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Gastrodin attenuates dextran sulfate sodium-induced colitis in mice *via* modulating gut microbiota and inhibiting NF- κ B signaling pathway

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ABSTRACT

Gastrodin (GAS), the principal bioactive composition of *Gastrodia elata* Blume, has potential for pharmaceutical applications. Several studies in recent years have shown that GAS may enhance neurotrophic benefits, reduce inflammation, and act as an antioxidant. In this study, we sought to identify the molecular mechanisms underlying the protective benefits of GAS against colitis induced by dextran sodium sulfate (DSS) in mice. GAS (200 mg/kg) significantly ameliorated the severity of colitis in mice caused by DSS, as evidenced by an increase in colon length, a reduction in disease activity index, a decrease in tissue damage, and a reduction in body weight loss. Additionally, GAS inhibited DSS-induced hyperactivation of inflammation-related NF- κ B signaling pathways to reduce the production of inflammatory mediators, thereby mitigating the inflammatory response in mice. Furthermore, the administration of GAS restored the function of the gastrointestinal barrier by increasing the count of goblet cells, as well as the levels of tight junction-associated proteins, including Zonula occludens-1 (ZO-1), Occludin, and Claudin-3. GAS also influenced the overall richness of the gut microbiota, as shown by 16S rRNA sequencing analysis, consequently boosting the proliferative rate of probiotic species, such as Lachnospiraceae and Muribaculaceae, while reducing the richness of harmful bacteria including *Escherichia Shigella*, Enterobacteriaceae, Bacteroidaceae, and *Bacteroides*. GAS (200 mg/kg) alleviated ulcerative colitis (UC) by modulating gut dysbiosis, as demonstrated by a fecal microbial transplantation (FMT) test. Furthermore, inflammatory damage induced by lipopolysaccharide (LPS) was averted in RAW264.7 cells by GAS administration, hence preventing the NF- κ B signaling pathway from being activated in these experimental conditions conducted *in vitro*. Overall, the data indicate that GAS treatment effectively reduces colitis caused by DSS by regulating gut microbiota, suppressing inflammation, and preserving the mucosal barrier integrity.

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1. Introduction

Gastrodia elata Blume, also called Tianma in Chinese, is a traditional herbal remedy used in Chinese medicine. Gastrodin (GAS), which is sometimes referred to as 4-hydroxybenzyl alcohol

4-*O*- β -D-glucoside, is the primary bioactive ingredient of *Gastrodia elata* Blume with a chemical formula of $C_{13}H_{18}O_7$ and a molecular weight of 286.278 Da (Fig. 1A). There is a wide range of pharmacological activities associated with GAS, with the neuroprotective effects being the most significant of all of them, according to studies^[1-2]. GAS neuroprotective benefits are based on its ability to improve inflammatory responses and oxidative stress, and suppress neuronal apoptosis, and can be utilized for the treatment of neuropathic pain, cognitive impairment, emotional disorders, Tourette syndrome, Alzheimer's disease, epilepsy, cerebral ischemia-reperfusion injury, and Parkinson's disease^[3-10]. Along with its neuroprotective benefits, GAS also exhibits cardiovascular protective properties, including anti-inflammatory, anti-oxidative, anti-cell-apoptotic, and anti-collagen synthesis, as well as a relaxation of the arterial ring, all of which alleviate myocardial ischemia-reperfusion injury, hypertension, and myocardial hypertrophy^[11-13]. In addition, GAS may alleviate inflammation and oxidative stress-induced damage to the liver, kidneys, and bones^[14-16]. In summary, GAS has been the topic of extensive pharmacological research, involving various complex animal models, dosages, and action mechanisms. Previous studies have shown that certain components of *Gastrodia elata* Blume may have therapeutic effects on UC^[17]. However, whether GAS has a protective effect on digestive system diseases, especially inflammatory bowel disease (IBD) remains unclear.

IBD, which is characterized by persistent and recurrent inflammation of the gastrointestinal system, consists of 2 major types, namely ulcerative colitis (UC) and Crohn's disease (CD)^[18]. Between them, UC is a lifelong inflammatory disorder

and constitutes the vast majority of IBD cases. In 2023, the prevalence of UC was estimated to be 5 million cases around the world, accompanied by an increase in the global incidence rate^[19]. UC usually manifests as rectal bleeding and related symptoms in over 90% of patients, depending on the severity of the disease^[20]. In addition, increased stool frequency and decreased stool consistency are also common. Presently, the precise cause of UC remains unknown. However, it is believed that a range of factors associated with the host genetics, immunity, gut microbiota, and environment disrupt the intestinal barrier function, leading to UC^[21-23]. Therefore, common mechanisms to control the degree of inflammation in the colon include modulating the infiltration levels of inflammatory mediators and cells, oxidative stress, the production of mucins and tight junction (TJ) proteins, the composition of the intestinal microbiota, and metabolites linked to microbes.

Individuals with genetic susceptibility are exposed to risk factors in the environment, causing intestinal epithelial cell death by decreasing the production of TJ proteins and other intestine-protective proteins, leading to impaired intestinal barrier integrity^[24]. The lamina propria is penetrated by symbiotic microbes and their associated metabolites *via* the intestinal epithelial barrier's damaged site, which in turn stimulates local inflammatory cells such as dendritic cells (DCs) and macrophages. These cells then release pro-inflammatory cytokines and chemokines, recruiting other types of inflammatory cells to the inflammatory site and amplifying the inflammatory response in the intestine. Intestinal oxidative stress, brought on by microbial invasion and inflammatory cell activation, may damage intestinal tissue and

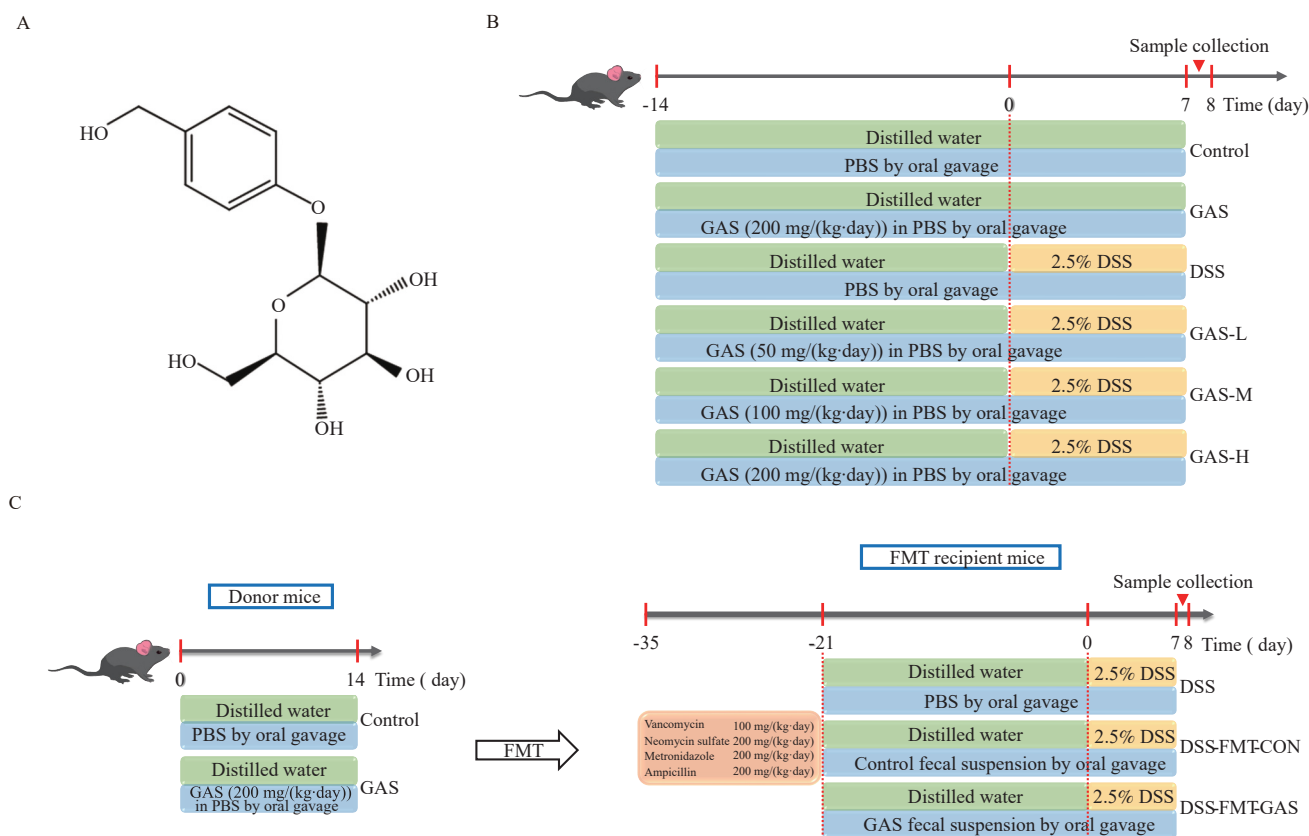


Fig. 1 Schematic diagrams of GAS, experimental design in DSS-treatment colitis, and FMT procedure. (A) GAS structural schematic. (B) Schematic diagram of the experimental design of GAS in DSS-treatment colitis. (C) Schematic diagram of the experimental design of FMT.

worsen inflammation. Therefore, common mechanisms influencing the severity of colonic inflammation include regulating the infiltration levels of inflammatory cells and mediators, the composition of gut microbiota, oxidative stress in the gut, the production of mucin and TJ proteins, and microbial-related metabolites^[25]. According to the above mechanisms, the current main treatment options include “conventional therapy” composed of aminosalicylates, glucocorticoids, and immunomodulators, “biological therapies” consist of tumor necrosis factor alpha (TNF- α) antibiotics, integrin antibiotics, and interleukin antibiotics, the “small molecules” consist of Janus Kinase inhibitors and sphingosine 1-phosphate modulators^[26], as well as 10%–20% of patients still require rectal resection for refractory types^[27–28]. Drug resistance and other adverse effects are among the clinical problems associated with these medications and therapies. Consequently, more effective and safer ways to treat UC are desperately needed. Natural product extracts, as naturally occurring bioactive substances derived from plants or herbaceous plants, have sparked great research interest due to their considerable adaptability and low toxic effects. Moreover, current research suggests that therapeutically altering the intestinal microbiota using prebiotics, probiotics, and fecal microbiota transplantation (FMT) as an alternative strategy seems more promising than common therapy^[29].

GAS, as a natural product extract, has been shown to achieve therapeutic effects by regulating gut microbiota in certain diseases^[30–32]. However, it is unclear whether this relationship applies to UC. Therefore, with the use of a DSS-treated colitis mouse model, this study seeks to evaluate the phenotype, histological manifestations, inflammatory response, colonic barrier damage, gut microbiota, and metabolome of the disease. Additionally, it focuses on elucidating the protective effects of GAS on colitis, its role in shaping different gut microbiota, and the possible underlying mechanism.

2. Materials and methods

2.1 Material

The Shanghai YuanYe Biotechnology Co., Ltd. (Shanghai, China) provided the GAS (purity $\geq 98\%$). MP Biomedicals (Santa Ana, CA, USA) provided dextran sodium sulfate (DSS) (36–50 kDa).

2.2 Animals and experimental design

Liaoning Changsheng Biotechnology Co., Ltd. (Benxi, China) provided 36 male C57BL/6 mice (weighing 21 ± 2 g; 7 weeks old). The mice were provided free access to water and food and were housed in a 12-h light/12-h dark cycle. Following a week of acclimation to their new environment, they were randomized into 6 groups ($n = 6$): Control (Control), GAS (high dose, 200 mg/kg), DSS-treated UC model (DSS), GAS-L (DSS + low dose GAS, 50 mg/kg), GAS-M (DSS + median dose GAS, 100 mg/kg), and GAS-H (DSS + high dose GAS, 200 mg/kg). The reason for the design of the GAS group is to demonstrate that the inflammatory changes in the animal model are not caused by the drug, and that the drug does not cause significant damage to the body. Previous research served as the basis for the selection and adjustment of these dosages^[31,33–34]. The experiment lasted 21 days.

