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ORIGINAL RESEARCH

Wuda Granule Alleviates DSS-Induced Colitis in Mice by Inhibiting Inflammation, Protecting Intestinal Barrier and Reducing Oxidative Stress Through Nrf2/Keap1/HO-1 Pathway

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Background: Colitis, a inflammatory disorder of the colon, causes significant morbidity and declines quality of life. Despite treatment advancements, effective therapies remain limited. Wuda Granules (WDG), a traditional medicine formula, is clinically used for intestinal obstruction and gastrointestinal recovery through its anti-inflammatory effects.

Methods: Network pharmacology and molecular docking were employed to identify the active compounds, key targets, and associated signaling pathways of WDG against Colitis. A mouse model of DSS-induced colitis was established to evaluate the therapeutic potential of WDG. Colitis-related symptoms, including weight loss, DAI score, spleen index, and colon length, were measured. Histopathological changes were analyzed using H&E staining. To assess intestinal barrier integrity, goblet cell abundance was determined by AB-PAS staining, and the expression of related proteins was analyzed by IHC. Inflammatory and oxidative stress markers were measured by ELISA, biochemical assays, and Western blotting.

Results: Network pharmacology analysis identified the core therapeutic targets of WDG against UC is IL-6, TP53, AKT1, IL-1β, and TNF. WDG treatment significantly improved colitis-related symptoms, as evidenced by reduced weight loss, DAI score, and spleen index, as well as increased colon length. Histological analysis revealed preserved colon structure and reduced inflammatory infiltration. WDG suppressed the expression of pro-inflammatory mediators (IL-6, IL-1β, TNF-α, MPO) and enhanced the levels of anti-inflammatory cytokines (IL-10 and IL-22). In addition, WDG alleviated lung inflammation and inhibited M1 macrophage polarization. Intestinal barrier integrity was improved by increasing goblet cell numbers and upregulating the expression of MUC2, ZO-1, Occludin, Claudin-1. Antioxidant activity was enhanced, indicated by elevated SOD and CAT levels and decreased MDA content. Mechanistically, Western blot analysis showed that WDG activated the Nrf2/Keap1/HO-1 signaling pathway. Molecular docking further revealed one potential active compound, 6,7-Dimethoxy-2-(2-phenylethyl) chromone, which exhibited strong binding affinity with Nrf2/Keap1 complex.

Conclusion: WDG alleviates DSS-induced colitis by inhibiting inflammation, enhancing intestinal barrier integrity, and reducing oxidative stress through activation of the Nrf2/Keap1/HO-1 pathway.

Keywords: wuda granule, colitis, anti-inflammatory, antioxidant, intestinal barrier protection

Introduction

Inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease, are chronic disorders that cause considerable morbidity and a reduced quality of life.[1,2] Patients with UC are often found with weight loss, hematochezia, diarrhea, and fatigue, which may be accompanied by extraintestinal manifestations, including

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arthropathies, mucocutaneous and ophthalmological influenced conditions.[3] The etiology of IBD is multifactorial, involving genetic, environmental, immune system, and microbial factors.[4] Conventional treatments, including aminosalicylates (eg, sulfasalazine and 5-aminosalicylic acid), corticosteroids (eg, budesonide and hydrocortisone), immunomodulators (eg, azathioprine and methotrexate) and biologics (eg, vedolizumab and ustekinumab), aim to control inflammation and induce remission.[5–7] However, these treatments are frequently accompanied by adverse effects and incomplete response.[8] The imbalance between pro- and anti-inflammatory cytokines directly drives inflammation propagation and intestinal tissue damage. Pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, activate downstream signaling pathways like nuclear factor-kappa B (NF-κB), leading to the hyperactivation of inflammatory cells and the cascading release of additional inflammatory mediators, perpetuating a vicious cycle of inflammation.[9] Conversely, insufficient secretion of anti-inflammatory cytokines, such as IL-10, compromises the intestinal protective mechanisms, further aggravating disease severity.[10] Balancing pro- and anti-inflammatory cytokines is therefore critical for restoring immune homeostasis in the gut.

Additionally, oxidative stress is usually accompanied by inflammation. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense systems, leading to the accumulation of free radicals.[11] This further leads to direct epithelial cell damage and activates inflammatory response. Oxidative stress can activate the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, which further promotes inflammation and ultimately disrupts the colonic mucosal structure, triggering IBD.[12] The antioxidant defense system consists of enzymatic and non-enzymatic mechanisms, including enzymes like superoxide dismutase and catalase, as well as non-enzymatic antioxidants such as glutathione, vitamin C, and vitamin E. It is also regulated by key pathways, including the Nrf2/Keap1 axis, which helps maintain cellular protection against oxidative stress.[13,14]

Among the pathological characteristics of colitis, disruption of the intestinal epithelial barrier is commonly considered a central mechanism, frequently associated with dysregulated expression of tight junction proteins (TJPs).[15] These proteins, including Occludin and ZO-1, are essential for maintaining epithelial integrity by regulating intercellular adhesion and serving as a barrier to prevent the translocation of toxins and pathogens.[16] Downregulation of TJPs increases intestinal permeability, promoting the leakage of pro-inflammatory cytokines and subsequently intensifying the inflammatory response.

Traditional Chinese medicine (TCM) has gained significant attention as a complementary therapeutic approach for inflammatory bowel disease (IBD) due to its multi-targeted and holistic mechanisms.[17] Wuda Granules (WDG) (formerly Xiangbin prescription), a TCM formulation developed by the Guangdong Provincial Hospital of Traditional Chinese Medicine (Approval No. Z20180010), is currently applied clinically to enhance intestinal peristalsis, restore normal intestinal function, relieve abdominal distension and pain caused by obstruction, and facilitate defecation and gas expulsion.[18,19] Furthermore, it serves as an adjunct therapy before and after surgery, supporting the recovery of intestinal function.[20–22] The main ingredients include Amomum villosum Lour. (Sharen), Lindera aggregata Kosterm (Wuyao), Panax ginseng C.A.Mey. (Renshen), Prunus persica (L). Batsch (Taoren) and Areca catechu L. (Binlang). The synergistic combination of these five herbs contributes to warming the stomach, strengthening the spleen, and promoting bowel relaxation. Ginsenoside Rc, Rd, Rg1, quercetin, quercitrin, isoquercitrin, laetrile, norisoboldine, linderane, and arecoline were quantified in WD using LC-MRM-MS analysis.[23] Additionally, UPLC-QQQ-MS/MS was used to identify alkaloids, arecoline, arecaidine, norisoboldine, and boldine in WD.[24] Our group identified 20 potential biomarkers, including LysoPCs, LysoPEs, 11-HETE, leucine, glutamine, tryptophan, and arachidonic acid that associated with WD-related rapid recovery after laparoscopic surgery, through UPLC/Q-TOF-MS/MS-based metabolomic analysis.[25]

However, the therapeutic effects and mechanisms of WDG in treating colitis remain unclear. This paper aims to explore the therapeutic potential and mechanisms of WDG in DSS-induced colitis mice model. We hypothesize that WDG may exert therapeutic effects against colitis through the inhibition of inflammatory responses, protection of intestinal barrier integrity, and reduction of oxidative stress. By examining the underlying pathway, this research helps to clarify the potential of WDG as a novel treatment option for managing inflammatory bowel disease.

Materials and Methods

Preparation of WD

Wuda granule is comprised of *Amomum villosum Lour*. (Sharen) 300 g, *Lindera aggregata Kosterm* (Wuyao) 500 g, *Panax ginseng C.A.Mey*. (Renshen) 450 g, *Prunus persica (L). Batsch* (Taoren) 500 g, and *Areca catechu L*. (Binlang) 500 g. The names of the herbs were verified through The Plant List (http://www.theplantlist.org). *Amomum villosum Lour*. was soaked in 5 volumes (w/v) of water for 2 hours, followed by steam distillation for 4 hours to extract the essential oil. The residual aqueous phase was collected and reserved for subsequent use. Separately, *Panax ginseng* was extracted with 10 volumes (w/v) of 60% ethanol in three rounds, each lasting 0.5 hours. The filtrates were combined for later use. *Lindera aggregata Kosterm, Prunus persica (L). Batsch* and *Areca catechu L*. were decocted in water four times, each for 0.5 hours. The resulting decoctions were filtered and combined with the previously reserved aqueous extract of *A. villosum*, the *P. ginseng* extract was then added, and the mixture was stirred thoroughly. Finally, the *A. villosum* essential oil and suitable pharmaceutical excipients were incorporated. The final mixture was processed into the desired pharmaceutical dosage form. It is produced in batches of 1000 g and packaged in units of 10 g per bag.[25] Batch number: 230501.

Animals

Five to six weeks old C57BL/6 male mice (20–22 g) were purchased by Zhuhai BesTest Bio-Tech Co,.Ltd. The Institutional Ethical Committee for Animal Research of Institute of Zoology, Guangdong Academy of Science approved the animal experiments (No. GIZ20240408-02), which were carried out in compliance with the ARRIVE guidelines. The mice were housed in the SPF animal breeding room under controlled environmental conditions, including a temperature of 23±2 °C, humidity of 60 ± 10% and 12:12 hours light-dark light cycle. All mice were provided free access to food and water.

