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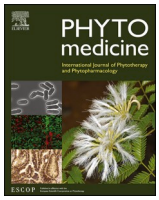

Original Article

Qi-Huang decoction alleviates DSS-induced colitis with *Candida albicans* dysbiosis by enhancing innate immune response through Dectin-1-associated signaling

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ABSTRACT

Background: Ulcerative colitis (UC) is a chronic inflammatory disease of the gastrointestinal tract. *Candida albicans*, a common commensal fungus in the human gut, has been increasingly implicated in UC pathogenesis. Qi-Huang decoction (QHD), a traditional Chinese herbal formula known for its spleen-invigorating and purgative properties, is commonly used to restore gastrointestinal function.

Purpose: This study investigates the therapeutic potential of QHD in treating colitis exacerbated by *C. albicans* and explores the underlying mechanisms of action.

Methods: A mouse model of colitis was induced using dextran sulfate sodium combined with gavage of *C. albicans*. Following QHD treatment, colitis severity was evaluated by measuring survival rate, body weight, disease activity index, colon length, and fungal burden, and through histopathological analysis using hematoxylin-eosin staining. The expression of proinflammatory genes IL-1β and TNF-α was quantified, alongside protein levels of key molecules involved in Dectin-1 signaling, including Syk, CARD-9, NLRP-3, Raf-1, and NF-κB. Barrier integrity markers, such as Occludin and Claudin-1, were also examined. To further elucidate QHD's mechanisms, Dectin-1 was inhibited using laminarin. In vitro experiments assessed QHD's antifungal activity against three *Candida* strains through microdilution, spot assays, and time-kill tests. RAW 264.7 macrophages were employed to study the exposure of fungal cell wall β-glucan and subsequent phagocytosis. Molecular docking simulations predicted interactions between QHD's active compounds and the Dectin-1 receptor.

Results: QHD significantly mitigated colitis severity and reduced fungal burden in vivo. QHD enhanced β-glucan exposure on the fungal cell wall, thereby stimulating phagocytosis by RAW264.7 macrophages. QHD effectively activated Dectin-1-mediated signaling pathways and increased proinflammatory levels in RAW 264.7 cells. In colitis mice, QHD treatment markedly reduced inflammation and Dectin-1 signaling following fungal clearance. However, Dectin-1 inhibition with LAM neutralized QHD's therapeutic effects, highlighting the pathway's importance in mediating QHD's efficacy. Interestingly, QHD alone elevated Dectin-1, NF-κB, TGF-β, and IL-10

Abbreviations: ANOVA, analysis of variance; BSA, bovine serum albumin; C. albicans, Candida albicans; CARD-9, caspase recruitment domain protein 9; CFU, colony-forming units; CLEC7A, C-type lectin domain containing 7A; CLSI, Clinical and Laboratory Standards Institute; DAI, disease activity index; Dectin-1, dendritic cell-associated C-type lectin-1; DMEM, Dulbecco's modified Eagle medium; DSS, dextran sulfate sodium; HE staining, hematoxylin-eosin Staining; IDA, information-dependent acquisition; IL-1β, interleukin-1 beta; IBD, inflammatory bowel disease; LAM, laminarin; MES, mesalazine; MS, mass spectrometry; MIC, minimum inhibitory concentration; MOI, multiplicity of infection; NF-κB, nuclear factor kappa-B; NLRP-3, NOD-like receptor protein 3; PBS, phosphate-buffered saline; QHD, Qi-Huang decoction; RPMI, Roswell Park Memorial Institute; S1PR, sphingosphine 1-phosphate receptor; SD, standard deviation; SDS, sodium dodecyl sulfate; Syk, spleen tyrosine kinase; TCMSP, traditional Chinese medicine systems pharmacology database and analysis platform; T-K test, time-killing test; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TNF-α, tumor necrosis factor-α; UC, ulcerative colitis; UHPLC, ultra-high-performance liquid chromatography; WHO, World Health Organization; YPD, yeast peptone dextrose.

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levels, whereas reduced IL-1β and TNF-α expression, suggesting a dual modulatory role in inflammation. Molecular docking confirmed a potential direct interaction between QHD's bioactive components and the Dectin-1 receptor.

Conclusion: QHD demonstrates promising therapeutic potential for managing *Candida* colitis by modulating immune responses and targeting Dectin-1 signaling pathways in clinical settings.

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) that predominantly affects the rectum and colon, often leading to significant morbidity. Recent estimates suggest a global prevalence of approximately 5 million cases in 2023 (Le Berre et al., 2023). UC has a multifactorial etiology, involving genetic predispositions, environmental triggers, dysbiosis of gut microbiota, and a dysregulated immune response (Le Berre et al., 2023). Although intestinal fungi (mycobiota) account for only 0.01%–0.1% of the gut microbiome (Li et al., 2019), increasing evidence suggests that mycobiota dysbiosis is more pronounced in IBD patients compared with healthy individuals (Sokol et al., 2017).

Candida albicans is one of the most prevalent opportunistic dimorphic fungi in the human gut and has been classified as a "critical priority pathogen" by the World Health Organization (WHO) (https://www.who.int/publications/i/item/9789240060241). *C. albicans* is estimated to constitute approximately 40%–80% of *Candida* isolates in hospital-associated infections and is a primary agent of mucosal infections (e.g., oropharyngeal candidiasis and vulvovaginal candidiasis) and systemic diseases such as candidiemia (Katsipoulaki et al., 2024). Beyond infections, *C. albicans* overgrowth has been linked to severe health conditions, including cancer and neurological disorders such as Alzheimer's disease (Wang et al., 2023; Wu et al., 2023). In UC, recent studies have highlighted a high genetic diversity of *C. albicans* in the colonic mucosa of affected patients, with elevated *Candida* spp. detected in those with active endoscopic disease compared with those in remission (Hsia et al., 2023; Li et al., 2022c). Murine models of colitis induced by chemical agents such as dextran sulfate sodium (DSS) and 2,4,6-trinitrobenzene sulfonic acid (TNBS) have shown that exogenous gavage of *C. albicans* exacerbates colitis. Similarly, overcolonization by *C. albicans* disrupts the intestinal microbial balance and alters gut microRNA profiles, further aggravating disease severity (Cheng et al., 2023; Xu et al., 2024).

Although traditional UC therapies such as 5-aminosalicylic acid drugs and corticosteroids remain mainstays, newer alternatives like Janus kinase inhibitors (e.g., tofacitinib), sphingosphine 1-phosphate receptor (S1PR) modulators (e.g., ozanimod and etrasimod), and selective IL-23 antagonists (e.g., mirikizumab) have expanded the therapeutic arsenal (Chang et al., 2024; Fudman et al., 2024). However, these treatments are often associated with significant side effects, including cardiovascular risks, thromboembolism, bacterial and viral infections, and skin conditions like acne, which restrict their full therapeutic efficiency (Fudman et al., 2024). Furthermore, whether these drugs effectively address *Candida*-associated UC remains unclear, underscoring the urgent need for alternative therapies targeting fungi-associated UC.

Qi-Huang decoction (QHD), (formulated by Prof. Qingsheng Yu at The First Affiliated Hospital of Anhui University of Chinese Medicine), is a traditional Chinese herbal remedy comprising eight herbs: *Astragalus membranaceus, Rheum officinale, Lagehead atractylodes* rhizome, *Codonopsis pilosula, Fructus aurantii immaturus, Mangnolia officinalis, Salvia Miltrorrhiza,* and *Radix scutellariae*. QHD is derived from two classical formulations: Buzhong Yiqi decoction from *Treatise on the Spleen and Stomach* (1249 A.D.) by Dongyuan Li and Dachengqi decoction from *Treatise on Febrile Diseases* (200–210 A.D.) by Zhongjing Zhang respectively. Prof. Yu and colleagues have demonstrated that QHD enhanced the recovery of intestinal immune barriers, enteric nervous system functions, and gastrointestinal motility in gastrectomized rats (Peng et al., 2018; Qi et al., 2022). Clinically, it is widely employed to aid gastrointestinal recovery following gastrectomy. This study investigates the therapeutic effects of QHD in a DSS-induced murine colitis model with *C. albicans* infection. By examining its ability to alleviate colitis and exploring the underlying mechanisms, this study aims to establish an experimental foundation for QHD's potential clinical application in managing *Candida*-associated UC.

Materials and methods

Strains and cells

The *C. albicans* SC5314 strain was kindly provided by Prof. Yuanying Jiang from the Second Military Medical University (Shanghai, China). Fluconazole-resistant clinical strains *C. albicans* Z4935 and Z5172 were obtained from the Dermatology Department of the First Affiliated Hospital of Anhui University of Chinese Medicine (Hefei, China). The strains were initially cultured overnight at 37 °C in liquid Sabouraud medium (HB0379, Hopebio, Qingdao, China) and subsequently propagated in yeast peptone dextrose (YPD) broth (HB5193–1, Hopebio) at 37 °C for 12–16 h to attain the exponential growth phase. RAW264.7 cells were procured from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China). These cells were routinely cultured at 37 °C in a humidified atmosphere with 5% CO_2. The culture medium used was Dulbecco's modified Eagle medium (DMEM, ca002–500 mL, SparkJade, Shandong, China), supplemented with 10% fetal bovine serum (KY-01000S, Kangyuan Biologicals, Anhui, China) and 1% penicillin/streptomycin (C0222, Beyotime, Shanghai, China) for proper propagation.