GAS was administered by oral gavage to the mice in the GAS and 3 GAS-treated groups at doses of 200, 50, 100, and 200 mg/(kg·day),

respectively. Conversely, oral gavage of phosphate-buffered saline (PBS) was given to the other 2 groups. For the first 14 days, standard drinking water was provided to all groups. From the 14th day, apart from the Control and GAS group, the drinking water of mice in other groups was replaced with distilled water containing 2.5% DSS for 7 days to establish a mouse UC model (Fig. 1B). Throughout the experiment, each mouse's disease activity index (DAI) and body weight were measured daily. After consuming the DSS solution for 7 days, under a moderate amount of ether the mice were cervical dislocated to euthanize them with moderate amount of ether, after which, samples of stool, blood serum, and colon tissues were obtained. Subsequently, 4% formaldehyde was added to some colon tissues. For further examination, the remaining fecal samples, blood serum, and colon tissues were kept refrigerated at -80°C . The Technology Ethics Committee of Jilin Engineering Normal University approved the protocol for the animal experiment (Permit number: 2023SY10307).

2.3 Cell culture

DMEM with 10% FBS was used to culture RAW264.7 cells from Haixing Biosciences (Suzhou, China) in an incubator with 5% CO_2 concentration at 37°C . Cells were inoculated into different cell culture dishes overnight, GAS-pretreated for 1 h, and then treated with LPS (1 $\mu\text{g/mL}$). These cells were subjected to the experimental conditions as follows: Control, GAS (40 $\mu\text{mol/mL}$), LPS (0.2 mg/mL, 50 μL), LPS+GAS (10, 20, and 40 $\mu\text{mol/mL}$), the concentration selection of GAS is based on the maximum concentration without significant cytotoxicity in the cell viability assay, and a concentration gradient is set downwards. Thereafter, the cells were obtained for further studies following treatment.

2.4 Cell viability assay

The CCK-8 assay was performed to examine the effect of GAS treatment on cell viability (Yeasten, Shanghai, China). Each well was supplied with 10 μL of CCK-8 reagent for 2 h after 24 h of treatment with GAS (2.5, 5, 10, 20, 40, 80, 160 mmol/L) or an equivalent volume PBS control. The absorbance at 450 nm was subsequently measured utilizing a microplate reader.

2.5 Assessment of the DAI and body weight changes

As reported previously, DAI was obtained by calculating the arithmetic mean of the 3 scores of body weight change, stool consistency, and rectal bleeding^[35]. The specific scoring rules are listed in Table 1.

Table 1
DAI scoring.

Score	Weight loss (%)	Fecal property	Rectal bleeding
0	0	Normal	Normal
1	1–5	Soft stool (+)	Occult blood (+)
2	6–10	Soft stool (++)	Occult blood (++)
3	11–15	Moderate diarrhea	Bloody feces (+)
4	> 15	Watery diarrhea	Bloody feces (++)

The degree of weight changes is calculated by the following formula:

$$\text{Body weight change (\%)} = \frac{\text{Body weight (}m_n\text{)}}{\text{Body weight (}m_0\text{)}} \times 100$$

Where body weight (m_n) represents the weight of a mouse on the n^{th} day after modeling, and body weight (m_0) represents the initial weight of the mouse.

2.6 Evaluation of histological injury

Sections were prepared from colon samples after dehydration in 4% formaldehyde, embedding in paraffin, and processing. Afterward, the number of goblet cells was quantified by periodic acid-Schiff (PAS) staining and an evaluation of tissue damage was carried out using hematoxylin-eosin (H&E) staining^[36]. Histological damage was scored using the criteria given in Table 2.

2.7 Immunofluorescence staining

Before dehydration using an ethanol gradient, tissue slices underwent deparaffinization with diethyl ether, after which they were boiled in sodium citrate buffer to facilitate antigen recovery and rinsed 3 times (5 min per wash) at room temperature using PBS. Donkey serum was subsequently used to block the sections for 1 h, and then they were rinsed thrice using PBS as described earlier. Next, the samples were subjected to incubation at 4 °C for an entire night with primary antibodies against ZO-1 (1:100; Proteintech Group, Wuhan, China) and Claduin-3 (1:100; Proteintech Group, Wuhan, China). Thereafter, sections were then rinsed thrice with PBS and probed for 1 h with secondary goat anti-rabbit IgG (1:2 000; Santa Cruz) at room temperature. Additional three washes were conducted before staining the nuclei with 4',6-diamidino-2-phenylindole (DAPI).

2.8 Quantitative PCR (qPCR) assay

The isolation of total RNA from RAW264.7 cells and colon tissue were performed with the help of TRIzol reagent. Then, an analysis of

the expression of interleukin-6 (*IL-6*), cyclooxygenase-2 (*COX-2*), interleukin-1 beta (*IL-1β*), inducible nitric oxide synthase (*iNOS*), and *TNF-α* was performed using qPCR. Table 3 contains a listing of the primers that were utilized.

2.9 Enzyme-linked immunosorbent assay (ELISA)

ELISA kits (Proteintech Group, Wuhan, China) were used to determine the levels of *TNF-α*, *IL-6*, and *IL-1β* in blood serum, as directed by the manufacturer.

2.10 Myeloperoxidase (MPO) activity assay

A procedure outlined in a previous study was followed when conducting MPO activity analysis^[37]. In brief, the colon tissues were ground with N-2-hydroxyethyl piperazine-*N*-ethane-sulfonic acid (HEPES) solution and centrifuged. Subsequently, 0.5% cetyltrimethylammonium chloride (CTAC) was added to grind the precipitate once again in the same proportion as HEPES. The samples were then centrifuged to obtain the supernatant. The supernatant and substrate were added to the plate. Eventually, 2 mol/L sulfuric acid was introduced to stop the reaction, after which the absorbance was determined at 450 nm.

2.11 Western blot

The RIPA buffer (Beyotime, Shanghai, China) was employed to isolate the total protein from cells and colon tissues. A BCA kit (Yeasten, Shanghai, China) was subsequently used for protein analysis, followed by separating the proteins via 10% or 12% SDS-PAGE and transferring them onto PVDF membranes (Millipore, Darmstadt, Germany). Thereafter, the blots were blocked and treated with appropriate primary antibodies (1:1 000) for 1 night at 4 °C. Following five 10-min washes using Tris-buffered saline/Tween 20 (TBS-T) buffer, the samples were allowed to incubate at room temperature for 1 h with secondary antibodies that were either goat anti-rabbit or HRP-linked goat anti-mouse IgG (1:10 000). The electrochemiluminescence (ECL) test kit (Beyotime) was employed

Table 2
Scoring of histological damage.

Score	Goblet cell loss	Mucosa architecture disruption	Crypt abscess formation	Muscle thickening	Inflammation severity
0	Non-existence	Non-existence	Normal	Normal	Normal
1	Existence	Existence	Mild	Mild	Mild
2	-	-	Moderate	Moderate	Moderate
3	-	-	Severe	Severe	Severe

Note: -, The score situation does not exist.

Table 3
Primer sequences for qPCR.

Gene	Forward (5'→3')	Reverse (5'→3')
<i>GAPDH</i>	GCATTGCTGACAATCTTGAGTGAG	TGTCTTCACCACTGGAGAAG
<i>IL-6</i>	TTGTATGAACAACGATGATGCACT	TGTTCTTCATGTACTCCAGGTAGC
<i>IL-1β</i>	GTAGTGCAAGTTGTCTAATGGGAACG	GCTACCTGTGTCTTTCCCGTGGA
<i>TNF-α</i>	GGGCCATAGAAGTATGATGAGAGGG	TCCAGAAGTCCAGGCGGT
<i>iNOS</i>	CACCCAGAAGAGTTACAGC	GGAGGGAAGGGAGAATAG
<i>COX-2</i>	GGAGGGAAGGGAGAATAG	CTTGATAGTAGGCTTAAACATAG

to identify protein bands on a developer after five 10-min washes with TBS-T. The ImageJ software was utilized to calculate the protein bands, and the results were normalized with respect to β -actin.

2.12 16S rRNA high throughput sequencing

To detect microorganisms in the intestines of mice with induced UC, 16S rRNA gene sequencing was performed on stool samples. The Magnetic Soil and Stool DNA Kit (TianGen, China; Catalog #: DP712) was utilized to extract bacterial DNA from feces. We used 1% agarose gel electrophoresis to check the quality of the DNA extraction. Below are the primer sequences utilized to amplify the 16S rRNA gene's V3-V4 region: forward primer (5'-CCTAYGGGRBGCASCAG-3') and reverse primer (5'-GGACTACNNGGTATCTAAT-3'). The NEB Next® Ultra™ II FS DNA PCR-free Library Prep Kit (New England Biolabs, USA; Catalog #: E7430L) was employed to produce sequencing libraries which were then indexed in compliance with the protocols stipulated by the manufacturer. The library was checked with Qubit and qPCR for quantification and bioanalyzer for size distribution detection. Thereafter, Illumina platforms were used to pool and sequence quantified libraries based on the data amount required and effective library concentration. Bioinformatics analysis was conducted using the NovoMagic platform.

2.13 Short-chain fatty acids (SCFAs) analysis

Gas chromatography was conducted to identify the SCFAs in the cecum. The experiment included collecting 200 mg of fresh mouse fecal matter, mixing it with 1 mL of deionized water, and then centrifuging it for 10 min at 12 000 r/min. The next step was to mix 1 mL of the supernatant with 100 μ L of concentrated hydrochloric acid. Thereafter, ether (5 mL) was added to this mixture, mixed thoroughly, and extracted for 20 min at room temperature. The sample was utilized for SCFA identification after centrifuging the organic phase extract for 10 min at 3 500 r/min. We used a DB-FFAP capillary column (30 m $\times$ 0.25 mm, 0.25 μ m) (Agilent Technologies) to conduct gas chromatography. Standard SCFA curves were prepared using hexanoic, butyric, valeric, propionic, and acetic acids.

2.14 FMT trial

At random, 10 male C57BL/6 donor mice were classified into 2 groups of 5: GAS and Control. For 2 weeks, each group drank water with either PBS (Control) or GAS (200 mg/kg). Donor mice's fresh stool samples were obtained on day 14. The feces from each donor group were mixed, weighed, homogenized, and washed with PBS for 3 times. The next step was to filter the suspension 2 times in PBS after centrifuging it at 8 000 r/min, 4 °C for 5 min to obtain the total bacteria. Eventually, a final concentration of 20% was achieved by mixing the final bacterial suspension with an equivalent amount of 40% sterile glycerol (BioFroxx, Germany), after which the sample was frozen at -80 °C for subsequent FMT.

Three groups (each consisting of 6 male C57BL/6 recipient mice) were established at random: DSS, FMT-CON, and FMT-GAS groups. Before FMT, recipient mice were administered an antibiotics cocktail (vancomycin, 100 mg/kg; neomycin sulfate, metronidazole, and ampicillin, 200 mg/kg) once daily for 14 days. Subsequently, fecal

microbiota from the CON and GAS donors were administered by oral gavage to the FMT-CON and FMT-GAS groups, respectively, for the subsequent 21 days. For 7 consecutive days, 2.5% DSS was administered to every mouse, while the FMT groups continued to receive fecal microbiota gavage (Fig. 1C). The colonies' lengths were assessed on day 8 after euthanizing the recipient mice. Four percent formaldehyde was used to preserve some colon samples. For subsequent testing, the colon samples that were not used were stored at -80 °C.

2.15 Statistical analysis

In this study, all data are represented as mean $\pm$ standard error of the mean (SEM). Statistical differences between the groups were examined via one-way ANOVA conducted in GraphPad software 8.0.2 (GraphPad, CA, USA). To ascertain the location of significant pairwise differences in cases when overall *F*-tests yielded significant results ($P < 0.05$), *post-hoc* comparisons using Tukey's method of adjustment were performed.