DSS-Induced IBD and Drug Treatment

After one week of adaptive feeding, the mice were randomly divided into six groups: control, DSS, WDG (0.75, 1.5 and 3 g/kg) + DSS group and positive control 5-aminosalicylic acid (5-ASA) (200 mg/kg) + DSS group. The number of mice in each group was eight. Mice in the control group were given only water, while mice in the DSS, DSS + WDG and DSS + 5-ASA group received 2.5% of DSS dissolved in the drinking water for 7 days.[26] During the DSS intervention, mice in the WDG groups were administered WDG orally via gavage at daily doses of 0.75, 1.5, and 3 g/kg, while the positive control group received 5-ASA at a daily dose of 200 mg/kg. The mice were sacrificed on day 8. All investigators involved in data collection and analysis were blinded to the group allocations.

Disease Activity Index (DAI)

DAI score used to assess the severity of DSS-induced colitis was shown in Table 1. Fecal occult blood was assessed using a qualitative test kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Negative (-) (no color development within 3 min); weakly positive (+) (30s-60s, blue); positive (++) (immediately blue-green); strongly positive (+++) (immediately dark blue).

The DAI score is calculated according to the following formula: DAI score = (weight loss score + stool consistency score + fecal occult blood score) / 3.

Table 1 Calculation of the DAI Score

Scores	Weight Loss (%)	Stool Consistency	Fecal Occult Blood
0	0	Normal	(-)
1	<5		(+)
2	5~10	Loose stool	(++)
3	10~15		(+++)
4	>15	Diarrhea	Visible bleeding with blood

Spleen Index

After sacrifice, the spleen is carefully excised from the mice and weighed using a precision scale. The spleen index is calculated according to the following formula: Spleen index = Spleen weight (mg) / Body weight (g).

Histological Analysis

The tissue samples were fixed in 4% paraformaldehyde for 24 hours, followed by dehydration in gradient ethanol, wax infiltration, and embedding. Sections of 4 μm were cut using a paraffin microtome (Leica HistoCore MULTICUT, Wetzlar, Germany) and subsequently stained with H&E, AB-PAS and Masson (Solarbio, Beijing, China). Histopathological examination and imaging were performed using a microscope (EVOS M7000, Thermo Fisher Scientific, USA).

Immunohistochemical Analysis of Colonic Tissues

Immunohistochemical staining was performed to detect the expression of MUC-2, Occludin, and ZO-1. Following dewaxing and rehydration, 4 μm colonic tissue sections were subjected to antigen retrieval with a sodium citrate solution. Color development was performed using two-step detection kit (PV-9000, ZSGB-BIO, Beijing, China) and DAB Chromogenic Kit (ZLI-9018, ZSGB-BIO, Beijing, China). The primary antibodies used were MUC-2 (27,675-1-AP, Proteintech Group, Wuhan, China), Occludin (13409-1-AP, Proteintech Group, Wuhan, China), and ZO-1 (21,773-1-AP, Proteintech Group, Wuhan, China).

Measurement of Inflammation-Related Proteins in Colonic Tissue

Colonic tissues were homogenized in ice-cold homogenization buffer using a high-speed low-temperature tissue grinder (Servicebio, Wuhan, China). The homogenates were centrifuged at 12,000 g for 10 minutes at 4 °C, and the supernatants were collected for the measurement of IL-6, TNF-α, MPO, and IL-10 levels following to the manufacturer's instructions (Jianglai Biology, Shanghai, China).

Measurement of Oxidation Levels in Colonic Tissue

Supernatants from colonic tissue lysates were collected for assessing the activities of catalase (CAT), superoxide dismutase (SOD), and the levels of malondialdehyde (MDA), nitric oxide (NO) using commercially available kits, following the manufacturer's instructions (Beyotime, Shanghai, China).

Network Pharmacology Analysis

Intersection Analysis of Disease and WDG Targets

TCMSP (http://tcmspw.com/tcmsp.php) was used to predict the targets of the active ingredients present in WDG. Candidate compounds from WDG with oral bioavailability (OB) ≥ 20% and drug-likeness (DL) ≥ 0.10 were selected for further analysis. To predict the targets associated with UC, the term "ulcerative colitis" was used to search in the GeneCards database (http://www.genecards.org/), OMIM (https://omim.org/), DrugBank (https://go.Drugbank.com/), and TTD (http://db.idrblab.net/ttd/) to obtain the disease target of UC. The standard gene name of the target protein was unified through the Unitprot database (http://www.Unitprot.org/). The duplicate data were removed.

Construction of PPI Network

Venny tool (2.1.0) was used to draw the Venn diagram of the "drug-disease" targets, with the intersection targets identified as the potential targets of WDG for treating ulcerative colitis. These intersection targets were imported into the STRING database (version 12.0), with a medium confidence threshold (>0.7) set for protein-protein interactions (PPI). The results were saved and subsequently imported into Cytoscape 3.7.2 for PPI network visualization.

Construction of WDG-Active Ingredients-Target Network

The active ingredients of WDG and the drug target information were imported into the Cytoscape 3.7.2 software after removing the duplication for analysis, and constructing WDG-active ingredients-target network. Network ANalyzer, the

built-in tools of Cytoscape 3.7.2, was used to analyze the topological parameters of effective compounds and target nodes in the network, including betweenness centrality, closeness centrality and degree. Based on these parameters, the core targets and the core active ingredients of the drug were identified.

Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway and Gene Ontology (GO) Analysis

The core targets were imported into the DAVID database, with the identifier set to "official gene symbol" and the species selected to "Homo sapiens". KEGG pathway and GO analysis were conducted to investigate biological processes (BP), cellular components (CC) and molecular functions (MF). The results were visualized using the Microbioinformatics platform (https://www.bioinformatics.com.cn/).

Western Blotting

Colonic tissues were homogenized in ice-cold homogenization buffer containing protease inhibitors to extract the total protein. The homogenates were centrifuged at 12,000 g for 10 minutes at 4 °C, and the supernatants were collected. The protein samples from colonic tissues were heated to 95°C for 10 minutes and separated by 10% SDS-PAGE gels. Then, proteins were transferred from the gel to PVDF membranes at 100 V for 2 hours. After transfer, the membranes were blocked with 5% non-fat milk for 1 hour at room temperature, and then incubated overnight at 4°C with primary antibody, including anti-Nrf2 (#12721, Cell Signaling Technology, Massachusetts, USA), anti-HO-1 (#43966, Cell Signaling Technology, Massachusetts, USA), anti-keap1 (80,744-1-RR, Proteintech Group, Wuhan, China) and anti-β-actin (ZB15001-HRP-100, Servicebio, Wuhan, China). After washed for three times with TBST, each in 10 minutes, the membranes were incubated with secondary antibody (Anti-mouse IgG, HRP-linked Antibody, #7076, Anti-rabbit IgG, HRP-linked Antibody, #7074, Cell Signaling Technology, Massachusetts, USA) at room temperature for 1 hour. Following three additional washes, the immunoreactive bands were detected using Tanon™ High-sig ECL Western Blotting Substrate (Tanon, Shanghai, China).

Molecular Docking

The program AutoDock was used to dock the core active ingredients in WDG to the crystal structure of Keap1/Nrf2 complex (1X2R). Using PyMOL 2.4.0 software, proteins were processed by removing water and ligands. AutoDockTools 1.5.7 was then employed to add hydrogen atoms, neutralize charges, and make other necessary modifications to the proteins. Molecular docking was performed between the proteins and ligand molecules, and binding affinity was evaluated based on the free energy of binding. Finally, PyMOL was used for further analysis and visualization.

Statistical Analysis

All data are expressed as the mean ± standard deviation (SD). Prior to statistical testing, the data were first tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test. Data that conformed to normal distribution and equal variance were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

Results

Network Pharmacological Analysis of WDG Against UC

Wuda Granules (WDG) contains Areca catechu, Panax ginseng, Amomum villosum, Prunus persica, and Lindera aggregata. As shown in Figure 1A, through the TCMSP database, 72 active components were identified in WDG, with *Areca catechu* contributing 8 compounds, *Panax ginseng* 22, *Amomum villosum* 10, *Prunus persica* 23, and *Lindera aggregata* 9. Using databases GeneCard, OMIM, DrugBank, and TTD, 3,661 gene targets related to ulcerative colitis were identified. Among these disease-associated targets, 139 were found to overlap with the compound targets of WDG. In the PPI network, nodes with darker colors and larger diameters indicate stronger associations, with the top 5 core targets identified as IL-6, TP53, AKT1, IL-1β, and TNF. IL-6, IL-1β (Figure 1B), and TNF-α are key pro-inflammatory cytokines in colitis, exacerbating intestinal barrier damage through activation of inflammatory pathways such as JAK/STAT3 and NF-κB.[27] These cytokines are significantly elevated in colitis patients, correlating with disease stage and

