



Gardenia jasminoides extract without crocin improved atopic dermatitis-like skin lesions via suppression of Th2-related cytokines in Dfe-induced NC/Nga mice

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ABSTRACT

Ethnopharmacological relevance: Atopic dermatitis (AD) is a pruritic, chronic, relapsing inflammatory skin disease. *Gardenia jasminoides* extract (GJE) has been used as a traditional remedy for the treatment of various inflammatory diseases, including AD. The specific effects of the extract components, which include crocin, geniposidic acid, and gardenoside, on inflammatory responses in AD are not entirely clear.

Aim of the study: We determined the effects of *G. jasminoides* extract with crocin removed (GJE-C) on AD-like skin lesions in *Dermatophagoies farina* crude extract (Dfe)-treated NC/Nga mice, a well-known AD mouse model. **Materials and methods:** To prepare the mice, 150 µl of 4% sodium dodecyl sulfate (SDS) was applied to the shaved dorsal skin or ear of NC/Nga mice 1 h before application of 100 mg Dfe. After 7 d, GJE-C was applied every day for 14 d. We performed behavior, histological, ELISA, assays to evaluate chemokines, cytokines, and skin barrier proteins in skin or serum samples from treated and untreated NC/Nga mice.

Results: Topical application of GJE-C improved the severity scores of the AD-like skin lesions, frequency of scratching, and ear swelling in Dfe-treated NC/Nga mice similar to the complete GJE. In addition, GJE-C also reduced serum IgE and chemokine levels as well as the inflammatory response. Topical application of GJE-C also resulted in decreased infiltration of inflammatory cells, such as mast cells, via reduction of Th2 inflammatory mediators, including interleukin (IL)-4, IL-5, and IL-13, pro-inflammatory cytokines, and chemokines, and increased skin barrier protein expression in Dfe-treated NC/Nga mice. The GJE components geniposidic acid and gardenoside inhibited the production of atopic-related chemokines in HaCaT cells, but inclusion of crocin dampened this inhibition of chemokine production.

Conclusions: Together, these findings indicate that GJE-C may improve AD-like lesions by inhibiting the Th2 inflammatory response and expression of chemokines while increasing the expression of skin barrier proteins. These data provide experimental evidence that GJE-C may harbor therapeutic potential for AD.

1. Introduction

Atopic dermatitis (AD) is a major inflammatory skin disorder characterized by severe itching and relapsing eczematous lesions. The pathogenesis of AD has been attributed to a complex interaction between a thickened epidermis, skin barrier defects, and immunologic abnormality. Skin barrier proteins, include filaggrin, loricrin, and involucrin, are essential proteins in the defense against the development and aggravation of AD. Destruction of skin barrier proteins is an

important process in the pathogenesis of AD, and such destruction is the result of sensitive inflammatory reactions accompanied by erythema, lichen sores, itchiness, and subsequent scratching of the skin (Abramovits, 2005). Immunologically, an abnormal Th2-cell immune response induces elevated immunoglobulin E (IgE) levels and infiltration of AD effector cells, such as mast cells (Choi et al., 2017), potentially aggravating the disease and triggering systemic Th2 responses, including respiratory allergies (Lee et al., 2015; Choi et al., 2014b). Thymus and activation regulated chemokine (TARC) and interleukin

Abbreviations: AD, atopic dermatitis; GJE, *Gardenia jasminoides*; GJE-C, *Gardenia jasminoides* extract with crocin removed; Dfe, *Dermatophagoies farina* crude extract; EP, epidermis; DE, dermis; HF, hair follicle; KIOM, Korea Institute of Oriental Medicine

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(IL)-4, -5, and -13, which are produced by Th2 cells, are strongly associated with the onset and development of AD (Yamada et al., 2018). Many chemokines and inflammatory cytokines, such as IL-8, regulated on activation normal T cell expressed and secreted (RANTES), and tumor necrosis factor alpha (TNF- α), affect the skin barrier damage and the inflammatory mechanisms (Cha et al., 2017), further complicating the relationship between the skin barrier defect and the inflammatory response in AD.

NC/Nga mice harbor a mutation on chromosome 9, leading to increased IgE production and a Th2-dominant inflammation. Many studies used Dfe-induced in NC/Nga mice as atopic dermatitis animal model (Oshio et al., 2009; Yamamoto et al., 2007). These pathophysiological characteristics in NC/Nga mice strongly resemble human AD, and thus, this mouse strain has served as a useful model to analyze the pathological mechanisms of human AD (Lee et al., 2015; Yagi et al., 2002; Suto et al., 1999). In agreement with the results of the mutation in these mice, previous studies demonstrated the involvement of Th2 cells in the development of AD-like skin lesions in NC/Nga mice (Cha et al., 2017; Kim et al., 2011).

Traditionally, *Gardenia jasminoides* was used in many Asian countries for the treatment of inflammatory disorders, and ulcers of the skin, suggesting its anti-inflammatory effects (Ma et al., 2017). Indeed, studies reported that *G. jasminoides* inhibits expression of inflammatory-related factors, such as TNF- α , IL-4, and CD68, in inflammatory disease models, including asthma or allergies (Deng et al., 2013; Zang et al., 2018; Sung et al., 2014). *G. jasminoides* extract (GJE) contains iridoid glycosides and their aglycones, including gardenoside, geniposide, and crocin (also called gardenia yellow; Yu et al., 2009). In previous studies, we reported that geniposide, but not crocin, is the active GJE component that suppresses histamine release *in vitro* (Sung et al., 2014). Therefore, we aimed to determine whether application of GJE with the crocin removed (GJE-C) suppresses the Th2 cytokine production required for the serum IgE response, infiltration of mast cells, and defective skin barrier in AD in NC/Nga mice.

2. Materials and Methods

2.1. Animals and ethics

Male NC/Nga mice (8 weeks old) were purchased from Central Lab Animal Inc. (Seoul, Korea). The mice were fed a standard laboratory diet (Purina 38057, Cargill Agri Purina Inc., Sungnam, Korea) and water *ad libitum* and were provided with a natural corn cob bedding material (BioCOB 10384197, Bio System Corporation Pte Ltd, Peninsula Plaza, Singapore). All procedures conformed to regulatory standards based on the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at the Korea Institute of Oriental Medicine (KIOM).

2.2. Animal experimental design

The skin dermal barrier was disrupted via application of 150 μ l 4% sodium dodecyl sulfate (SDS) to the shaved dorsal skin or ear of NC/Nga mice 3 h before application of 100 mg of crude *Dermatophagoies farina* extract (Dfe; Biostir Inc., Kobe, Japan). Mice were treated with Dfe two times each week for 3 weeks (Fig. 1A). Then, 7 d after final Dfe application, GJE or GJE-C dissolved in acetone:olive oil (4:1 v/v) was applied every day for 14 d. The NC/Nga mice were randomly divided into five groups: normal (untreated), AD-control (Dfe only), 100 mg of AD-GJE, or AD-GJE-C, and tacrolimus (0.1% protopic ointment, positive control). For the normal and control group, the same volume of vehicle without GJE or GJE-C was applied.

2.3. Preparation of GJE and GJE-C

The *G. jasminoides* was obtained from Mega Herb, Co. (Cheonbuk,

Korea). A voucher specimen (no. KIOM 32/KIOM 51) was deposited at the herbarium of the Herbal Medicine Research Division at the KIOM. *G. jasminoides* dried plants (100 g) were extracted in 1 L of 70% ethanol for 120 min followed by filtration sequentially through 5- μ m and 1- μ m filters. The extract was dried under reduced pressure and subsequently stored at -20°C until use. The extract yield was 87.3%.

To prepare the GJE-C, *G. jasminoides* dried plants were extracted with 70% ethanol for 120 min and followed by sequential filtration through 5- μ m and 1- μ m filters. The filtrate was incubated with activated carbon. After filtration through a 1- μ m filter, the extract was dried under reduced pressure with a yield of 57.3%.

