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ORIGINAL RESEARCH

# Cucurbitacin E Alleviates Colonic Barrier Function Impairment and Inflammation Response and Improves Microbial Composition on Experimental Colitis Models

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**Purpose:** Cucurbitacins, which are found in a variety of medicinal plants, vegetables and fruits, were known for their diverse pharmacological and biological activities, including anticancer, anti-oxidative and anti-inflammatory effects. Cucurbitacin E, one of the major cucurbitacins, was recently proved to inhibit inflammatory response.

**Methods:** To explore the therapeutic effects of cucurbitacin E on colitis and the underlying mechanisms, male mice drunk water containing 2.5% dextran sulfate sodium (DSS) to establish colitis model and administrated with cucurbitacin E during and after DSS treatment. The disease activity index was scored and colonic histological damage was observed. Intestinal tight junction and inflammatory response were determined. 16S rRNA and transcriptome sequencing were performed to analyze gut microbiota composition and gene expression, respectively.

**Results:** We found that cucurbitacin E alleviated DSS-induced body weight loss and impaired colonic morphology. Cucurbitacin E decreased the expression of inflammatory cytokines and cell apoptosis, and maintained barrier function. Additionally, cucurbitacin E retrieved DSS-induced alterations in the bacterial community composition. Furthermore, a variety of differentially expressed genes (DEGs) caused by cucurbitacin E were enriched in several pathways including the NFκB and TNF signaling pathways as well as in Th17 cell differentiation. There was a close relationship between DEGs and bacteria such as *Escherichia-Shigella* and Muribaculaceae.

**Conclusion:** Our results revealed that cucurbitacin E may exert protective effects on colitis via modulating inflammatory response, microbiota composition and host gene expression. Our study supports the therapeutic potential of cucurbitacin E in colitis and indicates that gut microbes are potentially therapeutic targets.

**Keywords:** barrier function, colitis, cucurbitacins, inflammation, microbiota

## Introduction

Although factors such as damaged integrity, impaired immune ability, microbial disturbance, genetic susceptibility, and dietary modes have been shown to induce chronic inflammatory responses and cause inflammatory bowel diseases (IBD) including colitis,[1] its etiology remains to be fully elucidated. Nevertheless, the consensus is that the inhibition of intestinal inflammatory responses is effective in alleviating the symptoms of colitis.[2] Therefore, various dietary nutrients with strong anti-inflammatory effects can be used to prevent and treat IBD.

Cucurbitacin, which are found in a variety of vegetables and fruits, including cucumber, squash, watermelon, and melon, are attracting increasing interest owing to their wide spectrum of pharmacological and biological activity.[3,4] Cucurbitacin E, an extract produced in abundance from Cucurbitaceae plants, exerts many beneficial effects, including

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anti-angiogenesis,[5] immunosuppressive[6,7] and antitumor[8] effects. Recently, its potential effects on the inflammatory response have been explored. Cucurbitacin E can inhibit inflammation via decreasing the cytochrome c oxidase subunit II expression and the production of TNF-α, IL-6, and IL-1β in RAW264.7 cells and bronchial epithelial cells treated with lipopolysaccharides.[9,10] Cucurbitacin E also decreases the TNF-α and INF-γ levels by inhibiting the NF-κB pathway in Jurkat T cells.[11] Although these results suggest that cucurbitacin E can directly affect inflammation in vitro, the underlying mechanisms remain to be elucidated. Recently, several studies have reported that the gut microbiota is critically involved in the development and treatment of colonic inflammation. However, the effects of cucurbitacin E on colitis and gut microbes have rarely been reported.

Therefore, we conducted this study to explore the effects of cucurbitacin E on colitis caused by dextran sulfate sodium (DSS). Additionally, multi-omics analyses, including gut microbiota profiling and transcriptome sequencing, were applied to explore the effects of cucurbitacin E on the intestinal microbial composition and intestinal function. Furthermore, the interaction between intestinal gene expression and gut microbes was determined to elucidate how cucurbitacin E alleviates intestinal inflammation.

# Materials and Methods

## Experimental Design

Eight-week-old male C57BL/6j mice were purchased from the SLAC laboratory (Beijing, China). All mice were kept in plastic cages at the temperature of 23 ± 2 °C and the relative humidity of 50 ± 10%. The animals were randomly allocated into three groups (seven mice in each group): 1) mice drunk running water for 14 days (CONT group); 2) mice drunk water containing 2.5% (wt/vol) DSS with a molecular weight of 36–50 kDa (MP Biomedicals, Shanghai, China) for 7 days and then drunk running water for another 7 days (DSS group); 3) mice drunk water containing 2.5% DSS for 7 days and then drunk running water for another 7 days, and mice were orally gavaged with 10 mg cucurbitacin E/kg body weight (CUCE group) once a day during the 14 days. Cucurbitacin E was purchased from Aladdin (Shanghai, China). During the experiment, mice can freely access to water and feed. On day 14, the experiment was ended and all animals were deeply anesthetized with 60 mg/kg sodium pentobarbital. Then, all mice were sacrificed and samples were collected and stored for further analysis. The experimental protocol was approved by the Review Committee of the Hospital of Shandong University (20221209), and the mice were sampled according to the animal care guidelines of the Hospital of Shandong University.

## Disease Activity Assessment

The disease activity index (DAI) was scored according to a previously described scoring system[12] based on the changes of body weight, stool consistency and rectal bleeding (Table S1). DAI was calculated by adding the score of these three indices together.

## Histological Analyses

Colonic tissues were obtained and immediately fixed in 4% paraformaldehyde. Then, the samples were embedded in paraffin and sliced into sections for hematoxylin-eosin (HE) staining and morphology observation. The histological index of colitis was scored as previously described.[1]

## Determination of Immunoglobulin, Myeloperoxidase (MPO), Eosinophil Peroxidase (EPO), and Inflammatory Cytokine Level

The level of IgA, IgG, IgM, TNF-α, IL-6 and IL-8 in plasma, and MPO and EPO level in colonic tissue were determined by using commercially available kit (Boyan Biological Technology Co., Ltd., Nanjing, China) according to the manufacturer's instructions.

