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Research Article

# Korean Red Ginseng alleviates dextran sodium sulfate-induced colitis through gut microbiota modulation in mice

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ABSTRACT

*Background:* There is a growing interest in understanding the association between the gut microbiota and inflammatory bowel disease (IBD). Natural compounds, such as Korean Red Ginseng (KRG), show promise for IBD treatment because of their ability to influence gut microbiota. This study explored the effects of KRG on gut microbiota modulation and subsequent intestinal epithelial cell regeneration in an experimental colitis model.
*Method:* Using a mouse model of colitis induced by 2 % dextran sodium sulfate, the study administered 200 or 400 mg/kg/day of KRG to evaluate its biological effects. Colitis symptoms were assessed through body weight, disease activity index, colon length, and histological analysis. The microbial composition in the fecal was determined using 16S rRNA sequencing. To evaluate regeneration signals in the colon, western blotting and immunohistochemistry assays were conducted.
*Result:* Administration of KRG effectively mitigated colitis symptoms in mice, as indicated by histological examination showing alleviated epithelial damage and inflammation, along with increased mucus production. Microbiota analysis showed that KRG significantly altered microbial diversity, favoring beneficial taxa and suppressing harmful taxa. Moreover, ameliorated β-catenin/transcription factor-4 protein expression, a key signal associated with epithelial cell regeneration, was observed in the KRG treated groups, accompanied by improved intestinal linings.
*Conclusion:* These findings suggest that KRG exerts biological effects in colitis by modulating gut microbiota and creating a favorable intestinal environment, thereby reducing regenerative signals. Further research is warranted to elucidate the cellular and molecular mechanisms underlying the interaction of KRG with gut microbiota and pave the way for effective IBD therapies.

## 1. Introduction

Inflammatory bowel disease (IBD) is a chronic and recurrent disease characterized by persistent and widespread inflammation affecting the mucosal lining and submucosa of the intestine, resulting in severe abdominal pain and bloody diarrhea, among other symptoms [1]. Although several factors, such as genetic predisposition, mucosal immune response, and dysbiosis of the gut microbiota, have been implicated in the pathogenesis of IBD [2,3], the precise etiology remains complex and uncertain.

Intestinal homeostasis is maintained by specialized host cells and the gut microbiota [4]. The gut microbiota, in symbiosis with the host, forms mucosal and systemic immune systems [5]. When the gut microbiota is disrupted, harmful bacteria in the gut rapidly increase, enterotoxins are released, intestinal mucosal permeability increases, and immunosuppressive proteins are produced, leading to immune

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dysfunction [6]. In addition, the overgrowth of certain bacteria affects metabolism and energy balance, leading to intestinal inflammation [7]. Therefore, maintaining the balance of the gut microbiota is crucial for preserving a healthy gut environment, and for this reason, modulating the gut microbiota has been proposed as a potential therapeutic strategy for treating IBD.

In areas of damaged mucosa, there is an increase in the proliferation of epithelial cells to replace damaged cells and prevent erosion caused by mechanical damage or pathogen exposure [8]. In this process, β-catenin plays a crucial role in regulating cell proliferation and tissue homeostasis by stimulating the expression of target genes that promote cell cycle progression through the activation of transcription factor (TCF) [9]. Under normal physiological conditions, this signaling is kept low, resulting in a lower rate of cell turnover. However, when injury occurs, it is temporarily activated, initiating a regenerative program. In the context of IBD, the expression of β-catenin/TCF signaling facilitates the healthy regeneration of epithelial cells, aiding in the restoration of a healthy intestinal envrionment.

Recent studies have shifted focus to the interactions between gut microbiota and various natural compounds [10,11]. The gut microbiota can significantly affect biological metabolic processes and may influence the pharmacological functions of various drugs. Korean Red Ginseng (KRG; *Panax ginseng* Meyer), known for its major active pharmacological components, steroidal saponins (ginsenosides), possesses diverse physiological activities [12]. Its therapeutic potential in modulating gut microbiota and enhancing intestinal barrier function is well-established and known to alleviate the symptoms of various diseases [13–15]. Although KRG has been identified as a promising candidate for IBD treatment owing to its anti-inflammatory effects, its influence on the composition of the gut microbiota in colitis is not completely understood. Therefore, this study aimed to investigate the potential gut-improving effects of KRG against IBD in a dextran sodium sulfate (DSS)-induced colitis mouse model.

## 2. Materials and methods

### 2.1. Chemicals and reagents

DSS was purchased from MP Biomedicals (MW, 36−50 kDa; Irvine, CA, USA). KRG was prepared by the Korean Ginseng Corporation (Daejeon, Republic of Korea). Enzyme-linked immunosorbent assay (ELISA) kits for interleukin (IL)-1β, IL-6, and interferon (IFN)-γ were purchased from RayBiotech Inc. (GA, USA), ELISA kits for immunoglobulin (Ig)A and IgG were purchased from Sigma-Aldrich (St. Louis, MA, USA). Myeloperoxidase (MPO) activity assay kit was purchased from Abcam (Cambridge, MA, USA). For total DNA extraction from fecal samples, QIAamp PowerFecal Pro DNA Kit was purchased from Qiagen (Hilden, Germany). The antibodies for flow cytometric analysis, PerCP-CD4, APC-CD8a, FITC-CD25, and PE-FoxP3 were purchased from BD Biosciences (San Jose, CA, USA). The primary antibodies used were as follows: total (t)-phosphatidylinositol 3-kinase (PI3K), phosphor (p)-PI3K, t-protein kinase B (AKT), p-AKT, β-catenin, TCF-4, Ki67, cyclin D1, proliferating cell nuclear antigen (PCNA), Notch1, Activated Notch1, and hairy and enhancer of split-1 (Hes1) were purchased from Abcam, and β-actin was purchased from Cell Signaling Technology (Beverly, MA, USA).

### 2.2. Animals and experimental procedure

Four-week-old C57BL/6 male mice were purchased from Samtaco (Osan, Republic of Korea), quarantined, and acclimated for 1 wk. All animal experiments were approved by the Animal Care and Use Committee of the Chungnam National University (202209A-CNU-173).

The mice were randomly divided into four groups as follows: 1) normal control (NC, vehicle orally (p.o.)), 2) DSS (only 2 % DSS ad libitum in drinking water), 3) DSS + KRG200 (2 % DSS + KRG 200 mg/kg p.o.), and 4) DSS + KRG400 (2 % DSS + KRG 400 mg/kg p.o.). Each group was comprised of eight mice. KRG was dissolved in phosphate-buffered saline (PBS), and administered orally at two doses (200 and 400 mg/kg) 4 wks before DSS treatment until sacrifice. DSS was administered ad libitum at a concentration of 2 % in the drinking water for 7 days. Fresh fecal samples were collected for microbiome analysis once every 2 wks starting before KRG administration. All fecal samples were stored at −80 °C until analysis. Body weight was measured daily after DSS administration, and euthanasia was performed 3 days after DSS treatment.

