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Effects of Green Tea and Java Pepper Mixture on Gut Microbiome and Colonic MicroRNA-221/222 in Mice with Dextran Sulfate Sodium-Induced Colitis

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ABSTRACT: In this study, we aimed to investigate the regulatory effects of a green tea and java pepper mixture (GTP) on the gut microbiome and microRNA (miR)-221/222 expression in mice with dextran sulfate sodium (DSS)-induced colitis. Male C57BL/6J mice were divided into four groups: DSS−, DSS+, GTP50, and GTP100. In the GTP50 and GTP100 groups, GTP was orally administered to mice at doses of 50 and 100 mg/kg body weight, respectively, every day for 2 weeks, and colitis was induced in the DSS+, GTP50, and GTP100 groups by adding 3% DSS to their drinking water for 1 week. GTP was found to mitigate the severity of inflammation and the damage to goblet cells caused by DSS-induced colitis. The results showed that compared with the DSS− group, the relative abundance of *Bacteroidetes* was increased and that of *Proteobacteria* and *Candidatus Melainabacteria* was decreased in the GTP100 group. The ratio of *Firmicutes* to *Bacteroidetes* was also reduced in the GTP100 group. However, GTP administration did not modulate the microbial diversity. GTP administration upregulated the mRNA and protein levels of occludin and zonula occludens 1. In addition, GTP effectively downregulated the expression of miR-221 and miR-222. Overall, GTP altered the gut microbiota composition and downregulated colonic miR-221/222 expression in mice with DSS-induced colitis.

Keywords: colitis, microbiota, microRNAs, piper, tea

INTRODUCTION

Ulcerative colitis (UC) is a type of inflammatory bowel disease (IBD) characterized by persistent inflammation of the colon and rectum (Ungaro et al., 2017). The incidence of UC is increasing globally and is positively correlated with the risk of developing colorectal cancer (Xiong et al., 2022). Microbiota dysbiosis and abnormalities in intestinal barrier function have been shown to play significant pathogenic roles in UC onset (Ungaro et al., 2017).

The gut microbiota includes bacteria, viruses, and fungi, that reside within the digestive tract of organisms and regulate metabolism and gut immune homeostasis (Hou et al., 2022). The gut microbiome is predominantly composed of the phyla *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*, with *Bacteroidetes* and *Firmicutes* accounting for the majority (Hou et al., 2022). Microbiome dysbiosis is the abnormal composition of the major bacterial phyla in the gut and is a key factor in the progression of IBD (Hooper et al., 2012). In particular, abnormal abundance of *Proteobacteria* is a hallmark of dysbiosis in patients with IBD and mice with dextran sulfate sodium (DSS)-induced colitis (Shin et al., 2015; Munyaka et al., 2016).

The intestinal barrier plays a crucial role in preventing the penetration of substances into the lumen and maintaining intestinal homeostasis (McCole, 2014). Moreover, it consists of epithelial cells and tight junctions (TJs), which regulate cell adhesion and barrier permeability (Zihni et al., 2016). TJs are composed of the tetraspan membrane proteins occludin (OCLN) and adaptor protein zonula occludens 1 (TJP1), and their interaction determines the integrity of the barrier (Lee et al., 2023). The downregulation of OCLN and TJP1 expression in the intestinal barrier increases intestinal permeability and promotes the pathogenesis of DSS-induced colitis (Kuo et al., 2019).

MicroRNAs (miRs) are small (18−22-nucleotide long) non-coding RNAs that bind to the complementary sequences of target mRNA and regulate gene expression

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(Ravegnini et al., 2019). miRs influence various developmental and physiological processes, including inflammation, metabolism, and cell differentiation (Vienberg et al., 2017; Jiang et al., 2022). Furthermore, miRs regulate TJP expression, thereby affecting the epithelial barrier (Cichon et al., 2014). miR-21, miR-146a, miR-155, and miR-221 contribute to intestinal epithelial barrier dysfunction by damaging junctional complexes (Hübenthal et al., 2019). In human colorectal cancer (CRC) cells, miR-221 and miR-222 specifically form a positive feedback loop, leading to the activation of the nuclear factor kappa B (NF-κB) and signal transducer and activator of transcription 3 pathways (Liu et al., 2014). In patients with cancer, high expression of miR-221 and miR-222 is correlated with a lower overall survival rate (Ravegnini et al., 2019). Therefore, miR-221 and miR-222 may serve as target biomarkers for colitis-related diseases by influencing the intestinal epithelial barrier function.

Green tea (*Camellia sinensis*) is consumed as a beverage worldwide. It is rich in catechins, which include bioactive polyphenols, such as epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epicatechin (EC). Green tea catechins are known to have an anti-inflammatory effect (Truong and Jeong, 2022) and can improve mucosal barrier function (Wu et al., 2021) and gut microbiota dysbiosis (Pérez-Burillo et al., 2021).

