



20-O- β -D-glucopyranosyl-20(S)-protopanaxadiol-fortified ginseng extract attenuates the development of atopic dermatitis-like symptoms in NC/Nga mice

Jong Rhan Kim^{a,c,1}, Jinhwan Choi^{a,1}, Jiyoung Kim^{a,b,c,1}, Heejeung Kim^a, Heerim Kang^a, Eun Hye Kim^d, Jeong-Hwa Chang^e, Yeong-Eun Kim^e, Young Jin Choi^{b,d}, Ki Won Lee^{a,b,c,f,*}, Hyong Joo Lee^{a,b,c,*}

^a WCU Biomodulation Major, Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Republic of Korea

^b Center for Food and Bioconvergence, Seoul National University, Seoul 151-921, Republic of Korea

^c Advanced Institutes of Convergence Technology, Seoul National University, Suwon 443-270, Republic of Korea

^d Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Republic of Korea

^e CJ Foods R&D, CJ Cheiljedang Corporation, Seoul 152-051, Republic of Korea

^f Research Institute of Bio Food Industry, Institute of Green Bio Science and Technology, Seoul National University, Pyeongchang 232-916, Republic of Korea

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ABSTRACT

Ethnopharmacological relevance: Ginseng and ginsenosides are frequently used in the treatment of chronic inflammatory diseases. Recently, 20-O- β -D-glucopyranosyl-20(S)-protopanaxadiol (GPD), the main metabolite of ginsenosides, was reported to have both anti-allergic and anti-pruritic effects. The immunomodulatory effects of GPD-fortified ginseng extract (GFGE) on atopic dermatitis (AD)-like symptoms in mice were investigated. This study was designed to investigate the preventive effect of GFGE on AD-like symptoms.

Materials and methods: The effects of orally administered GFGE on *Dermatophagoides farinae* body extract (DFE)-induced AD-like symptoms in NC/Nga mice were assessed by analyzing dermatitis score, ear thickness, scratching time, skin histological changes, and serum level of macrophage-derived chemokine (MDC). In addition, splenocytes were isolated from the mice and stimulated with anti-CD3 and anti-CD28 monoclonal antibodies to produce cytokines.

Results: Oral administration of GFGE significantly attenuated DFE-induced increases in dermatitis score, ear thickness, scratching time, and severity of skin lesions in NC/Nga mice. GFGE treatment also reduced level of MDC in serum, infiltration of eosinophils and mast cells in skin, and production of cytokines in splenocytes.

Conclusions: These results suggest that GFGE might ameliorate DFE-induced AD-like symptoms and be an alternative therapeutic agent for the prevention of AD.

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1. Introduction

Atopic dermatitis (AD) is a chronic, relapsing, pruritic, and inflammatory skin disorder (Leung et al., 2004). Skin lesions associated with AD exhibit severe rash, edema, hemorrhage, and desquamation (Leung and Bieber, 2003b). Pathological changes associated with AD include epidermal thickening and the infiltration of inflammatory cells, such as eosinophils and mast cells

(Imokawa et al., 1991). Findings from several studies suggest that AD is the cutaneous manifestation of a systemic disorder that also gives rise to allergic rhinitis, food allergies, and asthma (Leung and Bieber, 2003a; Leung et al., 2004; Spergel and Paller, 2003). Although it is not life-threatening, AD requires early treatment and management to prevent recurrence (Cho and Cho, 2013).

The pathogenesis of AD is predominantly driven by T-helper cell (Th) type 2 immune responses, along with increased immunoglobulin E production. Th2-mediated cytokines, such as interleukin (IL)-4, IL-5, and IL-13, are found in skin lesions during the acute phase of AD (Ong and Leung, 2006). On the other hand, the dysregulated immune responses are accompanied by a combination of both Th2 and Th1 responses (Ong and Leung, 2006) during the chronic phase of AD. Increased levels of interferon (IFN)- γ ,

* Corresponding authors at: WCU Biomodulation Major, Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Republic of Korea. Tel.: +82 2 880 4661/+82 2 880 4853; fax: +82 2 878 6178/+82 2 873 5095.

E-mail addresses: kiwon@snu.ac.kr (K.W. Lee), leehyjo@snu.ac.kr (H.J. Lee).

¹ Authors contributed equally to this work.

IL-12, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) are observed in chronic skin lesions of AD (Ong and Leung, 2006). AD is also associated with mechanical tissue injury, which triggers the production of pro-inflammatory cytokines, including tumor necrosis factor (TNF)- α , GM-CSF (Homey et al., 2006), and pro-inflammatory chemokine such as macrophage-derived chemokine (MDC) (Homey et al., 2006).

Ginseng (*Panax ginseng* C.A. Meyer) is frequently used as a traditional remedy for allergies (Choo et al., 2003). The major components of ginseng are ginsenosides, which contain an aglycone with a dammarane skeleton (Choo et al., 2003; Shibata et al., 1963). The pharmacological actions of ginsenosides have been explained on the basis of the biotransformation of ginsenosides by human intestinal bacteria (Akao et al., 1998a, 1998b; Choo et al., 2003). Ginseng and its ginsenosides have been reported to exhibit immunomodulatory, anti-inflammatory and anti-allergic effects (Choo et al., 2003; Rhule et al., 2006; Yu et al., 2005). Ginseng and red ginseng have shown to ameliorate the development of AD-like symptoms in animal model (Cho and Cho, 2013; Choi et al., 2013).

