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Effect of topical application of quercetin-3-O-(2''-gallate)- α -l-rhamnopyranoside on atopic dermatitis in NC/Nga mice

Eun Joo Park^a, Ji-Yun Kim^b, Mi Sook Jeong^b, Kui Young Park^b, Kwan Hee Park^c,
Min Won Lee^c, Seong Soo Joo^d, Seong Jun Seo^{b,*}

^a College of Medicine, Department of Dermatology, Hallym University College of Medicine, Anyang, South Korea

^b Institute of Atopic Dermatitis, Department of Dermatology, Chung-Ang University College of Medicine, Seoul, South Korea

^c College of Pharmacy, Chung-Ang University, Seoul, South Korea

^d Division of Marine Molecular Biotechnology, Gangneung-Wonju National University, Gangneung, South Korea

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ABSTRACT

Background: Quercetin-3-O-(2''-gallate)- α -l-rhamnopyranoside (QGR) is a new quercetin derivative which is isolated from the leaves of *Acer ginnala* Maxim, a native plant of Korea. Quercetin has several biological effects including antioxidative, anti-inflammatory, and anti-allergic effects. However, the topical effect of QGR on atopic dermatitis (AD) like skin lesion in NC/Nga mice has not been studied. **Objective:** To evaluate the anti-inflammatory and anti-allergic effect of QGR in a murine model of atopic dermatitis.

Methods: We measured inducible nitric oxide synthase (iNOS) and cyclooxygenase -2(COX-2) level in RAW264.7 cell with QGR treatment. And after induction of AD like skin lesions with *Dermatophagoides farina* (Df) ointment, mice were treated with QGR and control drugs. Clinical scores, interleukin (IL) 4, 5, and 13, serum IgE, eosinophil levels, iNOS and COX-2 level were evaluated.

Results: Results show that mRNA level of iNOS and COX-2 *in vitro* were decreased after QGR treatment. Topical QGR markedly decreased the iNOS and COX-2 mRNA expressions in the skin. QGR also significantly suppressed the increase in the level of total plasma IgE and eosinophils. In addition, topical application of QGR down-regulated the expressions of the cytokines, IL-4,5 and 13, which were induced by Df ointment stimulation.

Conclusions: In the present study, we showed that topical application of QGR ameliorated Df-induced AD-like inflammatory responses in NC/Nga mice. These results demonstrate that QGR might be beneficial in the treatment of AD.

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1. Introduction

Atopic dermatitis (AD) is the most common, relapsing allergic disease of the skin. It is characterized by pruritic and eczematous skin lesions. AD is caused by the complex interaction of genetic and environmental factors that result in immune system dysregulation [1]. In AD, type 2 helper T (Th2) cells show increased production of interleukin (IL)-4, which stimulates plasma cells to increase IgE production and promotes further Th2 cell development [2]. Other studies have shown that in addition to IL-4, IFN- γ production by Th1 cells at both mRNA and protein levels is induced in the lesions of AD patients [3,4]. These reports suggest that both Th1 and Th2

subsets contribute to the pathogenesis of AD [5]. In addition to the Th1/Th2 paradigm, role of Th17 have been highlighted. Phenotypic analysis of peripheral blood mononuclear cells derived from AD patients exhibited a marked increase in the IL-17 + CD4+ T-cell population. A high percentage of IL-17-producing cells was found in severe AD, which suggests a direct correlation between the presence of Th17 cells and disease severity [6]. To date, AD has been regarded as an intractable dermatological disorder, whose treatment modalities consist mainly of corticosteroids, anti-histamines, and immune suppressants. However, these therapeutic agents are of limited value because of the frequent occurrence of adverse effects and recurrence after cessation of therapy. Therefore, alternative and compensatory agents need to be developed promptly to treat this condition. Recently, phytochemicals have been tested as potential therapeutic agents for the treatment of AD [7–9].

* Corresponding author. Tel.: +82 2 6299 3088; fax: +82 2 823 1049.
E-mail address: drseo@cau.ac.kr (S.J. Seo).

The flavonoid quercetin and its derivatives are known to exert several antioxidative [10,11] and anti-inflammatory effects. Previous studies showed that quercetin inhibited proinflammatory cytokine expression through modulation of the activation of NF- κ B in a human mast cell line [12] and downregulated eosinophil and IL-5 production in a murine model of asthma [13]. In addition, a study showed the inhibitory effect of quercetin and tannic acid on angiogenesis and TARC expression in NC/Nga mice [14].