### 2.3. Colonic injury assessment and histopathological evaluation

Following DSS administration, the disease activity index (DAI) score was checked daily with body weight, stool consistency, and rectal bleeding to assess the extent of intestinal damage [16]. In addition, the length from the cecum to the rectum was measured at the time of euthanasia. To determine intestinal permeability, 200 μL of 4 kDa fluorescein isothiocyanate (FITC)-dextran (Sigma-Aldrich) at 8 mg/mL in PBS was administered orally 3–4 h before sacrifice. The collected blood was centrifuged, and the concentration of FITC-dextran in the serum was measured at Ex: 480 nm and Em: 530 nm using a VICTOR Nivo™ multimode plate reader (PerkinElmer, Inc., Waltham, MA, USA).

Colon tissue was fixed in 10 % neutral-buffered formalin, embedded, sectioned into 4 μm thick sections, and attached to slides. Tissue slides were stained with hematoxylin and eosin (H&E; TissuePro Technology, Gainesville, FL, USA) and evaluated for inflammatory cell infiltration, crypt depletion, and the epithelial barrier. Periodic acid-Schiff (PAS; Sigma-Aldrich) staining was performed to confirm a decrease in the number of goblet cells.

### 2.4. Flow cytometric analysis

Mesenteric lymph nodes (MLN) were collected from each mouse, washed with stain buffer (BD Biosciences), filtered through a 40 μm cell strainer. The resulting solution was centrifuged, the supernatant was discarded, and the cell pellet was resuspended in the staining buffer at a concentration of $1 \times 10^6$ cells/mL. Initially, the cells were stained with PerCP-CD4, APC-CD8a, and FITC-CD25 antibodies for 30 min at 4 °C in the dark. Cells were then washed with stain buffer, and subjected to fixation and permeabilization for 30 min. Following they were stained with an PE-FoxP3 antibody for 30 min at 4 °C in the dark. Data were collected and analyzed using a BD Accrui™ C6 Plus Flow Cytometer (BD Biosciences).

### 2.5. ELISA

Various relevant indices were assessed to evaluate the extent of the inflammatory response in the colitis state. Blood was centrifuged to obtain serum, and the concentrations of IL-1β, IL-6, IFN-γ, IgA, and IgG levels were measured using ELISA kits, according to the manufacturer's protocol.

Neutrophil infiltration, considered a biomarker of colitis, was assessed using a MPO activity assay kit. 10 mg of colon tissue were homogenized in the assay buffer and processed in accordance with the protocol. The concentrations of each marker were measured at 450 nm using a spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA).

### 2.6. 16s rRNA sequencing and gut microbiome analysis

Total DNA was extracted from fecal samples using the DNA extraction kit, following the manufacturer's protocol. V4 sections of the 16s rRNA gene were amplified using the following primers:

515F : 5’ – TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTAA – 3’

806R : 5’ – GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT – 3’

The amplicons were sequenced on a MiSeq platform (Illumina). All the sequences were processed using the Mothur (v.1.48.0) pipeline (https://mothur.org/wiki/miseq_sop) [17,18]. Rarefaction curves and Venn diagrams were generated using the Mothur command (rarefaction.single, Venn). Alpha diversity was evaluated using the Sobs and Chao indices for community richness, and the Shannon and Invsimpson indices for community diversity [19]. Statistical analysis was performed using GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA). Beta diversity was assessed using the J-class index to evaluate community membership similarity and the Thetayc index to evaluate community structure similarity. Statistical significance was evaluated using Mothur’s analysis of molecular variance (AMOVA) [20]. Linear discriminant analysis (LDA) effect size (LEfSe) was used to identify significant taxanomic differences between groups. LEfSe analysis was conducted using the following parameters: the *p*-value for the Kruskal-Wallis test and Wilcoxon test between classes was set at 0.05, and the threshold on the logarithmic LDA score for distinguishing features was set at 3.0 [21]. Phylogenetic investigation of communities by reconstruction of unobserved state 2 (PICRUSt2) was implemented to infer functional differences between the gut microbiota and was predicted based on the MetaCyc pathway [22,23]. Statistical significance was assessed and visualized using STAMP version 2.1.3 [24].

### 2.7. Immunohistochemistry (IHC) analysis

To visualize the expression levels of Ki67, PCNA, and cyclin D1, a VECTASTAIN Elite ABC Kit (Vector Laboratories, Burlingame, CA, USA) was used, following the manufacturer’s protocol. Anti-Ki67 and cyclin D1 antibodies were probed with goat anti-rabbit IgG, and anti-PCNA was probed with goat anti-mouse IgG as the secondary antibody. 3,3′-Diaminobenzidine chromogen was used with Harris’s hematoxylin for counterstaining. Ten random areas per slide were selected for microscopic examination (Leica, Wetzlar, Germany).

### 2.8. Immunoblotting

Frozen colon tissues were homogenized in tissue lysis buffer (Sigma-Aldrich) containing a protease/phosphatase inhibitor to extract intracellular proteins. Western blotting was performed to observe changes related to intestinal epithelial tissue regeneration, as previously described [25]. Relative protein expression levels were confirmed using a ChemiDoc imaging system (Bio-Rad Laboratories).

### 2.9. Statistical analysis

Data are expressed as mean ± standard deviation (SD), and one-way analysis of variance (ANOVA) was conducted to analyze the means of more than two groups, followed by Tukey’s post hoc test. Statistical significance was defined as *p*-values of less than 0.05, or 0.01, as highly significant, using GraphPad Prism version 8.0.1 (GraphPad Software).

## 3. Results

### 3.1. KRG ameliorated symptoms and colon histological damage in DSS-induced colitis mice

To evaluate the effects of KRG in DSS-induced colitis mice, the administration was conducted according to the schedule depicted in Fig. 1A. Significant weight changes began to manifest 6 days after DSS administration, with severe bleeding and diarrhea observed from 4 days after DSS treatment. KRG administration alleviated these weight changes and colitis symptoms (Fig. 1B and C). Additionally, upon assessing the decrease in intestinal barrier function, a common symptom in IBD animal models [26], it was found that the increase in FITC-dextran concentrations due to DSS administration was reduced with KRG treatment (Fig. 1D). Moreover, the overall colonic length from the cecum to the rectum, which was shortened by DSS treatment, increased depending on the dosage of KRG (Fig. 1E and F).

To assess the effects of KRG on the extent of epithelial damage in the colon, histological analysis of the colonic tissue was conducted using H&E and PAS staining (Fig. 1G and H). Compared to the NC group, DSS treatment resulted in shortened and lost crypts, infiltration of inflammatory cells, and a reduction in goblet cells in the colonic tissue of mice. Conversely, the KRG treatment group showed a protection effect against these epithelial damage.

