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# Genistein alleviates colitis by suppressing inflammation and modulating colonic *Marvinbryantia formatexigens* abundance and metabolites

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ABSTRACT

As an active ingredient of leguminous plants, genistein is extremely important for alleviating various human diseases. However, the regulatory effect of genistein on intestinal microbiota in alleviating enteritis is still unclear. In this study, the effect of genistein in alleviating dextran sodium sulfate (DSS)-induced colitis and the potential microbial metabolic regulation mechanism were explored. First, the effect of genistein on DSS-induced colitis was studied in mice. Then antibiotics were used to inhibit intestinal bacteria to verify that intestinal microorganisms play an important role in alleviating colitis of genistein. Finally, mice were administrated with live differential bacterium to confirm that genistein can regulate intestinal microorganisms to treat colitis. The results indicated that genistein alleviated DSS-induced colonic inflammation by inhibiting the Nuclear factor kappa-B and Cyclooxygenase-2/Prostaglandin E2 pathway. Genistein alleviated DSS-induced intestinal injury and decreased Mucin 2 secretion. Supplementation with genistein attenuated the DSS-induced decrease in the alpha diversity of gut bacteria. Genistein increased the abundance of *Lachnospiraceae* and *Marvinbryantia formatexigens*, increased the concentration of short chain fatty acids in colitis. After antibiotics depleted the intestinal bacteria, genistein lost the effect of relieving colitis, indicating that genistein must relieve colitis through the intestinal bacteria. Mice fed with living *Marvinbryantia formatexigens* increased short-chain fatty acids and relieved colitis. The present study demonstrates that genistein alleviated colonic inflammation by regulating intestinal bacterium of *Marvinbryantia formatexigens* and increasing short-chain fatty acid production.

## 1. Introduction

Inflammatory bowel disease (IBD), medically including ulcerative colitis and Crohn's disease, is a chronic inflammatory disease of the digestive system for which there is currently no ideal treatment. Their prevalence has been rising globally in recent years and is associated with high healthcare costs (Peery et al., 2019). IBD is closely tied to gut microbes disturbances (Franzosa et al., 2019), and a variety of short-chain fatty acids (SCFA)-producing bacteria are missing from the gastrointestinal tract of IBD patients (Wang et al., 2018).

The gut microbiota is extremely important for a determinant of human health and is a key factor regulating gastrointestinal physiology and pathophysiology (Han et al., 2022). In addition, previous evidence showed that SCFA as fermentation products of gut microbes, are key molecules in the regulation of host metabolism, and bacteria that produce SCFA have attracted widespread attention and have been applied to the treatment of IBD (Qin et al., 2025). Recently, the manipulation of the gut microbiota and its modulation through phytomedicine has potential for the treatment of IBD, such as the screening of probiotics and the application of plant active ingredients (Han et al., 2022). Furthermore, many epidemiological surveys have shown that plant active ingredients prevent and treat chronic diseases such as IBD (Liang et al., 2023); however, it is currently unclear how active ingredients mediate IBD by changing the composition of the microbiota.

Western populations have higher rates of IBD than Asians (Weng et al., 2018). Daily consumption of soy or its processed products in Asians, rich in soy isoflavones, has been shown to reduce the risk of IBD (Cao et al., 2019). Genistein, the main active isoflavone in legumes, has relieved inflammatory and has preventive benefits in a mouse model of colitis (Vanden et al., 2018). Gut microbes have also been reported to contribute to soybean isoflavone metabolism to regulate host immune function and cell development (Mace et al., 2019). The differences in the

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constitution of human gut microbes directly affect the metabolism and function of genistein (Zhou et al., 2019). Similarly, genistein also inhibits the occurrence of obesity mediated by gut microbes (López et al., 2018). In addition, previous study has shown that the interaction of gut microbes with host inflammation and intestinal epithelial cell development is critical for maintaining intestinal stem cell development and mucosal barrier function (Zhou et al., 2019). However, it is unknown whether gut microbes mediate the effect of genistein in relieving IBD.

Considering the potential interaction of gut microbiota and genistein, we hypothesized that genistein-mediated reduction of colitis is primarily mediated by modulation of gut microbiota, which subsequently attenuates mucosal damage and intestinal inflammation in the colon.

## 2. Materials and methods

### 2.1. Animals and diets

All animal studies involving 104 mice were performed in accordance with procedures approved by the Experimental Animal Welfare and Ethics Committee of China Agricultural University with the permission number of AW13099102-1-1.

In the test of genistein alleviating colitis, Genistein (purity ≥98%, catalog number: ZL0576) was purchased from Shaanxi Zelang Biotechnology Co., Ltd. and was added to mouse normal diets according to dosage requirements. The DSS was purchased from MP Biomedicals (Colitis Grade, molecular weight 36000–50000, MP Biomedicals, Shanghai, China). The DSS induction effect of each batch was not the same due to the inconsistent composition levels of each molecular weight, and a preliminary experiment is required to determine the dosage. Therefore, DSS used a 1% drinking water addition level for the first animal test, and a 2% drinking water addition level for the second and third animal trials based on the preliminary experiments, and adjusted the time when mice were sacrificed and sampled based on the actual situation. 32 six-week-old male C57BL/6J mice were randomly divided into 4 groups. The control group was fed a normal diet (CON, n = 8), The DSS-induced colitis group was fed a normal diet with dextran sulfate sodium-induced colitis at 10 weeks of age (DSS, n = 8). At the same time, the genistein group was fed genistein at 400 mg/kg diet (GEN, n = 8), and the DSS-induced colitis with the genistein group (DSS + GEN, n = 8). All mice were maintained with 4 mice *per* cage in the SPF animal facility of China Agricultural University, and free access to water and feed. The colitis model was induced by adding 1% DSS to the drinking water, while the diet was changed to a genistein-added treatment diet when DSS induced colitis. After 5 days of DSS drinking water, the DSS drinking water were replaced with normal drinking water. After 5 days of recovery, all mice were sacrificed by cervical dislocation after anaesthetization using isoflurane to collect colon samples and the colon contents. The mice were weighed every day, and the weight change curve was drawn by calculating the proportion of the weight change. The length of the colon was measured by using a ruler from the junction of the cecum and colon to the anus. Two approximately 0.5 cm colon of the proximal anal end was excised, one was fixed with paraformaldehyde for section staining, and the other was embedded in optimal cutting temperature (OCT) compound and stored at −80 °C for immunofluorescence detection of cryosections. The remaining colon samples were frozen grinding in liquid nitrogen and stored at −80 °C for later analysis. The fecal samples were stored at −80 °C for SCFA determination and DNA extraction.

In the antibiotic treatment trial, 32 six-week-old male mice were randomly divided into 4 groups, all of which were induced with the colitis model. The DSS-induced colitis group was fed a normal diet with DSS-induced colitis at 10 weeks of age (DSS, n = 8), the DSS-induced colitis and genistein-supplemented group was fed genistein at 400 mg/kg (DSS + GEN, n = 8), the broad-spectrum antibiotic (ABX) depleted gut microbiota group were fed a normal diet (ABX + DSS, n = 8), ABX-depleted gut bacteria and genistein group was fed genistein at 400 mg/kg (ABX + DSS + GEN, n = 8). ABX treatment (Neomycin: 1 g/L, Vancomycin: 500 mg/L, Metronidazole: 1 g/L, Ampicillin: 1 g/L) depleted gut microbiota by feeding antibiotics in water for 4 weeks according to previous method (Pathak et al., 2018). All mice were free to diet and drink. After 4 weeks of antibiotic treatment, the colitis model was constructed by adding 2% DSS to drinking water. After 5 days of DSS drinking water, the water was replaced by normal drinking water. After 2 days of recovery, all mice were sacrificed. Colon samples were stored and processed as in the first animal experiments.

In the bacteria perfusion test, forty 6-week-old male mice were randomly divided into 5 groups, the control group was fed a normal diet (CON, n = 8), the DSS-induced colitis group was given DSS-induced colitis (DSS, n = 8) at the 10th week of age, and DSS-induced colitis and live bacteria group (LB + DSS, n = 8), DSS-induced colitis and heat-killed bacteria group (HK + DSS, n = 8). Mice were fed with 0.1 mL of 1 × $10^9$ CFU/mL of viable bacteria once *per* day. Heat inactivated bacteria were heat treated at 85 °C for 10 min. The colitis model was induced by adding 2% DSS to the drinking water. After 5 days of drinking water, it was replaced with normal drinking water. After 4 days of recovery, all mice were sacrificed to collect colon samples and the samples were stored and processed as in the first animal experiments.

