Journal of Ethnopharmacology 156 (2014) 33–40

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Research Paper

The *Gardenia jasminoides* extract and its constituent, geniposide, elicit anti-allergic effects on atopic dermatitis by inhibiting histamine *in vitro* and *in vivo*

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ARTICLE INFO

Article history:
Received 14 April 2014
Received in revised form
23 July 2014
Accepted 25 July 2014
Available online 19 August 2014

Keywords:
Anti-allergic
Anti-inflammatory
Gardenia jasminoides
Geniposide
Histamine
Mast cells

ABSTRACT

Ethnopharmacological relevance: Gardenia jasminoides Ellis has been used in traditional medicine for treatment of inflammation, edema, and dermaitis. The aim of this study was to investigate the mechanism by which *Gardenia jasminoides* extract (GJE) elicits anti-allergic effects in mast cells and in mice with atopic dermatitis (AD).

Materials and methods: We investigated the effects of GJE and its fractions on compound 48/80-induced histamine release from MC/9 cells and *Dermatophagoides farinae*-exposed NC/Nga mice. The effects of its constituents on histamine release from MC/9 cells were also investigated.

Results: GJE and its ethyl acetate fraction (GJE-EA) inhibited compound 48/80-induced histamine release from MC/9 mast cells. The topical application of GJE or GJE-EA to *Dermatophagoides farinae*-exposed NC/Nga mice reduced the symptoms of AD, inhibited the infiltration of inflammatory cells, and lowered the serum levels of immunoglobulin E and histamine. Both GJE and GJE-EA reduced the expression of cytokines (interleukin [IL]-4, IL-6, and tumor necrosis factor-alpha) and adhesion molecules (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1) in ear lesions. In addition, the quantitative analysis of GJE and GJE-EA by high-performance liquid chromatography revealed the presence of crocin and geniposide. Geniposide, but not crocin, inhibited the release of histamine from mast cells, which may contribute to the anti-allergic effect of GJE and GJE-EA.

Conclusions: These results suggest that GJE and GJE-EA can suppress mast cell degranulation-induced histamine release, and geniposide may be potential therapeutic candidates for AD.

1. Introduction

Gardenia jasminoides Ellis (*Fructus gardenia*), which is listed in the Chinese, Korean, and Japanese pharmacopoeias, is widely used due to its homeostatic, antiphlogistic, analgesic, anti-inflammatory, and antipyretic effects (Sheng et al., 2006; Jung et al., 2008). The fruit of *Gardenia jasminoides* has been included in traditional medicine formulations for the treatment of inflammation, headache, edema, fever, hepatic disorders, and hypertension (Koo et al., 2006). Huang-Lian-Jie-Du-Tang is a prescription formulation that contains *Coptix chinensis*, *Scutellaria baicalensis*, *Phellodendron amurense*, and *Gardenia jasminoides*, and it is used to treat various inflammatory diseases, such as gastritis, dermatitis, and aphthous stomatitis (Ma et al., 2005). Importantly, *Gardenia jasminoides* inhibits tumor necrosis factor-alpha (TNF-α)-induced vascular inflammation in human umbilical vein endothelial cells (Hwang et al., 2010). The major constituents of *Gardenia jasminoides* are iridoid glycosides, such as geniposide, gardenoside, shanzhiside, scandoside methyl ester, deacetyl-asperulosidic acid methyl ester, and genipin-1-β-D-gentiobioside (Zhou et al., 2007). The anti-asthmatic effects of geniposide in a mouse model of ovalbumin-induced allergic airway inflammation have been recently reported (Deng et al., 2013). Although studies on the physiological functions of *Gardenia jasminoides*, including anti-inflammatory and anti-allergic effects, have been performed, the effectiveness of *Gardenia jasminoides* and its constituents in reducing allergic skin inflammatory reactions and improving atopic dermatitis (AD) symptoms have not been evaluated.

AD is a chronic, relapsing, and inflammatory skin disease that occurs most often in infants and children, and its prevalence is increasing (Spergel and Paller, 2003). It is characterized by elevated

Abbreviations: AD, atopic dermatitis; GJE, *Gardenia jasminoides* extract; GJE-BuOH, GJE *n*-butanol-soluble fraction; GJE-EA, ethyl acetate-soluble fraction of GJE; GJE-H_2O, water-soluble fraction of GJE; GJE-Hx, GJE *n*-hexane-soluble fraction; GJE-MC, GJE methylene chloride-soluble fraction; HPLC, high-performance liquid chromatography; ICAM-1, intercellular adhesion molecule-1; IgE, immunoglobulin E; IL, interleukin; Th, T-helper cell; TNF-α, tumor necrosis factor-alpha; VCAM-1, vascular cell adhesion molecule-1

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http://dx.doi.org/10.1016/j.jep.2014.07.060
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serum immunoglobulin E (IgE) levels, peripheral eosinophilia, and pruritic and relapsing eczematous skin lesions, which have been infiltrated by inflammatory cells, such as T lymphocytes, macrophages, and mast cells (Leung and Bieber, 2003). In response to the cross-linking of the IgE receptor by antigens, mast cells degranulate and release various inflammatory mediators, including histamine, proteases, chemokines, and cytokines (Galli et al., 1991). The pathogenesis of AD is complex and involves genetic, environmental, and immunological factors (Leung et al., 2004). Although the pathology of AD is not fully understood, recent studies have shown that the typical symptoms of AD involve increased levels of T-helper cell type-2 (Th2)-mediated cytokines and a deficiency in T-helper cell type-1 (Th1)-mediated cytokines (Sawada et al., 2012). Th2 cells produce interleukin (IL)-4, IL-5, and IL-13, which are important in the onset and development of AD in its acute phase. Th1 cells produce interferon (IFN)-γ, which contributes to pathogenesis during the chronic phase of AD (Leung et al., 2004).