## Determination of Gene Expression by Real-Time PCR

Total RNA was extracted using Trizol reagent (Sigma, Shanghai, China) and RT-qPCR assay was performed in a LightCycler® 480/LC480 (Roche, Shanghai, China) by using the Power SYBR Green RNA-to-CT 1-step Kit

(Invitrogen, Shanghai, China) in a volume of 10 μL system including 5.0 μL SYBR Green RT-PCR Mix, 0.08 μL RT Enzyme mix, 0.5 μL RNA template, 0.5 μL each of the primers and 3.42 μL RNase-free $H_2O$.[13] The expression of target genes relative to β-actin expression were calculated based on the comparative Ct value method. The primer sequences are showed in Table S2.

## Assessment of Apoptosis

The fixed colonic samples in 4% paraformaldehyde were embedded in paraffin and sliced into sections. TUNEL staining was performed using an in situ cell death detection Kit (Roche, Shanghai, China) to determine cell apoptosis. The representative pictures were photographed under a fluorescence microscope.

## Immunohistochemistry Staining of Colon Tissue

Sliced sections of colonic samples were used for immunohistochemical analysis of F4/80 and CD177 protein expression.[14] No specific binding sites were firstly block with 1% BSA (w/v) for 30 min at 23 ± 2 °C. Then, the sections were incubated with primary antibodies against F4/80 and CD177 (Abcam, Shanghai, China) at 4 °C for 2 h. The sections were incubated with HRP-conjugated IgG (Abcam) after washed with pre-cold PBS for five times. Finally, the sections were treated with gradient alcohol to dewater and then treated with xylene to increase transparency. A neutral balsam was used for mounting and the representative pictures were taken under a light microscope.

## FITC-Dextran Intestinal Permeability Assay

The animals were orally gavaged with 100 μL FITC-dextran (0.6 g per kg body weight). Whole blood was collected from the orbit vein 4 h later. The concentration of FITC-dextran was then determined at the wavelength of 488 nm to evaluate the intestinal permeability.

## Immunofluorescence Staining of Colon Tissue

Sliced sections of colonic samples were used for immunofluorescence analysis of Claudin-2 protein expression. Slices were first incubated with primary antibody Claudin-2 (Abcam, Shanghai, China) at 4 °C for 2 h. After washed with pre-cold PBS for five times, the sections were incubated with secondary antibody. Then, a reagent containing 4, 6'-diamidino-2-phenylindole was mounted on the slides. Finally, the representative results were collected under a fluorescent microscope.

## Gut Microbiota Profiling

Colonic content was collected and DNA was extracted by using stool DNA Kit (TianGen, Beijing, China). The distinct region of 16S (V3+V4) was amplified by using specific primers and conducted with Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Shanghai, China). Then, sequencing libraries were generated and quantified libraries were sequenced on Illumina platforms. Species annotation, multiple sequence alignment, alpha and beta diversity were performed using QIIME2 software. Furthermore, we performed linear discriminant analysis effect size (LEfSe) analysis to search the biomarkers.

## Transcriptome Sequencing

Colonic samples were used for RNA extraction and then mRNA was obtained by using poly-T oligo-attached magnetic beads. Those fragments of 370~420 bp in length were selected and purified for PCR performance after the first and second strand cDNA were synthesized. To generate 150 bp paired-ended reads, the library preparations were then sequenced on an Illumina Novaseq platform. Paired-end clean reads were aligned to the reference genome built by using Hisat2 v2.0.5. Quantification of gene expression level was performed by counting the reads numbers mapped to each gene by using featureCounts v1.5.0-p3. Then, the number of Fragments per Kilobase of transcript sequence per Millions base pairs sequenced was calculated for each gene. The DESeq2 R package (1.20.0) was performed to analysis differentially expressed genes (DEGs) (based on an adjusted $P$-value$\leq$ 0.05 and log2Fold change value$\geq$ 1 or $\leq -1$).[15] Finally, the clusterProfiler R package was used to analyze the statistical enrichment of DEGs in GO and KEGG Pathway. The correlation between intestinal microbiota composition and colonic gene expression was analyzed using Spearman correlation.

## Statistical Analysis

Statistical analyses are performed by a one-way ANOVA followed by SNK post hoc test (SPSS statistics 17.0 software). The results are presented as the mean ± SEM. Mean values are considered to be significantly different when a *P*-value is less than 0.05.

# Results

## Cucurbitacin E Relieved DSS-Induced Colitis Symptoms

We firstly explored the effects of cucurbitacin E on DSS-induced colitis symptoms. No animals were found dead during the experiment. DSS significantly decreased the body weight of mice from day 5, and cucurbitacin E alleviated this decrease (Figure 1A). Cucurbitacin E prevented the DSS-induced decrease in colon length and weight (Figure 1B and C), while no change was observed in the ratio of colon length and weight among the three treatment groups (Figure 1D). DSS induced an increase in DAI from day 4, whereas cucurbitacin E significantly decreased the DSS-induced increase in DAI from day 7 (Figure 1E). DSS induced obvious edema in the colonic mucosal layer of mice, whereas control mice and mice administered cucurbitacin E did not show such pathological changes (Figure 1F). The histological index was also significantly higher in mice treated with DSS than in the control mice or mice treated with cucurbitacin E (Figure 1G).

