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Malvidin-3-*O*-galactoside ameliorates small intestinal mucosal barrier function *via* Notch pathway

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ABSTRACT

The intestinal mucosa is the intestinal lumen tissue that protects the intestine from invasion, maintains intestinal barrier function, and participates in the immune response. Diseases such as inflammatory enteritis and intestinal infections can cause damage to the intestinal mucosal barrier and dysfunction. The aim of this study was to investigate the improvement mechanism of malvidin-3-*O*-galactoside (M3G) on small intestinal mucosal barrier function. C57BL/6J male mice were given dextran sodium sulfate (DSS) for 7 days to induce enteritis, and then were fed normally with or without M3G supplementation for another 7 days. The results showed that M3G supplementation significantly improved the disease activity index (DAI) score and small intestinal tissue injury in mice with DSS induced enteritis. M3G ameliorated the small intestinal mucosal mechanical barrier function by modulating the expression of mucin 2 (MUC2), zona occludens 1 (ZO-1), Occludin, Claudin-1, intestinal fatty acid binding protein (iFABP), and trefoil factor 3 (TFF3) in the small intestine mucosa, and the serum levels of *D*-lactic acid (*D*-LA), lipopolysaccharide (LPS), and diamine oxidase (DAO) were significantly decreased. Additionally, M3G also relieved the small intestinal immunologic barrier of mice by decreasing the immune protein levels of immunoglobulin A (IgA), immunoglobulin M (IgM), and immunoglobulin G (IgG) in serum, and secretory immunoglobulin A (SIgA) level in small intestine tissue. Furthermore, M3G inhibited the expression of Notch pathway-related proteins such as Notch1, Notch intracellular domain (NICD), delta-like ligand 4 (DLL4), delta-like ligand 1 (DLL1), and hairy/enhancer of split 1 (Hes1). In conclusion, the results demonstrated that M3G can improve intestinal mucosal barrier function by inhibiting Notch pathway.

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1. Introduction

Anthocyanins are a class of compounds composed of anthocyanidins and sugar, which are connected by glycosidic bond. Anthocyanins play important roles in anti-inflammatory, antioxidant, anti-tumor, protect vision, cardiovascular protection, and other physiological functions[1-4]. Therefore, they are widely used in food and medicine industries. Anthocyanins are rich in resources, widely existing in fruits, vegetables, and grains, and berries are the main source of anthocyanins, such as blueberries, strawberries and grapes[5].

The most widely existed anthocyanin monomers are cyanidin, delphinidin, petunidin, peonidin, pelargonidin and malvidin, which are combined with glucose, galactose and arabinose to form anthocyanins. Researches on the isolation of blueberry anthocyanins showed 13 kinds of anthocyanins were isolated from 28 blueberries, and malvidin-3-*O*-

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galactoside (M3G) was the highest content anthocyanin monomer in blueberries. At present, studies have found that M3G has antioxidant, anti-cancer, anti-inflammatory and other physiological functions[2,6-7]. Therefore, it is of great significance to continue to explore the biological activity of M3G and apply it to improve physiological health.

Intestinal mucosal barrier is an important defense line of the human body. Under normal circumstances, intestinal mucosal barrier can play its own shielding function to ensure the stability of the internal environment of the body. Injured intestinal mucosal barrier function due to various reasons such as infection, ischemia, and acute pancreatitis would cause increased intestinal mucosal permeability, further leading to intestinal bacterial translocation and a large number of pro-inflammatory factors release, thereby aggravating the primary disease and even inducing the occurrence of multiple organ dysfunction and failure[8].

At present, studies have shown that anthocyanins can alleviate colitis and intestinal mucosal barrier dysfunction mitigate[9-11]. Cheng et al.[12] found that M3G can regulate the intestinal flora structure of mice to a certain extent. However, whether M3G can improve the damage of intestinal mucosal barrier function and the potential mechanism is not clear. Therefore, dextran sodium sulfate (DSS) induced enteritis mouse model was used to observe the effect of M3G on the small intestinal mucosa barrier function and explore its mechanism of action in this study.

2. Materials and methods

2.1 Materials

M3G (CAS: 30113-37-2) was purchased from Xinyi Science and Technology Instrument Business Department, Weibin District, Baoji City, China (HPLC ≥ 98%).

2.2 Animal experiments

This animal study was approved by the Laboratory Animal Welfare Ethics Review Committee of Shenyang Agricultural University (Approval No.: 2022030304). Five-week-old male C57BL/6J mice (Wanlei Bio Co., Ltd., Shenyang, China) weighing 16−18 g were selected and given AIN-93M feed (Wanlei Bio Co., Ltd., Shenyang, China) feeding. Mice acclimated to the environment for 1 week, then were randomly divided into two groups: the Control group (n = 6) and the model group (n = 12). The model group mice were free to drink 2.5% DSS (CAS: 9011-18-1) solution on day 1−7, while the Control group mice were free to drink distilled water. After 7 days, the model group mice were equally divided into two groups: the DSS group (n = 6) and the DSS + M3G group (n = 6). The DSS + M3G group mice were given M3G (5 mg/(kg·day)) by gavage, and the Control group and the DSS group mice were given equal volume of distilled water by gavage. During the experiments, body weight, food consumption, stool consistency and bloody stool were measured every day. On day 15, the mice were sacrificed after 12 h of fasting, and the ileum, jejunum and blood samples of mice were collected.

2.3 Hematoxylin-eosin (HE) staining

HE staining method was referred to the previous report with some modifications[13]. Briefly, small intestinal tissue was embedded with paraffin and cut to sections, and then dewaxed and placed in water, following by stained with hematoxylin and eosin solution. The stained tissue was dehydrated and sealed for observation. The microscope (BX53, OLYMPUS Co., Ltd., Tokyo, Japan) was used to observed the villus height and crypt depth of the small intestine tissue and photographed using 100 × magnification (DP73, OLYMPUS Co., Ltd., Tokyo, Japan).