Quercetin-3-O-(2''-gallate)- α -L-rhamnopyranoside(QGR) is a new quercetin derivative, and the effect of QGR on atopic dermatitis has not been studied, so we investigated the therapeutic anti-inflammatory and anti-immunologic effects of QGR on atopic dermatitis like skin lesion on NC/Nga mice.

2. Materials and methods

2.1. Preparation of extraction and isolation of QGR

The 2 kg of the leaf of *Acer ginnala* collected from Korea National Arboretum (Pocheon, Korea) were extracted several times with 80% methanol at room temperature (for 72 h). After removing the acetone under vacuum, the aqueous solution was filtered through filter paper (Tokyo Roshi Kaisha Ltd, Tokyo, Japan). The filtrate was then concentrated (674 g) and applied to Sephadex LH-20 (2 kg, 10–25 μ m, 10 $\times$ 80 cm, GE Healthcare Bio-Science AB, Uppsala, Sweden) eluted with water containing increasing proportions of MeOH, to afford 3 sub-fractions. The 43 g of fraction 3 was subjected to MCI-gel CHP 20P (600 g, 75–150 μ m, 5 $\times$ 60 cm, Mitsubishi Chemical Co., Tokyo) with gradient elution system of water-methanol to yield quercetin-3-O-(2''-galloyl)- α -L-rhamnopyranoside (850 mg).

2.2. Structural identification of QGR

The structural identity of QGR was characterized by spectral analyses. The 1D nuclear magnetic resonance (NMR) findings such as, ^{1} H (600 MHz) and ^{13} C (150 MHz) NMR were recorded with Gemini 2000 (Varian, Palo Alto, CA, USA). Low-resolution fast atom bombardment mass spectrum (LRFAB-MS) was recorded with JMS-600W (Jeol, Tokyo, Japan).

Yellow amorphous powder, negative FAB-MS m/z : 599 [$M - H$] $^-$, ^{1} H NMR and ^{13} C NMR data (Fig. 1) [15].

2.3. Cell culture

The RAW 264.7 rat macrophage cell line was purchased from the Korea Cell Line Bank (Seoul, Korea). The cells were grown in Dulbecco's modified Eagle's medium (DMEM, Welgene, Daegu, Korea) supplemented with heat-inactivated fetal bovine serum (FBS, Welgene, Daegu, Korea), penicillin-streptomycin (100 U/ml, PS, Welgene, Daegu, Korea) in 5% CO $_2$ incubator at 37 $^\circ$ C. The cells were plated at a density of 2×10^6 cells/well in a 6-well plate or

1×10^6 cells/well in 24-well plate reached 80–90% confluence. The adherent macrophage were washed gently with phosphate buffered saline (PBS, Welgene, Daegu, Korea) and cultured in serum-free DMEM for 2 h. Then, the cells were incubated in the absence or presence of QGR (0.02, 0.2, 1 and 2 μ g/ml) with or without peptidoglycan (PGN, 1 μ g/ml, Sigma, Louis, MO). QGR dissolved in PBS was added to the medium 1 h prior to the addition of PGN. Experimental groups were divided into four as follow; normal control, PGN only (1 μ g/ml), PGN + QGR (0.02, 0.2, 1 and 2 μ g/ml) and PGN + ascorbic acid (100 μ M, positive group) as a positive control, and incubated for 24 h. We measured nitric oxide (NO) concentration and inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 mRNA in RAW 264.7 cell to evaluate the effect of QGR.

2.4. Nitric oxide (NO) production by macrophage

RAW 264.7 cells (1×10^6 cell/ml) were seeded on a 24-well plate and pre-incubated at 37 $^\circ$ C for 12 h to achieve stable attachment. The wells were then washed with PBS after pre-incubation, refreshed with 1% FBS-DMEM containing the sample PGN from *Staphylococcus aureus* (Sigma, Louis, MO), 100 μ M ascorbic acid, and QGR (0.02 to 2 μ g/ml) and incubated for 24 h. NO production was then monitored by measuring nitrite levels in the culture media using the Griess reagent (Promega, Madison, WI).

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

The total RNAs from RAW 264.7 cells stimulated by PGN were prepared by the Trizol method (Invitrogen, Carlsbad, CA). cDNA was synthesized from RNA by the reverse transcription of 1 μ g of total RNA using the Improm-II reverse transcription system (Promega, Madison, WI) and oligo dT primers in a total volume of 20 μ l. PCR amplification was performed using the primers shown in Table 1 (Bioneer, Deajeon, Korea). The PCRs were carried out at the preheating condition of 94 $^\circ$ C for 2 min, denaturation at 94 $^\circ$ C for 30 s, annealing at 57–62 $^\circ$ C for 30 s, extension at 72 $^\circ$ C for 30 s for 28 cycles and final extension at 72 $^\circ$ C for 10 min. The PCR products were analyzed on a 1.2% agarose gel containing ethidium bromide. The band intensities of the amplified DNAs were compared to the gel documentation system (MiniBIS Pro, Jerusalem, Israel).