Java pepper (*Piper retrofractum*), belonging to the Piperaceae family, is mainly cultivated in Asia for use as a spice (Amaliyah et al., 2020). The major component in java pepper is piperine, a natural alkaloid (Derosa et al., 2016). Piperine has anti-inflammatory effects (Hu et al., 2015) and modulates the components of the intestinal microbiota in mice with DSS-induced colitis (Hu et al., 2023). The anti-inflammatory effect of EGCG is reportedly enhanced when consumed with piperine in DSS-induced colitis (Brückner et al., 2012), as piperine increases the bioavailability of EGCG (Lambert et al., 2004). In our earlier study, we found that a green tea and java pepper mixture (GTP) alleviated colitis through the regulation of miR-21 and NF-κB activity (Lee et al., 2022). GTP has also been reported to improve intestinal health by protecting TJs from secondary bile acid damage in mice with DSS- and deoxycholic acid (DCA)-induced colitis (Choi et al., 2023). However, the effects of GTP on the regulation of gut microbiota and colonic miR-221/222 expression in mice with DSS-induced colitis have not yet been elucidated. Therefore, we hypothesized that GTP alleviates gut microbiota dysbiosis and downregulates colonic miR-221/222 expression in mice with DSS-induced colitis.

MATERIALS AND METHODS

Preparation of green tea and java pepper mixture (GTP)

GTP, provided by Newtree, was a mixture of green tea extract (Naturex) and java pepper extracted with 70% ethanol in a ratio of 99:1 (w/w), as described in our previous study (Lee et al., 2022). The quantities of catechins and piperine in the GTP were analyzed using a Nanospace SI-2 high-performance liquid chromatography system (Shiseido Co.), as previously described (Lee et al., 2022). The mean±standard error (SE) quantity of total catechins (EGCG, ECG, EGC, and EC) in GTP from six replicated analyses was 791.99±15.50 mg/g (522.04±9.84, 111.22±2.06, 105.67±2.51, and 53.06±1.09 mg/g, respectively). The content of piperine in GTP was 2.05±0.13 mg/g.

Animals and experimental design

Six-week-old C57BL/6J male mice were purchased from Doo Yeol Biotech Co. and housed individually in cages. They were acclimated to a water and chow diet (2018S Teklad rodent diet; Envigo) under controlled conditions at 22°C±2°C, 55%±5% humidity, and a 12-h light/dark cycle for 1 week. Mice were randomly divided into four groups (6 animals/group), as described in our previous study (Lee et al., 2022): DSS− (control), DSS+ (3% DSS), GTP50 (DSS+50 mg/kg bw GTP), and GTP100 (DSS+100 mg/kg bw GTP). The DSS+, GTP50, and GTP100 groups were allowed to freely drink water with added DSS (molecular weight=36,000 to 50,000 Da; MP Biomedicals) from week 1 to induce colitis, as shown in Fig. 1. The GTP50 and GTP100 groups were orally administered GTP at doses of 50 and 100 mg/kg bw, respectively, once a day for 2 weeks. Feces from the DSS−, DSS+, and GTP100 groups were collected at week 2 and stored at −30°C until use. All animals were fasted for 12 h and then sacrificed using a mixture of Zoletil 50 (50 mg/kg, Virbac Corporation) and Rompun (10 mg/kg,

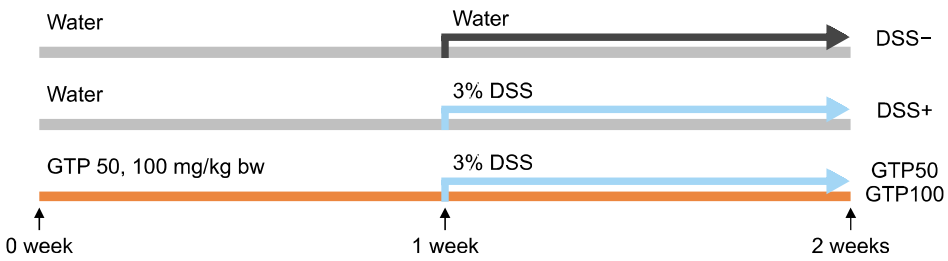

Fig. 1. Schematic diagram of the experimental design. GTP, green tea and java pepper mixture; DSS, dextran sulfate sodium; bw, body weight.

Bayer Korea). The colon tissues were fixed in 10% formalin overnight or frozen in liquid nitrogen and stored at −80°C until analysis. Animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Ewha Womans University (IACUC No. 20-013).