2.4 Periodic acid-Schiff (PAS) staining

Small intestinal tissue was paraffin embedded and cut to sections, and then dewaxed and placed in water, following by stained with Schiff and hematoxylin solution. The stained tissue was dehydrated and sealed for observation[14]. The mucosal thickness of the small intestine was observed, and the number of goblet cells was recorded. The stained small intestinal tissue was observed under a microscope and photographed using 100× magnification.

2.5 Immunohistochemical (IHC)

The number of Panzer cells in the small intestine tissue was detected by IHC method[15]. Small intestinal tissue was embedded with paraffin and cut into sections, and then dewaxed using xylene and ethanol. The microwave antigen repair method was used. The sections were incubated in 3% hydrogen peroxide for 15 min. After incubating with the primary antibody at 4 ℃ overnight, tissue were incubated with horseradish peroxidase (HRP) labeled secondary antibody at 37 ℃ for 60 min. Finally, the stained tissue was dehydrated and sealed for observation after color reaction with diaminobenzidine (DAB) chromogen and counterstained by hematoxylin. The stained sections were observed under a microscope and photographed using 400 × magnification.

2.6 Real-time polymerase chain reaction (PCR)

The expression of mucin 2 (*MUC2*) in small intestine was detected by real-time PCR[16]. Total RNA was extracted from small intestine tissues, and the concentration in samples was determined using an ultraviolet spectrophotometer (NANO 2000, Thermo, USA). Then, Transcriptor First Strand cDNA Synthesis Kit (Roche Co., Ltd., Basel, Switzerland) was used to synthesize the single-stranded cDNA from the extracted RNA (0.1 μL). The primer sequences used in the experiments are shown in Table 1. Exicyler™ 96 (BIONEER, Korea) was used for fluorescence quantitative analysis of cDNA. The reaction conditions were as follows: 94 ℃ for 5 min, 94 ℃ for 10 s, 60 ℃ for 20 s, 72 ℃ for 30 s, then followed by 2 cycles for 40, incubate at 72 ℃ for 2 min 30 s, 40 ℃ for 1 min 30 s, melting from 60 ℃ to 94 ℃, and incubated at 25 ℃ for 1−2 min.

Table 1
Primer sequences used in the quantitative real-time PCR analysis.

Gene	Prime	Sequence (5′-3′)	Size (bp)
MUC2	Forward	TGTGCCTGGCTCTAATA	17
	Reverse	AGGTGGGTTCTTCTTCA	17
β-actin	Forward	CTGTGCCCATCTACGAGGGCTAT	23
	Reverse	TTTGATGTCACGCACGATTTCC	22

2.7 Western blot

The protein expression levels of Claudin-1, Occludin, zona occludens 1 (ZO-1), intestinal fatty acid binding protein (iFABP), delta-like ligand 1 (DLL1), DLL4, Notch1, notch intracellular domain (NICD), hairy/enhancer of split 1 (Hes1) and trefoil factor 3 (TFF3) were determined by western blot. Methods was referred to the previous article with minor modifications[17]. Whole proteins from the small intestine (200 mg) were extracted using the Whole Cell Lysis Assay kit (BioTeke Co., Ltd., Beijing, China) according to the manufacturer's protocol. Briefly, ileum tissue was cut into very small pieces and mixed with phenylmethanesulfonyl fluoride (PMSF), and then the protein extraction reagents A and B were added to prepare the tissue homogenate. Then protein concentration was measured by Bradford assay using Bradford kit (BioTeke Co., Ltd., Beijing, China) according to the manufacturer's protocol. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The membranes were blocked with 5% non-fat dry milk in TBST buffer for 1 h, followed by incubating with the appropriate monoclonal primary antibody (Table 2) at 4 ℃ overnight. Then, membranes were washed and incubated with the secondary antibody (goat anti-rabbit IgG-HRP), 1∶5 000; Wanlei Bio Co., Ltd., Shenyang, China) at 37 ℃ for 45 min. Protein bands were detected by an enhanced chemiluminescence (ECL) Western blotting detection reagent (Wanlei Bio Co., Ltd., Shenyang, China) and visualized with a Gel Imaging System (Beijing Liuyi Biotechnology Co., Ltd., Beijing, China).

Table 2
Details of the primary antibodies used in the experiment.

Primary antibody	Dilution ratio	Manufacturer
Claudin-1	1:500	Wanlei Bio Co., Ltd. (China)
Occludin	1:500	Wanlei Bio Co., Ltd. (China)
ZO-1	1:500	Wanlei Bio Co., Ltd. (China)
iFABP	1:1 000	ABclonal Technology Co., Ltd. (China)
DLL1	1:500	Wanlei Bio Co., Ltd. (China)
DLL4	1:1 000	ABclonal Technology Co., Ltd. (China)
Notch1	1:500	Wanlei Bio Co., Ltd. (China)
NICD	1:1 000	Affinity Biosciences Co., Ltd. (China)
Hes1	1:1 000	ABclonal Technology Co., Ltd. (China)
TFF3	1:1 000	Affinity Biosciences Co., Ltd. (China)
β-actin	1:1 000	Wanlei Bio Co., Ltd. (China)

2.8 Enzyme-linked immunosorbent assay (ELISA)

ELISA kits were used to detect the levels of *D*-lactic acid (*D*-LA), lipopolysaccharide (LPS), diamine oxidase (DAO), immunoglobulin A (IgA), IgM, and IgG in serum. The level of secretory immunoglobulin A (SIgA) in small intestine tissues was detected by SIgA ELISA kit. The ELISA kits used in the experiment are shown in Table 3, and the experimental methods referred to the kit instructions.