The plant name was checked at www.theplantlist.org on Nov 8, 2018.

2.4. Ear thickness/scratching

Scratching behavior and ear thickness were measured twice each week. Using a scratching behavior test, scratching frequency of the rostral back and of the caudal back was measured for 20 min. Ear thickness was measured using a digital caliper (©CAS, Seoul, Korea).

2.5. Evaluation of dermatitis severity

The severity of dermatitis was evaluated in ear and skin lesions according to erythema/hemorrhage, edema, scarring/dryness, and excoriation/erosion. The total clinical dermatitis severity score for each mouse was defined as the sum of the individual scores (0, no symptoms; 1, mild; 2, moderate; and 3, severe), yielding an overall dermatitis score ranging from 0 to 12. At the start of this experiment, the score for each group was 0.

2.6. Analysis of IgE, histamine, and TARC

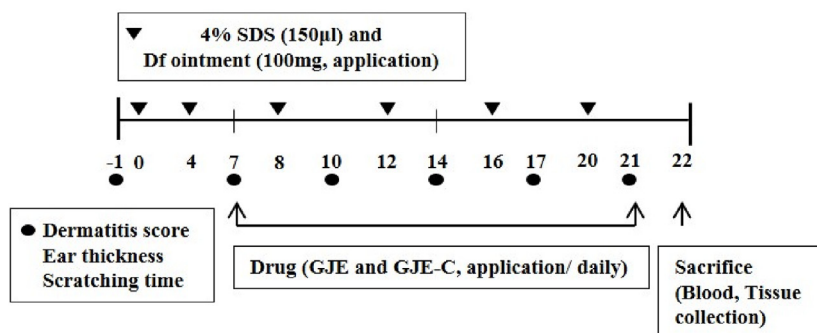
Serum from mice was collected and stored at -80°C until use. The levels of IgE, histamine, and TARC, IL-4, IL-5, IL-13, TNF- α were assessed using ELISA kits (Abcam, Cambridge, UK; Oxford Biomedical Research Inc., Oxford, MI, USA; and R&D Systems Inc., Minneapolis, MN, USA, respectively) according to the manufacturers' protocols. The absorbance was measured using a Thermo MultiSkan GO Microplate Reader (Thermo Fisher Scientific, Waltham, MA, USA).

HaCaT cells (3×10^5 cells) were seeded in wells of a 24-well culture plate and incubated for 24 h. The following day, the cells were stimulated with TNF- α (10 ng/ml, R&D Systems) and interferon (IFN)- γ (10 ng/ml, R&D Systems). The cells were subsequently incubated with GJE or GJE-C for 24 h. The production of MDC, RANTES, TARC and TSLP in the supernatants was measured using ELISA kits (R&D systems) according to the manufacturer's protocol.

2.7. Histopathological analysis

All trimmed ear and dorsal back skin tissues were re-fixed in 10% neutral-buffered formalin (NBF) for 24 h for histopathological observation. Following fixation, paraffin blocks were prepared for all samples, and one individual ear and one individual dorsal back skin sample from the same animal were located in one block. Then, 3-4- μ m serial sections were prepared as three serial sections. Representative sections were stained with hematoxylin and eosin (H&E) for general histopathology and with Toluidine blue (TB) stain for mast cells according to previously established methods (Chiba et al., 2012; Kim et al., 2012, 2013; Kawahara et al., 2017). Then, histological profiles of individual sections of the ear and dorsal back skin were observed under a light microscope (Model Eclipse 80i, Nikon, Tokyo, Japan). To examine changes in more detail, epidermal thicknesses (μ m) and numbers of infiltrated inflammatory cells (cells/mm² of dermis) in the ear and dorsal back skin were calculated using a computer-assisted image analysis program (iSolution FL ver 9.1, IMT i-solution Inc., Vancouver,

A



B

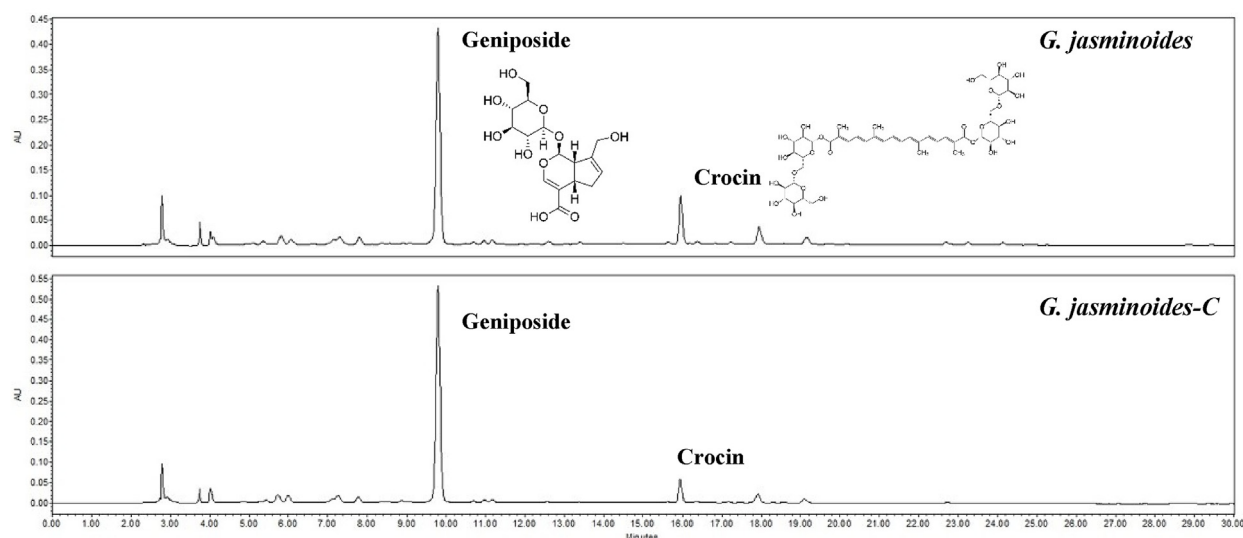


Fig. 1. Experimental protocol and HPLC analysis. (A) Experimental protocol, To induce AD, 150 µl of 4% sodium dodecyl sulfate (SDS) was applied to the dorsal skin or ears of NC/Nga mice. After 1 h, 100 mg of Dfe was topically applied to the dorsal back skin and ears of NC/Nga mice. After 1 week of Dfe application, the mice were treated with GJE or GJE-C every day for 14 d. (B) Analysis of GJE-C by HPLC reveals the chemical constituents. HPLC chromatogram of GJE and GJE-C at 260 nm.

Quebec, Canada). In addition, mean mast cell numbers in the ear and dorsal back skin dermis (cells/mm² of dermis) were also measured using a computer-assisted image analysis program according to previously published methods (Kim et al., 2013; Kim et al., 2015a,b; Kawahara et al., 2017).

2.8. Western blot analysis

Ear or back skin samples were homogenized in PRO-PREB protein extraction buffer (iNtRON Biotechnology, Seongnam, Korea) to obtain protein extracts. The proteins were fractionated via 10% SDS-PAGE and transferred to a mini nitrocellulose membrane via a Trans Blot® TURBO Transfer pack (Bio-Rad, Hercules, CA, USA). The membranes were incubated with primary antibodies against involucrin and loricrin (Abcam) and β-actin (Cell Signaling Technology, Beverly, MA, USA) in blocking buffer at 4 °C overnight. The membrane was then incubated for 1 h with horseradish peroxidase-conjugated secondary antibody (Cell Signaling Technology). The blot was developed using an ECL substrate solution (GE Healthcare Life Sciences, Buckinghamshire, UK) for 5 min until satisfactory intensity of chemiluminescence was achieved. The band intensities were measured using an ImageQuant LAS 4000 apparatus (GE Healthcare Life Sciences).