The NC/Nga mouse is an inbred strain of Japanese fancy mice (Nishiki-Nezumi) that was the first mouse model of AD-like skin disease to be reported (Matsuda et al., 1997). These mice spontaneously develop AD-like skin lesions when placed in conventional areas, not in specific pathogen-free conditions (Choi et al., 2013). NC/Nga mice exhibit frequent scratching, elevated serum IgE levels, and the infiltration of inflammatory cells into skin lesions (Vestergaard et al., 1999). The topical application of an extract of *Dermatophagoides farinae* (DfE), which is a major species of house dust mite, increases IgE production and AD-like skin lesions, as well as Th2 responses, in NC/Nga mice (Yamamoto et al., 2007).

To evaluate the anti-allergic effects of *Gardenia jasminoides*, we examined several fractions of *Gardenia jasminoides* extract (GJE) and demonstrate that its constituents could modulate the allergic immune response through the inhibition of histamine release and cytokines in mast cells and AD mice.

2. Materials and methods

2.1. Preparation of ethanolic extract and various fractions from Gardenia jasminoides fruits

The dried fruits of *Gardenia jasminoides* were purchased from Omniherb Co. (Yeoungcheon, Korea) and authenticated based on the macroscopic characteristics provided by the Classification and Identification Committee of the Korea Institute of Oriental Medicine (KIOM). A voucher specimen (No. 000599) was deposited in the herbarium of the Department of Herbal Resources Research at the KIOM. The dried fruits of *Gardenia jasminoides* (250.54 g) were soaked three times in 3.0 L of 70% ethanol in an ultrasonic bath (8510 model, 25 CW, 44 kHz; Branson Co., Danbury, CT, USA) for 90 min. This residue was filtered and evaporated *in vacuo* to yield the total, 70%-ethanol extraction (59.82 g). The extract was then suspended in water, followed by several partitioning processes with various extraction solvents, including *n*-hexane, methylene chloride, ethyl acetate, and *n*-butanol. The resulting fractions were GJE-Hx (the *n*-hexane-soluble fraction), GJE-MC (the methylene chloride-soluble fraction), GJE-EA (the ethyl acetate-soluble fraction), GJE-BuOH (the *n*-butanol-soluble fraction), and GJE-H_2O (the water-soluble fraction).

2.2. Reagents and cell culture

Compound 48/80, L-glutamine, and 2-mercaptoethanol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The MC/9 mouse mast cell line was purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were maintained in Dulbecco's Modified Eagle's Medium, which was supplemented with 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol, 10% rat T-STIM (Becton Dickinson, Franklin Lakes, NJ, USA), and 10% fetal bovine serum (FBS), at 37 °C in a 5%-CO_2 incubator.

2.3. Histamine assay in mast cells

Histamine levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Oxford Biomedical Research Inc., Oxford, MI, USA), according to the manufacturer's instructions. MC/9 mast cells were incubated with GJE or its fractions (0–800 μg/ml) for 30 min at 37 °C, after which compound 48/80 (25 μg/ml) was added and incubated for an additional 30 min. The optical density of each well was measured at 650 nm using a Benchmark-plus microplate spectrophotometer (BioRad, Hercules, CA, USA).

2.4. Animals

Male NC/Nga mice (8 weeks old) were purchased from SLC, Inc. (Hamamatsu, Japan) and housed in an air-conditioned animal room with a 12-h light/dark cycle at 22 ± 1 °C and 50 ± 10% humidity. Mice were provided access to a standard laboratory diet and water *ad libitum*. All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication No. 85-23, revised 1996) and were approved by the Institutional Animal Care and Use Committee of the KIOM.

2.5. Induction of AD in NC/Nga mice

Induction of AD-like skin lesions using mite antigen was performed as described previously (Sung et al., 2011). The schematic of the experimental procedure is shown in Fig. 1A. Mice were anesthetized with ether, and hair on the upper back of each mouse was removed with a clipper and a shaver 1 day before the experiments. Barrier disruption was achieved using 4% sodium dodecyl sulfate (150 μl) on the shaved dorsal skin and both surfaces of each ear. After 3 h, the skin was treated with 100 mg ointment that was prepared from a crude extract of DfE (Biostir-AD, Biostir, Kobe, Japan). Control mice were treated with the ointment base (hydrophilic petrolatum). Sodium dodecyl sulfate was applied to the skin twice a week for 3 weeks, and then 100 mg DfE ointment or hydrophilic petrolatum was reapplied.

Mice were randomly divided into six groups: (1) normal controls that did not receive DfE application, (2) DfE-treated control group (100 mg/mouse), (3) DfE-treated mice that received 400 μg GJE, (4) DfE-treated mice that received 400 μg GJE-EA, (5) DfE-treated mice that received 400 μg GJE-H_2O, and (6) DfE-treated mice that received 100 μg Protopic ointment containing 0.1% tacrolimus (Astellas Pharma Inc., Deerfield, IL, USA) as a positive control. The powder of GJE or it fractions was dissolved in the vehicle (acetone: distilled water = 3:1, v/v) solution. The same volume of vehicle was applied to the normal and DfE-treated control groups without GJE or its fractions. After the second DfE application on day 7, GJE or its fractions were applied daily for 14 days. On each day of DfE application, GJE or its fractions were applied 1 h before the addition of the antigen. Ear thickness was measured twice a week using a micrometer (Mitutoyo Corporation, Kanagawa, Japan). Mice received the last GJE treatment on day 21 and were sacrificed on day 22 to evaluate immunological and histological changes. The mice were anesthetized with ether and blood samples were obtained from the inferior vena cava of each mouse.