Animals

Female C57BL6/J mice (age: 6–8 weeks, weight: 18–22 g, License: SCXK2024–0004) and male Sprague Dawley (SD) rats (age: 6–8 weeks, weight: 180–220 g, License: SCXK2020–0001) were purchased from Changsheng (Liaoning, China). All animals were housed under controlled conditions with a 12-h light-dark cycle and were provided ad libitum access to tap water and standard rodent chow during a 7–10 day acclimatization period. All experimental procedures and housing conditions were approved by the Animal Ethics Committee of Anhui University of Chinese Medicine (Animal Ethics Number: AHUM-mouse-2,024,172, AHUM-rats-2,024,166, issued October 23, 2024). For animal maintenance and treatment, the study complied with the principles of the Institutional Animal Ethics Committee of the Chinese Center for Disease Control and Prevention and conformed to the Chinese National Guidelines on the Care and Use of Laboratory Animals.

Drug serum preparation

QHD was prepared using 234 g of eight medicinal herbs: *Astragalus membranaceus* (40 g), *Rheum officinale* (20 g), *Lagehead atractylodes* rhizome (40 g), *Codonopsis pilosula* (40 g), *Fructus aurantii immaturus* (20 g), *Mangnolia officinalis* (20 g), *Salvia Miltrorrhiza* (30 g), and *Radix Scutellariae* (24 g). As described previously, the herbal mixture was soaked in 500 mL of distilled H_2O and boiled for approximately 30 min (Peng et al., 2018; Qi et al., 2022). After filtration, the decoction was concentrated to a final concentration of 1.0 g/mL and stored at 4 °C until use. To prepare drug serum, 40 SD rats were evenly divided into four groups and administrated QHD intragastrically at doses of 2.5, 5, and 10

g/kg daily for 7 consecutive days. Following QHD treatment, the animals were anesthetized with an intraperitoneal injection of 2 mL urethane ($C_3H_7NO_2$, 200 mg/mL). Blood was collected via the abdominal aorta, left at room temperature for 2 h, and centrifuged at 3000 rpm for 15 min. The serum was pooled, heat-inactivated in a water bath (56 °C for 30 min), filtered through a 0.22-μm filter, and stored at −80 °C for subsequent experiments.

UHPLC-MS/MS analysis

The chemical composition of QHD was analyzed through ultrahigh-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) conducted by Biotree (Shanghai, Chinese). The analysis employed a Vanquish UHPLC system (Thermo Fisher Scientific) equipped with a Waters UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm). The sample operated at a flow rate of 0.4 mL/min with a sample injection volume of 5 μL. The mobile phase consisted of 0.1% formic acid in water (phase A) and 0.1% formic acid in acetonitrile (phase B). The multi-step linear elution gradient was as follows: 0–3.5 min, 95%–85% A; 3.5–6 min, 85%–70% A; 6–6.5 min, 70%–70% A; 6.5–12 min, 70%–30% A; 12–12.5 min, 30%–30% A; 12.5–18 min, 30%–0% A; 18–25 min, 0%–0 % A; 25–26 min, 0%–95% A; 26–30 min, 95%–95% A. MS and tandem mass spectrometry (MS/MS) data were acquired using an Orbitrap Exploris 120 MS instrument coupled with Xcalibur software in an information-dependent acquisition mode. During each acquisition cycle, the mass range was 100–1500 *m/z*, and the top four of every cycle were screened and the corresponding MS/MS data were further acquired. The MS conditions were as follows: sheath gas flow rate: 30 Arb, aux gas flow rate: 10 Arb, ion transfer tube temperature: 350 °C, vaporizer temperature: 350 °C, full mass resolution: 60,000, MS/MS resolution: 15,000, collision energy: 16/32/48 in NCE mode, and spray voltage: 5.5 kV (positive) or −4 kV (negative).

Antifungal tests in vitro

Microdilution test

The minimum inhibitory concentrations (MICs) of QHD against planktonic cells were determined using 96-well flat-bottomed microplates, following the Clinical and Laboratory Standards Institute (CLSI) M60 protocol (Pfaller et al., 2020). Serial two-fold dilutions of QHD were prepared at concentrations ranging from 2–1024 μg/mL. Fluconazole at 0.25–128 μg/mL for SC5314 and 2–1024 μg/mL for Z4935 and Z5172 was used as positive control. Candida cells were inoculated at an initial density of 2×10^3 cells/mL and incubated with the drugs at 37 °C for 24 h. The MIC was defined as the lowest drug concentration that visibly inhibited cell growth.

Time-killing (T-K) detection

Candida cells were prepared at a concentration of 2×10^3 colony-forming unit (CFU)/mL and co-incubated with QHD at final concentrations of 256, 512, and 1024 μg/mL in a shaker set at 37 °C. At predetermined time intervals (0, 2, 4, 8, 16, and 24 h), aliquots of the fungal solution were centrifuged at 3000 rpm for 5 min, washed three times with sterile PBS, and serially diluted. The diluted fungal solutions were plated on YPD agar (Hopebio) containing 100 U/mL penicillin and 0.1 mg/mL streptomycin (Biosharp, Shanghai, China). After 48 h of incubation at 37 °C, the number of colonies was counted to determine CFU/mL.

Spot assay

QHD was dissolved in YPD medium at final concentrations of 256, 512, and 1024 μg/mL. *Candida* cells were spotted in 5 μL volumes onto the YPD medium in serial dilutions corresponding to inoculum sizes of 1×10^2, 1×10^3, and 1×10^4 CFU/mL. Plates were incubated at 37 °C for 48 h, and fungal growth was visually recorded.

DSS-induced colitis model

The DSS-induced colitis model was established in C57BL6/J mice by following our previous methods (Xu et al., 2024). The mice were orally given 3% DSS (Zeping, Beijing, China) in drinking water daily, while *C. albicans* SC5314 (1×10^8 CFU per animal) was intragastrically administered via gavage on days 1, 3, 5, and 7 in the model group. Experimental groups received either QHD (10, 20, and 40 g/kg), mesalazine (MES, 0.2 g/kg), laminarin (LAM, 300 mg/kg, Sigma-Aldrich; Merck KGaA; Dectin-1 receptor antagonist) or their combinations. Sham-treated mice received only saline. QHD and MES were intragastrically administered daily via oral gavage, while LAM was injected intraperitoneally for 2 consecutive days before DSS exposure. In the established colitis model, disease activity index (DAI), body weight, and colon length were measured to evaluate colitis severity, as described elsewhere (Xu et al., 2024).

Hematoxylin-eosin staining

The experimental procedures were performed as described previously with fewer modifications (Xu et al., 2024). Briefly, colonic tissues were fixed in 10% formalin (Zhenwo Biomedical, Guangzhou, China), embedded in paraffin (Haotian, Jiangsu, China), and sectioned at a thickness of 4 μm. The sections were stained with hematoxylin-eosin (HE) (Leagene, Beijing, China) following standard procedures. Pathological changes were observed under an optical microscope (Olympus BX51, Japan).

Fungal burden

Colonic tissues were cut and homogenized. Fecal samples were suspended in sterile PBS and centrifuged at 3000 rpm for 5 min. RAW264.7 cells were washed twice with sterile PBS and lysed with 2% sodium dodecyl sulfate (Macklin, Shanghai, China) for 10 min at room temperature. Sample solutions containing *Candida* were plated on YPD agar supplemented with 100 U/mL penicillin and 0.1 mg/mL streptomycin. The plates were incubated at 37 °C for 48 h. Fungal burden was quantified and recorded as CFU/gram.

β-glucan exposure

C. albicans (1.8×10^6 CFU/mL) was incubated with QHD at concentrations of 1, 0.1, 0.01, 0.001, and 0.0001 g/mL, or with caspofungin at 0.005 μg/mL, in RPMI-1640 medium at 37 °C overnight. The fungal cells were collected through centrifugation at 3000 rpm for 5 min, washed with sterile PBS, and blocked with 2% bovine serum albumin (BSA, 9048–46–8, BioFRoxx, Shanghai, China) for 1 h. The cells were then incubated sequentially with a monoclonal anti-β-glucan antibody (1:300, 400–2, Bioscience Supplies, Australia) for 4 h and Cy3-labeled goat anti-mouse IgG (1:100, A22210, Abbkine, Shanghai, China) for 1 h at 4 °C. After pooling, fluorescence was observed under a fluorescence microscope (OLYMPUS IX 81, OLYMPUS, Japan) and analyzed through flow cytometry (FACSCelesta, BD, USA) by using a FL2 channel at an excitation and emission wavelengths of 488 and 570 nm, respectively. For in vivo exposure, the colon tissues were cut into small 3–5 mm squares and digested in 0.25% trypsin (prewarmed to 37 °C) for 30–60 min at 37 °C. After the tissues were centrifuged at 800 rpm for 6–8 min, the supernatant was aspirated. The remnants were rinsed several times with PBS and filtered through a nylon mesh or stainless steel mesh (size: 100 μm) to remove larger pieces of undigested tissue. After the filtrate was properly washed and combined, the digestion was blocked with 3% BSA for 1 h, followed by centrifugation at 3000 rpm for 5 min and three washes in sterile PBS. The harvested fungal cells (possibly containing small tissue fractions) were incubated with monoclonal anti-β-glucan antibody (1:800) on ice for 1.5 h. After primary antibody treatment, the fungal cells were washed and treated with diluted Cy3-labeled goat anti-

Table 1
Primers for PCR.

Genes	Sequence (5′ to 3′)
TNF-α	Forward AAAGGACACCATGAGCACTGAAAG
	Reverse AGGAGAAGAGGCTGAGGAACAAG
IL-1β	Forward ATGGCTTATTACAGTGGCAATGAGG
	Reverse AGTGGTGGTCGGAGATTCGTAG
TGF-β	Forward CAGTCAGCTGGCCTTGGTC
	Reverse CAACTTCTTCTCCCCGCCAT
IL-10	Forward GGGGCCAGTACAGCCGGGAA
	Reverse CTGGCTGAAGGCAGTCCGCA
β-actin	Forward ATCTGGCACCACACCTTCTACAATG
	Reverse CACGCTCGGTCAGGATCTTCATG

mouse IgG (1:300) for 20 min on ice. The average fluorescent intensity of the exposed fungal cells (n = 10,000) was measured through flow cytometry and visualized using an inverted fluorescence microscope (OLYMPUS IX 81, Japan).