### 2.2. Histological staining

The fixed colon tissue was dehydrated with graded alcohol and embedded in paraffin. Tissues were cut crosswise into 5 μm thickness and stained with hematoxylin and eosin for pathological scoring. Pathology scores were assessed according to the previous evaluation criteria (Dong et al., 2020). The basic polysaccharide secreted by goblet cells was stained with periodic acid-Scheff reaction (PAS).

### 2.3. Immunofluorescence

The OTC compound embedded frozen intestines were cut crosswise into 7 μm thick sections. The antigens were retrieved using 50 mM citrate buffer. The tissue was then permeabilized for 30 min using 0.5% triton-100 in phosphate buffered saline (PBS). The sliced tissue was blocked with 5% goat serum in PBS. Then the tissue was incubated by primary antibodies overnight at 4 °C. Afterwards, the sliced tissues were washed three times with PBS for 5 min *per* time, then incubated with fluorescently-labeled secondary antibody for 2 h in the dark at room temperature. The slides were washed 3 times with PBS and mounted with anti-quencher containing DAPI. Intestinal proliferating cells in the three animal tests were stained using 5-ethynyl-2' -deoxyuridine (EdU). Mice were injected with 40 mg/kg body weight at 2 h before sacrifice, and intestinal tissue proliferating cells were detected using EdU cell proliferation kit (Shanghai Beyotime, Shanghai, China). Goblet cells were stained with FITC-labeled UEA-1. Fluorescence signals were acquired using a confocal laser microscope SP8 (Leica Microsystems GmbH, Mannheim, Germany). All antibody information and dilutions are shown in Table 1 of the Supplementary Materials.

### 2.4. Western blot

Colon tissue proteins were extracted by radio immunoprecipitation assay lysis buffer added phenylmethylsulfonyl fluorid at 1 mg/mL (Shanghai Beyotime, Shanghai, China) in a tissue homogenizer. Then the supernatant protein solution was separated by centrifugation at 12,000×*g* for 10 min at 4 °C. The protein content was measured by the BCA protein assay kits (Thermo Fisher Scientific, Beijing, China). Denatured proteins were separated using 8–12% polyacrylamide gel electrophoresis. The separated protein bands were then transferred to polyvinylidenedifluoride membrane. Membranes were blocked using quick block blocking buffer (Shanghai Beyotime, Shanghai, China). Then the membrane was incubated with the primary antibody overnight

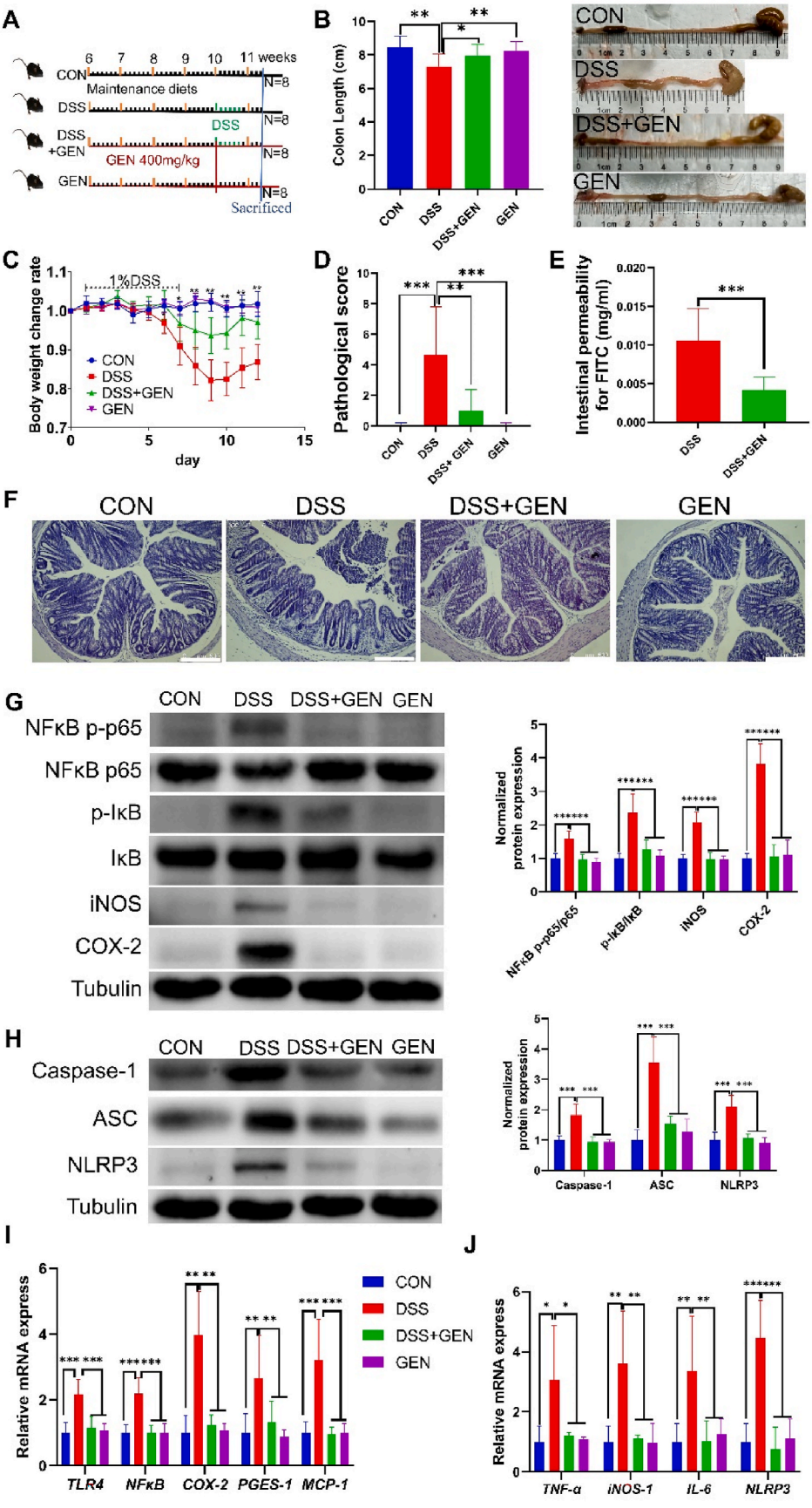


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**Fig. 1.** Genistein attenuated DSS-induced colonic inflammation. (A) Schematic diagram of the animal test timeline. (B) Genistein alleviates colonic atrophy caused by colitis. (C) Genistein ameliorated DSS-induced colitis-induced weight loss in mice. (D) Genistein reduces colitis pathology scores. (E) Genistein reduces the increase in intestinal permeability caused by colitis. (F) HE staining showed that genistein reduces inflammatory damage in the colon ( × 100 magnification; scale bar: 500 μm). (G) Genistein alleviates DSS-induced colonic inflammation by inhibiting NF-κB/IκB phosphorylation and Cox-2 signaling pathway. (H) Genistein inhibited the NLRP3 signaling pathway and alleviated the inflammatory response. (I) Genistein inhibited TLR4/NFκB and its regulated Cox-2/PGES-1 gene expression and alleviated DSS-induced colonic inflammation. (J) Genistein inhibited NLRP3 and its regulated TNF-a/iNOS-1 gene expression and alleviated DSS-induced colonic inflammation. The number of mice in each treatment group was n = 8.

at 4 °C. After that the membrane were washed three times with TBST (20 mM Tris, 150 mM NaCl, 0.05% Tween 20, pH 7.4) followed by incubation with horseradish peroxidase-conjugated secondary antibody for 2 h. Subsequently, the membrane bands were washed 3 times with TBST. After that, the proteins were developed using chemiluminescent solution BeyoECL Plus (Shanghai Beyotime, Shanghai, China), and the gray values of the bands were quantified using ImageJ software.