2.6. Animals

Female 3-week-old NC/Nga mice were purchased from SLC Japan (Yokohama, Japan) and were maintained for 2 weeks under specific pathogen-free (SPF) condition environment. These mice were housed in polycarbonate cages ($n = 5$ per cage) in a room with controlled temperature (23 ± 2 $^\circ$ C) and humidity ($55 \pm 7\%$) with a 12 h light/dark cycle. The hair on their backs was depilated by treatment with a skin-hair-remover. Hair removal was performed every 2 weeks. All experiments with mice were performed in accordance with the regulations.

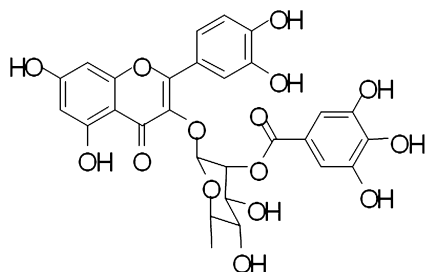


Fig. 1. Chemical structure of quercetin-3-O-(2''-gallate) α -L-rhamnopyranoside (QGR).

Table 1
Primer sequences.

Gene	Sequences	Product size (bp)
COX-2	F: 5'-GCCTGACAAGTTCCAAGA-3' R: 5'-CTTTGCGATGCCGAGTCT-3'	123
iNOS	F: 5'-CCCCACAGTCAAAGACT-3' R: 5'-CTCATCACCCTCAGGAT-3'	191
β -Actin	F: 5'-AATTTGGCATTGTGGAAGG-3' R: 5'-ACACATTGGGGTAGGAACA-3'	291

2.7. Induction of AD-like skin lesions in NC/Nga mice

At 5 weeks of age, mice were anesthetized with ether and then the hair on their dorsal area was shaved once a week. 100 mg *Dermatophagoides farinae* ointment (Df, Biostir AD, Hiroshima, Japan) was treated on the shaved dorsal skin each mouse twice a week, for 4 weeks.

2.8. Topical application of QGR

After induction of atopic dermatitis like skin lesion, QGR (1%) were administered by topical application, everyday for 4 weeks with Df ointment. The experiment were divided into five groups as follow; normal group, 1% base cream group (negative control group), 0.1% hydrocortisone ointment group (HDS, positive control group), 1% *Acer ginnala* extract (AGE) group, 1% quercetin (QGR) group.

2.9. Clinical observation of AD

Clinical observation on the back of NC/Nga mice was measured once a week for 8 weeks. To compare the skin lesions of the mice, the clinical severity of the dermatitis was scored using previously described macroscopic diagnostic criteria, which are normally used for human AD. In brief, the severity of dermatitis was evaluated once a week. The development of (1) erythema/hemorrhage, (2) scarring/dryness, (3) edema, (4) excoriation/erosion was scored as 0 (none), 1 (mild), 2 (moderate) and 3 (severe) respectively. The sum of the individual scores was taken as the dermatitis score [16].

2.10. Measurement of eosinophil count in blood

Blood samples were collected after the induction of AD-like skin lesions and at the end of experiment. Eosinophil counts were conducted on 30 μ L of capillary blood diluted six fold with 150- μ L saline. Microscopic differential counts were performed using Wright-Giemsa stained blood smears. Eosinophil counts were calculated from differential counts.

2.11. Measurement of serum IgE in plasma

The amount of total IgE in the serum was determined by enzyme-linked immunosorbent assay (ELISA) method as previously described [17]. Plasma samples were obtained by centrifuging at 12,000 rpm for 10 min and stored at -80°C until required for assay. Total plasma IgE levels were determined by ELISA using a method involving the capture and detection of monoclonal antibody pairs, as suggested by BD Pharmingen (Pharmingen, San Diego, CA) [17,18].

2.12. Cytokine measurement

Blood samples were collected at 13 weeks of age. Th2 cytokines (IL-4,5,13) were quantified using a mouse cytokine enzyme immunoassay kit (R&D Systems, Minneapolis, MN) [19].