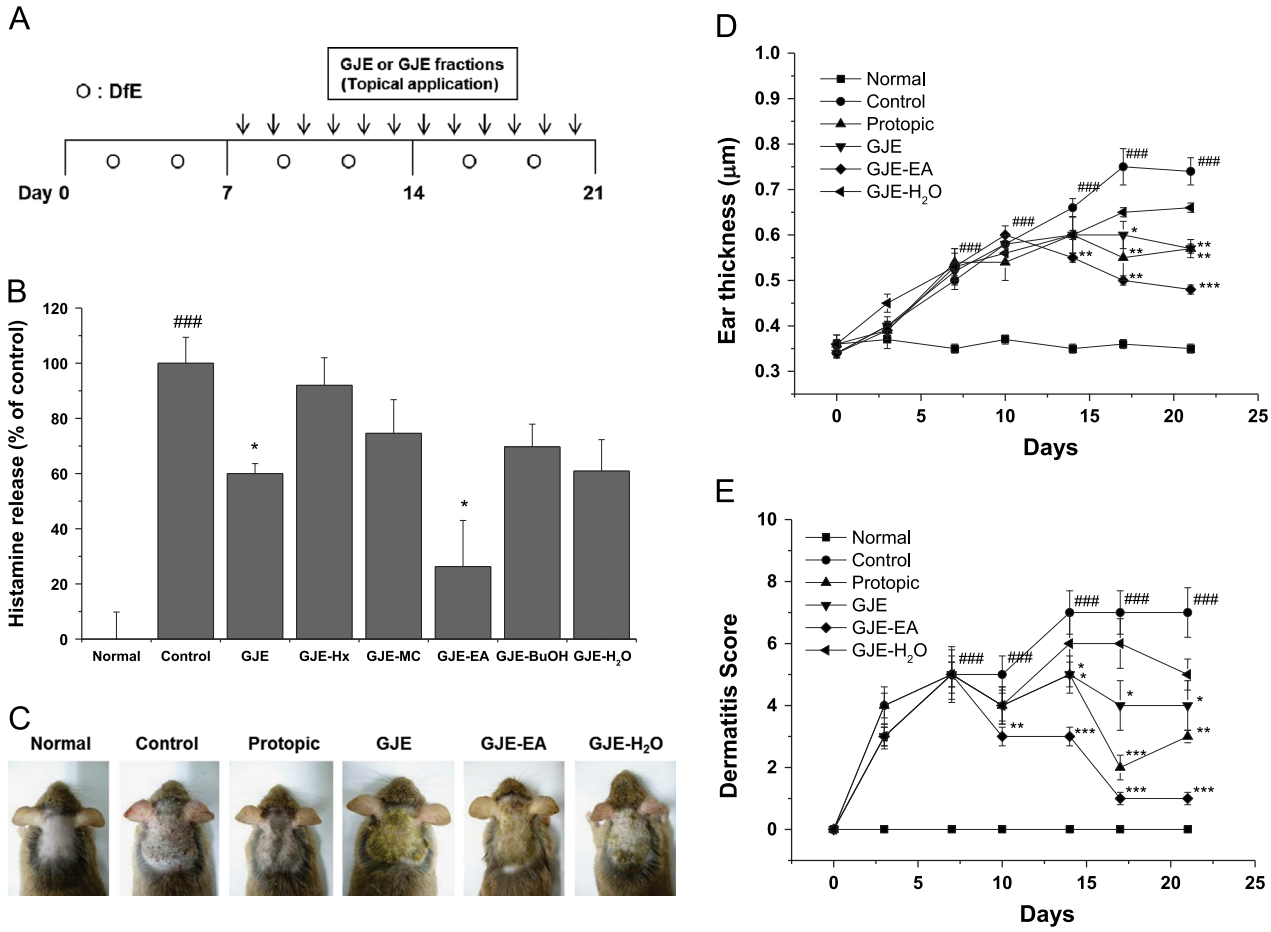

Fig. 1. Effects of *Gardenia jasminoides* extract (GJE) and its fractions on histamine release from mast cells and *Dermatophagoides farinae* (DfE)-induced atopic dermatitis in NC/Nga mice. (A) Experimental induction of atopic dermatitis-like skin lesions. (B) Release of histamine from MC/9 mast cells. MC/9 cells (2×10^5 cells/ml) were pretreated with GJE or its fractions (200 μg/ml) at 37 °C for 30 min prior to the addition of compound 48/80 (25 μg/ml). (C) Representative photographs of clinical features on day 21. (D) Dermatitis severity score. (E) Ear thickness. Results are expressed as mean ± SD ($n=6$). Normal, untreated group; control, DfE-treated group; tacrolimus, tacrolimus-treated group; GJE, GJE-treated group; GJE-EA, GJE-ethyl acetate fraction-treated group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. normal; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control.

2.6. Evaluation of dermatitis severity

The severity of dermatitis on the ear and dorsal regions was evaluated twice a week. The development of erythema/hemorrhage, scarring/dryness, edema, and excoriation/erosion was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). The sum of the individual scores comprised the dermatitis severity score (Matsuda et al., 1997).

2.7. Measurement of serum IgE, histamine, and IL-4 levels

The blood samples were centrifuged at 2000*g* for 20 min at 4 °C, then the serum was collected and stored at −70 °C for further investigations. Serum total IgE, histamine, and IL-4 levels were measured using ELISA kits from Shibayagi (Gunma, Japan), Oxford Biomedical Research Inc., and R&D Systems (Minneapolis, MN, USA), respectively, according to the manufacturers' instructions.

