

Gardenia jasminoides extract ameliorates DfE-induced atopic dermatitis in mice through restoration of barrier function and T-helper 2-mediated immune response

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ABSTRACT

Atopic dermatitis (AD) leads to skin barrier abnormalities and immune dysfunction. As the topical steroids commonly used to treat AD have side effects from long-term use, research into safer treatments for AD is greatly needed. The medicinal herb *Gardenia jasminoides* improves AD symptoms via skin barrier activation and T helper 2-mediated immune response regulation. Crocin, a bioactive component within the extract, is dispensable for its restorative effects. As such, this work explored the effects of *Gardenia jasminoides* extract without crocin (GjexCr) on AD symptoms in a DfE-induced AD model in 6-week-old male NC/Nga mice (25.0 ± 0.25 g, $n = 10$ each, 6 groups). Using histological and behavioral assays, the effects of GjexCr on dermatitis scores, scratching behavior, skin barrier activation, and serum levels of IgE, chemokines, and cytokines were analyzed. In addition, the major components from the GjexCr extract were analyzed by high-performance liquid chromatography and validated in the AD model. GjexCr reduced ear thickness due to hyperkeratosis, dermal thickening, and scratching behavior and restored dermatitis scores in AD-induced mice. GjexCr administration also decreased inflammation and mast cell infiltration, as well as modulated skin barrier recovery by upregulating the production of epidermal proteins. Moreover, GjexCr administration attenuated imbalanced immune responses. Furthermore, geniposide, the main component of GjexCr, improved AD symptoms in DfE-treated NC/Nga mice. Thus, GjexCr could be a suitable treatment for protecting the skin barrier in AD-like skin lesions and a potential therapy for AD.

1. Introduction

Atopic dermatitis (AD) is a common chronic or relapsing inflammatory skin disease that often precedes asthma and allergy disorders [1, 2]. Several different types of cells participate in the immune response of AD, which can be divided into two phases according to the chronicity of inflammation. The acute phase is mainly driven by T helper 2 (Th2) cells that actively secrete various cytokines, including interleukins -4, -5, and -13 (IL-4, IL-5, and IL-13) [3,4]. As the percentage of Th2 cells increases, AD skin lesions express higher levels of Th2 cytokines and chemokines compared to normal skin [5]. Other studies have demonstrated that levels of IL-4, IL-5, IL-13, and IL-10 are elevated in the skin of AD patients and that IL-5 and IL-13 levels are further increased in patients with

elevated immunoglobulin E (IgE) levels [1]. Interestingly, transgenic mice overexpressing IL-4, IL-5, and IL-13 spontaneously develop AD with elevated IgE levels, increased inflammation, and skin lesions [5]. Th2 cytokines suppress the expression of terminal differentiation proteins in keratinocytes (e.g., filaggrin, loricrin, involucrin), thereby destabilizing cutaneous barrier function [6].

AD has a complex etiology based on genetic, immunological, and environmental factors that cause skin barrier abnormalities and immune dysfunctions crucial to the pathogenesis of AD [7]. Skin barrier dysfunction is the first step in the development of AD [8]. Abnormal expression of epidermal differentiation-related molecules, such as filaggrin, loricrin, and involucrin, has been demonstrated in AD patients, and data suggest that these molecules affect homeostasis of the

Abbreviations: AD, Atopic dermatitis; *G. jasminoides*, *Gardenia jasminoides*; GjexCr, *Gardenia jasminoides* extract with Crocin removed; DfE, *Dermatophagoides farinae* crude extract; EP, epidermis; DE, dermis; HF, hair follicle; IgE, Immunoglobulin E; Th2, T helper 2; H&E, hematoxylin and eosin; TB, toluidine blue; MDC, Macrophage-derived chemokine; TSLP, Thymic stromal lymphopoietin; RANTES, Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted; SEM, standard error of the mean.

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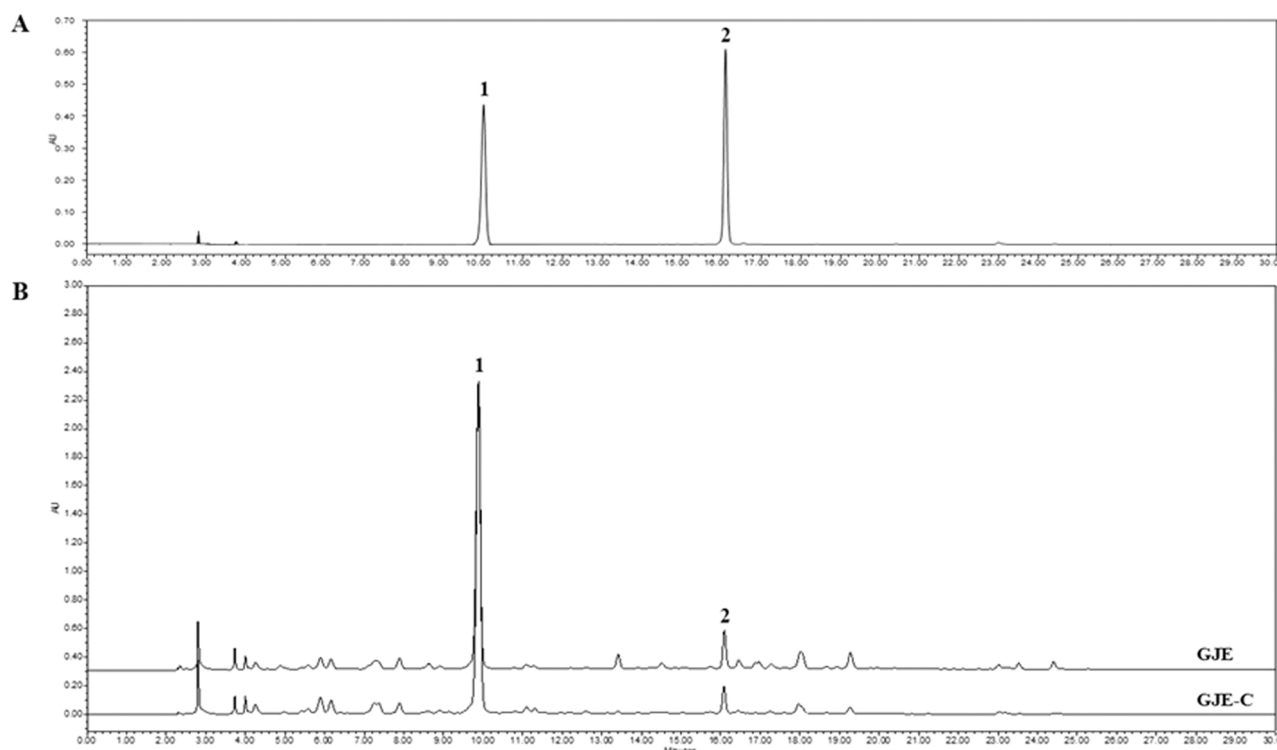


Fig. 1. Chemical composition of *G. jasminoides* extract. HPLC chromatograms of standard mixture (A), *G. jasminoides* extract (B, top trace), and *G. jasminoides* extract with crocin removed (GjexCr) (B, bottom trace). The compounds are geniposide (1) and crocin (2).