2.13. Quantitative real-time PCR analysis

We synthesized the PCR primers using data in the Gene Bank. Primers were chemically synthesized using a DNA synthesizer. After the application of Drugs for 8 weeks, skin lesions from the back were removed, frozen immediately in liquid nitrogen, and stored at -80°C . Total RNA was isolated from tissue using TRIzol reagent (Invitrogen, Carlsbad, CA). The cDNA was synthesized from equal amounts of total RNA with reverse transcriptase, and

polymerase chain reaction (PCR) was performed with Taq DNA polymerase. Equal amounts of cDNA were mixed with SYBR Green I (Bio-Rad, Hercules, CA).

2.14. Western blotting

The dorsal skin was heated at 95°C for 5 min and briefly cooled on ice. Following centrifugation at 13,000 rpm for 20 min, 20 μ L aliquots were resolved by 10% SDS-PAGE. After electrophoresis, the protein was transferred to nitrocellulose membranes, then, membranes were incubated overnight with human iNOX and COX-2 polyclonal antibodies (1:1000 in 5% bovine serum albumin, Chemicon, CA, USA). Next, membranes were incubated in a secondary murine anti-rabbit peroxidase conjugated antibody (1:2000 in blocking solution, Chemicon, CA, USA). Finally, the protein bands were visualized using enhanced chemiluminescence assay purchased from BioRad Co. (Bio-Rad, Hercules, CA, USA) following the manufacturer's instructions.

2.15. Statistical analysis

All data are expressed as the mean and SD. Statistical difference between the groups were determined by a factorial analysis of variance (ANOVA) followed by an unpaired t-test. Statistical significance was accepted for P value of <0.05 .

3. Results

3.1. QGR inhibits production of NO in PGN-stimulated macrophage

To evaluate the effect of QGR on NO secretion in PGN stimulated RAW 264.7 cells, we measured nitrite concentrations in culture media using the Griess reagent. RAW264.7 cells were treated with 0–2 μ g/ml QGR for 24 h and stimulated with PGN for 24 h. NO secretion was increased by treatment of PGN alone; QGR significantly reduced NO levels in PGN-stimulated cells in a dose-dependent manner (Fig. 2). Interestingly, treatment group of QGR resulted in similarly decreased NO secretion compared with that of positive group. The Cytotoxic effects of different concentrations of QGR (0.02–2 μ g/ml) on RAW 264.7 cells were determined after incubation for 24 h. In these experiments, QGR did not affect the viability of RAW 264.7 cells at concentrations ≤ 60 μ g/ml. Hence, the inhibitory effect of QGR on the PGN-induced RAW 264.7 macrophages was deemed not to be attributable to cytotoxicity.

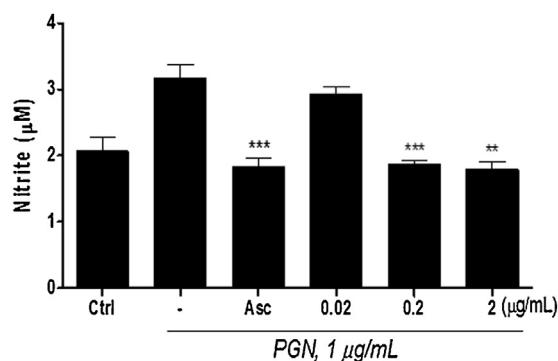


Fig. 2. NO production in RAW 264.7. The NO production was assayed from the culture medium of cells stimulated with PGN in the presence of QGR for 24 h. The results are expressed as mean(SD). Ctrl: normal control, Asc: ascorbic acid, PGN: peptidoglycan. ** $P < 0.01$ versus normal control, *** $P < 0.001$ versus normal control.

3.2. QGR decrease iNOS and COX-2 expression in PGN-stimulated RAW 264.7 cells

To determine, whether QGR inhibits pro-inflammatory repressors (iNOS and COX-2) at the gene level, RAW 264.7 cells were stimulated by PGN, and iNOS and COX mRNA were examined in the presence of QGR. Ascorbic acid, an inhibitor of NO generation from arginine was included for comparison. As shown in Fig. 3 mRNA expression levels of iNOS and COX-2 were inhibited with QGR and the levels of inhibition were superior to that produced by ascorbic acid (100 μ M) (Fig. 3).

3.3. Topical application of QGR suppresses the development of AD-like skin lesions in Df-treated NC/Nga mice

In this study, all animals were weighed on a weekly schedule. In Fig. 4a, Base group of mice showed a slight decrease in weight after 5 weeks. But, as a side effect, significant weight loss was observed in these hydrocortisone-treated mice as a positive group (Fig. 4a). However, we could not find a QGR related decrease of body weight compared with control group. Df-stimulated back skin of NC/Nga mice developed AD-like skin lesions (Fig. 4b). As expected, application of 0.1% HDS ointment significantly inhibited the skin symptoms to an extent greater than those symptoms observed after the administration of QGR (Fig. 4c).

