

Oral PHGG ameliorates atopic dermatitis-like dermatitis with concurrent modulation of colonic IgA and cecal microbiota: implications for the gut–skin axis

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Abstract

BACKGROUND: Atopic dermatitis (AD) is increasingly recognized as a systemic inflammatory disorder linked to intestinal immune and microbiome dysregulation. However, whether dietary galactomannan fibers can mitigate AD through coordinated modulation of the gut–skin axis remains unexplored. This study investigated the anti-atopic potential of orally administered partially hydrolyzed guar gum (PHGG), a low-viscosity, fermentable galactomannan enriched in low-mass oligosaccharides (mannose/galactose ratio of 1.87).

RESULTS: In TNF- α /IFN- γ -stimulated HaCaT keratinocytes, PHGG (100–400 $\mu\text{g mL}^{-1}$) suppressed IL-6, IL-8, and MCP-1 secretion and down-regulated AD-relevant mediators, including *TARC*, *MDC*, *RANTES*, and *TSLP*. In TNF- α -challenged Caco-2 cells, PHGG reduced IL-8 and MCP-1 and partially restored barrier-associated transcripts (*TJP1*, *TJP2*, *OCLN*, *CLDN1*) and *MUC2*. In 2,4-dinitrochlorobenzene (DNCB)-induced AD BALB/c mice, oral PHGG (100–400 $\text{mg kg}^{-1} \text{d}^{-1}$) attenuated lesion severity, epidermal hyperplasia, collagen loss, and mast cell infiltration without adverse effects on body weight or organ weights. PHGG reduced circulating immunoglobulins and inflammatory cytokines, suppressed skin type 2 helper T-cell (Th2)-associated mediators, and restored colonic immune homeostasis, as evidenced by increased immunoglobulin A (IgA) and decreased interleukin 1 beta (IL-1 β) and IL-6. Cecal 16S rRNA profiling revealed that PHGG shifted dysbiotic microbial signatures toward fiber-responsive Bacteroidales lineages (e.g., Muribaculaceae, Alistipes, Rikenellaceae) and partially normalized community structure in beta-diversity analyses.

CONCLUSION: These findings demonstrate that oligosaccharide-enriched PHGG ameliorates AD-like inflammation through concurrent modulation of cutaneous immune responses, colonic mucosal immunity, and gut microbial community structure, supporting a gut–skin axis mechanism. Future studies quantifying microbial metabolites are warranted to establish causal mediators of gut-to-skin signaling.

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INTRODUCTION

Atopic dermatitis (AD) represents a chronic relapsing inflammatory condition of the skin, distinguished by compromised barrier function, aberrant immune responses, and persistent inflammatory cascades.^{1–3} While historically conceptualized as a disorder confined to cutaneous tissues, emerging scientific evidence indicates that AD pathogenesis encompasses systemic immune perturbations and bidirectional communication with the intestinal ecosystem.^{4,5} Notably, the predominance of type 2 helper T-cell (Th2) immunity, heightened circulating immunoglobulin levels, and compromised integrity of keratinocyte tight junctions that constitute cardinal pathophysiological hallmarks driving AD development and exacerbation.^{3,6}

Recently, in contemporary dermatological research, the bidirectional communication between intestinal ecology and cutaneous physiology – termed the gut–skin axis – has emerged as a pivotal conceptual paradigm for understanding the interplay between gastrointestinal homeostasis and dermatologic health.^{7,8} Perturbations in intestinal barrier function, alterations in intestinal mucosal immune surveillance, and compositional shifts in the resident gut microbiome have been proposed as key modulators of both peripheral inflammatory mediators and skin-localized immune reactivity.^{9,10} While numerous clinical investigations and laboratory-based studies have documented correlations between microbial imbalance in the gastrointestinal tract and the clinical severity of AD, the majority of published research remains descriptive in nature, emphasizing therapeutic trials of microbial supplementation or characterization of isolated bacterial populations.^{11,12} Mechanistic data elucidating how dietary macromolecules influence intestinally-derived immunoregulatory signals and their subsequent impact on cutaneous inflammatory pathways remain limited.

Partially hydrolyzed guar gum (PHGG) is a soluble, low-viscosity dietary fiber derived from enzymatic or chemical depolymerization of native guar gum, yielding galactomannan oligomers with enhanced colonic fermentability.^{13,14} Compared to native guar gum, the hydrolytic processing of PHGG confers critical functional advantages, including markedly reduced solution viscosity that facilitates oral administration and dietary incorporation, alongside significantly enhanced colonic fermentability that amplifies prebiotic efficacy and short-chain fatty acid (SCFA) production.^{13,15,16} These structural modifications enable PHGG to exert robust biological effects at lower dosages while minimizing gastrointestinal discomfort associated with the high viscosity of unmodified guar gum, thereby expanding its therapeutic applicability beyond traditional fiber supplementation.^{17,18} PHGG has been extensively studied for its prebiotic properties, including modulation of gut microbiota composition,^{15,19–21} improvement of intestinal motility,¹⁷ regulation of lipid metabolism and immune function,²² and therapeutic efficacy in irritable bowel syndrome (IBS).^{14,18} Despite this growing evidence for gastrointestinal benefits, the role of PHGG in extraintestinal inflammatory conditions – particularly cutaneous disorders such as AD – remains unexplored. Critically, no prior study has investigated whether PHGG-induced changes in gut microbiota, intestinal barrier function, and mucosal immunity can influence skin inflammation or epidermal barrier homeostasis, representing a significant knowledge gap in the context of the gut–skin axis.

Thus, it was hypothesized that oral PHGG supplementation would mitigate AD-like cutaneous inflammation and preserve epidermal barrier integrity. It was further hypothesized that these

effects would be accompanied by gut-associated immunomodulation, reflected by coordinated changes in colonic immune markers and cecal microbiota profiles. Accordingly, this study aimed to evaluate the anti-inflammatory and barrier-modulatory effects of PHGG using integrated *in vitro* (HaCaT and Caco-2) and *in vivo* (2,4-dinitrochlorobenzene (DNCB)-induced AD BALB/c mice) models, together with analyses of skin lesion severity, immune and signaling biomarkers, colonic immune mediators, and cecal microbiota composition.

MATERIALS AND METHODS

PHGG preparation

PHGG used in this study was supplied by HIEMS NATURA Inc. (Seongnam, South Korea). PHGG was produced from food-grade guar gum via controlled enzymatic hydrolysis using β -mannanase to depolymerize the galactomannan backbone by cleaving β -(1 $\rightarrow$ 4) glycosidic linkages, thereby reducing solution viscosity and improving water solubility. After hydrolysis, the reaction mixture was subjected to decolorization and filtration to remove process-related impurities. The resulting filtrate was then sterilized by ultra-high temperature (UHT) treatment to ensure microbiological safety, concentrated to increase solids content, and finally converted to a powder by spray drying.

Analytical methods

Monosaccharide composition analysis using PMP–sugar derivatization of PHGG

PHGG were hydrolyzed with trifluoroacetic acid (Sigma-Aldrich, St Louis, MO, USA), and the released monosaccharides were derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP; Sigma-Aldrich). The PMP–sugar derivatives were separated on an Agilent 1120 high-performance liquid chromatography (HPLC) system (Agilent Technologies, Palo Alto, CA, USA) equipped with a YMC Triart C18 column (250 $\times$ 4.6 mm, 5 μ m; YMC Co., Ltd, Kyoto, Japan) and detected using a diode array detector (DAD; Agilent Technologies). Isocratic elution was performed with 0.1 mol L^{−1} phosphate buffer (pH 6.7) and acetonitrile (83:17, v/v) at a flow rate of 1.0 mL min^{−1}. The injection volume was 20 μ L, and detection was performed at 257 nm.

Molecular distribution determination

The molecular weight distribution of PHGG was characterized using ultrahigh-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS²) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Detailed operating conditions for UHPLC–MS² and MALDI-TOF/MS are provided in Supporting Information Tables S1 and S2, respectively.

Assessment of *in vitro* anti-inflammatory effect of PHGG

Cell culture and cytotoxicity assessment

HaCaT human keratinocytes (CLS Cell Line Service, Eppelheim, Germany) and Caco-2 human intestinal epithelial cells (ATCC, Manassas, VA, USA) were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS; Corning Life Sciences, Manassas, VA, USA) and 1% penicillin–streptomycin (PS; Corning Life Sciences) at 37 °C in a humidified atmosphere containing 5% carbon dioxide (CO₂). For cytotoxicity assessment, HaCaT (1.0 $\times$ 10⁵ cells mL^{−1}) and Caco-2 (6.0 $\times$ 10⁵ cells mL^{−1}) cells were seeded in 96-well plates (200 μ L well^{−1}) and incubated to reach

approximately 80% confluence (HaCaT, 22 h) or complete confluence (Caco-2, 48 h). Cells were washed twice with phosphate-buffered saline (PBS) and serum-starved in serum-free DMEM (SFM; 200 $\mu\text{L well}^{-1}$) for 24 h (HaCaT) or 4 h (Caco-2). Cells were then pretreated with PHGG (at the indicated concentrations) for 1 h and subsequently stimulated with TNF- α and IFN- γ (HaCaT) or TNF- α alone (Caco-2) for 24 h. TNF- α (5 ng mL^{-1}) was used to induce low-grade inflammatory stress in Caco-2 monolayers, as TNF- α alone is sufficient to disrupt tight-junction integrity without excessive cytotoxicity. Cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 0.5 mg mL^{-1}) for 30 min. After removal of the MTT solution, formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 550 nm.

Enzyme-linked immunosorbent assay (ELISA) and quantitative real-time polymerase chain reaction (qPCR)

Following PHGG pretreatment and inflammatory stimulation for 24 h, culture supernatants were collected and analyzed for cytokine/chemokine levels using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions (Table S3). For gene expression analysis, total RNA was isolated from cells, reverse-transcribed to complementary DNA (cDNA), and quantified by quantitative real-time polymerase chain reaction (qPCR) as described previously.²³ Primer sequences are provided in Table S4.