Phagocytosis

An inoculum of 1.8 × 10^6 CFU/mL of *C. albicans* SC5314 cells was pretreated with a gradient of QHD at 1, 0.1, 0.01, 0.001, 0.0001 g/mL, and its serum at 2.5, 5, and 10 g/kg overnight. After centrifugation, the fungal cells were resuspended in DMEM complete medium to a final concentration of 9 × 10^5 CFU/mL before incubation with RAW264.7 (3 × 10^5 cells/mL) at a multiplicity of infection (MOI, *C. albicans*:macrophage) = 3:1 for 3 h. After serial dilutions, an aliquot of 50 μL of the dilution was smeared on YPD plates and cultured at 37 °C for 24–48 h. The phagocytosis was recorded as CFU/mL. To evaluate the effect of the Dectin-1 receptor, RAW264.7 cells were pre-treated with 1 mg/mL of LAM (Sigma-Aldrich, St Louis, MO, USA) for 30 min before the subsequent experiments.

Immunoblotting

The experimental procedures were reported in our previous report, albeit with no modifications (Xu et al., 2024). In this study, the antibodies used included anti-Dectin-1 (1:1000, BIOSS, bs-2455R), anti--CARD9 (1:2000, Affinity, DF8387), anti-NLRP3 (1:800, Zenbio, 381,

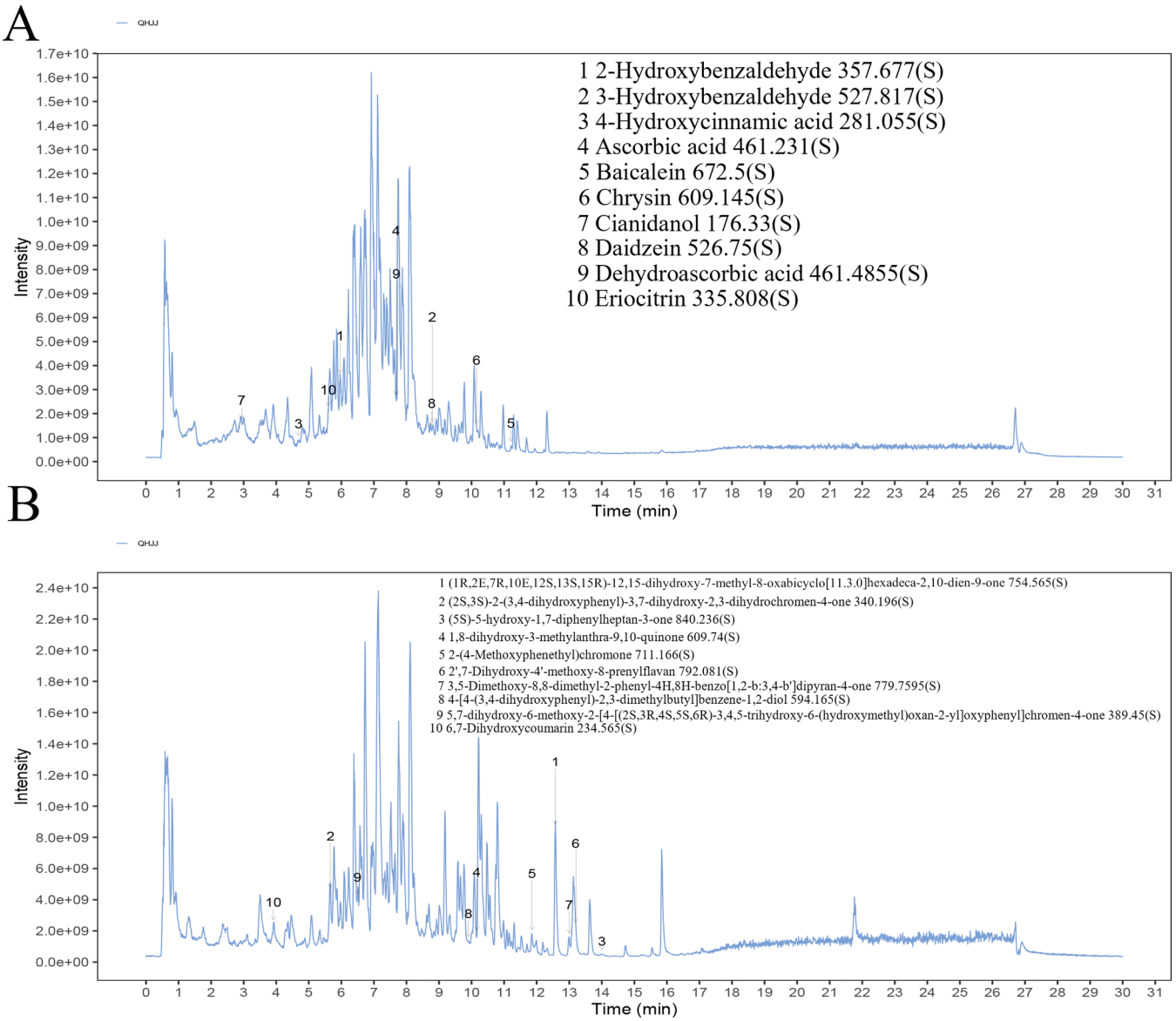

Fig. 1. Total ion chromatograms of QHD by UHPLC-MS/MS. (A) Positive ion mode. (B) Negative ion mode.

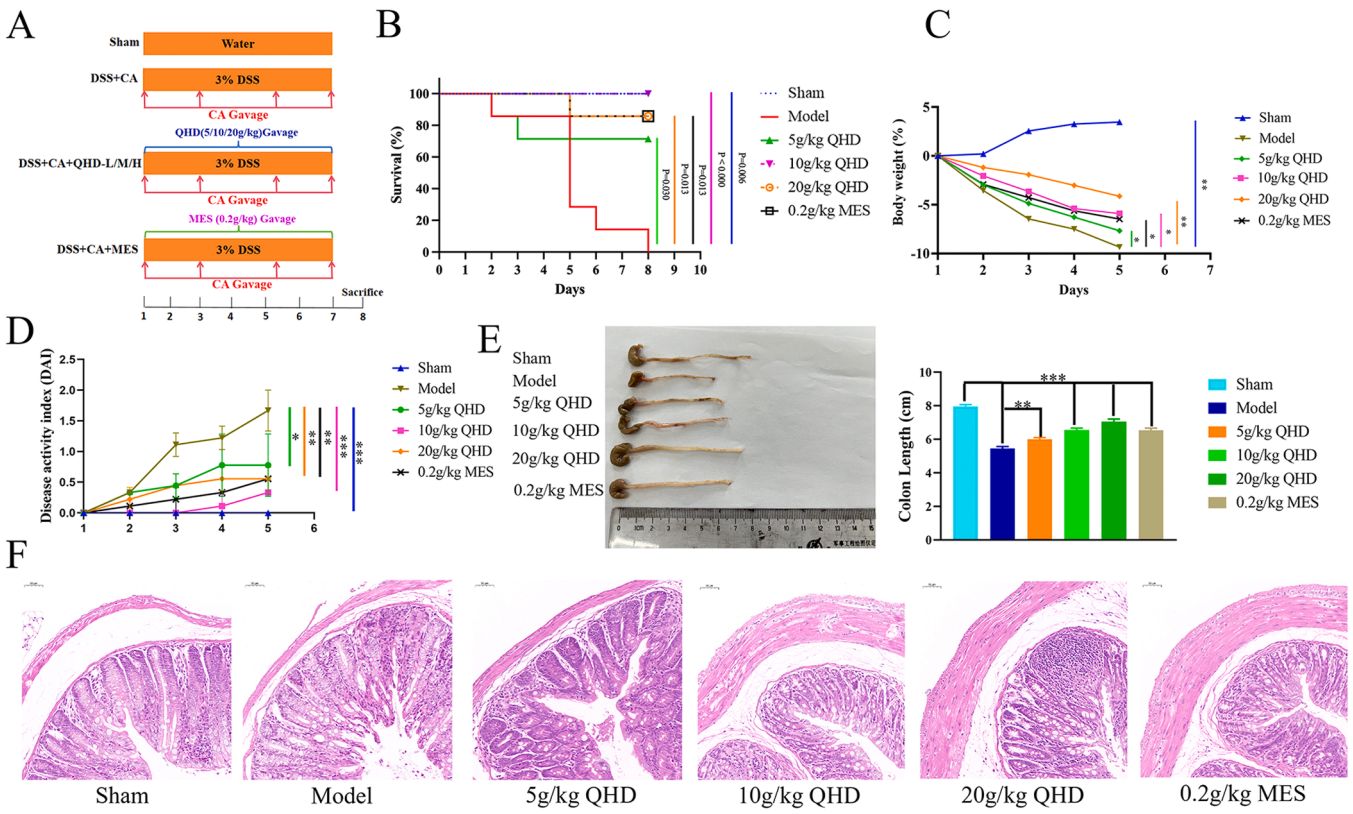

Fig. 2. QHD is protective against the DSS-induced colitis model with *C. albicans* supplementation. (A) A flowchart depicting the animal experiment process. A total of six groups of C57BL/6 mice are used. Of them, the sham mice drank only saline; the model mice were orally given 3% DSS everyday and 1×10^8 CFU of *C. albicans* SC5314 fungal cells every other day; the experimental mice intragastrically received three gradients of QHD at 10, 20, and 40 g/kg, and mesalazine at 0.2 g/kg. (B) Survival rate ($n = 7$). (C) Body weight in five days ($n = 3$). (D) DAI score ($n = 3$). (E) Colon length ($n = 5$). (F) Representative images of HE staining ($n = 3$). Scale bar: 50 μm. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

207), anti-SYK (1:800, Zenbio, 821,293), anti-NF-κB (1:1000, Zenbio, R25149), anti-Raf1 (1:1000, Zenbio, R25538), anti-Claudin1 (1:1000, Abcam, EPR25359–48), anti-Occludin (1:1000, Biodragon, RM4965), and anti-β-actin (1:5000, Servicebio, GB15003). The secondary antibodies contained anti-mouse IgG (1:10,000, Zenbio, 511,103) and anti-rabbit IgG (1:10,000, Zenbio, 511,203). After development, the target protein bands were quantified by using the Tanon 5200 device (Shanghai, China) and ImageJ 2.1.0 software, respectively.