3.4. QGR decreases the level of eosinophil in blood

As shown in Fig. 5a, eosinophil levels were decreased after treatment of the mice with QGR. Interestingly, topical application of QGR results in decrease of eosinophil level compared with that in HDS group. This indicated that QGR might be associated with improvement of eosinophil-related allergic diseases (Fig. 5a).

3.5. QGR down-regulated IgE level in plasma

The increased level of IgE is also a diagnostic indicator for AD. Df significantly upregulated total plasma IgE production induced by Df stimulation. As shown in Fig. 5b, QGR treatment, topically, significantly down-regulated the IgE levels in the NC/Nga mice (Fig. 5b).

3.6. QGR suppresses the production of Th2 cytokine

The rate of suppression of Th2-related cytokines such as IL-4, IL-5 and IL-13 was measured in serum. In the QGR-treated group, the expression of cytokines was significantly inhibited. These results suggest that QGR might exert an immunoregulatory function associated with AD (Fig. 6).

3.7. QGR decrease iNOS and COX-2 expression in the skin lesions of Df-treated NC/Nga mice

To investigate whether QGR affects cytokine mRNA expression in skin, we examined the gene and protein expression levels of inflammatory factors on back skin of NC/Nga mice using real-time PCR analysis and Western blotting. The mRNA and protein expression levels of iNOS and COX-2 were inhibited in the QGR-treated group (Fig. 7). These results suggested that QGR might be effective in exerting anti-inflammatory activity in NC/Nga mice.

4. Discussion

Atopic dermatitis (AD) is a highly pruritic inflammatory skin disease characterized by skin hypersensitivity in response to various environmental and genetic factors [1]. There are several treatments for AD. The currently used steroids, anti-histamines, and immunosuppressants have shown limitations in their efficacy, and it is therefore imperative that alternative and compensatory agents be developed promptly to treat this condition. To develop an appropriate treatment agent for AD using a natural substance, we performed *in vitro* and *in vivo* experiments using NC/Nga mice to assess the anti-AD effect of QGR, a new quercetin derivative, isolated from the leaf of *Acer ginnala*. Quercetin is known to have various biological activities, including antioxidation [10,11], anticarcinogenic [20–22], and anti-inflammatory effects through the modulation of Th1–Th2 responses in an asthma murine model [23]. Granado-Serrano et al. [24] reported that quercetin inhibits the tumor necrosis factor (TNF)-induced COX pathway, suggesting that this flavonoid might act as an anti-inflammatory agent in the liver. Recent studies showed that quercetin effectively inhibited the secretion of histamines, leukotrienes, and prostaglandin in cultured human mast cells; reduced IL-6 release in a dose-dependent manner; and effectively inhibited IL-8 and TNF release from mast cells stimulated by substance P [12,25]. Therefore, we hypothesized that quercetin may inhibit AD development and investigated the therapeutic effect of new quercetin derivative, QGR on AD-like skin lesions.

Although the pathogenesis of AD is not completely understood, abnormalities in Th1 and Th2 cytokines are one of the important etiologies. Especially, Th2 cytokines have been reported to be key mediators of AD development [3,26]. IL-4 and IL-13 induce IgE secretion from activated B cell [27], and IL-5 stimulates the differentiation of eosinophils and prolongs eosinophil survival [28]. Although Th1 cytokines such as IL-12, IL-18, and IL-11 play a role in AD, they are mainly involved in the maintenance of chronic AD. As an experimental period of 8 weeks, including 4 weeks for skin lesion induction and 4 weeks for treatment, is considered the acute phase, we focused on the assessment of Th-2 cytokine levels. In the QGR-treated group, the expressions of Th2 cytokines such as IL-4, IL-5, and IL-13 were significantly inhibited (Fig. 6). Thymic stromal lymphopoietin (TSLP) induces Th2 cytokines including thymus and activation regulated chemokine (TARC), and is involved in allergic response, IgE production and eosinophilia. Therefore we will examine the level of TSLP and TARC in further study.

IgE mediates mast cell activation, which culminates in the release of preformed mediators such as histamines and cytokines, including those promoting Th-2 polarized T-cell responses. Thus, IgE can contribute to AD development through the activation of mast cells localized to the skin after induction by various allergens [29]. An elevated serum IgE level was associated with AD-like skin lesions and correlated with the severity of AD [30,31]. In our mouse study, assessment of serum IgE expression showed that it was downregulated in the group receiving QGR treatment compared with the AD control group (Fig. 5b).