Assessment of *in vivo* anti-AD effect of PHGG

Animal care and induction of AD

BALB/c mice (female, 5 weeks old; Dooyeol Biotech, Seoul, South Korea) were maintained under specific pathogen-free conditions (12-h light/dark cycle; controlled temperature and humidity) with *ad libitum* access to standard chow and water. After a 7-day acclimation period, mice were randomly assigned to the normal control (NOR), DNCB-treated control (DNCB), positive control (PC), or PHGG-treated groups and handled in accordance with institutional guidelines. The NOR and DNCB groups received normal saline, the PC group received dexamethasone (1 mg $\text{kg}^{-1} \text{d}^{-1}$), and the PHGG groups received PHGG (100, 200, or 400 mg $\text{kg}^{-1} \text{d}^{-1}$) by oral gavage. Oral administration was initiated during the pretreatment phase (days 1–14) and continued throughout the DNCB sensitization/challenge period (days 15–25), as summarized in the experimental timeline (Supporting Information Fig. S1). To induce AD-like dermatitis, dorsal hair was shaved on day 14. Sensitization was performed by topical application of 1% (w/v) DNCB (Sigma-Aldrich) to the shaved dorsal skin twice at 3-day intervals (days 15 and 18). Mice were euthanized on day 26 by gradual-fill CO_2 inhalation in a dedicated chamber, and tissues were collected for subsequent analyses. All procedures were approved by the Institutional Animal Care and Use Committee of Chung Ang University (IACUC No. 202501020096) and are reported in accordance with the ARRIVE guidelines.

Assessment of biomarkers from serum, skin and colonic tissues

After euthanasia, whole blood was collected from the inferior vena cava and centrifuged at 2000 $\times g$ for 10 min to obtain serum. For tissue biomarker analyses, dorsal skin and colonic tissues (150 mg each) were homogenized in self-prepared RIPA lysis buffer (1 mL) using a bead beater (1 min $\times$ 5 cycles with 1-min cooling intervals). Lysates were centrifuged at 13 000 $\times g$ for 15 min at 4 $^{\circ}\text{C}$, and the resulting supernatants were collected.

Protein concentrations were determined using a bicinchoninic acid (BCA) assay. Levels of immunoglobulins and cytokines in serum and tissue extracts were quantified using ELISA kits according to the manufacturers' instructions (Table S3). In addition, inflammation-related signaling proteins and epithelial barrier-related proteins in skin and colonic extracts were analyzed by Western blotting as described previously,²³ using the antibodies listed in Table S5.

Histomorphometric analysis

Following euthanasia, the shaved dosal skin of each mouse was collected and fixed with 10% (v/v) neutral-buffered formalin (Sigma-Aldrich) for a day, washed for 12 h, processed into a paraffin block using an automatic tissue processor (Leica Biosystems, Wetzlar, Germany), and then prepared into a tissue section (3–5 μm). Sections were individually stained with hematoxylin and eosin (H&E) dye (Sigma-Aldrich), Masson's Trichrome dye (Sigma-Aldrich), and Toluidine Blue dye (Sigma-Aldrich). After subsequent processing (washing, dehydration, rewashing, and mounting), a histomorphometric analysis was performed with an automated digital slide scanner (Konfoong Biotech International Co., Ltd, Ningbo, China).

Cecal short-chain fatty acid content determination

SCFAs in cecal contents were analyzed using HPLC with an ion-exclusion column. Cecal contents were collected immediately after euthanasia and stored at -70°C until analysis. For extraction, fecal samples (200 mg) were suspended in phosphoric acid–acidified deionized water (pH 2.5) to maintain SCFAs in their protonated form, followed by the addition of methanol to achieve a final concentration of 90% (v/v). The mixture was sonicated for 1 h and incubated at 4 $^{\circ}\text{C}$ overnight to enhance extraction efficiency. The extracts were centrifuged at 15 600 $\times g$ for 10 min and filtered through a 0.22 μm polyvinylidene fluoride (PVDF) syringe filter (Pall Corporation, Port Washington, NY, USA) prior to analysis. SCFA analysis was performed using an Ultimate 3000 HPLC system (Thermo Dionex, Sunnyvale, CA, USA) equipped with a refractive index (RI) detector (RefractoMAX520; ERC, Tokyo, Japan). Chromatographic separation was achieved using an ICsep Coregel-87H3 column (7.8 $\times$ 300 mm; Transgenomic Inc., Omaha, NE, USA) maintained at 40 $^{\circ}\text{C}$, with 0.01 eq L^{-1} sulfuric acid (Sigma-Aldrich) as the mobile phase at a flow rate of 0.5 mL min^{-1} . The injection volume was 10 μL . Individual SCFAs were identified by comparing retention times with those of authentic standards and quantified using external calibration curves. All samples were processed under identical conditions to ensure reliable comparison among experimental groups.

Cecal microbiome analysis

Cecal contents were collected aseptically at the end of the experimental period ($n = \text{six per group}$; total $n = 30$) from five groups (NOR, DNCB, and PHGG 100, 200, and 400 mg $\text{kg}^{-1} \text{d}^{-1}$), immediately snap-frozen, and stored at -80°C until analysis. Microbial DNA was extracted from cecal contents using the *i*-Genomic Clinic DNA Extraction Kit (iNtRON Biotechnology, Seongnam, South Korea) according to the manufacturer's instructions. 16S rRNA metagenome sequencing was conducted on an Illumina NextSeq platform with 2 $\times$ 300 bp paired-end reads (30 samples, five groups). Quality criteria for sample retention were Q30 $\geq 65\%$ and survived reads $\geq 10\,000$ read pairs. Raw FASTQ files were demultiplexed and subjected to quality control. Denoising included adapter removal, quality trimming, read-level error

correction, and chimera removal, followed by taxonomic classification with removal of mitochondrial/chloroplast-derived sequences. Representative sequences (features/ASVs) and a feature table were generated. Alpha-diversity was assessed using Shannon's diversity index with rarefaction curves, and beta-diversity was evaluated using weighted and unweighted UniFrac distances with principal coordinates analysis (PCoA). Group differences in beta-diversity were tested using permutational multivariate analysis of variance (PERMANOVA), and alpha-diversity was compared using appropriate parametric or non-parametric tests depending on data distribution. Differential taxonomic abundance was analyzed using linear discriminant analysis effect size (LEfSe), which incorporates non-parametric Kruskal–Wallis and Wilcoxon rank-sum tests. To control for multiple testing and reduce the likelihood of false positives, false discovery rate (FDR) correction was applied using the Benjamini–Hochberg method.

Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD) for *in vitro* experiments and as mean $\pm$ standard error of the mean (SEM) for *in vivo* studies. The number of biological replicates (*n*) for each experiment is indicated in the corresponding figure legends. Statistical analyses were performed using SPSS Statistics software (version 26; IBM Corp., Armonk, NY, USA). Differences among multiple groups were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's multiple range test for *post hoc* comparisons. Sample sizes were determined based on previous studies using similar experimental models and preliminary data. A formal *a priori* power analysis was not performed. All statistical tests were conducted using independent biological replicates, and a *P*-value < 0.05 was considered statistically significant.

RESULTS

Chemical characteristics and molecular weight distribution of PHGG

The monosaccharide composition of PHGG consisted primarily of mannose (64.4 mol%) and galactose (34.4 mol%), with a minor amount of glucose (1.2 mol%), yielding a mannose/galactose ratio of 1.87 (Table 1 and Fig. S2). The molecular weight profile of PHGG was further evaluated by UHPLC–MS² and MALDI-TOF-MS. The UHPLC–MS² total ion chromatogram showed a dominant peak at 0.98 min (Fig. 1(A)), and the corresponding mass spectrum displayed a series of ions consistent with oligosaccharide species (Fig. 1(B)). MALDI-TOF-MS analysis indicated that PHGG

predominantly contained low-mass oligosaccharides, with major signals observed below *m/z* 2000, suggesting a distribution of galactomannan-derived oligomers with varying degrees of polymerization (Fig. 1(C)).

Inhibitory effects of PHGG on pro-inflammatory responses and barrier dysfunction *in vitro*

To define a non-cytotoxic concentration range, cell viability was assessed after PHGG treatment in HaCaT (Fig. 2(A)) and Caco-2 (Fig. 3(A)) cells. PHGG up to 400 $\mu\text{g mL}^{-1}$ showed no apparent cytotoxicity in either model, with viability remaining $\geq 97\%$ relative to the stimulated control. Therefore, concentrations up to 400 $\mu\text{g mL}^{-1}$ were used for subsequent experiments. In TNF- α and IFN- γ (T + I)-stimulated HaCaT cells, T + I markedly increased the secretion of IL-6 (6.9-fold), IL-8 (6.5-fold), and MCP-1 (16.6-fold) compared with the unstimulated control (Fig. 2(B)). PHGG significantly and dose-dependently suppressed these responses. At 400 $\mu\text{g mL}^{-1}$, PHGG reduced IL-6 from 244.5 to 96.3 pg mL^{-1} (60.6% reduction), IL-8 from 1434.2 to 673.1 pg mL^{-1} (53.1% reduction), and MCP-1 from 4293.6 to 1307.8 pg mL^{-1} (69.5% reduction) *versus* the T + I-treated group. Consistently, PHGG treatment (100–400 $\mu\text{g mL}^{-1}$) significantly down-regulated the messenger RNA (mRNA) expression of atopic inflammation-related mediators, including *MDC1* (86.5–93.6% inhibition), *RANTES* (65.5–96.9% inhibition), *TARC* (72.6–95.9% inhibition), *MDC* (60.6–90.8%), and *TSLP* (74.0–96.4% inhibition), compared to the T + I control group (Fig. 2(B)).

In TNF- α -stimulated Caco-2 cells, TNF- α significantly increased IL-8 and MCP-1 secretion by 5.3- and 5.0-fold, respectively (Fig. 3(A)). PHGG significantly suppressed both cytokines, with inhibition rates of 28.4–90.1% for IL-8 and 18.9–72.9% for MCP-1 across 100–400 $\mu\text{g mL}^{-1}$. Moreover, TNF- α down-regulated barrier-associated gene expression (*TJP1*, *TJP2*, *OCLN*, *CLDN1*, and *MUC2*) to 0.41–0.50-fold relative to the unstimulated control (Fig. 3(B)). PHGG treatment significantly improved this barrier dysfunction. Specifically, PHGG treatment significantly recovered the expression of *TJP1* (7.9–32.2%), *TJP2* (9.5–28.9%), *OCLN* (22.1–31.2%), and *CLDN1* (14.4–42.9%) in a concentration-dependent manner, compared to the TNF- α control group.