Table 3
ELISA kit list.

Kit name	Model number	Manufacturers
D-LA kit	E-BC-KOO2-M	Elabscience Co., Ltd. (China)
LPS kit	IEB526Ge	Uscnk Co., Ltd. (China)
DAO kit	EM0980	Wuhan Fine Biotech Co., Ltd. (China)
IgA kit	EK274	Multi Sciences Lianke Biotech Co., Ltd. (China)
IgM kit	EK276	Multi Sciences Lianke Biotech Co., Ltd. (China)
IgG kit	EK271	Multi Sciences Lianke Biotech Co., Ltd. (China)
SIgA kit	EM1362	Wuhan Fine Biotech Co., Ltd. (China)

2.9 Statistical analysis

Data are expressed as mean ± standard deviation (SD). Differences between two groups were assessed using Student's *t* test, and comparisons among more two groups were analyzed by one-way ANOVA followed by Tukey's post hoc multiple comparison test. The results were considered statistically significant at $P < 0.05$. Data were analyzed using GraphPad Prism 8.0 (Graph Pad Software, San Diego, CA, USA) and SPSS 17.0 (IBM Corporation, Armonk, NY, USA).

3. Results

3.1 Effects of M3G on the body weight, disease activity index (DAI) score, and food intake of mice with DSS induced enteritis

The body weight, body weight before sacrificed, DAI score and food intake of mice in each group were shown in Fig. 1. The weight of the mice increased in the Control group during experiment, while decreased in the DSS group, and gradually increased after the administration of M3G on day 8 (Fig. 1A). Compared with the Control group, the body weight before sacrificed of the mice in the DSS group was significantly reduced ($P < 0.01$). The body weight before sacrificed of mice in the DSS group significantly decreased ($P < 0.01$) compared with that in the Control group, and M3G supplementation significantly increased the body weight gain before sacrificed of the mice ($P < 0.01$) (Fig. 1B). The mice in the Control group had semi-formed feces without weight loss or bloody stools, and DAI score of the mice in the DSS group was stability during experiment. DAI score of the mice in the DSS group increased rapidly first and then decreased slowly, while the DAI score of the mice in the DSS + M3G group increased rapidly first and then began to decrease rapidly on the day 7. At day 14, the DAI score of the mice in the DSS group was significantly higher ($P < 0.01$) compared with that of the Control group, and M3G supplementation significantly decreased ($P < 0.01$) DAI score of the mice compared with the DSS group, but the DAI score of the mice in the DSS + M3G group was still significantly higher ($P < 0.05$) compared with that of the Control group (Fig. 1C). Food intake of the mice was stability in the Control group during experiment, was gradually decreased in the DSS group, and after supplementing M3G on day 8, food intake of the mice was gradually recovered. On day 14, food intake of mice was no significant difference ($P > 0.05$) between the Control group and the DSS + M3G group, and food intake of the mice in the DSS group was significantly lower ($P < 0.05$) than that of the Control group and the DSS + M3G group. (Fig. 1D). These results indicated that M3G can restore loose stools, bloody stools and weight loss in mice with DSS induced enteritis.

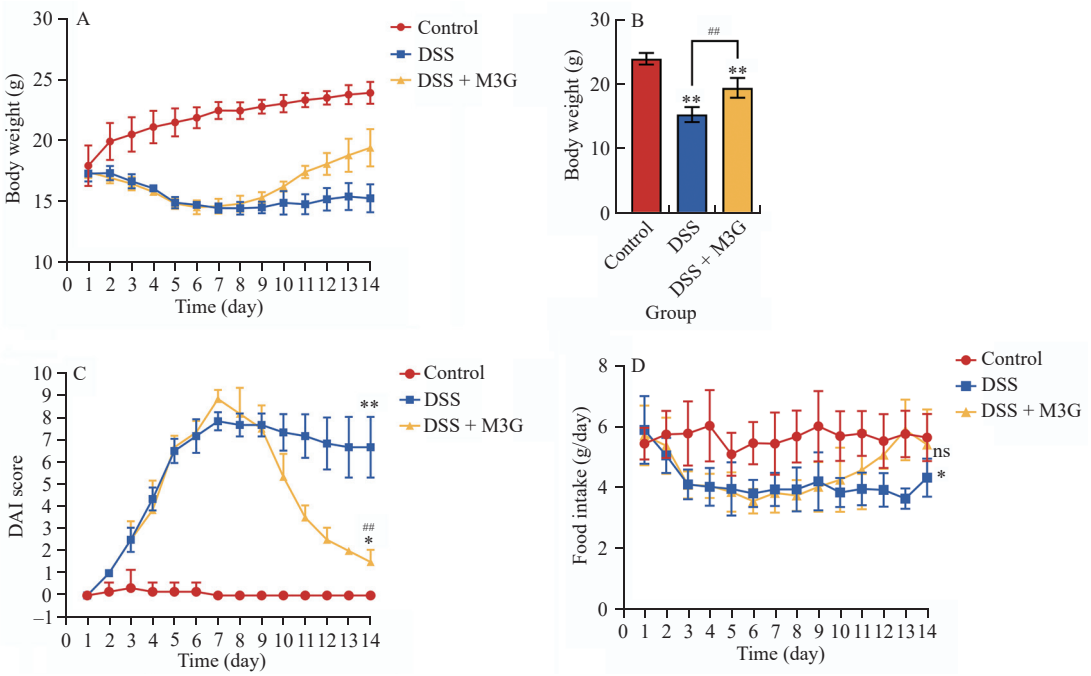

Fig. 1 Effect of M3G on body weight, DAI score, and food intake in mice with DSS-induced enteritis. (A) Body weight curve. (B) Body weight before sacrificed. (C) DAI score. Fecal consistency: 0 = normal, 1 = semi-formed, 2 = soft stool, 3 = diarrhea or watery stool; bloody stool: 0 = occult blood negative, 1 = occult blood positive, 2 = visible bloody stool, 3 = massive hemorrhage; weight loss: 0 = 0%, 1 = 1%−5%, 2 = 6%−10%; 3 = 11%−15% reduction. (D) Food intake. $n = 6$ per group. Results are expressed as the mean ± SD. ns = no significant, $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the Control group; $^{\#\#}P < 0.01$ compared with the DSS group.