3. Results

3.1 GAS alleviates the symptoms of DSS-treated colitis in mice

To determine the effect of GAS on colitis, mice were fed 2.5% DSS to construct a UC model, and then, the effect of GAS on colitis symptoms was evaluated (Fig. 1B). The impact of GAS on the DAI score, colon length variation, and weight changes, which are the most obvious indicators of UC severity, was determined. In comparison to the control group, the DSS model mice had substantial weight loss, increased DAI scores, and a reduction in colon length. According to the results, treatment with GAS (50, 100, and 200 mg/kg) substantially decreased the body weight loss (Fig. 2B), lowered DAI scores (Fig. 2D), and increased the colon length (Figs. 2A, C) in DSS-treated mice. These results add up to a strong case for GAS as a potential symptomatic reliever of colitis caused by DSS in mice.

3.2 Impact of GAS on colonic histological alterations caused by DSS in mice

The H&E staining technique was used to determine the histological alterations taking place in the colon. In comparison to the control mice, the DSS-treated animals had submucosal edema, infiltration of inflammatory cells, and loss of crypt structures, as determined by H&E staining (Fig. 2E). Conversely, mice that were treated with GAS demonstrated considerably reduced levels of crypt degradation, infiltration of inflammatory cells, and epithelial injury. Additionally, the histology score of these mice was considerably lower than that of the colitis group treated with DSS (Fig. 2G).

The findings of the PAS staining analysis indicate that the mucosal tissues of the mice treated with GAS were better than those of the DSS group (Fig. 2F). Quantitative analysis indicated that GAS-treated mice exhibited a higher number of goblet cells compared to the DSS group (Fig. 2H). These findings indicate that the treatment with GAS ameliorated the symptoms of colonic tissue damage in mice treated with DSS.

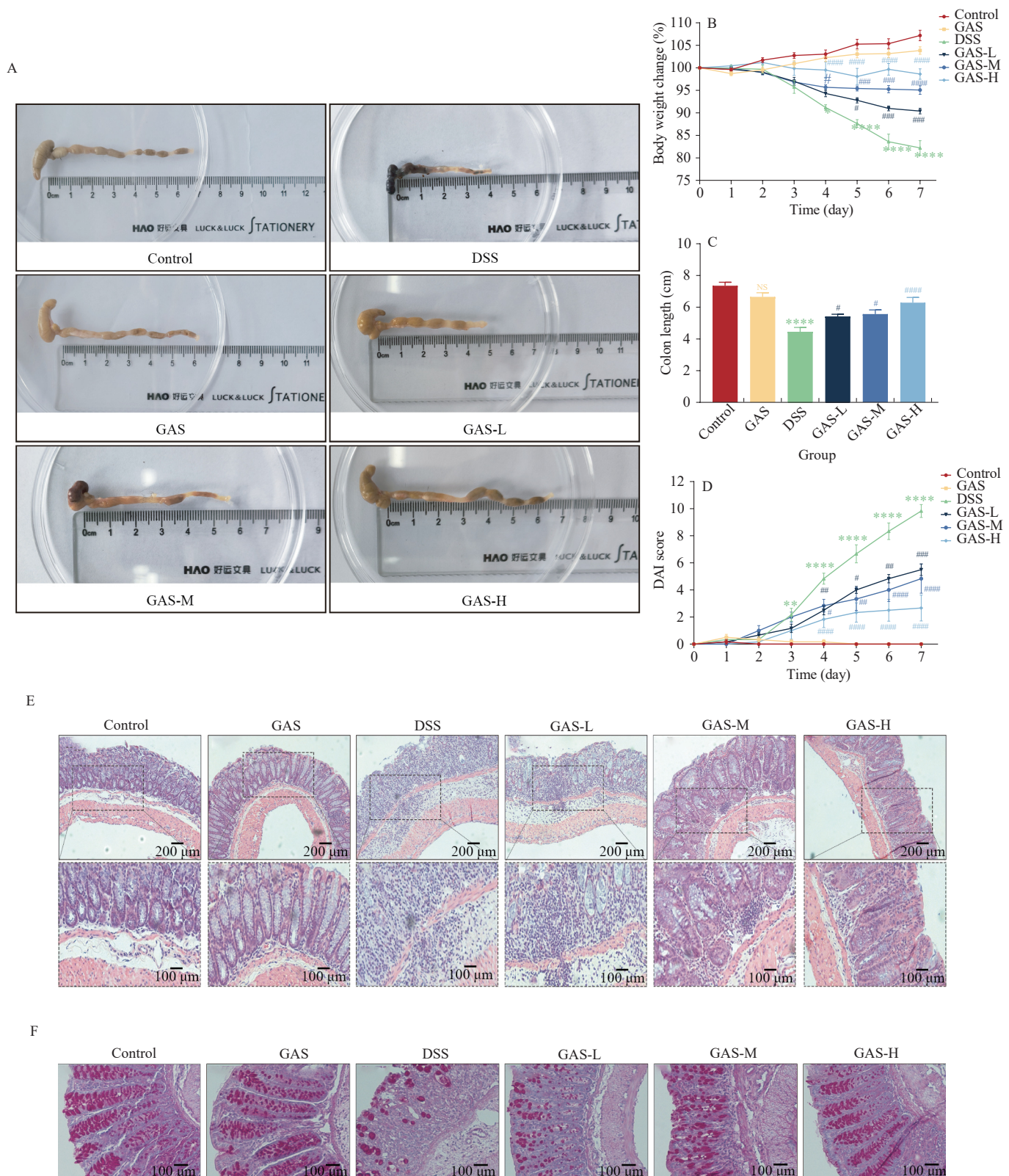


Fig. 2 Effect of GAS on DSS-induced colitis symptoms and pathological damage in mice. (A) Representative images of colon samples from each treatment group. (B) Average weight values for mice in the indicated treatment groups. (C) Analysis of changes in colon length. (D) Changes in the DAI in mice after DSS treatment. (E) Representative micrographs of H&E staining of the colonic tissue. (F) Representative micrographs of PAS staining of the colon tissues. (G) Pathological injury score of the colon tissues. (H) Number of goblet cells in the colon. All data are expressed as mean $\pm$ SEM ($n = 6$). NS: no statistically significant difference vs. Control group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ vs. Control group. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, and #### $P < 0.0001$ vs. DSS-treated group. The same below.

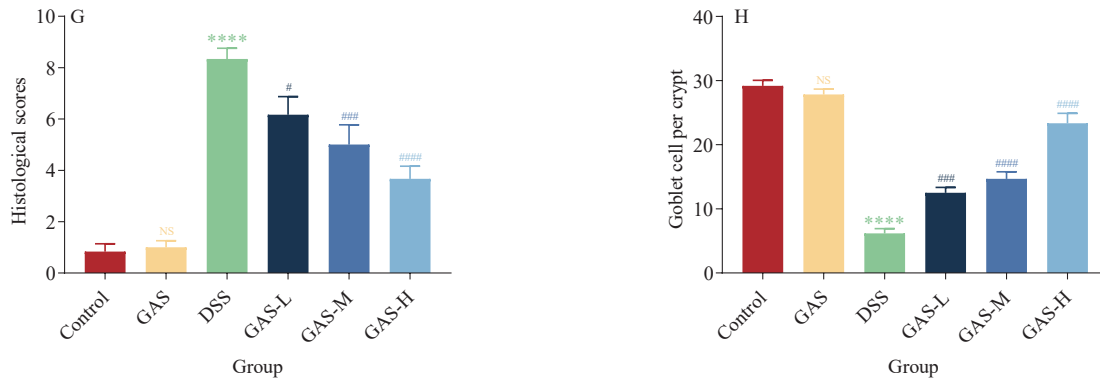


Fig. 2 (Continued)

3.3 GAS alleviates the colonic inflammatory responses in mice treated with DSS

The levels of enzymes (iNOS, COX-2, and MPO) and pro-inflammatory indicators (IL-6, TNF- α , and IL-1 β) were quantified in colon tissues to assess the anti-inflammatory effect of GAS. The iNOS,

COX-2, MPO, IL-6, TNF- α , and IL-1 β levels in the colon samples of mice treated with DSS were elevated in comparison to those of the Control arm. Nevertheless, GAS given at dosages of 50, 100, and 200 mg/kg remarkably decreased the levels of iNOS, IL-1 β , MPO, COX-2, and IL-6 (Fig. 3). According to these observations, GAS treatment could mitigate colonic inflammation in mice treated with DSS.

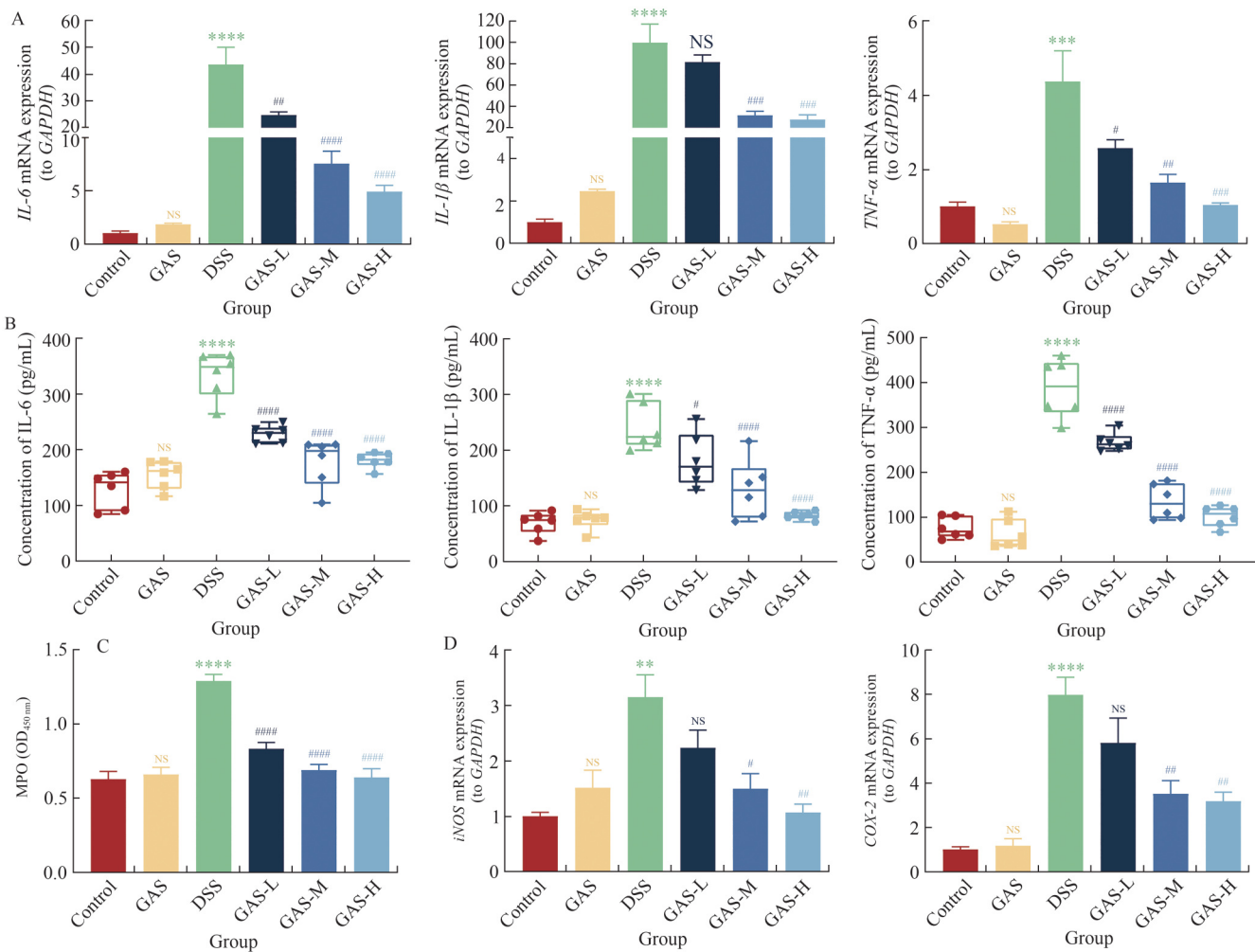


Fig. 3 Effect of GAS administration on inflammation in the colon tissues of mice with UC. The expression levels of inflammatory cytokines (IL-6, IL-1 β , and TNF- α) in colon tissues were detected by (A) qPCR and (B) ELISA. (C) MPO activity in colon tissues. (D) The expression levels of iNOS and COX-2 in the colon tissues were detected by qPCR. (E) Representative bands of Western blot analysis of COX-2 and iNOS in colon tissues. (F) Results of the gray-scale analysis of the corresponding proteins. β -Actin served as the loading control. All data are expressed as mean $\pm$ SEM ($n = 6$).