2.9. Cell culture

The spontaneously immortalized human HaCaT keratinocyte cell line (a gift from Dr. H. K. Shin, KIOM, Daejeon, Korea) was cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco BRL, Gaithersburg, MD, USA) with 10% fetal bovine serum (Gibco BRL), 100 U/ml penicillin (Gibco BRL), and 100 µg/ml streptomycin (Gibco BRL) at 37 °C in a humidified 5% CO₂ atmosphere.

2.10. Cell viability

Cells were seeded in 96-well, flat-bottom plates (5 × 10⁴ cells/well) and incubated in the presence of GJE and GJE-C, geniposide, crocin for 24 h. MTT solution (0.5 mg/ml) was added to each well for 2 h (5% CO₂ at 37 °C), and 100 µl of dimethyl sulfoxide (DMSO) was added to solubilize the purple formazan crystals. Absorbance at 540 nm was recorded by microplate reader (Thermo Fisher Scientific).

2.11. Data analysis

Values are expressed as means ± standard error of the mean (SEM). Statistical comparisons were performed using unpaired

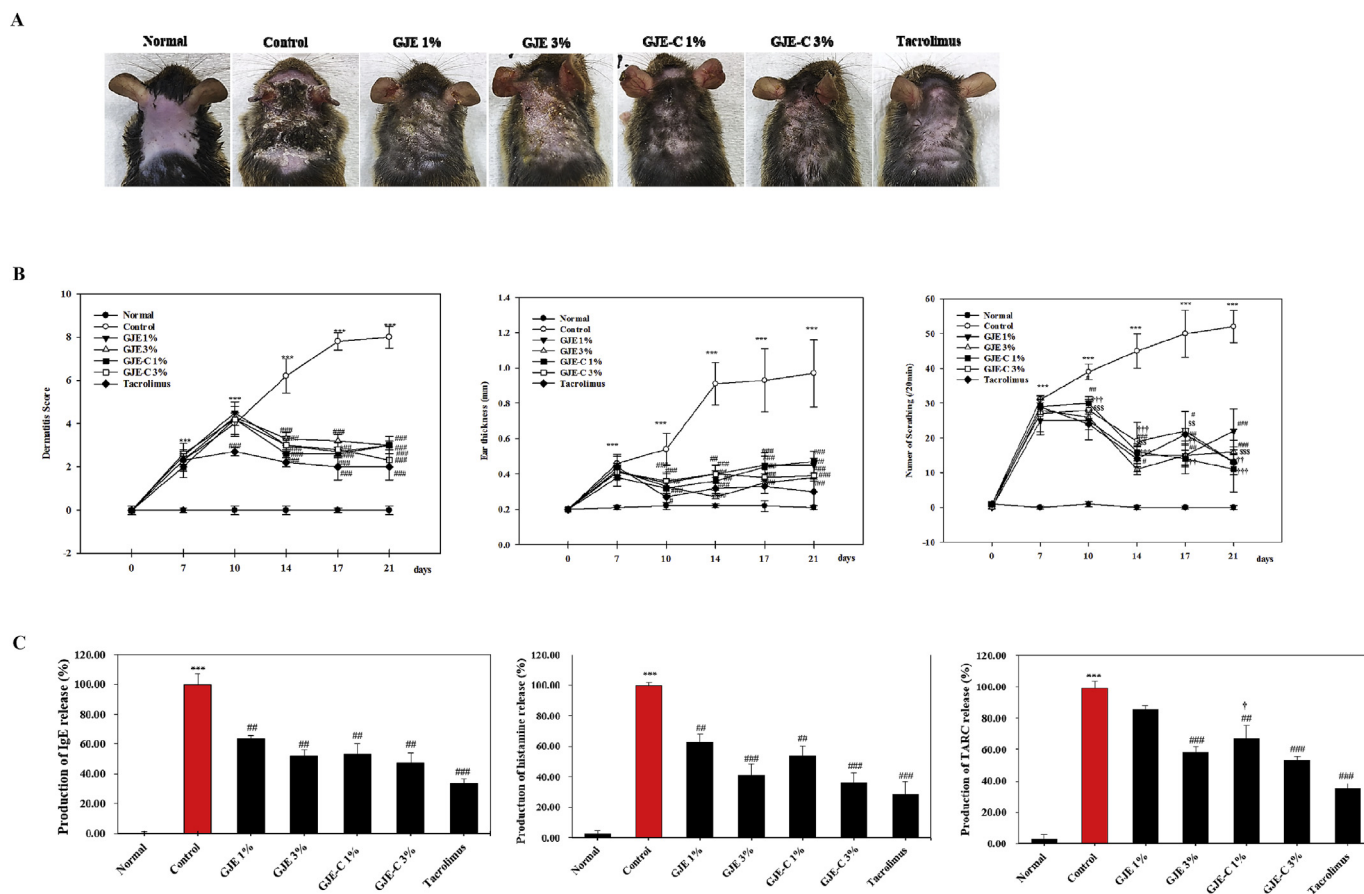


Fig. 2. Protective effects of GJE-C and GJE on the severity of AD-like skin lesions in Dfe-induced NC/Nga mice. (A) Photographs of AD-like back skin and ear. (B) Overall dermatitis scores were determined from the sum of all individual scores. Ear thickness was measured two times each week, while the frequency of scratching was measured for 20 min. (C) The concentrations of total IgE, histamine, and TARC serum levels were measured by ELISA. Data are presented as mean $\pm$ SEM. *** p < 0.001 vs. normal; # p < 0.05, ## p < 0.01, ### p < 0.001 vs. control; † p < 0.01, †† p < 0.001 vs. control; § p < 0.05, §§ p < 0.01, §§§ p < 0.001 vs. control.

Student's *t*-test and analysis of variance (ANOVA) for repeated measures using Sigma Plot software version 13.0, 2016 (Systat Software Inc. San Jose, CA, USA). Differences were considered significant at p < 0.05.

3. Results

3.1. Standardization of GJE/GJE-C by HPLC

We have HPLC analysis on the GJE. In the previous study, the proper chemical profile of GJE was identified [chemical composition of *Gardenia jasminoides* fruit; geniposide acid, corocetin] (Koo et al., 2006; Yamauchi et al., 2011). Therefore, we component measured in GJE and GJE-C by HPLC analysis was crocin with retention time of approximately, 15.9 min. The detection wavelength was set at 260 nm for the standard and the samples. Calibration curves for compound showed linearity (r^2 > 0.9999) using the described HPLC. The amounts of crocin in GJE and GJE-C were 8.2 mg/g, 5.0 mg/g, respectively (Fig. 1B).

3.2. GJE-C abrogates the severity of AD-like skin lesions and barrier dysfunction in the Dfe-induced NC/Nga mice

We determined whether GJE or GJE-C suppresses AD-like lesion development and barrier dysfunction in the Dfe-treated NC/Nga mouse AD model. The Dfe-induced AD mouse showed an increased frequency of scratching, greater ear thickness, and higher dermatitis scores than normal untreated mice. In contrast, application of GJE resulted in

decreased frequency of scratching, ear thickness, and dermatitis scores compared to control Dfe-induced AD mice. Moreover, treatment with GJE-C yielded more potent suppression of the AD-like lesions (dermatitis score at 21 d, F [6, 41] = 56.42, p < 0.001), ear thickness (21 d, F [6, 41] = 31.96, p < 0.001), and scratching (21 d, F [6, 41] = 131.46, p < 0.001; Fig. 2A–B) than the GJE.