### 2.5. Intestinal permeability test

Mice were fasted but did not stop drinking water for 8 h before sacrifice. 200 μL of 4 kDa Mw fluorescein isothiocyanate (FITC) -dextran (Thermo Fisher Scientific, Waltham, MA) 1 mg/mL PBS was administered to each mouse at the 4 h fast. Then, the mice were sacrificed after continued starvation for 4 h. The blood samples were collected into PE tubes by enucleating the eyeball. Serum from blood was separated by centrifugation at 3000×*g* for 10 min. The FITC fluorescence values in serum were measured using SpectraMax i3X microplate reader with 485 excitation/528 emission (Molecular Devices, San Jose, CA, USA). At the same time, the FITC concentration was calculated according to the established standard curve.

### 2.6. Quantitative real-time PCR

Total RNA of colon tissue was extracted with trizol reagent according to the instructions (GenStar, Beijing, China). The mRNA concentrations were detected using nanodrop. The cDNA was obtained by reverse transcription by StarScript II First-strand cDNA Synthesis Kit (GenStar, Beijing) and used as a PCR template using a kit. QPCR was performed in a 20 μL reaction system containing 10 μL 2 × RealStar Green Fast Mixture (GenStar, Beijing, China), 0.8 μL each upstream primer and downstream primer, 7.5 μL sterile enzyme-free water, and 0.5 μl cDNA template. The reaction program and the relative gene expression was calculated according to previous method (He et al., 2017). Primer sequences of all tested genes are shown in Supplementary Table 2.

### 2.7. Bacteria preparation and growth conditions

*Marvinbryantia formatexigens* bacteria were purchased from German Collection of Microorganisms and Cell Cultures (DSMZ), and the culture method was cultured according to the recommended medium. The bacterial cells were obtained by centrifugation of bacterial solution at 3000×*g* for 10 min. The cells were washed once with aqueous 0.9% NaCl solution and diluted for administration to mice. The mice were gavaged with bacteria once a day, and the gavage volume was 100 μL of $1 \times 10^9$ CFU/mL.

### 2.8. Fecal microbiota 16s rDNA sequencing and SCFA analysis

The fecal DNA was extracted using QIAamp DNA Isolation Kit (Qiagen, Hilden, Germany). The V3-4 region of the bacterial alternative spliceosome was amplified using PCR. Each sample was then uniquely tagged by PCR. Libraries were created by mixing equal amounts of PCR products from each sample. Libraries are sequenced on the platform. Sequencing data was analyzed using QIIME2 software according to our previous method (He et al., 2021). SCFA in feces were measured using a gas chromatograph according to the previous instrument parameters (He et al., 2017). The feces were homogenized in advance with 5 times ultrapure water (w/v), then centrifuged at 10,000×*g* for 10 min, and the supernatant was used for SCFA analysis, the detailed procedures are in the supplementary materials.

### 2.9. Data analysis

All data are expressed as mean ± standard deviation, and the significant differences between groups were analyzed by two-way ANOVA followed by the Tukey test, with significance marked as $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

## 3. Results

### 3.1. Genistein attenuates DSS-induced colonic inflammation

Compared with the DSS colon group, oral administration of genistein significantly alleviated colitis, mainly as genistein significantly alleviated DSS-induced body weight loss (Fig. 1B), shortened colon length (Fig. 1C), and increased pathological scores (Fig. 1D). Intestinal permeability analysis further demonstrated that genistein alleviated DSS-induced impairment of intestinal barrier function (Fig. 1E). Not only that, pathological analysis showed that oral genistein could alleviate inflammatory cell infiltration and mucosal damage (Fig. 1F).

After oral administration of genistein, phosphorylated Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor (IκB) had a significantly reduced in the colon of DSS-treated mice, as were the downstream protein levels of Nitric oxide synthase (iNOS) and cyclooxygenase-2 (Cox-2) (Fig. 1G). DSS-induced colitis significantly raised the phosphorylation level of inflammatory proteins NF-κB and IκB, as well as iNOS and Cox-2 protein expression (Fig. 1G). Not only that, DSS treatment could significantly increase NLR family pyrin domain containing 3 (NLRP3) inflammasome, as well as apoptosis-associated speck-like protein (ASC) and Caspase-1 proteins bound to the inflammasome. In contrast, genistein reduced DSS-induced NLRP3 inflammasome, ASC and Caspase-1 protein (Fig. 1H). Consistent with the protein results, supplementation with genistein suppressed the increased expression of Toll-like receptor 4 (*TLR4)* upstream of NF-κB and the increased expression of *prostaglandin E synthases-1* (PGEs-1) and *Monocyte chemoattractant protein-1* (MCP-1) downstream of *Cox-2* caused by DSS treatment (Fig. 1I). Meanwhile, DSS-induced colitis could increase the expression of tumor necrosis factor-alpha (TNF-α), *iNOS-1, Interleukin-6* (IL-6) and *NLRP3* genes, and genistein supplementation abolished DSS-induced inflammation and inflammasome elevation (Fig. 1J).

### 3.2. Genistein improves mucosal barrier and epithelial dysfunction caused by colitis

DSS-induced colitis could significantly reduce goblet cells and mucin 2 secreted by goblet cells, and genistein could alleviate DSS-induced goblet cell loss and mucin secretion (Fig. 2A). Not only that, intestinal epithelial tight junction gene expression was measured. DSS treatment significantly inhibited the expression of *Claudin1, Occludin* and *ZO-1* genes, and pre-feeding genistein alleviated the decrease in tight junction gene expression induced by DSS (Fig. 2B).

DSS-induced colitis can significantly increase the number of proliferating cell markers EdU-positive cells and proliferating cell nuclear