Ginsenosides Rb1, Rb2, and Rc are transformed by human intestinal microflora to form 20-O- β -D-glucopyranosyl-20(S)-protopanaxadiol (GPD, also known as compound K), which has anti-allergic properties (Choo et al., 2003; Park et al., 2003; Wakabayashi et al., 1997). GPD potently inhibits β -hexosaminidase release from rat basophil leukemia cells and reduces the passive cutaneous anaphylaxis reaction in mice (Choo et al., 2003). The inhibitory activity of GPD is more potent than that of sodium cromoglycate, which is a commercially available anti-allergic drug (Choo et al., 2003). GPD has also been shown to have an anti-pruritic effect on the scratching behavior of mice treated with compound 4/80, substance P, and histamine (Shin and Kim, 2005). These observations suggest that GPD may prevent or alleviate the characteristics of AD.

In this study, we tested the effects of GPD-fortified ginseng extract (GFGE), which contains 8.29% GPD, on *Dermatophagoides farinae* body extract (DFE)-induced AD-like symptoms in NC/Nga mice. Dermatitis score, ear thickness, scratching time, and histological changes in skin were measured. The effects of GFGE on the production of pro-inflammatory cytokines in splenocytes and MDC in serum were also examined.

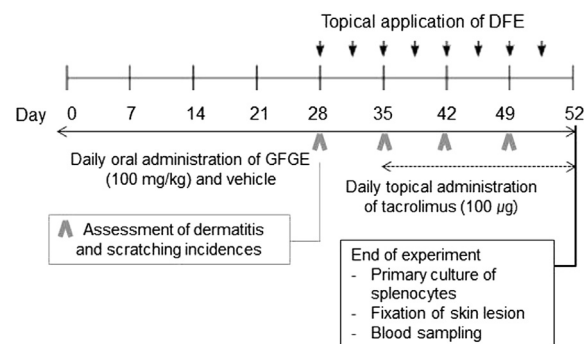
2. Materials and methods

2.1. Animals

Female NC/Nga mice (5 weeks old) were purchased from Japan SLC (Shizuoka, Japan). Mice were maintained under specific pathogen-free conditions and a 12-h light-dark cycle at 22 ± 2 °C. All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Washington DC, USA) and were approved by the Institutional Animal Care and Use Committee of Seoul National University, Seoul, Korea (SNU-110211-2).

After 2 weeks of acclimation, mice were divided into four groups ($n=10$ –11 per group): non-induction + vehicle, DFE + vehicle, DFE + GFGE, and DFE + tacrolimus. Mice were treated with GFGE (100 mg/kg/day, 150 μ l/mouse/day) or vehicle (10% ethanol and 10% tween 20 in distilled water, 150 μ l/mouse/day) by oral administration for 52 d. On day 28, the mice received topical applications of DFE (Biostir, Hiroshima, Japan) twice a week for 2 weeks. Tacrolimus (0.1% Protopic® Ointment, Astellas Pharma, Tokyo, Japan; 100 μ g/mouse/day) was topically applied onto shaved dorsal surfaces after 7 d of DFE application. The experimental design is summarized in Fig. 1.

Schedule of experiment



Groups and treatment

- Non-induction+vehicle: oral administration of vehicle from d 0
- DFE+vehicle: oral administration of vehicle from d 0
topical application with DFE from d 28
- DFE+GFGE: oral administration of GFGE (100 mg/kg) from d 0
topical application with DFE from d 28
- DFE+tacrolimus: topical application with DFE from d 28
topical administration of tacrolimus (100 μ g) from d 35

* Vehicle: 10% ethanol and 10% Tween 20 in distilled water, 150 μ l

Fig. 1. The experimental design is shown. NC/Nga mice were divided into four groups: non-induction + vehicle, DFE + vehicle, DFE + GFGE, and DFE + tacrolimus. DFE + vehicle and DFE + GFGE mice were treated with vehicle or GFGE daily for 52 days and applied with DFE topically, twice a week, starting from day 28. DFE + tacrolimus mice received daily topical applications of tacrolimus from days 35 to 52.

2.2. Sample preparation

GFGE was prepared from the fine roots of 4-year-old Korean ginseng (*Panax ginseng* C.A. Meyer, Poonggi Ginseng Nonghyup, Poonggi, Korea) (Kim et al., 2013). Raw white ginseng extract was manufactured by the Vitrosys Inc. (Poonggi, Korea) using water extraction method (extracted in 80 °C water for 4 h and concentrated using Vacuum Rotary Evaporator (BUCHI Labortechnik AG, Flawil, Switzerland)). GFGE was obtained by enzymatic biotransformation as follows. Reaction mixture (pH 4.3) containing 7 °Brix of the ginseng extract with 2% (v/v) Cytolase PCL5® (DSM, Delft, Netherlands) was incubated for 84 h in 57 °C. After incubation, the mixture was adjusted to pH 6.5 with sodium tricitrate (RZBC Co. Ltd., Shandong, China) and heated for 5 min in 95 °C. After the reaction mixture was centrifuged, the supernatant was passed through a Diaion HP-20 column (Iontec Corp., Seongnam, Korea) with water and 95% ethanol. The ethanol eluate was evaporated in a rotary evaporator (BUCHI Labortechnik AG). The residual eluate was lyophilized in a freeze-dryer (HETO, Oliveira de Azeméis, Portugal) to make powders.