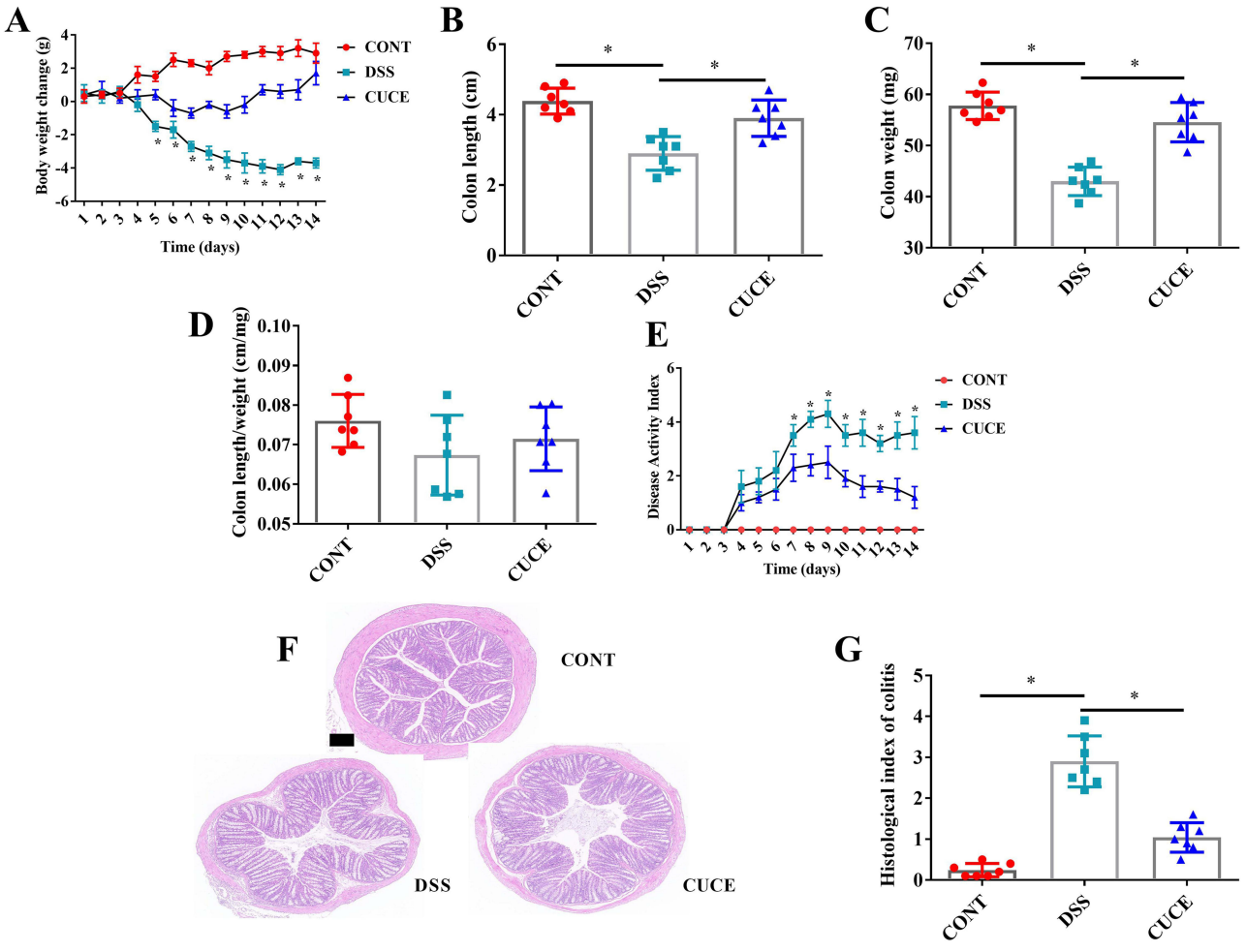


**Figure 1** Cucurbitacin E relieves DSS-induced colitis symptoms. (**A**) Body weight change; (**B**) Colon length; (**C**) Colon weight; (**D**) Colon length/weight; (**E**) Disease activity index; (**F**) Representative results of HE staining of colon morphology (Scale bar, 200 μm); (**G**) Histological index calculated according to the results of HE staining. Data are presented as mean ± SEM. *P<0.05, n=7. CONT, control mice; DSS, mice treated with dextran sulphate sodium; CUCE, mice treated with dextran sulphate sodium and cucurbitacin E.

## Cucurbitacin E Alleviated DSS-Induced Inflammation and Apoptosis

We then explored the effects of cucurbitacin E on DSS-induced inflammation and apoptosis. The results showed that the DSS treatment caused a significant decrease in the plasma IgA, IgG, and IgM concentrations, which was alleviated by cucurbitacin E (Figure 2A). The concentration of TNF-α, IL-6, and IL-8 in plasma, as well as their gene expression in the colon of DSS-treated mice increased significantly, while cucurbitacin E retrieved these changes (Figure 2B and C). The DSS treatment induced cell apoptosis in the colonic tissue, while cucurbitacin E prevented apoptosis (Figure 2D and E). The DSS treatment increased the protein expression of F8/40 and CD177, while cucurbitacin E decreased their expression (Figure 2F–H).

## Cucurbitacin E Improved DSS-Induced Intestinal Permeability

We further explored the effects of cucurbitacin E on DSS-induced intestinal permeability. Compared with the control mice, the MPO and EPO levels in the colons of mice in the DSS group were significantly higher, whereas no significant change was observed in the CUCE group (Figure 3A and B). FITC-dextran permeability was used to assess intestinal permeability. It showed that the FITC-dextran content of mice in the DSS group was significantly higher than that of mice in the CONT group, while no significant difference was observed between mice in the CONT and CUCE groups (Figure 3C). The DSS treatment significantly decreased the expression of barrier function-related genes including *Muc1, Muc2* (Figure 3D), *Claudin-1, Tjp-1*, and *Occludin* (Figure 3E), whereas the cucurbitacin E treatment increased their expression. Furthermore, immunofluorescence staining showed a significantly higher expression of Claudin-2 protein in mice in the DSS group than in mice in the CONT and CUCE groups (Figure 3F and G).

