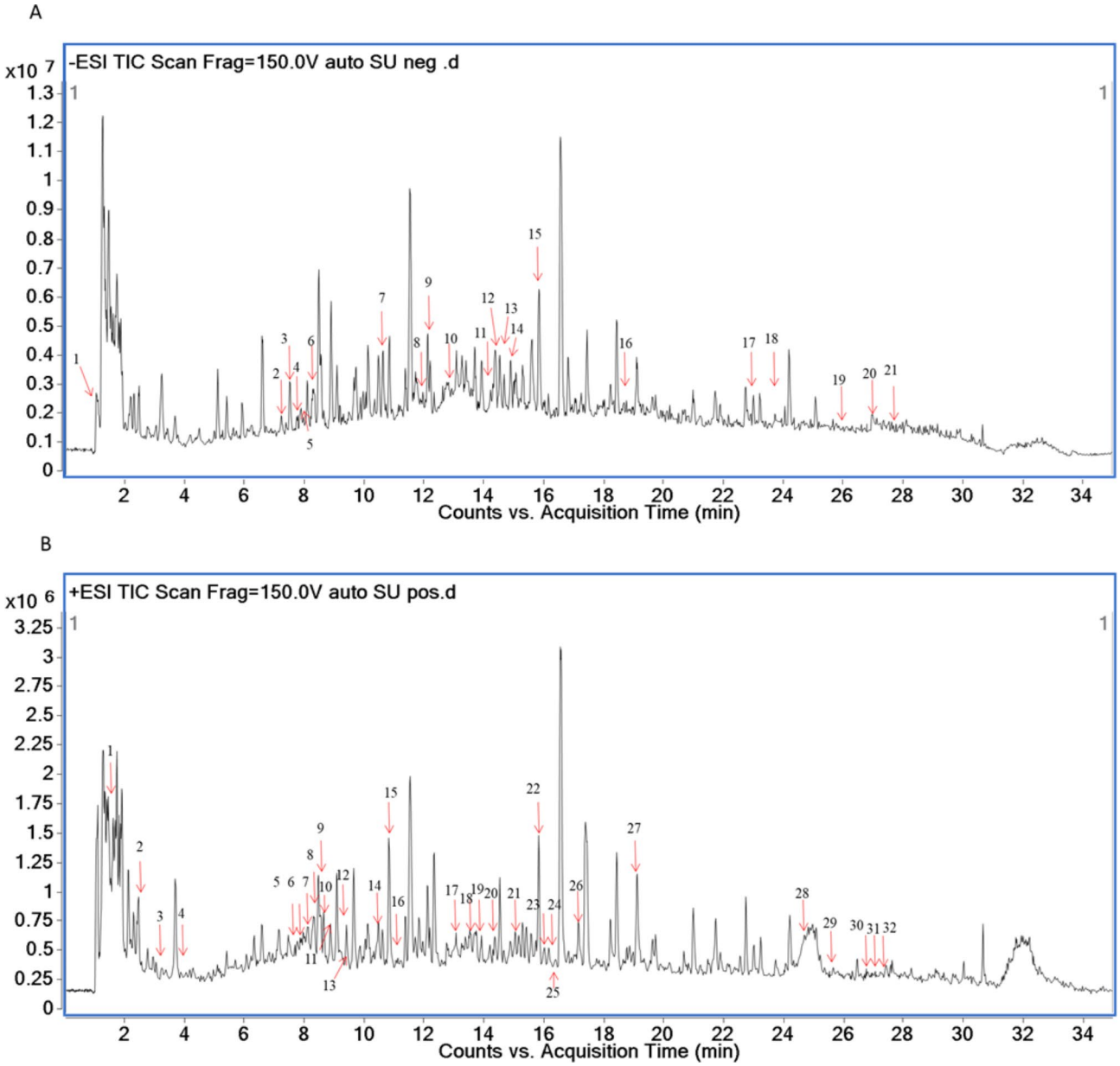

Fig. 11. Compounds identification for SXHGF by UHPLC-QTOF-MS. (A) Negative model. (B) Positive ion model.

It is reported that the occurrence and development of enteritis are closely related to liver diseases[40]. Non-alcoholic fatty liver disease is frequently detected in UC patients, and UC is commonly accompanied by secondary liver injury and cholecystitis[41]. Anti-hepatitis drugs would have the potential to inhibit colitis, because of the close relation between liver disease and intestinal disease[42]. SXHGF is a TCM compound commonly used in the clinic, which has good efficiency in treating acute and chronic hepatitis, cholecystitis, and fatty liver diseases. Therefore, it was speculated that SXHGF probably had the function of treating intestinal inflammation. This study for the first time revealed the therapeutic efficiency of SXHGF against UC in mice, while the underlying mechanism remained unclarified.