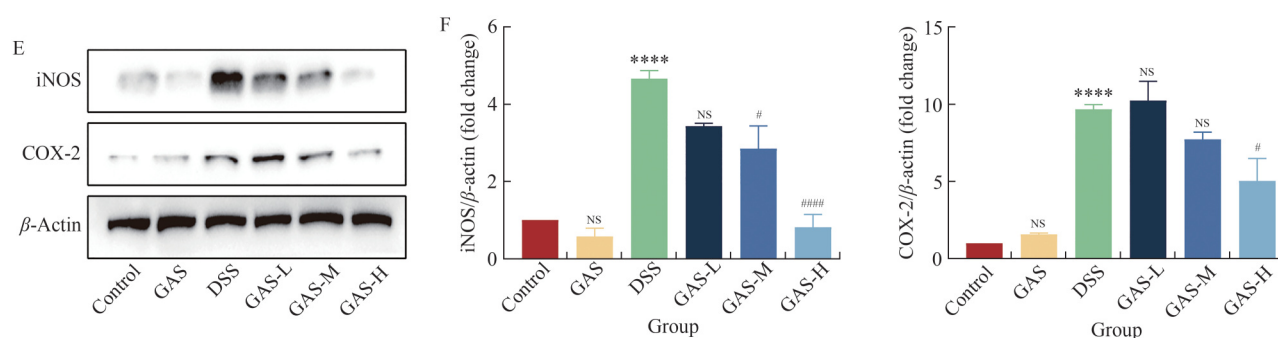


Fig. 3 (Continued)

3.4 The intestinal barrier is protected by GAS treatment in mice treated with DSS

Colitis is characterized primarily by the degradation of the intestinal barrier. The regulation of intestinal mucosal permeability and the bridging of cell gaps are critical functions of TJ proteins. The primary components of TJs are proteins from the claudin family, ZO, and Occludins. To examine the correlation between GAS therapy and the integrity of TJs in the intestines, we then performed Western blotting and immunofluorescence staining to assess the levels of Occludin, ZO-1, and Claudin-3 proteins. As shown in Figs. 4A-F, the levels of these 3 proteins in mice treated with DSS mice were maintained by GAS administration alone, in contrast to the decreased levels observed in the DSS model group. The above analysis illustrates that the increase of TJ protein expression in the colon tissues is linked to the protective properties of GAS against DSS-treated colitis.

3.5 GAS treatment suppresses DSS-treated NF- κ B signaling activity

The major inflammatory mechanism linked to UC is the NF- κ B signaling pathway. Subsequently, to examine the process by which GAS administration ameliorated colitis induced by DSS in mice, we examined NF- κ B signaling pathway protein activation (Fig. 4G). Mice treated with DSS showed significantly elevated levels of NF- κ B p65 and inhibitor of kappa B (I κ B) phosphorylation in their colon tissues in comparison to the Control mice (Figs. 4G-I). Nonetheless, the results of this research showed that GAS effectively inhibited the NF- κ B p65 and I κ B phosphorylation induced by DSS. Therefore, our analysis confirmed that the mitigative effect of GAS on colonic inflammation in mice is associated with the inhibition of NF- κ B pathways.

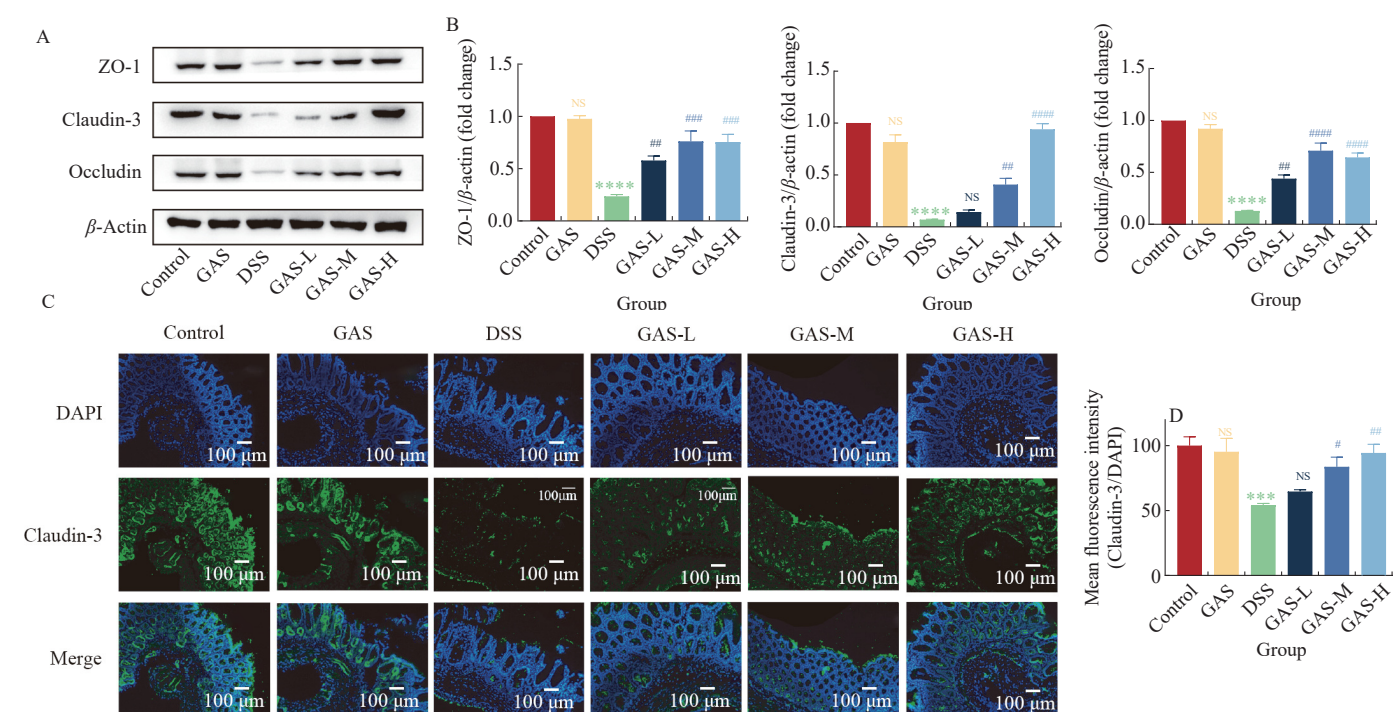


Fig. 4 Effect of GAS on DSS-treated destruction of colonic barrier integrity and activation of NF- κ B signaling in mice. (A) Representative bands of Western blot analysis of ZO-1, Claudin-3, and Occludin in colon tissues. (B) Results of the gray-scale analysis of the corresponding proteins. β -Actin served as the loading control. (C) Representative immunofluorescence pictures of Claudin-3 in the colon tissues. (D) Results of the mean fluorescence intensity analysis of Claudin-3. (E) Representative immunofluorescence pictures of ZO-1 in the colon tissues. (F) Results of the mean fluorescence intensity analysis of ZO-1. (G) Protein levels of p65, p-p65, I κ B, and p-I κ B in the colon tissues. (H, I) Results of the gray-scale analysis of the corresponding protein bands. All data are expressed as mean $\pm$ SEM ($n = 6$).

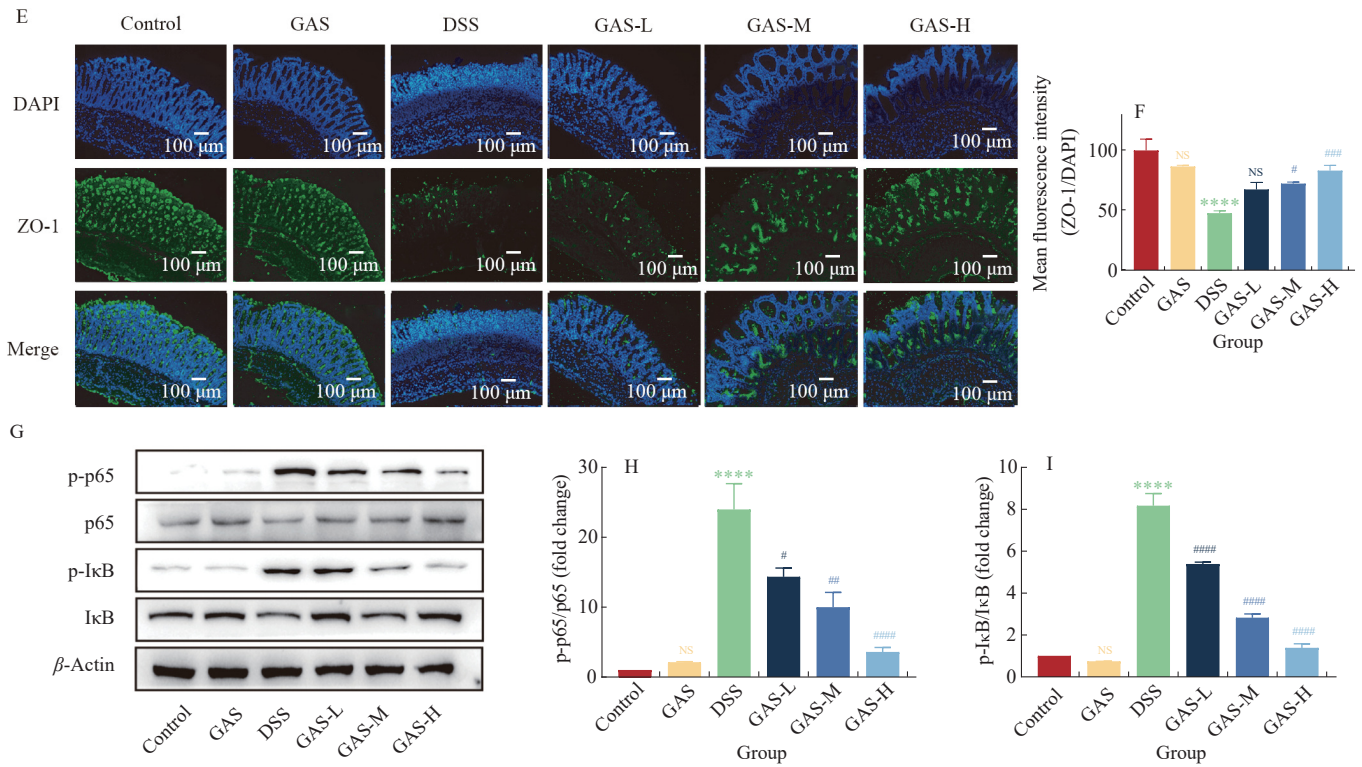


Fig. 4 (Continued)

3.6 GAS regulates the diversity of the intestinal microbiome in colitis mice treated with DSS

Extensive research points to dysbiosis in the gut microbiome as a key contributor to UC pathogenesis. By sequencing 16S rRNA from mouse fecal matter, we determined if the positive impacts of GAS on colitis were associated with the modulation of gut microbiome. With increasing sequence numbers, the rarefaction curve representing the levels of amplicon sequence variants (ASVs) in the gut microbiota of mice gradually reached a saturation plateau, indicating that the depth of the sequencing was enough to cover the majority of bacterial communities (Figs. 5A, B). The Venn diagram showed that 519, 284, and 289 unique ASVs were detected in the Control group, DSS group,

and GAS-H group, respectively (Fig. 5C). The rank-abundance curve reflects a uniform distribution of species in the 3 groups.