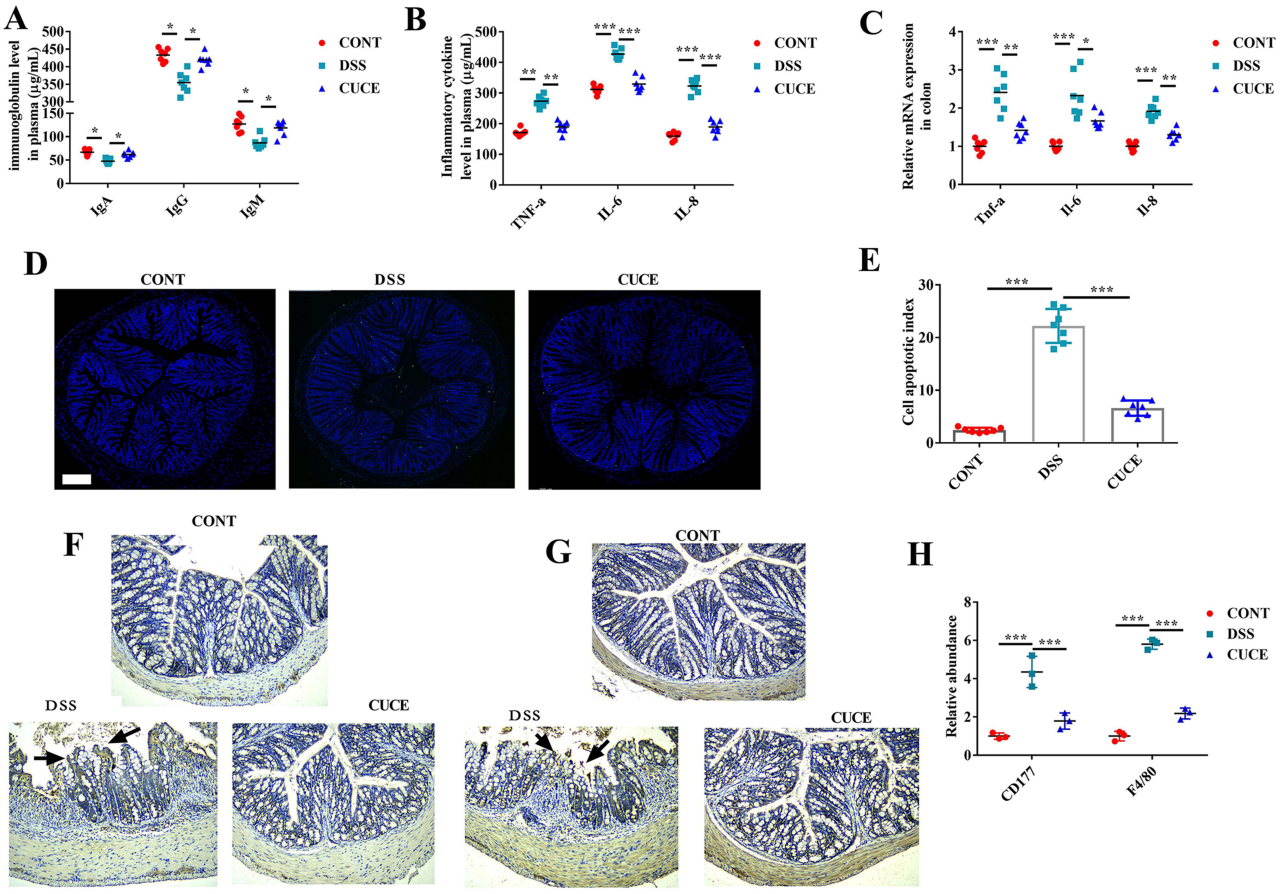


**Figure 2** Cucurbitacin E alleviates DSS-induced inflammation and apoptosis. (**A**) Immunoglobulin level in plasma; (**B**) Inflammatory cytokine level in plasma; (**C**) Relative mRNA expression in colon; (**D**) Representative results of TUNEL staining of apoptosis (Green, apoptotic cells; Scale bar, 200 μm); (**E**) Cell apoptotic index calculated according to the results of TUNEL staining. n=7. (**F** and **G**) Representative results of immunofluorescence staining of CD177 and F4/80 protein (yellow, expression of target proteins; black arrows, representative areas of target protein expression). (**H**) Relative abundance of CD177 and F4/80 expression (n=3). Data are presented as mean ± SEM. *P<0.05, **P<0.01, ***P<0.001.