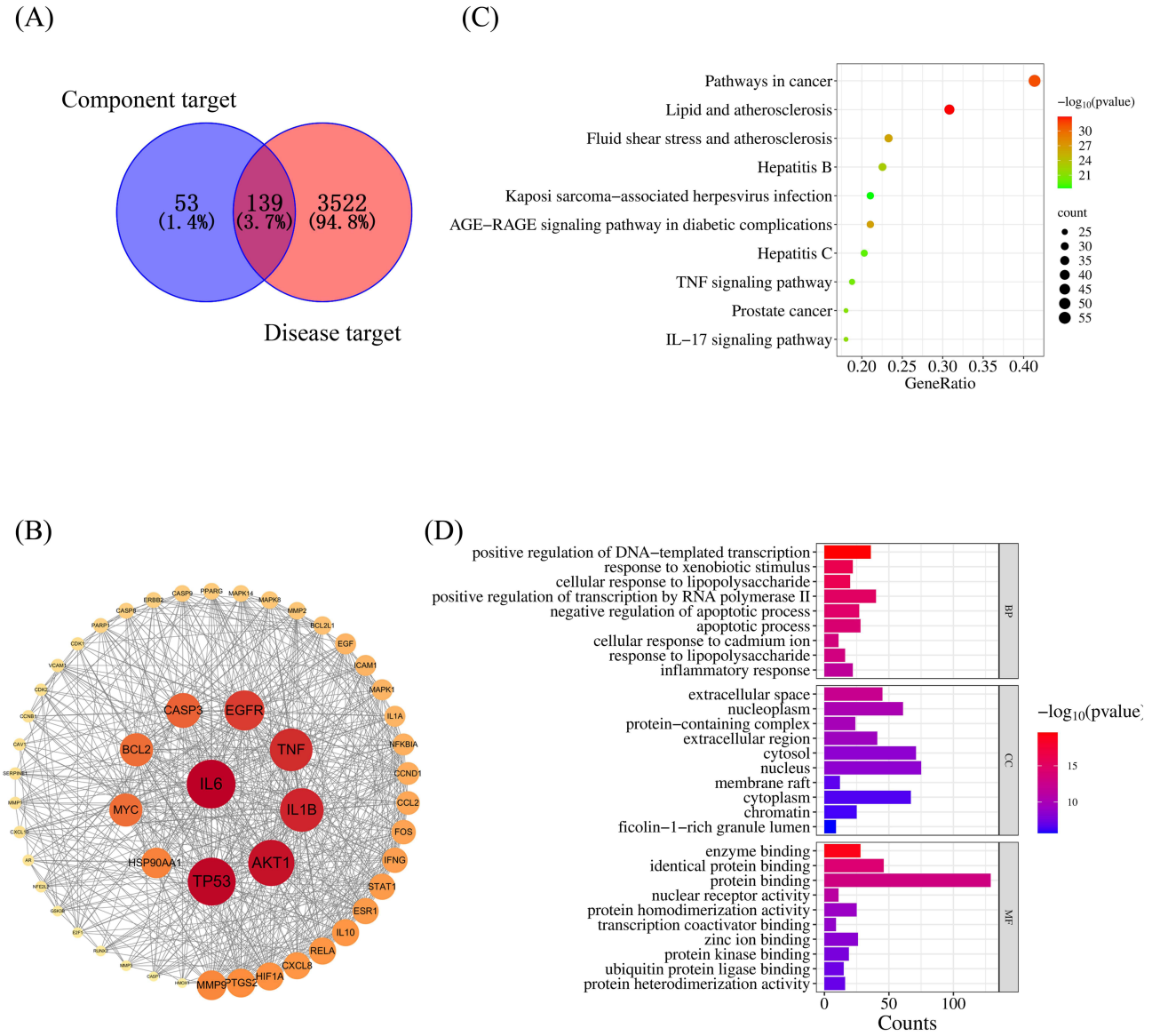
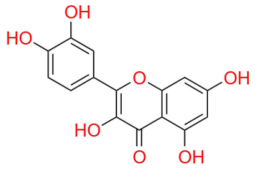
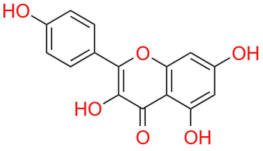
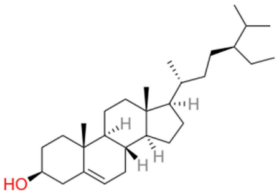
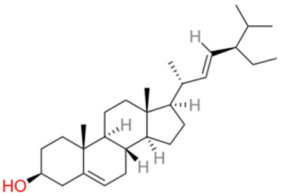
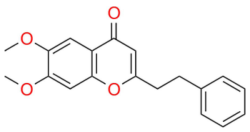


Figure 1 Network pharmacological analysis of WDG against UC. (**A**) Venn diagram of related targets of WDG and UC. The overlapping targets representing the potential therapeutic targets of WDG against UC. (**B**) PPI network of compound targets against UC. Larger and more centrally located nodes indicate proteins with higher interaction degrees, suggesting their potential role as core targets. (**C**) Bubble chart of Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment of core targets. (**D**) Bar chart of gene ontology (GO) function enrichment of core targets.

patient characteristics.[28] Top 5 core active ingredients in WDG against UC is Quercetin, Kaempferol, β-sitosterol, Stigmasterol and 6,7-dimethoxy-2-(2-phenylethyl) chromone (Table 2). Studies have shown that Quercetin, Kaempferol, β-Sitosterol, and Stigmasterol possess therapeutic potential in the treatment of colitis.[29–32] However, the potential of 6,7-Dimethoxy-2-(2-phenylethyl) chromone in the treatment of colitis remains unknown.

The intersecting targets were analyzed for KEGG pathway and GO enrichment using the DAVID database, which led to the identification of 640 biological processes (BP), 69 cellular components (CC), 149 molecular functions (MF), and 170 KEGG pathways. As shown in Figure 1C, key enriched BP terms include positive regulation of transcription by RNA polymerase II, positive regulation of DNA-templated transcription, and positive regulation of gene expression. The MF analysis indicated that WDG targets are primarily enriched in protein binding, identical protein binding, enzyme binding, and zinc ion binding. The CC analysis shows that WDG targets are mainly located in the nucleus, cytosol, cytoplasm, nucleoplasm, and extracellular matrix. The KEGG pathway analysis identified multiple signaling pathways

Table 2 Top 5 Core Active Ingredients in WDG Against UC

Rank	Ingredients	Molecule ID	Degree	Herb	2D Structure
1	Quercetin	MOL000098	134	Lindera aggregata Kosterm (Wuyao)	
2	Kaempferol	MOL000422	54	Panax ginseng C.A.Mey. (Renshen)	
3	β-sitosterol	MOL000358	31	Amomum villosum Lour. (Sharen), Panax ginseng C. A.Mey. (Renshen), Prunus persica (L.) Batsch (Taoren), Lindera aggregata Kosterm (Wuyao)	
4	Stigmasterol	MOL000449	29	Amomum villosum Lour. (Sharen), Panax ginseng C. A.Mey. (Renshen)	
5	6,7-dimethoxy-2-(2-phenylethyl) chromone	MOL010495	26	Lindera aggregata Kosterm (Wuyao)	

related to inflammation, including pathways in cancer, lipid and atherosclerosis, and fluid shear stress and atherosclerosis (Figure 1D).

WDG Improves the Survival Quality of DSS-Induced Colitis Mice

In order to investigate the effect of WDG on colitis, 2.5% DSS was used to induce colitis in the mice model, and different doses of WDG were administered by gavage. 5-ASA was used as the positive control (Figure 2A). In terms of body weight changes, all treatment groups showed a significant trend of weight loss compared to the control group. Among them, the DSS group exhibited the most severe weight loss percentage. However, the WDG groups were observed a significantly slower rate of weight loss compared to the DSS group (Figure 2B). The disease activity index (DAI) score is

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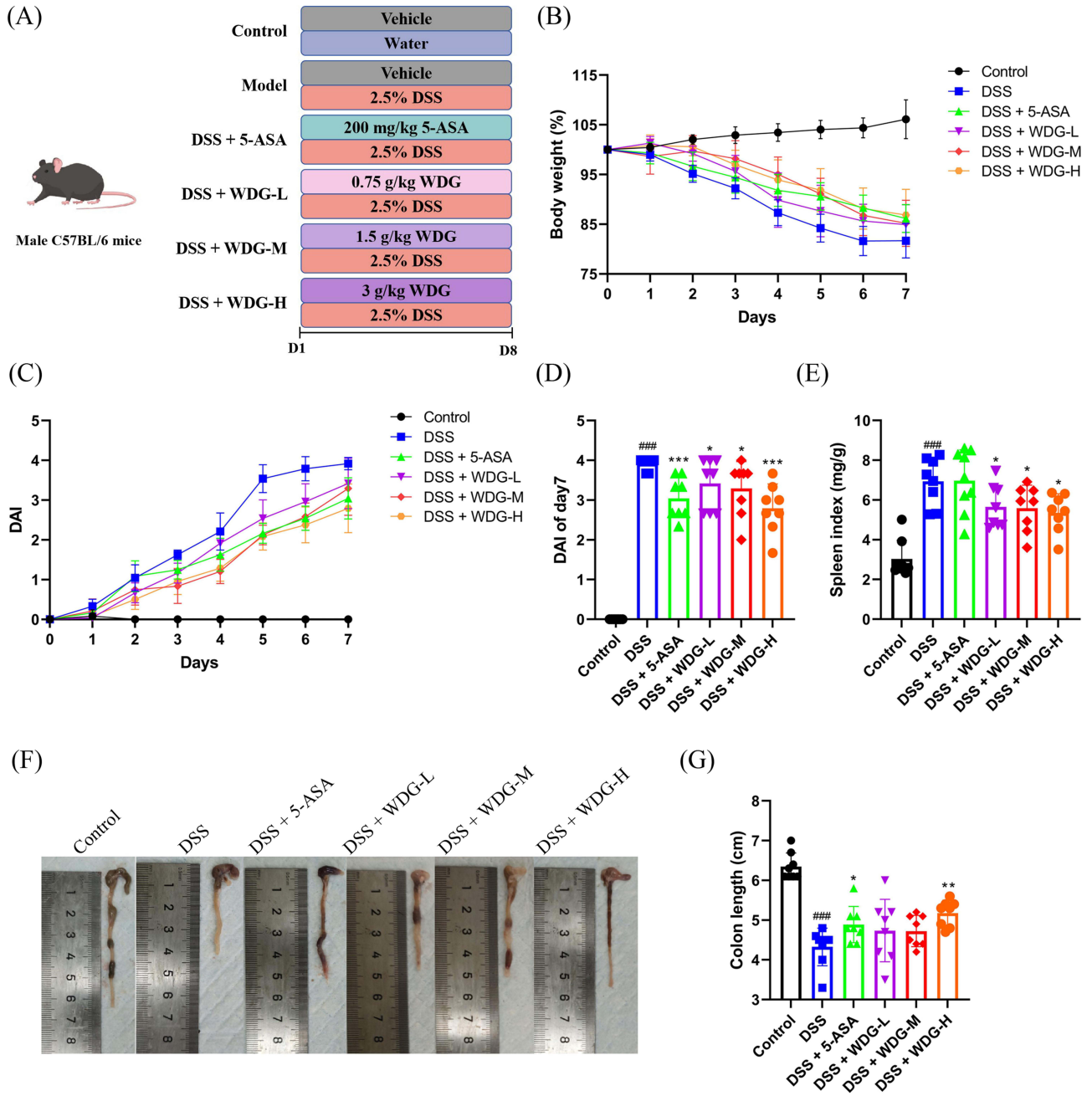