TCM compound has the characteristics of synergistic effect combining with multi-components, -targets, and -pathways, exhibiting unique advantages in the treatment of complex diseases while elaborating its detailed molecular mechanism is a complicated work. Network pharmacology can connect drug components with disease genes through a powerful database, forming a "drug-ingredient-gene-disease" network, identifying the most relevant targets, and the pathway of drug action, and clarifying the mechanism from the molecular biological level[43,44]. In this study, the mechanism of SXHGF in the treatment of UC was studied by network pharmacology via constructing a drug ingredient-gene network, searching out key nodes of protein interaction, among which the top nodes were IL6, AKT1, IL1β, PTGS2, MMP9, STAT3, CCL2, HIF1A, HMOX1, and ICAM1. These targets

have been reported to regulate the development of inflammation and oxidative stress and are closely related to the treatment of UC[25,45].

The pathogenesis of UC is complex and a high level of inflammation is the classic feature[46]. Bioinformatics analysis showed that some inflammatory factors, such as IL-6, IL-1β, and STAT3, may be important targets of SXHGF in the treating UC, and pro-inflammatory signaling pathways, such as TNF, IL-17, and NF-κB, might be the key pathways, which were verified in our experimental studies. Oxidative stress is considered to be one of the most important pathogenic factors of UC. In an inflammatory environment, abnormally accumulated ROS can lead to protein dysfunction and DNA damage, increase the expressions of MDA, 8-OHDG, and OGG1, and further promote inflammation and oxidative injury[47]. Keap1/Nrf2/HO-1 signaling acts the key role of oxidative stress, during which the complex of Nrf2 and Keap1 are separated, and Nrf2 transfers into the nucleus, resulting in induction of HO1, NQO1, and SOD2 expressions[48]. Nrf2/HO1 also enhances the expression of intestinal tight junction protein and protects the intestinal barrier, thereby mitigating colon injury[49]. In this study, through GO and KEGG analysis, the mechanism of SXHGF against UC was speculated to be associated with the regulations of ROS, ROS metabolism process, and oxidative stress. Hence, in the experimental study, we focused on the effect of SXHGF on oxidative stress-related mechanisms. As a result, SXHGF reduced the high levels of MDA, 8-OHDG, OGG1, and Keap1, and increased the levels of SOD2, Nrf2, NQO1, and HO1, suggesting the good effect of SXHGF on inhibiting the oxidative injury in UC mice.

The main chemical components of SXHGF were identified using UPLC-Q-TOF/MS, which served to validate the findings of network pharmacology-related analyses. Through UHPLC-QTOF-MS analysis, the predominant constituents of SXHGF were identified as Liquiritin, Quercetin, Coumarin, and Paeoniflorin. Network pharmacology analysis also suggests that these substances may be the main active components in the treatment of UC with SXHGF. Furthermore, previous studies have also shown that these active ingredients can treat UC by inhibiting inflammatory responses or oxidative damage pathways. For example, Paeoniflorin can play a role in treating UC by inhibiting the abnormal activation of the TLR4/NFκB/NLRP3 signaling pathway[50]. Liquiritin has been shown to attenuate the secretion of inflammatory cytokines, such as MPO and IL-6, in UC mice while also restoring intestinal barrier function[51]. Quercetin has been shown to improve intestinal barrier damage in experimental colitis mice through its anti-inflammatory and antioxidant mechanisms[52].

Altogether, this study showed that SXHGF could strongly alleviate UC by suppressing the transduction of IL6/JAK2/STAT3 and LPS/TLR4/NF-κB/MyD88 pro-inflammatory pathways to protect the intestinal barrier, and inhibiting ROS production, up-regulating the expressions of Nrf2, HO1, NQO1 and SOD2, and reducing the levels of 8-OHDG and OGG1, thereby resisting oxidative stress injury. However, the limitation of this study is that the bioactive components in SXHGF were not quantitatively analyzed. Additionally, further investigation is required to elucidate the absorbed active compounds and the drug metabolic process. Moreover, more systematic and in-depth in vitro and in vivo experiments are necessary to further validate the mechanism by which SXHGF alleviates UC.