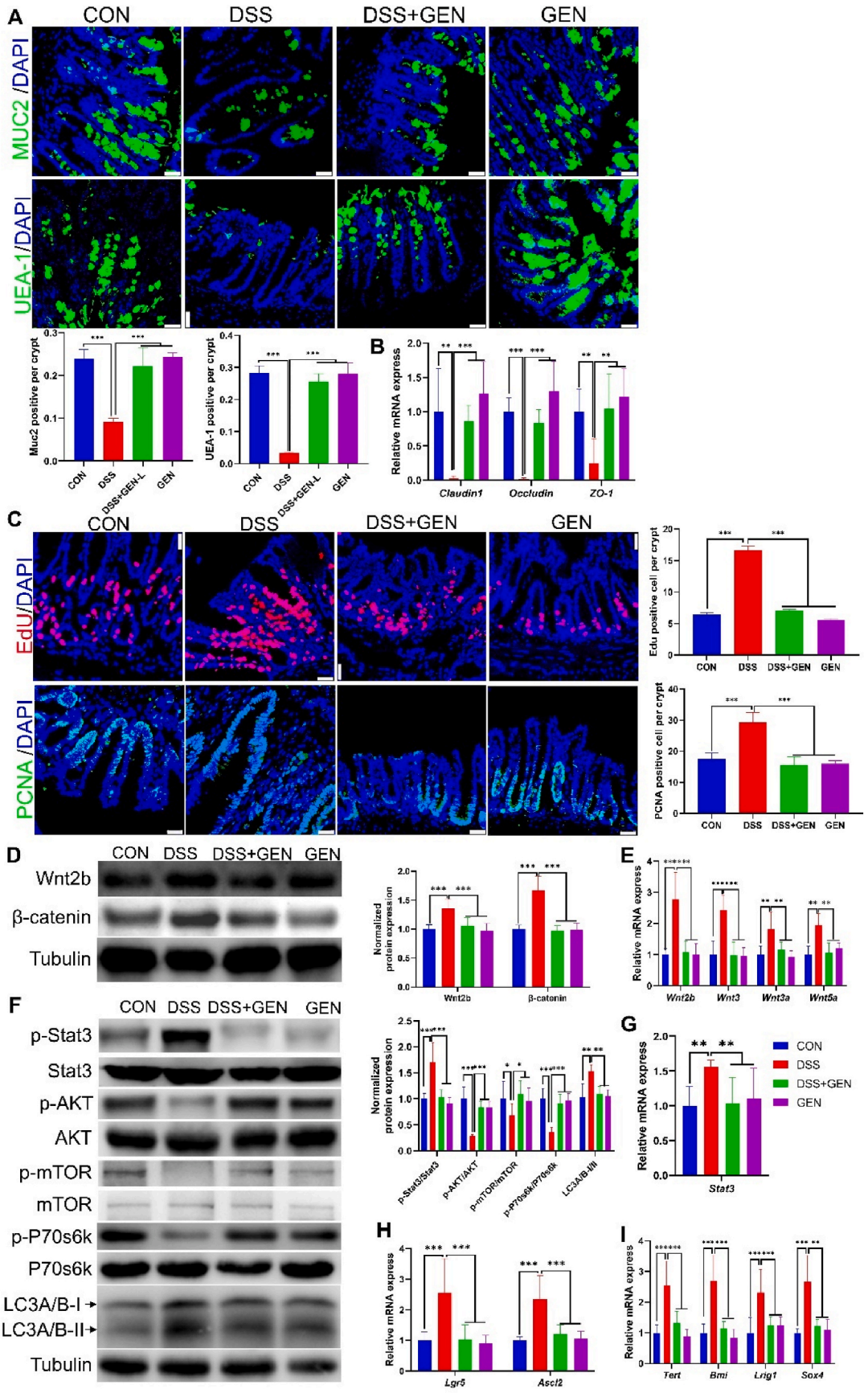


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**Fig. 2.** Genistein improves mucosal barrier and epithelial dysfunction caused by colitis. (A) Genistein alleviates colitis-induced decrease in MUC2 protein and decrease in goblet cell marker UEA-1 ( × 400 magnification; scale bar, 50 μm). (B) Genistein relieves the expression of tight junction genes *Claudin1, Occludin* and *ZO-1* caused by colitis. (C) Genistein inhibits colitis-induced increases in Edu and PCNA positive cells, markers of intestinal proliferative cells ( × 400 magnification; scale bar: 50 μm). (D) Genistein inhibition inhibits the increase of wnt2b/β-catenin protein expression in colitis. (E) Genistein inhibits colitis-induced increase in Wnt gene expression. (F) Genistein inhibits autophagy caused by colitis-induced decreased expression of AKT/mTOR/P70S6K. (G) Genistein increase Stat3-mediated apoptosis in colitis. (H) Genistein inhibits the elevated expression of *Lgr5* and *Ascl2* in colitis. (I) Genistein reduced the elevated expression of slow-proliferating genes *Tert, Bmi, Lrig1* and *Sox4* caused by colitis. The number of mice in each treatment group was n = 8.

antigen (PCNA)-positive cells during the recovery phase. Compared with the DSS group, pre-fed genistein was able to inhibit cell proliferation in the recovery phase of colitis induced by DSS (Fig. 2C). Correspondingly, DSS-induced inflammation can up-regulate the wingless-type MMTV integration site family (Wnt)2b/β-catenin signaling pathway that regulates intestinal epithelial cell differentiation, and genistein can alleviate the up-regulation of this pathway caused by inflammation (Fig. 2D). Not only that, genistein could alleviate the elevated expression of *Wnt2b, Wnt3, Wnt3a* and *Wnt5a* genes caused by colitis (Fig. 2E). Autophagy promotes compensatory cell proliferation. In this study, DSS-induced colitis can promote autophagy by inhibiting the protein kinase B (AKT)/mammalian target of rapamycin (mTOR)/p70 ribosomal protein S6 kinase (P70S6K) signaling pathway and increasing the phosphorylation of Stat3 protein. Compared with the inflammatory group, genistein supplementation in mice could promote the AKT/mTOR/P70S6K signaling pathway to inhibit the autophagy induced by colitis (Fig. 2F). This was confirmed in the autophagy marker LC3A/B-II protein, and genistein supplementation suppressed the elevated expression of microtubule-autophagy-related protein1 light chain 3 (LC3A/B)-II protein caused by colitis (Fig. 2F). Consistent with protein expression, colitis was able to enhance *Stat3* gene expression, and genistein reversed this inhibition (Fig. 2G). In terms of stem cell marker genes, DSS-induced colitis could increase the expression of rapid proliferation marker genes Leucine-rich repeat-containing G-protein coupled receptor 5 (*Lgr5)* and Achaete scute complex like 2 (*Ascl2)* (Fig. 2H), and at the same time, increased the expression of slow proliferation genes telomerase reverse transcriptase (*Tert), polycomb complex protein bmi-1 (Bmi), leucine rich repeats and immunoglobulin like domains protein 1 (Lrig1)* and sex determining region Y-box 4 (*Sox4)* (Fig. 2I). Genistein prevents the elevation of stem cell marker genes caused by colitis.

### 3.3. Genistein regulates gut microbiota composition and promotes SCFA production in the colon

In the α-diversity index, DSS-induced colitis significantly decreased Chao1, observed species, PD-whole-tree and Shannon index, and genistein reversed the decrease in α-diversity index caused by colitis (Fig. 3A).

At the phylum level, Firmicutes and Bacteroidetes are the dominant phyla for colonic contents (Fig. 3B). At the genus level, *Lactobacillus, Ruminococcus* and *Streptococcus* are the main dominant genera (Fig. 3C). The colitis group fed with genistein was able to significantly increase the number of *Streptobacteriaceae* and *Akkermansia* (Fig. 3B). Not only that, we compared the main differential strains between the colitis group and the genistein-supplemented colitis group, the latter significantly increased the relative abundance of bacteria *Alloprevotella* and *Marvinbryantia*, and suppressed the other 10 strains (Fig. 3D). Principal coordinates analysis (PCoA) based on Bray-Curtis distance revealed differences in gut bacterial structure among the groups (Fig. 3E).

DSS-induced colitis could significantly reduce fecal acetate, butyrate and total SCFA (Fig. 3F). Pre-feeding genistein reversed the reduction in SCFA. There were no significant differences in propionic acid concentrations between treatment groups. Therefore, changes in the structure of intestinal bacteria may be an important mediator for genistein to relieve colitis.

### 3.4. Gut bacteria depletion abrogates genistein benefits

In order to verify genistein mediated the alleviation of colitis through gut bacteria, antibiotic depletion of the gut bacteria was implemented. A schematic diagram of the specific experiment is shown in Fig. 4A. It was shown that antibiotic depletion of gut bacteria and genistein supplementation did not alleviate weight loss (Fig. 4B), colon shortening (Fig. 4C) and inflammatory pathology scores (Fig. 4D) induced by DSS-induced colitis. Pathological staining showed that after intestinal antibiotics depleted the intestinal bacteria, with or without addition of genistein, inflammatory cell infiltration and epithelial damage were manifested in DSS-induced colonic delay, which was opposite to the group without antibiotics (Fig. 4E).

### 3.5. Marvinbryantia formatexigens attenuates DSS-induced colonic inflammation

Antibiotic depletion of gut microbiota in the present study had demonstrated that genistein alleviates colitis *via* gut microbiota. In the colitis model group, the addition of genistein significantly increased the relative abundance of *Marvinbryantia formatexigens*. Therefore, *Marvinbryantia formatexigens* was used to verify whether genistein alleviated colitis by up-regulating the bacteria, as shown in Fig. 5A. Administration of live *Marvinbryantia formatexigens* alleviated DSS-induced colonic inflammation. It was shown that the induction of DSS colitis after gavage with *Marvinbryantia formatexigens* alleviated body weight loss (Fig. 5B), shorten the colon (Fig. 5C) and increase the colonic pathological score (Fig. 5D), compared with the DSS-induced colitis group. Not only that, the neutral mucus staining by PAS indicated the DSS-induced inflammation significantly reduced the positive cell number, while gavage with *Marvinbryantia formatexigens* alleviated the decrease in neutral mucus secretion caused by colitis (Fig. 5E). Gavage of heat-killed *Marvinbryantia formatexigens* did not relieve colitis. This shown that only live bacteria had the alleviating function of genistein.

Administration of *Marvinbryantia formatexigens* could inhibit the phosphorylation of NF-κB and IκB proteins in mice with colitis, and the downstream protein levels of iNOS and Cox-2 were also significantly reduced (Fig. 5F). Similar to the first animal study results, DSS-induced colitis significantly increased the phosphorylation of NF-κB and IκB, as well as iNOS and Cox-2 protein expressions (Fig. 5F). Not only that, DSS treatment could significantly increase the expression of NLRP3 inflammasome, as well as ASC and Caspase-1 proteins bound to the inflammasome (Fig. 5G). In contrast, intragastric administration of *Marvinbryantia formatexigens* to mice inhibited the DSS-induced increase in NLRP3 inflammasome, ASC and Caspase-1 protein expression (Fig. 5G). Consistent with the protein results, *Marvinbryantia formatexigens* was able to inhibit the increased expression of TLR4 upstream of NF-κB and the increased expression of *PGEs-1* and *MCP-1* downstream of *Cox-2* caused by DSS treatment (Fig. 5H). Antibiotic depletion of gut microbiota abrogated the alleviating effect of genistein on colitis, as evidenced by antibiotic depletion of gut microbiota and genistein feeding not inhibiting the increased phosphorylation levels of inflammatory proteins NF-κB and IκB induced by DSS colitis. Likewise, gavage of antibiotics was able to reverse the inhibitory effect of genistein on the NLRP3 inflammasome in colitis (Figs. S1A and B).