Effects of oral PHGG on AD-like skin lesions and safety-related indices in DNCB-induced AD mice

Representative dorsal skin images showed that repeated DNCB application induced pronounced AD-like lesions (erythema, scaling, and crust formation) at 5 and 9 days after stimulation, whereas PHGG-treated groups displayed visually attenuated lesion severity, with a clearer improvement trend at 100–400 $\text{mg kg}^{-1} \text{d}^{-1}$ (Fig. 4(A)). Body weight trajectories remained comparable across all groups throughout the 26-day experimental period, indicating no apparent effect of PHGG on body weight gain (Fig. 4(B)). At study termination, major organ weights (heart, kidney, liver, and spleen) did not show PHGG-associated increases compared with controls; notably, liver weight in PHGG-treated groups was comparable to NOR and significantly lower than the DNCB group ($P < 0.05$), while heart, kidney, and spleen weights were largely within the control range without a consistent dose-dependent pattern (Fig. 4(C)).

PHGG ameliorates DNCB-induced histopathological alterations in dorsal skin

Histological staining (H&E for epidermal thickness and inflammatory cell infiltration, Masson's Trichrome for dermal collagen

Table 1. Monosaccharide composition analysis of partially hydrolyzed guar gum (PHGG)

Type	Monosaccharide	Mol%
Pentose	Arabinose	—
	Xylose	—
Deoxyhexose	Rhamnose	—
	Fucose	—
Hexose	Glucose	1.2 $\pm$ 0.0
	Galactose	34.4 $\pm$ 2.8
	Mannose	64.4 $\pm$ 4.5
Uronic acid	Glucuronic acid	—
	Galacturonic acid	—

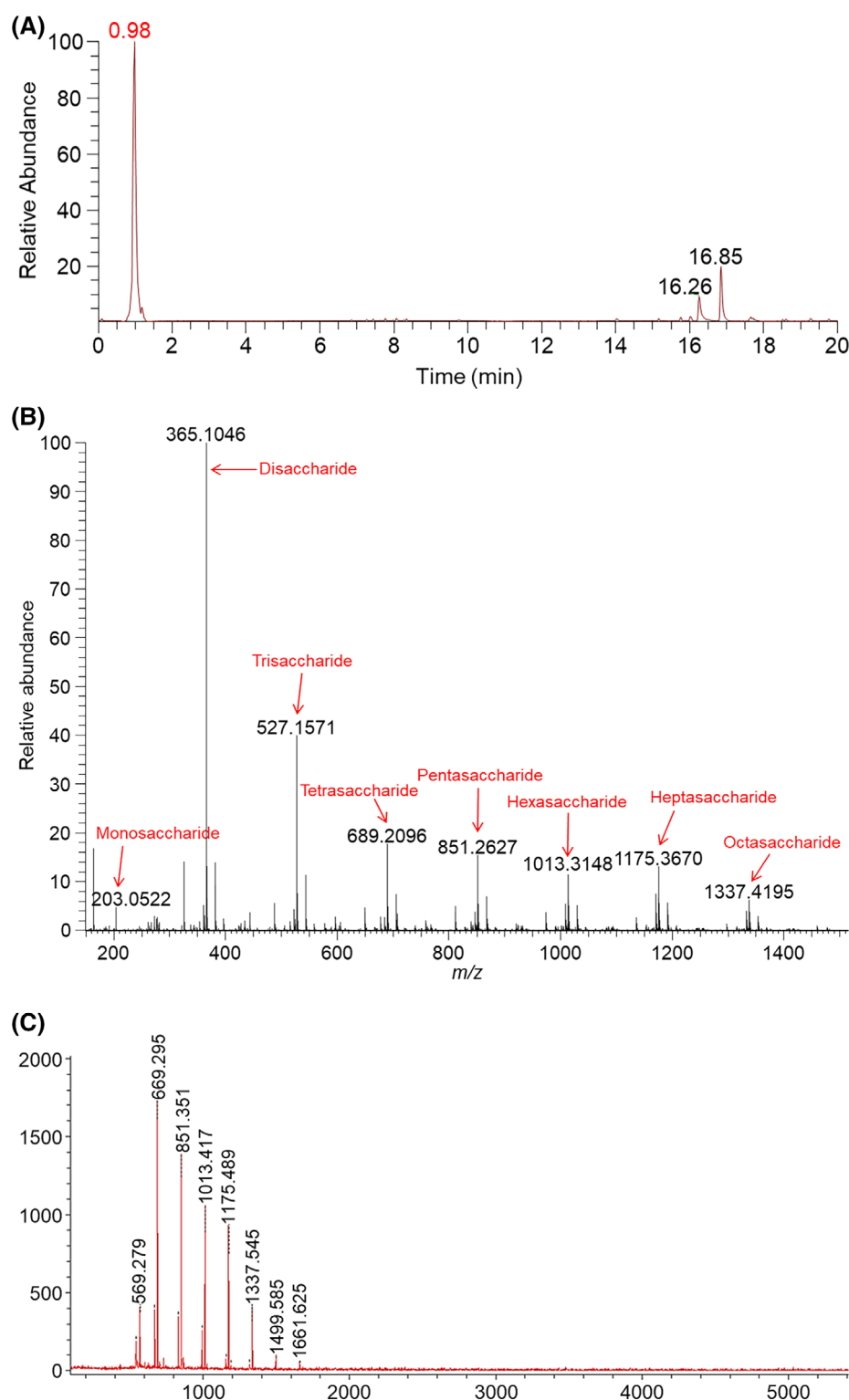


Figure 1. Molecular weight distribution analysis of PHGG determined by UHPLC-MS² and MALDI-TOF-MS. (A) Total ion chromatogram (TIC) of PHGG obtained by UHPLC-MS² analysis. (B) MS mass spectrum corresponding to the dominant TIC peak at 0.98 min. (C) MALDI-TOF-MS mass spectrum of PHGG.

distribution, and Toluidine Blue for mast cell infiltration) showed that DNCB challenge markedly induced epidermal hyperplasia with inflammatory cell infiltration, increased dermal collagen-occupied region, and increased mast cell accumulation, whereas these pathological changes were attenuated by oral PHGG administration (Fig. 5(A)). Consistent with these observations,

quantitative analyses (Fig. 5(B)) indicated that DNCB significantly increased epidermal thickness (20.6 μm in NOR *versus* 143.7 μm in DNCB), while PHGG reduced epidermal thickness variably across doses (100.2, 58.5, and 52.9 μm at 100, 200, and 400 mg kg^{-1} , respectively). Dermal collagen-occupied region was significantly increased by DNCB (0.24 mm^2 *versus* 1.33 mm^2)

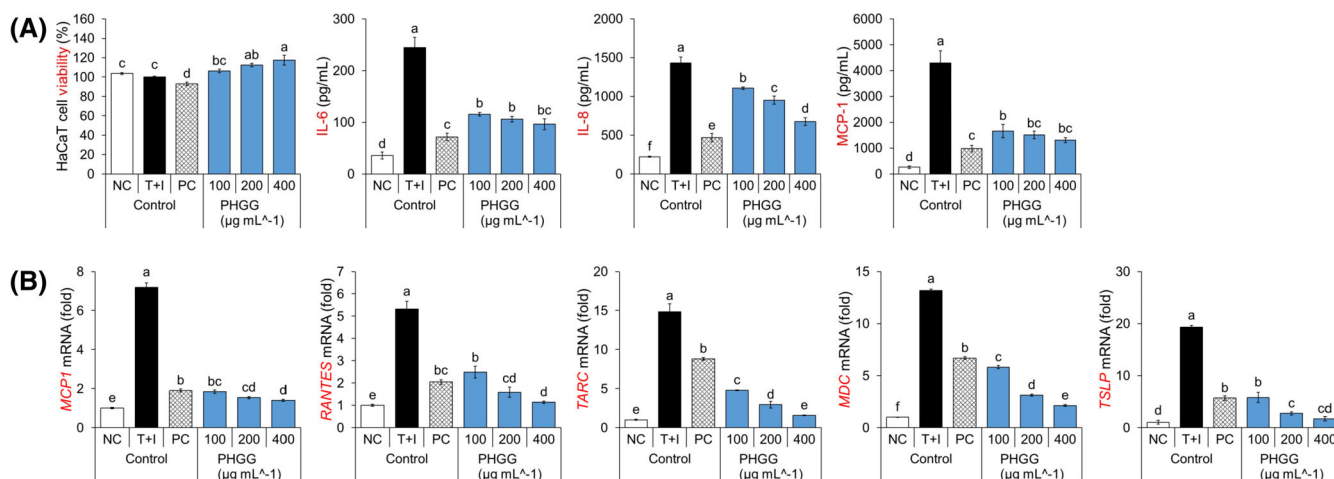


Figure 2. Effects of PHGG on inflammatory responses in a keratinocyte cell model under pro-inflammatory conditions. HaCaT keratinocytes were treated with PHGG, followed by stimulation with TNF- α and IFN- γ (T + I). (A) Cell viability was assessed using the MTT assay, and inflammatory cytokine secretion were measured by ELISA. (B) Messenger RNA expression levels of inflammatory mediators were analyzed by qPCR. Data are presented as mean $\pm$ SD ($n = 3$). Different letters indicate statistically significant differences among groups ($P < 0.05$, Tukey's multiple range test).