3.2 Effect of M3G on pathological morphology of the small intestine of mice with DSS induced enteritis

The HE staining, PAS staining, and IHC methods were used to observe the pathological morphology of the small intestine of mice, and determine small intestinal tissue damage score, number of Paneth cells, villus height, crypt depth, and intestinal mucosal thickness of mice in each group. In the Control group, the small intestinal mucosa was intact, villi were arranged normally without damage. Lamina propria disappeared and large area ulcers formed in the DSS group, and M3G supplementation made partial recovery of the small intestinal morphology damage, such as intestinal mucosa was elevated, villi were lodging to both sides, part of villi tips was fell off, and ulcers were reduced (Fig. 2A). The small intestinal tissue damage score of the mice in the DSS group was significantly higher ($P < 0.01$) than that of the Control group, and the damage was serious. M3G supplemented mice exhibited significantly lower ($P < 0.01$) small intestinal tissue damage score compared with the DSS group (Fig. 2D). The villi were long and the crypts were shallow the small intestine of the mice in the Control group, DSS caused the villi to be significantly shorter ($P < 0.01$) and the crypts depth to be significantly deeper ($P < 0.01$) compared with those of the Control group. After M3G treatment, the villi were significantly longer ($P < 0.01$) and the crypt depth significantly shallower ($P < 0.01$) than that of the DSS group (Figs. 2E and F). Compared with the Control group, the small intestinal mucosal thickness was significantly reduced in the DSS group ($P < 0.01$), and significantly increased after M3G supplementation (Figs. 2B and G). The number of Paneth cells of the mice in the DSS group was significantly higher ($P < 0.01$) compared with that in the Control group. M3G treatment significantly decreased ($P < 0.01$) the number of Paneth cells compared with the DSS group (Figs. 2C and H). The above results indicated that M3G could significantly improve the small intestinal tissue damage in mice with DSS-induced enteritis. The damage degree of small intestine of the mice in the DSS + M3G group was less than that in the DSS group.

3.3 Effects of M3G on the small intestinal mucosal barrier function of mice with DSS induced enteritis

The mRNA expression level of *MUC2* in the small intestine was determined (Fig. 3). Compared with the Control group, DSS significantly down-regulated ($P < 0.01$) the mRNA expression level of MUC2, which was significantly increased by M3G supplementation ($P < 0.01$). However, the expression level of MUC2 in the DSS + M3G group was still significantly lower ($P < 0.01$) than that of the Control group.

Subsequently, ZO-1, Occludin, Claudin-1, iFABP, and TFF3 protein expression levels in the small intestine were measured. The expression levels of ZO-1, Occludin, and Claudin-1 were significantly lower ($P < 0.01$) in the DSS group in comparison with those of the Control group. M3G supplementation significantly increased the expression levels of ZO-1, Occludin, and Claudin-1 (Figs. 4B-D). The expression level of iFABP in the DSS group were significantly higher ($P < 0.01$) than the Control group, and it exhibited a significantly reduced ($P < 0.01$) expression in the DSS + M3G group compared with the DSS group (Fig. 4E). Compared with the DSS group, M3G supplementation significantly increased ($P < 0.01$) TFF3 expression level (Fig. 4F). However, there were still existed significant differences ($P < 0.01$) in ZO-1, Occludin, Claudin-1, iFABP, and TFF3 protein expression levels in the small intestine between the DSS + M3G and the Control groups. Based on the above results, it can be seen that M3G has a significant recovery effect on the changes of small intestinal mucosa caused by DSS induced enteritis.