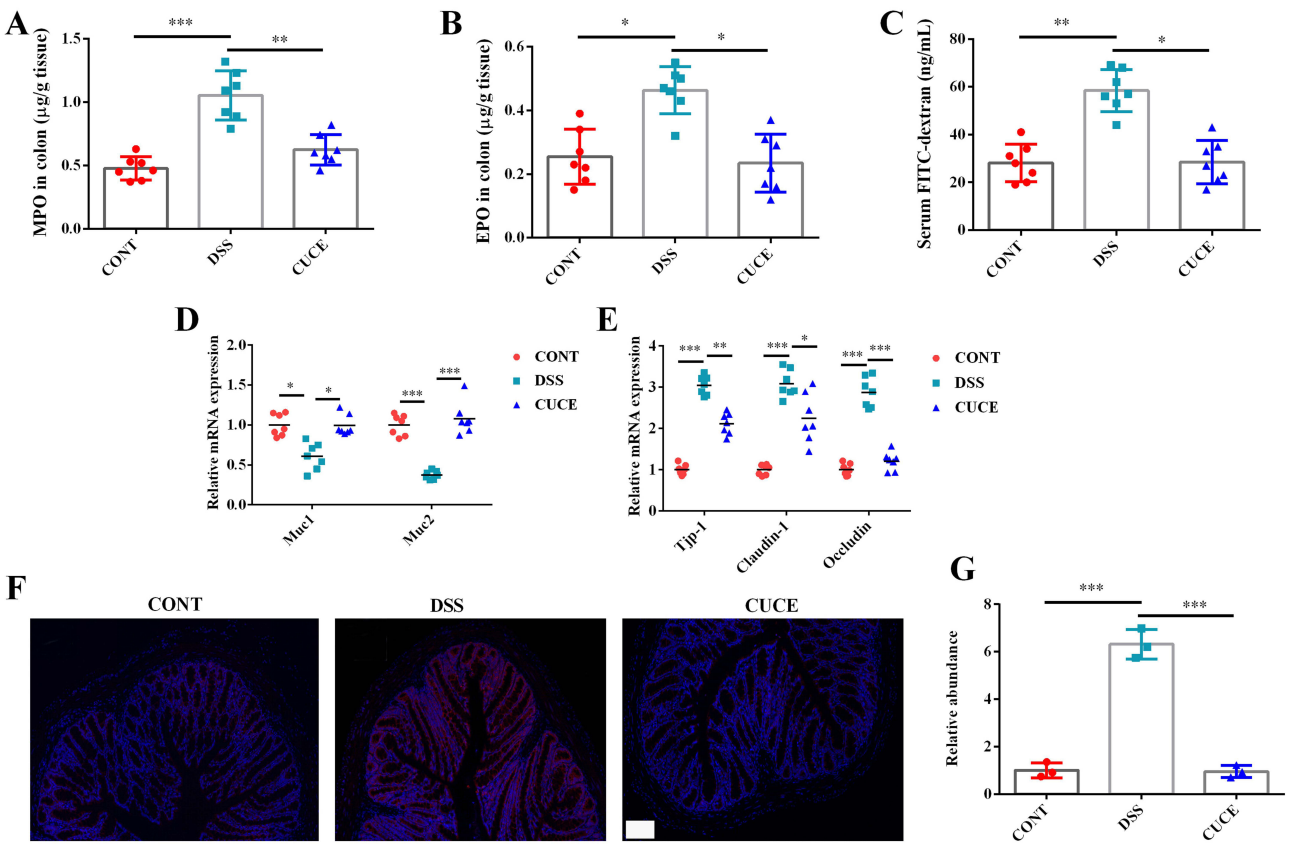


**Figure 3** Cucurbitacin E improves DSS-induced intestinal permeability. (**A**) MPO level in colon; (**B**) EPO level in colon; (**C**) Serum FITC-dextran level; (**D**) Relative mRNA expression of *Muc1* and *Muc2*; (**E**) Relative mRNA expression of *Tjp-1*, *Claudin-1* and *Occludin*; n=7. (**F**) Representative results of immunohistochemistry staining of Claudin 2 protein (Red, Claudin 2 protein; Scale bar, 100 μm). (**G**) Relative abundance of Claudin 2 expression (n=3). Data are presented as mean ± SEM. *P<0.05, **P<0.01, ***P<0.001.

## Cucurbitacin E Improved the Microbiota Composition in DSS-Treated Mice

To explore the effects of cucurbitacin E on intestinal microbiota composition, we performed 16S rRNA sequencing on the colonic content. Although there was no significant difference in the Chao1 index and observed OTUs among the three treatment groups (Figure 4A and B), the alpha diversity of the gut microbiota communities, as indicated by the Simpson and Shannon indices (Figure 4C and D), was significantly decreased in mice treated with DSS and was restored by cucurbitacin E supplementation. The PCoA results based on the weighted UniFrac analysis revealed that the overall microbial structure of control mice was clearly separated from that of DSS-treated mice, whereas the microbial structure of cucurbitacin E-supplemented mice was intermediate between those of the control and DSS-treated mice (Figure 4E). At the phylum level, the DSS treatment significantly increased the abundance of Firmicutes, Proteobacteria, and Verrucomicrobiota, while decreasing the abundance of Bacteroidetes (Figure 4F). At the genus level, the DSS treatment significantly increased the abundance of *Streptococcus, Escherichia-Shigella*, and *Akkermansia* and decreased the Muribaculaceae abundance (Figure 4G). As expected, the abundance of these microbes at the phylum or genus level in the cucurbitacin-E-supplemented mice was in the middle of that in the control and DSS-treated mice. Based on LEfSe in the microbiome date, biomarkers in DSS-treated mice included Firmicutes, Streptococcaceae, *Streptococcus*, Bacilli, Enterobacteriaceae, Enterobacterales, *Escherichia_Shigella*, Gammaproteobacteria, and *Lachnospiraceae_NK4A136_group*. Biomarkers in mice supplemented with cucurbitacin E included Verrucomicrobiota, Verrucomicrobiae, Verrucomicrobiales, Akkermansiaceae, *Akkermansia*, Erysipelotrichales, and *Rikenellaceae_RC9_gut_group*. Biomarkers in control mice included Bacteroidales, Bacteroidia, Bacteroidota, Muribaculaceae, Prevotellaceae, and *Alloprevotella* (Figure 4H).