The ginsenosides were quantitatively determined at a wavelength of 203 nm by the high-performance liquid chromatography (HPLC) system (Agilent 1260 infinity quaternary system; Agilent Technologies, Palo Alto, CA, USA) with a C18-bonded reversed-phase silica column (Venusil XBP C18, 4.6 mm $\times$ 250 mm, 5 μ m, Bonna-Agela technologies, Wilmington, DE, USA) performed at a flow rate of 1.0 ml/min of the mobile phase composed of water and acetonitrile. HPLC condition was as follows: solvent A, water; solvent B, acetonitrile; gradient, 0–7.5 min (20–21% B), 7.5–15 min (21–32% B), 15–20 min (32–36% B), 20–30 min (36–37% B), 30–50 min (37–75% B), 50–51 min (75–95% B), 51–60 min (95–95% B).

Each standard ginsenoside were injected for the HPLC analysis, and peaks were assigned by comparing their retention times with that of each reference compound. Rg1, Re, Rb1, Rc, Rd (Wako Pure Chemical Industries, Osaka, Japan), Rf, Rg2, Rh1, Rg3(s), Rg3(r) (AMBO Institute, Daejeon, Korea), Rb2, F2 (BTGIN, Daejeon, Korea), and GPD (Chromadex, Irvine, CA, USA) were used as standard ginsenosides (98% purity). White ginseng extract was composed of ginsenosides Rg1, Re, Rf, Rb1, Rc, Rb2, and Rd, but not GPD (Fig. 2A). After enzymatic treatment, GFGE was composed of ginsenosides Rg1, Re, Rf, Rg2, Rh1, F2, Rg3(s), Rg3(r), and GPD (82.91 ± 7.47 mg/g) (Fig. 2B).

2.3. Induction of AD-like symptoms

NC/Nga mice were anesthetized with 2% isoflurane (Hana Pharm, Seoul, Korea) and dorsally shaved using an electric clipper (Daito Thrive, Tokyo, Japan) and cream (Reckitt Benckiser, Cedex, France). For the disruption of skin barrier, 150 μ l of 4% sodium dodecyl sulfate (Sigma, St. Louis, MO, USA) was topically applied. After 3 h, 100 mg Biostir cream containing DFE was applied to the shaved dorsal surfaces, twice a week for 2 weeks, to induce AD-like symptoms.

2.4. Assessment of skin lesions and dermatitis score

Mice were anesthetized with 2% isoflurane, and images of the skin lesions were taken once a week using a digital camera (Canon SX40 HS, Canon, Tokyo, Japan). The dermatitis score was measured once a week, according to a slight modification of the criteria described previously (Leung et al., 1990). Scores of 0 (none), 1 (mild), 2 (moderate), and 3 (severe) were given for each of the four symptoms (erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness). A total dermatitis score of clinical severity was defined as the sum of all scores.

2.5. Measurement of scratching time and ear thickness

Scratching time for each mouse was observed once a week for 20 min. Ear thickness of each mouse was measured and recorded

three times per week using a vernier caliper (Mitutoyo, Kanagawa, Japan).

2.6. Histopathological examination

The dorsal skin lesions of each mouse were fixed with 10% neutral-buffered formalin, embedded in paraffin, cut into sections (4 μ m), and transferred onto slides. Deparaffinized skin sections were stained with Congo red or toluidine blue to detect eosinophils or mast cells, respectively. Tissue sections were examined using an Olympus AX70 light microscope (Olympus, Tokyo, Japan), and the numbers of eosinophils and mast cells per 0.025 mm² skin were counted at 400 $\times$ magnification.

2.7. Measurement of serum MDC

Blood samples were collected from anesthetized mice by cardiac puncture on the last day of the experiment and serum obtained from the whole blood was stored at -80°C until use. The total level of MDC in the serum was determined using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions.

2.8. Cytokine production by splenocytes

To measure cytokine production in cultured splenocytes, the spleen was aseptically removed from each mouse and homogenized. Red blood cells (RBCs) were lysed using RBC lysis buffer (Qiagen, Hilden, Germany), and the resulting lysate was centrifuged at 1500 $\times$ g for 10 min at 4°C . The prepared splenocytes (5×10^6 cells/ml) were placed in RPMI-1640 media (Welgene, Daegu, Korea) containing 10% FBS (Sigma) and stimulated with plate-bound anti-CD3 (1 mg/ml) and anti-CD28 (3 mg/ml) monoclonal antibodies (mAbs) (BD Bioscience, San Jose, CA, USA) at 37°C under 5% CO₂ for 48 h. Afterwards, the supernatants were collected and stored at -80°C until use. Levels of Th1 (IL-2, IL-12, and IFN- γ), Th2 (IL-4 and IL-5), regulatory T cell (Treg) (IL-10), and pro-inflammatory (TNF- α and GM-CSF) cytokines were determined using the multiplex protein analysis (Bio-Plex Pro, Bio-Rad Laboratory, Hercules, CA, USA), according to the manufacturer's instructions.

