ORIGINAL ARTICLE

Asian Pacific Journal of
Allergy and Immunology

APJAI

Oleuropein attenuates inflammation and regulates immune responses in a 2,4-dinitrochlorobenzene-induced atopic dermatitis mouse model

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Abstract

Background: Olive (*Olea europaea Linn*) leaves contain a phenolic compound oleuropein (Ole) has antioxidant, anti-inflammatory, and immunomodulatory activities. However, whether Ole might be an effective treatment for atopic dermatitis (AD) remains unknown.

Objective: This study investigated the functional role of oleuropein in a 2,4-dinitrochlorobenzene-induced AD-like mouse model, with a focus on allergic inflammation.

Methods: We evaluated cytokine gene expression, COX-2 inflammatory protein production, and Th2 related cytokine regulation of mast cells and eosinophils that infiltrated AD-like skin lesions.

Results: A topical application of Ole significantly reduced Th2-related cytokine gene expression (IL-4 and IL-5) and inflammatory COX-2 protein production in AD-like skin lesions. Additionally, Ole suppressed serum IgE levels. Furthermore, Ole effectively reduced ear swelling and epidermal and dermal thickening.

Conclusion: These results suggested that, mechanistically, Ole treatment improved allergic inflammation by blocking the Th2-driven inflammatory axis. In conclusion, our findings indicated that Ole showed promise in treating AD by regulating serum IgE and Th2 cytokine levels. Although the effects of Ole on AD in humans require clinical trials, our results provided insights into how AD treatments might be improved.

Key words: Atopic dermatitis, oleuropein, IgE, mast cells, eosinophils, allergic inflammation

Citation:
Huang, W. C., Liou, C. J., Shen, S. C., Hu, S., Chao, J. C. J., Huang, C. H., Wu, S. J. (2025). Oleuropein attenuates inflammation and regulates immune responses in a 2,4-dinitrochlorobenzene-induced atopic dermatitis mouse model. *Asian Pac J Allergy Immunol*, 43(3), 486-497. https://doi.org/10.12932/ap-200122-1309

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Introduction

Atopic dermatitis (AD), also known as eczema, is the most common chronic inflammatory skin disease. In developed countries, its prevalence is up to 20% in children and 10% in adults.[1] AD is related to various allergies, including food allergies, asthma, allergic rhinitis, Etc. Its pathological process involves genetic factors, epidermal dysfunction, and T cell-driven immune-inflammatory pathways.[2] Skin lesions in AD are intense, itchy, red, erythematous patches with exudation, blistering, and crusting in the early stages and scaling fissuring, and thickening in the later stages.[3] These manifestations lead to sleep loss and a lower quality of life, putting psychological pressure on patients and their families. In addition, recent studies have found that environmental factors, notably air pollution, have increased the incidence and severity of AD.[4]

AD pathology mainly arises from an immune imbalance dominated by immunoglobulin E (IgE), which indicates the type 2 T-helper cell (Th2)-immune response. Th2 cells secrete inflammatory cytokines, including interleukin-4 (IL-4), IL-5, and IL-13, which directly activate sensory nerves and cause itching.[5] IL-5 and IL-13 also activate and attract the accumulation of eosinophils, which causes severe local inflammation. In addition, IL-4 and IL-13 activate receptors linked to the signal transducer and activator of transcription, which stimulates B cells to switch from the IgM class to the IgE class. Then, serum IgE increases and contributes to skin inflammation.[6] Scratching damage leads to a reduction in the diversity of skin surface microbes, which induces a large number of dendritic cells to express high-affinity IgE receptors.[7] Moreover, invading allergens bind to IgE and activate mast cells, which induces the release of inflammatory cytokines and leads to severe skin inflammation.[2]