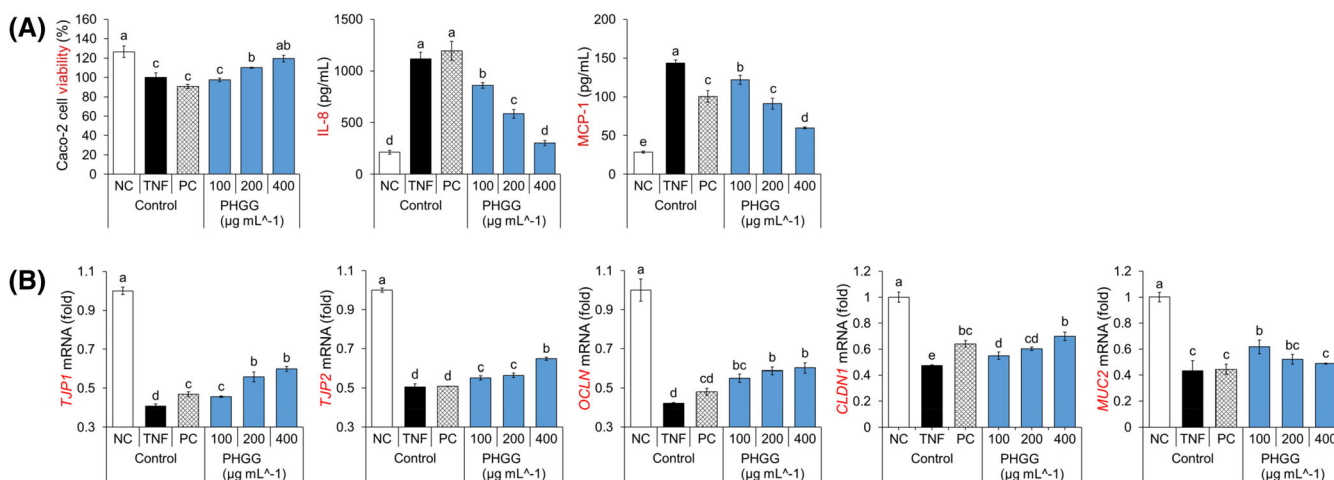


Figure 3. Effects of PHGG on inflammatory responses in an intestinal epithelial cell model under pro-inflammatory conditions. Caco-2 intestinal epithelial cells were treated with PHGG, followed by stimulation with TNF- α (TNF). (A) Cell viability was assessed using the MTT assay, and inflammatory cytokine secretion were measured by ELISA. (B) Messenger RNA expression levels of tight junction- and mucus-related gene were analyzed by qPCR. Data are presented as mean $\pm$ SD ($n = 3$). Different letters indicate statistically significant differences among groups ($P < 0.05$, Tukey's multiple range test).

and was significantly restored by PHGG in a dose-dependent manner (31.3–73.7% decreases). Mast cell numbers increased from 1.8 to 35.0 cells mm^{-2} and were significantly reduced by PHGG administration to 17.4 and 10.0 cells mm^{-2} at 200 and 400 mg kg^{-1} , respectively.

PHGG modulated serum immunoglobulin and cytokine profiles in DNCB-induced AD mice

DNCB challenge significantly increased circulating immunoglobulins, elevating total IgG (1900.9 $\mu\text{g mL}^{-1}$ in NOR $\rightarrow$ 7457.9 $\mu\text{g mL}^{-1}$ in DNCB), IgG1 (1.2 $\rightarrow$ 2.6 $\mu\text{g mL}^{-1}$), IgG2a (0.8 $\rightarrow$ 1.7 $\mu\text{g mL}^{-1}$), and IgE (0.1 $\rightarrow$ 16.1 $\mu\text{g mL}^{-1}$) (Fig. 6(A)). Oral PHGG administration markedly attenuated these elevations, reducing total IgG (7109.2–5492.6 $\mu\text{g mL}^{-1}$), IgG1 (1.4–1.0 $\mu\text{g mL}^{-1}$), IgG2a (1.0–0.8 $\mu\text{g mL}^{-1}$), and IgE (2.6–2.4 $\mu\text{g mL}^{-1}$). Consistent with systemic immune activation, DNCB induction significantly increased multiple serum cytokines, including IL-1 β (0.1 $\rightarrow$ 1.8 pg mL^{-1}), IL-4 (4.7 $\rightarrow$ 25.8 pg mL^{-1}), IL-5

(0.8 $\rightarrow$ 11.1 pg mL^{-1}), IL-6 (10.7 $\rightarrow$ 17.2 pg mL^{-1}), TNF- α (0.1 $\rightarrow$ 1.7 pg mL^{-1}), MCP-1 (35.4 $\rightarrow$ 84.7 pg mL^{-1}), and IFN- γ (14.2 $\rightarrow$ 37.0 pg mL^{-1}) (Fig. 6(B)). PHGG supplementation significantly reduced circulating IL-4 (6.9–9.0 pg mL^{-1}), IL-6 (11.0–13.0 pg mL^{-1}), and IFN- γ (19.2–22.7 pg mL^{-1}) across doses, and the 400 mg kg^{-1} PHGG group showed the most pronounced suppression of IL-5 (3.3 pg mL^{-1}) and MCP-1 (58.6 pg mL^{-1}) relative to DNCB. For IL-1 β , reductions were evident at $\geq 200 \text{ mg kg}^{-1}$ (0.8–1.0 pg mL^{-1}), whereas 100 mg kg^{-1} did not differ significantly from the DNCB group. TNF- α showed a decreasing trend following PHGG administration, although the difference did not reach statistical significance at any dose tested.

PHGG alleviated local inflammatory responses in skin and colon tissues of DNCB-induced AD mice

To examine whether oral PHGG is associated with coordinated immune changes in the skin and colon, key mediators were quantified in skin (Fig. 7(A)) and colonic tissues (Fig. 7(B)). In the skin,

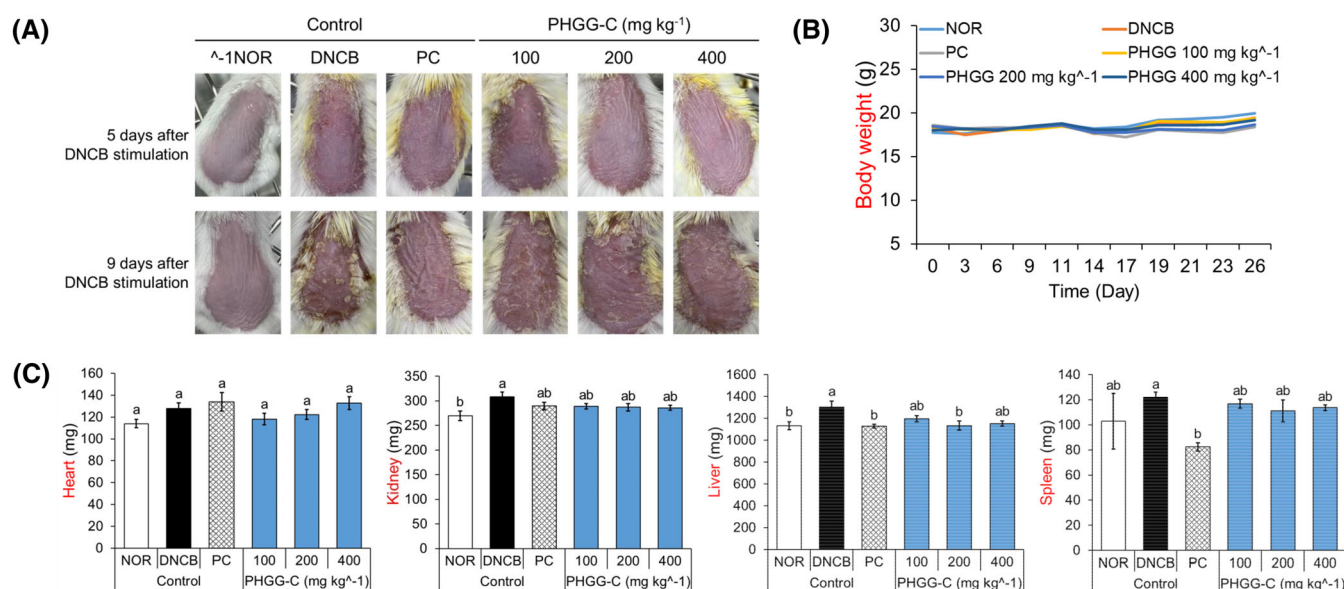


Figure 4. Effects of oral administration of PHGG on skin lesions, body weight, and organ weights in DNCB-induced AD BALB/c mice. (A) Representative photographs of dorsal skin lesions were monitored at 5 and 9 days after DNCB stimulation. (B) Body weight was monitored every 3 days throughout the experimental period in normal control (NOR), DNCB-treated control (DNCB), dexamethasone-administered positive control (PC), and PHGG-administered groups. (C) Weights of major organs, including heart, kidney, liver, and spleen, were measured at the end of the experimental period. Data are presented as mean $\pm$ SEM ($n =$ six per group). Different letters indicate statistically significant differences among groups ($P < 0.05$, Tukey's multiple range test).

DNCB challenge markedly increased Th2-associated mediators, including IL-4 ($1.3 \rightarrow 3.1 \mu\text{g g}^{-1}$), IL-5 ($0.2 \rightarrow 2.0 \mu\text{g g}^{-1}$), and MCP-1 ($3.5 \rightarrow 12.5 \mu\text{g g}^{-1}$), compared with the NOR group. Oral PHGG supplementation produced a marker-specific response: IL-4 remained elevated at 100 and 200 mg kg⁻¹ and was significantly reduced only at 400 mg kg⁻¹ ($1.8 \mu\text{g g}^{-1}$), while MCP-1 was decreased at 100 mg kg⁻¹ ($10.5 \mu\text{g g}^{-1}$) and further reduced at 400 mg kg⁻¹ ($7.9 \mu\text{g g}^{-1}$). Skin IL-5 showed a partial decrease at 100 mg kg⁻¹ but did not significantly differ from the DNCB control. In parallel, DNCB challenge disrupted mucosal immune homeostasis in the colon, evidenced by reduced IgA ($109.6 \rightarrow 37.0 \text{ mg g}^{-1}$) and increased IL-1 β ($3.7 \rightarrow 4.3 \mu\text{g g}^{-1}$) and IL-6 ($8.4 \rightarrow 19.8 \mu\text{g g}^{-1}$). PHGG significantly restored colonic IgA ($53.9\text{--}82.1 \text{ mg g}^{-1}$) across doses and reduced IL-1 β (3.8 , 3.3 , and $2.6 \mu\text{g g}^{-1}$ at 100, 200, and 400 mg kg⁻¹, respectively). Colonic IL-6 was significantly suppressed at 200–400 mg kg⁻¹ (12.2 and $8.4 \mu\text{g g}^{-1}$), whereas 100 mg kg⁻¹ showed no difference from the DNCB group. Collectively, these parallel tissue-level changes are consistent with a potential gut-skin immune linkage, although causal relationships require further mechanistic confirmation.