Masson's trichrome (M/T) and periodic acid-Schiff (PAS) staining

The fixed colon tissues were embedded in paraffin blocks and then cut into 5-μm-thick slices. To observe histological changes, paraffin-embedded tissue samples were stained with M/T (ScyTek Laboratories) according to the manufacturer's instructions. Other slides were stained with PAS (ScyTek Laboratories) according to the manufacturer's instructions to identify goblet cells. The stained slides were observed at ×400 magnification under an Olympus microscope. Histological score was obtained by summing the scores for the severity of inflammation (0, none; 1, mild; 2, moderate; 3, severe), extent of inflammation (1, mucosa; 2, mucosa and submucosa; 3, transmural), and degree of crypt damage (0, none; 1, 1/3 crypts damaged; 2, 2/3 crypts damaged; 3, crypts lost but surface and epithelium present; 4, crypt and surface epithelium lost) (Cooper et al., 1993) in M/T-stained colon tissue. Goblet cell density was assessed by quantifying the proportion of PAS-positive cells in PAS-stained colon tissue. The percentage of stained area was measured using ImageJ 1.51k software (United States National Institutes of Health).

Microbiota analysis

DNA was extracted from the feces of mice using a DNeasy power soil kit (Qiagen) and quantified using Quant-IT PicoGreen (Invitrogen). DNA was amplified using a universal primer pair with an Illumina adapter overhang sequence (V3 forward primer, 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3' and V4 reverse primer, 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATCC-3'). PCR products were purified and quantified using the KAPA Library Quantification Kit (Kapa Biosystems Inc.) for the Illumina sequencing platforms. Quantification was conducted using TapeStation D1000 ScreenTape (Agilent Technologies). Subsequently, paired-end sequencing was performed by Macrogen using the MiSeq™ platform (Illumina) to cluster operational taxonomic units (OTUs) and conduct diversity analysis.

Real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA from colon tissue was extracted using TRIzol reagent (GeneAll Biotechnology) and reverse transcribed into cDNA using M-MLV reverse transcriptase (Bioneer) for mRNA analysis (Jung et al., 2021) and using a cDNA synthesis kit with poly (A) polymerase tailing (ABM Inc.) for miR analysis. RT-qPCR was performed on a Rotor-Gene Q thermocycler (Qiagen) by adding Greenstar qPCR Master Mix (Bioneer) to the cDNA. The primer sequences were designed using the Primer3 program (Rozen and Skaletsky, 2000) and were as follows: OCLN, 5'-CCT CCA CCC CCA TCT GAC TA-3' (forward) and 5'-TCG CTT GCC ATT CAC TTT GC-3' (reverse); TJP1, 5'-CAA GCC AGC AGA GAC CTT GA-3' (forward) and 5'-CGA GGT TGG TAG GGC TGT TT-3' (reverse); β-actin, 5'-GGA CCT GAC AGA CTA CCT CA-3' (forward) and 5'-GTT GCC AAT AGT GAT GAC CT-3' (reverse). mRNA expression levels were normalized using β-actin as the internal control. Primers specific for miR-221, miR-222, and U6 were purchased from ABM Inc., and U6 expression was used as a control to normalize the expression of the miRs. For RT-qPCR relative quantification, we used the $2^{-\Delta\Delta Ct}$ method (Lee and Kim, 2018), and the results were expressed as fold changes in the DSS+ group.

Immunohistochemistry (IHC) analysis

The fixed slides were boiled in Tris-EDTA buffer (pH 8.0) for 40 minutes for antigen retrieval of OCLN and TJP1. Peroxide blocking solution (200 μL) was added to the slides, and the slides were incubated for 15 min at room temperature. After blocking, the slides were incubated at 4°C overnight with primary antibodies against OCLN (ab216327, Abcam) and TJP1 (40-2200, Invitrogen), each diluted 1:200, and then incubated with a Polink-2 HRP Plus Rabbit DAB Detection System (D39-18, Golden Bridge International). The incubated slides were developed using a DAB Chromogen/Substrate Kit (High Contrast; Scytek Laboratories) and counterstained with hematoxylin. Digital images were acquired at ×400 magnification using an Olympus microscope. The OCLN and TJP1 staining intensities were measured using ImageJ and expressed as fold changes in the DSS+ group.

Statistical analysis

Statistical analysis was performed using SPSS software version 25 (IBM Corp.). Values were expressed as mean± standard error from six mice per group. Statistical differences were analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc test, and a *P*-value of <0.05 was considered statistically significant.