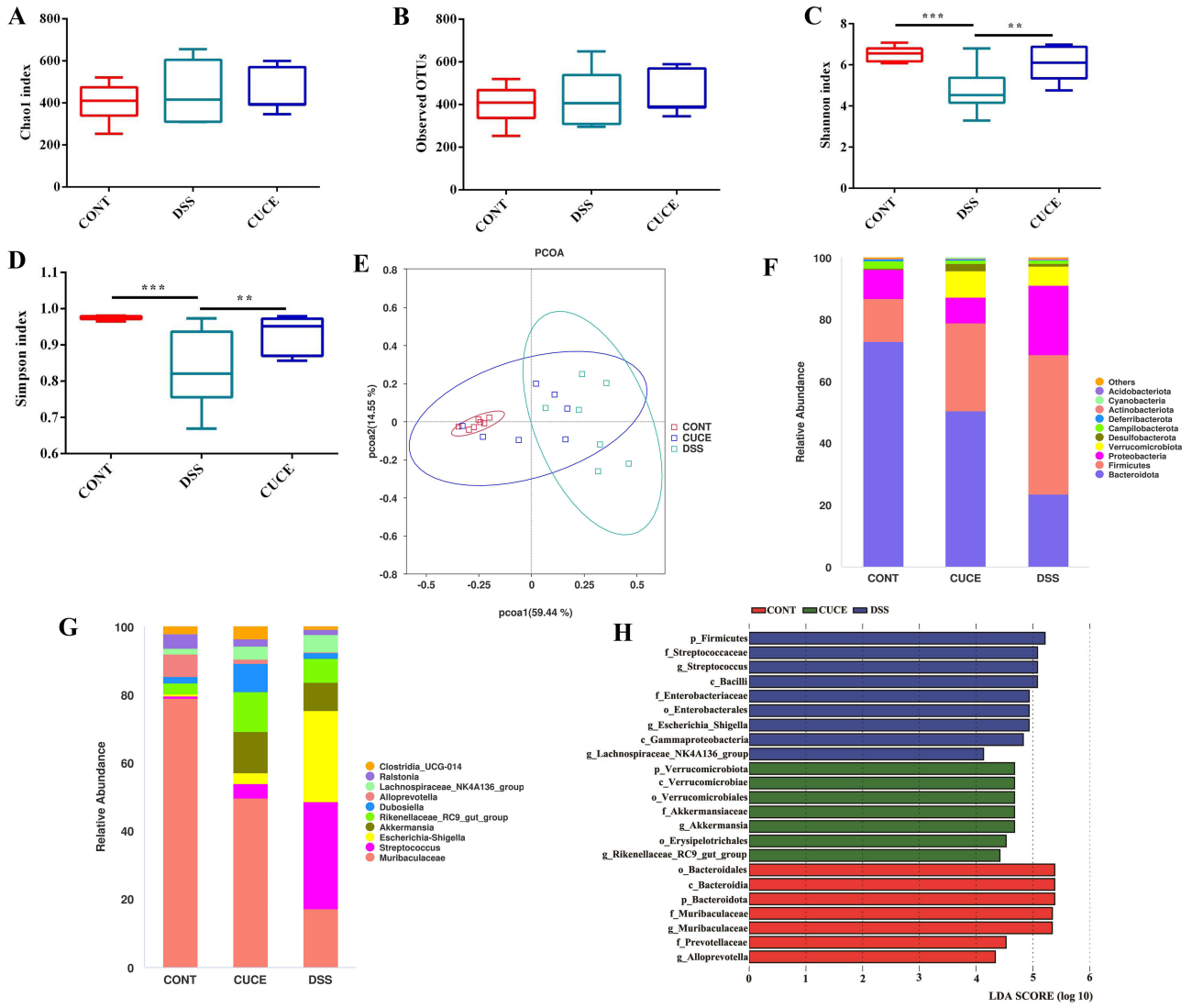


**Figure 4** Cucurbitacin E improves microbiota composition in DSS-treated mice. (**A**) Chao1 index; (**B**) Observed OTUs; (**C**) Shannon index; (**D**) Simpson index; (**E**) PCoA plot of the microbiota based on a weight UniFrac metric. Relative abundance of predominant bacteria at the phylum (**F**) and genus (**G**). (**H**) Bacterial biomarkers in each treatment group (LDA score value > 4 or < −4). Data are presented as mean ± SEM. **P<0.01, ***P<0.001, n=7.

## Cucurbitacin E Modulated Colonic Gene Expression in DSS-Treated Mice

To explore the effects of cucurbitacin E on colonic gene expression, we performed transcriptome analysis of colonic tissue samples. The DEGs (P<0.05, $log_2$Foldchange>1) between the libraries are presented in Figure S1. The data for the DSS group versus the CONT group revealed 850 upregulated and 1075 downregulated DEGs. The data for the CUCE group versus the CONT group revealed 17 upregulated and 65 downregulated DEGs. Data for the CUCE group versus the DSS group produced 157 upregulated and 1027 downregulated DEGs. Hierarchical clustering revealed more similar gene expression between mice in the CUCE and CONT groups than between mice in the DSS and CONT groups (Figure 5A). GO enrichment analysis identified biological processes, including the regulation of the inflammatory response and positive regulation of cytokine production, and molecular functions, including cytokine binding, cytokine receptor activity, cytokine activity, and cytokine receptor binding (Figure 5B). Furthermore, the KEGG metabolic pathway analysis indicated that the DEGs were involved in pathways including the TNF signaling pathway, cytokine-cytokine receptor interaction, Th17 cell differentiation, and NFκB signaling pathway, which were closely related to inflammatory bowel diseases (Figure 5C). Notably, DEGs including *Cxcl2, Cxcl3, Il1b, Ptgs2, Cxcl1, Ccl4, Cd14, Icam1, Card14, Il17a, Il6, Stat3, Il17f, Il4ra, Runx1, Jak3, Hif1a, Nfkbia, Il22, Tgfb1, Mmp3, Mmp9, Cxcl5, Tnfrsf1b, Socs3,*