### 3.2. KRG influenced intestinal microbiota composition in DSS-induced colitis mice

The rarefaction curve indicated that the sequencing data is suitable for assessing microbial diversity. The total number of operational taxonomic units (OTUs) in the control group was higher than that in the other groups (Fig. 2A). This indicates that DSS treatment reduces the biodiversity of intestinal microbial communities [27]. Using alpha diversity analysis, dysbiosis of the intestinal microbiota following DSS treatment was observed. Based on the Shannon index, which considers both richness and evenness, microbial diversity showed a significant decreased in the DSS group compared to the control group. Interestingly, this decrease was further accentuated following the intake of KRG (Fig. 2B). Other indices supported this finding (Fig. S1). The Jaccard index, which measures the differences among the members of the microbial communities, indicated significant differences among the members of the gut microbial communities (Fig. 2C). Similarly, the Thetayc index, which gauges differences in the microbial community structure (the number and relative abundance of species constituting the community), showed similar results (Fig. S2). Collectively, DSS treatment reduced the alpha diversity (both richness and evenness) of the gut microbiota, and the KRG consumption perturbed the species composition in each group compared to that in DSS alone group.

### 3.3. KRG altered specific bacterial taxa in DSS-induced colitis mice

To assess the effect of KRG on the composition of intestinal microbes, we compared the OTUs present in each experimental group. Initial

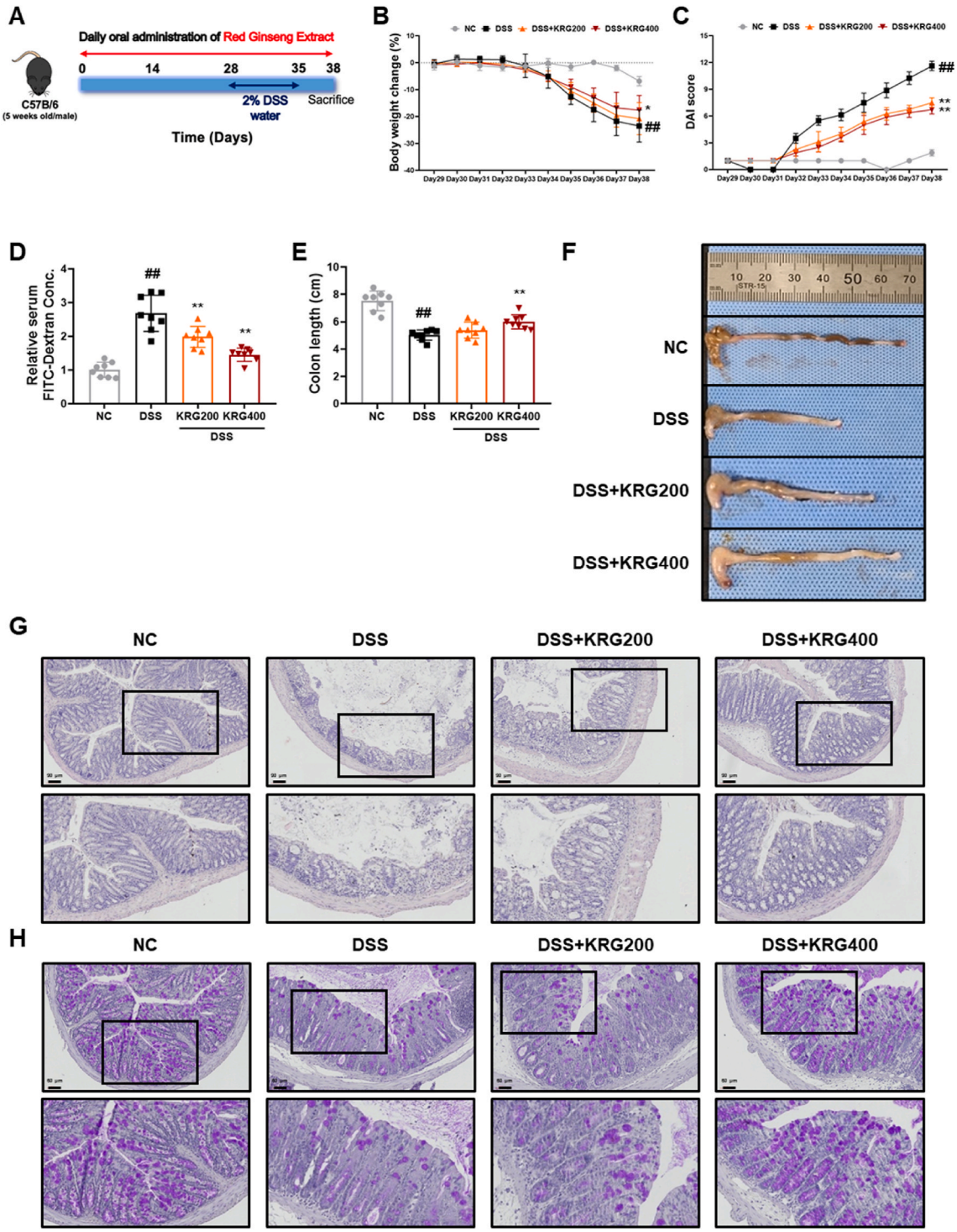


**Fig. 1.** Effects of KRG ingestion on the symptoms and colon histological damage in DSS-induced colitis mice. (A) Experimental scheme illustrating the administration of KRG and DSS in this study. Changes resulting the administration of KRG in (B) body weight change, (C) DAI score, (D) relative serum FITC-Dextran concentration, (E) colon length, and (F) representative photographs of the entire colon. Representative images of (G) H&E staining (Bar = 90 μm) and (H) PAS staining (Bar = 60 μm) of colon tissue. NC, normal control mice; DSS, 2 % dextran sodium sulfate ad libitum in drinking water mice; DSS + KRG200, DSS + oral administration of 200 mg/kg/day of KRG; DSS + KRG400, DSS + oral administration of 400 mg/kg/day of KRG. Values: means ± SD ($n = 8$). Significance: $^{\#\#}p < 0.01$ vs NC; *, **p < 0.05, 0.01 vs DSS, respectively.

comparisons revealed that, relative to the DSS group, the groups treated with KRG exhibited 69 unique OTUs and lacked 288 OTUs (Fig. 3A). A comparison of the overall gut microbiota composition at the phylum level revealed a decrease in *Proteobacteria*, which had increased after DSS administration in all KRG-treated groups (C: 1.12 %, D: 4.99 %, KRG200: 2.81 %, KRG400: 2.70 %). Interestingly, *Actinobacteriota*, which were nearly absent, increased by approximately 3.5 times only in the DSS + KRG400 group (Fig. S3). When comparing the compositional changes in the intestinal microbiome at the family level, the abundance of bacteria such as *Lactobacillaceae* and *Prevotellaceae* that had decreased in the DSS group compared to those in the control group, increased in the KRG group. Conversely, the abundance of bacteria such as