3.3. GJE-C suppresses the inflammatory response in Dfe-induced NC/Nga mice

To determine whether GJE and GJE-C suppress the inflammatory response, including inhibition chemokines and cytokines, in the Dfe-treated NC/Nga AD mouse model, we first measured the levels of the chemokine TARC, histamine, and IgE in the serum of the treated animals. The Dfe-treated AD mice exhibited increased TARC, histamine, and IgE; however, these levels were reduced by GJE-C or GJE application (TARC: F [6,20] = 81.91, p < 0.001; histamine: F [6,20] = 37.12, p < 0.001; IgE: F [6,20] = 48.30, p < 0.001; Fig. 2C). We next performed skin and ear histological analyses of H&E-stained samples. GJE-C application reduced hyperkeratosis, as evidenced by epidermal thickness, and inflammatory cell infiltration in back skin and ears of Dfe-treated NC/Nga mice, and GJE induced similar effects (epidermal thickness back skin: F [6,41] = 131.46, p < 0.001; inflammatory cells back skin: F [6,41] = 52.58, p < 0.001; epidermal thickness ear: F [6,41] = 131.14, p < 0.001; inflammatory cells ear: F [6,41] = 188.01, p < 0.001; Fig. 3). In addition, we examined the infiltration of mast cell using TB stain. Dfe-treated AD mice displayed

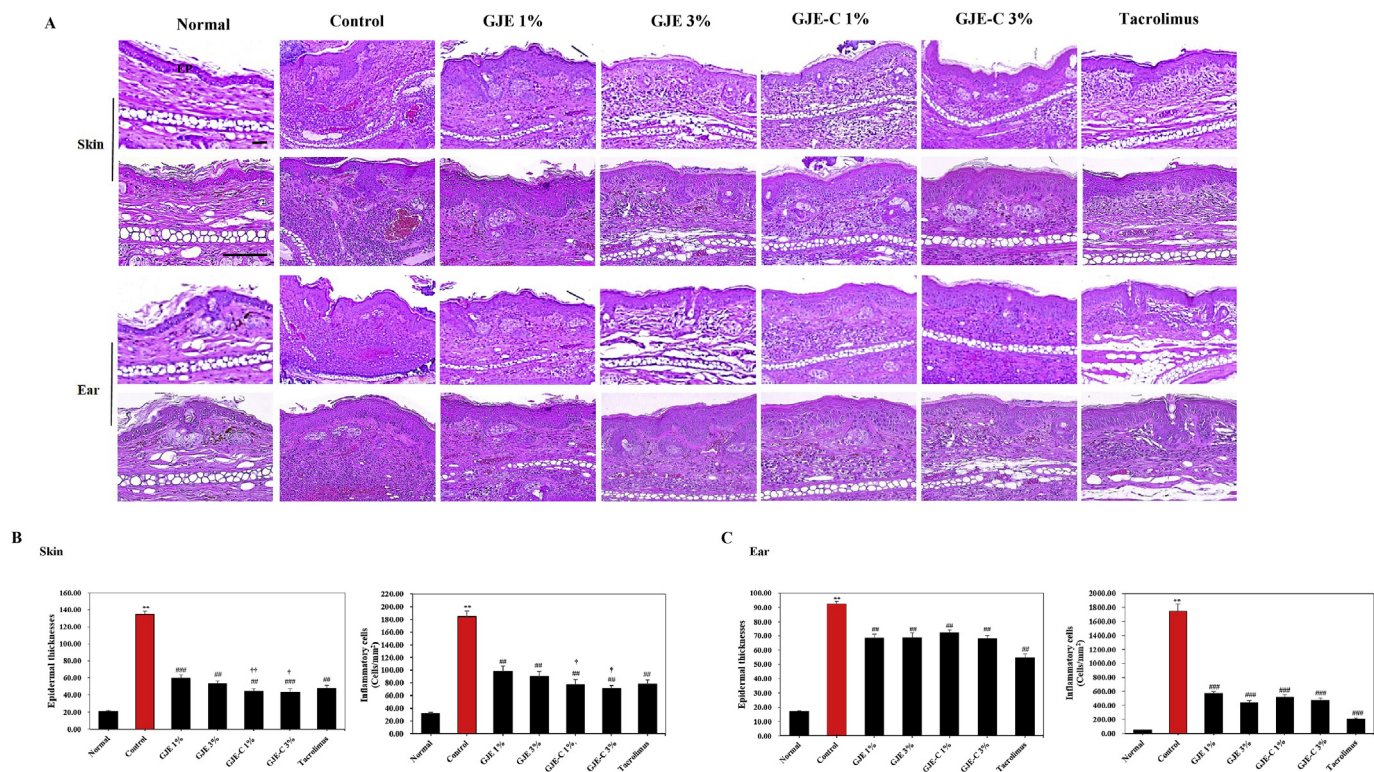


Fig. 3. GJE-C and GJE reduced the histological features in back skin and ears of Dfe-induced NC/Nga mice. (A) H&E-stained skin samples from the indicated mice. Analysis of epidermal thickness (μm) and inflammatory cells (cells/mm² of dermis) in (B) skin and (C) ears in Dfe-induced NC/Nga mice. Data are presented as mean ± SEM. ***p* < 0.01 vs. normal; ###*p* < 0.01, ###*p* < 0.001 vs. control; ††*p* < 0.01, †††*p* < 0.001 vs. control; \$\$*p* < 0.01, \$\$\$*p* < 0.001 vs. control. Scale bars = 80 μm. EP, epidermis.

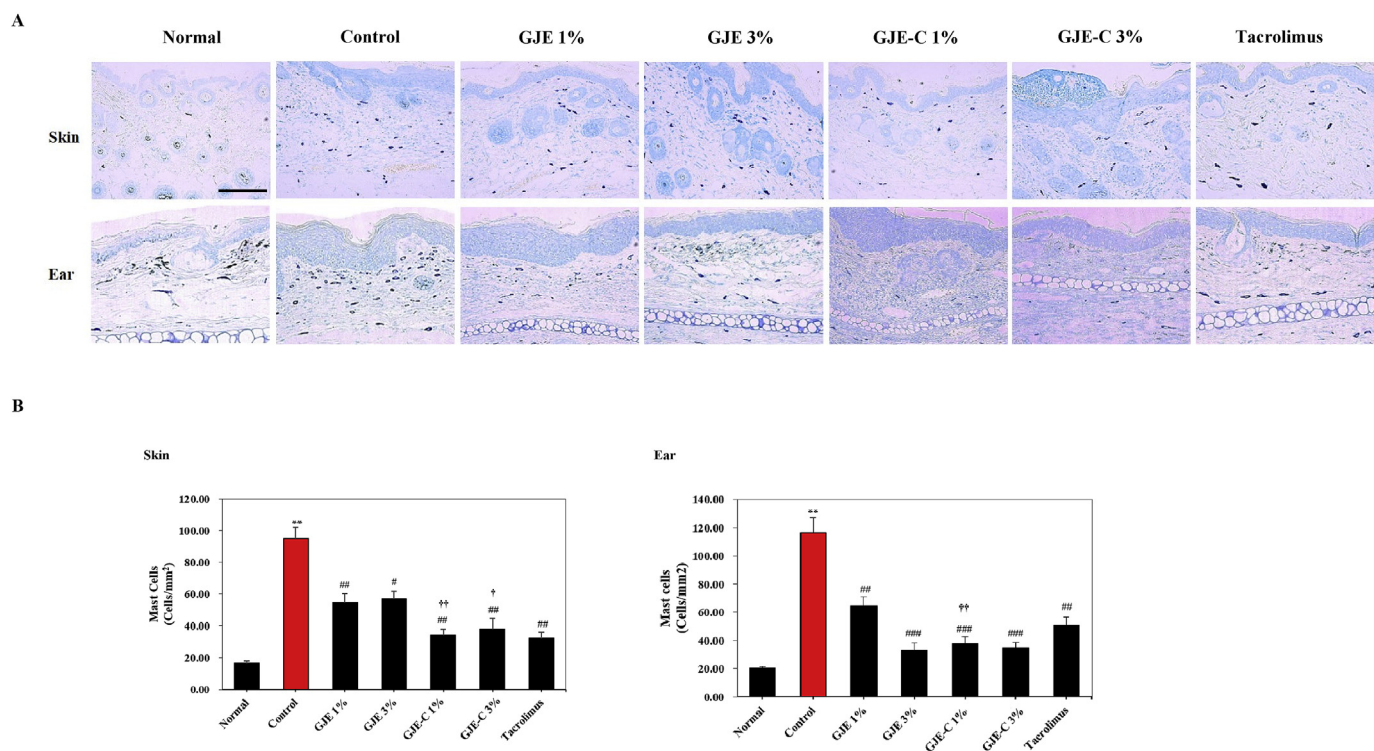


Fig. 4. GJE-C and GJE reduced the infiltration of mast cells in back skin and ears of Dfe-induced NC/Nga mice. (A) Infiltration of mast cells in the indicated mice is shown by TB stain. (B) Analysis of infiltration of mast cells in skin or ears of Dfe-induced NC/Nga mice. Data are presented as mean ± SEM. ***p* < 0.01 vs. normal; #*p* < 0.05, ###*p* < 0.01, ###*p* < 0.001 vs. control; ††*p* < 0.01, †††*p* < 0.001 vs. control; \$\$*p* < 0.01 vs. control. Scale bars = 80 μm.