Reverse-transcription quantitative real-time PCR (RT-qPCR)

The experimental procedures were reported in our previous report, albeit with no modifications (Xu et al., 2024). The primers used in this study were synthesized by Sangon (Shanghai, Chinese) and listed in Table 1. All of the gene expressions were calculated by using the $2^{-\Delta\Delta Ct}$ method and normalized to the β-actin expression.

Network pharmacology

The target prediction of QHD constituents was performed using TCMSP database (https://old.tcmsp-e.com/tcmsp.php), PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and Swiss Target Prediction database (http://swisstargetprediction.ch/), according to a reported document with some modifications (Li et al., 2022b).

Molecular docking

AutoDock4 software was applied to predict the interaction between the QHD constituents with the maximum targets identified by network pharmacology and the Dectin-1 receptor. The procedures were performed as described previously (Li et al., 2022b).

Statistic analysis

All data were analyzed by SPSS 23.0 (SPSS Inc, Chicago, IL, USA), and expressed as the mean ±standard deviation (SD). All samples were tested for normality by Shapiro—Wilk test and homogeneity of variance by the Levene test. For comparison between two groups, a student's *t*-test was performed, while for multiple groups comparison, a one-way analysis of variance (ANOVA) was conducted. $p < 0.05$ was statistically deemed as the significance threshold.

Results

QHD ameliorates DSS-induced colitis in mice supplemented with C. albicans

Using HPLC-MS technology, the primary constituents of QHD were analyzed, with the 10 most abundant components identified and presented in Fig. 1 and Table S1. To evaluate the therapeutic potential of QHD, we established a conventional colitis model in mice (Fig. 2A). Supplementation with *C. albicans* remarkably reduced the survival rate, body weight, and colon length of the mice, while markedly increasing the DAI score and exacerbating histopathological damage. After treatment with low, medium, and high dosages of QHD, survival rates improved markedly, body weight increased significantly, DAI scores decreased, colon length extended, and histopathological features showed substantial improvement (Fig. 2 B–F). Further analysis via plate counting revealed that all three QHD dosages significantly inhibited *C. albicans* growth in the liver, kidney, spleen, stomach, colon, and feces

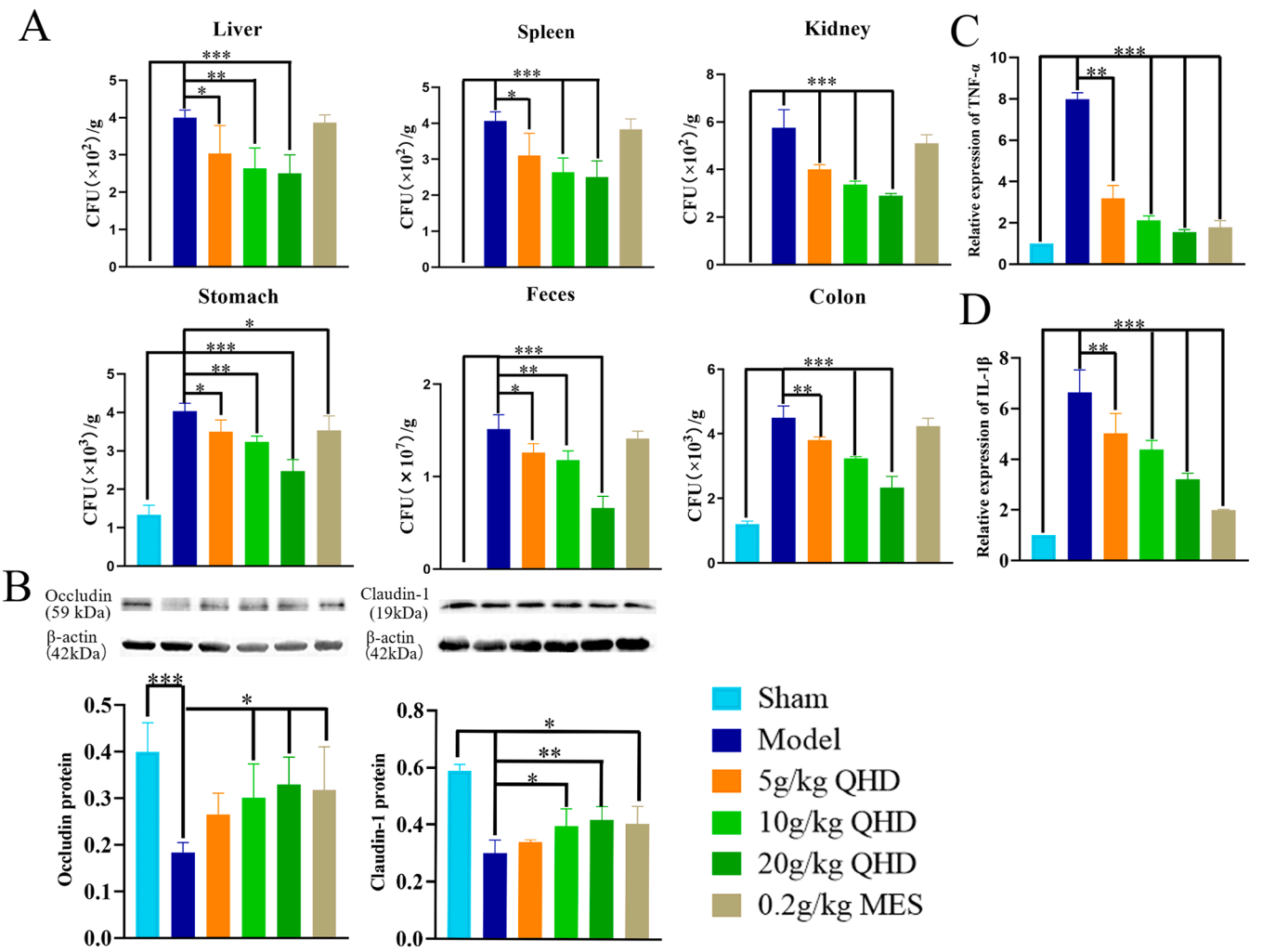

Fig. 3. QHD inhibits the growth of intestinal *C. albicans*, improves intestinal mucosal barrier integrity, and reduces proinflammatory cytokine expression. (A) Fungal capacity in the liver, kidney, spleen, stomach, colon, and feces ($n = 3$). The organic homogenates and fecal suspensions were smeared on YPD plates after proper dilution. (B) Immunoblotting analysis of occludin and claudin-1 in the colon tissues. Relative gene expressions of (C) IL-1β and (D) TNF-α in the colon tissues. All Western blots and gene analyses are representative of at least three independent experiments from three biological replicates. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

(Fig. 3A). Moreover, QHD treatment restored mucosal barrier integrity, as evidenced by increased expression of tight-junction proteins Occludin and Claudin-1 (Fig. 3B and S1). The QHD treatment also dose-dependently reduced the expression of the two critical proinflammatory cytokines TNF-α and IL-1β compared to the model group (Fig. 3C, D).

QHD activates macrophages by inducing fungal β-glucan exposure

As shown, the MICs of fluconazole were 2 μg/mL against SC5314, ≥1024 μg/mL against Z4935 and Z5172, respectively (Fig. 4A). Given QHD's inhibitory effect on fungal growth in the colitis model, we hypothesized that it processes antifungal properties. However, microdilution testing revealed that QHD, even at concentrations as high as 1024 μg/mL, failed to completely inhibit the growth of the three *C. albicans* strains tested (Fig. 4A). Similarly, the spot assay and T-K tests confirmed that fungal growth was not significantly suppressed, even at the highest tested QHD concentration (Fig. 4B and 4C). These results demonstrate that QHD exhibits only weak direct antifungal activity against these *Candida* strains. Interestingly, when co-cultured with RAW264.7 macrophages, both QHD and QHD serum significantly enhanced fungal clearance in a dose-dependent manner. This effect was negated by the Dectin-1 inhibitor LAM, suggesting that QHD activates macrophages through the Dectin-1 signaling pathway (Fig. 5A and 5B). Many antifungal drugs (e.g., caspofungin and fluconazole) and traditional Chinese medicine monomers (e.g., sodium houttuyfonate and berberine) are known to induce β-glucan exposure on the fungal cell wall. This β-glucan exposure is recognized by Dectin-1 receptors, enhancing macrophage-mediated fungal phagocytosis (Liu et al., 2020; Pradhan et al., 2019; Wheeler and Fink, 2006). As expected, caspofungin at 0.005 μg/mL robustly induced β-glucan exposure in *C. albicans*. Similarly, both QHD and QHD serum promoted *in-vitro* β-glucan exposure in a concentration-dependent manner (Fig. 6A, 6B and S2A, S2B). Furthermore, gavage with QHD strongly induced *in-vivo* β-glucan exposure in the colons of Candida colitis mice (Fig. 6C, 6 D and S2C).