Species diversity was lower in the DSS-treated animals relative to the control mice; however, this disparity was ameliorated after GAS administration (Fig. 5D). The specifications accumulation boxplot demonstrated that the sample size was sufficient and the data analysis was convincing (Fig. 5E). An investigation of α -diversity was carried out to assess how GAS impacted the diversity and abundance of the intestinal microbiome. A remarkable reduction in the Chao1 and Observed_otus index was observed in the DSS-treated mice in comparison to those of the Control mice; however, GAS administration showed an upward trend in these indices. Furthermore, the Shannon and Simpson index of the DSS-treated animals showed a

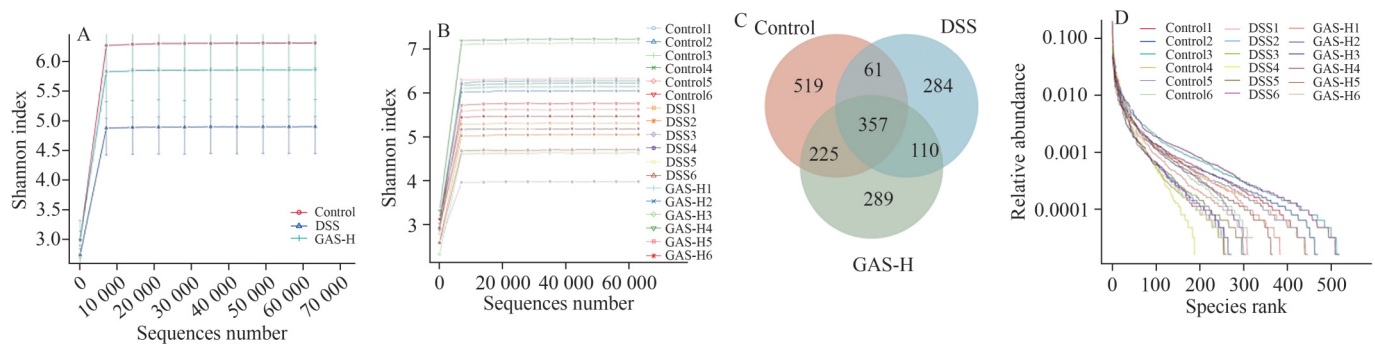


Fig. 5 Effect of GAS administration on the diversity and structure of intestinal flora in mice with colitis induced by DSS. (A) Inter-sample and (B) inter-group rarefaction curves based on Shannon of intestinal flora. (C) Venn diagram shows common and unique ASVs between groups. (D) Rank abundance curves. (E) Species accumulation boxplot. (F) α -Diversity based on the Observed_otus, Chao1, Shannon and Simpson indices. (G) β -Diversity heatmap. (H) Principal co-ordinates analysis intestinal flora based on the unweighted UniFrac distance. (I) NMDS analysis of intestinal flora based on Bray-Curtis distance. (J) Evolutionary cladistic diagram of LefSe analysis. (K) LDA of LefSe analysis (LDA > 4, $P < 0.05$). All data are expressed as mean $\pm$ SEM ($n = 6$). NS, Not significant ($P > 0.05$).

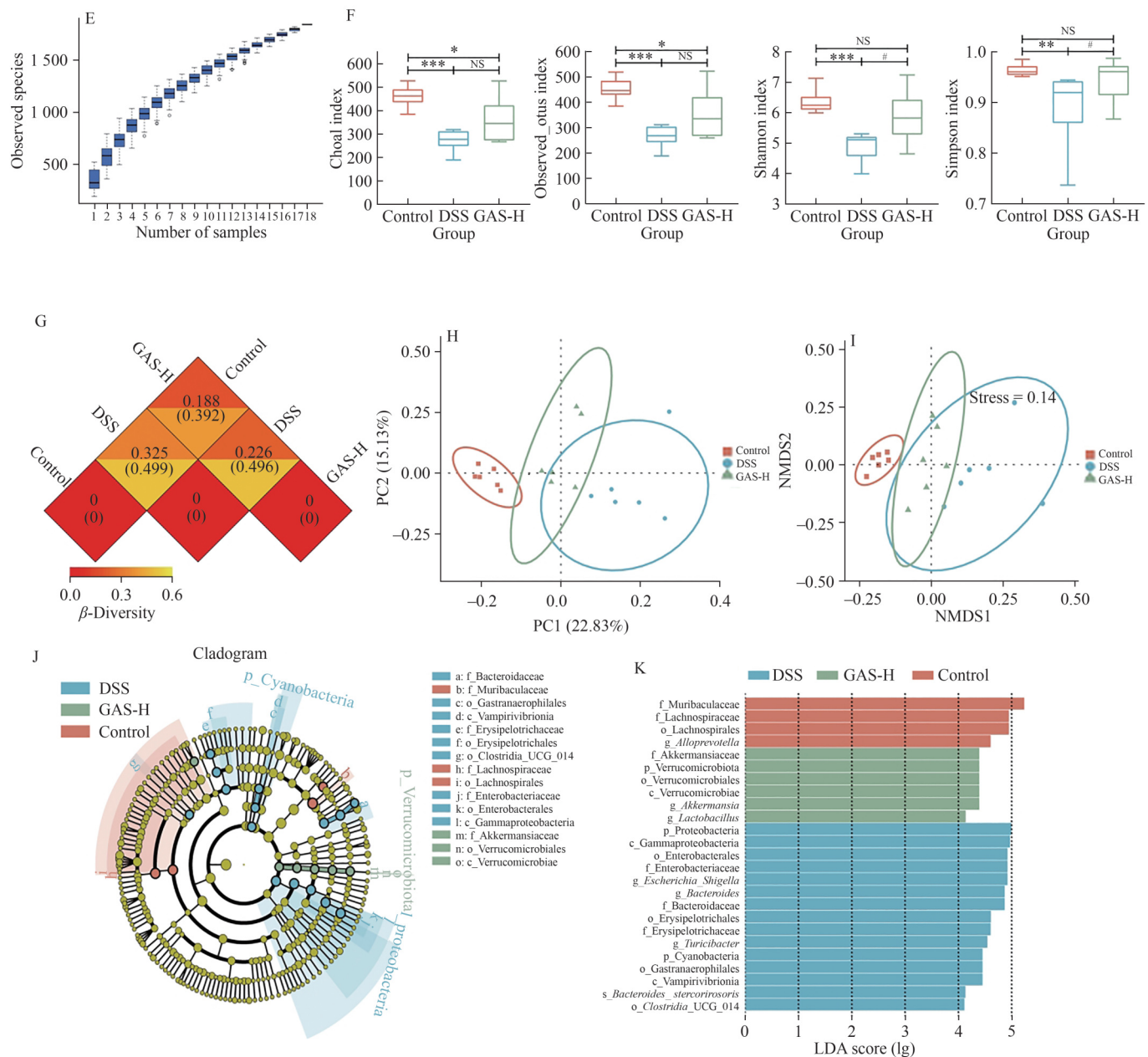


Fig. 5 (Continued)

remarkable decrease in comparison to those of the Control mice, but administering GAS significantly raised these indices (Fig. 5F), suggesting that the treatment with GAS can potentially enhance microbial diversity. The distance matrix heatmap shows that the species diversity of the DSS-treated mice differs from that of the Control and GAS-H mice (Fig. 5G). Next, β -diversity, which including principal coordinates analysis (PCoA) and non-metric multidimensional scaling (NMDS), was used to evaluate the degree of similarity across microbial populations. The findings demonstrated that the gut microbiota of the Control and the DSS mice were significantly different, while GAS administration mitigated this DSS-induced shift (Figs. 5H, I). In summary, the results show that the gut microbiome in mice treated with DSS is regulated by GAS treatment.

3.7 GAS regulates the diversity of the intestinal microbiome in DSS-treated mice

We used linear discriminant analysis (LDA) effect size (LEfSe) to evaluate the influence of GAS treatment on the diversity of microbes in the gut, which was based on an investigation of the diversity of the gut microbiome. The findings demonstrated that the dominant flora in the GAS-H mice were *Akkermansia* and *Lactobacillus*, but in the DSS mice, it was *Escherichia Shigella*, *Bacteroidaceae*, and *Turicibacter* (Figs. 5J, K). Analyzing alterations in the gut microbiome across groups at the phylum, family, and genus levels helped to better elucidate the impact of GAS treatment on associated microbiota composition (Figs. 6A-F). At the family level, GAS administration significantly increased the relative abundance of

Muribaculaceae and Lachnospiraceae, while decreasing the relative abundance of Enterobacteriaceae and Bacteroidaceae in DSS-treated mice (Fig. 6D). At the genus level, *Bacteroides* and *Escherichia-Shigella* were shown to be considerably less abundant in DSS-treated animals after GAS treatment (Fig. 6F). Additionally, we used Tax4Fun to create a clustering heat map of functional annotations for stool specimens and to predict their functions in our study (Fig. 6G). In stool specimens, we found functional information for 35 different bacterial species. Notably, 14 species were substantially increased in the DSS mice and the detected functional information included biosynthesis of other secondary metabolites, aging, drug resistance, endocrine system, metabolism, lipid metabolism, metabolism of terpenoids and polyketides, energy metabolism, xenobiotics biodegradation and metabolism, amino acid metabolism, cell growth and death, cellular community-prokaryotes, metabolism of other amino acids, infectious diseases. In the GAS-H group, we detected a

significant increase of 3 species with functional information on folding, sorting and degradation, carbohydrate metabolism, and poorly characterized. In conclusion, GAS administration may adjust the composition and function of the intestinal microbiome in mice treated with DSS.

3.8 The impact of GAS on the gut microbiome of SCFAs in mice treated with DSS

To ascertain if the beneficial effects of GAS against UC were linked to the synthesis of SCFAs, we further quantified the SCFA content in the feces. When compared to control mice, the synthesis of propionic, valeric, acetic, and hexanoic acids was remarkably enhanced by DSS treatment, but acetic acid synthesis was remarkably reduced by GAS treatment (Fig. 6H). Based on these results, reduced synthesis of certain SCFAs in the feces may have contributed to the protective impact of GAS against UC.