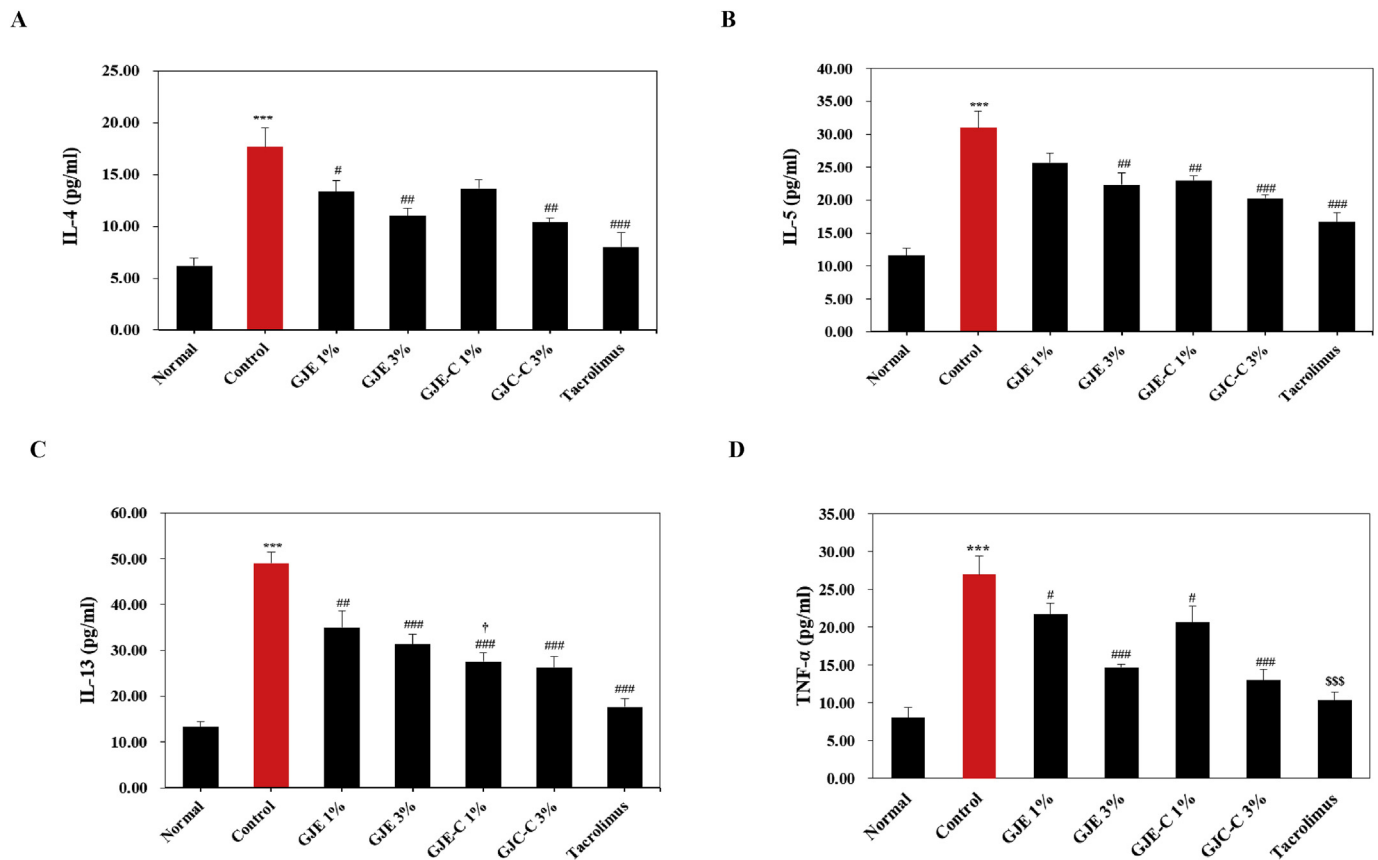


Fig. 5. GJE-C and GJE suppressed inflammatory mediator response in serum of Dfe-induced NC/Nga mice. The levels of Th1/2-related cytokines and AD-related chemokines (A) IL-4, (B) IL-5, (C) IL-13, and (D) TNF- α in serum were determined by ELISA. Data are presented as mean $\pm$ SEM. *** p < 0.001 vs. normal; # p < 0.05, ## p < 0.01, ### p < 0.001 vs. control; † p < 0.01, †† p < 0.001 vs. control; \$ p < 0.05, \$\$ p < 0.01, \$\$\$ p < 0.001 vs. control.

infiltration of mast cell in back skin and ears, but application of GJE-C or GJE decreased these histological features of AD (mast cell skin: F [6,41] = 29.00, p < 0.001; mast cell ear: F [6,41] = 32.49, p < 0.001, Fig. 4). These results suggest that GJE-C application results in suppression of mast cell infiltration, cytokine expression, and overall inflammatory response similar to that of GJE application in this AD mouse model.

3.4. GJE-C suppresses AD-related chemokines and Th1/2 immune response in the Dfe-induced NC/Nga mice

To determine whether GJE-C also suppresses Th2-related cytokines, chemokines, and Th1-related cytokines in the Dfe-treated NC/Nga AD mouse model, we measured the chemokines and Th1 and Th2 cytokines in serum from Dfe-treated NC/Nga mice using ELISA. All examined chemokines and Th1 and Th2 cytokines increased in the serum samples from the Dfe-treated NC/Nga mice. GJE-C or GJE application inhibited the production of Th2 cytokines, Th1 cytokines, and chemokines in the serum of treated mice (IL-4: F [6,20] = 18.84, p < 0.001; IL-5: F [6,20] = 25.63, p < 0.001; IL-13: F [6,20] = 39.11, p < 0.001; TNF- α : F [6,20] = 26.99, p < 0.001; Fig. 5A–D).

3.5. GJE-C increases the expression of skin barrier proteins in Dfe-induced NC/Nga mice

To evaluate the effect of GJE-C or GJE on skin barrier protein expression in the Dfe-treated NC/Nga AD mouse model, we measured the expression of involucrin and loricrin protein using western blotting. The Dfe-induced AD mice exhibited reduced involucrin and loricrin protein expression; however GJE-C or GJE application significantly increased

the involucrin and loricrin protein levels (involucrin skin: F [6,20] = 6.86, p < 0.001; loricrin skin: F [6,20] = 87.67, p < 0.001; involucrin ear: F [6,20] = 12.92, p < 0.001; loricrin ear: F [6,20] = 19.89, p < 0.001; Fig. 6). These result suggest that GJE-CR like as GJE application caused a protective allergic response via suppressed of inflammatory cytokines and mediators and upregulated of skin barrier protein expression.

3.6. GJE-C contains geniposidic acid, but the removal of crocin slightly suppressed production of chemokines in vitro

To evaluate the suppression of AD-related chemokine production by GJE, we examined the effects of this extract on HaCaT cells using ELISA analysis. The GJE compound geniposidic acid significantly reduced production of the AD-related chemokines RANTES (F [6,20] = 103.24, p < 0.001), TARC (F [6,20] = 54.16, p < 0.001), MDC (F [6,20] = 106.90, p < 0.001), and thymic stromal lymphopoietin (TSLP; F [6,20] = 31.55, p < 0.001; Fig. 7); however, crocin induced a slightly lesser effect. These results suggest that the GJE compounds geniposidic acid act to reduce atopic dermatitis symptoms via decreasing the inflammatory response.