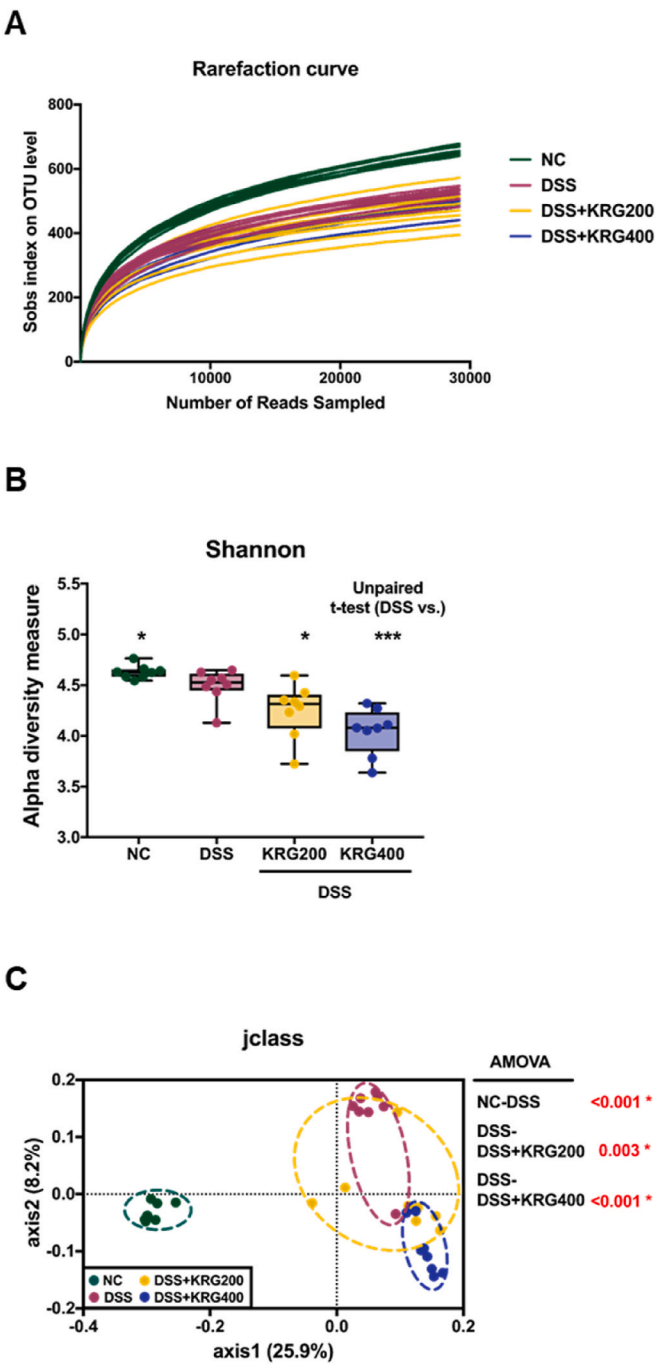


**Fig. 2.** KRG ingestion changes the gut microbial community member in DSS-induced colitis mice. (A) Rarefaction curves of all samples on Day 35. (B) Comparison of alpha diversity (Shannon) within groups on Day 35. It is determined by the community diversity (richness and evenness). Unpaired t-tests (two-tailed) were used to analyze variation between the two groups (*, **, ***$p < 0.05$, 0.01, and 0.001). (C) Principal Coordinate Analysis (PCoA) plots of jclass metric that assess the similarity in community member. Statistical significance was evaluated using Mothur's analysis of molecular variance (AMOVA). Red values indicate that there is a significant difference between DSS and each group. NC, normal control mice; DSS, 2 % dextran sodium sulfate ad libitum in drinking water mice; DSS + KRG200, DSS + oral administration of 200 mg/kg/day of KRG; DSS + KRG400, DSS + oral administration of 400 mg/kg/day of KRG.

*Clostridia_UCG-014_fa*, *Tannerellaceae*, *Marinifilaceae*, *Clostridia_vadinBB60_group_fa* and *Oscillospiraceae* that had increased in the DSS group compared with those in the control group, decreased in the KRG group (Fig. 3B). In addition, the group treated with KRG showed an increase in beneficial probiotic bacteria [28,29] (Fig. 3C), whereas harmful bacteria that causes intestinal inflammation in both acute and chronic colitis were reduced [30] (Fig. 3D). The Firmicutes/Bacteroidota ratio, which is associated with the maintenance of intestinal homeostasis, exhibited a significant increase in the DSS group compared to the control group, with a tendency to decrease in the DSS + KRG200 group (Fig. S4). Furthermore, we conducted LEfSe to identify specific OTUs that differed between the DSS- and KRG-treated groups. A logarithmic LDA score cutoff of 3.0 was employed to discern taxonomic differences between the groups (Fig. S5). Accordingly, we compared the relative abundances of specific bacteria at the genus level (Fig. S6).

### *3.4. KRG altered the functional pathways of the microbial community in DSS-induced colitis mice*

We performed PICRUSt2 analysis using the MetaCyc database to predict functional changes associated with gut microbiota. Based on these predictions, significant functional differences were observed in the DSS + KRG200 and DSS + KRG400 groups on day 35. The functional abundance of some pathways, such as D-glucarate degradation, L-lysine degradation, purine nucleotide degradation, and fermentation to butanoate, was higher in the DSS group than in the DSS + KRG200 and DSS + KRG400 groups. In contrast, the functional abundances of certain pathways, such as polysaccharide degradation, lactate fermentation, and glycolysis, were higher in the DSS + KRG200 and DSS + KRG400 groups than in the DSS group (Fig. 4). Descriptions of each pathway and information on the superclasses have been described (Table S1).

### *3.5. KRG improved regulatory T ($T_{reg}$) cells in MLN and inflammatory response in DSS-induced colitis mice*

Gut microbiota can promote the production of $T_{reg}$ cells that regulate intestinal homeostasis [31], and in this regard, flow cytometry analysis showed that the ratio of $CD4^+CD25^+FoxP3^+$ was reduced in DSS alone group compared to NC group, and this trend increased with KRG administration (Fig. 5A and B). We also investigated whether KRG administration in DSS-induced colitis mice affected changes in the expression levels of inflammatory mediators in serum or colon tissue homogenates. Upon DSS treatment, all mice had significantly elevated levels of IL-1β, IL-6, and IFN-γ in their serum. These elevations tended to decrease with KRG consumption (Fig. 5C–E). In addition, the concentrations of IgA and IgG, which play a fundamental role in maintaining the interaction between microorganisms living on the mucosal surface and the host, were significantly reduced in DSS alone group compared to NC group (Fig. 5F and G). The activity of MPO enzyme, which is activated during inflammation, was increase by DSS treatment, but decreased in KRG treatment groups (Fig. 5H).