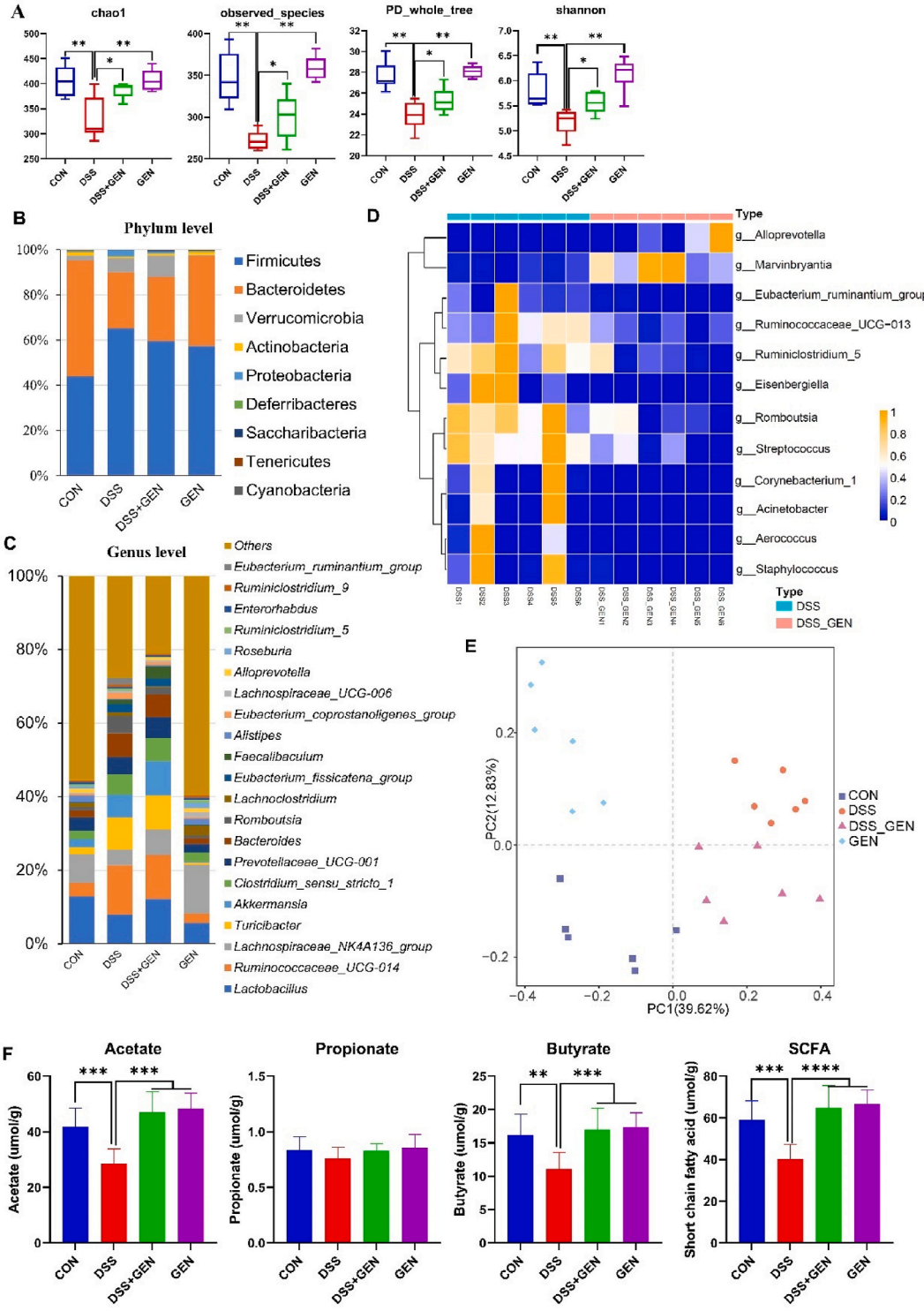


**Fig. 3.** Genistein regulates gut bacteria composition and promotes SCFA production. (A) Genistein relieves colitis by reducing the alpha diversity index of gut bacteria. (B) Effects of genistein on gut bacteria at the phylum level. (C) Effects of genistein on gut bacteria at the genus level. (D) Genistein was able to increase the relative abundance of *Marvinbryantia formatexigens* in colitis. (E) Principal coordinates analysis (PCoA) showing significant clustering across treatment groups. (F) Genistein increases the concentrations of acetate, butyrate and SCFA in colitis stool. The number of mice in each treatment group was n = 6.

### *3.6. Marvinbryantia formatexigens improves mucosal barrier and epithelial function damage caused by colitis*

Antibiotic depletion of the gut microbiota showed that genistein no longer relieved colitis mucosal damage and decreased mucin secretion (Fig. S2A). At the same time, depletion of the microbiota caused genistein to lose its function of enhancing the expression of the tight junction genes *Occludin* and *Muc2* in colitis (Fig. S2B). Gavage of live *Marvinbryantia formatexigens* attenuates mucosal damage and decreased mucin 2 secretion caused by DSS-induced colonic inflammation. Concomitant administration of live *Marvinbryantia formatexigens* increased the expression of *Claudin1* and *Occludin* genes in the tight junction of colitis (Fig. 6B).