QHD requires Dectin-1 signaling to alleviate Candida colitis severity

Dectin-1, a natural and specific receptor for fungal β-glucan recognition (Taylor et al., 2007), is hypothesized to play a role in QHD's protective effects against colitis exacerbated by *C. albicans*. The gavage of *C. albicans* significantly elevated NLRP3, Raf1, Syk, NF-κB, CARD9, and Dectin-1 protein levels in colonic tissues. QHD treatment effectively suppressed these protein levels to varying degrees compared with the model group (Fig. 7A–G and S3). To further investigate the role of Dectin-1 signaling, we used LAM to block the Dectin-1 receptor and

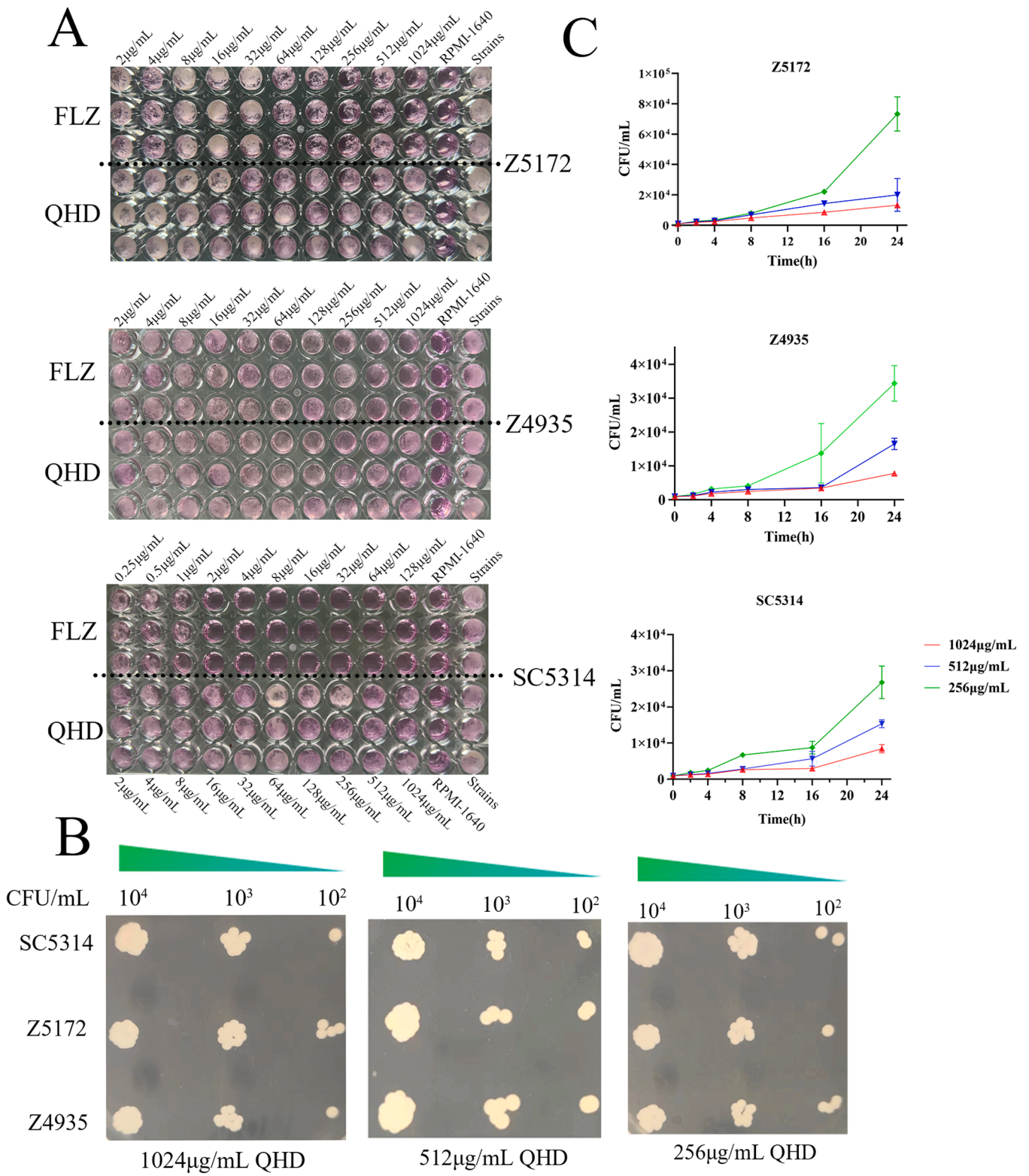

Fig. 4. QHD exhibited weak in vitro antifungal activity. (A) Microdilution detection. An inoculum of 2×10^3 cells/mL of the three fungal strains (SC5314, Z4935, and Z5172) is respectively incubated with a two-fold dilution of QHD and fluconazole till no visible fungal growth was seen. (B) Spot assay. The three fungal strains at a gradient of 1×10^2, 1×10^3, and 1×10^4 CFU/mL were incubated with three QHD concentrations (256, 512, and 1024 μg/mL). (C) Time-kill test. An inoculum of 2×10^3 CFU/mL of the three fungal strains was incubated with QHD at the final concentrations of 256, 512, and 1024 μg/mL. The fungal growth was monitored at 0, 2, 4, 8, 16, and 24 h through plate counting. All tests were performed in three independent experiments. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

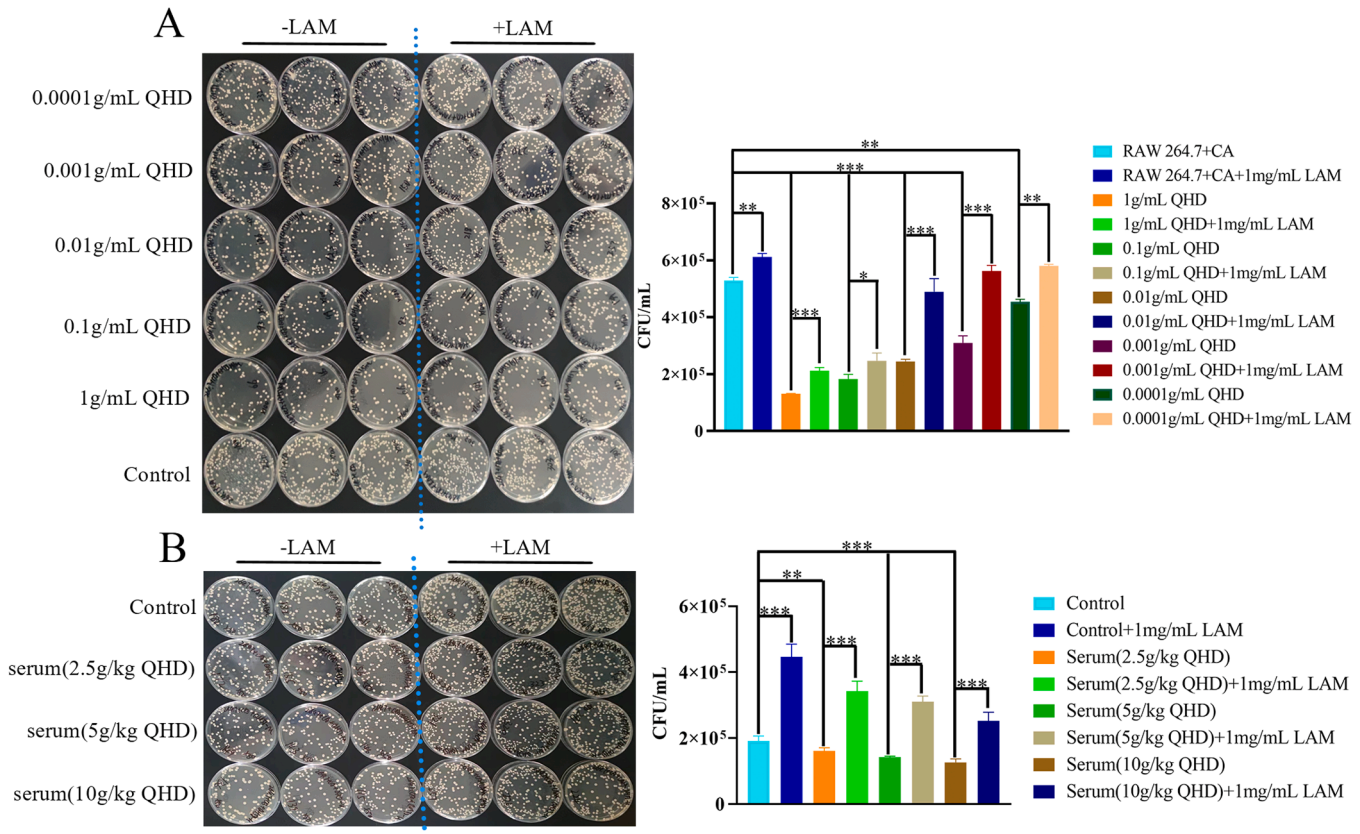

Fig. 5. RAW264.7 was primed to inhibit the growth of *C. albicans* in the presence of (A) QHD and (B) QHD serum. The QHD concentrations used were 0.0001, 0.001, 0.01, 0.1, and 1 g/mL. The QHD serum was prepared from SD rats orally treated with 2.5, 5, and 10 g/kg QHD. A concentration of 1 mg/mL of LAM was used to suppress the expression of Dectin-1 in RAW 264.7 for 30 min. All tests were performed in three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

assessed its impact on QHD's therapeutic efficacy. The blockage of Dectin-1 receptor significantly negated the ameliorative effects of QHD in a dose-dependent manner, as reflected in body weight maintenance (Fig. 8A), reduced DAI scores (Fig. 8B), longer colon lengths (Fig. 8C), and improved histopathology (Fig. 8D). Additionally, fungal burdens across various organs and tissues (liver, kidney, spleen, stomach, colon, and feces; Fig. 9A) remained elevated following Dectin-1 inhibition, along with suppressed expression of tight-junction proteins (Occludin and Claudin-1; Fig. 9B). Inflammatory cytokines IL-1β and TNF-α in colonic tissues also remained significantly elevated when Dectin-1 was inhibited (Fig. 9C, D and S4). These findings suggest that QHD mitigates *Candida*-associated colitis severity through both Dectin-1/Syk-dependent and independent signaling pathways.