### *3.6. KRG affected the expression of regenerative and proliferation signaling proteins in DSS-induced colitis mice*

An increase in regeneration-related signaling proteins facilitates recovery from DSS-induced damage. As shown in Fig. 6A and B, Western blot showed increased protein expression levels of PI3K/AKT-mediated β-catenin/TCF-4 in the DSS alone group compared to the NC group. However, this increase was significantly reduced in a dose-dependent manner in the KRG treatment groups. However, the protein expression levels of Notch1/NICD/Hes1, another regeneration-related signal, did not differ between the groups (Fig. S7).

The effects of KRG on epithelial cell proliferation markers in DSS-induced colitis mice were evaluated using IHC staining (Fig. 6C–E). The protein expression of Ki67, PCNA, and cyclin D1 in colonic

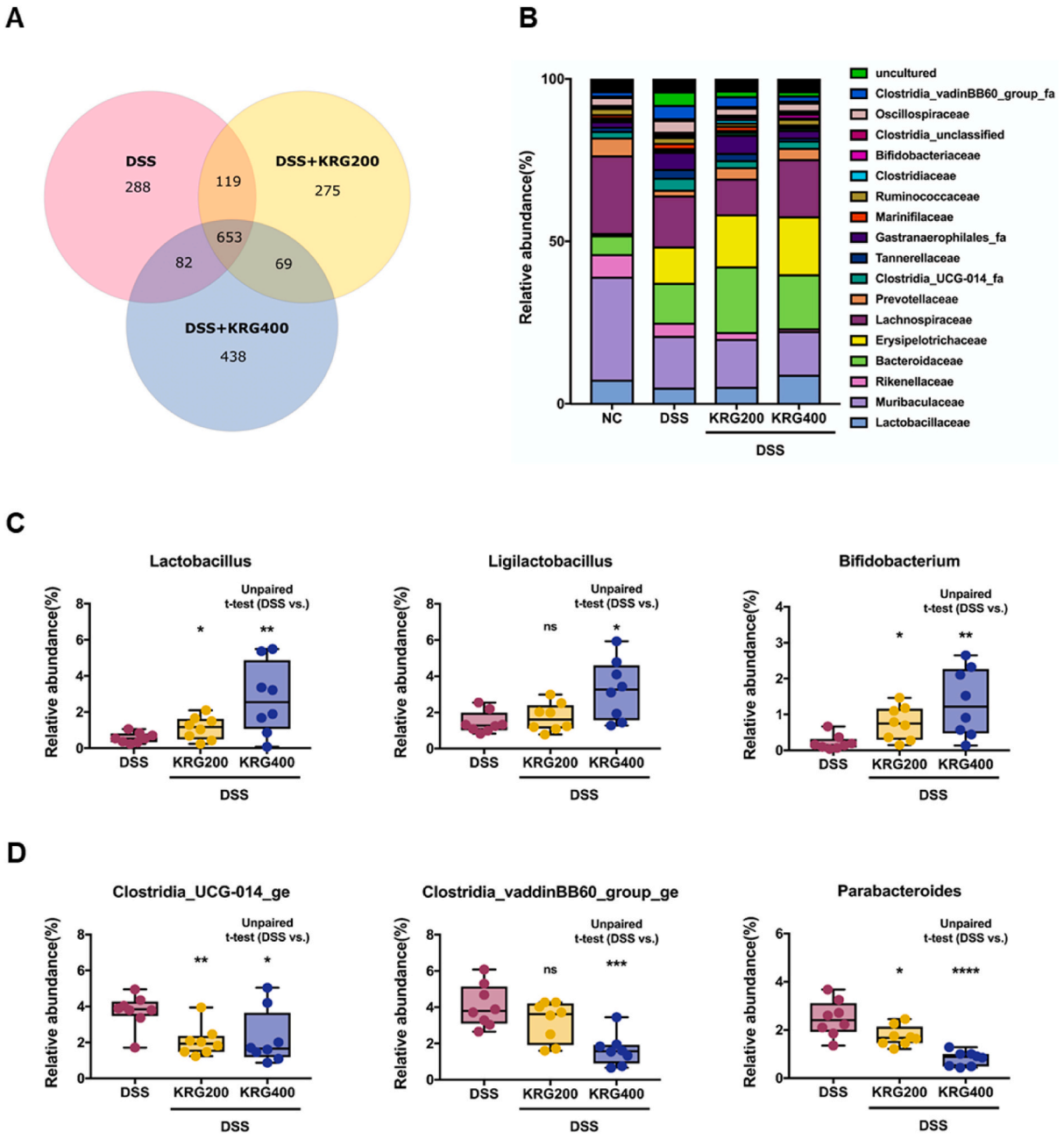


**Fig. 3.** Changes in specific bacterial taxa with KRG ingestion in DSS-induced colitis mice. (A) Venn diagram indicating the number of different OTUs in each group and the number of common OTUs between groups (Day 35). (B) Relative abundance bar chart at the family level. Relative abundance values less than 1 % are not shown. (C) Comparison of relative abundance of representative beneficial bacteria. (D) Comparison of relative abundance of representative harmful bacteria. Unpaired t-tests (two-tailed) were used to analyze variation between the two groups (*, **, ***, ****$p < 0.05$, 0.01, 0.001, and 0.0001, ns means no significance). DSS, 2 % dextran sodium sulfate ad libitum in drinking water mice; DSS + KRG200, DSS + oral administration of 200 mg/kg/day of KRG; DSS + KRG400, DSS + oral administration of 400 mg/kg/day of KRG.

epithelial cells was significantly increased by DSS administration compared to the NC group. However, a decrease in the number of positive cells for these markers was observed following KRG treatment.

## 4. Discussion

Recent research has suggested associations between microbiota composition and microbial-mediated intestinal metabolism in IBD, as indicated by both animal models and clinical treatments [32]. Natural compounds have emerged as promising candidates for the treatment of several diseases because they regulate the gut microbiota, thereby modulating host physiology and inflammatory immune responses to alleviate disease pathology [33]. In this study, to validate the potential biological effects of KRG against colitis, we investigated its effect on the structure of the gut microbiota and the subsequent regeneration factors of intestinal epithelial cells.

KRG treatment alleviated the symptoms of colitis in mice, stimulating the growth of specific microbial groups, thereby influencing both the abundance and evenness of the species. Structural differences, considering the number and relative abundance of members and species within the intestinal microbial community in the KRG treatment group, also supported our hypothesis. DSS-induced colitis mice showed a significant increase in *Proteobacteria* and a decrease in *Actinobacteriota* [34], both of which were effectively offset by KRG consumption.