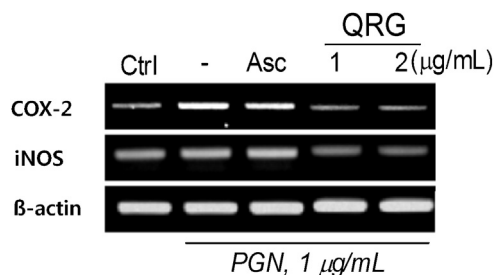


Fig. 3. Effect of QGR (1, 2 μ g/ml) on the mRNA expression of COX-2 and iNOS in RAW 264.7 cells stimulated with PGN. Ctrl: normal control, Asc: ascorbic acid, PGN: peptidoglycan.

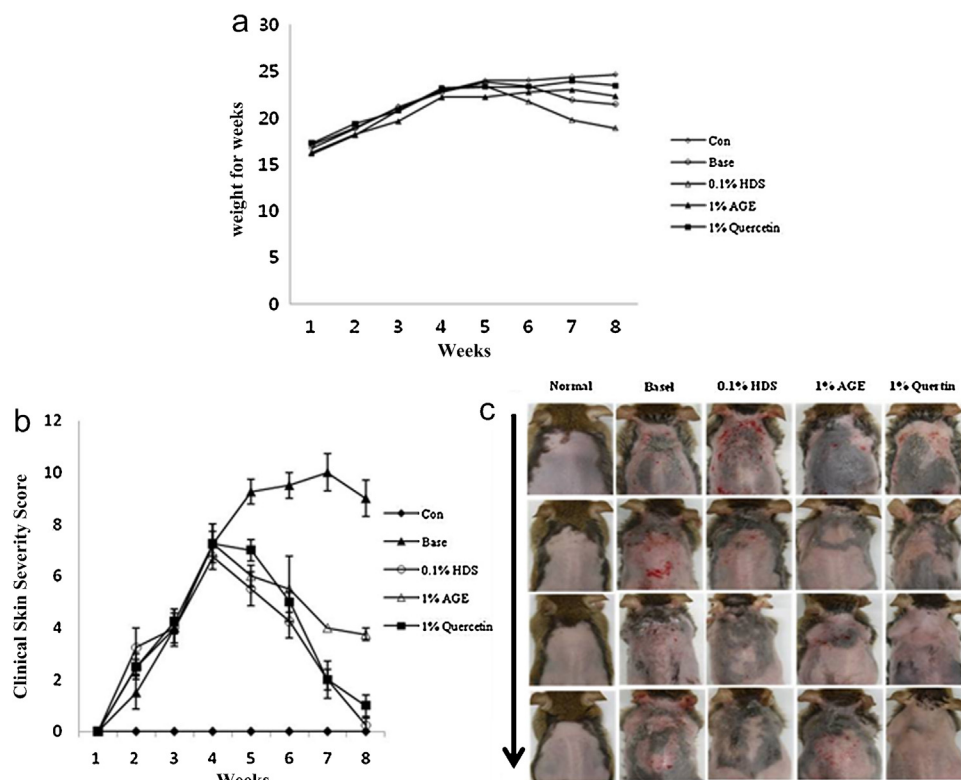


Fig. 4. Clinical observation of AD-like skin lesion induced NC/Nga mice during experimental periods. QGR treatment significantly reduced the severity of AD-like lesions induced in NC/Nga mice. (a) Body weights in each group of NC/Nga mice were monitored once a week starting week 1. (b) Clinical skin severity scores were monitored twice a week for 8 weeks. Values represent the mean $\pm$ SD of the total score from 5 mice for each group. (c) This photograph of the mice was taken twice a week for 8 weeks. Values represent the mean $\pm$ SD of 5 mice for each group. Con: normal, base: base cream (negative group), HDC: Hydrocortisone (positive group), AGE: *Acer ginnala* extract.

Eosinophils play a crucial role in aggravating AD symptoms and are correlated with AD severity [32]. These cells infiltrate the dermis in response to allergens and subsequently secrete eosinophil cationic protein (ECP), which mediates the migration of other immune cells into the forming skin lesions [28]. Eosinophil levels were decreased after treatment with QGR in mice. This indicated that QGR might help improve eosinophil-related allergic diseases (Fig. 5a).