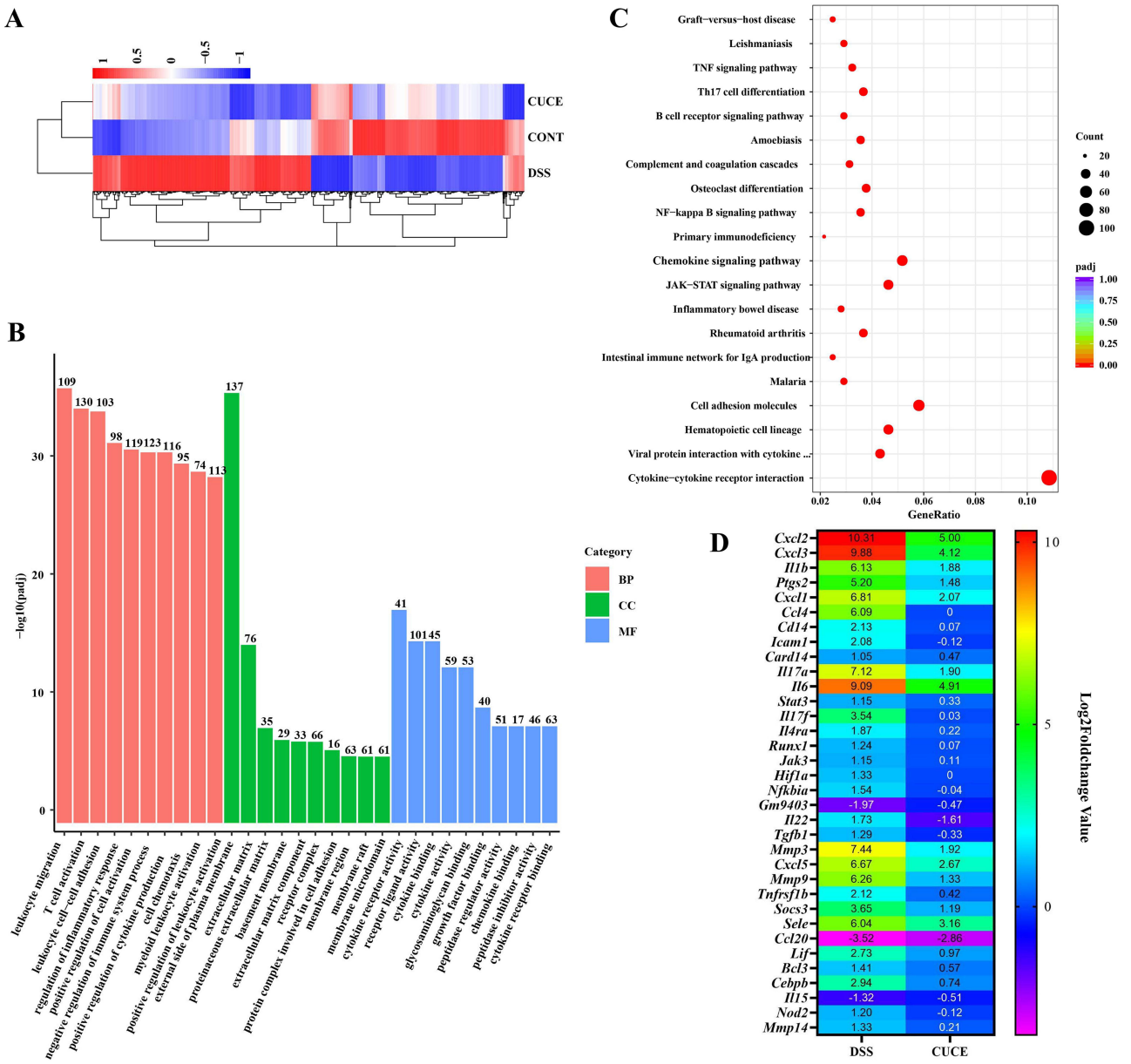


**Figure 5** Cucurbitacin E modulates gene expression in DSS-treated mice. (**A**) Heatmap showing differentially expressed genes; (**B**) GO analysis for differentially expressed genes; (**C**) KEGG analysis for differentially expressed genes. (**D**) The relative expression of differently expressed genes enriched in the TNF signaling pathway, Th17 cell differentiation and NFκB signaling pathway in DSS and CUCE group when compared with those in CONT group.

*Sele, Lif, Bcl3, Cebpb, Nod2* and *Mmp14* enriched in the abovementioned inflammation-related pathways were significantly increased by DSS treatment while they were decreased by cucurbitacin E treatment (Figure 5D).

## Associations Between Microbes and Intestinal Gene Expression

We selected DEGs involved in Th17 cell differentiation, the NFκB signaling pathway, and the TNF signaling pathway to determine the correlation between these DEGs and the characterized bacteria in the three treatment groups (Figure 6). Characterized bacteria in DSS-treated mice included Streptococcaceae, *Streptococcus*, and *Escherichia_Shigella* that had significantly positive correlations with seven DEGs enriched in the NFκB signaling pathway, 11 DEGs enriched in Th17 cell differentiation, and 18 DEGs enriched in the TNF signaling pathway. Characterized bacteria in control mice included *Muribaculaceae, Prevotellaceae*, and *Alloprevotella* that had significantly negative correlations with eight DEGs enriched in the NFκB signaling pathway, 11 DEGs enriched in Th17 cell differentiation, and 20 DEGs enriched in the TNF signaling pathway.

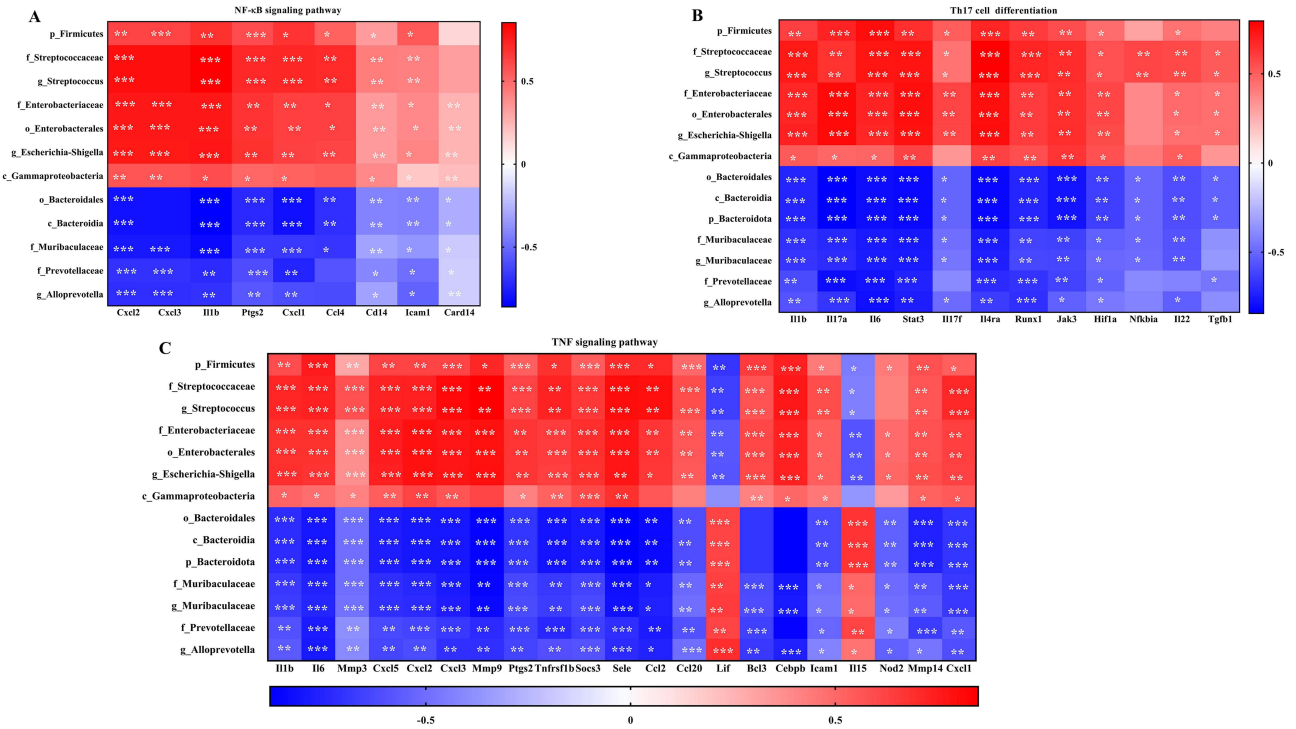


**Figure 6** The association between bacterial biomarkers and differentially expressed genes enriched in major pathways. The correlation between bacterial biomarkers and major differentially expressed genes enriched in NFκB signaling pathway (**A**), Th17 cell differentiation (**B**), and TNF signaling pathway (**C**). *P<0.05, **P<0.01, ***P<0.001.