QHD exhibits anti-inflammatory potential by directly interacting with Dectin-1

Although network pharmacology analysis revealed no direct involvement of Dectin-1 signaling in QHD's traditional role in treating UC (data not shown), this may be due to Dectin-1's primary association with antifungal responses rather than UC-specific mechanisms. However, PCR results unveiled that a high dose of QHD (20 g/kg) significantly upregulated the relative TGF-β (Fig. 10A) and IL-10 expressions (Fig. 10B) in the company of downregulated pro-inflammatory cytokines such as IL-1β (Fig. 10C) and TNF-α (Fig. 10D) in colonic tissues. These results indicate that QHD exerts a potent anti-inflammatory effect which might be also associated with Dectin-1 receptor. To further explore QHD's interaction with Dectin-1, we screened its eight herbal components against potential UC drug targets (Table S2–9). Among these, molecular docking analysis revealed strong physical interactions between Dectin-1 and several active compounds, including quercetin from *Astragalus membranaceus*, taraxerol from *Codonopsis pilosula*, luteolin from *Fructus aurantii immaturus*, wogonin from *Radix Scutellariae*, and α-amyrin from *Salvia Miltrorrhiza.* These compounds exhibited binding energies ranging from -5.83 ± 0.26 to -7.72 ± 0.21 kcal/mol (Fig. S5). Thus, QHD's ability to interact with the Dectin-1 receptor, likely mediated by its active components, warrants further experimental validation to fully elucidate its mechanisms of action.

Discussion

Because the overproliferation of *C. albicans* is a major contributor to the exacerbation of UC, targeting and controlling this fungus is essential for restoring intestinal microbial homeostasis and improving UC outcomes. While various herbs in QHD are known to contain antifungal compounds, such as baicalin and baicalein from *Scutellaria baicalensis* (Li et al., 2022a; Yang et al., 2014), and rhein and chrysophanol from *Rheum officinale* (Agarwal et al., 2000), our study findings were surprising. Despite the presence of these antifungal agents, QHD demonstrated no significant in vitro antifungal activity (Fig. 4). In both the murine UC model (Fig. 3) and the phagocytosis test (Fig. 5), the reduction in *C. albicans* colonization and growth was not attributable to a direct antifungal effect of QHD. We hypothesize that QHD likely inhibits *C. albicans* by activating the immune response, thereby mitigating UC progression. A key component of the *Candida* cell wall is β-glucan, a well-characterized pathogen-associated molecular pattern (PAMP), which is specifically recognized by the C-type lectin receptor Dectin-1 (Hameed et al., 2021). This interaction triggers an innate immune response against *C. albicans*. Numerous external factors, including antifungal drugs, lactate, pH, and oxygen/CO_2 levels, can cause remodeling of the fungal cell wall, exposing buried β-glucan (Avelar et al., 2024; Ballou et al., 2016; Pradhan et al., 2018; Sherrington et al.,

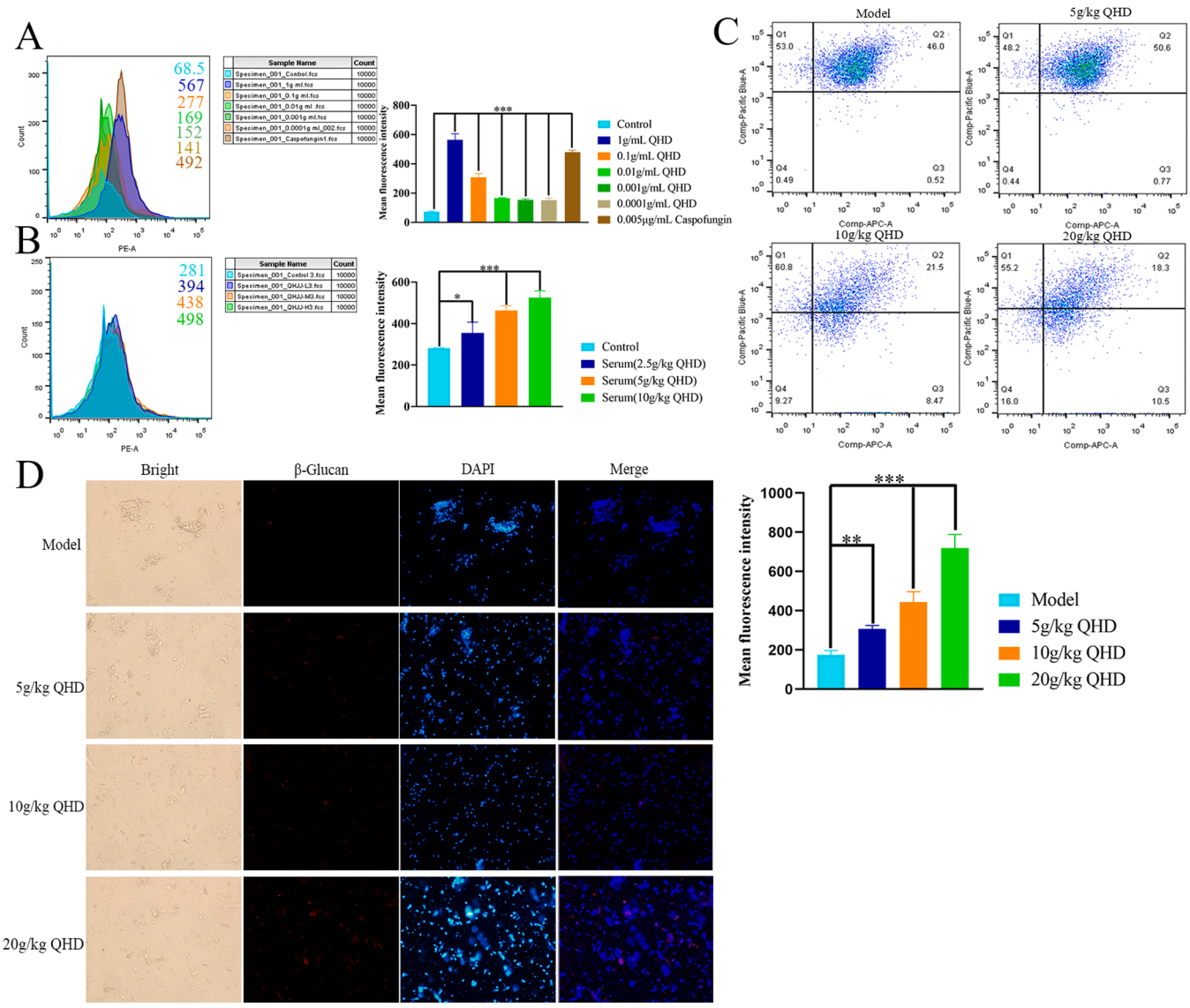

Fig. 6. QHD induces fungal cell wall β-glucan exposure in the presence of (A) QHD and (B) QHD serum, as well as (C-D) in DSS-induced *Candida* colitis mice. In the cellular test, fungal β-glucan exposure was induced by 0.0001, 0.001, 0.01, 0.1, and 1 g/mL QHD or a gradient of 2.5, 5, and 10 g/kg of QHD serum, which was detected and quantified through flow cytometry. Caspofungin (0.005 μg/mL) was used as a positive control. All profiles are representative of at least three independent experiments. In the animal experiment, the mice received 3% DSS and 1×10^8 cells/mL *C. albicans* orally for 5 consecutive days. The mice were treated with QHD at 5, 10, and 20 g/kg from day 3 for 3 consecutive days. On day 5, the colonic tissues were stained with DAPI and monoclonal anti-β-glucan antibody after sacrificing the mice. β-glucan exposure was detected and quantified through flow cytometry. Fluorescence in colonic tissues was observed under a fluorescence microscope. The images are representative of at least three independent experiments from two biological replicates. Magnification: × 200. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

2017; Wagner et al., 2023). We previously observed that certain remarkable exposure of *Candida* β-glucan by several monomers from traditional Chinese monomers, such as sodium houttuyfonate, cinnamaldehyde, and berberine, can expose *Candida* β-glucan (Liu et al., 2020). In this study, we demonstrated that QHD and its corresponding drug serum can unmask *C. albicans* β-glucan (Fig. 6).

Dectin-1 is expressed on the surface of various innate immune cells, including dendritic cells, macrophages, neutrophils, and monocytes. De Vries et al. revealed increased Dectin-1 expression in immune cells involved in the inflammatory response in IBD patients (de Vries et al., 2009). Moreover, a polymorphism in the CLEC7A gene encoding Dectin-1 has been strongly linked to UC in humans (Iliev et al., 2012). These findings underscore the critical role of Dectin-1 in regulating colitis associated with mycobiota dysbiosis. Our results showed that QHD can inhibit *C. albicans* growth and alleviate DSS-induced colitis in mice through Dectin-1-dependent signaling pathways (Fig. 7). Notably, the Dectin-1 inhibitor LAM significantly neutralized these protective effects (Figs. 8 and 9). The Dectin-1 signaling pathways, including Dectin-1/Syk/CARD9, Dectin-1/Syk/NLRP3, and Dectin-1/Raf-1, are key mediators of the antifungal immune response against *C. albicans* (Gringhuis et al., 2009; Gross et al., 2006; Gross et al., 2009). In this study, we observed that QHD administration alone promoted Dectin-1 and NF-κB expression in healthy mice (Fig. 10 A and 10 B), suggesting that QHD has immune-enhancing effects that protect against fungal growth through Dectin-1 signaling.