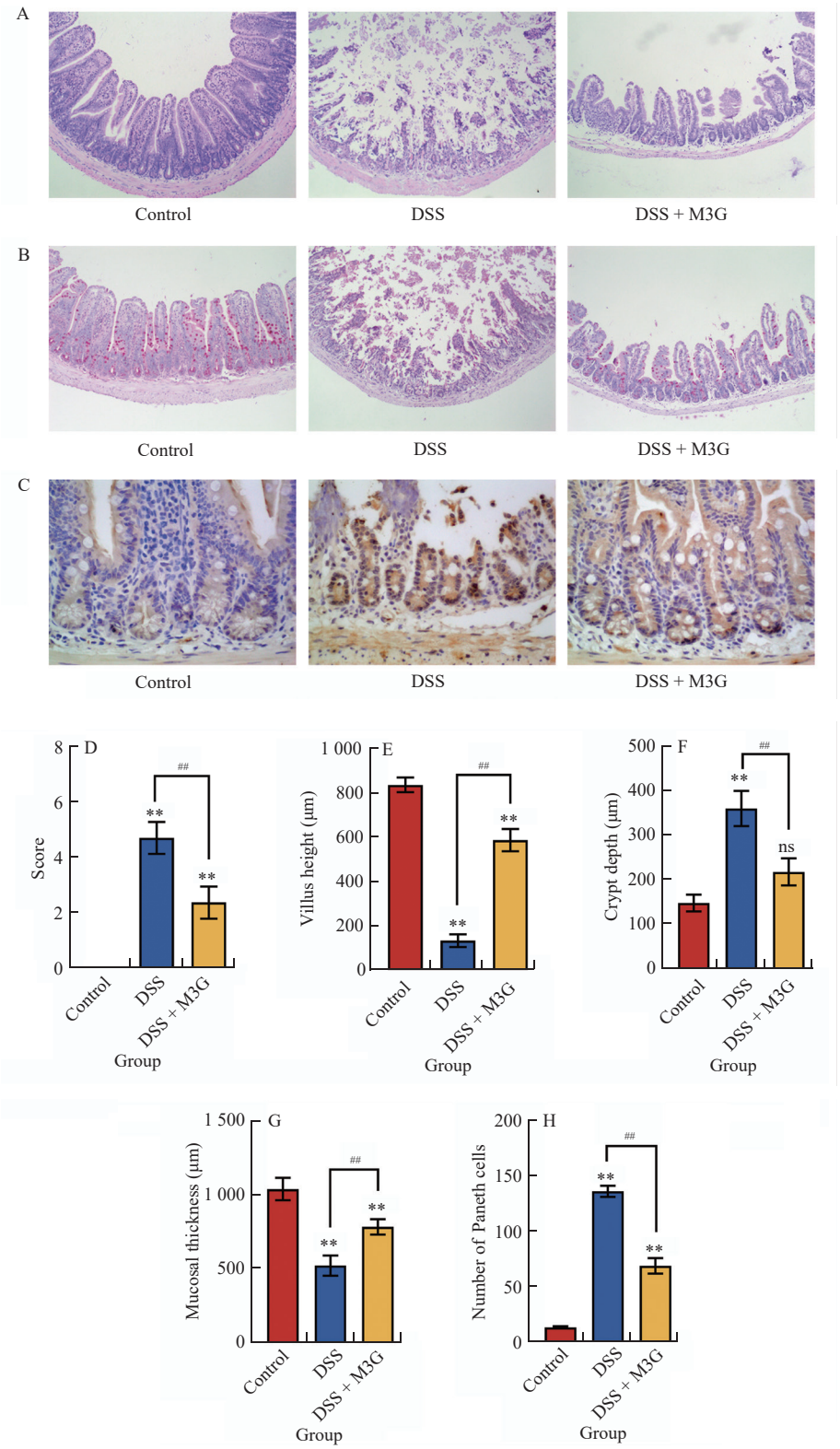

Fig. 2 Effect of M3G on pathological morphology of small intestinal tissue in mice. (A) HE staining of small intestine. Original magnifications: 100×. (B) PAS staining of small intestine. Original magnifications: 100×. (C) IHC. Original magnifications: 400 ×. (D) HE staining of small intestinal tissue damage score. 0 = normal mucosal villus morphology; 1 = the presence of Gruenhagen's space subcutaneous above the mucous membrane in the middle axis of the hair, often accompanied by capillary congestion; 2 = intestinal mucosa from lamina propria epithelial elevation and expansion of the subepithelial space; 3 = large intestinal mucosal epithelium was elevated, villi lodging to both sides, and part of the tip of villi was shed; 4 = shedding of villi and membrane propria, dilatation of exposed capillaries, and increased composition of membrane propria cells; 5 = membrana propria metamorphosis or digestion, bleeding or ulceration. (E) The villus height of small intestine. (F) The crypt depth of the small intestine. (G) The mucosal thickness of the small intestine. (H) Number of Paneth cells in small intestine. $n = 6$ per group. Results are expressed as the mean ± SD. ns = no significant, $^{**}P < 0.01$ compared with the Control group; $^{\#\#}P < 0.01$ compared with the DSS group.

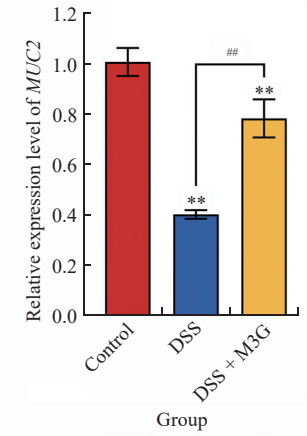

Fig. 3 mRNA expression level of *MUC2*. $n = 6$ per group. Results are expressed as the mean ± SD. $^{**}P < 0.01$ compared with the Control group; $^{\#\#}P < 0.01$ compared with the DSS group.

The serum levels of *D*-LA, LPS, and DAO in mice were shown in Fig. 5. Compared with the DSS group, M3G supplementation significantly decreased ($P < 0.01$) serum levels of *D*-LA, LPS, and DAO.

3.4 Effects of M3G on the small intestinal immune barrier of mice with DSS induced enteritis

The immune protein levels of serum and small intestinal mucosa of mice were determined. Compared with the Control group, the levels of IgA, IgM, and IgG in serum, and SIgA level in small intestine tissue were significantly decreased ($P < 0.01$) in the DSS group, but were significantly increased by M3G supplementation ($P < 0.01$) (Fig. 6). It showed that M3G can enhance the immune barrier of mice with DSS induced enteritis.

3.5 Effects of M3G on the small intestinal mucosal barrier function via Notch pathway

Notch1, NICD, DLL4, DLL1, and Hes1protein expression levels in the small intestine were measured. The expression levels of Notch1, NICD, DLL4, DLL1, and Hes1 in the DSS group were significantly higher ($P < 0.01$) than the Control group, and all of them exhibited a significantly reduced ($P < 0.01$) expression in the DSS + M3G group in comparison with the DSS group (Fig. 7). The results showed that M3G inhibited intestinal mucosal injury caused by DSS *via* suppressing Notch pathway, thereby improved intestinal mucosal barrier function.