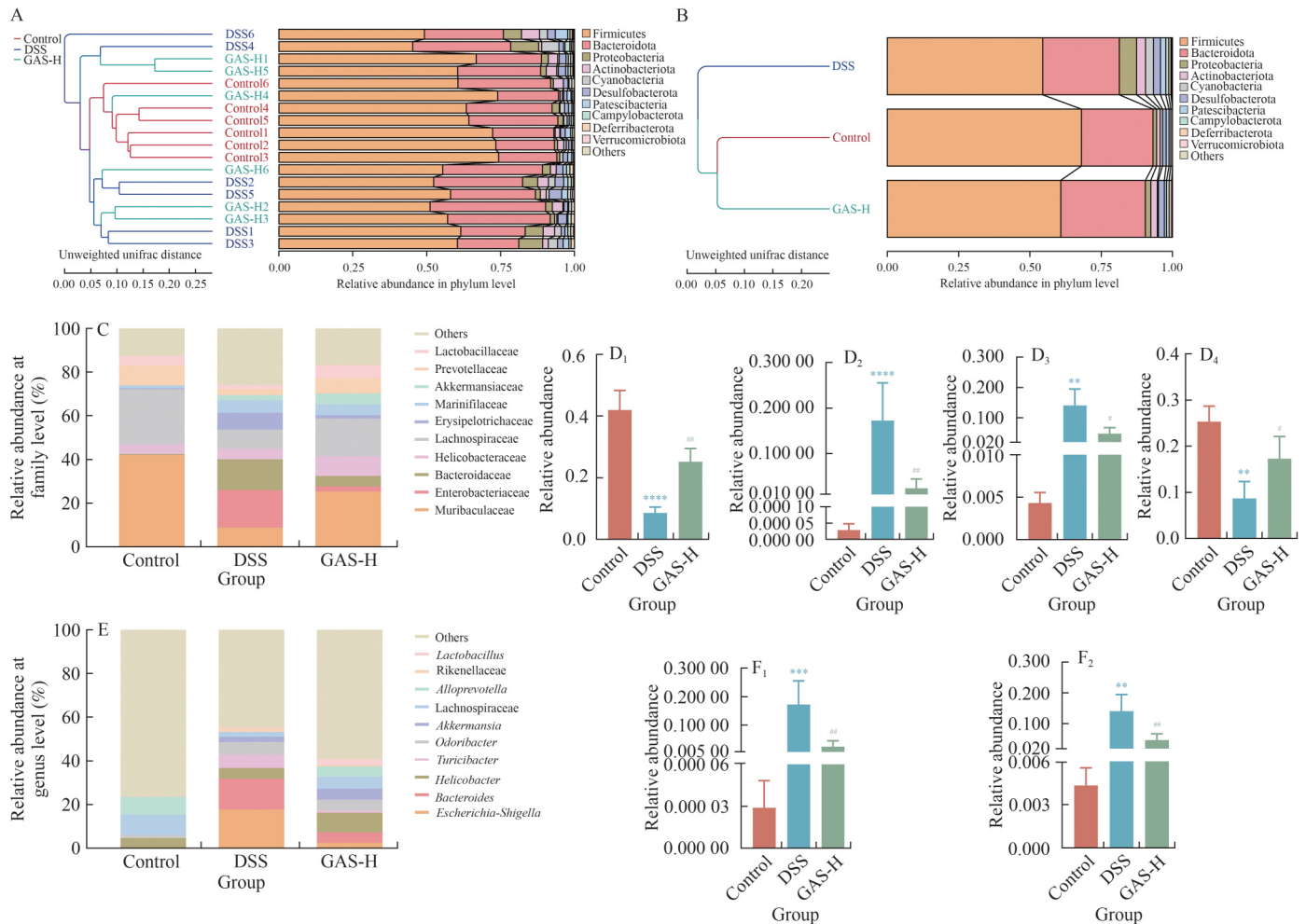


Fig. 6 Effect of GAS administration on the relative abundance of specific intestinal bacteria and the metabolites in DSS-treated mice. (A) Inter-sample and (B) inter-group unweighted pair group method with arithmetic mean (UPGMA) cluster trees at the phylum level. (C) The top 10 family in colon samples from the 3 treatment groups. (D) Difference in the relative abundance of specific intestinal bacteria at the family level between groups. 1-4. Muribaculaceae, Enterobacteriaceae, Bacteroidaceae, Lachnospiraceae. (E) The top 10 genus in colon samples from the 3 treatment groups. (F) Difference in the relative abundance of specific intestinal bacteria at the genus level between groups. 1-2. *Escherichia-Shigella* and *Bacteroides*. (G) Tax4fun functional annotations clustering heat map. Red and blue represent the higher and lower relative abundances of a function in the groups, respectively. (H) Effect of GAS on DSS-treated of intestinal flora of SCFA in mice. Violin plot shows fecal levels of in mice with different groups. (I) Correlation among gut microbiota, pro-inflammatory mediators, colitis indices, TJs and SFCAs based on Spearman's rank correlation analysis. The red color represents a positive correlation, and the blue color represents a negative correlation. All data are expressed as mean $\pm$ SEM ($n = 6$).

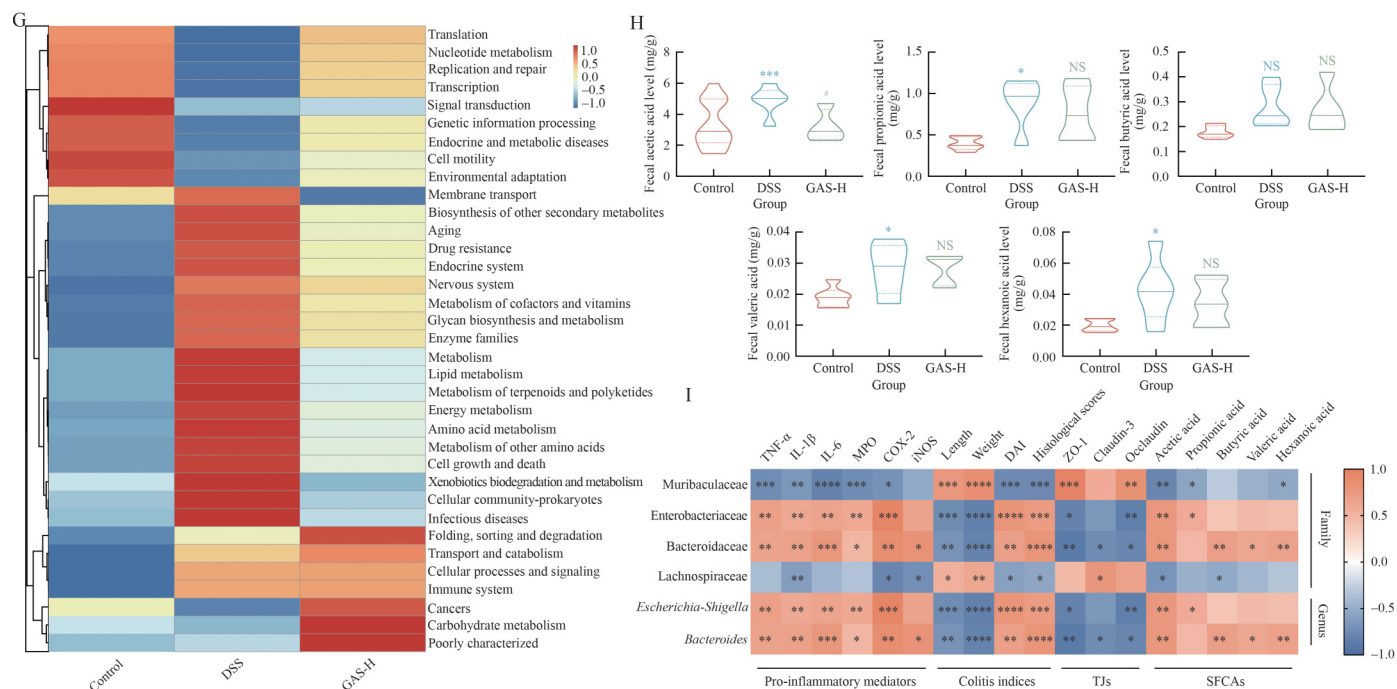


Fig. 6 (Continued)

3.9 The relationship between colitis and the intestinal microbiota

A Spearman's rank correlation analysis was applied to examine the link between gut microbiome, proinflammatory cytokines, colitis parameters, TJs, and SCFAs (Fig. 6I). Muribaculaceae and Lachnospiraceae were negatively related to the levels of inflammatory mediators, certain SCFAs, histological score, and DAI; conversely, they were positively linked to changes in body weight, variations in the length of the colon, and expression of TJ proteins, as determined at the family level. Enterobacteriaceae and Bacteroidaceae showed positive correlations with DAI, histological score, certain SCFAs, and proinflammatory mediators, respectively, and inverse correlations with changes in body weight, variations in the length of the colon, and expression of TJ proteins. Positive correlations were observed between certain SCFAs, proinflammatory cytokine levels, the DAI, histological score, and *Bacteroides* and *Escherichia-Shigella* at the genus level. Conversely, negative correlations were seen with other indices. Therefore, by regulating

specific gut microbes, GAS could alleviate colitis caused by DSS, according to these findings.

3.10 The impact of FMT from GAS-treated to DSS-treated mice

The procedure outlined in Fig. 1C was used to investigate the impacts of FMT from GAS-treated mice on DSS animals. Following the FMT experiment, the average body weight was increased from day 3 to day 7 (Fig. 7A), and the DAI scores were reduced from day 4 to day 10 (Fig. 7B) in the FMT-GAS mice relative to the DSS and FMT-CON mice. The FMT-GAS mice had noticeably longer colons and poorer histology scores relative to the DSS animals (Figs. 7C, D, F). The FMT-GAS group exhibited considerably mitigated crypt degradation, epithelial damage, and infiltration of inflammatory cells caused by DSS, as determined by H&E staining. However, the FMT-CON group showed significant alleviation only of the inflammatory cell infiltration (Fig. 7E). An increase in glycogen levels was observed in mice that were treated with feces from the GAS donors, as

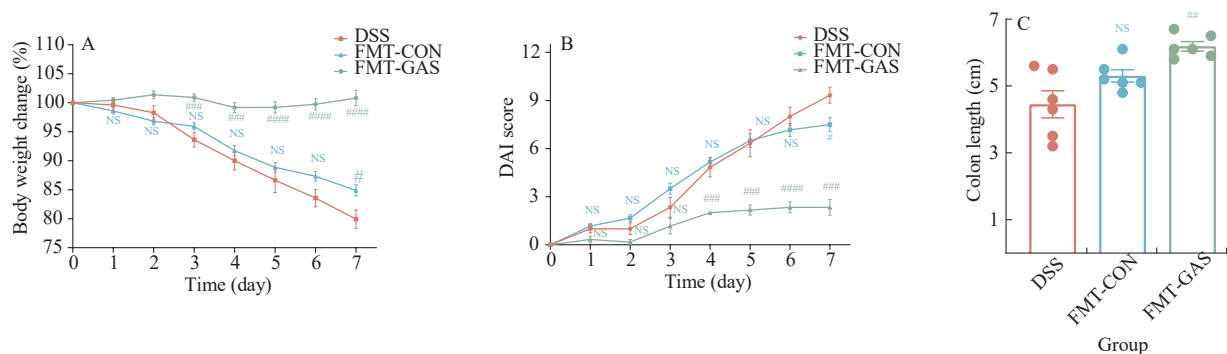


Fig. 7 Effect of FMT treated on DSS-induced colitis symptoms and pathological damage in mice. (A) Average weight values for mice in the indicated treatment groups. (B) Changes in the DAI in mice after DSS treatment. (C) Analysis of changes in colon length. (D) Representative images of colon samples from each treatment group. (E) Representative micrographs of H&E staining of the colonic tissue. (F) Pathological injury score of the colon tissues. (G) Representative micrographs of PAS staining of the colon tissues. (H) Number of goblet cells in the colon. All data are expressed as mean $\pm$ SEM ($n = 6$).

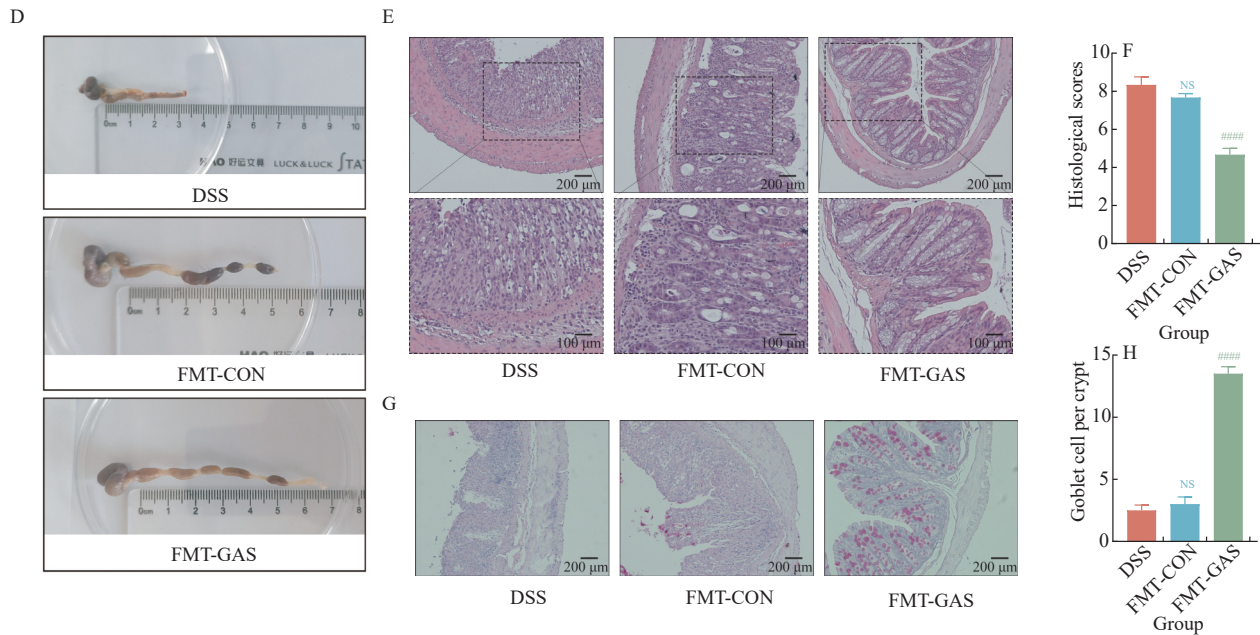


Fig. 7 (Continued)

determined by PAS staining, suggesting that the introduction of fecal bacteria from GAS donor mice may enhance the intestinal barrier's integrity and goblet cell abundance. In contrast, the concentration of glycogen remained unaffected by the FMT-CON treatment (Figs. 7G, H). Therefore, it may be deduced that GAS alleviated colitis by regulating the gut microbiota.