Figure 2 WDG improves the quality of life in DSS-induced colitis mice (n = 8). (**A**) The animal experimental design of this study. (**B**) Changes of body weight of mice in different treatment groups. Body weight (%) = (Body weight on day X / Body weight on day 0) * 100% (**C**) DAI score of mice in different treatment groups. (**D**) DAI score of mice in different treatment groups on day7. (**E**) spleen index of mice in different treatment groups. (**F**) The representative colon photos of mice in different groups. (**G**) The colon length of mice in different treatment groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$ vs DSS group, $^{\#\#\#}p < 0.005$ vs Control group.

used to evaluate the severity of colitis based on body weight loss, stool consistency, and the presence of rectal bleeding. The DAI scores showed the most significant increase from day 0 to day 7 in the DSS group (Figure 2C). On the final day, the WDG groups showed a significant reduction in DAI scores compared to the DSS group (Figure 2D). In parallel, the spleen index also showed a similar trend (Figure 2E). In term of colon length, the WDG group significantly attenuated the DSS-induced shortening of colon length (Figure 2F and G). The above results indicate that WDG improves the quality of life in DSS-induced colitis mice.

WDG Protects Intestinal Mucosal Layers and Alleviates Inflammation of DSS-Induced Colitis Mice

H & E staining was performed to examine the pathological changes in the colon.[33] The colon tissue from the mice in the control group was intact, with normal crypts. There was no significant inflammatory cell infiltration (Figure 3A). Following the DSS intervention, the crypts and mucosal layer of the colon tissue were destroyed, with submucosal edema and extensive infiltration of inflammatory cells. However, the structure of the colon tissue and damage symptoms were relieved after WDG treatment, with the most substantial remission observed in the DSS + WDG-H group.

To assess the anti-inflammatory effect of WDG in DSS-induced colitis mice, the levels of IL-6, IL-1β, TNF-α, MPO, IL-10 and IL-22 in colon tissues were measured by ELISA (Figure 3B–G). IL-6, IL-1β, and TNF-α are pro-inflammatory cytokines that promote inflammation and immune system activation.[34] In contrast, IL-10 and IL-22 are anti-inflammatory cytokines that regulate immune responses and suppress excessive inflammation.[35] MPO is a marker of inflammation, particularly in the damaged tissue.[36] After DSS intervention, the levels of IL-6, TNF-α, and MPO were increased, while the level of IL-10 was decreased compared to the control group. The WDG treatment significantly suppressed the upregulation of IL-6, IL-1β, TNF-α, and MPO, and increased the level of the anti-inflammatory cytokine IL-10 and IL-22 in the colon tissue. Taken together, WDG could protects intestinal mucosal layers and alleviates inflammation of DSS-induced colitis mice.

WDG Attenuates Lung Injury of DSS-Induced Colitis Mice

Under the experimental conditions, the heart, liver, and kidney tissues showed no significant pathological changes when examined with 200x HE staining, and their tissue structures remained normal (Figure 4A), indicating good safety for use. In addition, general observation revealed no apparent adverse effects in WDG-treated mice when compared to the model group. Notably, DSS intervention triggers pulmonary inflammation,[37] such as prominent inflammatory cell infiltration, thickening of alveolar walls, and alveolar structure destruction (Figure 4B). After WDG treatment, these pulmonary symptoms were alleviated. Masson staining was performed to assess the effect of compound WDG on collagen deposition in lung tissue. Compared to the control group, the DSS group exhibited a significant increase in collagen deposition in lung tissue, whereas collagen deposition was reduced following treatment with WDG. M1 macrophages, characterized by a pro-inflammatory phenotype, have been implicated in the pathogenesis of various pulmonary diseases, such as acute lung injury, acute respiratory distress syndrome, pulmonary fibrosis and chronic obstructive pulmonary disease. M1 macrophages promote the secretion of inflammatory cytokines, including IL-6, IL-1β, TNF-α, and IL-12. Inducible nitric oxide synthase (iNOS) and Arginase 1 (Arg-1) are widely recognized as biomarkers for M1 and M2 macrophages, respectively, in numerous inflammatory disorders.[38] To evaluate the effect of WDG on macrophage polarization in lung tissue, we performed immunohistochemical (IHC) staining of iNOS and Arg-1. As shown in Figure 4B, compared to the control group, iNOS expression was significantly elevated in the model group, while Arg-1 expression was reduced. Treatment with WDG resulted in a notable decrease in iNOS expression and an increase in Arg-1 expression. These findings suggest that WDG inhibits M1 macrophage activation in lung tissue, thereby alleviating DSS-induced pulmonary complications and inflammation.

WDG Protects Against Intestinal Epithelial Barrier Damage of DSS-Induced Colitis Mice

AB-PAS staining was used to assess changes in mucin-secreting cells and their secretions, providing insights into potential damage to the intestinal epithelial barrier. The AB-PAS staining results showed a reduction in the proportion of goblet cells and massive loss of mucin in the DSS group, the WDG treatment significantly increased the proportion of goblet cells and ameliorated colon injury (Figure 5A). ZO-1, occludin, MUC2, and claudin-1 were used to assess the integrity and function of the intestinal epithelial barrier. MUC2 is a mucin secreted by goblet cells that plays a protective function in the gastrointestinal tract. As a primary component of the intestinal mucus layer, it helps form a physical barrier that protects intestinal epithelial cells from pathogens, toxins, and mechanical damage.[39] According to the immunohistochemistry (IHC) results for MUC2 protein (Figure 5B), DSS significantly downregulated MUC2 expression