PHGG modulates cecal microbiota signatures and community structure in DNCB-induced AD mice

To determine whether DNCB-induced AD is accompanied by gut microbial alterations and whether oral PHGG modulates these changes, cecal 16S rRNA sequencing data were analyzed using LEfSe (Fig. 8) and diversity metrics (Figs 9 and 10). LEfSe identified taxa that significantly discriminated among groups and highlighted group-specific microbial signatures, while the cladogram visualized phylogenetic lineages enriched in each group (Fig. 8). The NOR group was characterized by *Firmicutes*-associated lineages, including *Lachnospiraceae* and *Ruminococcaceae*, with enrichment of *Roseburia*. In contrast, the DNCB group showed increased abundance of taxa associated with dysbiosis,

including *Deferribacteres*-related lineages (e.g., *Deferribacterales/Deferribacteraceae*, *Mucispirillum*), *Prevotellaceae*-associated taxa, and *Cyanobacteria*-related features. PHGG supplementation produced dose-dependent microbial signatures: the 100 mg kg⁻¹ group was associated with *Bacteroidaceae/Bacteroides* and additional discriminative taxa, whereas the 200 and 400 mg kg⁻¹ groups were enriched in *Bacteroidales*-related lineages such as *Muribaculaceae*, *Alistipes*, and *Rikenellaceae*. Across alpha-diversity indices, Shannon and Simpson values were largely maintained among groups, suggesting no overt collapse of community diversity (Fig. 9). Richness- and phylogenetic breadth-related indices (observed features, Chao1, and Faith's phylogenetic diversity) showed a modest decreasing tendency in the DNCB group, with partial recovery trends in PHGG-treated groups. Beta-diversity assessed by PCoA demonstrated clear separation between NOR and DNCB communities (Fig. 10(A)–(C)). PHGG-treated groups displayed a distinct community distribution from DNCB controls and showed a directional shift toward the NOR cluster across Bray-Curtis (abundance-based) and UniFrac (phylogeny-based) distance metrics, indicating that PHGG partially remodeled DNCB-associated microbial community restructuring.

PHGG modulates cecal SCFA profiles in DNCB-induced AD mice

To investigate whether PHGG influences gut microbial metabolic outputs associated with the gut-skin axis, cecal SCFAs were quantified (Table 2 and Fig. S3). DNCB challenge markedly increased cecal SCFA levels compared with the NOR group. Acetic acid increased from 356.2 to $565.2 \mu\text{g mL}^{-1}$ (~ 1.6 -fold), while butyric acid increased from 241.4 to $393.1 \mu\text{g mL}^{-1}$ (~ 1.6 -fold). The PC group exhibited the highest acetic acid ($649.2 \mu\text{g mL}^{-1}$) and butyric acid ($500.3 \mu\text{g mL}^{-1}$). Oral PHGG supplementation modulated these elevated SCFA levels in a dose-dependent or dose-responsive manner. Acetic acid was reduced across all doses ($466.8\text{--}423.4 \mu\text{g mL}^{-1}$), showing a dose-dependent trend, while

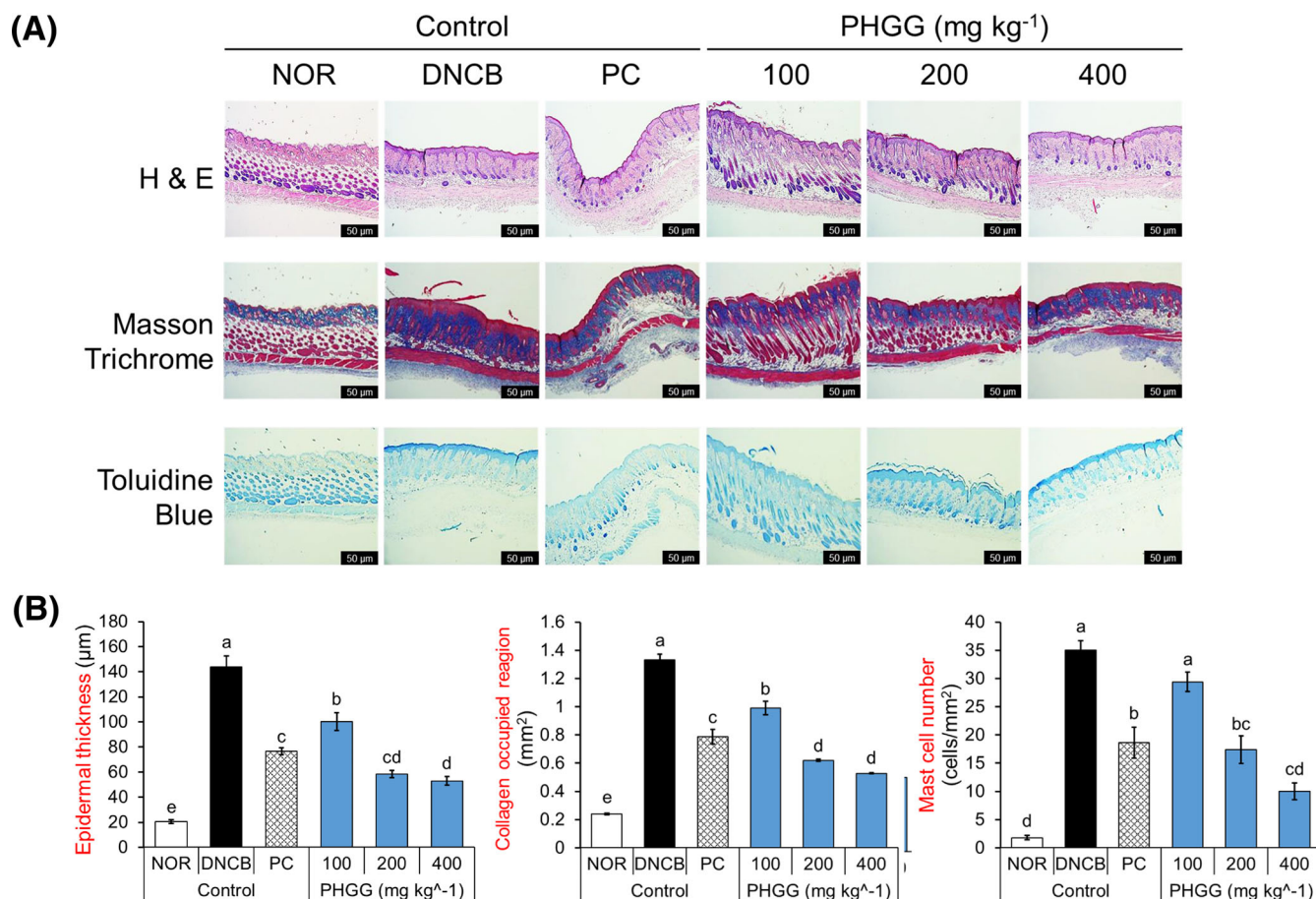


Figure 5. Histological evaluation of skin tissue in DNCB-induced AD BALB/c mice following oral administration of PHGG. (A) Representative histological images of dorsal skin sections stained with hematoxylin and eosin (H&E), Masson's Trichrome, and Toluidine Blue. (B) Quantitative analysis of epidermal thickness, collagen area (percent of dermis), and mast cell number (cells mm⁻²) based on the corresponding histological sections. Scale bar = 100 µm. Data are presented as mean ± SEM (*n* = three per group). Normal control (NOR), DNCB-treated control (DNCB), dexamethasone-administered positive control (PC), and PHGG-administered groups. Different letters indicate statistically significant differences among groups (*P* < 0.05, Tukey's multiple range test).

butyric acid exhibited a partial normalization, with the 100 mg kg⁻¹ group approaching NOR levels and higher doses showing intermediate values (282.2–337.5 µg mL⁻¹).

DISCUSSION

The present study demonstrates that oral administration of PHGG mitigates AD-like phenotypes in a DNCB-induced mouse model, accompanied by coordinated changes in cutaneous inflammation, systemic immune markers, and the intestinal ecosystem. Collectively, the data suggest that PHGG may act through a multi-level mechanism that includes reinforcement of intestinal barrier-related programs, attenuation of Th2-skewed immune responses, and modulation of gut microbial community structure. Using integrated *in vitro* (keratinocyte and intestinal epithelial models) and *in vivo* (DNCB-induced AD mice) approaches, we evaluated epithelial inflammatory responses, barrier-associated markers, systemic and tissue immune mediators, and cecal microbiota profiles to assess the potential relevance of a gut–skin axis framework.

The compositional and structural characteristics of the PHGG used in this study support that it retains a galactomannan backbone (mannose-dominant with substantial galactose) while being

partially depolymerized (Table 1 and Fig. 1).^{13,15,24} Consistent with this, the MS profiles indicated that the preparation was enriched in relatively low-mass oligosaccharide species (predominantly below *m/z* 2000), suggesting a substrate pool with potentially enhanced fermentability compared with higher-molecular-weight PHGG preparations. This feature may represent an important point of differentiation from conventional PHGG formulations frequently reported in clinical and prebiotic studies (often described as having average molecular weights in the ~20–50 kDa range),^{15,25} the present PHGG formulation exhibited a substantially lower molecular weight distribution enriched in oligosaccharide species. From a functional standpoint, a 'lower-viscosity/higher-fermentability' profile provides a plausible mechanistic basis for how PHGG could influence mucosal immunity and microbiota composition at physiologically feasible doses.^{13,14,26} However, because the present study did not directly quantify fermentation outputs (e.g., SCFAs), this interpretation should be regarded as a biologically plausible hypothesis that warrants direct experimental validation rather than a confirmed pathway within this work.¹⁹

The *in vitro* results further suggest that PHGG can directly modulate inflammatory and barrier-associated responses in both keratinocytes and intestinal epithelial cells, providing a mechanistic