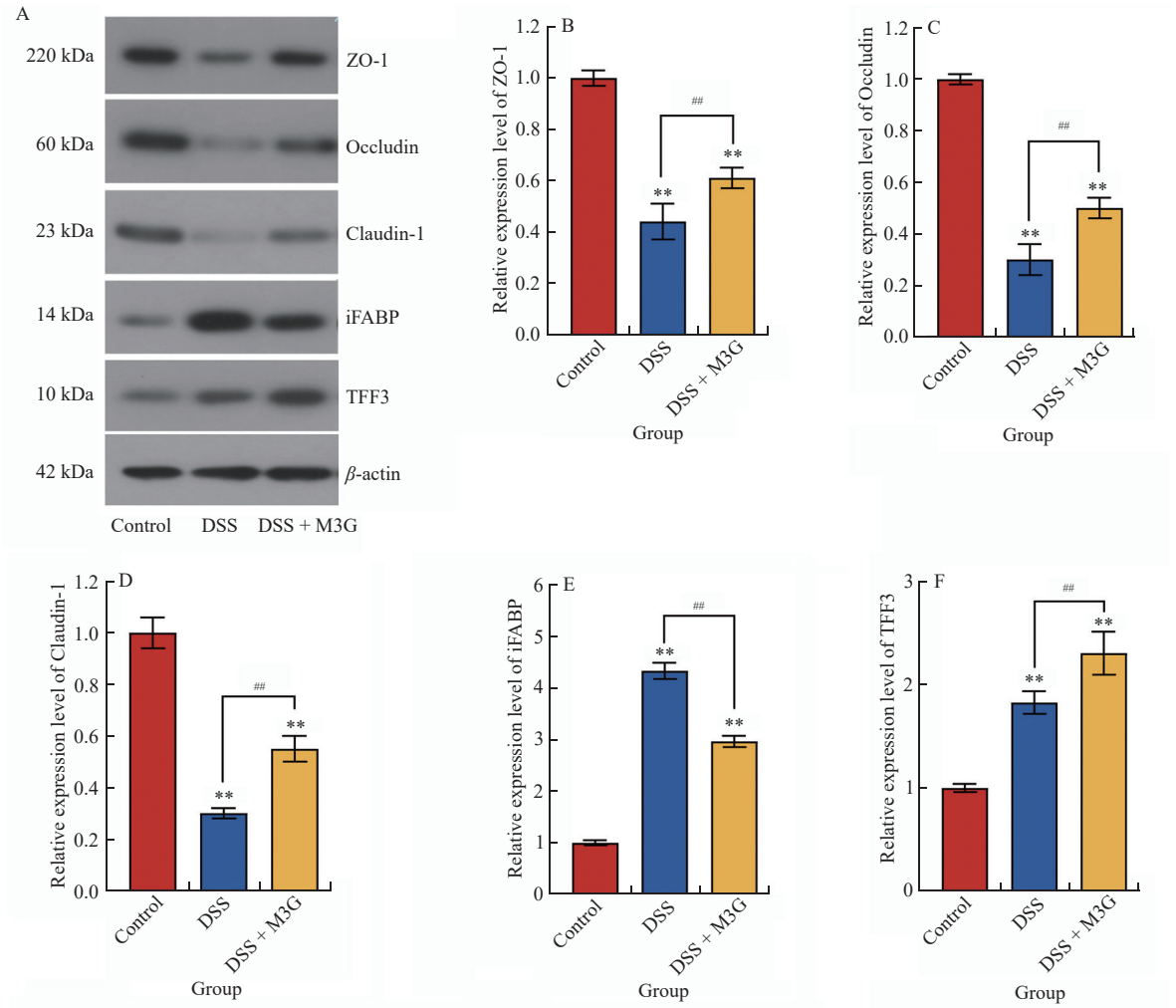

Fig. 4 Protein expression levels of ZO-1, Occludin, Claudin-1, iFABP, and TFF3 in small intestine mucosa. (A) Western blot assay results. (B) The protein expression level of ZO-1. (C) The protein expression level of Occludin. (D) The protein expression level of Claudin-1. (E) The protein expression level of iFABP. (F) The protein expression level of TFF3. $n = 6$ per group. Results are expressed as the mean ± SD. $^{**}P < 0.01$ compared with the Control group; $^{\#\#}P < 0.01$ compared with the DSS group.

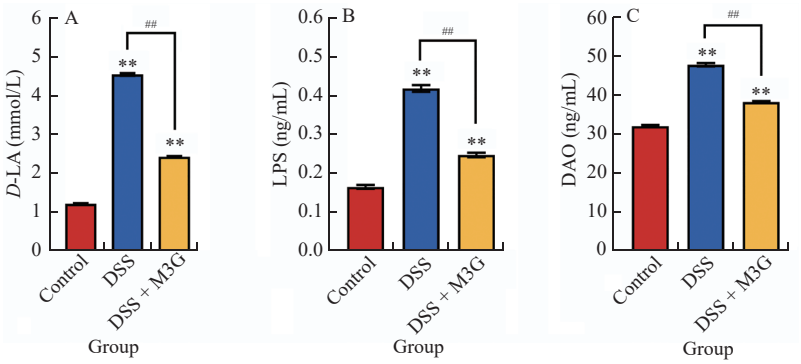

Fig. 5 Levels of *D*-LA, LPS and DAO in serum. (A) *D*-LA. (B) LPS. (C) DAO. $n = 6$ per group. Results are expressed as the mean ± SD. $^{**}P < 0.01$ compared with the Control group; $^{\#\#}P < 0.01$ compared with the DSS group.