4. Discussion

AD is a chronic inflammatory skin disease that is associated with cutaneous hyperreactivity to environmental triggers, resulting in eczematous lesional skin and high IgE production (Yang et al., 2017). The AD results from interactions between susceptibility genes, the environment, defective skin barrier function, and immunologic responses (Han et al., 2014). The basic pathogenic mechanism associated with the

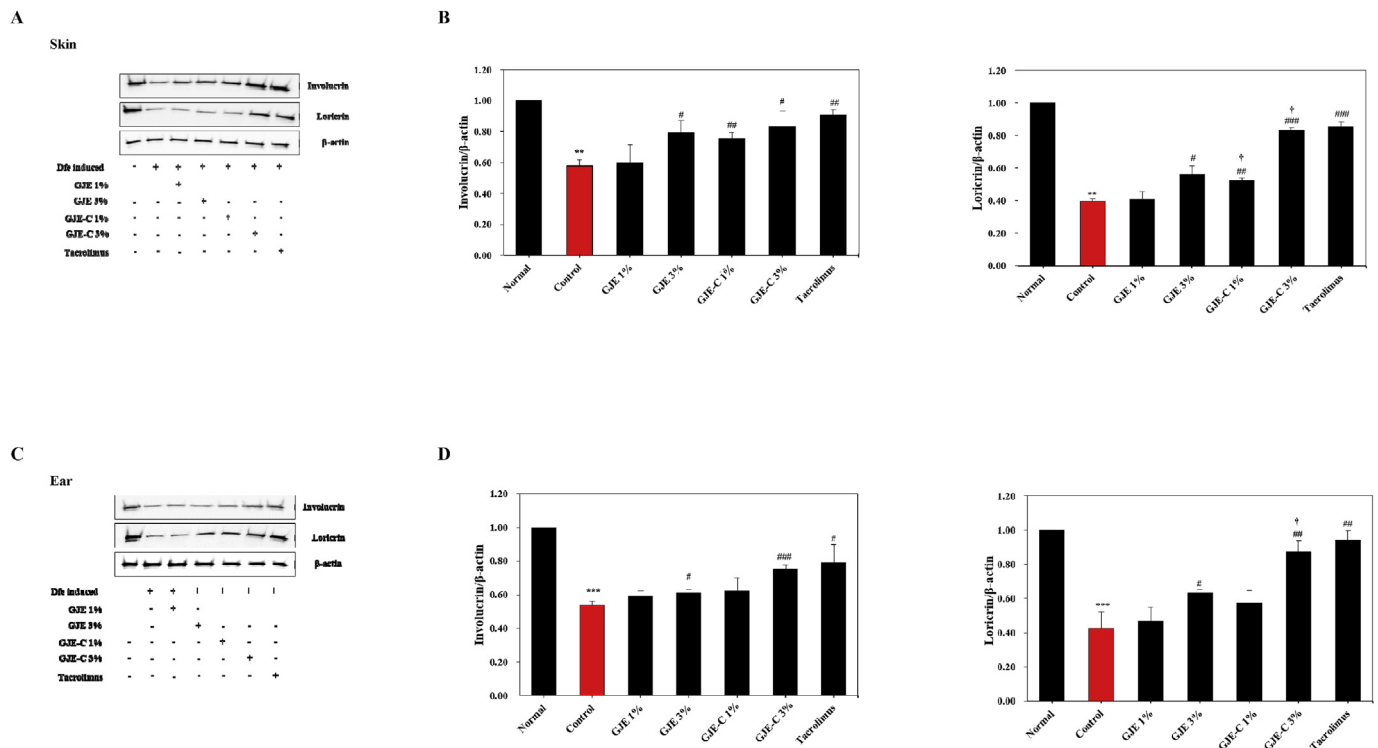


Fig. 6. GJE-C and GJE increased expression of skin barrier protein in back skin and ears of Dfe-induced NC/Nga mice. Involucrin and loricrin protein levels in the skin (A) and ears (C) were analyzed by western blot. The western blot densitometry of involucrin and loricrin protein levels in back skin (B) and ears (D) was analyzed. Data are presented as mean $\pm$ SEM. ^{***} p < 0.01, ^{***} p < 0.001 vs. normal; [#] p < 0.05 vs. control; [†] p < 0.05, ^{††} p < 0.01, ^{†††} p < 0.001 vs. control; ^{\$} p < 0.05, ^{\$\$} p < 0.01, ^{\$\$\$} p < 0.001 vs. control.

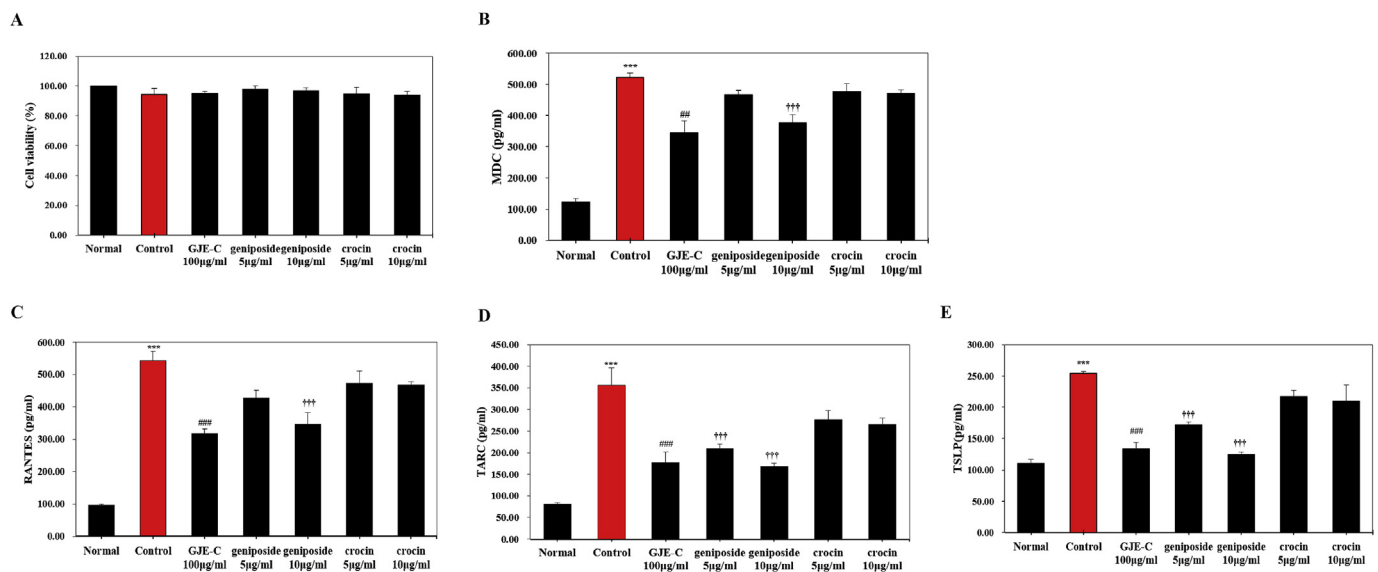


Fig. 7. GJE-C and GJE contain chemical constituents that suppressed production of chemokines in HaCaT cells. (A) HaCaT cell viability was measured by MTT assay. The expression of (B) MDC, (C) RANTES, (D) TARC, and (E) TSLP were assessed by ELISA. Data are presented as mean $\pm$ SEM. ^{***} p < 0.001 vs. normal; ^{##} p < 0.01, ^{###} p < 0.001 vs. control; [†] p < 0.05, ^{††} p < 0.01, ^{†††} p < 0.001 vs. control.

development of AD is an imbalance in T helper cells with allergy sensitization and acquisition of an inflammatory response to a specific allergen (Choi et al., 2014a). Furthermore, allergen-induced IgE synthesis can trigger mast cells to release cytokines for the differentiation of Th cells into Th2 cells to secrete IL-4, IL-5, and IL-13 (Wong et al., 2001).