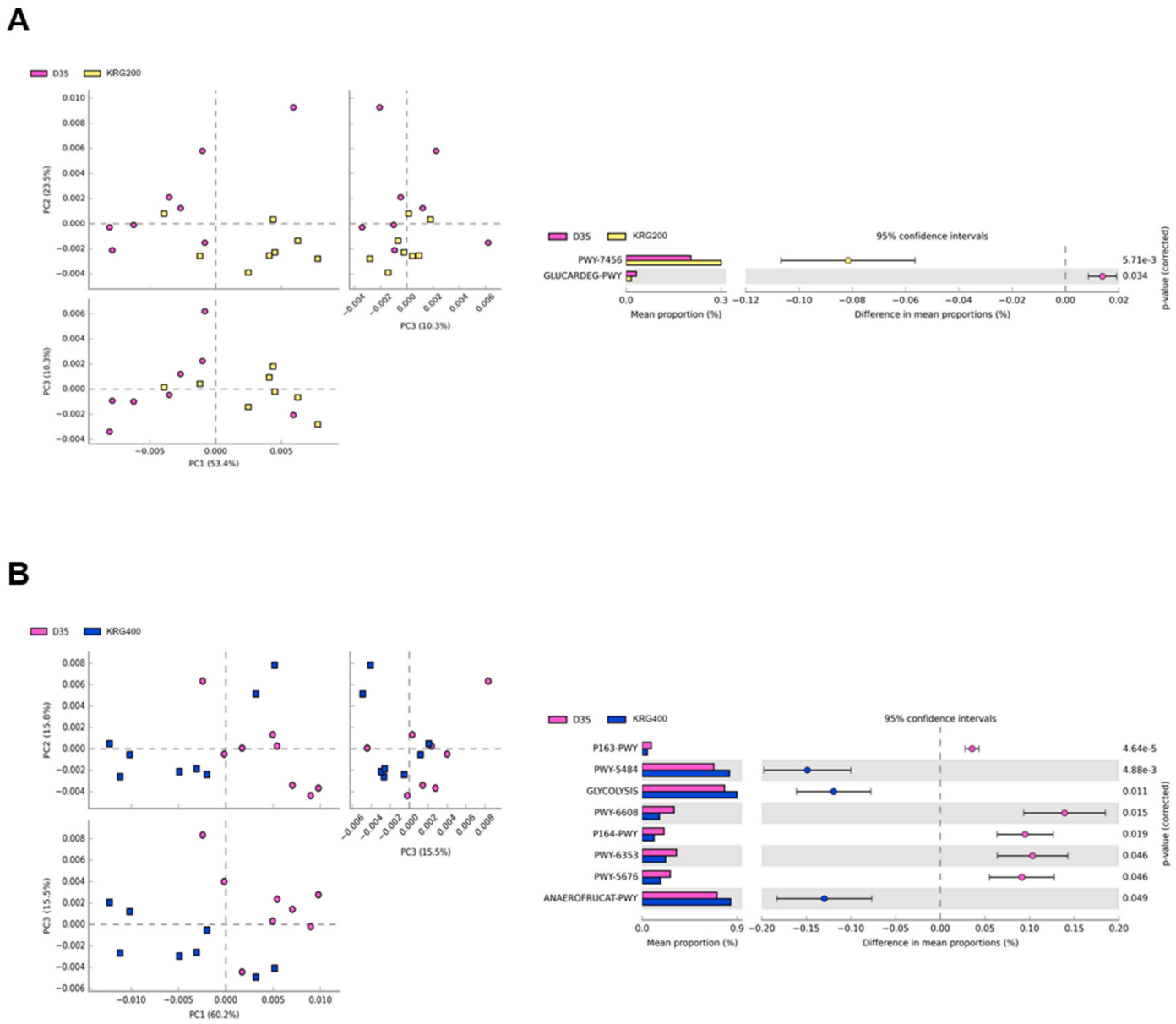


**Fig. 4.** Comparison of microbial functional prediction by using PICRUSt between each group. (A) The PCA plot and the extended error bar chart of DSS vs DSS + KRG200. The pink color indicates DSS on Day 35 and the yellow color indicates DSS + KRG200 on Day 35. (B) The PCA plot and the extended error bar chart of DSS vs DSS + KRG400. The pink color indicates DSS on Day 35 and the blue color indicates DSS + KRG400 on Day 35. The extended error bar chart showed the statistical difference using a Welch's *t*-test (two-sided) with Bonferroni correction in the predicted functional pathways between the 2 groups. The middle value represents the mean differences between the 2 groups (upper-lower bar value), and the error bar represents the 95 % confidence intervals with the effect size of difference in mean proportion. The *p*-value at the side indicates the significance between the upper and lower bars. DSS, 2 % dextran sodium sulfate ad libitum in drinking water mice; DSS + KRG200, DSS + oral administration of 200 mg/kg/day of KRG; DSS + KRG400, DSS + oral administration of 400 mg/kg/day of KRG.

Furthermore, at the family level, KRG consumption not only recovered the decreased levels of *Lactobacillaceae* and *Prevotellaceae* induced by DSS administration but also reversed the increased levels of *Clostridia_UCG-014_fa*, *Tannerellaceae*, and *Oscillospiraceae*. The abundance of *Lactobacillaceae* and *Prevotellaceae* was downregulated in rats with DSS-induced colitis, and increased when consuming *Holothuria leucospilota* polysaccharides, which have immunomodulatory effects [35]. *Tannerellaceae* and *Oscillospiraceae* were reported to be increased in mice with DSS-induced colitis [36,37].

In addition, LEfSe results showed that at the genus level, the abundance of taxa, including *Alloprevotella* of the phylum *Bacteroidota*, *Prevotellaceae_UCG-001*, *Romboutsia* of the phylum *Firmicutes,* and *Coriobacteriaceae_UCG-002* of the phylum *Actinobacteriota,* significantly increased in all KRG consumption groups compared to the DSS group. These genera play an important role in maintaining intestinal homeostasis and promote the growth of beneficial intestinal microorganisms in the host through fiber degradation [38]. They are also involved in the production of short-chain fatty acids (SCFA) and are dominant in healthy individuals [39]. SCFA plays beneficial roles in the regulation of both intestinal and systemic inflammatory responses [40]. Specifically, butyrate influences the regulation of genes involved in cell proliferation, differentiation, and inflammatory responses, contributing to intestinal homeostasis [41]. In line with the microbiome analysis, KRG-treated groups demonstrated a restoration in $T_{reg}$ cells populations in MLN compared to the DSS only group. $T_{reg}$ cells play a crucial role in the regulation and maintenance of intestinal mucosal homeostasis by suppressing the immune response during the pathological process of DSS-induced colitis [42]. Additionally, the increased markers of