RESULTS

Effects of GTP on histological damage in the colon

We evaluated histopathological changes in the tissues of

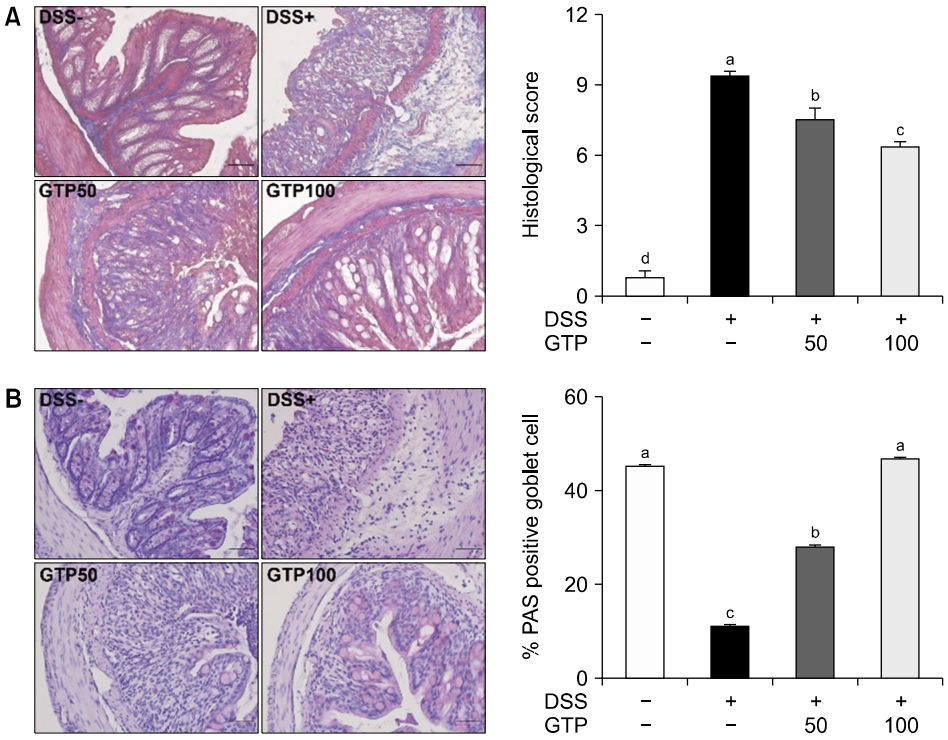

Fig. 2. Effects of GTP on colon tissue histology. (A) Representative images of Masson's trichrome-stained colon tissues and histological scores. (B) PAS-stained colon tissues and the percentage of PAS-positive goblet cells. Images are at ×400 magnification, scale bar=50 μm. Values are presented as mean± standard error (n=6). Different letters (a–d) indicate significant differences (P<0.05) among groups, as determined using one-way ANOVA with Tukey's multiple comparison tests. DSS, dextran sulfate sodium; GTP, green tea and java pepper mixture; PAS, periodic acid-Schiff reaction.

mice with DSS-induced colitis using M/T and PAS staining. A greater increase in the histological scores and a more advanced loss of goblet cells were observed in the DSS+ group compared with those in the DSS− group (P<0.05, Fig. 2A and 2B). The histological scores in the GTP50 and GTP100 groups were reduced by 19.64% and 32.14%, respectively, compared with that in the DSS+ group (P<0.05, Fig. 2A). The GTP50 and GTP100 groups showed 2.56- and 4.27-fold more goblet cells, respectively, compared with the DSS+ group (P<0.05, Fig. 2B).

Effects of GTP on gut microbial diversity

The effects of GTP on microbial diversity are shown in Fig. 3. Alpha diversity (OTUs, Choa1, Shannon, and inverse Simpson indices), which indicates the richness of species within a habitat, was significantly lower in the DSS+ group than in the DSS− group (P<0.05); however, no significant difference was observed in the alpha diversity between the DSS+ and GTP100 groups (Fig. 3A–D). The non-metric multidimensional scaling plots showed that beta diversity, which indicates the differences in species diversity between ecosystems, was not different between the DSS+ and GTP100 groups (Fig. 3E).

Effects of GTP on gut microbiome composition

The composition of the gut microbiome at the phylum level is shown in Fig. 4. *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*, which are the major components of the intestinal microbiome, were the most abundant phyla in the fecal microbial communities of the DSS− (55.16%, 31.11%, and 1.82%, respectively), DSS+ (35.63%, 45.38%, and 13.93%, respectively), and GTP100 (46.91%, 43.43%, and 4.39%, respectively) groups (Fig. 4A). The relative abundances of *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Tenericutes*, *Deferribacteres*, *Candidatus Melainabacteria*, and *Actinobacteria* were significantly changed by DSS treatment compared with the DSS− group (P<0.05, Fig. 4B, 4C, 4E–4I). Among the bacterial phyla, the relative abundance of *Bacteroidetes* was increased 1.29-fold in the GTP100 group compared with the DSS+ group (P<0.05, Fig. 4B). In addition, GTP reduced the relative abundance of *Proteobacteria* and *Candidatus Melainabacteria* by 36.56% and 41.28%, respectively, compared to the DSS+ group (P<0.05, Fig. 4E and 4H). In the DSS-induced colitis mice, the ratio of *Firmicutes* to *Bacteroidetes* was 2.35 times higher than that in the DSS− group. However, GTP treatment resulted in a 69.32% reduction in the ratio of *Firmicutes* to *Bacteroidetes* compared to the DSS+ group (P<0.05, Fig. 4D).