Previous studies indicated that IgE-mediated activation of mast cells and eosinophils contributed to AD. Moreover, the IgE level was associated with the severity of skin barrier defects in AD.[8] Mast cells express high-affinity IgE receptors on their surfaces. When activated by IgE, mast cells release various cytokines and chemokines. In addition, mast cells can act on dendritic cells to affect antigens Th1/Th2 polarization response. Therefore, mast cells affect the development of AD skin lesions. Eosinophils are found in dermal perivascular inflammatory infiltrates in acute and chronic AD. Moreover, eosinophils promote the Th2-like immune response in chronic AD lesions.[9] Furthermore, IL-33 and thymic stromal lymphopoietin (TSLP) aggravate the inflammation and itching signaling pathways, which are potential factors in severe epidermal barrier defects, including filaggrin gene mutations.[10] In patients with AD, epidermal barrier dysfunction leads to a loss of skin moisture, increased skin permeability, and changes in skin lipid composition.[11] In addition, epidermal barrier defects reduce microbial diversity, which allows the proliferation of *Staphylococcus aureus* and yeast (*Malassezia*) on the skin surface. *S. aureus* and *Malassezia* can aggravate epidermal barrier defects and increase AD recurrence.[12]

AD symptoms induce an itch-scratch cycle. Damage to the skin barrier causes epithelial cell release of TSLP. TSLP activates immune cells to produce inflammatory cytokines, which stimulate sensory neurons. Stimulated sensory neurons cause itching, which damages the skin barrier. Moreover, Th-2 cytokines stimulate mast cells and basophils to release histamine. Th-2 cytokines, including IL-4, IL-5, and IL-13, are closely related to itching in patients with AD.[13] Depending on the cytokine signals received at the time of antigen recognition, $CD4^+$ cells will terminally differentiate into Th1 or Th2 cells. Th1 and Th2 cells secrete cytokines to activate immune responses upon exposure to antigen. Th1 cells secrete IL-2, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and lymphotoxin (LT). These cytokines induce immunoglobulin-γ-2a (IgG2a) antibodies to defend against intracellular pathogens. In contrast, Th2 cells secrete IL-4, IL-5, IL-9, and IL-13. These cytokines promote different antibody classes (IgE, IgG1) to defend against extracellular pathogens.[14] When IgE crosslinks with an antigen, it stimulates cells to release potentially toxic products, which cause the symptoms of AD. In AD, IgE antibodies bind to surface receptors present on a wide variety of cells, most importantly mast cells and eosinophils.[15]

Oleuropein (Ole) is a bioactive phenolic compound derived from olive (*Olea europaea Linn*) leaves. Ole has extensive biological properties, including anti-inflammatory, anti-oxidation, anti-obesity, anti-diabetes, anti-cancer and hepatoprotection, which could significantly treat metabolic syndrome.[16] Furthermore, studies have shown that olive leaf extracts (OLEs) ameliorated gut microbiota dysbiosis and had immunomodulatory properties in a high-fat diet-induced inflammatory mouse model.[17] Previous results showed that OLEs act as modulators of the human immune response to increase the numbers of $CD8^+$ and NK cells to promote IFN-γ production and maintain the T regulatory cells and Th17 cells equilibrium.[18] Only a few related studies on Ole for immune-mediated diseases. In addition, Ole is currently most used in wound healing, prevents apoptosis in irradiated keratinocytes, improves skin elasticity, Etc.[19,20] However, Ole treatment in atopic dermatitis has not been reported. Therefore, we evaluated the effects of oleuropein on AD and investigated the mechanisms that regulated allergic inflammation in AD. We found that Ole reduced Th2-mediated mast-cell and eosinophil infiltrations and regulated the balance of Th2 and Th1 cytokines. Macroscopically, Ole reduced the epidermal and dermal thicknesses of ear and skin lesions in a 2,4-dinitrochlorobenzene (DNCB)-induced AD mouse model.

Materials and Methods

Chemical reagents and animals

Figure 1A shows the structure of Ole. We purchased Ole (from coumarins ≥ 98%), acetone, olive oil, and DNCB from Sigma-Aldrich (St. Louis, MO, USA). We purchased 8-week-old female BALB/c mice from the National Laboratory Animal Center (Taiwan). Mice were housed in a 12-h light-dark cycle at a consistent temperature (23 ± 2°C) and 55 ± 15% humidity at the Animal Center of Chang Gung University. All animal experiments were conducted according to international guidelines and regulations for the use and care of animals. This study was approved by the Laboratory Animal Care Committee of Chang Gung University of Science and Technology and Chang Gung University (IACUC approval number: 2018-007; 29 December 2018).