3.11 GAS's influence on inflammatory markers and cell viability

An analysis of the *in vitro* impacts of GAS administration on RAW264.7 cells demonstrated that it exhibited no toxicity within a restricted dosage range (2.5–40.0 mmol/L) (Fig. 8A). Cells were then treated for 1 h with GAS, after which they were stimulated with LPS for 24 h, and respective analyses of mRNA and protein expression were then conducted. The treatment with GAS effectively and dosage dependently suppressed the elevation of pro-inflammatory markers caused by LPS, such as *IL-6* (Fig. 8B), *IL-1 β* (Fig. 8C), and *TNF- α* (Fig. 8D), as shown by qPCR analysis. Furthermore, we used qPCR and Western blotting to evaluate the impact of GAS intervention on the expression profiles of iNOS and COX-2 in these cells, which illustrated that GAS effectively inhibited the LPS-induced elevation of both iNOS (Figs. 8E, G, H) and COX-2 (Figs. 8F, G, I).

3.12 Impact of GAS on cellular NF- κ B signaling pathways

To enhance our comprehension of the pathways via which GAS may impact inflammatory signaling processes, we assessed NF- κ B signaling pathway protein levels in RAW264.7 cells (Fig. 8J). NF- κ B p65 and I κ B phosphorylation elevations caused by LPS in these cells were considerably reduced by GAS treatment (Figs. 8K, L), which indicated that the suppression of NF- κ B pathways is linked to the mitigative impact of GAS on LPS-induced cell inflammation.

4. Discussion

UC is a non-specific and chronic inflammation that often occurs in the rectum and colon, with a long course and poor prognosis, seriously affecting patients' quality of life. UC mainly affects the mucosa and submucosa of the colon, manifesting as open ulcers or characteristic ulcers, and characterized by symptoms such as diarrhea, weight loss, abdominal pain, and mucopurulent bloody stool. Inadequate management may result in the progression to cancer associated with the condition. The treatment plan ranges from conventional drug therapy to targeted drugs, and even surgical intervention^[19]. The symptoms caused by DSS in mice are similar to those of UC patients, including bloody stools, weight loss, and colon shortening^[38]. Therefore, according to current research, DSS is considered the most effective method for establishing experimental colitis models, and this disease induction model has been widely used to study the molecular mechanisms and treatment methods of UC^[39].

In this study, the impact of GAS treatment on colitis mice and the underlying *in vivo* processes were assessed, as well as the effect on lipopolysaccharides (LPS)-induced RAW264.7 cells inflammation model and possible *in vitro* mechanisms^[40]. The findings demonstrated that the treatment with GAS in mice might mitigate the adverse effects of DSS, including weight loss, colon shrinkage, increased DAI, and increased histopathological scores. Mechanistically, GAS can inhibit the activation of NF- κ B related pathway proteins and the expression of pro-inflammatory mediators, thus promoting the expression of TJ proteins and restoring the integrity of the intestinal barrier in DSS-induced colitis mice. In addition, we studied the function of GAS in modulating gut microbiota in a DSS-treated UC mouse model through the FMT experiment. *In vitro* experiments showed that GAS treatment can also inhibit the NF- κ B related pathway proteins activation and the expression of pro-inflammatory markers in cellular inflammation models. In summary, the findings of this study suggest that GAS mitigates colitis caused by DSS in mice *via* modulating the NF- κ B

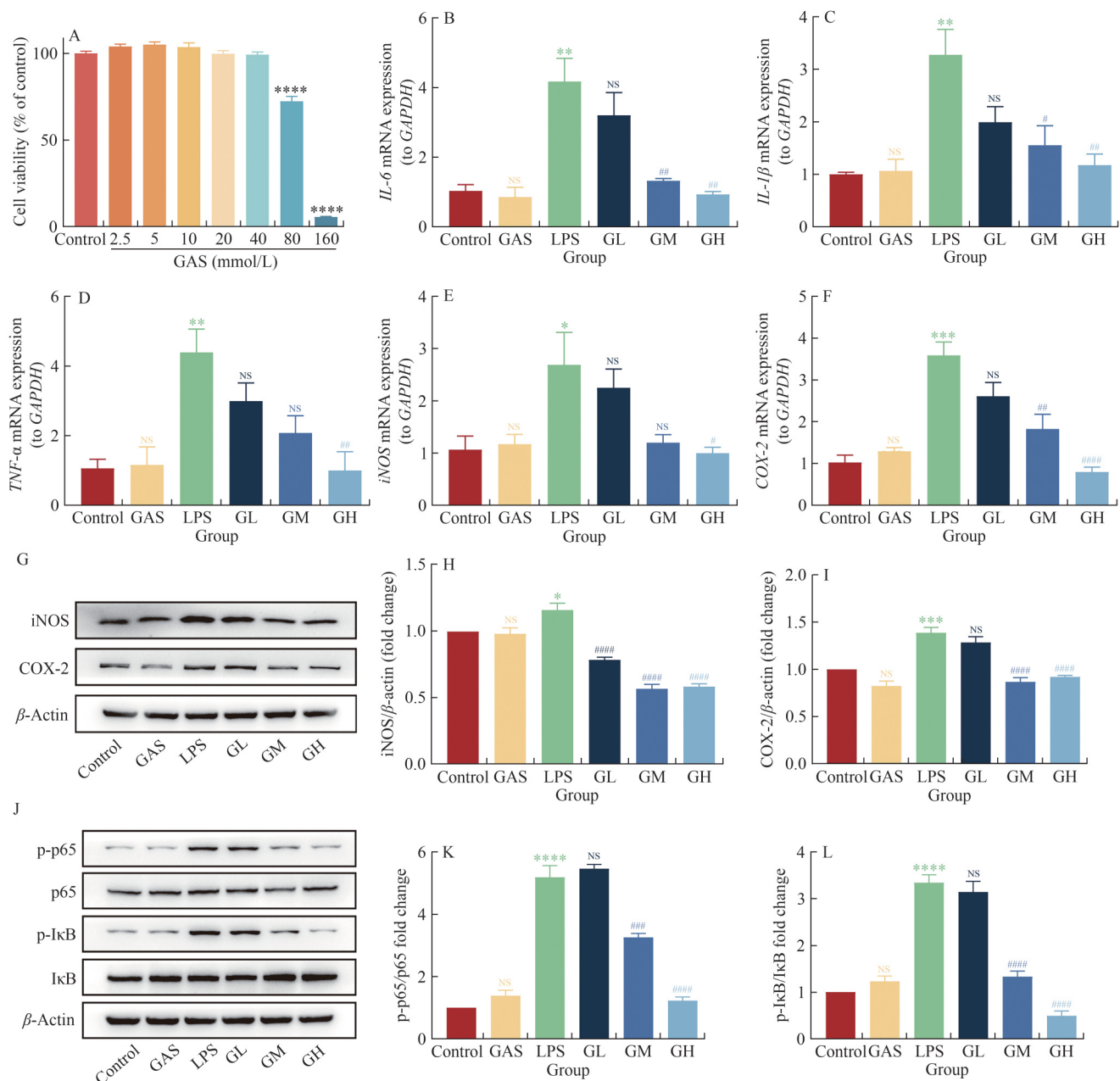


Fig. 8 GAS treatment suppresses LPS-induced pro-inflammatory cytokine production by RAW264.7 cells and inhibits NF-κB signaling. (A) The impact of GAS of gradient concentration treatment on RAW264.7 cell viability was assessed. (B-F) *IL-6*, *IL-1β*, *TNF-α*, *iNOS* and *COX-2* expression in treated cells were expressed by qPCR. (G-I) *COX-2* and *iNOS* protein levels were measured by Western blot following a 24 h treatment with LPS. (J) Protein levels of p65, p-p65, IκB, and p-IκB in the cells. (K, L) Results of the gray-scale analysis of the corresponding protein bands. All data are expressed as mean ± SEM ($n = 3$).

signaling pathway, which is responsible for cellular inflammatory response inhibition, intestinal barrier integrity maintenance, and intestinal microbiota structure adjustment. Therefore, we hypothesize that GAS administration can effectively treat UC (Fig. 9).

Inflammatory cell infiltration, loss of crypts, diminished goblet cells, and mucosal destruction are histological features of colitis caused by DSS in mice that closely resemble the clinical manifestations of UC in humans^[41]. GAS considerably reduced the elevation in histopathological scores and DAI induced by DSS in this study, especially in the high-concentration group (200 mg/kg). Research has demonstrated that goblet cells are essential for controlling intestinal permeability due to their capacity to produce

and secrete mucus. Intestinal mucosal injury and goblet cell loss are intimately associated with UC onset and progression. Our findings demonstrated that in comparison to the control mice, the DSS mice exhibited a substantially decreased proportion of goblet cells. Nevertheless, the goblet cell loss caused by DSS was remarkably attenuated after GAS treatment.

Research has linked pro-inflammatory factors to intestinal and mucositis^[42], and there is mounting evidence that immune responses generating local inflammation interact with gut tissue damage in susceptible individuals^[19]. Therefore, possible treatment options for this condition include preventing the generation of pro-inflammatory factors in the gut microenvironment. Earlier literature has

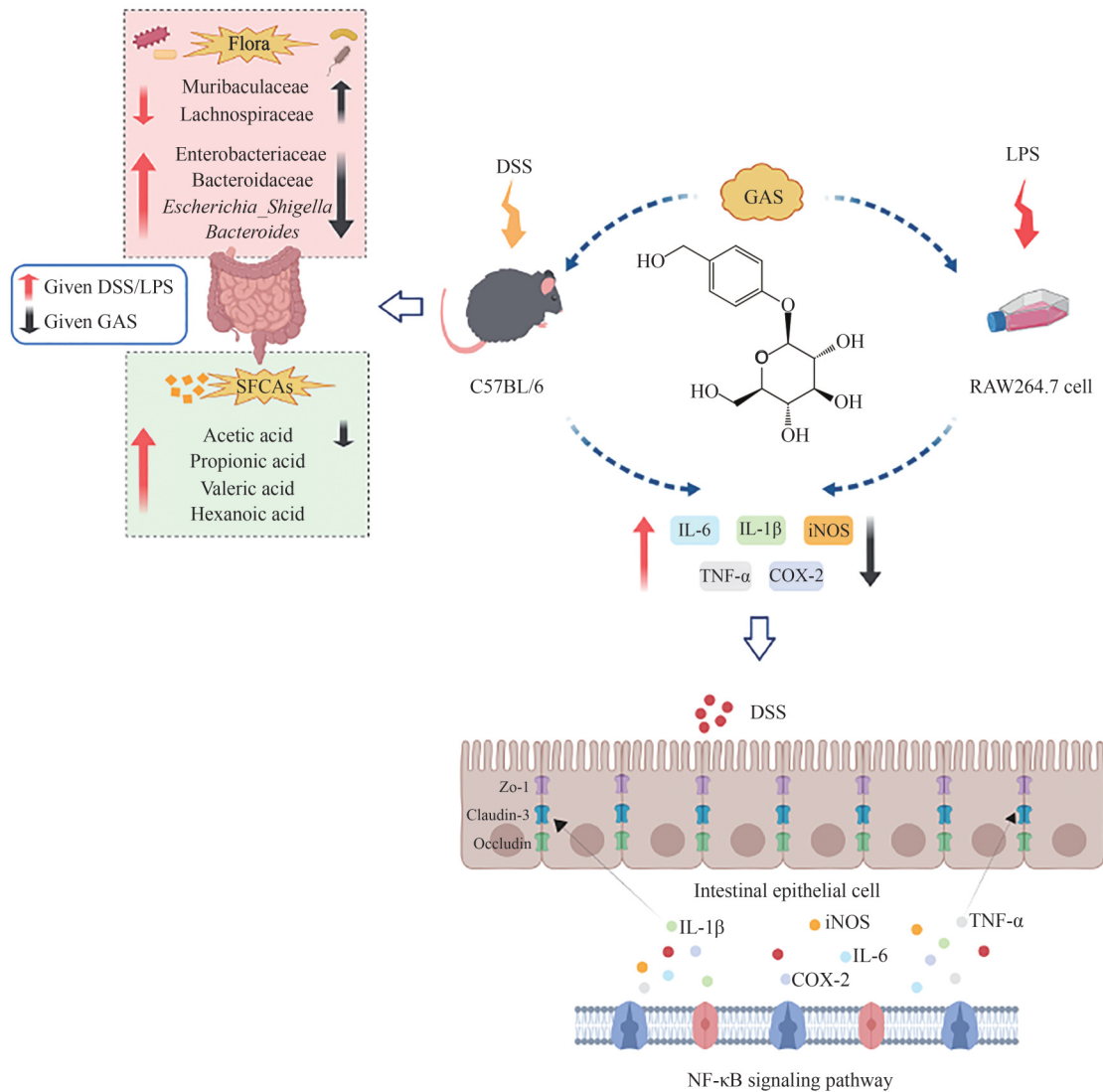


Fig. 9 Schematic for the effects and mechanisms of GAS bioactivity in DSS-induced UC mice and LPS-induced cells.