Table 1

Calibration curves, LOD, and LOQ of standard compounds.

Compound	Linearity			LOD ^a (μg/mL)	LOQ ^b (μg/mL)
	Equation	R ²	Range (μg/mL)		
Geniposide	$y = 12446x + 3843$	0.9998	3.13–200.00	0.01	0.02
Crocin	$y = 15527x - 10369$	1.0000	3.13–200.00	0.01	0.01

^a LOD (Limit of detection) = $3.3 \times \text{SD}/S$.

^b LOQ (Limit of quantification) = $10 \times \text{SD}/S$. (SD: standard deviation of the blanks, S: slope of the calibration curve).

permeability barrier [9,10]. Recent evidence supports a role for Th2-associated factors in regulating filaggrin expression. In human skin models and keratinocyte cultures, histamine and cytokines, including IL-4, IL-17, IL-22, IL-25, and IL-31, have been shown to decrease filaggrin production or repress filaggrin gene expression [11]. Interestingly, previous studies have shown that IgE-mediated sensitization will result in AD. The “outside-in” hypothesis proposes that disruption of the skin barrier via either an intrinsic genetic defect in epidermal skin barrier formation or an environmental alteration leads to sensitization and

atopic disease [1].

Recently, valuable complementary and integrative medical approaches, such as herbal medicine, have been practiced more frequently in Eastern and Western countries globally. Although solid evidence is

Table 3

Contents of the two compounds in GJE and GJE-C.

Samples	Geniposide		Crocin	
	Mean (mg/g) ± SD	RSD (%)	Mean (mg/g) ± SD	RSD (%)
GJE	108.66	0.06	8.55	0.56
GJE-C	140.79	0.22	5.82	0.44

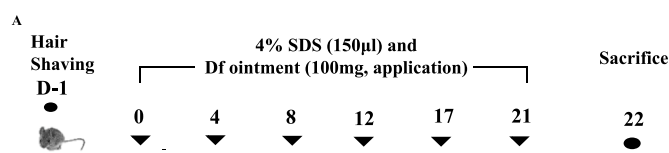


Fig. 2. Schematic of experimental induction of AD. After hair removal, mice were treated twice weekly for 3 weeks with ointment containing DfE to induce AD.

Table 2

Precision and accuracy of standard compounds.

Compound	Intraday precision		Interday precision		Accuracy		
	Concentration (μg/mL)	RSD (%)	Concentration (μg/mL)	RSD (%)	Spike levels (%)	Recovery (%)	RSD (%)
Geniposide	12.5	1.28	12.5	1.33	50	99.21	0.11
	25.0	0.66	25.0	1.87	100	100.24	0.27
	50.0	0.20	50.0	0.94	200	97.84	0.34
Crocin	12.5	0.37	12.5	0.39	50	98.09	0.42
	25.0	0.31	25.0	1.60	100	100.21	0.08
	50.0	0.42	50.0	2.09	200	101.80	0.64

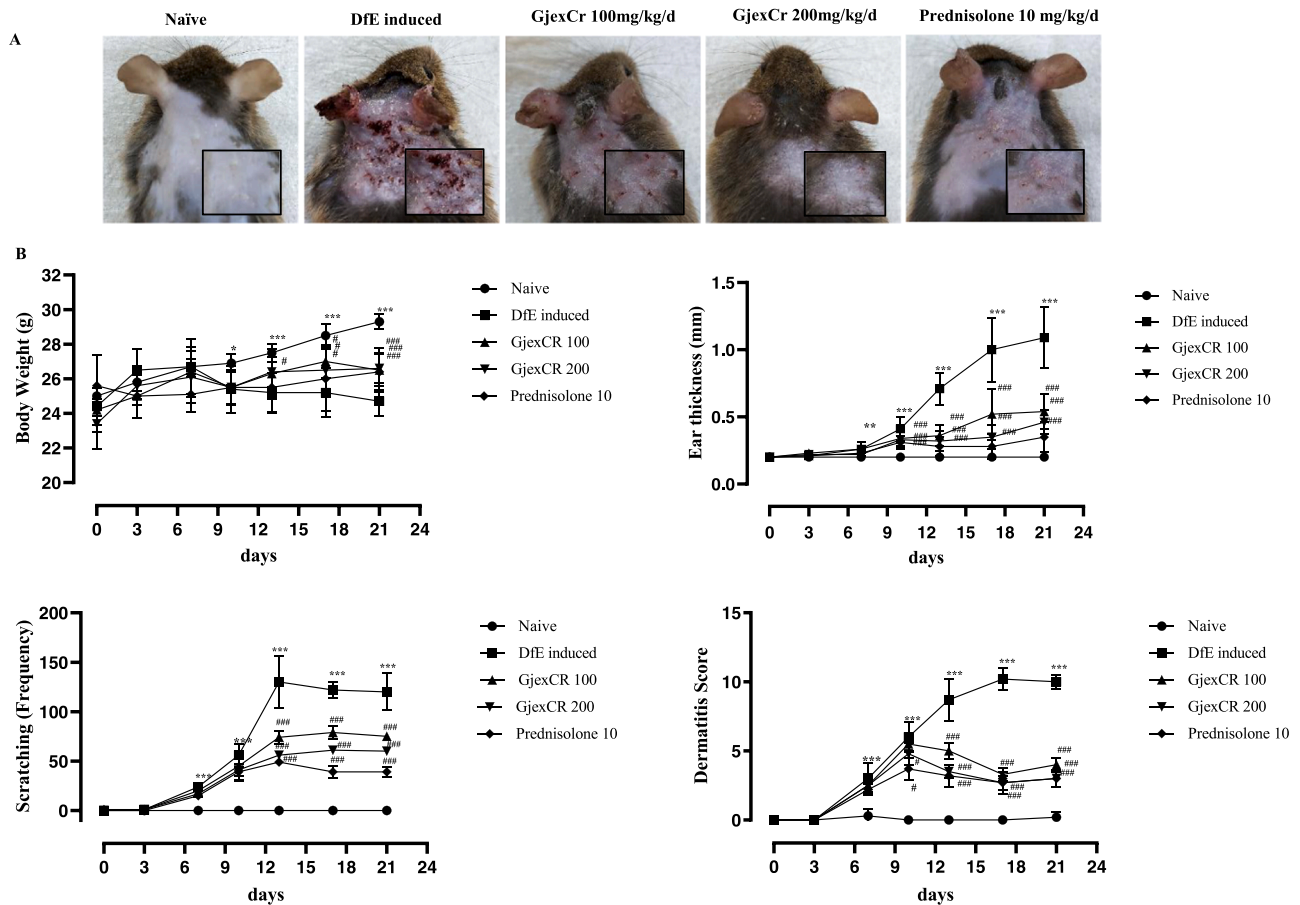


Fig. 3. GjexCr improves AD-like symptoms. (A) Photographic images of skin lesions from AD mice on the last day of treatment. (B) Body weight, ear thickness, scratching, and dermatitis scores of mice. Results are expressed as the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. naïve; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. DfE induced.