Nitric oxide (NO) plays a role in vasodilation, neurotransmission, blood coagulation, and immune regulation. An excessive NO presence aggravates the inflammatory response. iNOS is a major producer of NO when it is activated by various cytokines or bacterial lipopolysaccharides, and symptoms of AD such as scratching can be exacerbated by iNOS-derived NO [33]. COX-2 expression is increased in inflammation-related cells in response to stimulation with cytokines during the immune reaction. As

such, quantifying iNOS and COX-2 synthesis is considered the universal method for evaluating the anti-inflammatory effect of pharmacological agents [34,35]. Macrophages are known to be involved in homeostasis through various host response, and are crucial in host defense in early inflammatory phase by producing NO and numerous cytokines in inflammatory response. Patients with AD have frequent bacterial and viral skin infections. Several cofactors, such as an impaired skin barrier function, modifications of the immune system, and a complex genetic background, direct the course of atopic dermatitis. Within this complex network, macrophages play a pivotal role in enhanced susceptibility to cutaneous infections and act as central connecting components in the pathogenesis of AD on the cellular level. During the early and short inflammatory phase in AD, macrophages exert proinflammatory functions like antigen-presenting phagocytosis and the production of inflammatory cytokines and growth factors that facilitate the

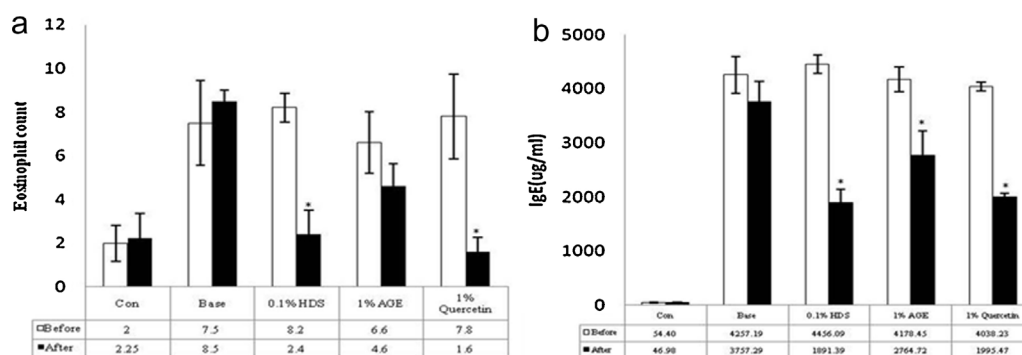


Fig. 5. Effect of topical QGR on eosinophil count and IgE. (a) Eosinophil levels were decreased after treatment of the animals with QGR. (b) QGR treatment, topically applied, significantly down-regulated the IgE levels in NC/Nga mice. The results are expressed as mean (SD) (* $P < 0.05$ versus each group before treatment with sample) ($n = 5$).

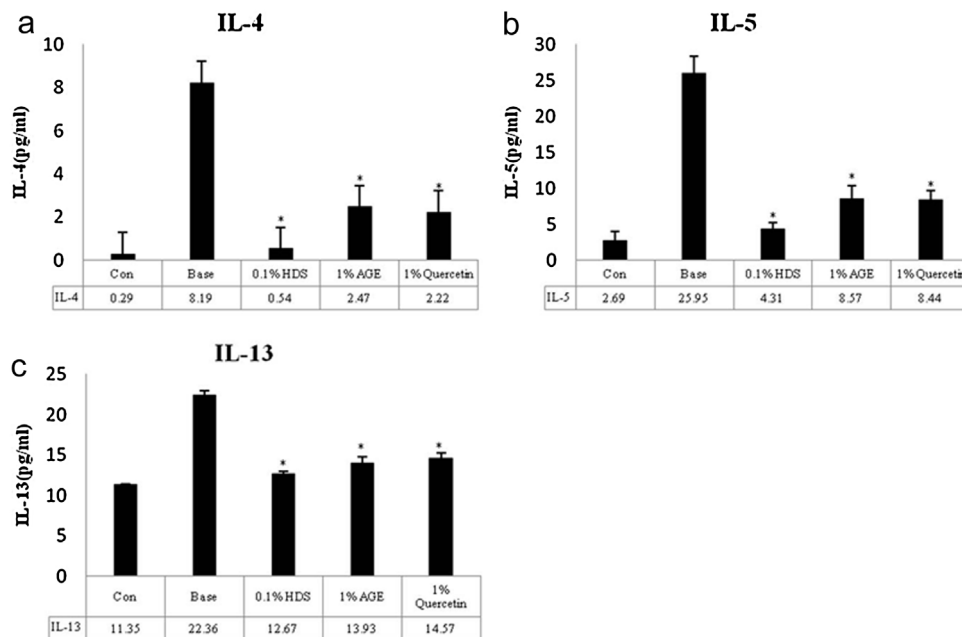


Fig. 6. Effects of topical application of QGR on serum Th2 cytokine levels. In the QGR treated group, the expression of Th2-related cytokines were significantly inhibited. These results suggest that QGR might play an immunoregulatory role associated with AD. The results are expressed as mean (SD). (* $P < 0.05$ compared with the base cream group) ($n = 5$).