2.9. Statistical analysis

Statistical analysis was performed with SPSS Statistics Software. The data were expressed as means $\pm$ standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey's HSD test was used for comparing differences between multiple groups. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Oral administration of GFGE attenuated the clinical severity of DFE-induced AD-like symptoms in NC/Nga mice

To investigate the effects of GFGE on DFE-induced AD-like symptoms in NC/Nga mice, we assessed the severity of the skin lesions and evaluated the dermatitis score. On day 52, the dorsal lesions of DFE-treated mice displayed severe erythema, erosion, and dryness (Fig. 3A). However, co-treatment with GFGE or the medicinal ointment, tacrolimus, markedly decreased the severity of the lesions (Fig. 3A).

The dermatitis score was significantly increased in DFE-treated mice on days 42 (5.36 ± 0.31) and 49 (6.36 ± 0.41), compared with the non-induction+vehicle group (1.10 ± 0.18 and 0.90 ± 0.18 ,

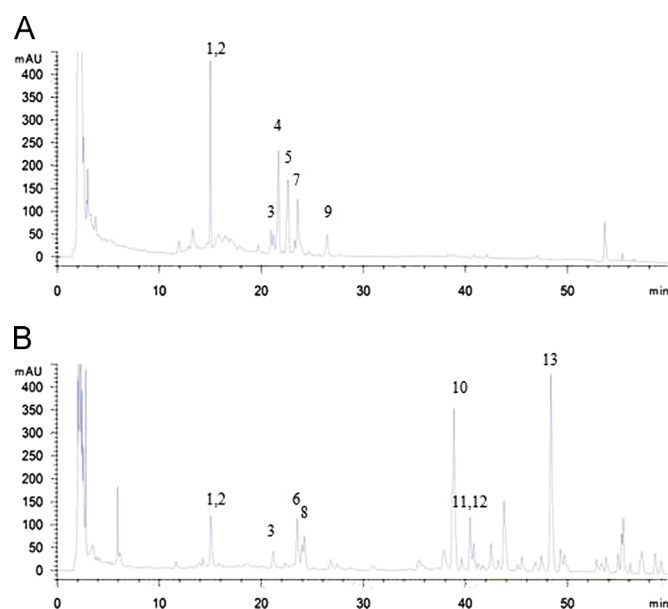


Fig. 2. High-performance liquid chromatography pattern of ginsenosides in ginseng extract used in this study. (A) Conventional white ginseng extract. GPD was not found in white ginseng extract. (B) GFGE (enzyme-treated white ginseng extract). GPD was found in GFGE (82.91 ± 7.47 mg/g). Peaks: 1, 2 ginsenoside Rg1+Re; 3, Rf; 4, Rb1; 5, Rc; 6, Rg2; 7, Rb2; 8, Rh1; 9, Rd; 10, F2; 11, Rg3(s); 12, Rg3(r); 13, GPD.

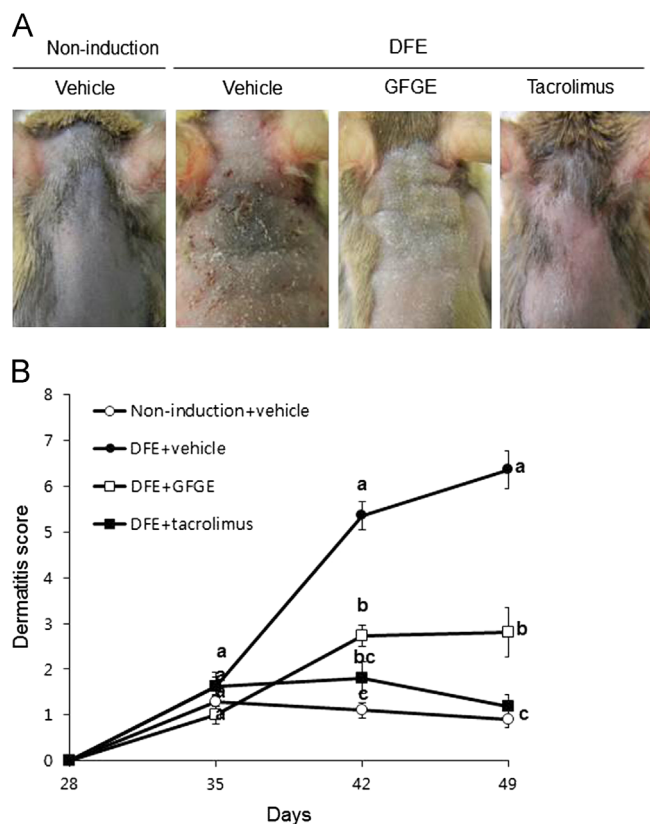


Fig. 3. Effects of GFGE on DFE-induced clinical severity of AD-like symptoms in NC/Nga mice. (A) Images of dorsal lesions from the groups of mice were taken on day 52. (B) Dermatitis scores were evaluated weekly from days 28 to 49. Data represent the mean $\pm$ SEM ($n=10\sim11$). Means with letters (a–c) within a graph are significantly different from each other at $p < 0.05$ (one-way ANOVA, Tukey's HSD test).

respectively) (Fig. 3B). Treatment with GFGE (2.73 ± 0.24 and 2.82 ± 0.54) or tacrolimus (1.82 ± 0.35 and 1.18 ± 0.26) significantly lowered the DFE-induced increases in dermatitis score on days 42 and 49 (Fig. 3B).