## Discussion

Recently, the anti-inflammatory effects of cucurbitacin E have been reported in several in vitro cell models[10,16,17] and in vivo mouse models of neuroinflammatory injury.[18,19] However, its beneficial effects on intestinal inflammation have not been studied. In this study, cucurbitacin E was administered to mice treated with DSS, a well-accepted experimental model of colitis. We found that cucurbitacin E alleviated the symptoms of DSS-induced colitis. Importantly, we found that cucurbitacin E not only protected DSS-treated mice from the overaccumulation of inflammatory cytokines and increased apoptosis, but also decreased intestinal permeability and increased the expression of genes encoding tight junction proteins. Furthermore, the results showed that cucurbitacin E decreased infiltration of inflammatory cells as indicated by decreased $CD177^{+}$ neutrophils and $F4/80^{+}$ macrophages (14). These results suggest that the possible mechanism of action of cucurbitacin E in colitis may be mediated by its anti-inflammatory effects and beneficial effects on maintaining the integrity of colonic barrier function.

DSS-induced colitis is also commonly associated with gut dysbiosis. A previous study reported decreased microbial community evenness,[1] which is consistent with our results, as indicated by decreased Shannon and Simpson indices. Furthermore, a shift in the bacterial community composition at the phylum level was observed in the present study as the abundance of Firmicutes increased, whereas the abundance of Bacteroidota decreased. However, these changes varied in different experiments.[12,20,21] These inconsistent results indicate that the effects of DSS on gut microbes may also affect by host microbial composition and other factors. Nevertheless, cucurbitacin E supplementation alleviated the alterations caused by the DSS treatment. Specifically, the abundance of *Escherichia-Shigella* and *Streptococcus*, which are potentially harmful bacteria, increased by the DSS treatment as previous studies reported.[20,22] Additionally, the DSS treatment decreased the abundance of Muribaculaceae, which are commonly considered to be "commensal bacteria". Muribaculaceae could not only produce short-chain fatty acids, but also secrete extracellular vesicles to inhibit pro-inflammatory cytokine accumulation and promote anti-inflammatory cytokine secretion.[23] Thus, Muribaculaceae was reported to be negatively correlated with inflammatory markers.[24,25] In the present study, cucurbitacin E supplementation prevented the DSS-induced decrease in Muribaculaceae abundance, indicating that it may alleviate inflammation via the gut microbes.

DSS-induced colitis is characterized by a chronic inflammatory response in the colonic tissue. We used transcriptional analysis of gene expression to determine host inflammation status. We found that most of the DEGs among the three treatment groups were enriched in inflammation-related pathways, including the regulation of the inflammatory response and cytokine production, based on GO analysis. These results suggest that cucurbitacin E and the DSS treatment significantly affected the inflammatory status of the host colonic tissue. Importantly, cluster analysis showed that the expression of these DEGs in the CUCE group was similar to that in the CONT group, indicating that cucurbitacin E supplementation alleviated the inflammatory response. The KEGG metabolic pathway analysis suggested that most DEGs were enriched in inflammation-related pathways. Among these pathways, the TNF signaling pathway was closely related to the development of colitis as TNF-α is a key molecule mediating DSS-induced inflammation.[26,27] IL17 cell differentiation[28–30] and the NF-κB pathway[31–33] are therapeutic targets for the prevention and treatment of colitis. These results suggest that cucurbitacin E alleviates DSS-induced colitis through a combination of multiple signaling pathways.

Gut microbes play critical roles in the development of intestinal inflammation. Alterations in the colonic microbiota composition have been observed following DSS treatment, and microbiota dysbiosis can further exacerbate intestinal inflammation in DSS-treated mice.[1,34] Thus, we analyzed the association between microbial alterations and host intestinal gene expression. Expectedly, the abundance of *Escherichia-Shigella* and *Streptococcus* was positively correlated with a variety of DEGs enriched in the NFκB signaling pathway, Th17 cell differentiation, and TNF signaling pathway, while the abundance of Muribaculaceae was negatively correlated with a variety of DEGs enriched in these three pathways. These results indicate that DSS may induce colonic inflammation by affecting *Escherichia-Shigella* and *Streptococcus* abundance, and that cucurbitacin E may target microbes majorly including Muribaculaceae to alleviate DSS-induced colitis. However, the specific mechanisms by which cucurbitacin E increases Muribaculaceae abundance and how Muribaculaceae prevents DSS-induced inflammation require further elucidation. Additionally, whether cucurbitacin E regulates host gene expression directly or indirectly mediated by gut microbiota remain to be elucidated. Future studies are needed to explore the indispensable role of gut microbiota in the preventing effects of cucurbitacin E on intestinal inflammation by using germ-free animals and fecal microbiota transplantation.

## Conclusion

In conclusion, our results showed that cucurbitacin E alleviated colitis symptoms, the inflammatory response, and apoptosis and improved intestinal permeability in DSS-treated mice. Furthermore, multi-omics analyses revealed that cucurbitacin E exerted protective effects by maintaining host-microbe interactions in the colon. Particularly, cucurbitacin E increased the abundance of Muribaculaceae and inhibited the activation of several pathways including Th17 cell differentiation, the NFκB signaling pathway, and the TNF signaling pathway, which are critically involved in inflammation. Notably, the abundance of Muribaculaceae are negatively correlated with most of the DEGs enriched in these three pathways. Our study supports the therapeutic potential of cucurbitacin E in colitis and indicates that gut microbes are potentially therapeutic targets.

## Abbreviations

IBD, inflammatory bowel diseases; DSS, dextran sulfate sodium; DAI, disease activity index; HE, hematoxylin-eosin; MPO, myeloperoxidase; EPO, eosinophil peroxidase; LEfSe, linear discriminant analysis effect size; DEGs, differentially expressed genes.

## Data Sharing Statement

The 16S rDNA gene sequence and transcriptome sequence data have been deposited in the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/) under accession numbers PRJNA995394 and PRJNA998211. Data are available upon reasonable request from the corresponding author.

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## Disclosure

The authors report no conflicts of interest in this work.

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