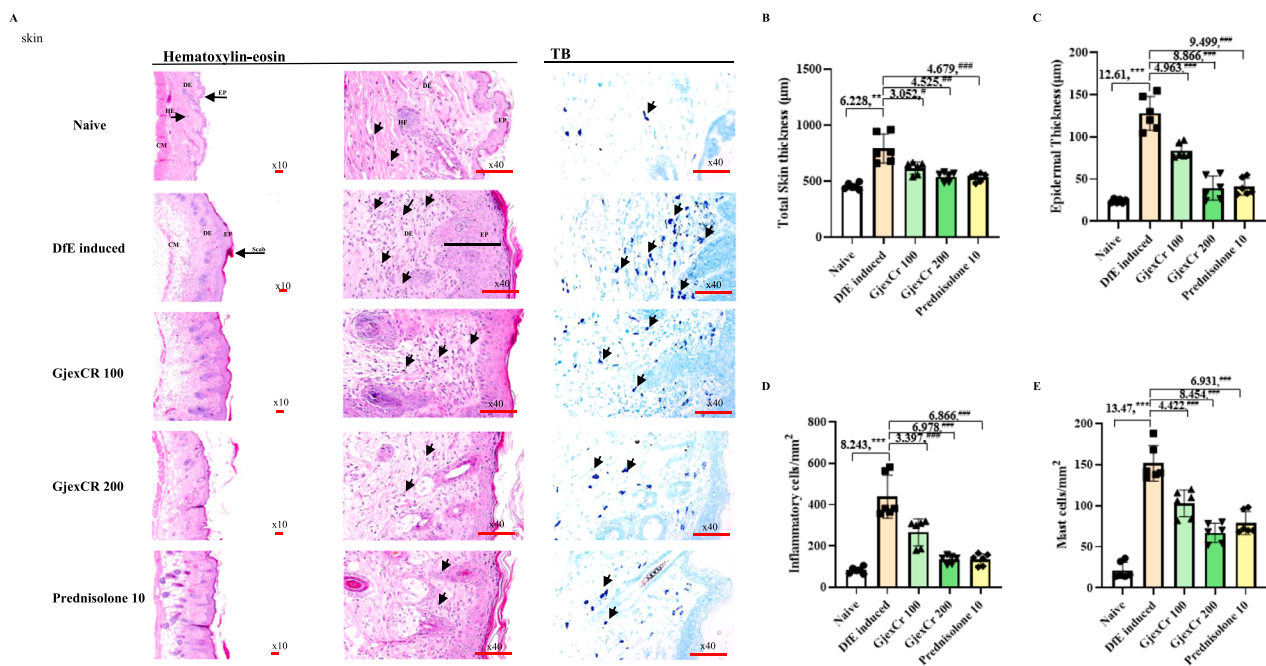


Fig. 4. GjexCr reduces immune cell infiltration in the skin following AD induction. (A) Staining with hematoxylin and eosin (H&E) or toluidine blue in the skin of AD mice. (B) Total skin thickness, (C) epidermis thickness, and accumulation of (D) inflammatory and (E) mast cells. Results are expressed as the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. naïve; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. DfE induced. HF = hair follicle, EP = epidermis, DE = dermis, SE = sebaceous gland, CA = ear cartilage, CM = cutaneous muscle. Scale bars = 80 μ m.

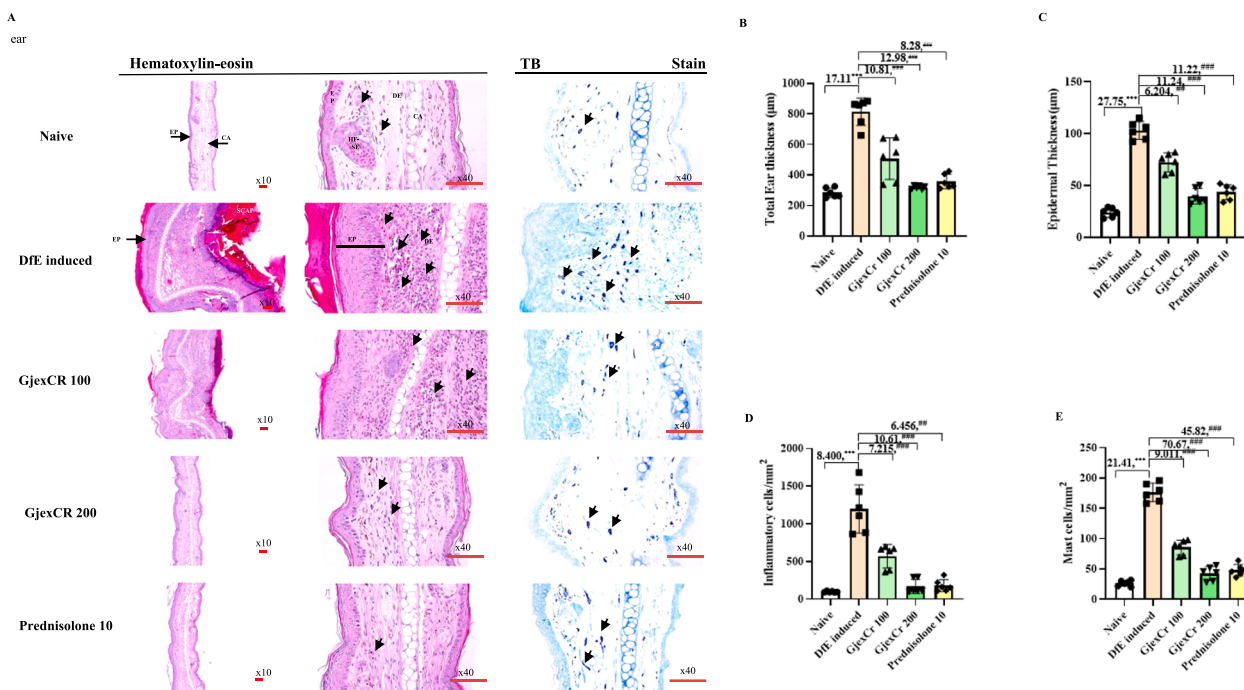


Fig. 5. GJexCr reduces immune cell infiltration in the ear following AD induction. (A) Staining with hematoxylin and eosin (H&E) or toluidine blue in the ear of AD mice. (B) Total skin thickness, (C) epidermis thickness, and accumulation of (D) inflammatory and (E) mast cells. Results are expressed as the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. naive; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. DfE induced. HF = hair follicle, EP = epidermis, DE = dermis, SE = sebaceous gland, CA = ear cartilage, CM = cutaneous muscle. Scale bars = 80 μ m.