2.8. Histopathological examination

Tissue specimens from the ear and dorsal skin of NC/Nga mice were removed, fixed in 10% formalin, embedded in paraffin, and serially sectioned at 2–3 μm. Tissue sections were then stained with hematoxylin and eosin or toluidine blue. Histopathological changes were examined by light microscopy (Olympus CX31/BX51, Olympus Optical Co., Tokyo, Japan) and photographed (Olympus DP70, Olympus Optical Co.).

2.9. Real-time reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from mouse ear tissues using an easy-BLUE total RNA extraction kit (Intron, Seoul, Korea). Reverse transcription was carried out in a 20-μl reaction with 2 μg total RNA, Maxime RT premix (Intron), oligo-dT primers, and diethylpyrocarbonate-treated water, and the solution was incubated at 45 °C for 60 min. The reaction was terminated by heat inactivation at 95 °C for 5 min. Gene expression was quantified by real-time PCR with SYBR green master mix (Qiagen, Tokyo, Japan). Amplification was carried out using the Rotor-gene 3000 (Corbett Research, Sydney, Australia), according to the following protocol: 94 °C for 2 min, followed by 35 cycles of 94 °C for 20 s, 60 °C for 20 s, and 72 °C for 30 s. The fold change in target gene expression relative to control mice was normalized to the internal control, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), using the $2^{-\Delta\Delta Ct}$ method. The primers for target genes were designed using the Primer 3 software (Table 1).

2.10. Quantitative high-performance liquid chromatography (HPLC) analysis

Quantitative analyses were performed using an Alliance 2695 model coupled with a photodiode array detector (Waters Corporation, Milford, MA, USA). Data processing was performed with

Table 1
Real-time RT-PCR primer sequences.

Genes	Forward	Reverse	Accession number	Length (bp)
IL-4	TCAACCCCCAGCTAGTTGTCA	CATCGAAAAGCCCGAAAGAG	NM_021283	313
IL-6	CCGGAGAGGAGACTTCACAG	TCCAGTTTGGTAGCATCCATC	NM_031168	220
TNF-α	CCTGTAGCCCACGTCGTAGC	TTGACCTCAGCGCTGAGTTG	NM_013693	373
IFN-γ	GCTACACACTGCATCTTGGCTTTG	CACTCGGATGAGCTCATTGAATGC	NM_008337	404
ICAM-1	CCTCTGCTCCTGGCCCTGGT	CGGACTGCTGTCCTCCCCGA	NM_010492	237
VCAM-1	TCGCGGTCTTGGGAGCCTCA	TCGCGGTCTTGGGAGCCTCA	NM_011693	213
GAPDH	AAGCTGTGGCGTGATGGCCG	TGGGCCCTCAGATGCCTGCT	NM_008084	228

Empower 2 (Waters Corporation, Milford, MA, USA), and the chromatographic separation was carried out on a Xselect™ HSS T3 C18 column (4.6 × 250 mm, 5-μm particle size, Waters Corporation, Milford, MS, USA) at ambient temperature. The mobile phase consisted of distilled water (A) and methanol (B), and the gradient was 0–50 min with 10–100% B. The ultraviolet (UV) wavelength was detected from 200 to 500 nm. Geniposide and crocin were monitored at 238 nm and 440 nm, respectively. The flow rate was 0.80 ml/min, and the injection volume was 10.0 μl. Sample peaks were compared to the retention time of standard compounds and UV spectra in the chromatogram. Analytical-grade crocin and geniposide (> 95.0%) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA) and Wako Pure Chemical Industries Ltd. (Osaka, Japan), respectively. Preparative and HPLC-grade chromatographic solvents were purchased from Daejung Chemicals & Metals Co., Ltd. (Gyeonggi-do, Korea) and J. T. Baker Inc. (Phillipsburg, NJ, USA), respectively.

2.11. Statistics

Results are expressed as mean ± standard error of the mean (S.E.M.). Statistical significance was determined using the one-way analysis of variance, followed by Dunnett's test. A value of $p < 0.05$ was used to indicate significant differences.

3. Results

3.1. Effects of GJE and it fractions on histamine release from MC/9 mast cells

Using the release of histamine by cell degranulation as an indicator of anti-allergic activity, we evaluated the effect of GJE and its fractions on compound 48/80-induced histamine release from MC/9 cells (Fig. 1B). GJE significantly inhibited histamine release. In addition, the ethyl acetate fraction of GJE, GJE-EA, significantly decreased histamine release from mast cells. The water fraction of GJE inhibited histamine release in a similar manner, although it was not statistically significant. Furthermore, GJE and its fractions did not affect cell viability and were not toxic to MC/9 cells (data not shown).

3.2. Effects of GJE and GJE-EA on NC/Nga mice that were exposed to dust mite allergens

Next, we investigated whether GJE and the GJE-EA fraction could ameliorate AD in mice. To establish a dust mite allergen-induced AD model, DfE was applied twice per week to the dorsal skin of NC/Nga mice. Skin conditions were evaluated twice per week using the dermatitis severity score. The clinical features, ear thickness, and dermatitis severity scores of the treatment groups are shown in Fig. 1C, D, and E. The repeated application of DfE induced skin dryness, followed by erythema, hemorrhage, edema, scarring, erosion, and excoriation (Fig. 1C). However, GJE and GJE-EA inhibited these symptoms of AD. Ear swelling increased gradually in DfE-treated control mice, and DfE-induced ear swelling was significantly reduced by GJE or GJE-EA, but not GJE-H_2O (Fig. 1D). Dermatitis severity scores increased rapidly in DfE-treated control mice and were significantly higher than those of normal controls after 3 days. However, the severity of dermatitis was significantly lower in GJE- and GJE-EA-treated mice compared with DfE-treated controls (Fig. 1E). GJE-H_2O also inhibited the dermatitis score, but this was not statistically significant. Among the experimental groups, the dermatitis severity score and ear thickness were most effectively inhibited by GJE-EA, and these inhibitory effects were significantly higher than those of GJE mice. These results demonstrate that GJE and GJE-EA suppressed DfE-induced AD in NC/Nga mice.