The complex etiology of AD has made the development of therapeutics difficult. For the past few decades, anti-histamine agents and

other immunosuppressive agents have commonly been used for AD; however, the safety and efficacy of these agents for long-term treatment remains unknown. Though new topical calcineurin inhibitors have recently begun to be used for the treatment of AD, their efficacy has proved insufficient to treat AD successfully (Yang et al., 2017; Yang et al., 2015). Therefore, further evaluation of the pathogenic mechanisms of this disease is warranted to further the potential for developing safe and more efficacious therapies against AD.

Thus, natural produce-based agents with good efficacy and low risks of side effects offer promising prevention and treatment strategies for inflammation-related disease. Many researchers are searching for alternative treatment agents to replace steroids for AD therapy (Dey et al., 2006; Yang et al., 2013). Here, we showed that GJE-C exhibited efficacy that was weaker than an immunosuppressive agent but that can be used as a rapidly effective and safe treatment for the management of AD via its anti-inflammatory effects and ability to increase expression of skin barrier proteins.

AD is a major allergic skin disease that results from skin inflammation and is characterized by erythema, edema, erosion, pruritus, and dryness. AD-like skin symptoms observed in NC/Nga mice are similar to those in human AD (Choi et al., 2014b; Jin et al., 2009). Thus, we first assessed frequency of scratching, ear swelling, and clinical skin dermatitis scores (including erythema/hemorrhage, edema, scarring/dryness, and excoriation/erosion) in these animals. GJE-C significantly reduced the abrogation of the AD-like clinical skin dermatitis score, including frequency of scratching and ear swelling in Dfe-induced NC/Nga mice. Moreover, GJE-C, as GJE, inhibited serum IgE, histamine, and production of chemokines. In previous studies, we reported that crocin, a component of GJE, was not effective in blocking histamine release (Sung et al., 2014), and these results suggested that GJE-C improved AD-like skin lesions in this mouse model by reducing production of IgE, histamine, and chemokines.

AD-like skin symptoms are induced by high-affinity IgE-mediated mast cell activation and Th cell inflammatory cytokine release (Kawakami et al., 2009). Indeed, inflammatory cell and mast cell skin infiltration is common in AD lesions, with Th cells most frequently constituting this infiltrate (Mekori and Metcalfe, 1999). The multivalent binding of antigen to receptor-bound IgE and the subsequent aggregation of the FcεRI provide triggers for mast cell activation. Furthermore, FcεRI aggregation results in the release of inflammatory mediators, including histamine, and mast cells can also produce a variety of cytokines, including TNF-α and Th2-associated cytokines such as IL-4, IL-5, IL-6, IL-10, and IL-13, which are all important players in the pathogenesis of allergic reactions (Masuda et al., 2002; Galli et al., 1993).

Dfe-induced NC/Nga AD model mice exhibited increased mast cell and inflammatory cell infiltration in the back skin or ears and also showed increased production of serum IgE, TNF-α, and the Th2-associated cytokines IL-4, IL-5, IL-6, IL-10, and IL-13. However, a clinical study showed that GJE-C attenuated AD-like lesions via histological effects on mast cells and inflammatory cell infiltration and suppressed Th2 cells cytokines. Interestingly, skin barrier function defects are critical for the development of AD, and recent evidence supports a role for Th2-associated factors in regulating filaggrin expression (Agrawal and Woodfolk, 2014). In human skin models and keratinocyte cultures, histamine and a variety of cytokines, including Th2 cytokines, and chemokines, such as RANTES and TARC, have been shown to decrease filaggrin production (Agrawal and Woodfolk, 2014; Cha et al., 2017). These results suggest that GJE-C improved development of AD-like skin symptoms by increasing the expression of skin barrier proteins and inhibiting the production of Th 2-associated cytokines and chemokines. In addition, the active compound of GJE-C (geniposidic acid) decreased production of chemokines in TNF-α/IFN-γ induced HaCaT cells, lending support to the anti-inflammatory role of this extract in AD.

5. Conclusions

Topical application of GJE-C prevents development of AD-like lesions in a mouse model via suppression of serum IgE and histamine production via effects on cytokines and chemokines and by increasing expression of skin barrier proteins. This finding provides a basis for additional investigations into such an alternative or complementary therapy for AD.

Conflicts of interest

None to report.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jep.2019.112015>.

References

- Abramovits, W., 2005. Atopic dermatitis. *J. Am. Acad. Dermatol.* 53, 86–93.
- Agrawal, R.I., Woodfolk, J.A., 2014. Skin barrier defects in atopic dermatitis. *Curr. Allergy Asthma Rep.* 14 (5), 433.
- Cha, K.J., Im, M.A., Gu, A., Kim, D.H., Lee, D., Lee, J.S., Lee, J.S., Kim, I.S., 2017. Inhibitory effect of *Patrinia scabiosifolia* Link on the development of atopic dermatitis-like lesions in human keratinocytes and NC/Nga mice. *J. Ethnopharmacol.* 2016, 135–143.
- Chiba, T., Takeuchi, S., Esaki, H., Yamamura, K., Kurihara, Y., Moroi, Y., Furue, M., 2012. Topical application of PPARα (but not β/δ or γ) suppresses atopic dermatitis in NC/Nga mice. *Allergy* 67 (7), 936–942.
- Choi, C.Y., Kim, Y.H., Oh, S., Lee, H.J., Kim, J.H., Park, S.H., Kim, H.J., Lee, S.J., Chun, T., 2017. Anti-inflammatory potential of a heat-killed *Lactobacillus* strain isolated from Kimchi on house dust mite-induced atopic dermatitis in NC/Nga mice. *J. Appl. Microbiol.* 123 (2), 535–543.
- Choi, J.H., Jin, S.W., Han, E.H., Park, B.H., Kim, H.G., Khanal, T., Hwang, Y.P., Do, M.T., Lee, H.S., Chung, Y.C., Kim, H.S., Jeong, T.C., Jeong, H.G., 2014a. Platycodon grandiflorum root-derived saponins attenuate atopic dermatitis-like skin lesions via suppression of NF-κB and STAT1 and activation of Nrf2/ARE-mediated heme oxygenase-1. *Phytomedicine* 21 (8–9), 1053–1061.
- Choi, J.K., Oh, H.M., Lee, S., Kwon, T.K., Shin, T.Y., Rho, M.C., Kim, S.H., 2014b. *Salvia plebeia* suppresses atopic dermatitis-like skin lesions. *Am. J. Chin. Med.* 42 (4), 967–985.
- Deng, Y., Guan, M., Xie, X., Yang, X., Xiang, H., Li, H., Zou, L., Wei, J., Wang, D., Deng, X., 2013. Geniposide inhibits airway inflammation and hyperresponsiveness in a mouse model of asthma. *Int. Immunopharmacol.* 17 (3), 561–567.
- Dey, M., Ribnick, D., Kurmukov, A.G., Raskin, I., 2006. In vitro and in vivo anti-inflammatory activity of a seed preparation containing phenethylisothiocyanate. *J. Pharmacol. Exp. Ther.* 317 (1), 326–333.
- Galli, S.J., Gordon, J.R., Wershil, B.K., 1993. Mast cell cytokines in allergy and inflammation. *Agents Actions Suppl.* 43, 209–220.
- Han, N.R., Kang, S.W., Moon, P.D., Jang, J.B., Kim, H.M., Jeong, H.J., 2014. Genuine traditional Korean medicine, *Naju Jjok* (Chung-Dae, *Polygonum tinctorium*) improves 2,4-dinitrofluorobenzene-induced atopic dermatitis-like lesional skin. *Phytomedicine* 21 (4), 456–460.
- Jin, H., He, R., Oyoshi, M., Geha, R.S., 2009. Animal models of atopic dermatitis. *J. Invest. Dermatol.* 129 (1), 31–40.
- Kawakami, T., Ando, T., Kimura, M., Wilson, B.S., Kawakami, Y., 2009. Mast cells in atopic dermatitis. *Curr. Opin. Immunol.* 21 (6), 666–678.
- Kawahara, T., Tsutsui, K., Nakanishi, E., Inoue, T., Hamazumi, Y., 2017. Effect of the topical application of an ethanol extract of quince seeds on the development of atopic dermatitis-like symptoms in NC/Nga mice. *BMC Complement Altern. Med.* 17 (1), 80.
- Kim, C.G., Kang, M., Lee, Y.H., Min, W.G., Kim, Y.H., Kang, S.J., Song, C.H., Park, S.J., Park, J.H., Han, C.H., Lee, Y.J., Ku, S.K., 2015a. Bathing Effects of Various Seawaters on Allergic (Atopic) Dermatitis-like Skin Lesions Induced by 2,4-dinitrochlorobenzene in Hairless Mice, vol 2015. pp. 179185.
- Kim, K.H., Park, S.J., Lee, Y.J., Lee, J.E., Song, C.H., Choi, S.H., Ku, S.K., Kang, S.J., 2015b. Inhibition of UVB-induced skin damage by exopolymers from *Aureobasidium pullulans* SM-2001 in hairless mice. *Basic Clin. Pharmacol. Toxicol.* 116 (2), 73–86.
- Kim, C.H., Cheong, K.A., Park, C.D., Lee, A.Y., 2011. Glucosamine improved atopic dermatitis-like skin lesions in NC/Nga mice by inhibition of Th2 cell development. *Scand. J. Immunol.* 73 (6), 536–545.
- Kim, C.H., Choi, Y.S., Cheong, K.A., Lee, A.Y., 2013. Mechanism underlying the effect of combined therapy using glucosamine and low-dose cyclosporine A on the development of atopic dermatitis-like skin lesions in NC/Nga mice. *Int. Immunopharmacol.* 15 (2), 424–432.
- Kim, C.H., Park, C.D., Lee, A.Y., 2012. Administration of poly(I:C) improved Dermatophagoides farinae-induced atopic dermatitis-like skin lesions in NC/Nga mice by the regulation of Th1/Th2 balance. *Vaccine* 30 (14), 2405–2410.
- Koo, H.J., Lim, K.H., Jung, H.J., Park, E.H., 2006. Anti-inflammatory evaluation of gardenia extract, geniposide and genipin. *J. Ethnopharmacol.* 103 (3), 496–500.
- Lee, H.J., Lee, N.R., Jung, M., Kim, D.H., Choi, E.H., 2015. Atopic March from atopic dermatitis to asthma-like lesions in NC/Nga mice is accelerated or aggravated by neutralization of stratum corneum but partially inhibited by acidification. *J. Invest.*