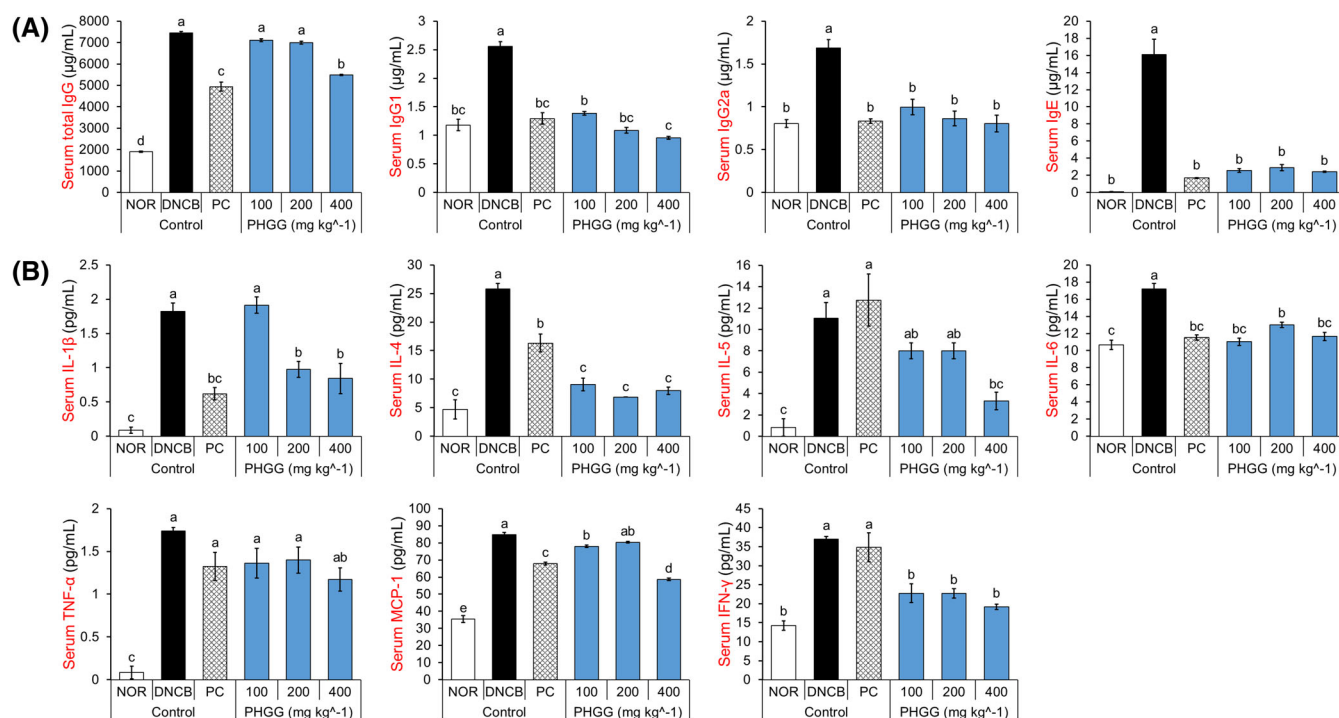


Figure 6. Effects of oral administration of PHGG on serum levels of (A) immunoglobulins and (B) cytokines in DNCB-induced AD BALB/c mice. Serum levels of immunoglobulins (total IgG, IgG1, IgG2a, and IgE) and inflammatory mediators (IL-1 β , IL-4, IL-5, IL-6, TNF- α , MCP-1, and IFN- γ) were quantified by ELISA at the end of the experimental period in normal control (NOR), DNCB-treated control (DNCB), dexamethasone-administered positive control (PC), and PHGG-administered groups. Data are presented as mean $\pm$ SEM ($n =$ three per group). Different letters indicate statistically significant differences among groups ($P < 0.05$, Tukey's multiple range test).

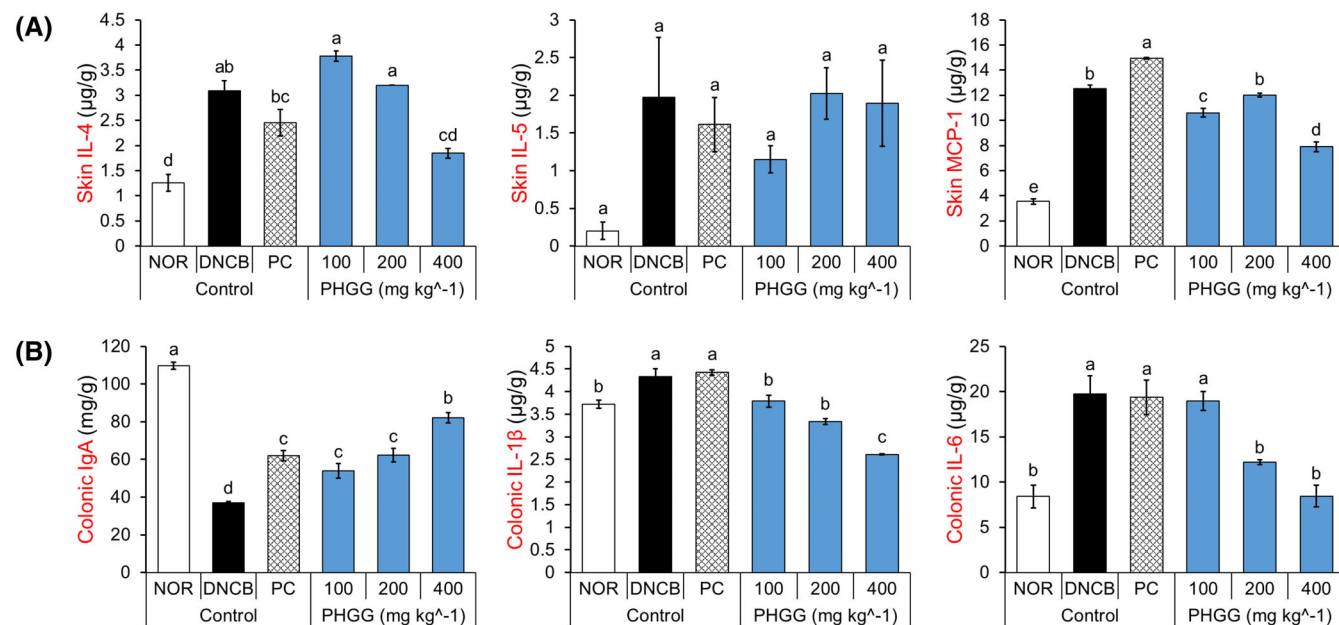


Figure 7. Effects of oral administration of PHGG on cutaneous Th2-associated mediators and colonic mucosal immune/inflammatory markers in DNCB-induced AD BALB/c mice. (A) Skin tissue levels of Th2-related cytokines and chemokines, including IL-4, IL-5, and MCP-1, were quantified by ELISA at the end of the experimental period in normal control (NOR), DNCB-treated control (DNCB), dexamethasone-administered positive control (PC), and PHGG-administered groups. (B) Colonic tissue levels of mucosal IgA and inflammatory cytokines (IL-1 β and IL-6) were measured by ELISA using colonic tissue extracts collected at termination from the same experimental groups. Data are presented as mean $\pm$ SEM ($n =$ three per group). Different letters indicate statistically significant differences among groups ($P < 0.05$, Tukey's multiple range test).

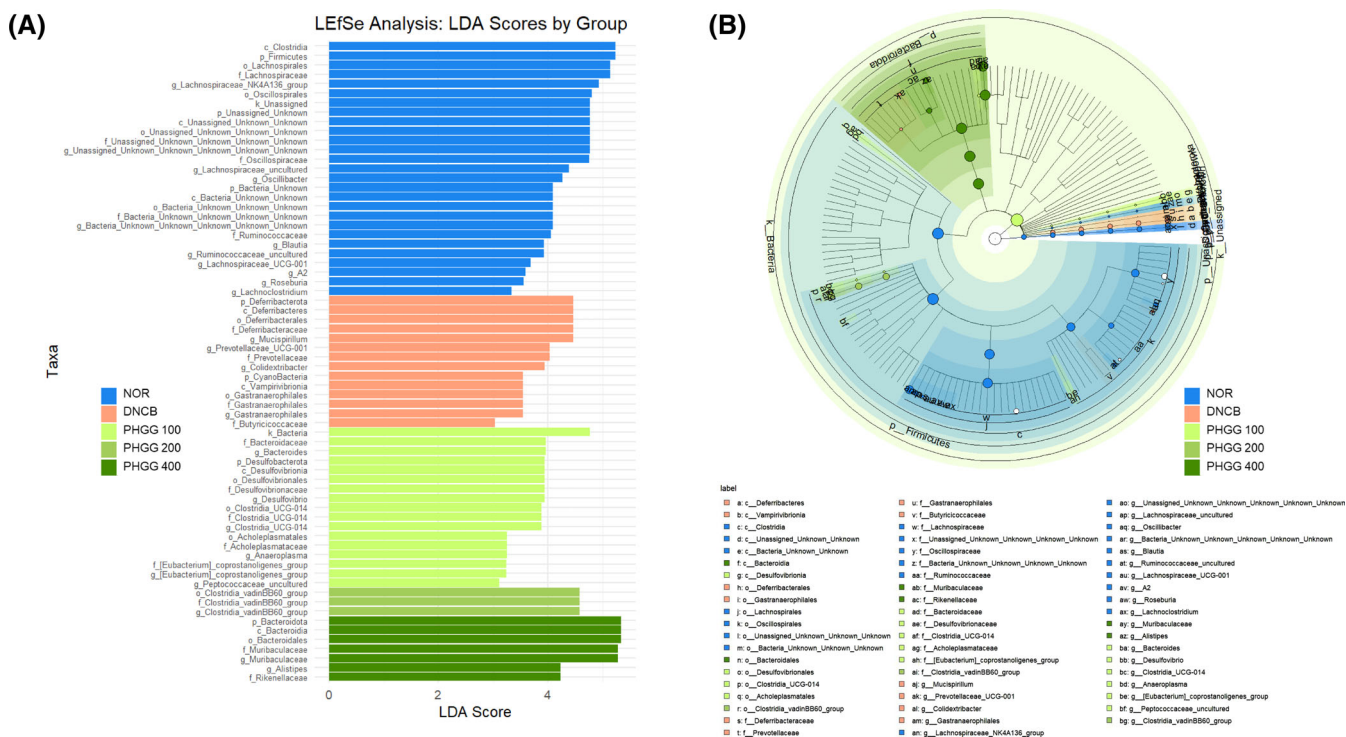


Figure 8. Linear discriminant analysis effect size (LEfSe) analysis of cecal microbiota in DNCB-induced AD BALB/c mice following oral administration of PHGG. (A) Bar plot showing differentially abundant bacterial taxa identified by LEfSe. Taxa with significant differences among experimental groups are displayed according to their linear discriminant analysis (LDA) scores, which indicate the magnitude of each discriminative feature. (B) LEfSe cladogram illustrating the phylogenetic relationships of significantly enriched taxa from phylum to genus levels. Colored nodes represent taxa that are significantly associated with each experimental group, including the normal control (NOR), DNCB-treated control (DNCB), and PHGG-administered groups. Node size reflects relative abundance, and colors indicate the group in which each taxon is most enriched. Only taxa meeting the predefined statistical significance and LDA score thresholds are shown. Statistical analysis was performed using $n =$ six samples per group. Taxa shown met the predefined significance threshold based on LEfSe analysis.