3.3. Effects of GJE and GJE-EA on serum IgE, IL-4, and histamine levels

Because an elevated serum level of IgE is a main feature of AD, we tested whether GJE and GJE-EA could affect the serum levels of IgE in DfE-treated NC/Nga mice. On day 22, the total IgE level in normal controls was 34.58 ± 4.03 ng/ml. DfE treatment elicited a 29-fold increase in IgE levels (995.34 ± 87.58 ng/ml). Treatment with GJE or GJE-EA decreased the DfE-induced increase in total IgE levels (Fig. 2A). In addition, GJE-EA decreased DfE-induced serum IL-4 levels (Fig. 2B). Histamine release from mast cell granules was significantly higher in DfE-treated controls than in normal mice, but treatments with GJE or GJE-EA significantly reduced DfE-induced increases in histamine levels (Fig. 2C).

3.4. Dose-dependent effects of GJE and GJE-EA on histamine release from MC/9 mast cells

Both GJE and GJE-EA inhibited the release of histamine from MC/9 mast cells in a dose-dependent manner (Fig. 2D and E). The IC_{50} values of GJE and GJE-EA were 400.44 μg/ml and 126.33 μg/ml, respectively, and the inhibitory effect on histamine release was 3.2-fold higher in GJE-EA-treated mice than in GJE-treated mice.

3.5. Histopathological features

The histopathological features of the ear and dorsal skin lesions are shown in Fig. 3A. Epidermal thickening by squamous cell hyperplasia (acanthosis) and inflammatory cell infiltration of the dermis were observed in DfE-treated control mice. However, treatment with GJE or GJE-EA inhibited these pathological changes. To evaluate DfE-induced mast cell infiltration, ear and skin sections were stained with toluidine blue. The number of mast cells in the dermis increased markedly in DfE-treated control mice compared with normal mice, but this increase was reduced by treatment with GJE or GJE-EA.

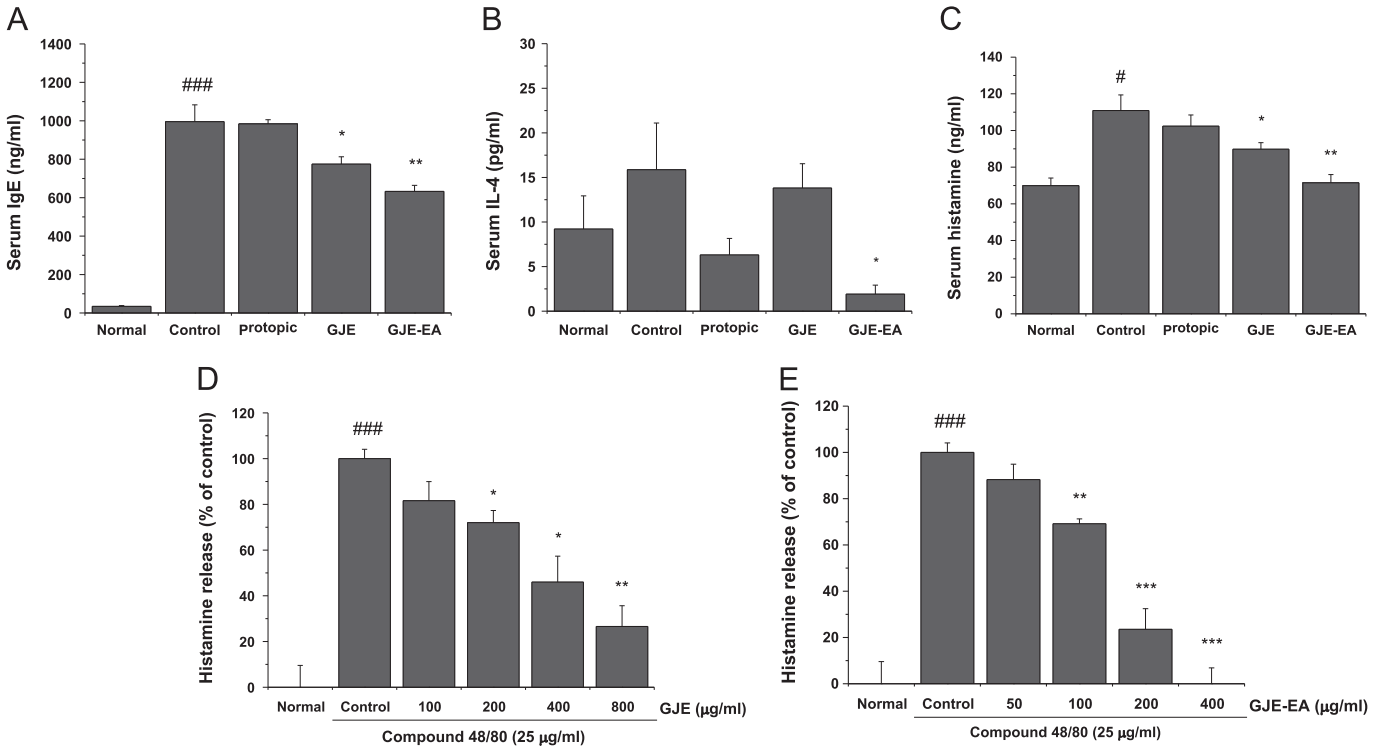

Fig. 2. Effect of *Gardenia jasminoides* extract (GJE) and the GJE-ethyl acetate (GJE-EA) fraction on serum levels of IgE, IL-4, and histamine in NC/Nga mice and histamine release from mast cells. (A) Serum total IgE, (B) IL-4, and (C) histamine levels were determined by ELISA. Results are expressed as mean ± SD ($n=6$). (D) Release of histamine from MC/9 cells. MC/9 cells (2×10^5 cells/ml) were pretreated with GJE or GJE-EA (0–800 μg/ml) at 37 °C for 30 min prior to the addition of compound 48/80 (25 μg/ml). The means ± SD of three independent experiments are shown. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. normal; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control.