Effects of GTP on tight junctions (TJs) in the colon

The effects of GTP on OCLN and TJP1 levels were assessed by IHC and qRT-PCR. The mRNA expression of OCLN and TJP1 in the GTP100 group was upregulated 1.58- and 1.35-fold, respectively, compared with the DSS+ group (P<0.05, Fig. 5A and 5B). The densities of OCLN and TJP1 observed under IHC were lower in the DSS+ group than in the DSS− group (P<0.05); however, they were increased in the GTP50 (2.90- and 2.08-fold, respectively) and GTP100 (8.81- and 3.21-fold, respectively) groups compared with the DSS+ group (P< 0.05, Fig. 5C and 5D).

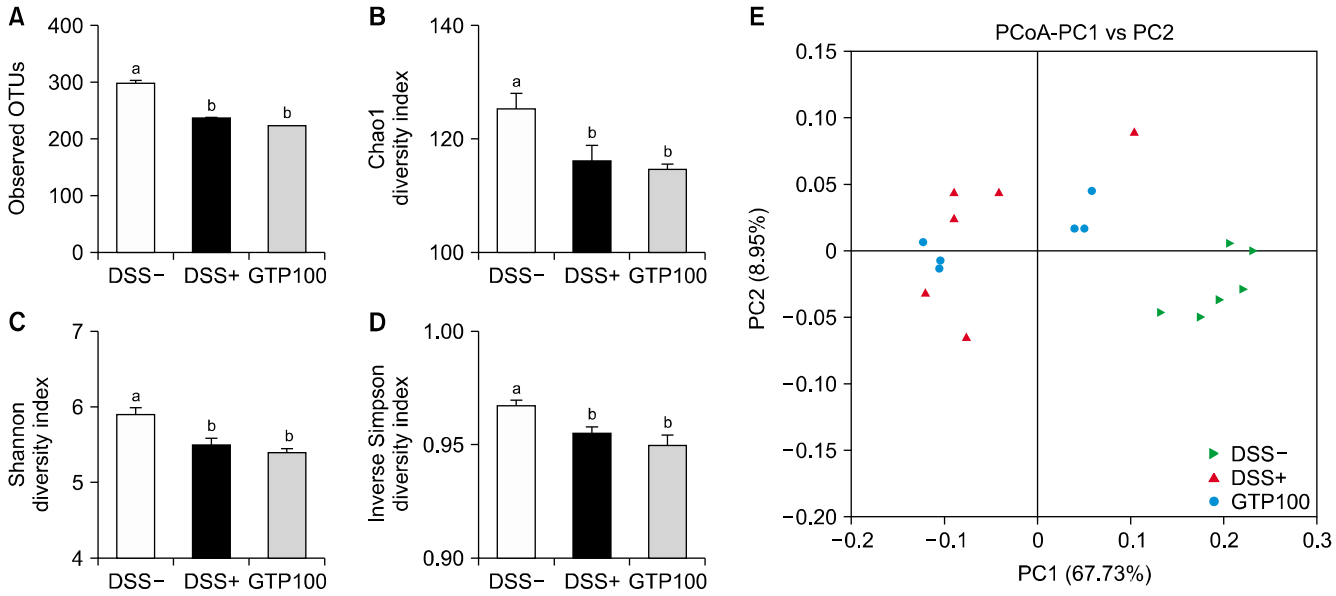

Fig. 3. Effect of GTP on gut microbial diversity. Observed OTUs (A), Chao1 index (B), Shannon index (C), Inverse Simpson index (D), and non-metric multidimensional scaling plot (E). Values are expressed as mean±standard error (n=6). Different letters (a, b) indicate significant differences (P<0.05) among groups, as determined using one-way ANOVA with Tukey's multiple comparison tests. DSS, dextran sulfate sodium; GTP, green tea and java pepper mixture; OTU, operational taxonomic unit; PCoA, principal coordinate analysis; PC, principal component.

Effects of GTP on colonic miR-221/222 expression

We confirmed whether GTP regulates the expression of miR-221 and miR-222. The expression of miR-221 and miR-222 was 2.28- and 1.70-fold higher, respectively, in the DSS+ group than in the DSS− group (P<0.05); however, the levels of miR-221 and miR-222 in the GTP50 (29.29% and 44.10%, respectively) and GTP100 (12.17% and 51.21%, respectively) groups were down-regulated compared to the DSS+ group (P<0.05, Fig. 6A and 6B).

DISCUSSION

UC is characterized by damage to the epithelial monolayer lining the colon, leading to weight loss, shortening of colon length, loosening of intestinal TJs, and microbiota dysbiosis (Chassaing et al., 2014). In this study, we investigated whether GTP alters the microbiome community and regulates the expression of colonic miR-221/222. GTP supplementation for 2 weeks reduced the DSS-induced increase in histological scores, which indicate the severity of crypt damage and inflammation in the colon. GTP also alleviated the DSS-induced reduction in the number of goblet cells, which secrete mucins that protect epithelial cells from luminal bacteria. In a previous study, green tea was shown to alleviate colon damage and restore the intestinal mucosa in mice with DSS-induced colitis (Liu et al., 2023). In another study, EGCG improved crypt damage, inflammatory cell infiltration, and goblet cell reduction in mice with DSS-induced colitis (Du et al., 2019). Furthermore, piperine has been found to decrease the histological score in mice with trinitrobenzene sulphonic acid-induced colitis (Guo et al., 2020). In our previous study, GTP improved the symptoms of colitis, including weight loss, colon shortening, and histological score (Lee et al., 2022). The results of the present study suggest that GTP might protect against DSS-induced intestinal mucosal damage and reduction of goblet cells.