Antibiotic depletion of gut microbiota abolishes genistein-induced

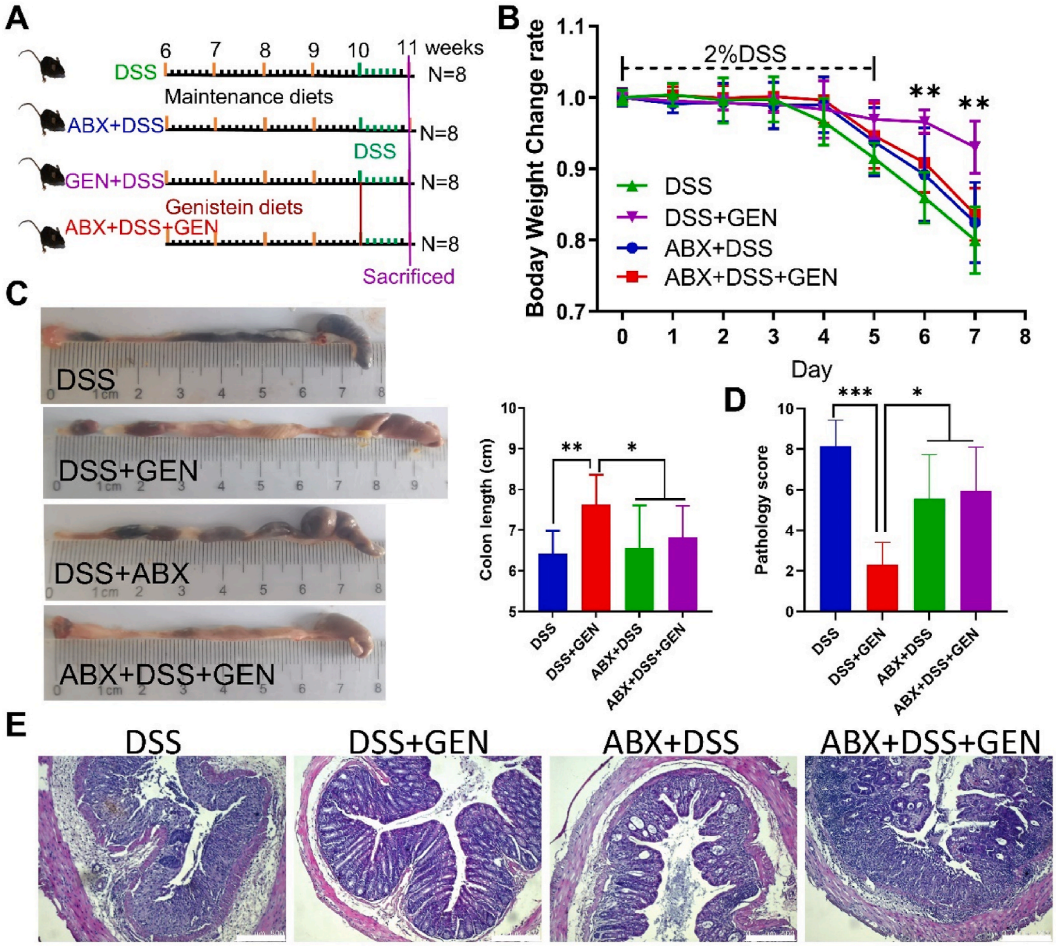


**Fig. 4.** Gut microbiota depletion abrogates genistein benefits. (A) Schematic timeline of the antibiotic-depleted gut microbiota assay. (B) After antibiotic pretreatment, genistein lost alleviation of colitis-induced weight loss. (C) After antibiotic pretreatment, genistein lost alleviation of colitis-induced weight loss. (D) Elevated pathological scores caused by genistein loss of remission colitis after antibiotic pretreatment. (E) After antibiotic pretreatment, genistein lost its function of relieving colitis ( × 100 magnification; scale bar: 500 μm). The number of mice in each treatment group was n = 6.

inhibition of intestinal cell proliferation and stem cell activity in colitis. It was shown that EdU and PCNA positive cells were not significantly different from the DSS-induced colitis group after genistein clearance in the gut bacteria (Fig. S3A). This is consistent with stem cell marker genes, genistein could not inhibit the increased expression of stem cell marker genes *Lgr5, Bmi, Tert, Lrig* and *Sox4* caused by colitis after clearing intestinal bacteria. Gavage of live *Marvinbryantia formatexigens* in mice can inhibit the number of EdU and PCNA positive cells in colitis. This may be due to the ability of live *Marvinbryantia formatexigens* to inhibit Wnt/β-catenin protein expression to inhibit stem cell proliferation (Fig. 6C and D). The *Marvinbryantia formatexigens* inhibited the expression of *Wnt2b, Wnt3, Wnt3a* and *Wnt5a* genes in colitis (Fig. 6E). In this study, administration of *Marvinbryantia formatexigens* to mice could promote AKT/mTOR/P70S6K signaling pathway to inhibit autophagy caused by colitis (Fig. 6F). This was confirmed in the autophagy marker LC3A/B-II protein, and *Marvinbryantia formatexigens* was able to suppress the elevated expression of LC3A/B-II protein caused by colitis (Fig. 6F). Consistent with the protein expression, *Marvinbryantia formatexigens* can reduce the expression of *Stat3* gene in colitis. Therefore, genistein modulation can improve colitis by modulating intestinal *Marvinbryantia formatexigens* (Fig. 6G). At the same time, *Marvinbryantia formatexigens* inhibited the expression of rapidly proliferating stem cell marker genes *Lgr5* and *Ascl2* in colitis, as well as the expression of slow proliferating stem cell marker genes *Tert, Bmi, Lrig1* and *Sox4* in colitis (Fig. 6H and I). Therefore, genistein regulation can improve colitis by regulating intestinal *Marvinbryantia formatexigens*. *Marvinbryantia formatexigens* promoted the increase of SCFA in feces (Fig. 6J).

## 4. Discussion

This study systematically explored the effect of genistein on DSS-induced colitis and verified the alleviation effect of differential bacterium on enteritis through intestinal clearance bacteria with antibiotics. Genistein relieves acute colonic inflammation, manifested by improved weight loss and colon shortening. Consistent with the results of this study, genistein was able to inhibit the inflammasome through TGR5-cAMP signaling (Chen et al., 2019). Not only that, previous study has shown that genistein can reduce pro-inflammatory cytokines in colon tissue to alleviate colon inflammation and has no adverse effects on the intestines of healthy animal (Zhang et al., 2017, 2020). Genistein increases the integrity of the colitis epithelium and reduces permeability, which is consistent with the findings in this study that genistein reduced colonic inflammation pathology scores and dextran permeability (Zhang et al., 2017). In addition, this study further explored the regulation of genistein on intestinal epithelial cell development and intestinal microbes in colitis, demonstrated that genistein alleviates colonic inflammatory response mediated by intestinal microbes.

DSS-induced acute colitis involves multiple pathogens and inflammatory responses induced by epithelial damage. Activated expression of NLRP3 involves two independent signals (He et al., 2016). The first being pathogen-mediated activation of NF-kB, which induces the synthesis and accumulation of precursor proteins including NLRP3 and