3.6. Expression of cytokines and adhesion molecules in ear lesions

We investigated the effects of GJE and GJE-EA on the mRNA levels of cytokines and adhesion molecules in ear lesions. Increases in the mRNA levels of Th2-derived (IL-4 and IL-6), pro-inflammatory (TNF-α), and Th1-derived (IFN-γ) cytokines were observed after DfE application (Fig. 3B). Treatment with GJE or GJE-EA attenuated the DfE-induced upregulation of IL-4, IL-6, and TNF-α,but not IFN-γ. The DfE-induced expression of adhesion molecules (intercellular adhesion molecule-1 [ICAM-1] and vascular cell adhesion molecule-1 [VCAM-1]) was also attenuated by GJE-EA. These results suggest that GJE and GJE-EA prevent skin inflammation by downregulating the expression of cytokines and adhesion molecules to reduce inflammatory cell infiltration.

3.7. Effects of single components (gardenoside and geniposide) on histamine release from MC/9 mast cells

To establish the fingerprint chromatogram and quantify the marker components, an optimized HPLC analysis was employed. The chromatogram patterns for GJE, GJE-EA, GJE-H_2O, and the standard compounds are shown in Fig. 4A and B. The retention times of geniposide and crocin were detected at approximately 20.9 min and 31.6 min, respectively. The yields of geniposide in GJE, GJE-EA, and GJE-H_2O were analyzed as 0.191 (±0.001) g/g, 0.330 (±0.002) g/g, and 0.010 (±0.0003) g/g, respectively. The yields of crocin in GJE, GJE-EA, and GJE-H_2O fractions were analyzed as 0.573 (±0.003) g/g, 0.071 (±0.002) g/g, and 0.569 (±0.003) g/g, respectively.

Next, we investigated the effects of geniposide and crocin on the release of histamine from MC/9 mast cells. The chemical structures of these compounds are shown in Fig. 4C. Geniposide, but not crocin, inhibited histamine release (Fig. 4D). This suggests that geniposide is the active component of GJE and GJE-EA that suppresses histamine release from mast cells.

4. Discussion

The results of this study demonstrate that GJE, GJE-EA, and geniposide exhibited anti-allergic properties, which were marked by the inhibition of mast cell degranulation. Furthermore, the topical application of GJE or GJE-EA to dust mite allergen-exposed NC/Nga mice significantly inhibited the development of AD-like skin lesions, as determined by the dermatitis severity score, histopathological analysis, levels of serum IgE and histamine, and the mRNA expression of cytokines and adhesion molecules in ear lesions.

Allergic and chronic inflammation are associated with the release of histamines, lipid mediators, chemical mediators, and cytokines in the tissues from mast cells after allergen-specific IgE antibodies bind to their high-affinity receptor, FcεRI, to activate mast cells (Metcalfe et al., 1997). Histamine exerts many effects that are related to the immediate phase of allergic inflammation, including vasodilation, increased vascular permeability, and tissue erythema (Guo et al., 1997). Compound 48/80 is a potent inducer of degranulation, and it stimulates the release of histamine and other chemical mediators from connective tissue-type mast cells (Guo et al., 1997). In this study, GJE and its ethyl acetate fraction significantly inhibited the release of histamine from MC/9 mast cells. Among all fractions, GJE-EA (400 μg/ml) fully inhibited compound 48/80-induced histamine release. This indicates that *Gardenia jasminoides* possesses anti-allergic properties by inhibiting the degranulation of mast cells.

The compounds that were extracted from GJE and GJE-EA include geniposide and crocin. Our results show that geniposide significantly inhibited histamine release from MC/9 mast cells.