still needed, some herbal medicines administered topically and orally have been shown to be effective in treating skin diseases such as AD [12]. For example, *Gardenia jasminoides* (*G. jasminoides*) is an important medicinal herb in traditional Korean medicine [13]. Several studies have reported that this plant species can elicit multiple biological and physiological benefits, such as antioxidant properties, hypoglycemic effect, inhibition of inflammation, antidepressant activity, and improved sleep quality [14]. Geniposide, a bioactive compound found in *G. jasminoides*, is used as an indicator in the quantitative determination of *G. jasminoides* in the Korean and Chinese Pharmacopoeia [15]. Recently, *G. jasminoides* has been found to be beneficial in treating AD-like skin lesions through its suppression of Th2-related cytokines. However, crocin did not show a significant effect on AD [16,17]. Here, this study investigates the role of a *G. jasminoides* extract without crocin (GJexCr) in improving skin damaged by AD in a mouse model in which AD is induced using *Dermatophagoides farinae* extract (DfE).

2. Materials and methods

2.1. Preparation of *G. jasminoides* Ellis extract without crocin (GJexCr)

The *G. jasminoides* Ellis fruit was obtained from Hanpoong Pharm. Co Ltd. (Wanju-gun, Jeollabuk-do, South Korea). Reflux extraction was used to obtain purified GJexCr as follows. The *G. jasminoides* Ellis fruit (100 g) was soaked twice in 1 L of 70% ethanol at 53–57 °C for a total 240 min (two 120 min soaks) followed by sequential filtration through 5 μ m and 1 μ m paper filters. To remove the crocin, the filtrate was incubated for 24 h stirred (Lab Stirrer, MS-6010D, Misung Scientific, Korea) with activated carbon. After stirred, centrifuged (Hanil Combi 514R, Korea) at 5332.8 g at 4 °C, 15 min and then evaporated and lyophilized. The extract yield was 16.92%. The plant name was verified at <http://www.theplantlist.org> on Dec 8, 2020.

2.2. High-performance liquid chromatography (HPLC) analysis

2.2.1. HPLC conditions

HPLC analysis was performed with a Waters e2695 liquid chromatography system (Waters Co., Milford, MA, USA), equipped with a Waters 2998 photodiode array detector. The Phenomenex Luna C18 column (250 mm $\times$ 4.6 mm; particle size 5 μ m; Phenomenex, Torrance, CA, USA) was used for chromatographic separation and detection at 260 nm. The mobile phase consisted of 0.1% aqueous acetic acid (A) and 0.1% acetic acid in acetonitrile (B). The elution conditions involved holding the starting mobile phase at 90% A and 10% B and applying a gradient of 50% A and 50% B for 30 min. A wash with acetonitrile was applied for 10 min, followed by equilibration at 90% A and 10% B for 10 min. The flow rate was 1.0 mL/min, and the injection volume for all the samples was 20 μ L.

2.2.2. Validation of HPLC method

The HPLC analytical method was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), precision (intraday and interday), and accuracy (recovery). The calibration curves were constructed by plotting the peak area (y) versus the concentration (x) of standard solutions and the linear regression equations were calculated in the form of $y = ax + b$. The LOD and LOQ were determined at signal-to-noise ratios of 3 and 10, respectively. To evaluate intraday precision, three different concentrations of standard solutions were analyzed within the same day, and interday precision was determined on three consecutive days. The recovery was evaluated by spiking the extracts with known amounts of standard solution at three different concentrations.

2.3. Animal care, AD induction, and GJexCr treatment

NC/Nga mice were purchased from Central Lab Animal Incorporated (Seoul, Korea) and allowed to acclimate for 1 week before experiments. The mice were randomly assigned into five groups and housed in cages