Conclusion

SXHGF is a TCM commonly used in the clinic to treat liver diseases. Our study for the first time revealed that SXHGF has excellent efficiency in treating UC. Network pharmacology analysis showed that the potential mechanism of SXHGF in treating UC was probably related to the regulations on inflammatory response, response to lipopolysaccharides, reactive oxygen species metabolic process, and oxidative stress. The animal study validated that SXHGF significantly enhanced the gut barrier via increasing the expressions of ZO1, Occludin, Claudin 1, and MUC2, and suppressed the inflammatory response of the colon via inhibiting the pro-inflammatory pathways of LPS/TLR4/NF-κB, IL6/JAK2/STAT3. Additionally, SXHGF significantly suppressed the oxidative stress and oxidative damage of the colon by reducing the levels of ROS, MDA, 8-OHDG, OGG1, and Keap1, and increasing the levels of SOD2, NRF2, NQO1, and HO1, which were consistent with the predicted results by network pharmacology approach. In all, this study indicated that SXHGF significantly alleviated UC in mice by suppressing the inflammatory response and oxidative injury, suggesting that SXHGF would be a new candidate for the treatment of UC in humans.

Data availability

All data are available from the corresponding author (thuxj@gdmu.edu.cn).

Received: 6 June 2024; Accepted: 4 November 2024
Published online: 08 November 2024

References

1. Ghosh, S. & Almadi, M. A. Inflammatory bowel disease: A Global Disease Saudi. *J. Gastroenterol.* **19** (1), 1–2 (2013).
2. Ng, S. C. et al. Population Density and Risk of Inflammatory Bowel Disease: a prospective Population-based study in 13 countries or regions in Asia-Pacific. *Am. J. Gastroenterol.* **114** (1), 107–115 (2019).
3. Kaplan, G. G. The global burden of IBD: from 2015 to 2025. *Nat. Reviews Gastroenterol. Hepatol.* **12** (12), 720–727 (2015).
4. Nieminen, U. et al. Inflammation and disease duration have a cumulative effect on the risk of dysplasia and carcinoma in IBD: a case-control observational study based on registry data. *Int. J. Cancer*. **134** (1), 189–196 (2014).
5. Wang, W. et al. Exploration of the potential mechanism of Banxia Xiexin Decoction for the effects on TNBS-induced ulcerative colitis rats with the assistance of network pharmacology analysis. *J. Ethnopharmacol.*, **277**(114197). (2021).
6. Dudzinska, E. et al. *Oxidative Stress and Effect of Treatment on the Oxidation Product Decomposition Processes in IBD* (Oxidative Medicine and Cellular Longevity, 2018). 2018(7918261).
7. Petkovic, M. et al. Dietary supplementation with sulforaphane ameliorates skin aging through activation of the Keap1-Nrf2 pathway. *J. Nutr. Biochem.*, **98**(108817). (2021).