IBD has been reported to correlate with gut microbiota dysbiosis symptoms, such as reduced bacterial diversity and increased bacterial instability (Larabi et al., 2020). DSS treatment exacerbates colitis by altering the gut microbiota (Chassaing et al., 2014). In addition, DSS-induced colitis decreases the relative abundance of *Bacteroidetes* (Nagalingam et al., 2011) and increases that of *Proteobacteria* (Cao et al., 2021) in the gut microbiota. *Bacteroidetes* produce short-chain fatty acids, which help maintain intestinal immune homeostasis (Yee et al., 2023). Meanwhile, *Proteobacteria* may reflect the unstable structure of the intestinal microbial community (Shin et al., 2015). We investigated whether GTP could regulate the fecal microbiome of mice with DSS-induced colitis. The results showed that GTP had no effect on alpha or beta diversity, but it did change the microbial composition (*Bacteroidetes*, *Proteobacteria*, and *Candidatus Melainabacteria*) and the ratio of *Firmicutes* to *Bacteroidetes*. In a previous study, oral EGCG administration had no significant impact on the alpha diversity in the gut of mice with DSS-induced colitis; however, the fecal microbial composition exhibited trends consistent with our find-

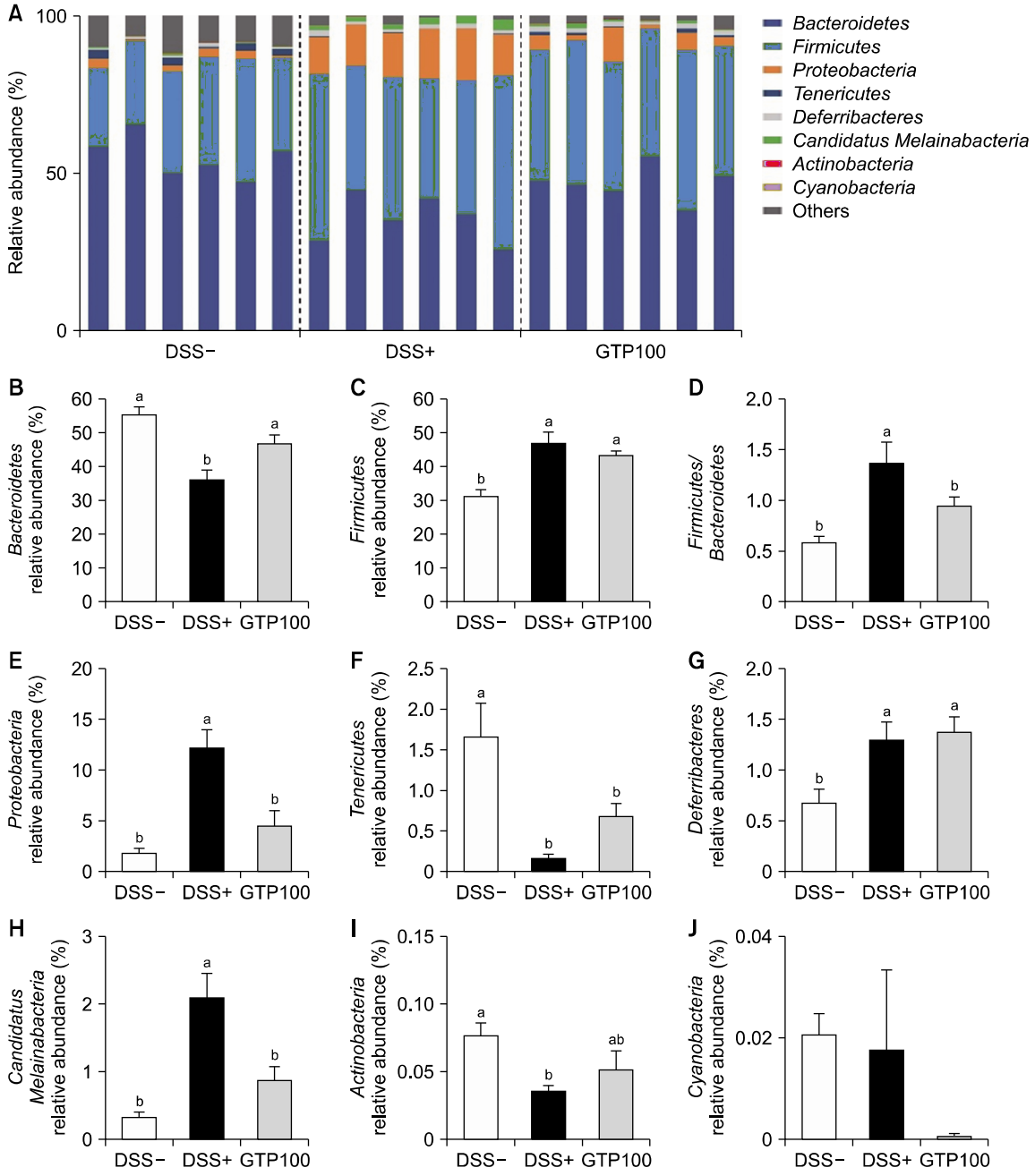

Fig. 4. Effect of GTP on gut microbial composition. (A) Relative abundance of fecal microbiota at the phylum level. Phylum-level taxonomy is presented as the percentage of total sequences. The relative abundance of *Bacteroidetes* (B), *Firmicutes* (C), *Proteobacteria* (E), *Tenericutes* (F), *Deferribacteres* (G), *Candidatus Melainabacteria* (H), *Actinobacteria* (I), and *Cyanobacteria* (J). (D) Ratio of *Firmicutes* to *Bacteroidetes* at the phylum level. Values are expressed as mean±standard error (n=6). Different letters (a, b) indicate significant differences ($P<0.05$) among groups, as determined using one-way ANOVA with Tukey's multiple comparison tests. DSS, dextran sulfate sodium; GTP, green tea and java pepper mixture.

ings (Wu et al., 2021). Taken together, our findings suggest that GTP affects the gut microbiota composition, but not its diversity, in DSS-induced colitis.

The integrity of the intestinal barrier is determined by the interaction of TJPs, which play an important role in intestinal epithelial barrier function and intestinal permeability (Gieryńska et al., 2022). OCLN forms two extracellular loops and one intracellular loop and has the potential to act as a regulator of barrier function (Kuo et al., 2022). TJP1 is a scaffolding membrane protein that has multiple domains specialized for protein interactions and binds claudins and OCLN (Kuo et al., 2022). Downregulation of OCLN and TJP1 expression leads to structural alterations in the junctional complexes, resulting in increased intestinal permeability and disruption of the intestinal barrier (Lee et al., 2018). In this study, the mRNA expression and protein levels of OCLN and TJP1 were upregulated by GTP. Similar to our results, GTP treatment increased mRNA expression of TJP1, OCLN, claudin-1, claudin-3, and claudin-4 in DSS+DCA-induced