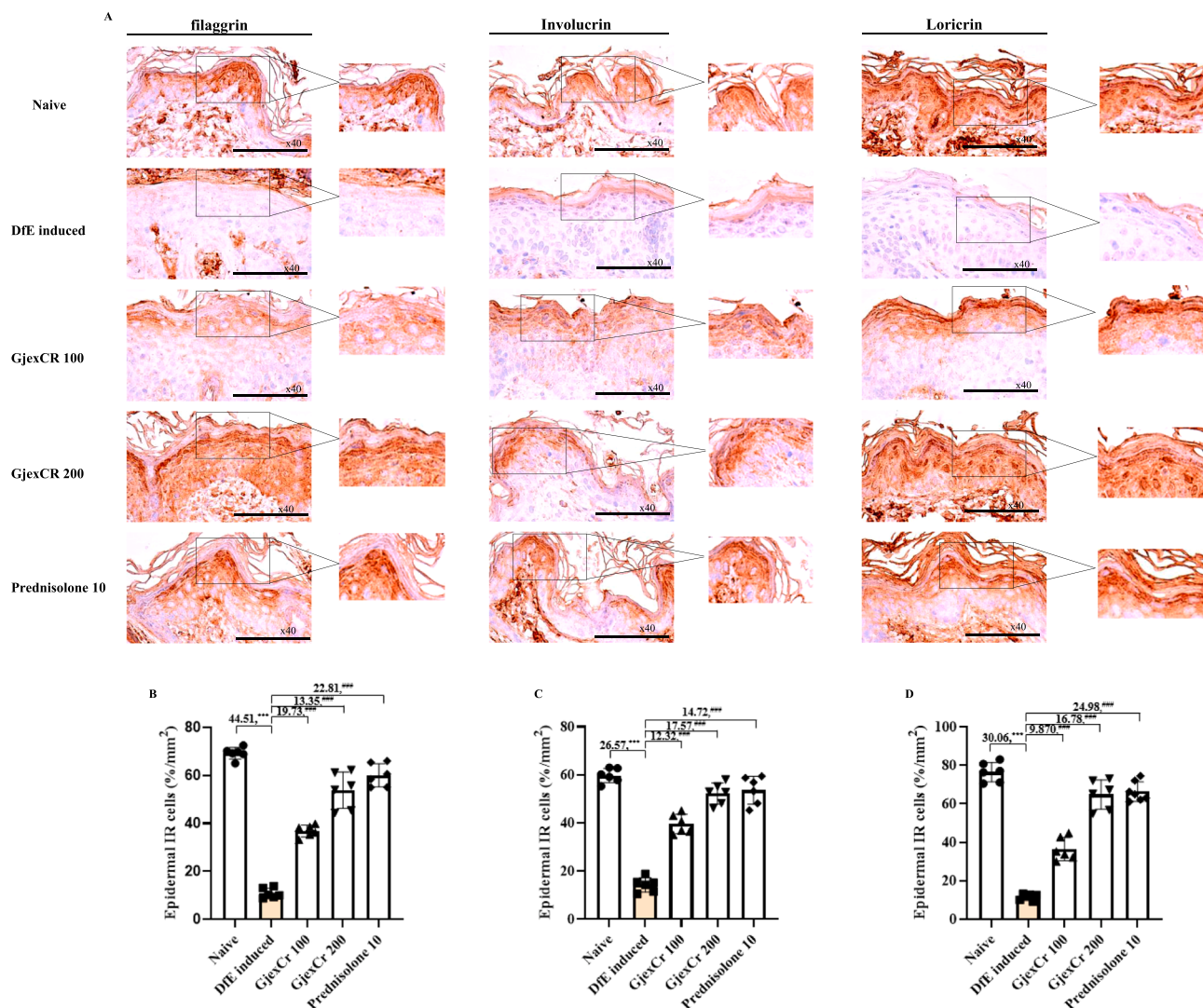


Fig. 6. GJexCr increases the expression of epidermal proteins in AD-like skin lesions. (A) Immunohistochemistry of filaggrin, involucrin, and loricrin in the skin of AD mice. (B) Filaggrin, (C) Involucrin, and (D) Loricrin Epidermal ImmunoReactive Analysis graphs are shown. Results are expressed as the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. naive; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. DfE induced. Scale bars = 80 μ m.

with ad libitum access to a nutritionally complete chow (Purina 38057, Cargill Agri Purina Inc., Sungnam, Korea) and water under alternating 12 h light/dark cycle conditions. The temperature and humidity were maintained at 23 ± 2 °C and $60 \pm 10\%$, respectively. Animal studies were performed in accordance with the Hungnam Korean Food and Drug Administration guidelines. All procedures conformed to regulatory standards and were approved by the Institutional Animal Care and Use Committee at the Korea Institute of Oriental Medicine according to the National Institutes of Health guidelines (Approval no. 19-012/20-069).

To induce AD via skin sensitization, the dorsal skin of each mouse was shaved using electric clippers and hair removal cream. Then, each animal was treated topically with an ointment containing 100 mg of crude *D. farinae* crude extract (Biostir Inc., Kobe, Japan) twice a week for 3 weeks as previously described [16]. After 7 days of DfE application, the mice were treated orally every day for 14 days with either GJexCr at concentrations of 30 mg/kg/d, 100 mg/kg/d, or 300 mg/kg/d, or geniposide at concentrations of 1 mg/kg/d or 3 mg/kg/d.

2.4. Body weight

Body weight was measured on days 0, 7, 10, 13, 17, and 21 using a Secura Precision Balance (Sartorius AG, Göttingen, Germany).

2.5. Ear thickness

Ear thickness was measured on the right ear of each mouse on days 0, 7, 10, 13, 17, and 21 using a digital caliper (CAS, Seoul, Korea).

2.6. Dermatitis scores

The severity of AD-like symptoms was evaluated by the SCORAD method [18]. Dermatitis severity in the dorsal skin/ear of DfE-sensitized AD mice was evaluated based on the occurrence of erythema/hemorrhage, edema, scarring/dryness, and excoriation/erosion. A total clinical dermatitis severity score for each mouse was determined as the sum of the individual scores to yield an Overall Dermatitis score between 0 and 11. Individual severity scores were defined as 0 (no symptoms), 1 (mild), 2 (moderate), and 3 (severe). At the start of this study, the score for each group was 0.

2.7. Assessment of epidermal irritation

Epidermal irritation was measured by examining scratching by the hind paw for 20 min. The conclusion of a scratching bout was defined as the time when a mouse either licked its hind paw or placed it back on the

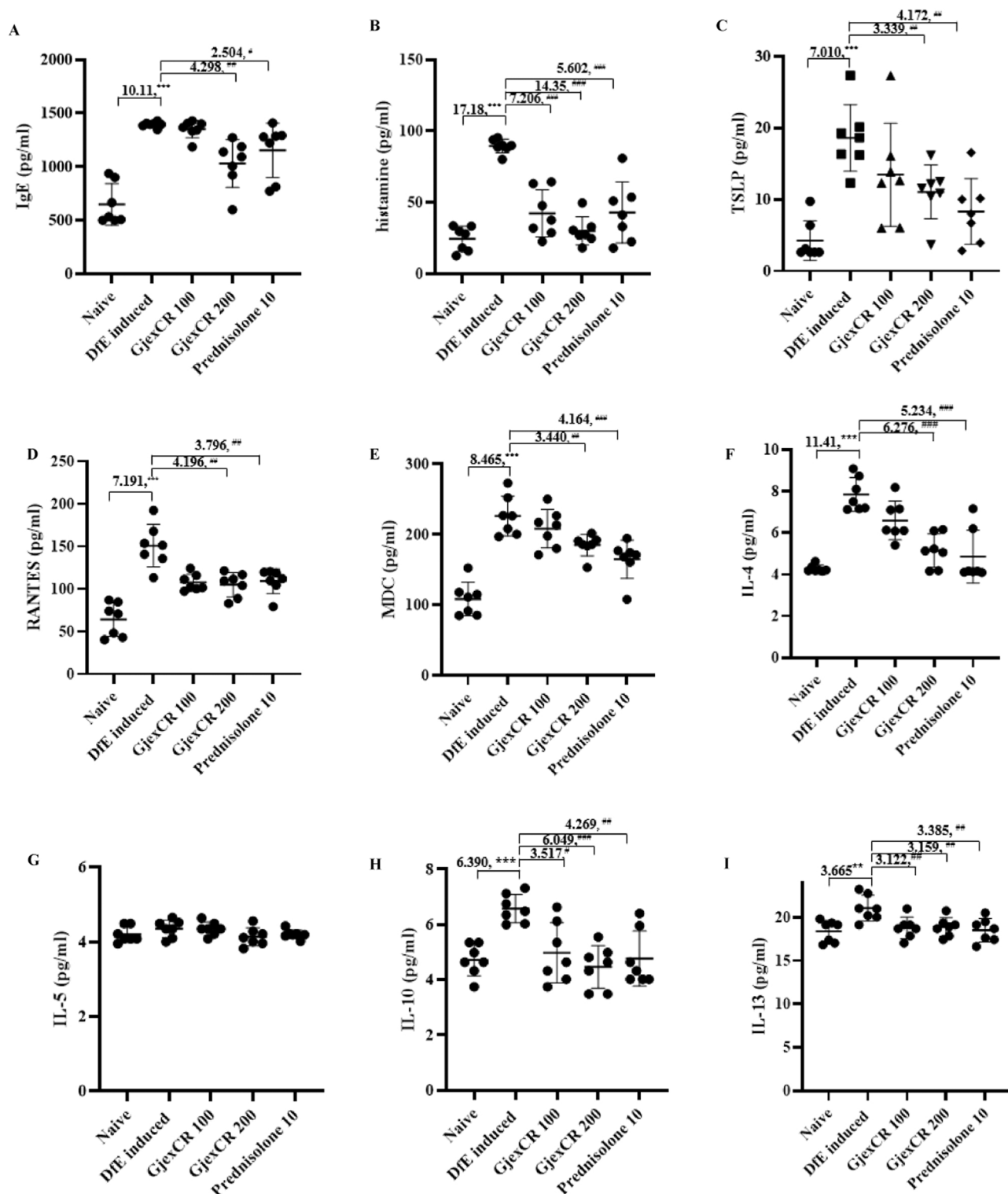


Fig. 7. GjexCr reduces the production of IgE and Th2-related inflammatory cytokines in the serum of AD mice. Production of IgE, histamine, TSLP, RANTES, MDC, IL-4, IL-5, IL-10, and IL-13. Results are expressed as the mean $\pm$ SEM. * P < 0.05, ** P < 0.01, *** P < 0.001 vs. naive; # P < 0.05, ## P < 0.01, ### P < 0.001 vs DfE induced.