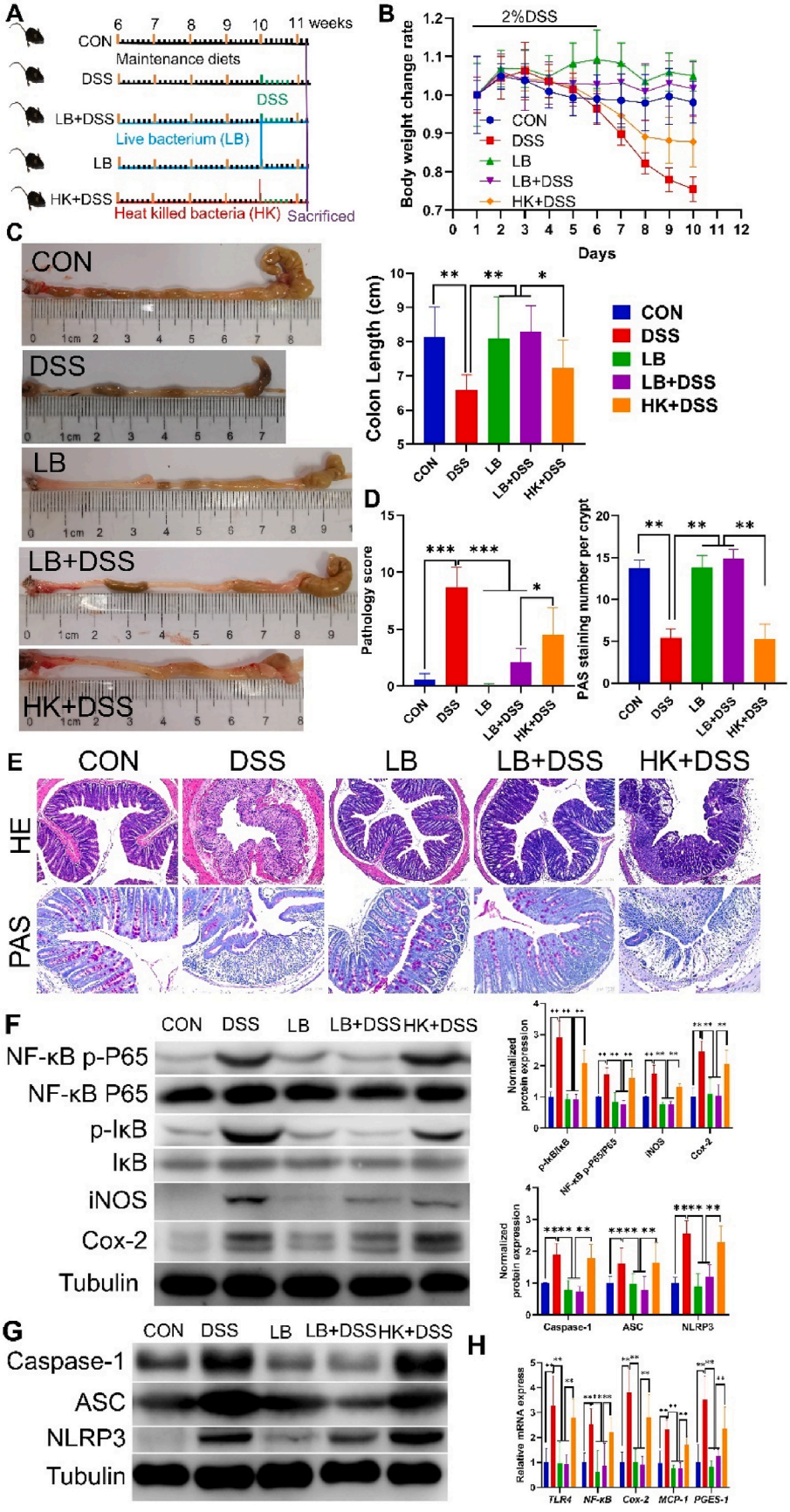


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**Fig. 5.** *Marvinbryantia formatexigens* attenuated DSS-induced colonic inflammation. (A) Schematic diagram of the time axis of the bacterium gavage test. (B) Gavage of *Marvinbryantia formatexigens* in mice alleviated colitis-induced body weight loss. (C) *Marvinbryantia formatexigens* is able to alleviate colonic atrophy induced by DSS-induced colitis. (D) *Marvinbryantia formatexigens* was able to alleviate the pathological score elevation caused by DSS-induced colitis. (E) *Marvinbryantia formatexigens* was able to alleviate DSS-induced colitis-induced increase in pathological score and decrease in the number of positive cells stained with neutral mucus ( × 100 magnification; scale bar: 500 μm). (F) *Marvinbryantia formatexigens* can inhibit DSS-induced colonic inflammation by inhibiting NF-κB/IκB phosphorylation-mediated Cox-2 signaling. (G) Gavage of *Marvinbryantia formatexigens* in mice can inhibit the NLRP3 signaling pathway and alleviate the inflammatory response. (H) *Marvinbryantia formatexigens* can suppress *TLR4/COX-2/PGE2* gene expression and alleviate DSS-induced colonic inflammation. The number of mice in each treatment group was n = 8.

IL-1β, leading to downstream expression of inflammatory genes (Toldo and Abbate, 2018). Typically, lipopolysaccharide on the bacterial wall of Gram-negative bacterial pathogens can activate the IκB/NF-κB signaling pathway through TLR4, thereby activating inflammatory responses including Cox-2, NLRP3 and iNOS (Sharma and Kanneganti, 2021). Cox-2 is the key rate-limiting enzyme for the synthesis of PGE2, which induces acute inflammatory responses. A second signal capable of activating NLRP3 is activation of NLRP3 mediated by damage-associated molecular patterns that recruit pro-caspase-1 through the adaptor protein ASC, which leads to autocatalytic processing to form activated Caspase-1 (Swanson et al., 2019). Intestinal inflammation is generally accompanied by an inflammatory response mediated by both pathogens and intestinal injury. Genistein can alleviate DSS-induced acute colitis by inhibiting the activation of NLRP3 by inhibiting the above two signals, namely pathogen-mediated and intestinal injury-mediated signals.

The intestinal mucus layer is formed by polymeric lamellae of densely glycosylated mucin, and the colon has a well-developed mucus layer that insulates the intestinal microbiota and epithelial cells. Depletion of the mucus layer is one of the most common features of IBD patients, bringing gut microbial components into close contact with host epithelial cells and triggering unregulated inflammation (van der Post et al., 2019). In this study, DSS-induced acute colitis could lead to decreased Muc2 protein secretion. Furthermore, it also decreased the amount of UEA-1, a marker of muc2 protein secreted by goblet cells. Loss of goblet cells is a major feature of host defense against pathogen invasion and intestinal epithelial damage, which results in decreased Muc2 protein secretion. Genistein supplementation can alleviate the loss of goblet cells and the decrease of Muc2 protein secretion and reduce intestinal epithelial damage. This was further confirmed in *claudin1* gene expression.

Different from other tissues, intestinal tissue has evolved to rapidly differentiate and proliferate to continuously renew to resist the external harsh and complex environment. Stem cells located at the base of colonic crypts gradually proliferate upward and differentiate into mature epithelial cells, which play an important role in repairing intestinal damage and maintaining epithelial homeostasis (Gehart and Clevers, 2019). Study has shown that intestinal injury or inflammation will lead to compensatory proliferation of intestinal stem cells to maintain epithelial integrity, and this compensatory proliferation, if uncontrolled, will further lead to colon cancer (Harnack et al., 2019). This study shows that in the recovery stage of DSS-induced acute colitis, colitis will lead to an increase in the number of proliferating cells manifested as an increase in the number of PCNA-positive cells and EdU-positive cells. Genistein did not increase the number of proliferating cells in colitis, suggesting that genistein can maintain intestinal homeostasis and avoid intestinal damage. Previous reports have shown that Wnt/β-catenin signaling is not only involved in promoting cell survival but also in the healing process of epithelial cells by acting on intestinal stem cells (Quandt et al., 2021). In this study, damaged epithelial cells can be repaired by activating Wnt/β-catenin signaling during recovery from acute colitis. Not only that, colitis led to increased expression of Wnt family genes and increased expression of stem cell marker genes for both rapidly proliferating cells and slow proliferating cells, which ultimately led to an increase in the number of proliferating cells. The changes in the number of proliferating cells in DSS-induced colitis were different, which may be due to the different stages of inflammation in which the mice were said to be, with a decrease in the number of proliferative cells during the peak inflammation period and an increase in the number of proliferative cells during the recovery period (Deng et al., 2018). Therefore, in this study, DSS-induced colitis can compensate for damaged intestinal epithelial tissue by activating Wnt/β-catenin signaling to increase stem cell activity during the recovery phase. Genistein can inhibit this pathway in colitis mice to maintain intestinal homeostasis and inhibit the compensatory proliferation of intestinal stem cells.

In addition to the proliferation of intestinal stem cells to repair epithelial damage, mammalian tissues can also degrade and recycle damaged organelles and misfolded proteins through autophagy to maintain a healthy cellular environment. Phosphorylation of AKT/mTOR/P70S6K is closely related to autophagy (Fan et al., 2015). Inhibiting the phosphorylation of this pathway can promote autophagy in tissues to remove damaged cells. A typical process of autophagy is that cellular LC3-I is converted to LC3-II (Shaikh et al., 2021). This study further proves that autophagy plays a role in repairing damage in the recovery stage of acute colitis. The body can promote the conversion of LC3-I to LC3-II by inhibiting the phosphorylation of AKT/mTOR/P70S6K to achieve the degradation of damaged cells. Genistein can alleviate autophagy caused by colitis due to its ability to relieve intestinal damage and inflammation.

Importantly, genistein significantly improved the structure and composition of intestinal bacteria in enteritis. Colitis can significantly reduce the α-diversity of colonic contents, which is consistent with previous findings that DSS-induced colitis disrupts intestinal microbiota homeostasis and reduces bacterial diversity (Jang et al., 2019). Supplementation with genistein increases the alpha diversity of gut microbiota in colitis. Not only that, PCOA analysis showed that genistein attenuated the intestinal bacteria of colitis and brought it closer to the control group. Interestingly, genistein was able to increase the abundance of *Marvinbryantia formatexigens* and *Alloprevotella*, which may be the microbial mechanism of its ability to relieve colon since *Marvinbryantia formatexigens* has been shown to produce acetic acid (Desai et al., 2016). In addition, genistein was able to reduce *Ruminiclostridium-5* and *Romboutsia*. bacteria in colitis, which can aggravate intestinal inflammation. Correspondingly, fecal SCFA concentrations were detected, and DSS-induced acute colitis was able to reduce SCFA concentrations. Genistein was able to significantly increase the concentrations of acetate, butyrate, and total SCFA in colitis. Therefore, genistein could increase the abundance of *Marvinbryantia formatexigens* bacteria and increase the metabolism of SCFA to alleviate colitis.