- Dermatol. 135 (12), 3025–3033.
- Ma, W.W., Tao, Y., Wang, Y.Y., Peng, I.F., 2017. Effects of Gardenia jasminoides extracts on cognition and innate immune response in an adult *Drosophila* model of Alzheimer's disease. *Chin. J. Nat. Med.* 15 (12), 899–904.
- Mekori, Y.A., Metcalfe, D.D., 1999. Mast cell-T cell interactions. *J. Allergy Clin. Immunol.* 104 (3pt1), 517–523.
- Masuda, A., Yoshikai, Y., Aiba, K., Matsuguchi, T., 2002. Th2 cytokine production from mast cells is directly induced by lipopolysaccharide and distinctly regulated by c-Jun N-terminal kinase and p38 pathways. *J. Immunol.* 167 (7), 3801–3810.
- Oshio, T., Sasaki, Y., Funakoshi-Tago, M., Aizu-Yokota, E., Sonoda, Y., Matsuoka, H., Kasahara, T., 2009. Dermatophagoides farinae extract induces severe atopic dermatitis in NC/Nga mice, which is effectively suppressed by the administration of tacrolimus ointment. *Int. Immunopharmacol.* 9 (4), 403–411.
- Sung, Y.Y., Lee, A.Y., Kim, H.K., 2014. The Gardenia jasminoides extract and its constituent, geniposide, elicit anti-allergic effects on atopic dermatitis by inhibiting histamine in vitro and in vivo. *J. Ethnopharmacol.* 156 (28), 33–40.
- Suto, H., Matsuda, H., Mitsuishi, K., Hira, K., Uchida, T., Unno, T., Ogawa, H., Ra, C., 1999. NC/Nga mice: a mouse model for atopic dermatitis. *Int. Arch. Allergy Immunol.* 120 (Suppl. 1), 70–75.
- Wong, C.K., Ho, C.Y., Ko, F.W., Chan, C.H., Ho, A.S., Hui, D.S., Lam, C.W., 2001. Proinflammatory cytokines (IL-17, IL-6, IL-18 and IL-12) and Th cytokines (IFN- γ , IL-4, IL-10 and IL-13) in patients with allergic asthma. *Clin. Exp. Immunol.* 125 (2), 177–183.
- Yagi, R., Nagai, H., Iigo, Y., Akimoto, T., Arai, T., Kubo, M., 2002. Development of atopic dermatitis-like skin lesions in STAT6-deficient NC/Nga mice. *J. Immunol.* 168 (4), 2020–2027.
- Yamada, K., Matsushita, K., Wang, J., Kanekura, T., 2018. Topical glucose induces claudin-1 and filaggrin expression in a mouse model of atopic dermatitis and in keratinocyte culture, exerting anti-inflammatory effects by repairing skin barrier function. *Acta Derm. Venereol.* 98 (1), 19–25.
- Yamamoto, M., Haruna, T., Yasui, K., Takahashi, H., Iduhara, M., Takaki, S., Deguchi, M., Arimura, A., 2007. A novel atopic dermatitis model induced by topical application with dermatophagoides farinae extract in NC/Nga mice. *Allergol. Int.* 56 (2), 139–148.
- Yamauchi, M., Tsuruma, K., Imai, S., Nakanishi, T., Umigai, N., Shimazawa, M., Hara, H., 2011. Crocetin prevents retinal degeneration induced by oxidative and endoplasmic reticulum stresses via inhibition of caspase activity. *Eur. J. Pharmacol.* 650 (1), 110–119.
- Yang, G., Choi, C.H., Lee, K., Lee, M., Ham, I., Choi, H.Y., 2013. Effects of Catalpa ovata stem bark on atopic dermatitis-like skin lesions in NC/Nga mice. *J. Ethnopharmacol.* 145 (2), 416–423.
- Yang, H., Jung, E.M., Ahn, C., Lee, G.S., Lee, S.Y., Kim, S.H., Choi, I.G., Park, M.J., Lee, S.S., Choi, D.H., Jeung, E.B., 2015. Elemol from Chamaecyparis obtusa ameliorates 2,4-dinitrochlorobenzene-induced atopic dermatitis. *Int. J. Mol. Med.* 36 (2), 463–472.
- Yang, Z., Liu, M., Wang, W., Wang, Y., Cao, B., Gao, Y., Chen, H., Li, T., 2017. Pseudolaric acid B attenuates atopic dermatitis-like skin lesions by inhibiting interleukin-17-induced inflammation. *Sci. Rep.* 7 (1), 7918.
- Yu, Y., Xie, Z.L., Gao, H., Ma, W.W., Dai, Y., Wang, Y., Zhong, Y., Yao, X.S., 2009. Bioactive iridoid glucosides from the fruit of Gardenia jasminoides. *J. Nat. Prod.* 72 (8), 1459–1464.
- Zang, C.X., Bao, X.Q., Li, L., Yang, H.Y., Wang, L., Yu, Y., Wang, X.L., Yao, X.S., Zhang, D., 2018. The protective effects of gardenia jasminoides (fructus gardenia) on amyloid- β -induced mouse cognitive impairment and neurotoxicity. *Am. J. Chin. Med.* 46 (1), 1–17.