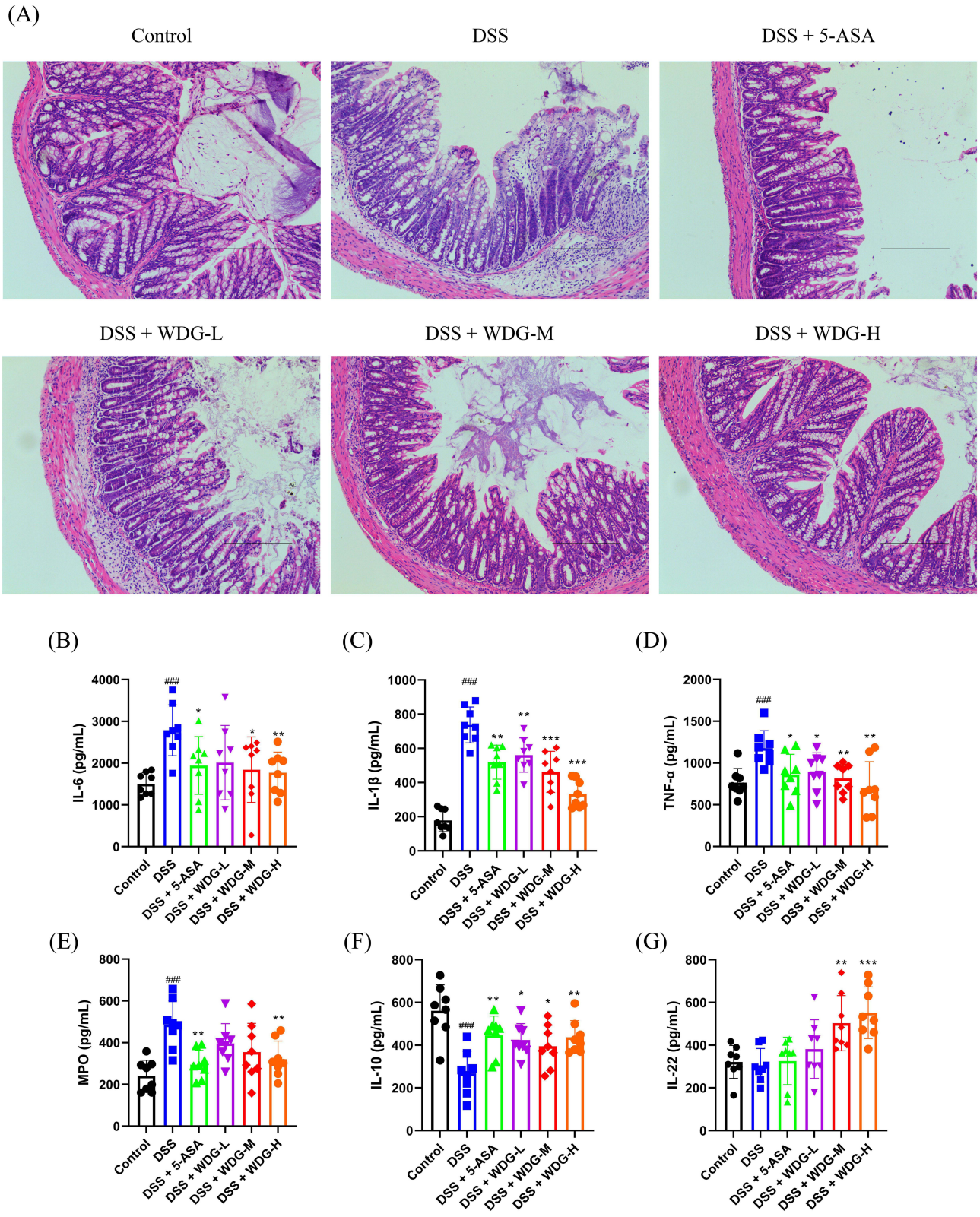

Figure 3 WDG alleviates inflammation and protects intestinal mucosal layers of DSS-induced colitis mice. (**A**) H&E staining from the colon of DSS-induced colitis mice. Scale bar, 100 μm. (**B**–**G**) Detection of inflammation-related markers (IL-6, IL-1β, TNF-α, MPO, IL-10, IL-22) in colon tissue by ELISA (n = 8). Scale bar, 100 μm. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$ vs DSS group, $^{\#\#\#}p < 0.005$ vs Control group.

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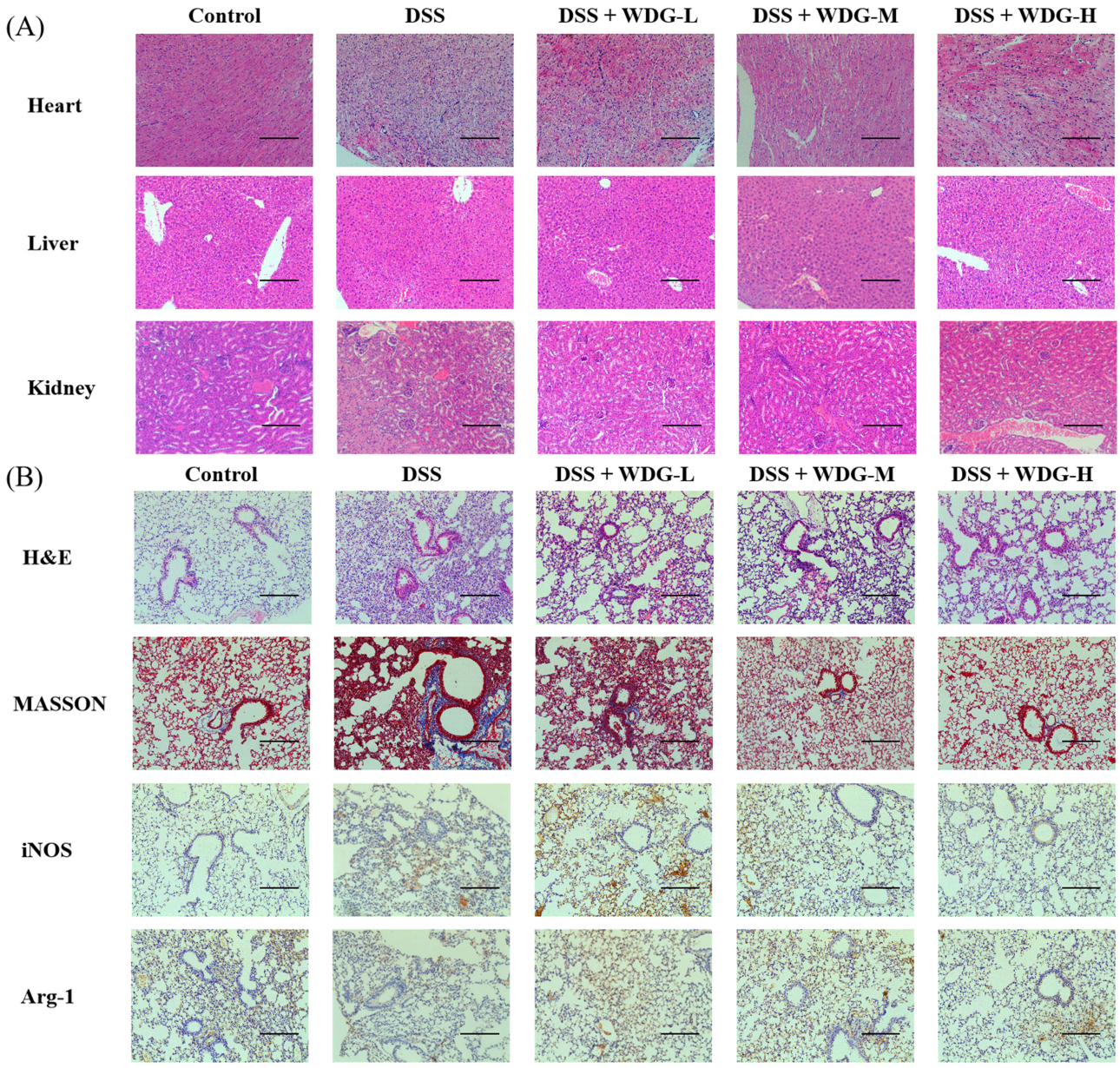

Figure 4 Histological evaluation of major organs and pulmonary inflammation after WDG treatment of DSS-induced colitis mice. (**A**) H&E staining of heart, liver and kidney tissue in DSS-induced colitis mice. No obvious histopathological changes were observed in WDG-treated groups, indicating the organ safety of WDG administration. (**B**) H&E, Masson and IHC staining of lung tissue. Scale bar, 200 μm. WDG treatment alleviated lung inflammation and fibrosis, as well as modulated macrophage polarization (reduced iNOS and increased Arg-1 expression).

compared to the control, whereas WDG treatment markedly upregulated MUC2 expression. These findings suggest that WDG may exert a protective effect by preserving mucus-secreting cell populations and maintaining mucus secretion, which are critical for the physical barrier function of the colon. Occludin, ZO-1 and claudin-1 proteins are key components of tight junction structures, playing crucial roles in regulating intercellular barrier function and permeability.[40] In the DSS group, the levels of Occludin, ZO-1 and claudin-1 proteins were significantly reduced (Figure 6A–C). After WDG treatment, the expression of these proteins was restored. These results imply that WDG improves intestinal barrier integrity by reinforcing tight junction protein expression, thereby reducing intestinal permeability and potential bacterial translocation.

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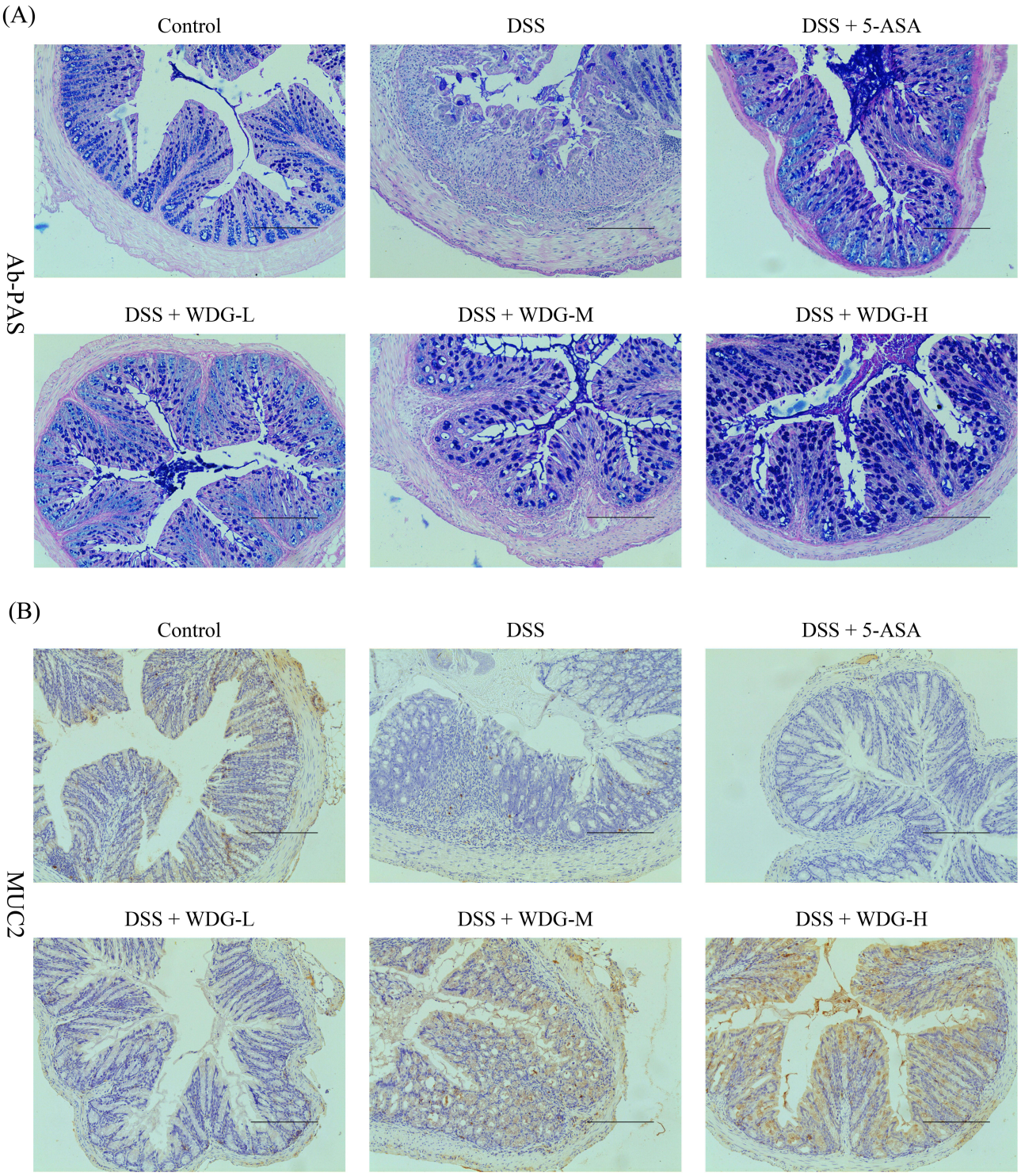