resolution of inflammation [36]. AD is complicated by their enhanced susceptibility to bacterial skin infections, especially with *S. aureus*, the recognition of its components might be crucial in the pathogenesis and aggravation of AD [37]. Therefore, activation of not only T lymphocytes, DCs, keratinocytes, mast cells, and eosinophils but also macrophages is characteristic of AD skin inflammatory responses. Thus, change of macrophage-induced cytokines would be another clue for AD treatment. In the present study, we measured NO concentration, and iNOS and COX-2 mRNA in macrophage to evaluate the effect of QGR. NO secretion and the mRNA levels of iNOS and COX-2 in RAW 264.7 cells were decreased in response to the

QGR treatment (Figs. 2 and 3). Furthermore, the mRNA and protein levels of iNOS and COX-2 were inhibited in the skin of the QGR-treated NC/Nga mice (Fig. 7). These results suggest that QGR might have anti-inflammatory activity in atopic dermatitis NC/Nga mice.

Our results showed that topical application of QGR to Df-treated NC/Nga mice inhibited the development of AD-like skin lesions, as shown by the significant decrease in the total skin severity scores and the reduction in the number of eosinophils, and IgE and IL-4, IL-5, and IL-13 production levels. In addition, the QGR treatment inhibited the expression of iNOS and COX-2 in the PGN-treated RAW 264.7 macrophages and NC/Nga mice.

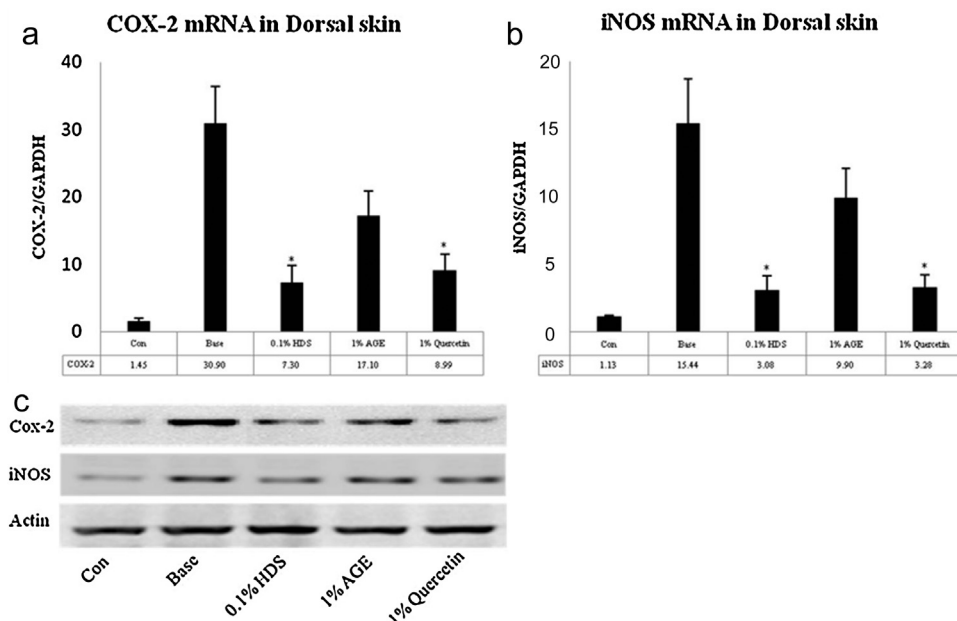


Fig. 7. Effects of topical application of QGR on mRNA and protein expression of iNOS and COX-2. (a and b) These were determined by realtime-PCR. The mRNA expression levels of iNOS and COX-2 were inhibited in the QGR-treated group. (c) These were determined by Western blotting. The protein expression levels of iNOS and COX-2 were inhibited in the QGR-treated group. These results suggested that QGR might be effective in exerting anti-inflammatory activity in NC/Nga mice. The results are expressed as mean $\pm$ SD (* $P < 0.05$) ($n = 5$).

The results demonstrate that topical application of QGR may improve AD-like skin lesions by suppressing inflammatory cytokines, indicating that QGR may be a potential therapeutic agent for AD.

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