3.2. Oral administration of GFGE reduced DFE-induced scratching incidence and ear swelling in NC/Nga mice

Intensive pruritus, a hallmark of AD, leads to extensive scratching (Barnes, 2010). Thus, the scratching time of NC/Nga mice was measured once a week for 20 min. On day 49, scratching time was markedly increased in DFE-treated mice (273.64 ± 40.99 s) compared with the non-induction+vehicle group (128.70 ± 22.68 s), whereas treatment with GFGE (107.18 ± 16.28 s) or tacrolimus (81.73 ± 12.00 s) reduced scratching incidence (Fig. 4A).

Ear thickness was measured on day 52. The ears of DFE-treated mice (0.45 ± 0.01 mm) were swollen and significantly thicker than those of the non-induction+vehicle group (0.26 ± 0.01 mm) (Fig. 4B). Treatment with GFGE markedly attenuated the DFE-induced increase in ear thickness to 0.34 ± 0.00 mm (Fig. 4B). Interestingly, treatment with tacrolimus (0.35 ± 0.00 mm) did not significantly affect ear thickness (Fig. 4B).

3.3. Oral administration of GFGE inhibited the DFE-induced infiltration of eosinophils and mast cells into skin lesions of NC/Nga mice

Congo red or toluidine blue was used to stain eosinophils or mast cells in the skin of NC/Nga mice, respectively (Fig. 5A). The number of Congo red-stained eosinophils in skin lesions of

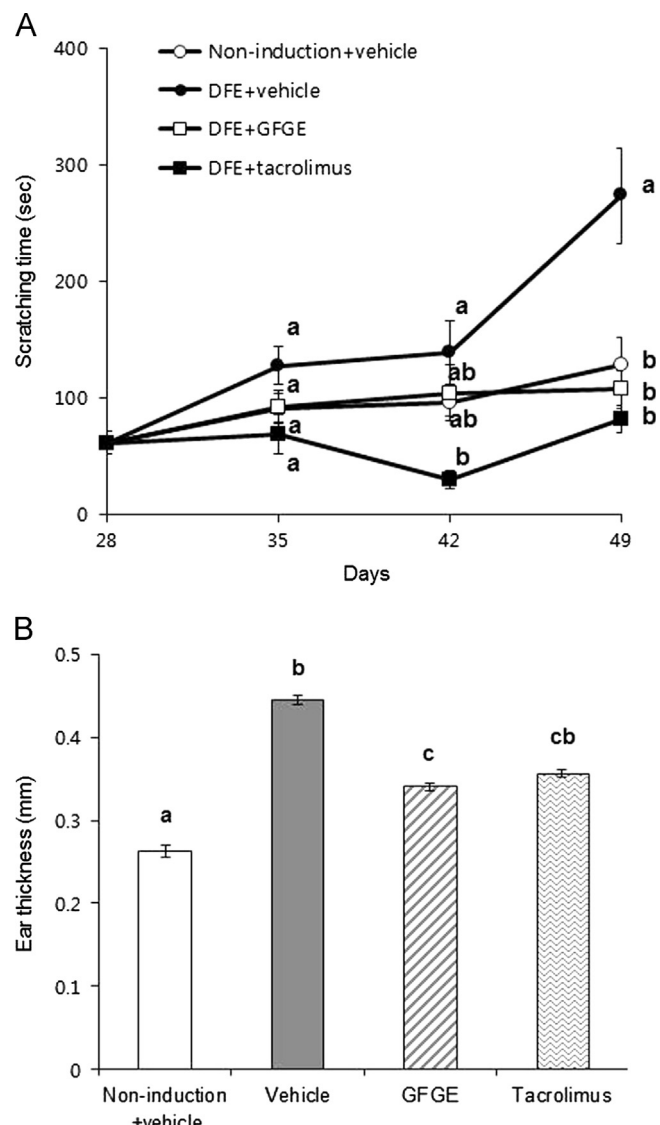


Fig. 4. Effects of GFGE on DFE-induced scratching incidence and ear thickness in NC/Nga mice. (A) Scratching times were evaluated weekly from days 28 to 49. (B) Ear thickness was measured on day 52. Data represent the mean $\pm$ SEM ($n=10\sim11$). Means with letters (a–c) within a graph are significantly different from each other at $p < 0.05$ (one-way ANOVA, Tukey's HSD test).

DFE-treated mice (974.55 ± 54.81 cells per mm^2) was significantly increased compared with that of the non-induction+vehicle group (44.00 ± 12.58 cells per mm^2) (Fig. 5A and B). Importantly, addition of GFGE (425.46 ± 38.24 cells per mm^2) or tacrolimus (654.55 ± 46.79 cells per mm^2) markedly lowered the number of eosinophils in the skin of DFE-treated mice (Fig. 5A and B).