floor. A series of one or more biting movements was counted as one episode that ended when the animal returned to a straight position.

2.8. Histopathological analysis

Immunohistochemistry experiments were performed as described

previously [16]. All trimmed ear and dorsal skin tissues were re-fixed in 10% neutral buffered formalin for 24 h. The fixed tissues were embedded in paraffin using an automated tissue processor (Shandon Cotadel 2000, Thermo Fisher Scientific, Waltham, MA, USA) and embedding workstation (Shandon Histostar, Thermo Fisher Scientific). An automated microtome (RM2255, Leica Biosystems, Nussloch,

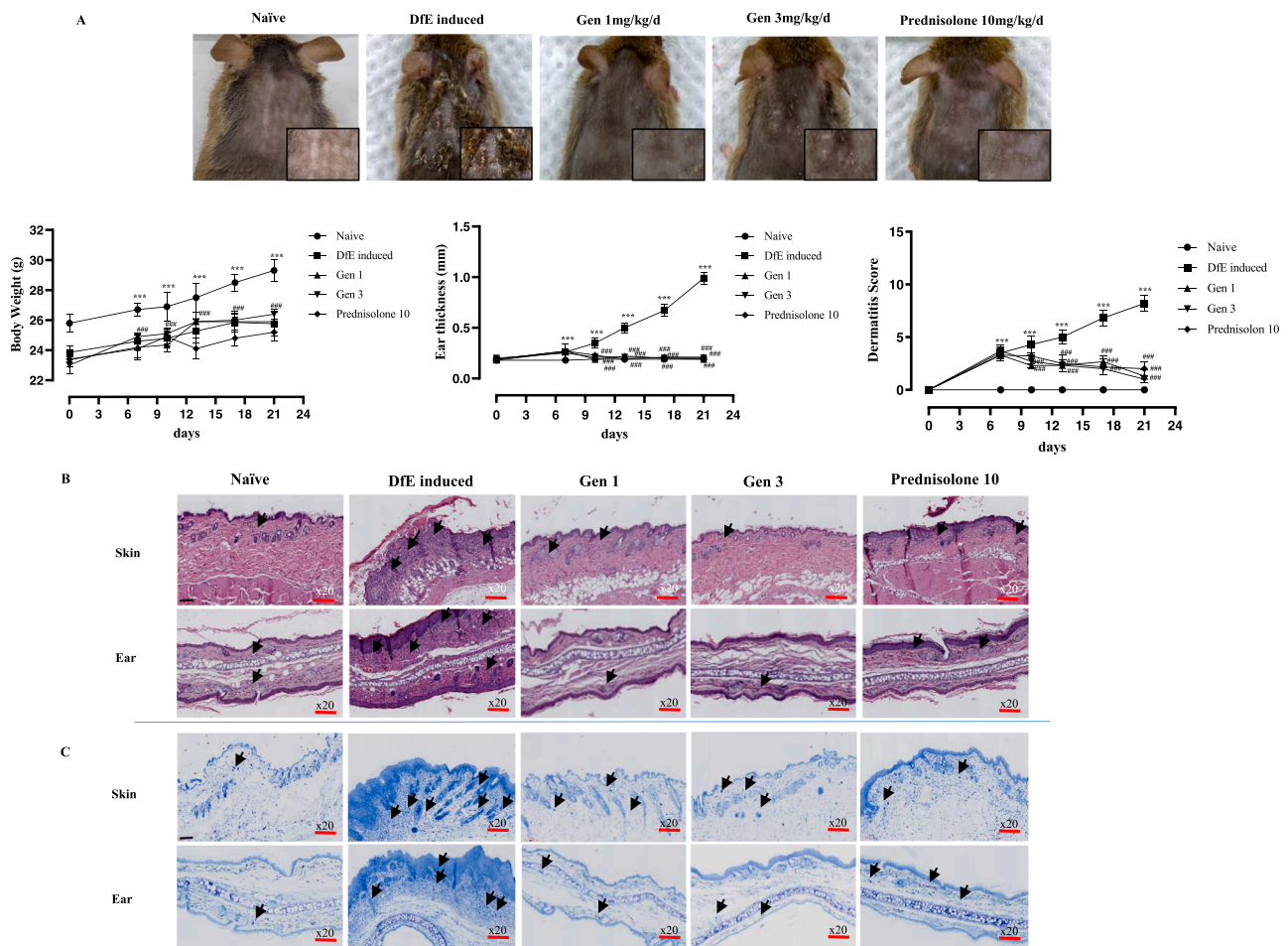


Fig. 8. Geniposide improves AD-like symptoms. (A) Photographic images of skin lesions in AD mice were taken on the last day of treatment. Body weight, ear thickness, and dermatitis scores of AD mice are shown. Staining of (B) inflammatory cells and (C) mast cells in the skin and ear of AD mice. Results are expressed as the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. naïve; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs DfE induced.

Germany) was used to produce 3–4- μ m thick serial sections of dorsal skin or ear tissue.

Representative sections were stained with hematoxylin and eosin (H&E) for general histopathology or toluidine blue (TB) for mast cell staining in the dorsal skin or ear according to previously established methods. After staining, the histological profiles of individual sections of the ear and dorsal skin were visualized by light microscopy (Eclipse 80i, Nikon, Tokyo, Japan). The total thickness of the ear, including cartilage, and dorsal skin, epidermis to dermis (μ m); mean ear interior and dorsal skin epidermal thickness (μ m); and the number of infiltrating inflammatory cells in the ear and dorsal skin dermis (cells/ mm^2 of dermis) were determined after H&E staining using a computer-assisted image analysis program (iSolution FL version 9.1, IMT i-solution Inc., Vancouver, Quebec, Canada). In addition, the mean numbers of eosinophils and mast cells located in the ear and dorsal skin dermis (cells/ mm^2 of dermis) were determined following TB staining using a computer-assisted image analysis program, according to previous methods [16].