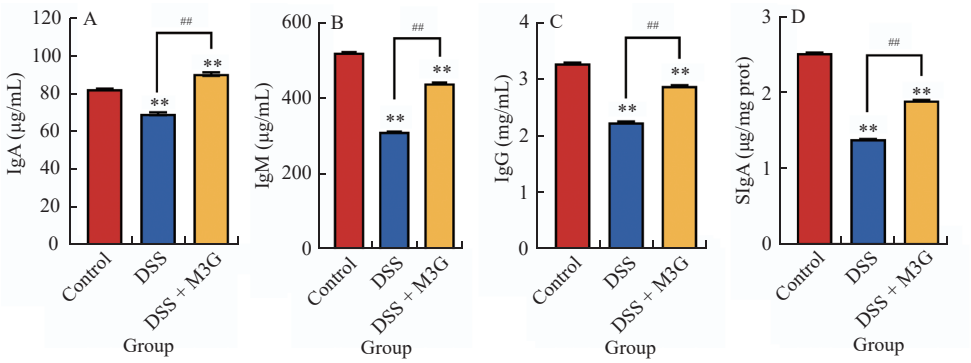

Fig. 6 Effect of M3G on immunoglobulin level in mice. (A) The level of IgA in serum. (B) The level of IgM in serum. (C) The level of IgG in serum. (D) The level of SIgA in small intestine. $n = 6$ per group. Results are expressed as the mean ± SD. $^{**}P < 0.01$ compared with the Control group; $^{\#\#}P < 0.01$ compared with the DSS group.

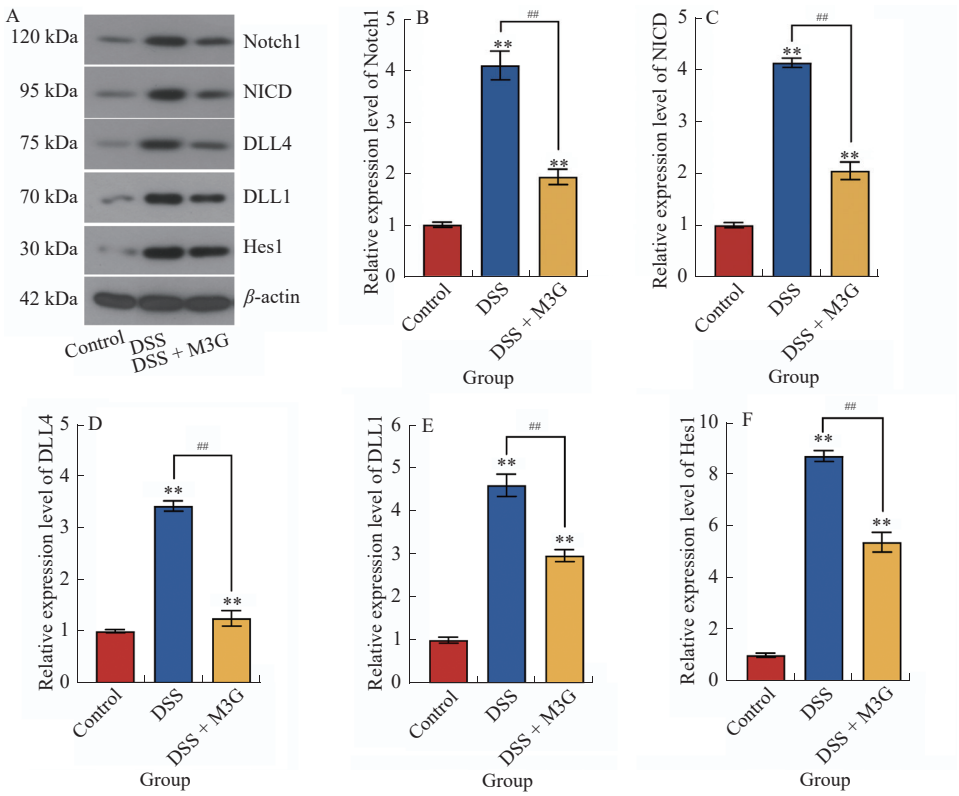

Fig. 7 Protein expression levels of Notch1, NICD, DLL4, DLL1, and Hes1. (A) Western blot assay results. (B) The protein expression level of Notch1. (C) The protein expression level of NICD. (D) The protein expression level of DLL4. (E) The protein expression level of DLL1. (F) The protein expression level of Hes1. $n = 6$ per group. Results are expressed as the mean ± SD. $^{**}P < 0.01$ compared with the Control group; $^{\#\#}P < 0.01$ compared with the DSS group.

4. Discussion

In this study, DSS was used to induce enteritis in mice, and M3G was given to enteritis mice to explore the effect of M3G on intestinal mucosal barrier function. The results showed that M3G could reduce the damage of intestinal mucosal tissue in mice by decreasing DAI score. In order to further research the effect of M3G on intestinal mucosal barrier function, the small intestinal mucosal mechanical barrier function factors and immune barrier factors were determined. The results exhibited that M3G could improve the expression levels of tight junction proteins such as ZO-1, Occludin, and Claudin-1, revealing that M3G could improve the integrity and stability of small intestinal mucosa in mice with DSS-induced enteritis. In order to explore how M3G affects mice with DSS-induced enteritis, the expression levels of Notch pathway related proteins were measured to explore the mechanism of the effect of M3G on intestinal mucosal barrier.