Furthermore, the number of toluidine blue-stained mast cells in skin lesions of DFE-treated mice (949.09 ± 115.44 cells per mm^2) was significantly increased compared to that of the non-induction+vehicle group (348.00 ± 40.46 cells per mm^2) (Fig. 5A and C). Treatment with GFGE (625.46 ± 48.62 cells per mm^2), but not tacrolimus (912.73 ± 61.93 cells per mm^2), significantly reduced the number of infiltrated mast cells in skin lesions of DFE-treated mice (Fig. 5A and C).

3.4. Oral administration of GFGE decreased DFE-increased level of serum MDC in NC/Nga mice

The serum level of MDC has been noted to be closely related with the severity of AD (Kakinuma et al., 2002). Thus, we

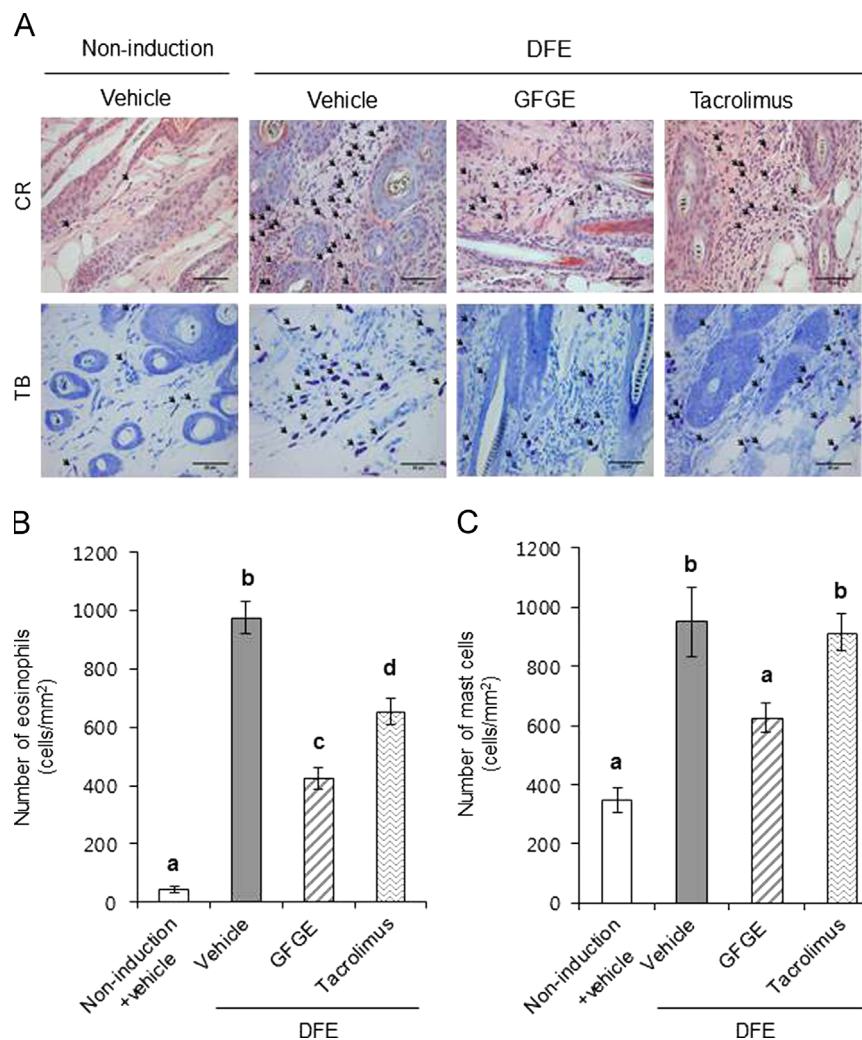


Fig. 5. Effects of GFGE on DFE-induced infiltration of eosinophils and mast cells in skin lesions. (A) Representative images depicting the histological features of skin lesions are shown. Staining with Congo red (CR) and toluidine blue (TB) were used to identify eosinophils and mast cells, respectively. The arrows indicate the CR-stained eosinophils and the TB-stained mast cells. Cells were counted under a microscope at 400 × magnification. Scale bar, 50 μm. The numbers of (B) eosinophils and (C) mast cells in 1 mm² of skin were calculated and graphed. Data represent the mean ± SEM ($n=10-11$). Means with letters (a–c) within a graph are significantly different from each other at $p < 0.05$ (one-way ANOVA, Tukey's HSD test). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

investigated whether GFGE alters the serum level of MDC in NC/Nga mice. We found that the level of MDC was markedly increased by DFE administration (279.88 ± 7.73 pg per ml), compared to non-induction+vehicle group (118.56 ± 3.57 pg per ml). However, treatment with GFGE and tacrolimus significantly reduced the level of MDC (204.48 ± 3.19 pg per ml and 221.29 ± 4.44 pg per ml, respectively) (Fig. 6).