Interestingly, Dectin-1 appears to exert dual roles in the gut (Iliev, 2015). On the one hand, Dectin-1 is indispensable for defense against fungal invasion and intestinal inflammation, and UC patients with mutations in Dectin-1 are more prone to colitis (Iliev et al., 2012). On the other hand, several studies have reported that Dectin-1 deficiency may alleviate colitis by downregulating the Syk signaling pathway and promoting regulatory T-cell expansion through gut *Lactobacillus* (Hang

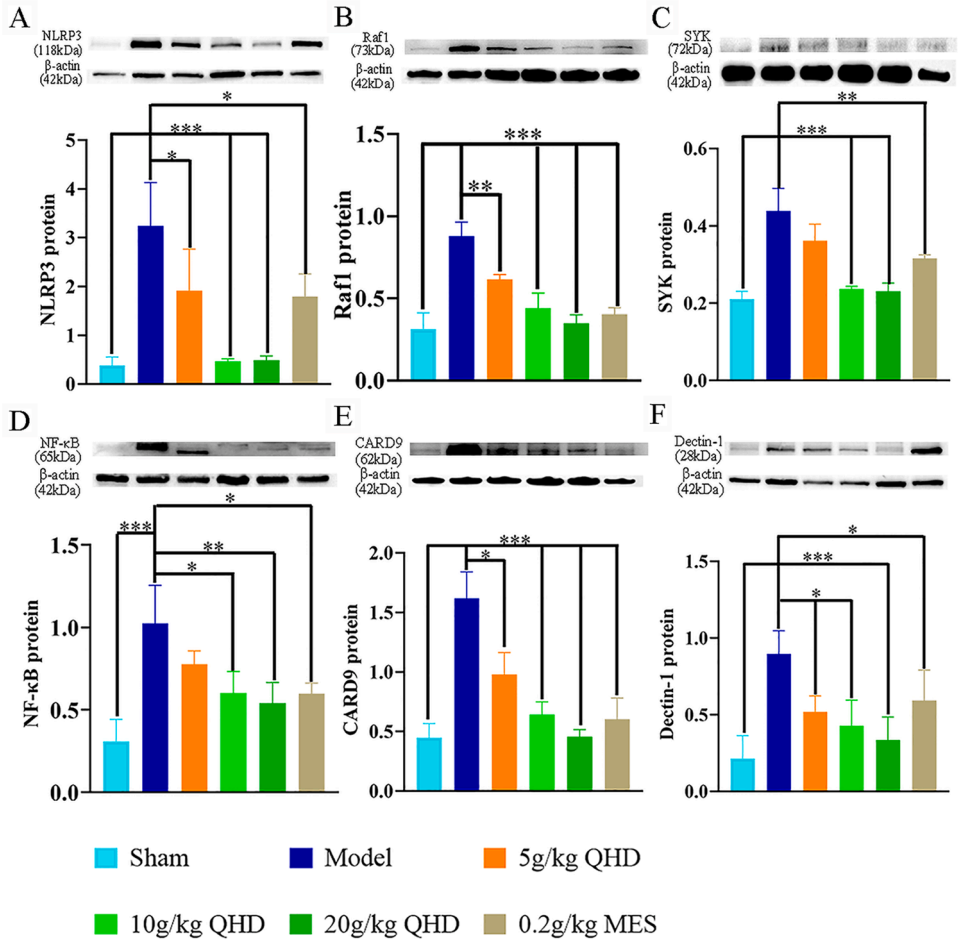

Fig. 7. QHD inhibits Dectin-1-associated signaling in DSS-induced colitis with *C. albicans* supplementation. (A–G) Immunoblot bands and quantification of NLRP3 (B), Raf-1 (C), SYK (D), NF-κB (E), CARD-9 (F), and Dectin-1 (G). The experimental conditions are the same as those in Fig. 2. The bands are representative of at least three independent experiments from three biological replicates. $*p < 0.05$, $**p < 0.01$ $***p < 0.001$.

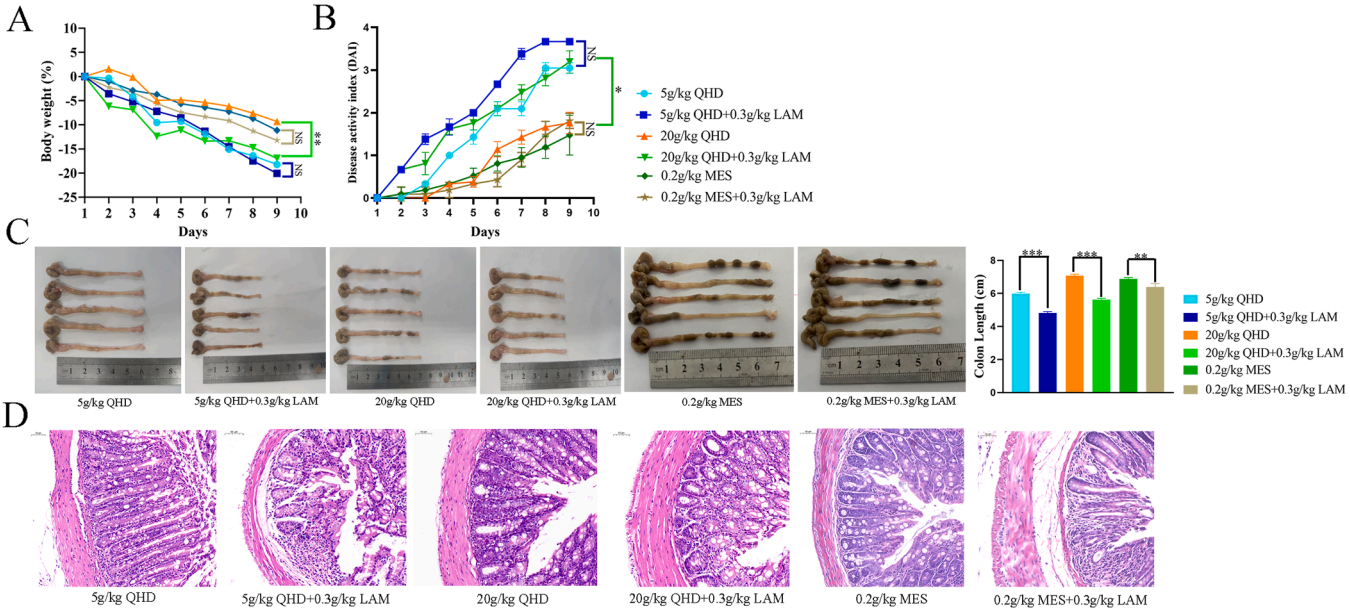

Fig. 8. Blockade of Dectin-1 receptor abrogates the protection of QHD against DSS-induced colitis with *C. albicans* supplementation. (A) Body weight ($n = 7$). (B) DAI score ($n = 7$). (C) Colon length ($n = 5$). (D) Representative images of HE staining ($n = 3$). Low and high doses of QHD (5 and 20 g/kg) were used, while laminarin (LAM) at 0.3/g/kg was injected intraperitoneally for 2 days before the colitis model was established. Mesalazine (MES) at 0.2 g/kg was used as a positive control. The experimental conditions are the same as those in Fig. 2. Scale bar: 50 μm. $*p < 0.05$, $**p < 0.01$ $***p < 0.001$, ns, no significance.