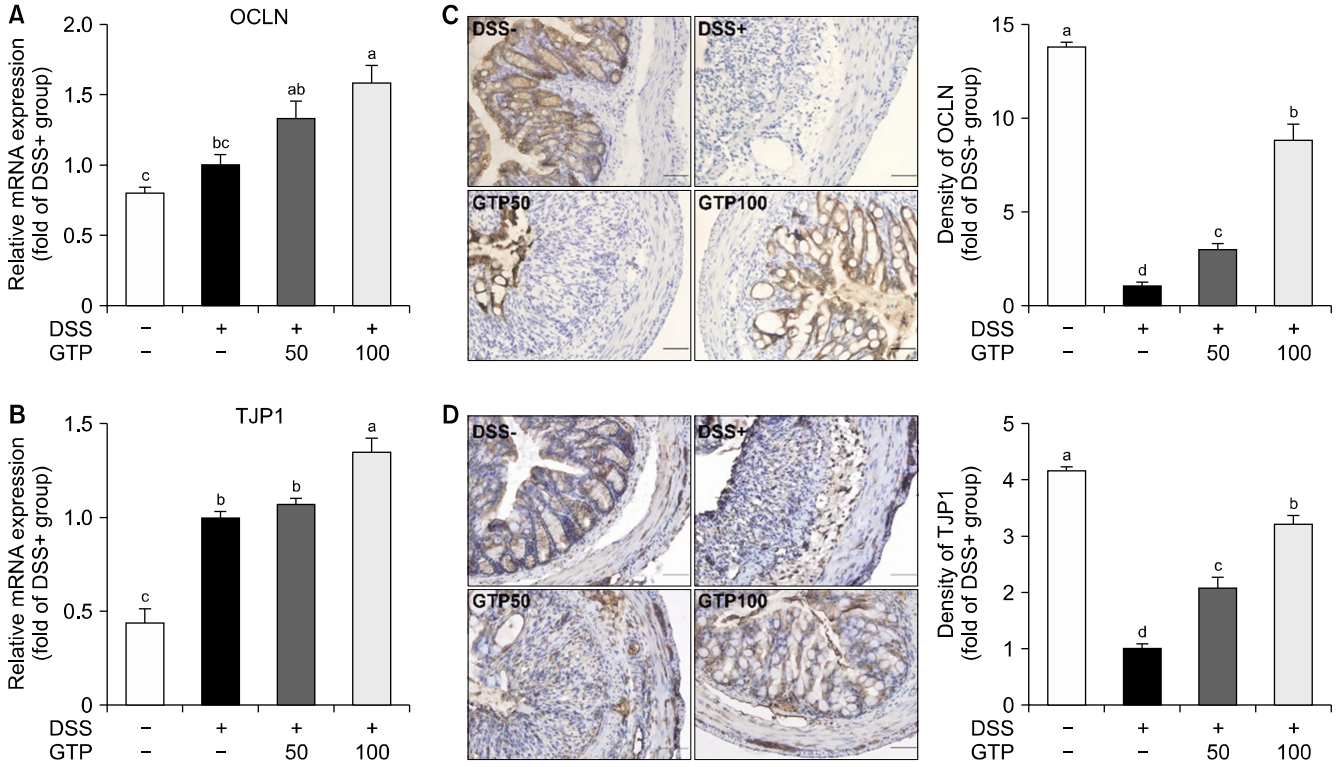

Fig. 5. Effects of GTP on tight junction protein regulation. The mRNA levels of OCLN (A) and TJP1 (B). (C) Representative images of colon tissues stained for OCLN by IHC and the density of OCLN. (D) Representative images of colon tissues stained for TJP1 by IHC and the density of TJP1. Images are at ×400 magnification, scale bar=50 μm. Values are presented as mean±standard error (n=6). Different letters (a-d) indicate significant differences (P<0.05) among groups, as determined using one-way ANOVA with Tukey's multiple comparison tests. DSS, dextran sulfate sodium; GTP, green tea and java pepper mixture; OCLN, occludin; IHC, immunohistochemistry; TJP1, zonula occludens 1.

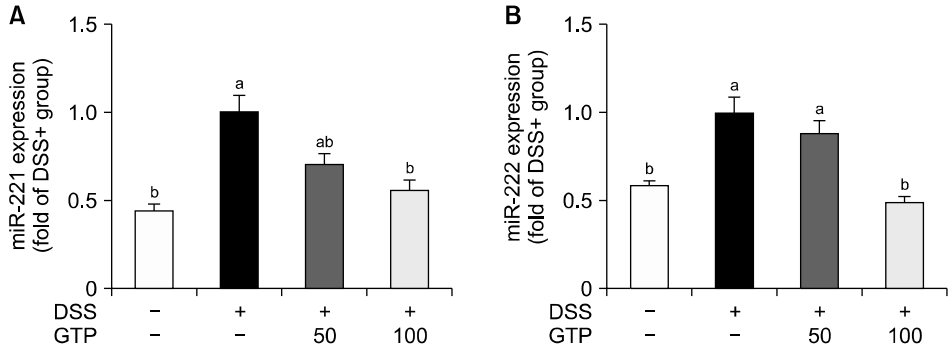

Fig. 6. Effects of GTP on the expression of miR-221 (A) and miR-222 (B). Values are presented as mean± standard error (n=6). Different letters (a, b) indicate significant differences (P<0.05) among groups, as determined using one-way ANOVA with Tukey's multiple comparison tests. DSS, dextran sulfate sodium; GTP, green tea and java pepper mixture; miR, microRNA.

colitis mice in a previous study (Choi et al., 2023). Treatment with EGCG and piperine, respectively, has previously been shown to upregulate the expression of TJP1 and OCLN in colitis animals (Guo et al., 2020; Diwan and Sharma, 2022). These observations suggest that GTP could potentially prevent DSS-induced intestinal leaks by upregulating OCLN and TJP1 expression.