In previous studies, DSS-induced enteritis mouse model showed weight loss, bloody stool, loose stool, intestinal tissue ulcers, and propria membrane shedding[18-19]. In this study, the mice in the DSS group also showed the above symptoms, indicating that the enteritis mouse model was successfully constructed. After 7 days of M3G treatment on DSS-induced enteritis mice, the body weight of the mice increased significantly, the DAI decreased significantly, the ulcer area in the small intestine tissue decreased, and the villi were repaired compared with the DSS group. It showed that M3G has a significant improvement effect on small intestinal injury in enteritis mice, which was consistent with previous research[20].

The absence of MUC2 in the intestine leads to the breakdown of the epithelial barrier, resulting in the abnormal morphology of the intestinal mucosa, and contributing to the onset and persistence of inflammatoty bowel disease (IBD)[21]. In this study, M3G supplementation increased the expression level of MUC2, which was decreased in the DSS group, indicating that M3G can repair the small intestinal epithelial barrier. Cremonini et al.[22] have found that anthocyanins supplementation can mitigated high-fat-diet (HFD)-induced reduction in the level of MUC2 in the intestine of mice, consumption of a HFD caused intestinal barrier damage.

Tight junction proteins are important components of the intestinal mucosal barrier, among which Claudins, Occludin, Zona occludens are representative[23]. ZO-1 is a tight junction protein related to intestinal mucosal epithelial integrity and a marker of intestinal mechanical barrier[24]. Occludin plays an important role in the stability of tight junction and barrier function of the intestinal mucosa[25]. Claudin-1 is a complete membrane protein, which is part of a tight junction chain[26]. iFABP is an intestinal fatty acid binding protein and an early serological marker of intestinal mucosal injury[27]. In this study, M3G supplementation significantly increased the expression levels of ZO-1, Occludin, and Claudin-1 in DSS-induced enteritis mice. At the same time, iFABP expression level in the DSS + M3G group was significantly reduced, indicating that M3G could repair small intestinal mucosal damage. This was consistent with the results in the study found that mulberry anthocyanins repaired intestinal integrity by up-regulating the expression of tight junction proteins[28]. The trefoil factor family plays an important role in intestinal mucosal protection and repair[29]. Research has found that the expression level of TFF3 was up-regulated in mice with DSS-induced enteritis to accelerate mucosal repair and protect the intestinal mucosa from additional damage[30]. The similar results were found in this study, the expression level of TFF3 in the DSS group was increased. After administration of M3G, the expression of TFF3 was significantly higher than that of the DSS group, and the results showed that M3G could promote the expression level of TFF3, thereby improving the small intestinal barrier function.

Intestinal permeability is the permeability of intestinal mucosa to substances. When intestinal permeability increases, the barrier function of intestinal mucosa will be destroyed, resulting in harmful substances entering the blood circulation system, causing immune response and inflammation of the body[31]. At present, the *D*-LA, LPS and DAO levels in serum can be used as indicators to evaluate the intestinal permeability[32]. *D*-LA can be used to assess the degree of intestinal permeability, and LPS is a kind of lipopolysaccharide existing in the bacterial cell wall and cause the immune and inflammatory response of the body[31-32]. In this study, M3G treatment reduced the increase levels of *D*-LA, LPS, and DAO in serum caused by DSS, and reduced intestinal permeability.

Immune proteins play an important role in the human immune system, and IgA is an immunoglobulin in mucosal surface and secretions[33]. IgM plays a pivotal role in both humoral and mucosal immunity, and IgG can assess the level of antibodies in the body and the stability of the immune system[33-34]. SIgA serves as the first line of defense in protecting the intestinal epithelium from enteric toxins and pathogenic microorganisms[35]. These indicators were jointly used to evaluate the intestinal mucosal barrier. Liu et al.[36] found that IgA production in serum and SIgA in the small intestine were promoted in mice by oral administration with anthocyanins. As expected, M3G can enhance the small intestinal mucosal immune, thereby promoting intestinal mucosal barrier function in this study.

Notch signaling is a key pathway that constitutes the stem cell signaling network, and is involved in the development and homeostasis of a variety of tissues and organs[37-38], and is critical for the development and homeostasis of normal intestinal epithelium. DLL1 and DLL4 are typical high-affinity Notch ligands that bind to the Notch receptor and initiate signal transduction. Studies have shown that, DLL1 or DLL4 is a key physiological ligand that mediates the basic epithelial Notch signaling in the intestinal tract of mice[39]. The activated Notch signaling pathway inhibits the differentiation of goblet cells and their mucus secretion, resulting in disruption of the intestinal mucosal barrier[40]. When the Notch ligand binds to the receptors on the surface of adjacent cells, a shear reaction occurs to produce the active fragment NICD, which is released into the nucleus and interacts with transcription factors to activate the transcription of effector genes such as Hes1. Ultimately, Hes1 regulates the expression of other genes, and then modulates the development and progression of related diseases[41-42]. In this study, the combination of DLL1 and DLL4 ligands with Notch1 activated the Notch pathway, causing an increase in NICD and Hes1 experssion levels in the mice in the DSS group. After M3G supplementation, the expression levels of Notch pathway related proteins were decreased, indicating that Notch pathway was inhibited. Therefore, it can be speculated that M3G improved small intestinal barrier function by inhibiting Notch pathway to reduce intestinal permeability and enhancing the immune function, stability, and integrity of the small intestinal mucosa.

5. Conclusion

The effect of M3G on the small intestinal mucosal barrier function was investigated in this study. The results have shown that

the improvement effect of M3G on the small intestinal mucosal barrier function may be mainly achieved by improving the permeability, stability, and integrity of the small intestinal mucosa, through regulating Notch pathway. In conclusion, M3G has a positive effect on the small intestinal mucosal barrier function, and M3G may be a potential functional component to prevent or reduce intestinal mucosal barrier function impairment in the future.

Conflict of interest

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

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