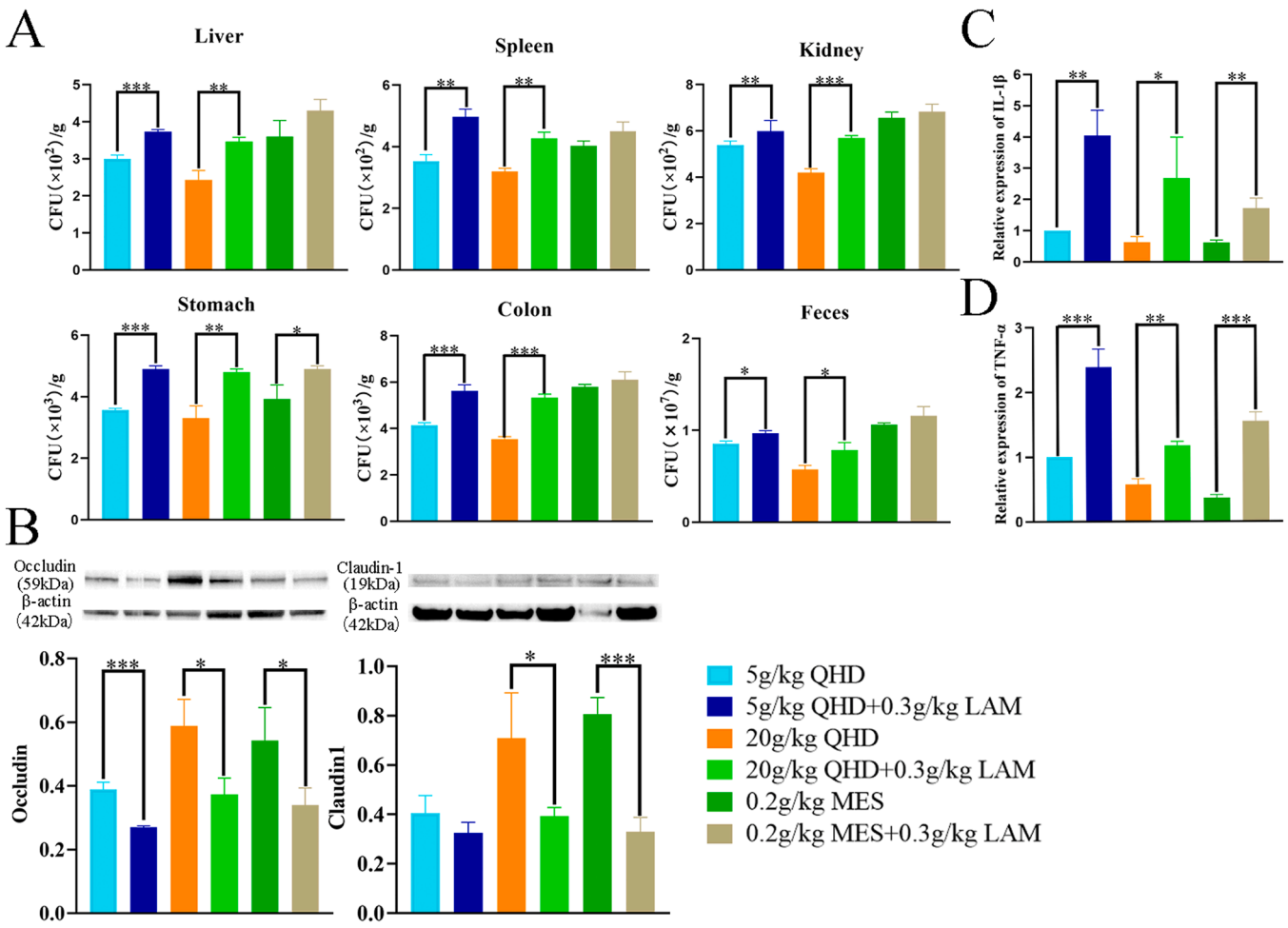

Fig. 9. Blockade of the Dectin-1 receptor abolishes the protection of QHD against the growth of intestinal *C. albicans* and intestinal mucosal barrier damage. (A) Fungal capacity in the liver, kidney, spleen, stomach, colon, and feces ($n = 3$). The organic homogenates and fecal suspensions were smeared on YPD plates after proper dilution. (B) Immunoblot analysis of occludin and claudin-1 in colonic tissues. Relative gene expression of (C) IL-1β and (D) TNF-α in colonic tissues. All Western blots and gene analyses are representative of at least three independent experiments from three biological replicates. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

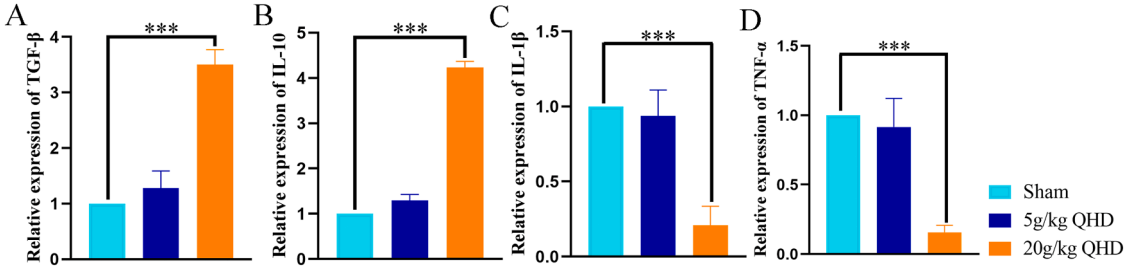

Fig. 10. QHD alone affects Dectin-1 and cytokine expressions in healthy mice. The C57BL/6 mice received 5 and 20 g/kg QHD orally for 7 consecutive days. On day 8, the mice were sacrificed. (A) Immunoblot analysis of the Dectin-1 receptor. Relative gene expression of (B) NF-κB, (C) TGF-β, (D) IL-10, (E) IL-1β, and (F) TNF-α. All Western blots and gene analyses are representative of at least three independent experiments from three biological replicates. $^{**}p < 0.01$ $^{***}p < 0.001$.

et al., 2016; Tang et al., 2015). In our cellular experiments, Dectin-1 signaling was activated by QHD-induced β-glucan exposure, thereby priming macrophages to phagocytose *C. albicans* (Fig. 5). As the fungal burden decreased, Dectin-1 signaling was downregulated in the murine UC model (Fig. 7). Furthermore, QHD administration alone enhanced the levels of anti-inflammatory cytokines (TGF-β and IL-10) while reducing proinflammatory cytokine levels (IL-1β and TNF-α) in colonic tissues (Fig. 10). These findings suggest that QHD-induced fungal β-glucan exposure may trigger a proinflammatory response, which is subsequently counterbalanced by QHD-mediated anti-inflammatory effects. Hang et al. demonstrated that Dectin-1 positively regulates the inflammatory phenotype of intestinal macrophages during colitis (Rahabi et al., 2020). Notably, Dectin-1, TGF-β, and IL-10 are characteristic markers of M2a macrophages, which are usually involved in tissue repair, angiogenesis, and fibrosis (Huang et al., 2018). Whether QHD activates M2a macrophage to promote anti-inflammatory responses and facilitate colonic mucosal healing warrants further investigation. Therefore, QHD is likely to exert antifungal response and anti-inflammatory effects during *Candida* colitis treatment by activating Dectin-1-associated signaling.

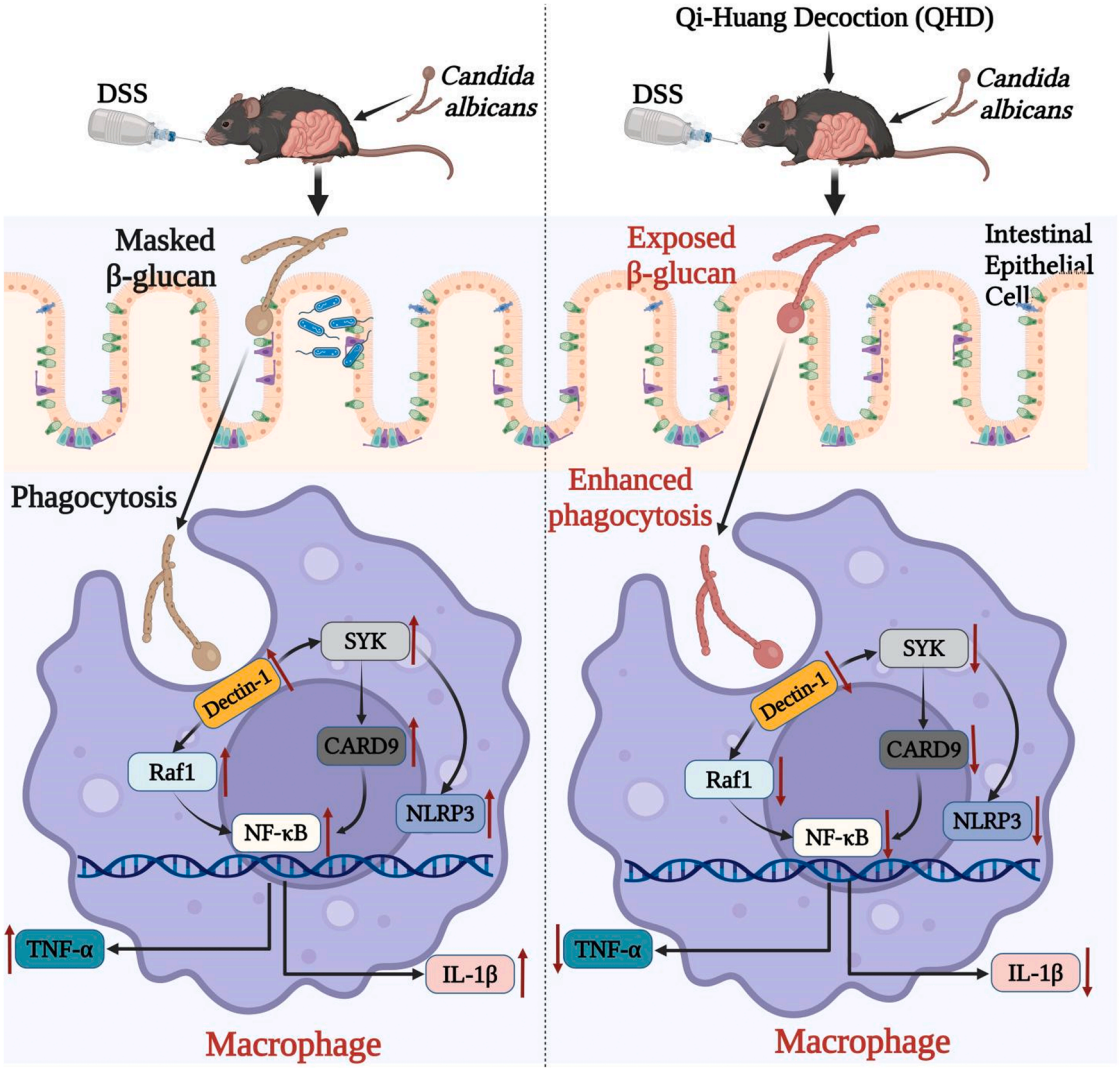

Fig. 11. Depiction of the schematic workflow in this study.

In conclusion, our study highlights the immunoprotective effects of QHD in ameliorating DSS-induced *Candida* colitis. The underlying mechanism may involve QHD-induced β-glucan exposure, which is recognized by Dectin-1 and activates both Syk-dependent and -independent pathways. Consequently, the fungal burden is reduced, intestinal inflammation is controlled, and mucosal barrier integrity is restored (Fig. 11). These findings provide solid experimental support for the potential of QHD in the clinical management of fungal colitis.

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CRediT authorship contribution statement

Chengcheng Liu: Methodology, Investigation, Formal analysis, Data curation. **Liu Yang:** Validation, Software, Investigation. **Zixu Wang:** Validation, Software, Investigation. **Hanyu Zhu:** Validation, Software, Investigation. **Qinai Luo:** Validation, Software, Investigation. **Daqiang Wu:** Visualization. **Tianming Wang:** Supervision. **Min Hu:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Changzhong Wang:** Resources. **Jing Shao:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2025.156613.

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