Since miRs regulate the expression of genes involved in various metabolic processes at the post-transcriptional level, many studies are being conducted to determine the role of miRs in the diagnosis and prognosis of diseases (Ravegnini et al., 2019). miR-221/222 expression shows a positive association with the progression of tumor nodal metastasis stage and local invasion in CRC cells (Sun et al., 2011; Song et al., 2017). Furthermore, miR-221 contributes to the disruption of the junctional complexes by damaging TJP1 (Hübenthal et al., 2019). In a previous study, EGCG treatment was reported to downregulate miR-221/222 expression in anaplastic thyroid carcinoma cells (Allegri et al., 2018) and triple-negative breast cancer cells (Lewis et al., 2018). In this study, we found that GTP supplementation downregulated the expression of colonic miR-221/222, which was increased due to DSS-induced colitis. These results suggest that GTP might inhibit the expression of miR-221/222 in DSS-induced colitis mice. In conclusion, our study demonstrated that GTP altered the composition of the gut microbiota and downregulated colonic miR-221/222 ex-

pression in a DSS-induced colitis model.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: YK. Analysis and interpretation: JL, MSL. Data collection: JL. Writing the article: JL. Critical revision of the article: JL, YK. Final approval of the article: JL, MSL, YK. Statistical analysis: JL, MSL. Obtained funding: YK. Overall responsibility: YK.

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