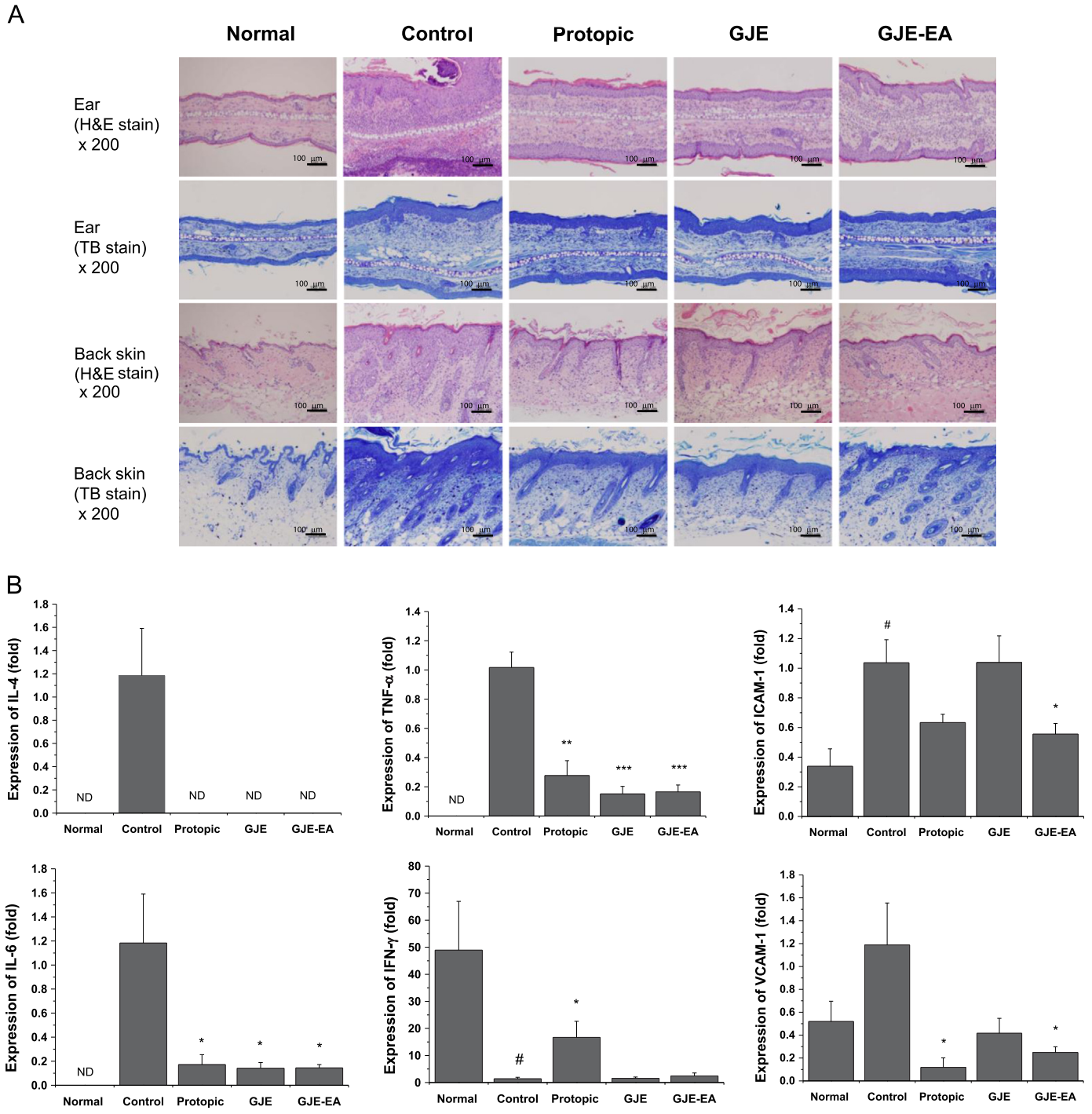

Fig. 3. Histopathological features and inflammatory gene expression in NC/Nga mice. (A) Representative photographs of ear and back skin sections, which were stained with hematoxylin and eosin or toluidine blue (original magnification: × 200). (B) Total RNA was isolated from ear tissues, and the expression of cytokines and adhesion molecules was evaluated by real-time RT-PCR. Results are expressed as mean ± SD ($n=6$). $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. normal; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. control.

Geniposide is the major iridoid glycoside constituent and the main active component of gardenia herbs. It elicits various pharmacological activities, such as the inhibition of ovalbumin-induced junction permeability in guinea pig tracheas and the recovery of ovalbumin-induced allergic airway hyperresponsiveness in mice, both of which highlight the potential of geniposide as an anti-asthmatic drug (Deng et al., 2013). Some reports have demonstrated that geniposide exhibits anti-inflammatory effects by inhibiting carrageenan-induced rat paw edema and acetic acid-induced vascular permeability in mice (Koo et al., 2006). In addition, geniposide blocks high glucose-induced cell adhesion through the nuclear factor-kappaB signaling pathway (Wang et al., 2010). Crocin, a natural carotenoid chemical compound, is a major colorant of gardenia fruits. Interestingly, it did not inhibit histamine release from mast cells. Furthermore, the EA fraction of GJE, which included many molecules of geniposide, effectively inhibited the release of histamine from mast cells, lowered serum histamine levels in AD mice, and consequently suppressed the development of AD-like skin lesions. These results suggest that geniposide may be responsible for the anti-allergic effects of *Gardenia jasminoides*. Future