8. Huang, C. et al. Polymorphisms of human 8-oxoguanine DNA glycosylase 1 and 8-hydroxydeoxyguanosine increase susceptibility to arsenic methylation capacity-related urothelial carcinoma. *Arch. Toxicol.* **90** (8), 1917–1927 (2016).
9. Lopez, A. & Peyrin-Biroulet, L. 5-Aminosalicylic acid and chemoprevention: does it work? Digestive diseases, **31**(2): pp. 248–253. (2013).
10. Berends, S. E. et al. Clinical pharmacokinetic and pharmacodynamic considerations in the treatment of Ulcerative Colitis. *Clin. Pharmacokinet.* **58** (1), 15–37 (2019).
11. Singh, S. et al. AGA Technical Review on the management of moderate to severe Ulcerative Colitis. *Gastroenterology*. **158** (5), 1465– (2020).
12. Cross, R. K., Lapshin, O. & Finkelstein, J. Patient subjective assessment of drug side effects in inflammatory bowel disease. *J. Clin. Gastroenterol.* **42** (3), 244–251 (2008).
13. Barnhill, M. S. et al. Hepatotoxicty of agents used in the management of inflammatory bowel disease: a 2020 Update. *Curr. Gastroenterol. Rep.* **22** (9), 47 (2020).
14. Pallavicino, F. et al. Inflammatory bowel diseases and primary sclerosing cholangitis: hepatic and pancreatic side effects due to azathioprine. *Eur. Rev. Med. Pharmacol. Sci.* **17** (1), 84–87 (2013).
15. Fang, S. et al. A high-throughput experiment- and reference-guided database of traditional Chinese medicine. *Nucleic Acids Res.* **49** (D1), D1197–D1206 (2021).
16. Yuan, Z. et al. [Method and application of proteomics in study of targets of traditional Chinese medicines]. Zhongguo Zhong Yao Za Zhi = Zhongguo zhongyao zazhi = China. *J. Chin. materia Med.* **45** (5), 1034–1038 (2020).
17. Lu, J. et al. Exploring the molecular mechanism of Yinao Fujian formula on ischemic stroke based on network pharmacology and experimental verification. *Heliyon*. **10** (1), e23742 (2024).
18. Zhou, Z. et al. *Applications of Network Pharmacology in Traditional Chinese Medicine Research* (Evidence-Based Complementary and Alternative Medicine, 2020). 2020(1646905).
19. Yang, L. et al. A recent update on the use of Chinese medicine in the treatment of inflammatory bowel disease. *Phytomedicine* 92(153709) (2021).
20. Kanehisa, M. & Goto, S. Kyoto Encyclopedia of genes and genomes. *Nucleic Acids Res.* **28** (1), 27–30 (2000).
21. Mehandru, S. & Colombel, J. The intestinal barrier, an arbitrator turned provocateur in IBD. *Nat. Reviews Gastroenterol. Hepatol.* **18** (2), 83–84 (2021).
22. Hansson, G. C., Kornberg, R. D. & Kornberg^Editors, R. D. Mucins and the Microbiome, in Annual Review of Biochemistry, pp. 769–793. (2020).
23. Iyer, S. S. et al. Necrotic cells trigger a sterile inflammatory response through the Nlrp3 inflammasome. *Proc. Natl. Acad. Sci. U.S.A.* **106** (48), 20388–20393 (2009).
24. Candelli, M. et al. Interaction between Lipopolysaccharide and Gut Microbiota in Inflammatory Bowel diseases. *Int. J. Mol. Sci.*, **22**(624212). (2021).
25. Pawlowska-Kamieniak, A., Krawiec, P. & Pac-Kozuchowska, E. Interleukin 6: biological significance and role in inflammatory bowel diseases. *Adv. Clin. Experimental Med.* **30** (4), 465–469 (2021).
26. Tian, T., Wang, Z. & Zhang, J. Pathomechanisms of oxidative stress in inflammatory bowel disease and potential antioxidant therapies. *Oxidative Med. Cell. Longev.*, (2017). 2017(4535194).
27. Pereira, C. et al. Oxidative stress and DNA damage: implications in inflammatory bowel disease. *Inflamm. Bowel Dis.* **21** (10), 2403–2417 (2015).
28. Balram, B. et al. Poor drug sustainability in inflammatory bowel disease patients in clinical remission on Thiopurine Monotherapy. *Dig. Dis. Sci.* **66** (5), 1650–1657 (2021).
29. Benchimol, E. I. et al. Epidemiology of Pediatric Inflammatory Bowel Disease: a systematic review of International Trends. *Inflamm. Bowel Dis.* **17** (1), 423–439 (2011).
30. Ng, S. C., Shi, H. Y. & Hamidi, N. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies (390, Pg 2769, 2018). *Lancet*. **396** (10256), E56–E56 (2020).
31. Ferro, J. M. & Santos, M. O. Neurology of inflammatory bowel disease. *J. Neurol. Sci.*, **424**(117426). (2021).
32. Rodriguez-Duque, J. C. et al. Increased risk of MAFLD and Liver Fibrosis in Inflammatory Bowel Disease Independent of Classic metabolic risk factors. *Clin. Gastroenterol. Hepatol.* **21** (2), 406– (2023).
33. Grivennikov, S. I. Inflammation and colorectal cancer: colitis-associated neoplasia. *Semin. Immunopathol.* **35** (2), 229–244 (2013).
34. Ortenzi, M. et al. Complications after bowel resection for inflammatory bowel disease associated cancer: a systematic literature review. *Minerva Surg.* **77** (3), 272–280 (2022).
35. Marzano, A. V. et al. Immune-mediated inflammatory reactions and tumors as skin side effects of inflammatory bowel disease therapy. *Autoimmunity*. **47** (3), 146–153 (2014).
36. Targownik, L. E. et al. Trends in Corticosteroid Use during the era of Biologic Therapy: a Population-based analysis. *Am. J. Gastroenterol.* **116** (6), 1284–1293 (2021).
37. Mocci, G. et al. Dermatological adverse reactions during anti-TNF treatments: focus on inflammatory bowel disease. *J. Crohns Colitis*. **7** (10), 769–779 (2013).
38. Salaga, M. et al. Chinese Herbal Medicines in the treatment of IBD and Colorectal Cancer: a review. *Curr. Treat. Options Oncol.* **15** (3), 405–420 (2014).
39. Wu, F. et al. Wu-Mei-Wan ameliorates chronic colitis-associated intestinal fibrosis through inhibiting fibroblast activation. *J. Ethnopharmacol.*, **252**(112580). (2020).
40. Adolph, T. E. et al. The metabolic nature of inflammatory bowel diseases. *Nat. Reviews Gastroenterol. Hepatol.* **19** (12), 753–767 (2022).
41. Sartini, A. et al. Non-alcoholic fatty liver disease phenotypes in patients with inflammatory bowel disease. *Cell Death Dis.*, (2018). 9(87).
42. Li, Y. et al. Bile acids and intestinal microbiota in autoimmune cholestatic liver diseases. *Autoimmun. rev.* **16** (9), 885–896 (2017).
43. Kanehisa, M. Toward understanding the origin and evolution of cellular organisms. *Protein Sci.* **28** (11), 1947–1951 (2019).
44. Kanehisa, M. et al. KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Res.* **51** (D1), D587–D592 (2023).
45. Opdenakker, G., Vermeire, S. & Abu El-Asrar, A. How to place the duality of specific MMP-9 inhibition for treatment of inflammatory bowel diseases into clinical opportunities? *Front. Immunol.*, **13**(983964). (2022).
46. Waldner, M. J. & Neurath, M. F. Master regulator of intestinal disease: IL-6 in chronic inflammation and cancer development. *Semin. Immunol.* **26** (1), 75–79 (2014).
47. Wang, Z. et al. Oxidative stress and Carbonyl Lesions in Ulcerative Colitis and Associated Colorectal Cancer. *Oxidative Med. Cell. Longev.*, (2016). 2016(9875298).
48. Kasai, S. et al. Regulation of Nrf2 by mitochondrial reactive oxygen species in Physiology and Pathology. *Biomolecules*, **10**(3202). (2020).
49. Liu, Y. et al. Extracellular Signal-regulated Kinase/Nuclear Factor-Erythroid2-like2/Heme Oxygenase-1 pathway-mediated Mitophagy alleviates traumatic Brain Injury-Induced Intestinal Mucosa damage and epithelial barrier dysfunction. *J. Neurotrauma*. **34** (13), 2119–2131 (2017).
50. Guo, Z. et al. Potential antidepressant effects of traditional Chinese botanical drug formula Chaihu-Shugan-San and its active ingredients. *Front. Pharmacol.* **15**, 1337876–1337876 (2024).