3.5. Splenocytes of DFE+GFGE-treated NC/Nga mice produced less cytokines compared to those of DFE-treated mice

To examine the effect of GFGE on the production of cytokines, splenocytes were isolated from treated or untreated NC/Nga mice and stimulated with anti-CD3 and anti-CD28 mAbs. Levels of pro-inflammatory molecules (TNF-α and GM-CSF) and cytokines derived from Th1 (IL-2, IL-12, and IFN-γ), Th2 (IL-4 and IL-5), and Tregs (IL-10) were determined in splenocyte supernatants. IL-12, IL-4, IL-5, IL-10, TNF-α, and GM-CSF levels were significantly increased with DFE treatment (IL-12, 235%; IL-4, 294%; IL-5, 338%; IL-10, 212%; TNF-α, 164%; GM-CSF, 163%) (Table 1). No differences in IL-2 and IFN-γ levels were observed (Table 1). Splenocytes derived from mice treated with both DFE and GFGE produced

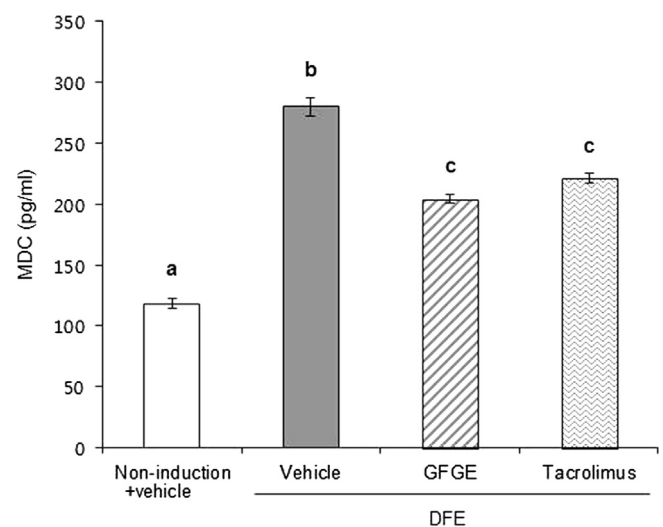


Fig. 6. Effects of GFGE on DFE-induced increase in the serum level of MDC in NC/Nga mice. The serum level of MDC was measured using an ELISA. Data represent the mean ± SEM ($n=7$). Means with letters (a–c) within a graph are significantly different from each other at $p < 0.05$ (one-way ANOVA, Tukey's HSD test).

Table 1
Effects of GFGE on the production of cytokines by cultured splenocytes.

Th1 cytokines	IL-2	IL-12	IFN- γ
Non-induction + vehicle	1399.17 $\pm$ 339.15 (100) ^a	26.69 $\pm$ 1.91 (100) ^a	2508.74 $\pm$ 579.13 (100) ^{ab}
DfE + vehicle	1426.32 $\pm$ 172.04 (102) ^a	62.61 $\pm$ 4.50 (235) ^b	2755.97 $\pm$ 173.40 (110) ^b
DfE + GFGE	770.63 $\pm$ 87.93 (55) ^{ab}	21.56 $\pm$ 2.48 (81) ^a	1313.97 $\pm$ 252.70 (52) ^{ac}
DfE + tacrolimus	428.23 $\pm$ 48.69 (31) ^b	23.41 $\pm$ 1.78 (88) ^a	500.79 $\pm$ 123.66 (20) ^c
Th2 cytokines	IL-4	IL-5	
Non-induction + vehicle	211.91 $\pm$ 23.05 (100) ^a	67.59 $\pm$ 10.84 (100) ^a	
DfE + vehicle	623.67 $\pm$ 39.15 (294) ^b	228.49 $\pm$ 24.85 (338) ^b	
DfE + GFGE	187.46 $\pm$ 27.50 (88) ^a	42.56 $\pm$ 10.79 (63) ^a	
DfE + tacrolimus	213.74 $\pm$ 19.16 (101) ^a	36.80 $\pm$ 6.82 (54) ^a	
Treg cytokines	IL-10		
Non-induction + vehicle	105.11 $\pm$ 10.62 (100) ^a		
DfE + vehicle	223.11 $\pm$ 23.36 (212) ^b		
DfE + GFGE	115.85 $\pm$ 18.04 (110) ^a		
DfE + tacrolimus	96.33 $\pm$ 18.58 (92) ^a		
Pro-inflammatory cytokines	TNF-α	GM-CSF	
Non-induction + vehicle	42.42 $\pm$ 3.56 (100) ^a	727.85 $\pm$ 80.52 (100) ^a	
DfE + vehicle	69.66 $\pm$ 6.64 (164) ^b	1188.91 $\pm$ 82.79 (163) ^b	
DfE + GFGE	33.96 $\pm$ 3.22 (80) ^{ac}	459.446 $\pm$ 85.50 (63) ^{ac}	
DfE + tacrolimus	22.65 $\pm$ 3.39 (53) ^c	232.88 $\pm$ 46.50 (32) ^c	

The pg/ml levels (percentage of non-induction + vehicle) of Th-derived and pro-inflammatory cytokines in splenocytes isolated from NC/Nga mice are shown. Splenocytes were stimulated with anti-CD3 and anti-CD28 mAbs in RPMI-1640 with 10% FBS for 48 h. Data represent the mean $\pm$ SEM ($n=8$). Means with letters (a–c) within a table are significantly different from each other at $p < 0.05$ (one-way ANOVA, Tukey's HSD test).

significantly lower levels of various cytokines (IL-12, 81%; IFN- γ , 52%; IL-4, 88%; IL-5, 63%; IL-10, 110%; TNF- α , 80%; GM-CSF, 63%), compared with those from mice treated with DfE and vehicle (Table 1). Similar effects on cytokine production were observed in splenocytes derived from mice treated with DfE and tacrolimus (IL-2, 31%; IL-12, 88%; IFN- γ , 20%; IL-4, 101%; IL-5, 54%; IL-10, 92%; TNF- α , 53%; GM-CSF, 32%) (Table 1).