Figure 5 Effect of WDG on goblet cell preservation and MUC2 expression in the colon of DSS-induced colitis mice in different treatment groups. (**A**) AB-PAS staining of colonic tissues to visualize goblet cells. DSS-induced colitis significantly reduced goblet cell numbers, while treatment with WDG restored goblet cell abundance. (**B**) IHC staining of MUC2, a major mucin secreted by goblet cells. DSS-induced colitis reduced MUC2 expression, which was markedly improved by WDG administration. Scale bar, 100 μm.

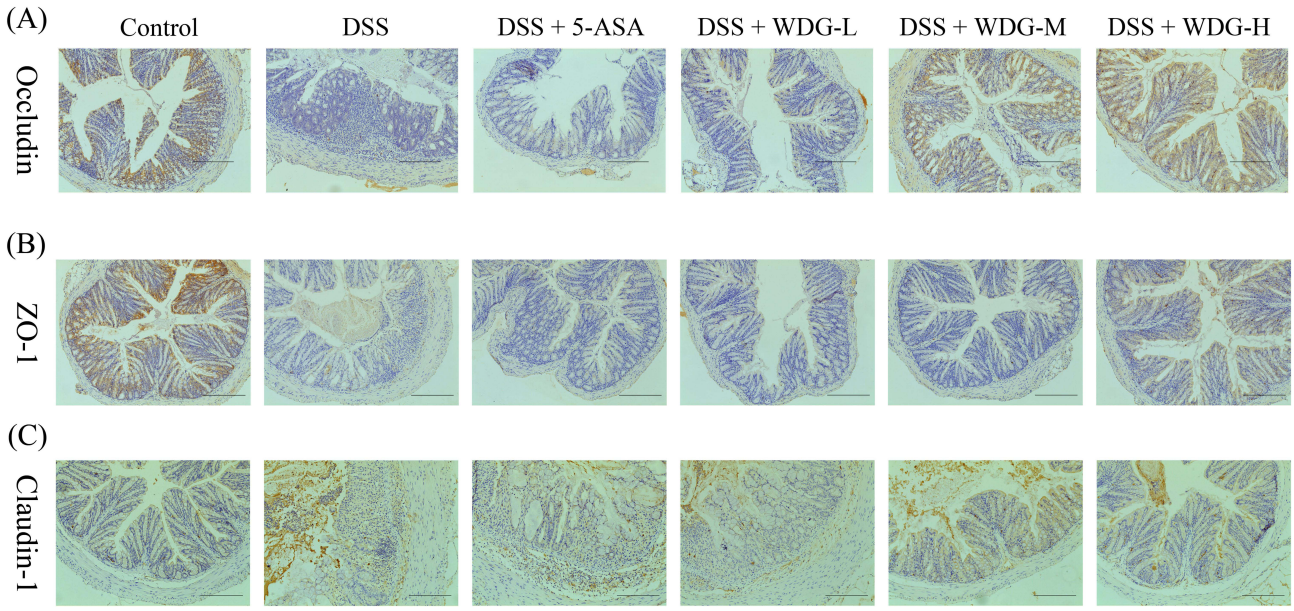

Figure 6 WDG enhances intestinal barrier integrity by restoring tight junction protein expression of DSS-induced colitis mice. (**A**) IHC staining of occludin, a key tight junction protein. DSS-induced colitis led to a marked reduction in occludin expression, while WDG treatment restored its expression. (**B**) IHC staining of ZO-1, a scaffold protein that anchors tight junction components. ZO-1 expression was disrupted in the DSS group and was significantly restored after WDG administration. (**C**) IHC staining of claudin-1, primarily distributed at the apical region of the epithelial cells, and its expression level reflects the integrity of the intestinal barrier. Scale bar, 100 μm.

WDG Reduces the Oxidative Stress and Activates the Nrf2/HO-1 Signaling Pathway of DSS-Induced Colitis Mice

To further evaluate colon damage, oxidative stress levels of colon tissues were measured.[41] CAT and SOD activities of the DSS group were lower than in the control group, while the WDG treatment alleviated this damage, with both CAT and SOD activities showing significant increased compared to the DSS group (Figure 7A and B). Additionally, MDA and NO levels in the WDG groups were significantly decreased compared to the DSS group (Figure 7C and D). The Nrf2/Keap1/HO-1 pathway regulates antioxidant gene expression, enhancing cellular defense and mitigating oxidative stress-induced damage.[42,43] The results of Western blot analysis (Figure 7E) indicated that the expression levels of Nrf2 and HO-1 in the colon tissue of the DSS group were significantly lower than those of the control group, while Keap1 expression was significantly increased in the DSS group. After WDG treatment, the expression levels of Nrf2 and HO-1 in the colon tissue significantly increased, and that of Keap1 significantly decreased (Figure 7F–H). These results indicate that WDG can enhance the antioxidant defense capability in the colon tissue and activate the Nrf2/Keap1/HO-1 pathway.

The docking results for top 5 core active ingredients in WDG with Keap1/Nrf2 complex (1X2R) were visualized, as shown in Figure 8. These ingredients were found to occupy spatial structures similar to that of Nrf2, residing within the same binding pocket (Figure 8B). Additionally, hydrogen bonding interactions were observed between all five molecules and the Keap1 protein. Specifically, Kaempferol (MOL000422) formed hydrogen bonds with SER-363, SER-602, ARG-380, and ARG-483 in the Keap1 protein. Stigmasterol (MOL000449) formed hydrogen bonds with ARG-380 in the Keap1 protein. 6,7-dimethoxy-2-(2-phenylethyl) chromone (MOL010495) formed hydrogen bonds with SER-555, SER-602, ASN-414, ARG-415, and ARG-414 in the Keap1 protein. Quercetin (MOL00098) formed hydrogen bonds with ASN-414 in the Keap1 protein. Lastly, β-sitosterol (MOL000422) formed hydrogen bonds with SER-363, SER-602, and ASN-382 in the Keap1 protein. (Figure 8C). The binding energy between both core active compounds and Keap1/Nrf2 complex is higher than −5kcal/mol (Table 3). Among them, Kaempferol had the highest binding energy (-8.1 kcal/mol). The results demonstrated that all five ingredients exhibited favorable binding affinities to the Keap1 protein. The mechanism of 6,7-Dimethoxy-2-(2-phenylethyl) chromone has not yet been reported. According to molecular docking results, the core active ingredients in WDG may activate the Nrf2/Keap1/HO-1 signaling pathway by inhibiting the binding between Keap1 and Nrf2.