DNCB-sensitized AD-like skin lesions and oleuropein treatment

Twenty-four mice were randomly divided into four groups (n = 6/group). The control group was challenged with normal saline; the DNCB-sensitized group was challenged with DNCB alone; the Ole-5 group was sensitized with DNCB and received topical treatment of 5 mg/kg Ole, and the Ole-10 group was sensitized with DNCB and received topical treatment of 10 mg/kg Ole. Briefly, all animals were shaved to expose the dorsal skin. DNCB was prepared in a solution of acetone and olive oil (3:1 ratio), as described previously.[21] On days 1-3, all groups, except the control group, were sensitized with 200 μL 0.5% DNCB applied to the shaved dorsal area of skin. Then, on experimental days 14, 17, 20, 23, 26, and 29, the three sensitized groups received 100 μL and 20 μL of 1% DNCB on the dorsal skin and each ear, respectively. On experimental days 14-29, the two treatment groups (Ole-5 and Ole-10) received topical Ole on the ears and dorsal skin daily before DNCB challenge 1 h. The experimental design is shown in **Figure 1B**.

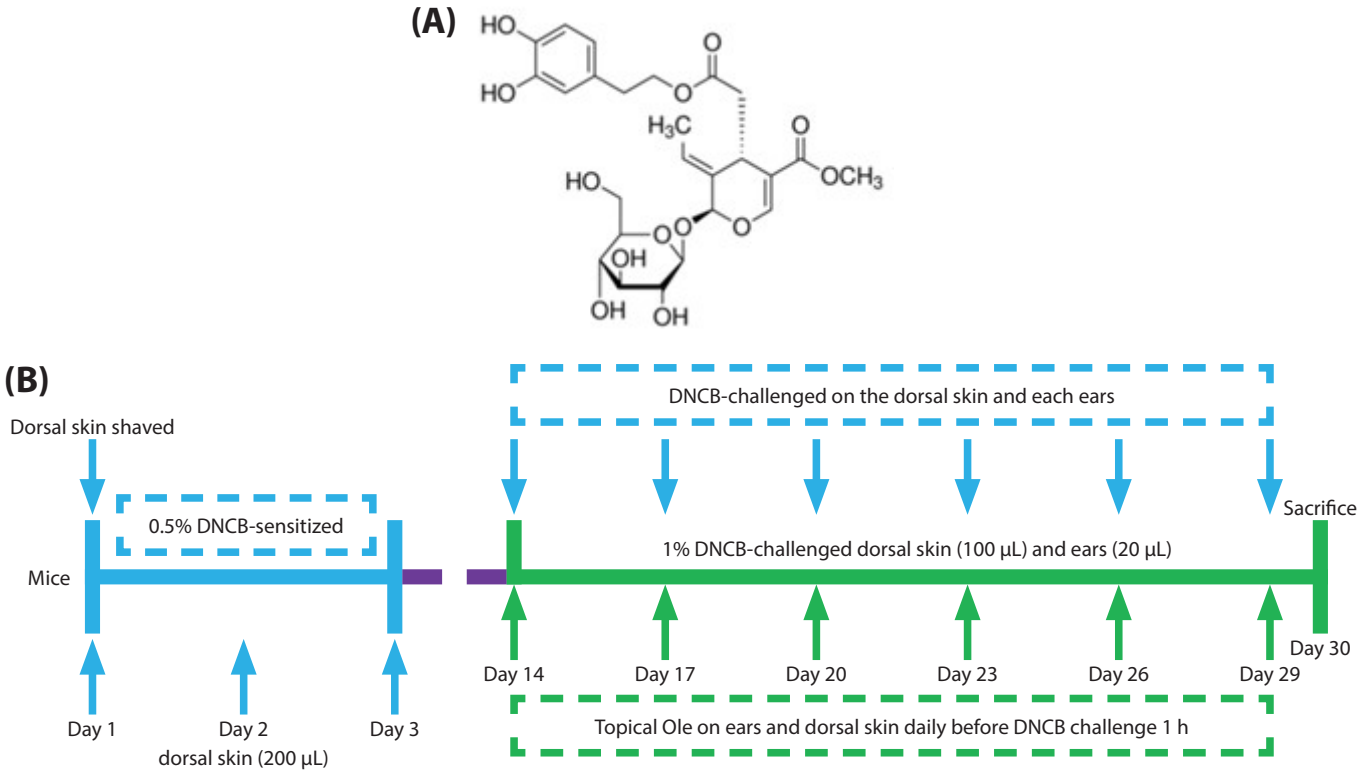
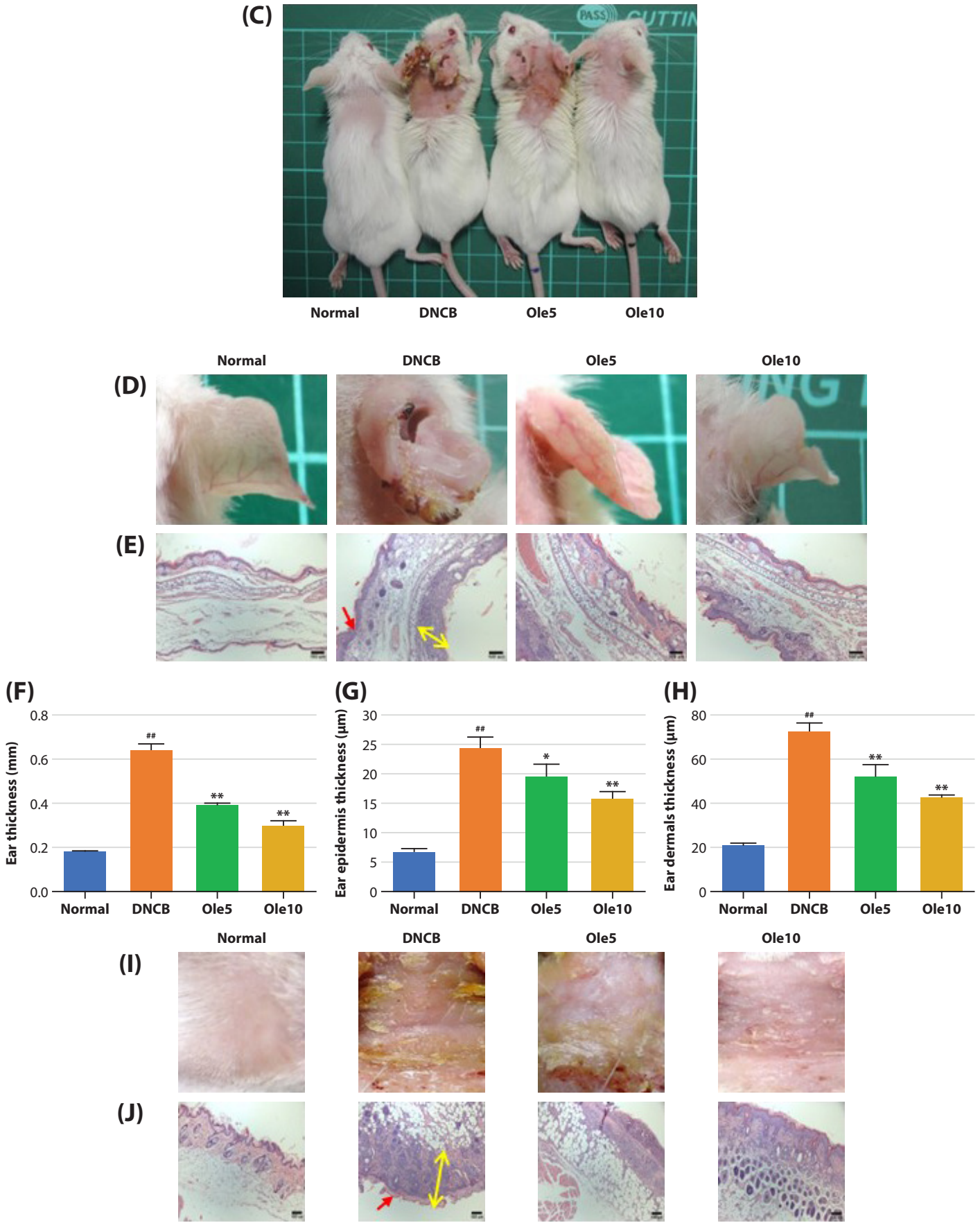