Changes in immunoreactivity of skin barrier-related epidermal proteins, such as filaggrin, involucrin, and loricrin, in the dorsal skin were observed using purified primary antibodies (LSBio Inc. Seattle, WA, USA) with an ABC and peroxidase substrate kit (Vector Labs, Burlingame, CA, USA). Primary antisera were treated overnight at 4 °C in a humidified chamber and then incubated with a biotinylated pan-secondary antibody and ABC reagents for 1 h at room temperature in a humidified chamber. Then, the sections were treated with a 3,3'-diaminobenzidine-based peroxidase substrate for 3 min at room temperature. Detection of each skin barrier-related epidermal protein (filaggrin,

involucrin, and loricrin) on the dorsal skin tissue was regarded as positive (+) compared to that of other background regions, and the mean number of cells staining positively for filaggrin, involucrin, and loricrin was calculated as %/ mm^2 using a computer-assisted image analysis program according to established methods.

2.9. Analysis of IgE, histamine, and chemokine levels by enzyme-linked immunosorbent assay

Mice were lightly anesthetized before collection of blood samples from the heart. Samples were placed in Serum Separator Gel tubes (BD Microtainer, Franklin Lakes, NJ, USA) on ice for 30 min before centrifugation at 13,000 rpm at 4 °C for 10 min. All samples were stored at –80 °C until use. The levels of IgE (FUJIFILM Wako Shibayagi, Ishihara, JAPAN), histamine (LDN, Nordhorn, Germany), Macrophage-derived chemokine (MDC), thymic stromal lymphopoietin (TSLP), and the cytokine RANTES (regulated upon activation, normal T cell expressed and presumably secreted) (R&D Systems Inc., Minneapolis, MN, USA) were measured using enzyme-linked immunosorbent assay kits according to the manufacturer's protocol. Absorbance was measured using a Thermo MultiSkan GO Microplate Reader (Thermo Fisher Scientific).

2.10. Data analysis

Values are expressed as the mean $\pm$ standard error of the mean (SEM). Statistical comparisons were performed using the unpaired Student's *t*-test and analysis of variance (ANOVA) on repeated

measurements using GraphPad Prism 8, 2020 (GraphPad Software, San Diego, CA, USA). Differences with a $p < 0.05$ were considered statistically significant.

3. Results

3.1. Chemical composition of *G. jasminoides*

The *G. jasminoides* extract was analyzed using HPLC to characterize the bioactive compounds in the extract. As previously described, *G. jasminoides* extract contains the bioactive compounds geniposide and crocin [16]. The levels of geniposide and crocin are shown in the chromatograms of the standard mixture (Fig. 1A), the *G. jasminoides* extract before crocin removal (Fig. 1B, top trace), and in GjexCr (Fig. 1B, bottom trace). The *G. jasminoides* extract contained 108.66 mg/g geniposide and 8.55 mg/g crocin, while GjexCr contained 140.79 mg/g geniposide and 5.82 mg/g crocin. The calibration curves showed good linearity ($R^2 > 0.999$) in the test ranges, and the LOD and LOQ values were in the range of 0.01–0.02 $\mu\text{g/mL}$ (Table 1). The RSD values of precision (intraday and interday) in were less than 2.09%. The recovery of the analytes was in the range of 97.84–101.80%, and RSD values were less than 0.64% (Table 2). Table 3 presents the contents of analytes in the *G. jasminoides* extract and GjexCr.

3.2. GjexCr treatment attenuates AD-like skin lesions and scratching behavior

To test if GjexCr treatment can alleviate symptoms of AD, mice were treated with DfE topically twice weekly to induce AD and then given oral doses of GjexCr daily for 2 weeks (Fig. 2). Repeated topical application of DfE aggravated the skin and caused AD-like symptoms, such as increased thickness of the ear and more frequent scratching behavior (Fig. 2A, B). Interestingly, daily oral administration of GjexCr for 2 weeks following DfE treatment reduced the ear and dermal thickening caused by hyperkeratosis as compared to the group treated with DfE alone [thickness, $F(24,315) = 30.11$ $p < 0.001$; scratching, $F(24,315) = 88.76$ $p < 0.001$]. Moreover, GjexCr treatment had an antipruritic effect, resulting in significantly reduced scratching behavior and restoration of dermatitis scores (Fig. 2B) [dermatitis scores, $F(24,315) = 94.87$ $p < 0.001$] (Fig. 3).

3.3. GjexCr treatment ameliorates AD-like skin inflammation and mast cell accumulation

To explore the mechanism by which GjexCr improves AD-like skin symptoms, skin samples were analyzed at the cellular level using H&E and toluidine blue staining. Topical application of DfE significantly increased total skin and epidermis thickness, as well as infiltration of inflammatory cells. Importantly, the administration of GjexCr restored the total skin and epidermal thickness and reduced the number of inflammatory cells in these tissues (Fig. 4, skin and Fig. 5, ear) [Ear, total skin, $F(4,25) = 47.07$ $p < 0.001$; epidermis thickness, $F(4,25) = 102.4$ $p < 0.001$; inflammatory cells, $F(4,25) = 45.28$ $p < 0.001$; Skin, total skin, $F(4,25) = 20.83$ $p < 0.001$; epidermis thickness, $F(4,25) = 69.12$ $p < 0.001$; inflammatory cells, $F(4,25) = 37.26$ $p < 0.001$]. Interestingly, the accumulation of mast cells was significantly increased in animals treated with DfE in both the skin and ear, which was restored to normal levels after administration of GjexCr to the dorsal skin of both (arrows, Figs. 4 and 5) [Ear mast cell, $F(4,25) = 176.8$; Skin mast cell, $F(4,25) = 59.33$].

3.4. GjexCr treatment restores defects in the epidermal barrier

To investigate the mechanism underlying the protective effect of GjexCr on the skin, we first examined the expression of epidermal proteins. Topical application of DfE disrupted the expression of filaggrin,

involucrin, and loricrin, suggesting that these proteins are involved in aggravating the skin barrier in AD. In contrast, GjexCr treatment resulted in increased expression of these proteins (Fig. 6), indicating their role in GjexCr-mediated skin barrier recovery from AD [filaggrin, $F(4,25) = 163.1$; involucrin, $F(4,25) = 112.7$; loricrin, $F(4,25) = 146.9$].