foundation relevant to the gut–skin axis concept in AD. Prior PHGG studies have largely emphasized gastrointestinal outcomes, including modulation of the gut microbiota,^{17,19} symptomatic improvement in IBS,^{14,18} and barrier-associated effects in inflammatory bowel models.²⁷ In contrast, our data (Figs 2 and 3) demonstrate concurrent anti-inflammatory effects in a keratinocyte model and barrier-supporting effects in an intestinal epithelial model within a unified experimental framework. In T + I-stimulated HaCaT cells, PHGG significantly reduced secretion of inflammatory mediators and down-regulated AD-relevant epithelial chemokines (*TARC/CCL17*, *MDC/CCL22*, and *RANTES/CCL5*) as well as *TSLP*, a cytokine implicated in initiating Th2 polarization through dendritic cell activation and contributing to the ‘atopic march’.^{28,29} Specifically, *TSLP* is recognized as a pivotal initiator of the atopic march, activating dendritic cells to drive Th2 cell differentiation and chronic itch.³⁰ In parallel, *TNF- α* alone was used in the Caco-2 model to reflect low-grade inflammatory conditions relevant to epithelial barrier dysfunction, whereas combined cytokine stimulation is more representative of severe intestinal inflammation. In *TNF- α* -challenged Caco-2 cells, PHGG partially restored expression of tight junction-related genes (*TJP1/2*, *OCLN*, *CLDN1*) and a mucus-related marker (*MUC2*), suggesting protection against inflammation-associated barrier impairment. The modest recovery observed suggests that PHGG may exert a direct but limited epithelial modulatory effect *in vitro*. Importantly, these epithelial effects were observed in the absence of live microbiota, indicating that PHGG may exert at least part of its activity through microbiota-independent mechanisms, potentially complementing

its well-characterized fermentation-dependent actions.^{19,31} These results provide preliminary *in vitro* support for the hypothesis that orally administered PHGG may influence cutaneous inflammation via the gut–skin axis. However, while the observed dual epithelial effects are mechanistically plausible, definitive validation of PHGG’s ability to modulate cutaneous inflammation through gut-mediated pathways required *in vivo* experimentation.

In vivo, PHGG administration attenuated the clinical and histopathological severity of AD-like dermatitis, supporting its potential efficacy in a systemic context. Using body surface-based dose conversion (Reagan-Shaw method), the highest dose used in this study ($400 \text{ mg kg}^{-1} \text{ d}^{-1}$) corresponds to approximately 2.24 g d^{-1} for a 70 kg adult. This dosage is consistent with human studies demonstrating biologically meaningful effects of low-dose PHGG supplementation.^{32,33} The stability of body weight trajectories and the absence of adverse shifts in major organ weights (Fig. 4) are consistent with the expected safety profile of PHGG as a dietary macromolecule, an important consideration for long-term functional food applications.^{13,18} PHGG administration significantly attenuated clinical AD manifestations (erythema, scaling, crust formation), reduced epidermal hyperplasia, restored dermal collagen integrity, and suppressed mast cell infiltration – all hallmark features of AD pathogenesis.^{1,3} Our data demonstrate that PHGG supplementation reversed the thickening across the administered doses, thereby partially restoring it toward a near-normal physiological state (Fig. 5). This structural recovery was further corroborated by Masson’s Trichrome staining, where PHGG partially restored dermal collagen distribution,

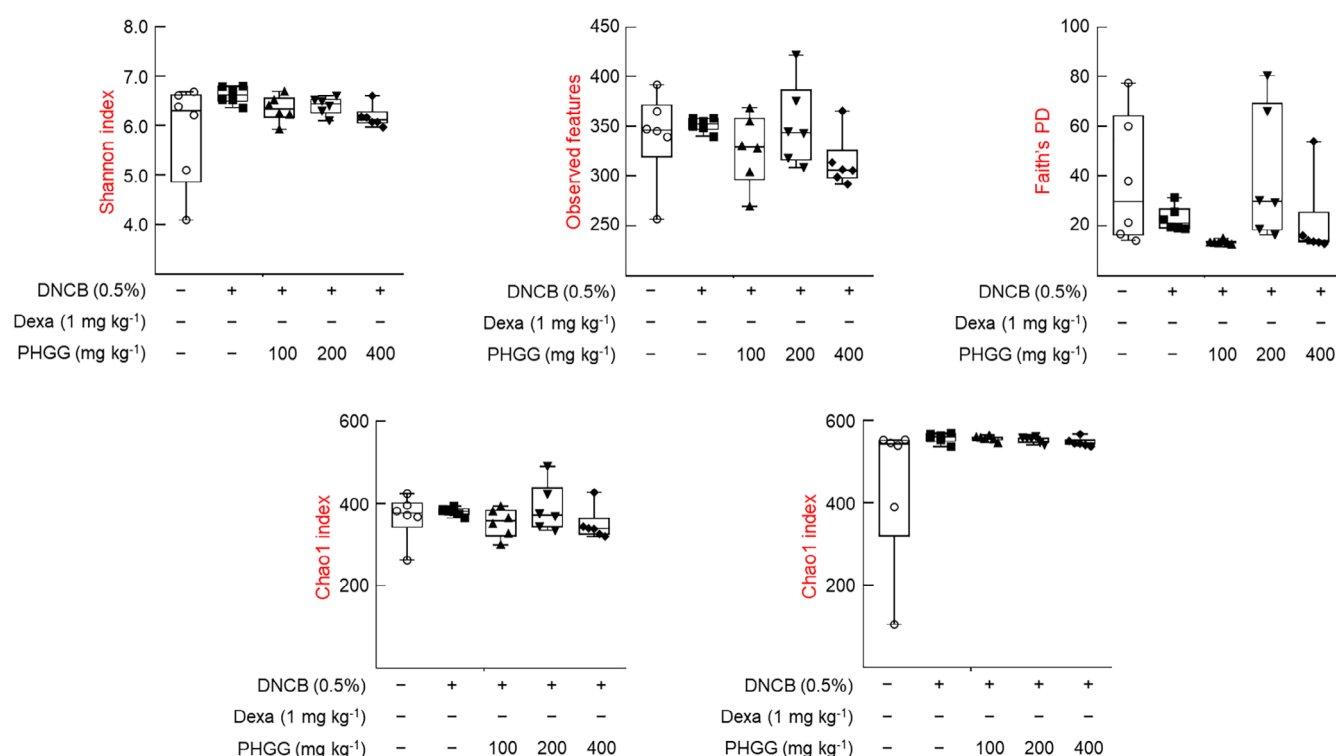


Figure 9. Alpha-diversity analysis of cecal microbiota in DNCB-induced AD BALB/c mice following oral administration of PHGG. Alpha-diversity of the cecal microbiota was assessed using the Shannon index, observed features, Faith's phylogenetic diversity (PD), Chao1 index, and Simpson index. Box plots depict the distribution of each alpha-diversity metric across experimental groups, including the normal control (NOR), DNCB-treated control (DNCB), and PHGG-administered groups. Alpha-diversity metrics were calculated using $n =$ six samples per group.

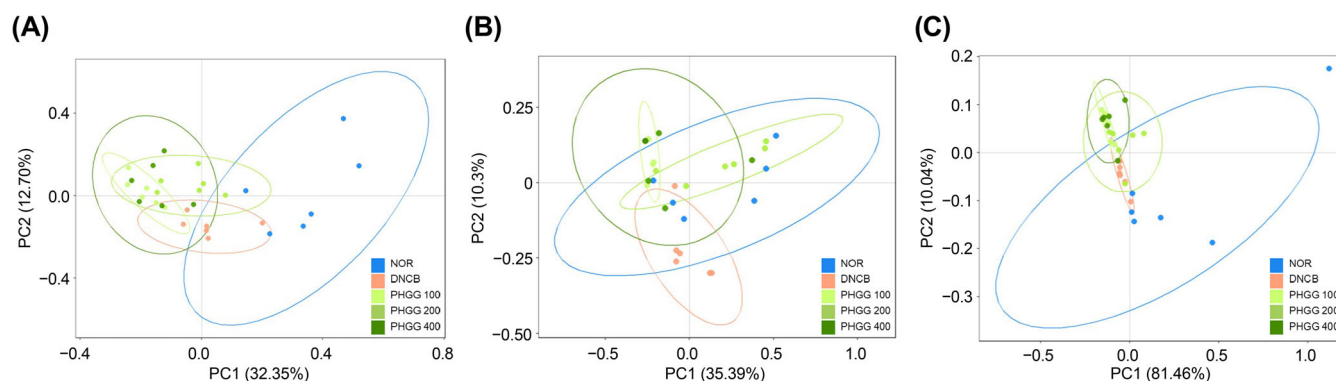


Figure 10. Beta-diversity analysis ((A) Bray-Curtis principal coordinate analysis (PCoA); (B) unweighted UniFrac PCoA; (C) weighted UniFrac PCoA) of cecal microbiota in DNCB-induced AD BALB/c mice following oral administration of PHGG. Beta-diversity of the cecal microbiota was evaluated using PCoA based on distance metrics. Each point represents an individual sample, and clustering patterns reflect differences in microbial community composition among experimental groups, including the normal control (NOR), DNCB-treated control (DNCB), and PHGG-administered groups. Ellipses indicate the 95% confidence interval for each group. Beta-diversity analysis was performed using $n =$ six samples per group. PC2 (10.04%).

indicating a suppression of chronic dermal remodeling and fibrotic changes.^{1,34} The significant reduction in mast cell numbers – a critical cellular mediator of AD pruritus and inflammation – suggests that PHGG may modulate not only innate immune responses but also adaptive Th2-skewed immunity, which drives mast cell recruitment and degranulation in AD lesions.^{35,36} Crucially, the significant reduction in Toluidine Blue-stained mast cells highlights a potent anti-allergic effect. Since mast cell degranulation is the primary driver of pruritus and the subsequent itch-scratch cycle that exacerbates AD, this cellular-level inhibition suggests that PHGG effectively disrupts the core

inflammatory loop of AD.³⁷ This partial restoration likely reflects the multifactorial nature of barrier disruption in AD.