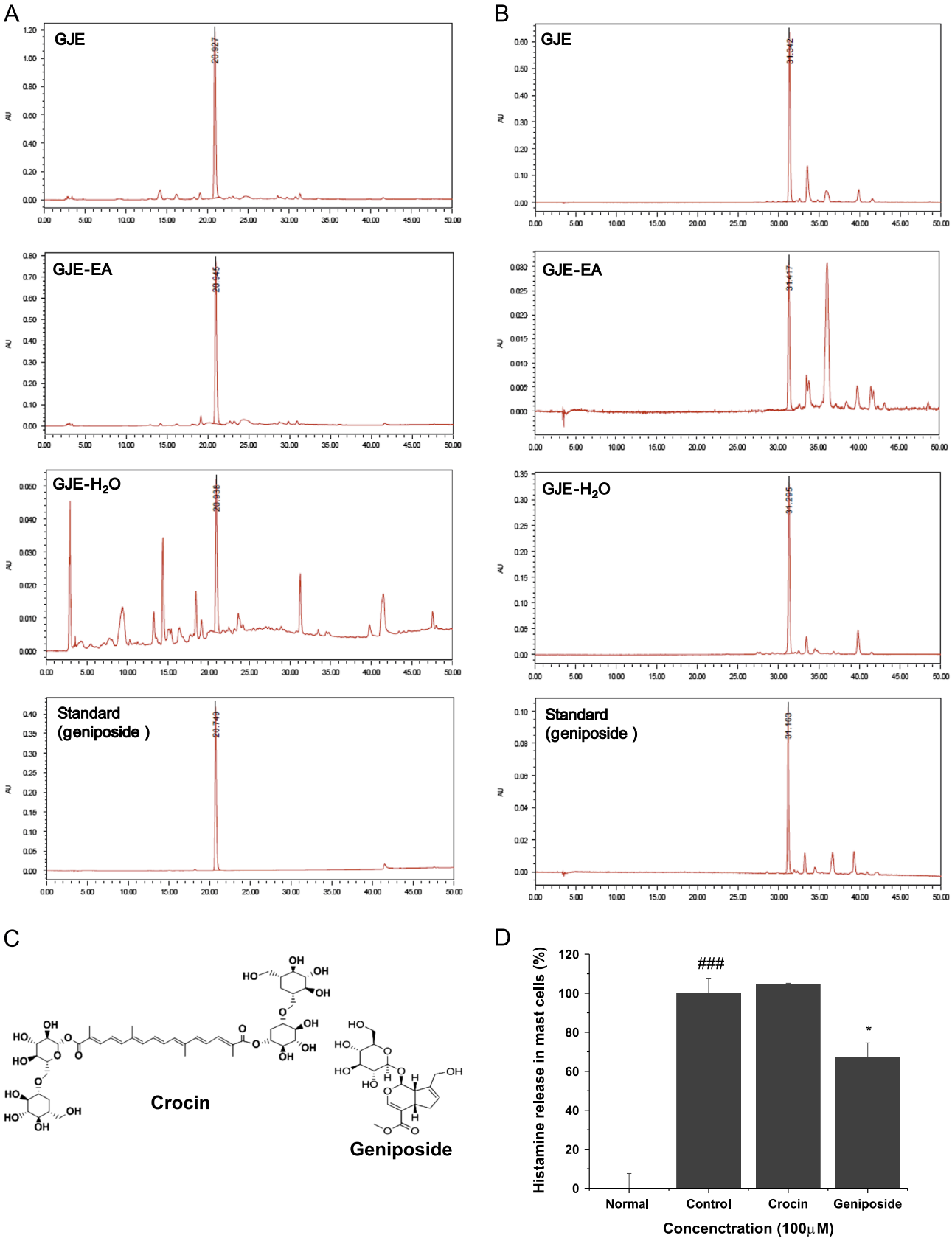

Fig. 4. Effect of crocin and geniposide on histamine release from MC/9 mast cells. (A) HPLC chromatograms of *Gardenia jasminoides* extract (GJE), the GJE-ethyl acetate fraction (GJE-EA), the GJE-water fraction (GJE-H_2O), and the geniposide standard at 238 nm. (B) HPLC chromatograms of GJE, GJE-EA, GJE-H_2O, and the crocin standard at 440 nm. (C) Chemical structures of compounds. (D) Release of histamine from mast cells. MC/9 cells (2×10^5 cells/ml) were pretreated with compounds (100 μM) at 37 °C for 30 min prior to the addition of compound 48/80 (25 μg/ml) The means ± SD of three independent experiments are shown. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. normal; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control.

studies using *in vivo* allergic AD models are necessary to verify that geniposide exerts its anti-allergic effect by inhibiting inflammatory mediators, such as histamine and cytokines.

In allergic inflammatory disease, Th2 cells secrete cytokines to mediate IgE-dependent mast cell degranulation and activation. IL-4 is a Th2 cell- and mast cell-secreted cytokine that is responsible for isotype switching in B cells for IgE synthesis and Th2 cell differentiation (Bergstedt-Lindqvist et al., 1988). The Th1 cell-secreted cytokine, IFN-γ, inhibits IgE synthesis and Th2 cell proliferation (Leung and Bieber, 2003). Dysregulation of IL-6 signaling contributes to the onset and maintenance of inflammatory diseases (Heinrich et al., 2003). IL-6, which is produced by macrophages, T cells, B cells, and keratinocytes in response to external immune stimuli (IL-1 and TNF-α), promotes Th2 differentiation by upregulating IL-4 expression and inhibits Th1 differentiation by upregulating the suppressor of cytokine signaling-1 expression (Diehl and Rincon, 2002). In our study, GJE-EA decreased the serum levels of total IgE, IL-4, and histamine, and it downregulated the mRNA expression of *IL-4*, *IL-6*, and *TNF-α*, but not *IFN-γ*, in ear lesions. This suggests that GJE-EA attenuates the development of AD by inhibiting Th2 cell responses.

ICAM-1 and VCAM-1 are cell-surface glycoproteins that are expressed on endothelial cells, and they promote leukocyte infiltration in immune responses by mediating the adhesion of leukocytes/endothelial cells (Roebuck and Finnegan, 1999). They also contribute to leukocyte accumulation in mice that are exposed to dust mite allergens (Kang et al., 2008), and the expression of these molecules in the keratinocytes of the skin lesions of AD is induced *via* mast cell degranulation-secreted TNF-α and histamine (Ackermann and Harvima, 1998). Importantly, GJE-EA decreased the DfE-induced upregulation of these molecules in our AD mouse model, thus suggesting that it inhibits the migration of leukocytes to the sites of inflammation in AD by downregulating the expression of adhesion molecules.

5. Conclusion

In this study, the topical application of GJE or GJE-EA inhibited the development of AD-like skin lesions and decreased serum IgE and histamine levels. The inhibition of histamine, cytokines, and adhesion molecules blocked inflammatory cell infiltration, which contributed to the anti-allergic effects of GJE and GJE-EA. We also reveal for the first time that the active compound of GJE was geniposide. These results indicate that *Gardenia jasminoides* may be useful in preventing or treating allergic disorders, such as AD.

Acknowledgments

This work was supported by the Construction of the Basis for Practical Application of Herbal Resources (Project K13020), the Korea Institute of Oriental Medicine (KIOM) to the Ministry of Science, ICT and Future Planning (MSIP), Korea. In addition, this work was also partially supported by Efficacy Study of Alter-native Herbal Medicine Resources (K14416), the Korea Institute of Oriental Medicine (KIOM) to the Ministry of Science, ICT and Future Planning (MSIP), Korea.

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