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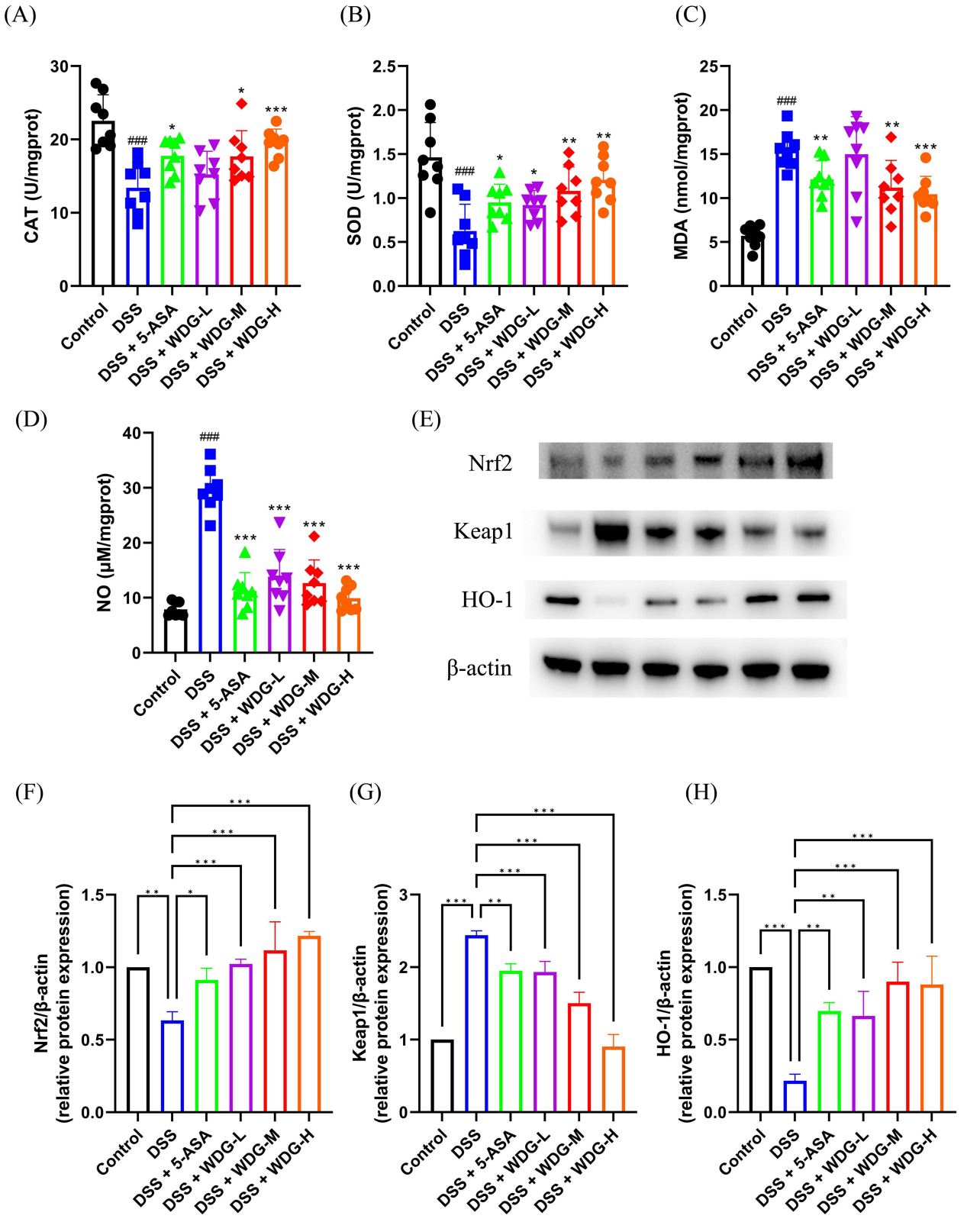

Figure 7 WDG improves the antioxidant defense system and activates the Nrf2/HO-1 signaling pathway. Effect of WDG on (**A**) catalase, (**B**) superoxide dismutase activities, and (**C**) malondialdehyde, and (**D**) nitric oxide levels in the colonic tissues. (**E**) Detection of Nrf2, Keap1, HO-1 protein expression in colonic tissues by Western blotting. (**F**–**H**) Quantitative analysis of Nrf2, Keap1 and HO-1 protein expression was performed. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$ vs DSS group, $^{\#\#\#}p < 0.005$ vs Control group.

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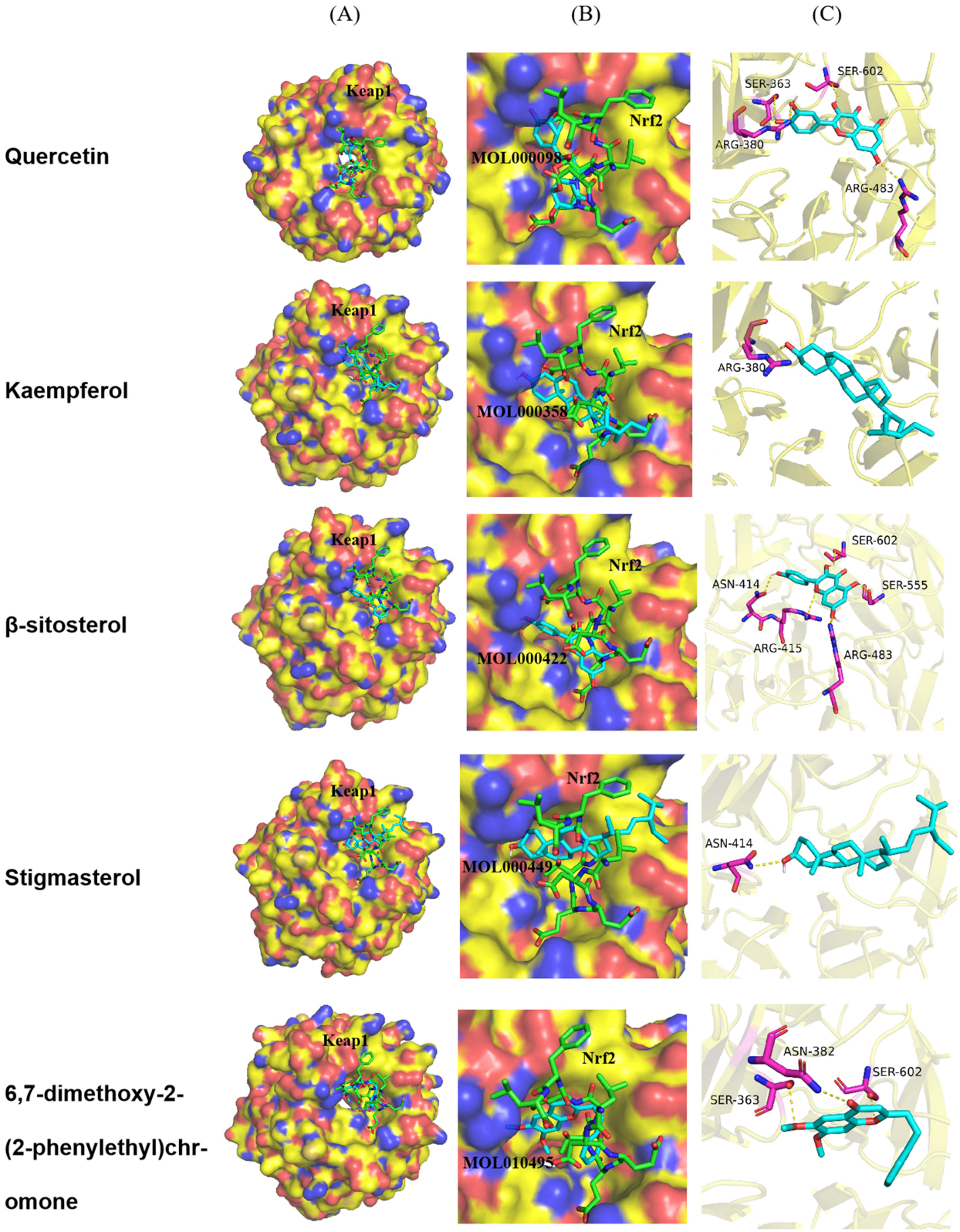

Figure 8 Docking of core active ingredients in WDG to Keap1/Nrf2 complex (PDB: 1X2R). (**A**) Schematic diagram of binding sites between compound (light blue-green sticks) and Keap1/Nrf2 complex. (**B**) Superimposed between compound and Nrf2 (green sticks). (**C**) The interaction between compound and Keap1 (purple sticks). Yellow dashed lines: Hydrogen bonds.

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Table 3 Binding Energy Between Core Active Ingredients and the Keap1/Nrf2 Complex

Rank	Molecule ID	Ingredients	Binding Energy(kcal/mol)
1	MOLO00422	Kaempferol	−8.1
2	MOLO00449	Stigmasterol	−7.9
3	MOLO10495	6,7-dimethoxy-2-(2-phenylethyl) chromone	−7.6
4	MOLO00098	Quercetin	−7.3
5	MOLO00358	β-sitosterol	−7.0

Discussion

Ulcerative colitis (UC) is a chronic inflammatory disease characterized by spontaneous inflammation and disruption of the intestinal mucosal barrier, resulting symptoms such as abdominal pain, diarrhea, and weight loss.[44] UC significantly affects patients' quality of life, and the inflammation often results in other tissue damage.[45] Patients with ulcerative colitis are at increased risk of developing primary sclerosing cholangitis, a chronic liver disease.[46] Current therapeutic options for colitis include aminosalicylates, corticosteroids, immunosuppressants and biologics. However, these treatments may lead to severe side effects and, in some cases, lose effectiveness over time.[47] Therefore, there is a growing need for alternative treatments.

Traditionally, WDG has been used to manage gastrointestinal motility disorders and related symptoms. By promoting gastrointestinal motility and reducing inflammation levels, WDG shows promise in alleviating these conditions.[18,19] The multi-active components of WDG enable it to target multiple molecular pathways involved in the inflammatory process, making it a potentially effective option for colitis treatment (Figure 1). Top 5 core active ingredients in WDG against UC is Quercetin, Kaempferol, β-sitosterol, Stigmasterol and 6,7-dimethoxy-2-(2-phenylethyl) chromone (Table 2). Quercetin ameliorates ulcerative colitis through multiple mechanisms, including activating the aryl hydrocarbon receptor to enhance intestinal barrier integrity by downregulating the cGAS-STING pathway and restoring the M2/M1 macrophage balance.[48,49] Transcriptomic analysis of colon tissue revealed that quercetin modulates several key genes, with the ERK1/2-FKBP and RXR-STAT3 pathways playing roles in the progression of IBD.[50] Kaempferol reduces IL-8 secretion and restores barrier function in a LPS-induced epithelial-endothelial coculture cell model by inhibiting the activation of the NF-κB signaling pathway.[51] In high-fat diet-induced mice, kaempferol alleviates obesity-related metabolic disorders, intestinal inflammation, and gut dysbiosis by enhancing barrier function and inhibiting the TLR4/NF-κB pathway. It also improves colitis by targeting the LPS-TLR4-NF-κB axis.[30,52] β-Sitosterol targets pathogenic bacteria, reduces colon shortening, macroscopic scores, and myeloperoxidase activity in colitis models. It inhibits proinflammatory cytokines production, COX-2 expression, and NF-κB activation.[53,54] Additionally, by suppressing NLRP3/Caspase-1/GSDMD-mediated pyroptosis and inflammation, β-sitosterol alleviates ulcerative colitis.[31] Stigmasterol alleviates colitis by restoring the Treg/Th17 cell balance through activation of the butyrate-PPARγ axis.[32] The potential of 6,7-Dimethoxy-2-(2-phenylethyl) chromone in the treatment of colitis remains unknown.