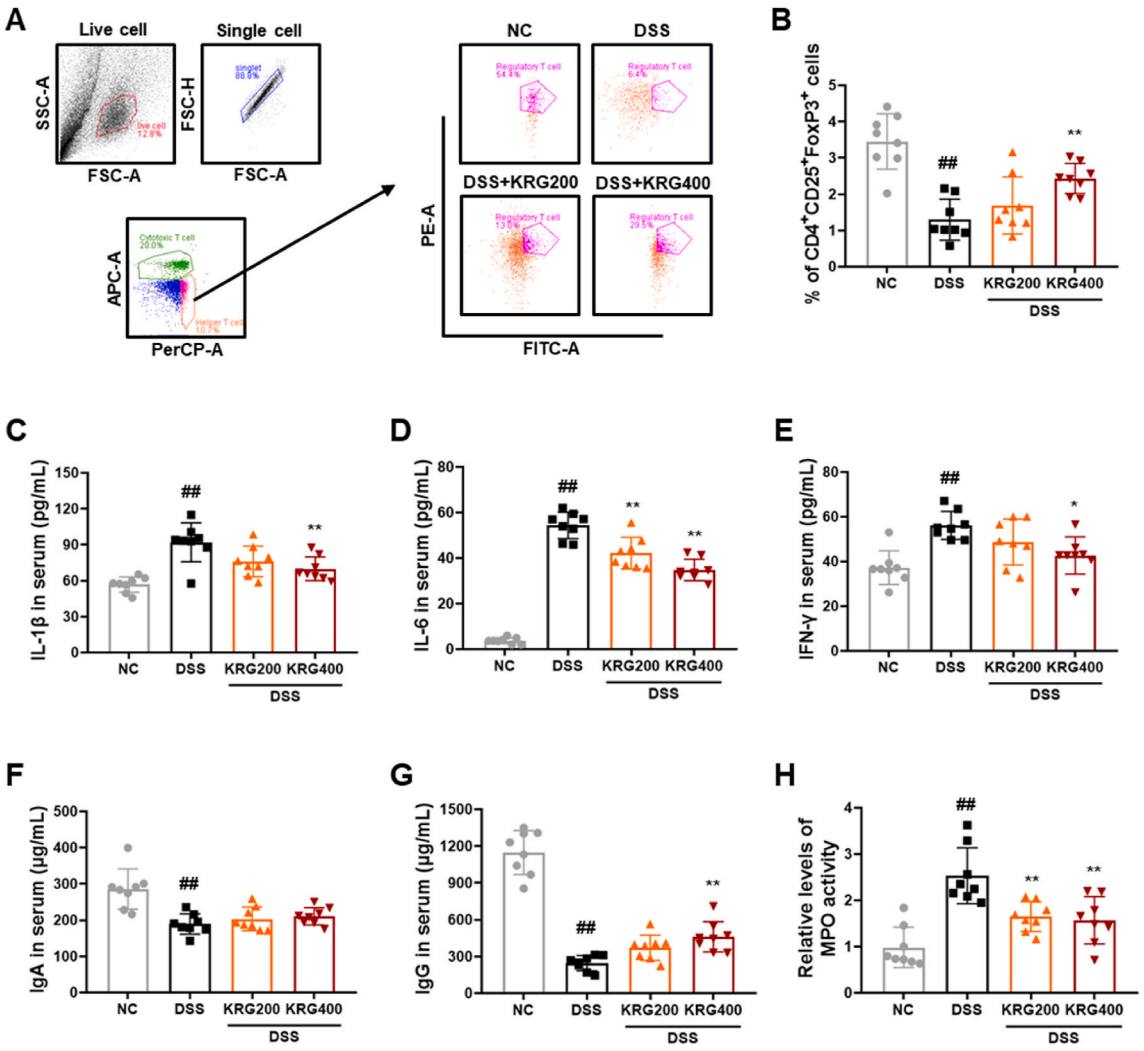


**Fig. 5.** Effects of KRG ingestion on regulatory T cells in MLN and inflammatory mediators in DSS-induced colitis mice. (A) Representative population dot plot and (B) quantification graph of regulatory T cells in MLN analyzed by flow cytometry. The levels of serum (C) IL-1β, (D) IL-6, (E) IFN-γ, (F) IgA, and (G) IgG measured by ELISA. (H) Relative levels of MPO activity in colon tissue. NC, normal control mice; DSS, 2 % dextran sodium sulfate ad libitum in drinking water mice; DSS + KRG200, DSS + oral administration of 200 mg/kg/day of KRG; DSS + KRG400, DSS + oral administration of 400 mg/kg/day of KRG. Values: means ± SD ($n = 8$). Significance: $^{\#\#}p < 0.01$ vs NC; *, $^{**}p < 0.05$, 0.01 vs DSS, respectively.

inflammatory response induced by DSS treatment were significantly reduced following KRG administration.

The analysis of PICRUSt2 was conducted to predict functional changes in the intestinal microbial community following KRG consumption. An increase in metabolic processes, such as mannan degradation, homolactic fermentation, glycolysis I (from glucose 6-phosphate), and glycolysis II (from fructose 6-phosphate), was observed. Meanwhile, processes such as D-glucarate degradation I, L-lysine fermentation to acetate and butanoate, purine nucleobase degradation I (anaerobic), acetyl-CoA fermentation to butanoate II, purine nucleotide degradation II (aerobic), and guanosine nucleotide degradation III were decreased. The glycolysis pathway has been reported to be associated with SCFA production [43], while the purine nucleobase degradation pathway is associated with the expression of PPKAB1, which has been implicated in the pathogenesis of Crohn's disease [44]. Additionally, the fermentation of acetyl-CoA to butanoate II pathway has been reported to be associated with the pericellular permeability biomarker, zonulin, in colonic cells [45].

Generally, increased epithelial cell proliferation is observed at the damaged mucosal edges during colitis, which facilitates sealing the wounds before they are replaced by normal intestinal epithelial cells [46]. Damage and inflammation temporarily alter the proliferation and differentiation of the intestinal epithelial cells, thereby triggering a regenerative program that restores the epithelial barrier. In the epithelial cells of crypts in the gut, AKT-dependent β-catenin activation promotes cell proliferation and initiates crypt budding [47]. Consistent with previous reports, the present study indicates an increased PI3K/AKT-mediated β-catenin/TCF-4 signaling to facilitate recovery from intestinal damage induced by DSS insults. Meanwhile, in the KRG treated groups, regenerative signals were dose-dependently reduced while showing improved intestinal lining compared to the DSS only group. This suggests that KRG administration prevented the exacerbation of damage by averting the imbalance in the gut microbiota, thereby suppressing the stimulation of regenerative signals in the colon. This was further supported by the IHC evaluation for cell proliferation factors, including Ki67, PCNA, and cyclin D1. Ki67 is a cell cycle-related protein predominantly present in the nucleus, appearing in all active stages of the cell cycle, except quiescent G0 cells [48]. PCNA and cyclin D1 are known to regulate epithelial cell progression through the G1/S phase [49]. Moreover, cyclin D1 has a binding site for TCF, enabling its direct regulation by the β-catenin/TCF-4 signaling pathway [50]. In contrast, the Notch signaling pathway, which controls the proliferation and differentiation of epithelial cells in the colon [51], was found to be relatively unaffected by the DSS insult and KRG treatment, which was