demonstrated that during UC, neutrophils and macrophages invading colon tissue may release several pro-inflammatory mediators, including $\text{TNF-}\alpha$, $\text{IL-1}\beta$, COX-2 , IL-6 , and iNOS , among others^[43]. Besides, MPO is an enzyme that is abundant in neutrophils and is considered an indicator of neutrophil infiltration into inflammatory tissue. The MPO released by neutrophils can promote the production of a potent oxidant that can cause colon tissue damage and inflammatory diseases^[44]. These mediators showed a significant upregulation in response to DSS treatment and a downregulation in response to GAS treatment in this study.

Another key mediator that contributes to the progression of UC is the disruption of the intestinal barrier. This mechanical barrier layer is made up of epithelial cells and the tight connections between them, which is essential for maintaining the barrier's integrity^[45]. Cytoplasmic scaffold proteins, like the ZO family, and transmembrane barrier proteins, such as occludin and claudin, are important components of TJs, which are critical for intestinal permeability^[46]. Consistent with the findings of our DSS group in this investigation, previous research has demonstrated that DSS dosing may increase gut permeability, decrease TJ protein levels, and disrupt

the TJ protein distribution^[36,47]. However, the levels of these proteins were remarkably increased in mice treated with DSS mice after GAS treatment, confirming that GAS aids in protecting the intestinal barrier integrity.

An extensive body of research has linked NF- κ B, one of the primary signaling pathways that regulate the expression of pro-inflammatory indicators including IL-6 , $\text{IL-1}\beta$, and $\text{TNF-}\alpha$, to the advancement of colitis^[48-50]. The NF- κ B family includes 5 related transcription factors: p50, p52, p65 (RelA), RelB and c-Rel^[51-52]. Under physiological conditions, the I κ B protein present in the cytoplasm inhibits NF- κ B. Pattern-recognition receptors (PRRs) activate I κ B kinase (IKK) through signal transduction mechanisms, then regulate NF- κ B pathway activated through I κ B phosphorylation, which leads to the activation of the p65/p50 complex, causing p65 phosphorylate and enters the nucleus to regulate transcription. This pathway regulates the production of pro-inflammatory cytokines and the recruitment of inflammatory cells, thereby promotes the inflammatory response^[53-54]. Hence, UC therapy could benefit from targeting the NF- κ B signaling pathway. Based on the above viewpoint, this study explores how GAS influences the NF- κ B

signaling pathway. The phosphorylation of proteins associated with NF- κ B was decreased by GAS, as was anticipated, which resulted in the inhibition of the inflammation.

Extensive research evidence has demonstrated that various diseases are linked to disorders in the gut microbiome, among which gut microbiota imbalance is intimately linked to the onset of IBD, including UC^[55]. A key pathogenic component of UC is an imbalance in the gut microbes, which could also trigger the body's immune response, impair the intestinal barrier, allow certain pathogens in the gut to pass through, activate the classic NF- κ B inflammatory pathway, and stimulate the production of mediators that promote inflammation (IL-6, IL-1 β , and TNF- α , etc.)^[56]. This can trigger uncontrollable inflammatory reactions, further damaging the intestines and causing a prolonged course of disease. Consequently, the ideal strategy to treat or prevent UC is to prevent or reverse changes in the gut microbiota.

Previous studies have found notable variations in the composition and diversity of gut microflora between healthy individuals and UC patients^[57-58]. Due to a certain concentration dependence of symptoms, degree of pathological damage, expression levels of inflammatory factors, and integrity of the colon barrier *in vivo* experiments, suggesting that group GAS-H is more representative in the treatment group. Therefore, GAS-H was measured for gut microbiota and SCFAs analysis. Consistent with earlier findings, this study examined the diversity of gut microflora and discovered that DSS model mice exhibited reduced bacterial abundance than control mice. However, after GAS treatment, α -diversity has been partially restored. Additionally, the gut bacteria of mice exposed to DSS differed significantly from that of the Control animals, according to NMDS and PCoA analyses. GAS (200 mg/kg) can alleviate the transfer of gut microbiota induced by DSS, illustrating the effects of GAS in alleviating the imbalance of the intestinal microbiota induced by DSS.

At the family level, GAS significantly increased the abundance of Muribaculaceae and Lachnospiraceae in UC mice. Muribaculaceae has a very significant advantage in the gut of rodents, and its abundance is also high in the intestines of humans^[59]. Previous research illustrated that the species abundance of Muribaculaceae in the intestine of mice is as high as 30%, with an average of at least 8 different species coexisting^[60-61]. This facilitates its functional versatility, allowing it to occupy different ecological niches in a common community. This versatility involves complex carbohydrate degradation, which is linked to the relief of inflammation^[60-61]. However, there is still controversy over its correlation with SCFAs. Some experiments have confirmed a positive correlation between this family of bacteria and the production of certain SCFAs^[62], while others have confirmed a negative correlation^[63], which concurs with this study's findings. Therefore, further experiments are needed to confirm the correlation and reasons between the two in organisms. Many species in the Lachnospiraceae family are associated with polysaccharide degradation and SCFA production, which can prevent various diseases, including colorectal cancer, IBD, and obesity, suggesting that Lachnospiraceae has anti-inflammatory and lipid-lowering effects^[64-65]. In addition, compared with DSS-treated mice, GAS considerably lowered the abundance of Enterobacteriaceae and Bacteroidaceae in colitis mice. These 2 bacteria exhibit positive correlations with the inflammation index of UC mice and negative correlations with TJs. Enterobacteriaceae exhibits high relative abundance in patients with IBD and can cause severe colitis. It is also considered a potential pathogen of DSS-treated mouse colitis^[66]. Bacteroidaceae is

an inflammation-related bacterium that significantly increases in abundance in colitis mice^[67]. Consequently, GAS treatment restored a healthy microbiome balance in mice with colitis at the family level.

The genus-level richness of *Escherichia_Shigella* and *Bacteroides* in colitis was remarkably decreased after GAS treatment. *Escherichia_Shigella* is a type of proteus that induces intestinal inflammation by penetrating epithelial cells, causing macrophage apoptosis, and releasing IL-1 β ^[68]. According to reports, some *Faeroseburia* can disrupt the mechanical barrier of the intestines and tend to correlate positively with symptoms of colitis^[69]. Early experiments provided evidence that as an important indicator of microbial changes, the ratio of Firmicutes to Bacteroidetes (F/B) was significantly decreased in the DSS mice^[70-71]. Our data demonstrated that the relative abundance of *Bacteroides* rose remarkably in the DSS mice, which was inhibited by GAS and indirectly increased the F/B. In addition, after DSS administration, changes were also observed in the abundance of several other colitis-related bacteria. The DSS group had an increased abundance of *Alloprevotella* and *Ligilactobacilli* and a decreased abundance of *Turicibacter* when compared to the Control animals. GAS administration improved this trend. According to previous reports, *Turicibacter* showed high abundance in the IBD mouse model, which can exacerbate intestinal damage and cause serious complications^[72]. Previous research has illustrated that *Alloprevotella* and *Ligilactobacilli* are associated with the onset of UC^[72]. Treatment with DSS remarkably raised the abundance of these microbes in our investigation, but treatment with GAS reversed the trend.

According to these findings, the positive effects of GAS in UC are associated with alterations in the gut microbiome. Therefore, FMT tests were performed to determine if GAS ameliorates DSS-induced UC *via* regulation of gut flora. These experiments involved transplanting feces from donor mice that had been treated with PBS or GAS into recipient mice that had been administered distilled water with 2.5% DSS. The findings demonstrated that FMT-GAS animals had considerably longer colons than DSS and FMT-CON mice, with much less inflammation and histological damage. These results indicate that the fecal treatment of GAS donor mice improved the levels of injury and inflammation in recipient UC mice.

In addition, gut homeostasis may also be influenced by microbial metabolites. For example, previous studies have reported that SCFAs can keep the intestinal epithelial barrier structurally intact and improve intestinal homeostasis^[73-74]. However, some reports suggest that excessive SCFAs may lead to pathological conditions such as diarrhea, which is consistent with the clinical manifestations of UC^[75-77]. Herein, substantial increases in the levels of acetic, propionic, valeric acid, and hexanoic acids were observed in the DSS group relative to the Control mice and this difference is strongly associated with diarrhea, loose stools, and other symptoms that were observed in the DSS group. After GAS administration, only acetic acid showed significant changes. Therefore, considering that although GAS can improve intestinal homeostasis, the SCFAs studied in this study may not be a key factor and effective target for this treatment regimen for UC. Further non-targeted metabolomics experiments are needed to determine the effective targets of drugs in microbial metabolites.

The intestinal epithelial barrier may be severely damaged by inflammatory substances that are produced by macrophages, which can then contribute to the development of UC. Moreover, in many chronic inflammatory disorders, macrophages are the primary source

of pro-inflammatory factors^[78-79]; hence, blocking these processes would be beneficial to alleviate UC symptoms. The activation of the NF- κ B signaling pathway is an important driving factor for the production of such inflammation and related cytokines^[51,80]. In this study, through *in vitro* experiments, we can confirm that GAS can not only inhibit the activation of the NF- κ B pathway in colon tissue and inhibit inflammatory factors but also inhibit the inflammatory changes in LPS-induced RAW264.7 cells. This suggests that the anti-inflammatory characteristics of GAS may arise from its capacity to suppress this signaling pathway in the context of inflammation.

5. Conclusion

To summarize, our research showed that GAS might inhibit the activation of the NF- κ B signaling pathway by reducing levels of I κ B and p65 phosphorylation, which prevented macrophage-mediated inflammatory responses and alleviated UC-related damage to the intestinal epithelium in this study. The fact that GAS might disrupt the composition of the gut microbiome and strengthen the intestinal barrier further makes it an attractive medicinal choice for the clinical treatment of UC.

According to these findings, GAS possesses anti-inflammatory properties and enhances the intestinal epithelial barrier. There is strong evidence that GAS is safe for use in therapeutic settings, and there have been reports of its use in the treatment of some psychiatric or neurological diseases. As such, GAS may be readily used in clinical settings. We contend that GAS offers a wide range of potential applications for UC treatment, based on the findings of this research. An independent evaluation of the impact that GAS has on UC will be possible when the follow-up research has been completed. We intend to enroll some volunteers for clinical trials, and it should be administered in conjunction with traditional therapeutic drugs. Furthermore, we believe that GAS is safer to use and may be administered for a long period in the treatment of chronic UC. It is important to note that this experiment is simply a preliminary investigation in mice and cells; hence, additional validation is required.

Data availability

Raw reads reported in this paper were deposited in the National Center for Biotechnology Information's Sequence Read Archive (SRA) database under the BioProject ID number PRJNA1014947.

Declaration of competing interest

All the authors declare that they have no competing interests.

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