The intestinal epithelial barrier functions as the primary defense mechanism against luminal antigens, pathogens, and toxins.[55] This barrier is maintained by a mucus layer secreted by goblet cells and tight junction proteins that regulate epithelial permeability. The mucus layer, composed primarily of mucins such as MUC2, provides a physical and biochemical barrier to microbial invasion.[56] In DSS-induced colitis, the depletion of goblet cells, and the reduction in mucin production lead to increased intestinal permeability and heightened immune activation.[57,58] Tight junction proteins include key components such as Claudins, Occludin, Junctional Adhesion Molecules (JAMs), Zonula Occludens (ZO) proteins, Tricellulin, and MarvelD3, which collectively affect cell-cell adhesion and barrier integrity.[59] Our results showed that WDG treatment restored goblet cell populations and MUC2 expression, as confirmed by AB-PAS staining and immunohistochemistry (Figure 4). Additionally, WDG upregulated the expression of Occludin, ZO-1 and claudin-1 (Figure 5). By increasing both mucus secretion and tight junction proteins, WDG mitigates the structural and functional disruption of the intestinal barrier, which is a key contributor to the pathogenesis of colitis.

Inflammation is mediated by the dysregulated activation of immune responses. Pro-inflammatory cytokines such as IL-6, TNF-α and IL-1β are central to the amplification of inflammatory signaling pathways. These cytokines recruit

immune cells, sustain the inflammatory response, and contribute to tissue damage. Additionally, MPO, an enzyme produced by activated neutrophils, is a biomarker of inflammation. The balance between pro-inflammatory and anti-inflammatory mediators, such as IL-10, is crucial for maintaining intestinal immune homeostasis. WDG treatment significantly suppressed the levels of pro-inflammatory markers (IL-6, TNF-α, IL-1β and MPO) while upregulating IL-10 and IL-22 expression, indicating its robust anti-inflammatory effects (Figure 3B–G). Furthermore, systemic inflammation, as evidenced by DSS-induced pulmonary inflammation, was ameliorated by WDG (Figure 3F), suggesting its potential to address the broader systemic effects of colitis through gut-lung axis modulation. Subclinical nasal and lung lymphocytosis is observed in ulcerative colitis patients, correlating with the severity of intestinal inflammation.[60] Lung injury, including cough, shortness of breath and expectoration, is a common extraintestinal complication of ulcerative colitis, highlighting the need for simultaneous treatment of the lungs and large intestine, as suggested by Traditional Chinese Medicine.[61]

Oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense systems, is a key contributor to various diseases.[62] It is worth noting that nitric oxide (NO) exhibits dual roles under pathological conditions. At high concentrations, NO reacts with superoxide anions to form peroxynitrite ($ONOO^-$), thereby exacerbating oxidative stress and tissue injury. In addition, excessive NO can activate pro-inflammatory signaling pathways, such as NF-κB, leading to the release of inflammatory cytokines and the aggravation of inflammatory responses. The Nrf2/Keap1/HO-1 pathway is a frontline defense mechanism preventing oxidative damage to proteins, lipids, and DNA. In liver ischemia/reperfusion injury, Nrf2 activation mitigates oxidative stress-induced cytotoxicity and promotes hepatic recovery.[63] Similarly, in neurodegenerative diseases such as Alzheimer's disease and amyotrophic lateral sclerosis, the activation of Nrf2 helps reduce oxidative damage and slow neuronal degeneration, thereby inhibiting disease progression.[64] In addition to its antioxidant properties, the Nrf2/Keap1/HO-1 pathway plays a vital role in mitigating inflammation, such as colitis, rheumatoid arthritis, and asthma.[65] In colitis mice models, the protective effects of Moringin were lost in Nrf2 knockout (Nrf2-/-) mice.[66] Modulation of the Nrf2/Keap1/HO-1 pathway has been identified as a promising strategy for alleviating oxidative stress in colitis.[67]

Nrf2 is a transcription factor that regulates the expression of a wide range of cytoprotective genes in response to oxidative and electrophilic stress.[68] Under basal conditions, Nrf2 is sequestered in the cytoplasm by Keap1, which targets it for ubiquitination and subsequent proteasomal degradation. However, upon exposure to oxidative stress, Keap1 undergoes conformational changes or becomes inactivated, leading to the stabilization and nuclear translocation of Nrf2.[69] Once translocated to the nucleus, Nrf2 binds to antioxidant response elements (AREs) in the promoter regions of target genes, such as HO-1, NQO1 and GSH, to regulate their expression.[70] HO-1, a downstream effector of Nrf2, is an inducible enzyme that catalyzes the degradation of heme into carbon monoxide, biliverdin and Fe^{2+}.[71] These byproducts are not only crucial for cellular metabolism but also exert potent anti-inflammatory, antioxidant, and cytoprotective effects. Biliverdin is rapidly converted to bilirubin, a strong antioxidant, while CO has anti-inflammatory and anti-apoptotic properties.[72,73]

WDG may exert additional pharmacological effects through other mechanisms, such as modulation of the gut microbiota or regulation of apoptotic pathways, which were not fully addressed in the current study. To support the clinical application of WDG in colitis treatment, further studies are required to assess their long-term efficacy and safety profile. In addition, clinical trials in colitis patients have not yet been conducted. These limitations highlight the need for future investigations to validate the therapeutic potential and translational applicability of WDG in colitis treatment.

Conclusion

WDG alleviated clinical symptoms and histopathological damage, suppressed inflammatory responses, restored intestinal barrier function, and enhanced antioxidant capacity. These effects were closely associated with the activation of the Nrf2/Keap1/HO-1 signaling pathway. Network pharmacology and molecular docking further supported the involvement of core targets such as IL-6, TNF, and AKT1, and identified 6,7-Dimethoxy-2-(2-phenylethyl) chromone as a potential bioactive compound. This study provides a theoretical foundation for the clinical application of WDG in the treatment of colitis. Additional studies, including further mechanistic investigations, long-term safety assessments and clinical trials, are required to validate the therapeutic potential and translational value of WDG.

Abbreviations

5-ASA, 5-aminosalicylic acid; AB-PAS, Alcian Blue-Periodic Acid Schiff Staining; ARE, Antioxidant Response Elements; BP, Biological Processes; CAT, Catalase; CO, Carbon Monoxide; CC, Cellular Components; DAI, Disease Activity Index; ELISA, Enzyme-Linked Immunosorbent Assay; GPx, Glutathione Peroxidase; HO-1, Heme Oxygenase-1; H&E, Hematoxylin and Eosin; IHC, Immunohistochemistry; IBD, Inflammatory Bowel Diseases; IL-10, Interleukin-10; IL-6, Interleukin-6; JAMs, Junctional Adhesion Molecules; Keap1, Kelch-Like ECH-Associated Protein 1; KEGG, Kyoto Encyclopedia of Genes and Genomes; MDA, Malondialdehyde; MUC-2, Mucin-2; MPO, Myeloperoxidase; NF-κB, Nuclear Factor-Kappa B; NLRP3, NOD-like receptor family pyrin domain containing 3; NO, Nitric Oxide; NQO1, NAD(P)H Quinone Oxidoreductase 1; Nrf2, NF-E2-Related Factor 2; ROS, Reactive Oxygen Species; SOD, Superoxide Dismutase; SPF, Specific Pathogen-Free; TCM, Traditional Chinese Medicine; TJP, Tight Junction Protein; TNF-α, Tumor Necrosis Factor-Alpha; UC, Ulcerative Colitis; UPLC-QqQ-MS/MS, Ultrahigh-Performance Liquid Chromatography Coupled with Triple Quadrupole Mass Spectrometry; WDG, Wuda Granules; ZO, Zonula Occludens.

Ethics Approval

The Institutional Ethical Committee for Animal Research of Institute of Zoology, Guangdong Academy of Science approved the animal experiments (No. GIZ20240408-02), which were carried out in compliance with the ARRIVE guidelines. The animal experiments were conducted in accordance with the Guiding Opinions on the Humane Treatment of Laboratory Animals issued by the Ministry of Science and Technology of the People's Republic of China, as well as the national standard GB/T 35892-2018 entitled Laboratory Animal—Guidelines for Ethical Review of Animal Welfare.

This study involves human data obtained from public databases. As GeneCards, OMIM, DrugBank, and the Therapeutic Target Database are all publicly accessible resources that allow users to freely download data for research and publication purposes. The above research content is exempt from ethical approval based on national legislation, specifically item 1 and item 2 of Article 32 of the *Ethical Review Measures for Life Science and Medicine Research involving Humans* (promulgated on February 18, 2023, China).

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Disclosure

The authors report no conflicts of interest in this work.

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