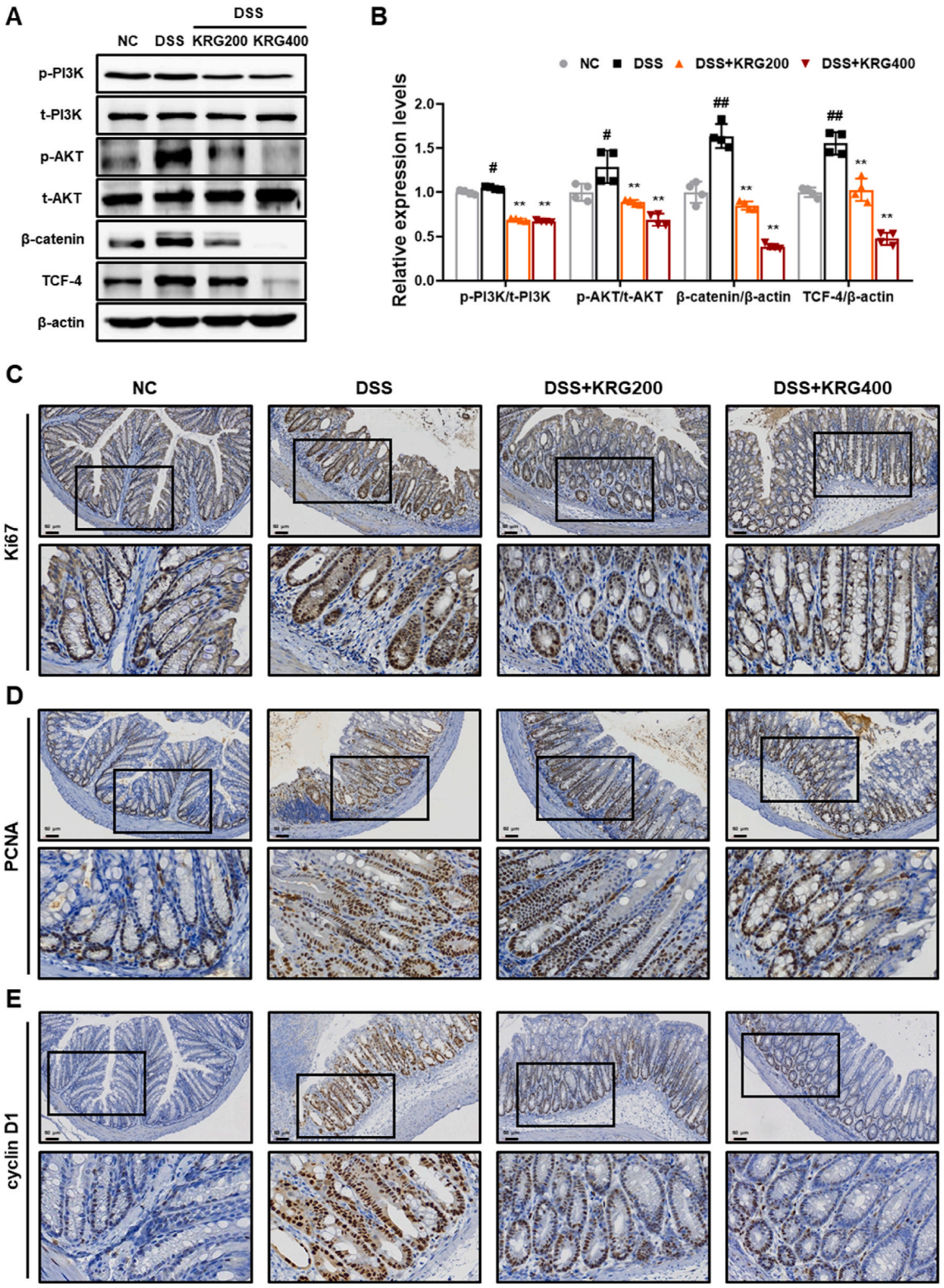


**Fig. 6.** Effects of KRG ingestion on colonic epithelial cell regeneration and proliferation in DSS-induced colitis mice. (A) Western blot analysis of the indicated proteins in colon and (B) graphs representing the densitometric values of protein expression. Representative images of IHC staining of (C) Ki67 (Bar = 60 μm), (D) PCNA (Bar = 60 μm), and (E) cyclin D1 (Bar = 60 μm) of colon tissue. NC, normal control mice; DSS, 2 % dextran sodium sulfate ad libitum in drinking water mice; DSS + KRG200, DSS + oral administration of 200 mg/kg/day of KRG; DSS + KRG400, DSS + oral administration of 400 mg/kg/day of KRG. Values: means ± SD ($n$ = 4). Significance: $^{\#, \#\#}p < 0.05$, 0.01 vs NC; $^{**}p < 0.01$ vs DSS, respectively.

comparable to that in the normal control group. Epithelial cells are reported to undergo rapid self-renewal [52], so further experiments at different time points are necessary to clearly interpret the regeneration-related effects of KRG in DSS-induced colitis mice. To assess the direct regenerative effects of KRG on the epithelial lining, a supporting *in vitro* study was conducted (Supplementary data). In the migration assay, the confluency of Caco-2 cells in the wounded area recovered in a KRG concentration-dependent manner compared to cells treated only with DSS. Additionally, transepithelial electrical resistance measurements confirmed that KRG positively affected the tight junctions of the Caco-2 cell monolayer.

Based on these findings, we propose that KRG could prevent colitis by addressing gut microbiota dysbiosis, preventing tissue damage, and ameliorating regenerative signaling stimulation. Gut bacteria can also metabolize ginsenosides in KRG by hydrolyzing glycosidic bonds or by stepwise cleavage of sugar moieties, thereby converting them into metabolites with enhanced biological activity [18]. Further research is needed to investigate the interaction between KRG and the gut microbiota at the cellular and molecular levels, using appropriate positive controls related to gut microbiota modulation, that can offer essential information on KRG's clinical application against IBD.

## CRediT authorship contribution statement

**Ji-Soo Jeong:** Methodology, Writing – original draft. **Ga-Hyeon Baek:** Methodology, Writing – original draft. **Jeong-Won Kim:** Methodology, Software. **Jin-Hwa Kim:** Methodology, Software. **Eun-Hye Chung:** Methodology, Software. **Je-Won Ko:** Conceptualization, Data curation. **Mi-Jin Kwon:** Investigation. **Sang-Kyu Kim:** Investigation. **Seung-Ho Lee:** Investigation. **Jun-Seob Kim:** Conceptualization, Writing – review & editing, All authors have checked and agreed to the final version of manuscript. **Tae-Won Kim:** Conceptualization, Writing – review & editing, All authors have checked and agreed to the final version of manuscript.

## Declaration of competing interest

All authors declare that they have no known competing conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2024.08.001.

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