3.5. GjexCr treatment regulates immunoglobulin and cytokine levels

To examine the role of GjexCr in reversing the symptoms of AD, we measured the levels of cytokines, histamine, chemokines, TSLP, Th2-mediated IgE, and the Th2-associated immune response in treated animals. Topical application of DfE resulted in increased levels of chemokines (e.g., MDC and RANTES), TSLP, IgE, and histamine [IgE, $F(4,30) = 19.9$ $p < 0.001$; histamine, $F(4,30) = 24.7$ $p < 0.001$; TSLP, $F(4,30) = 8.78$ $p < 0.001$; RANTES, $F(4,30) = 21.6$ $p < 0.001$; TSLP, $F(4,30) = 8.78$ $p < 0.001$; MDC, $F(4,30) = 23.7$ $p < 0.001$]. These AD-mediated increases were reduced by GjexCr treatment (Fig. 7A–E). In addition, we assessed the production of IL-4, IL-5, IL-10, and IL-13 and found that these Th2-related factors were significantly reduced by GjexCr administration compared with the DfE only group (Fig. 6F–I) [IL-4, $F(4,30) = 19.3$ $p < 0.001$; IL-5, $F(4,30) = 1.67$; IL-10, $F(4,30) = 7.41$ $p < 0.001$; IL-13, $F(4,30) = 5.16$ $p < 0.001$]. Collectively, our data demonstrate that GjexCr administration attenuated the imbalance of the immune response.

3.6. Geniposide treatment attenuates AD-like skin lesions and inflammatory response

To understand which components within GjexCr are responsible for the improvements on AD-like symptoms, a candidate approach was taken. Geniposide, a bioactive compound found in *G. jasminoides*, was treated orally every day given to mice after induction of AD-like symptoms by DfE treatment. The results demonstrate that ear thickness and dermatitis scores were significantly reduced by geniposide treatment compared to animals treated with DfE alone (Fig. 8A) [thickness, $F(20,138) = 140.4$ $p < 0.001$; dermatitis scores, $F(20,318) = 40.70$ $p < 0.001$]. Furthermore, geniposide reversed the skin and epidermal thickness, as well as the infiltration of inflammatory cells (Fig. 8B). Moreover, the accumulation of mast cells was significantly decreased by administration of geniposide to the dorsal skin of both ears (arrows, Fig. 8C). Taken together, these data indicate that extract from *G. jasminoides* is a potentially effective treatment for AD.

4. Discussion

AD, a chronic allergic inflammatory skin condition, occurs as a result of a complex interaction between immunological, environmental, and genetic mechanisms [19]. Due to its chronic nature and frequently occurring relapses, AD can substantially impact quality of life. This condition often requires long-term systemic treatment, especially in adult patients who frequently experience adverse reactions to topical treatments with mild to highly potent corticosteroids and/or calcineurin inhibitors [3]. Previous studies have shown that traditional herbal medicine can significantly improve the symptoms and severity of AD. Moreover, these treatments have been shown to be well-tolerated by patients. More rigorous clinical research and pharmacological studies of herbal medicine formulas are needed to obtain stronger evidence regarding the efficacy and safety of herbal medicine treatments for AD outside of the clinical trial setting. Furthermore, there is interest in developing a safe, curative herb-derived compound for treating AD based on combined knowledge of molecular biology and combinatorial chemistry technologies [20,21]. Previous studies demonstrated that crocin, a component of *G. jasminoides* extract, was not effective in blocking histamine release [16,17]. Moreover, when crocin is removed from the *G. jasminoides* extract, geniposide content increased. This study demonstrated an effect of the Korean traditional herbal medicine,

GjexCr, on skin barrier dysfunction and activation of Th2 cells in DfE-induced NC/Nga mice.

Elevated serum IgE levels are a major characteristic of AD, as several studies have demonstrated that serum IgE levels are elevated in patients with AD. Furthermore, serum IgE levels have been shown to be elevated in NC/Nga mice with AD-like skin lesions [22]. In this study, IgE levels are increased in DfE-treated NC/Nga mice. TSLP is expressed in the epidermis after mechanical stimulation and plays a principal role in the activation of Th2 cytokine-secreting natural killer cells and serum IgE, which are increased in AD skin. IgE production is associated with a predominantly Th2 cellular response [23,24]. In this study, AD-induced IgE, histamine, Th2-related cytokine and chemokine (e.g., IL-4, IL-5, IL-10, IL-13, RANTES, and MDC) production was reduced by GjexCr. In addition, this extract diminished the infiltration and degranulation of inflammation/mast cells in DfE-induced AD-like skin lesions. In allergic inflammation, Th2-derived cytokines can mediate mast cell homeostasis. Cytokines have been shown to play a role in mast cell hyperplasia during Th2-mediated inflammation, including IL-3, IL-4, and IL-10 [25], all of which are important players in the pathogenesis of allergic reactions [26]. Results from this study strongly support the hypothesis that GjexCr may regulate Th2 cell-related cytokine production by reducing the production of mast cells and IgE, which may further reduce the progression of AD.

In AD patients, reduced filaggrin expression could result from either mutations in the filaggrin gene or from the effects of Th2 cytokines [27, 28]. In addition, expression of epidermal differentiation-related molecules, such as filaggrin, loricrin, and involucrin, has been demonstrated in AD patients and data suggest that these molecules affect permeability barrier homeostasis [7]. These studies support the results that GjexCr reverses skin barrier dysfunction induced by AD possibly by re-localizing filaggrin, loricrin, and involucrin activity to the epidermis.

Previous studies have shown that protein antigens penetrating the damaged barrier further induce the Th2-shifted response in extrinsic AD [29]. Recent advances in our understanding of the pathophysiology of the epidermal barrier and its critical role in AD pathogenesis has led to increased interest in barrier repair therapies. Studies on AD and barrier repair treatment, either in animal models or in humans, showed that adequate lipid replacement therapy reduces inflammation and restores epidermal function [30]. Therapies targeting the skin barrier either directly (emollients) or indirectly (by targeting mast cells that induce itching or the cytokines that downregulate barrier genes) will most likely be beneficial [1]. These findings show that GjexCr protected the skin barrier by decreasing Th2-related cytokine, IgE, and mast cell levels in NC/Nga mice. Furthermore, the Gjex-containing component geniposide was also found to reduce skin inflammation and mast cell localization in the skin of AD-induced mice. Data from these studies will result in the development of improved therapies for treating AD and, as a consequence, lead to an improved quality of life for AD patients.

5. Conclusions

GjexCr reduced inflammatory symptoms in AD-like skin lesions by suppressing the production of Th2-related cytokines, mast cell infiltration, and the release of serum IgE. Moreover, GjexCr improved the expression of skin barrier factors. The findings provide support for the use of compounds derived from medicinal herbs as a topical treatment for AD.

CRedit author statement

HK Kim: Conceptualization, Methodology, Writing – original draft preparation. **SH Park:** Conceptualization, Methodology, Writing – original draft preparation, Investigation. **S Jang:** Investigation. All authors participated in the preparation of the manuscript and approved the final version submitted for publication.

CRedit authorship contribution statement

HK Kim and SH Park Conception and designed, discussed, and wrote the manuscript. SH Park performed the experiments. S Jang chemical constituents analysis. All authors participated in the preparation of the manuscript and approved the final version submitted for publication.

Conflict of interest statement

The author declare that there is no conflict of interests regarding the publication of this article.

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