4. Discussion

Because it exhibits potent anti-allergic properties, GPD has been suggested to be a potential therapeutic agent for the treatment of allergies (Choo et al., 2003). Results from the current study showed that GFGE, which contains 8.29% GPD, attenuates the DfE-induced AD-like symptoms in NC/Nga mice. GFGE treatment significantly reduced DfE-induced increases in the severity of skin lesions, dermatitis score, scratching incidence, and ear swelling. The DfE-induced infiltration of skin lesions by eosinophils and mast cells decreased with GFGE treatment. In addition, we observed that mice treated with both DfE and GFGE produce lower levels of MDC as well as cytokines, including IL-12, IFN- γ , IL-4, IL-5, IL-10, TNF- α , and GM-CSF, compared with those of DfE-treated mice.

The recruitment of eosinophils and mast cells in allergic diseases is regulated by several cytokines (Durham et al., 1992; Ying et al., 1997). For example, IL-5 has been suggested to play an important role in the production of eosinophils (Simon et al., 2004). IL-5-deficient mice are unable to develop eosinophilia under allergen exposure (Foster et al., 1996). Dent et al. (1990) demonstrated that IL-5 produces eosinophilia and induces the full pathway of eosinophil differentiation (Dent et al., 1990). GM-CSF is another regulator of eosinophil generation in the bone marrow (Nishinakamura et al., 1996). On the other hand, IL-4 is known to activate mast cells (Yamaguchi et al., 1999). Our findings showed that splenocytes of mice treated with DfE and GFGE produce lower amounts of IL-5, GM-CSF, and IL-4, compared with those of mice treated with DfE and vehicle. This finding suggests that the

effects of GFGE on reducing IL-5, GM-CSF, and IL-4 levels may be related to its effects on lowering cellular infiltration.

On the other hand, AD induction is primarily triggered by Th2 cytokines, although Th1 and non-Th pro-inflammatory cytokines are also involved in the disease process (Chen et al., 2004). In skin lesions of AD, significant increases in the number of cells expressing Th2 and Treg cytokines have been observed (Spergel et al., 1999; Szegedi et al., 2009). Transgenic mice that overproduce Th2 cytokines, such as IL-4 and IL-5, develop spontaneous AD (Lee and Flavell, 2004). The percentage and absolute number of Tregs producing IL-10 are elevated in AD patients (Szegedi et al., 2009). Furthermore, high levels of IFN- γ , a Th1 cytokine, are associated with the severity of AD (Grewe et al., 1998). Scratching of AD skin lesions causes mechanical injury, resulting in the production of IL-1, TNF- α , and GM-CSF (Homey et al., 2006). In addition to IL-4, IL-5, and GM-CSF, we found that levels of TNF- α and other Th1- and Treg-derived cytokines (IL-12 and IL-10) were decreased in splenocytes of mice treated with both DfE and GFGE. Interestingly, DfE itself did not appear to alter levels of either IL-2 or IFN- γ in the mice.

Steroids and anti-histamines are examples of commonly available drugs that are used to treat AD. However, they have moderate effects and may cause hypertension, osteoporosis, iatrogenic Cushing's disease, dizziness, and blurred vision (Leung et al., 2004). Recently, calcineurin inhibitors, such as tacrolimus and pimecrolimus, have been topically used and are considered to be quite safe (Breuer et al., 2005; Hultsch et al., 2005; Paul et al., 2006). Unfortunately, prolonged application of these drugs is also reported to cause skin cancer (Niwa et al., 2003). Therefore, the development of alternative therapeutic agents is required for long-term management of AD.

In conclusion, our results clearly demonstrate that oral administration of GFGE in NC/Nga mice attenuates DfE-induced AD-like symptoms, including increases in the severity of skin lesions, dermatitis score, scratching behavior, ear swelling, and eosinophil and mast cell infiltration. These effects were associated with decreases in the production of MDC in serum and pro-inflammatory/Th-derived cytokines, including IL-12, IFN- γ , TNF- α , GM-CSF, IL-4, IL-5, and IL-10, in splenocytes. Our findings suggest that GFGE

may be an effective therapeutic agent that can be used to prevent the development of AD.

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Glossary

- AD: atopic dermatitis;
 DFE: *Dermatophagoides farinae* extract;
 GFGE: GPD-fortified ginseng extract;
 GM-CSF: granulocyte-macrophage colony-stimulating factor;
 GPD: 20-O- β -D-glucopyranosyl-20(S)-protopanaxadiol;
 IFN: interferon;
 IL: interleukin;
 MDC: macrophage-derived chemokine;
 TNF: tumor necrosis factor.