The systemic and tissue-specific immune profiles observed in this study provide strong evidence for a coordinated immunoregulatory mechanism mediated by oral PHGG across the gut-skin axis. PHGG significantly reduced DNCB-induced elevations in circulating immunoglobulins (total IgG, IgG1, IgG2a, and IgE; Fig. 6 (A)) and pro-inflammatory cytokines (IL-1 β , IL-4, IL-5, IL-6, TNF- α , MCP-1, and IFN- γ ; Fig. 6(B)), while concurrently suppressing Th2-associated mediators (IL-4, IL-5, and MCP-1; Fig. 7(A)) in skin tissue and restoring mucosal immune homeostasis in the colon

Table 2. Effects of oral administration of partially hydrolyzed guar gum (PHGG) on cecal short chain fatty acids (SCFAs) in 2,4-dinitrochlorobenzene (DNCB)-induced atopic dermatitis (AD) BALB/c mice

	Type	Acetic acid ($\mu\text{g mL}^{-1}$)	Butyric acid ($\mu\text{g mL}^{-1}$)
Control	NOR	356.2 $\pm$ 2.5 ^e	241.4 $\pm$ 1.4 ^e
	DNCB	565.2 $\pm$ 47.5 ^b	393.1 $\pm$ 2.6 ^b
	PC	649.2 $\pm$ 8.9 ^a	500.3 $\pm$ 16.2 ^a
PHGG	100 mg kg ⁻¹	466.8 $\pm$ 17.4 ^c	248.9 $\pm$ 1.2 ^e
	200 mg kg ⁻¹	450.1 $\pm$ 8.5 ^c	282.2 $\pm$ 18.0 ^d
	400 mg kg ⁻¹	423.4 $\pm$ 9.1 ^d	337.5 $\pm$ 36.0 ^c

Note: Levels of SCFAs were quantified by high-performance liquid chromatography refractive index detector (HPLC-RID) at the end of the experimental period in normal control (NOR), DNCB-treated control (DNCB), dexamethasone-administered positive control (PC), and PHGG-administered groups. The data represent a single experiment that is representative of three independent replicates and are expressed as the mean $\pm$ standard deviation ($n = 3$). Different letters indicate statistically significant differences among groups ($P < 0.05$, Tukey's multiple range test).

(increased IgA, reduced IL-1 β and IL-6; Fig. 7(B)). Given that Th2-skewed humoral activation – particularly elevated IgE and IgG1 – is a hallmark of AD,^{3,38} the observed suppression of IgE together with reductions in IL-4 and IL-5 is consistent with attenuation of the allergic cascade.³⁹ Moreover, the parallel reduction of MCP-1 across serum and tissues suggests a broader dampening of chemokine-driven inflammatory cell recruitment.⁴⁰ Crucially, the simultaneous modulation of colonic mucosal immunity and cutaneous inflammation highlights the 'gut-derived' nature of PHGG's efficacy. The sharp decline in colonic IgA in DNCB-treated mice reflects a compromised mucosal defense system, which is often linked to increased intestinal permeability and systemic immune hyper-reactivity.⁴¹ These coordinated skin and gut immune changes are consistent with, but do not by themselves prove, a gut-skin axis linkage; identification of mediators connecting the gut to distal skin (e.g., SCFAs, Treg induction, or barrier-derived signals) will be necessary to establish causality.^{5,42}

While PHGG attenuates inflammatory signaling, it may not directly suppress all Th2-mediated cytokine activity or determinants of barrier disruption. Therefore, the observed effects are primarily modulatory rather than suppressive.

Microbiome analyses provide a critical missing link in the gut-skin axis paradigm, suggesting that the anti-atopic efficacy of PHGG is intrinsically tied to the strategic reorganization of the gut microbial ecosystem (Figs 8–10). The DNCB-induced dysbiosis observed in this study was characterized by an enrichment of *Mucispirillum* (Fig. 8), a genus within the phylum *Deferribacteres* known to thrive in pro-inflammatory environments and potentially degrade the intestinal mucus layer.^{43,44} This microbial shift likely exacerbates the intestinal barrier dysfunction (reduced MUC2 and tight junction expression) observed in our *in vitro* and *in vivo* data, facilitating the systemic translocation of inflammatory triggers. The administration of PHGG, particularly at 200 and 400 mg kg⁻¹, effectively counteracted this dysbiotic state by promoting the enrichment of *Muribaculaceae* and *Alistipes*. *Muribaculaceae* (formerly known as S24-7) is a dominant family in the murine gut specialized in the degradation of complex dietary carbohydrates, and its abundance is frequently correlated with increased production of SCFAs, particularly propionate and butyrate.⁴⁵ Similarly, members of the *Alistipes* genus have been identified as beneficial fermenters that contribute to immune homeostasis and the maintenance of the intestinal barrier.⁴⁶ The dose-dependent shift in community membership toward these specific fermenting taxa suggests that the

galactomannan oligomers of PHGG serve as a selective substrate, fostering a 'prebiotic niche' that favors beneficial commensals over pathobionts. The ecological stability reflected in the alpha-diversity indices (Fig. 9) further highlights that PHGG modulates the gut environment through subtle taxonomic shifts rather than a drastic community collapse. Although the richness-oriented indices (Chao1 and observed features) showed only a partial recovery trend, the beta-diversity patterns (Fig. 10) revealed a clear, directional restoration of the microbial community structure toward the NOR cluster. This reorganization of microbial membership is functionally significant; the enrichment of *Muribaculaceae* and the reduction of inflammatory markers like *Mucispirillum* likely drive the recovery of colonic IgA and the suppression of systemic Th2 cytokines (IL-4, IL-5, and IgE) observed in our immunological profiles.^{43–45}

Cecal SCFA levels were quantified to further explore the involvement of gut microbial metabolites in the gut-skin axis (Table 2). Notably, DNCB challenge markedly increased acetic acid and butyric acid levels compared with the NOR group. Although SCFAs are generally regarded as beneficial microbial metabolites that play key roles in host energy metabolism, immune regulation, and epithelial barrier function,⁴⁷ their elevation under inflammatory conditions has also been associated with microbial dysbiosis and altered fermentation patterns.⁴⁸ In particular, increased SCFA levels have been reported in certain pathological conditions, including gut dysbiosis, obesity, hypertension, and cardiometabolic risk,^{49,50} where they may reflect an imbalance in microbial composition or substrate utilization rather than improved gut health. In this context, PHGG supplementation attenuated these elevated SCFA levels, particularly acetic acid in a dose-dependent manner, suggesting a modulatory effect on gut microbial metabolic activity under inflammatory conditions. Importantly, rather than simply enhancing SCFA production, these findings suggest that PHGG may contribute to the normalization of DNCB-induced dysregulated fermentation. This interpretation is consistent with previous studies showing that fermentable fibers can restore microbial metabolic balance and functional ecology without necessarily increasing total SCFA concentrations.⁵⁰

To assess the translational relevance of the administered doses, the human equivalent dose was estimated using the body surface area normalization method proposed by Reagan-Shaw et al.⁵¹ Based on this approach, the highest dose used in this study (400 mg kg⁻¹ in mice) corresponds to approximately 32 mg kg⁻¹

in humans, equivalent to $\sim 2.2 \text{ g d}^{-1}$ for a 70 kg adult. Notably, this range falls within the commonly reported intake levels of PHGG in human studies, where daily doses of approximately 3–6 g have been shown to exert prebiotic and gastrointestinal benefits.^{13,33} These findings support the translational potential of PHGG and suggest that the effective doses used in this study are physiologically relevant for human application.

Taken together, our findings suggest that PHGG functions as a potent gut-microbiota-shaping agent. By promoting the proliferation of specialized carbohydrate-degrading bacteria and suppressing dysbiosis-associated taxa, PHGG may reinforce the intestinal biochemical barrier, including IgA- and mucin-associated defense systems, thereby dampening systemic inflammation and ultimately alleviating AD-like cutaneous lesions. Future studies focusing on the identification of specific microbial metabolites, besides SCFAs, and their direct causal links to skin-homing T-cell regulation will further strengthen the clinical relevance of PHGG within the gut–skin axis framework. Despite these findings, several technical limitations should be considered. First, SCFA levels were measured at a single endpoint using cecal contents, which may not fully capture dynamic changes in microbial fermentation during the experimental period. In addition, fecal or longitudinal SCFA monitoring was not performed, limiting the interpretation of temporal associations between SCFA modulation and disease progression. Second, histological and protein expression analyses were conducted using whole skin tissues, which may not fully reflect layer-specific or functional changes in epidermal barrier integrity. In addition, although the DNCB-induced model is widely used to mimic AD-like skin inflammation, it primarily reflects acute or sub-chronic contact hypersensitivity responses driven by hapten sensitization and does not fully recapitulate the chronic, relapsing, and multifactorial nature of human AD. Future studies employing chronic or spontaneous AD models would further strengthen the translational relevance of these findings. Finally, the detailed molecular mechanisms underlying the observed effects, including specific signaling pathways, were not directly investigated. Therefore, further studies incorporating time-resolved SCFA profiling, more sensitive analytical platforms, and mechanistic validation are needed to clarify the precise role of SCFAs in mediating the effects of PHGG and to confirm these findings in clinically relevant settings.

CONCLUSION

This study establishes a novel therapeutic paradigm for oligosaccharide-enriched PHGG as a systemic modulator of AD via the gut–skin axis. Distinct from conventional PHGG preparations – typically characterized by average molecular weights in the 20–50 kDa range – the PHGG utilized in this research exhibited a highly depolymerized profile, with a mass distribution predominantly below m/z 2000. The beneficial effects of PHGG observed in this study may be attributed, at least in part, to its low-molecular-weight, oligomer-enriched structure, which likely enhances its bioavailability and fermentability, thereby facilitating more effective modulation of gut microbial metabolism and associated immune responses. This structural distinctiveness provides a critical functional basis for its enhanced bioactivity. Integrating multi-level evidence from *in vitro* and *in vivo* models, we demonstrated that PHGG exerts its anti-atopic effects through a coordinated dual-barrier reinforcement strategy: cutaneous barrier that suppression of Th2-linked inflammatory cascades and pruritogenic signals in keratinocytes and intestinal barrier that

restoration of tight junction integrity and mucin expression in intestinal epithelial cells. Furthermore, oral administration of PHGG significantly attenuated systemic Th2-skewed immunity and cutaneous lesions in mice, which was fundamentally accompanied by a reorganization of the gut microbial community structure and the restoration of mucosal immune homeostasis (IgA). Collectively, these findings transition the mechanistic scope of PHGG from local gastrointestinal benefits to a systemic immunomodulatory profile. This study highlights highly depolymerized PHGG as a promising functional macromolecule candidate for the management of chronic inflammatory skin disorders. Future investigations focusing on the identification of specific microbial metabolites and their direct causal links to skin-homing T-cell regulation will further solidify the clinical applicability of PHGG within the gut–skin axis framework.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

Jung Cheul Shin is an employee of HIEMS NATURA Inc., which develops products related to the subject of this manuscript. HIEMS NATURA Inc. had no role in the study design, data collection, analysis, interpretation, or manuscript writing, except for providing study materials and general supervision. All other authors declare no competing financial or personal interests.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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