The *Marvinbryantia* genus belongs to the *Lachnospiraceae* family, contains only one known species, *Marvinbryantia formatexigens*, which was isolated from human feces. Genistein can increase the abundance of *Lachnospiraceae* in the gut of mice with colitis, which can degrade various carbohydrates to produce acetate and butyrate to promote intestinal health. Study has shown that *Lachnospiraceae* can alleviate intestinal damage and inhibit intestinal inflammation (Almeida et al., 2021). Administration of the bacteria to mice can increase the concentration of SCFA in colitis, indicating that the bacteria can relieve colitis by producing SCFA through fermentation. This may be one of the reasons why heat-killed *Marvinbryantia formatexigens* has no effect of alleviating colitis, the heat-killed *Marvinbryantia formatexigens* do not have the ability to ferment and produce short-chain fatty acids after inactivation. Different from the results of this study, previous studies have

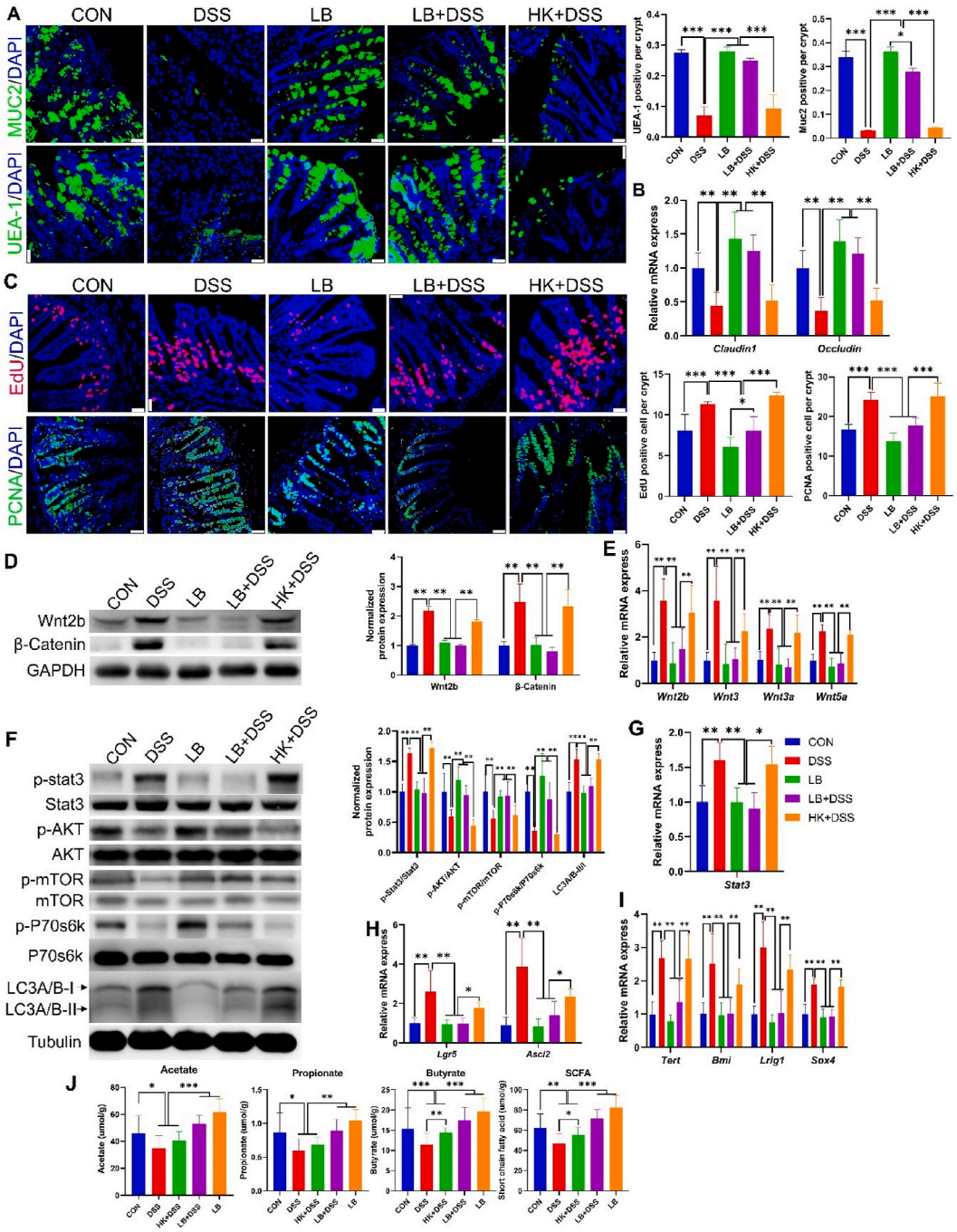


**Fig. 6.** *Marvinbryantia formatexigens* improved mucosal barrier and epithelial dysfunction caused by colitis. (A) *Marvinbryantia formatexigens* alleviate colitis-induced decrease in Muc2 protein and decrease in goblet cell marker UEA-1 ( × 400 magnification; scale bar: 50 μm). (B) *Marvinbryantia formatexigens* attenuates the expression of the tight junction genes *Claudin1* and *Occludin* caused by colitis. (C) *Marvinbryantia formatexigens* inhibits colitis-induced increases in Edu and PCNA-positive cells, markers of intestinal proliferative cells ( × 400 magnification; scale bar: 50 μm). (D) Inhibition of *Marvinbryantia formatexigens* inhibits the elevation of Wnt2b/β-catenin protein expression in colitis. (E) *Marvinbryantia formatexigens* inhibits colitis-induced increase in *Wnt* gene expression. (F) *Marvinbryantia formatexigens* inhibits autophagy caused by colitis-induced decreased AKT/mTOR/P70S6K expression. (G) *Marvinbryantia formatexigens* increases Stat3-mediated apoptosis in colitis. (H) *Marvinbryantia formatexigens* inhibits the elevated expression of *Lgr5* and *ASCL2* in colitis. (I) *Marvinbryantia formatexigens* inhibited the elevated expression of the slow-proliferating genes *Tert, Bmi, Lrig1* and *Sox4* caused by colitis. (J) *Marvinbryantia formatexigens* increased the concentration of SCFA in feces. The number of mice in each treatment group was n = 8.

shown that *Marvinbryantia formatexigens* can improve colon shortening in mice with DSS-induced colitis, but has no effect on pathological scores, which may be caused by the difference in the amount of the bacteria administered and the culture method (Wlodarska et al., 2017). Not only that, study has shown that the abundance of *Marvinbryantia formatexigens* in the gut is positively correlated with the content of dietary cellulose, which can degrade cellulose and methylcellulose to produce SCFA (Desai et al., 2016). In the present study, genistein was able to increase the relative abundance of *Marvinbryantia formatexigens*, whose fermentation produces SCFA to alleviate acute colitis.

## 5. Conclusion

Supplementation with genistein alleviated DSS-induced acute colitis, which is involves the increase of *Marvinbryantia*. Gavage of live *Marvinbryantia formatexigens*, but not heat-killed, alleviated DSS-induced acute colitis by increasing fecal SCFA concentrations. The alleviation process involved that genistein inhibited the loss of goblet cells in colitis, reduced MUC2 secretion, promoted the COX-2 pathway leading to inflammatory response.

## CRediT authorship contribution statement

**Yang He:** Investigation, Methodology, Data curation, Validation, Formal analysis, Writing – original draft. **Xiaoli Qin:** Conceptualization, Software, Writing – review & editing. **Chaoyong Liao:** Investigation, Methodology. **Rafaela Lameira Souza Lima:** Methodology. **Qihang Hou:** Methodology. **Jiaqi Lei:** Methodology. **Yujiao Lai:** Methodology. **Qiuyu Jiang:** Methodology. **Bo Wang:** Resources, Supervision. **Bingkun Zhang:** Funding acquisition, Project administration, Resources, Supervision, All data were generated in-house, and no paper mill was used, All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

## Data availability

The raw Illumina sequence data of fecal 16s rDNA in the present study are available in the sequence read archive (SRA) at NCBI under Bioproject accessions # PRJNA90871. The remaining data from the current study are available in the main text or in the supplementary information.

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## Declaration of competing interest

The authors declare that is no conflict of interest.

## Abbreviations:

AKT, protein Kinase B; ASC, apoptosis-associated speck-like protein containing card; COX-2, cyclooxygenase-2; DSS, dextran sodium sulfate; EdU, 5-ethynyl-2' -deoxyuridine; FITC, Fluorescein isothiocyanate isomer; IBD, inflammatory bowel disease; IL-1β, interleukin 1 beta; iNOS, inducible nitric oxide synthase; LC3I/II, Recombinant Microtubule Associated Protein 1 Light Chain 3; Lgr5, leucine-rich repeat-containing G protein-coupled receptor 5; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa-B; NLRP3, NOD-like receptor thermal protein domain associated protein 3; IκB, inhibitor of NF-κB; P70S6K, ribosomal protein S6 kinase, 70 kDa; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PGEs-1, Prostaglandin E1; SCFA, short-chain fatty acid; TERT, telomerase reverse transcriptase; TLR4,Toll-like receptors; Wnt, wingless-related integration site; ZO-1, zona occludens 1.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2025.101016.

## Data availability

Data will be made available on request.

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