51. Liu, J. et al. Prdx6-induced inhibition of ferroptosis in epithelial cells contributes to liquiritin-exerted alleviation of colitis. *Food Funct.* **13** (18), 9470–9480 (2022).
52. Lesjak, M. et al. Antioxidant and anti-inflammatory activities of quercetin and its derivatives. *J. Funct. Foods.* **40**, 68–75 (2018).

Acknowledgements
This work was financially supported by the National Natural Science Foundation of China (81503303, 81973548, and 81473401), Natural Science Foundation of Guangdong Province of China (2023A1515011116), Discipline Construction Project of Guangdong Medical University (4SG22009G and 4SG23282G), Guangdong Innovation Team Project for Regular Institutions of Higher Learning (2022KCXTD011), Guangdong Medical University Research Foundation (4SG22242G), Traditional Chinese Medicine Bureau of Guangdong Province, China (20231375, 20241159), Project of Medical Research Fund of Guangdong Province, China (A2022479), and Special Fund for Science and Technology Development of Zhanjiang (2022A01003).

Author contributions
Qiang Huang: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - review & editing. Weijie Peng: Investigation, Data curation, Writing - original draft. Qing Luo: Investigation, Formal analysis, Validation, Writing - review & editing. Wenchang Zhao: Software, Funding acquisition, Writing - review & editing. Weibo Dai: Supervision, Funding acquisition, Project administration, Writing - review & editing. Huifen Zeng: Supervision, Project administration, Resources, Writing - review & editing. Hoi Leong Xavier Wong: Software, Data curation, Validation, Writing - review & editing. Xianjing Hu: Conceptualization, Investigation, Supervision, Project administration, Resources, Writing - original draft, Funding acquisition, Writing - review & editing. All authors read and approved the final manuscript. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of the work, ensuring integrity and accuracy.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-78833-1.

Correspondence and requests for materials should be addressed to W.D., H.Z. or X.H.