Figure 1. Oleuropein (Ole) attenuated epidermal and dermal swelling in DNCB-sensitized AD mice. (A) The oleuropein chemical structure; (B) treatment schedule for DNCB-challenge and Ole treatment; (C) clinical features of DNCB-induced AD-like skin lesions in mice. (D) photographs show ears of mice; (E) histopathological images of H&E stained ear tissue; (F) ear thicknesses; (G) ear epidermal and (H) dermal thicknesses; (I) dorsal skin images (J) histopathological images of H&E stained dorsal skin tissue; (K) dorsal skin epidermal and (L) dermal thicknesses. Normal (unsensitized and untreated); DNCB-sensitized mouse; Ole 5 (DNCB-sensitized mouse topically treated with 5 mg/kg Ole); Ole 10 (DNCB-sensitized mouse topically treated with 10 mg/kg Ole) measured in each mouse group (n = 6/group). Data are presented as the mean ± SEM (n = 6 mice/group). $^{\#\#}P < 0.001$ versus normal mice, and $^{**}P < 0.01$, $^{*}P < 0.05$ versus DNCB-sensitized mice.



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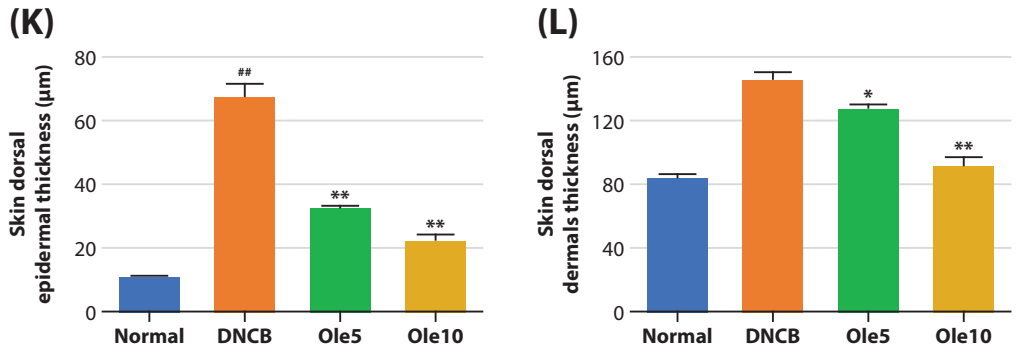

Figure 1. (Continued)

Table 1. Real-time PCR analyses of cytokines and ICAM-1 gene expression used primers.

Gene	Forward primer	Reverse primer
IL-4	TCCGTGCTTGAAGAAGAACTC	GTGATGTGGACTTGGACTCATT
IL-5	ATCCTCCTGCCTCCTCTTCC	GGTTCCATCTCCAGCACTTCA
IL-6	AGGACCAAGACCATCCAATTCA	GCTTAGGCATAACGCACTAGG
ICAM-1	AACAGAATGGTAGACAGCAT	TCCACCGAGTCCTCTTAG

Epidermal, dermal, and ear thickness measurements

Symptoms on the ear and dorsal skin were captured weekly with a digital camera (Coolpix, Nikon Inc., Tokyo, Japan). Animals were sacrificed on day 30. Ear thicknesses were measured with a dial gauge (Olympus, Tokyo, Japan). The ears and skin were stained with hematoxylin and eosin (H&E); then, the epidermal and dermal thicknesses were measured under a microscope with Image-Pro Plus software (version 6.0 for Windows).

Histopathological analysis

After animals were sacrificed, we collected ear and dorsal skin samples, fixed them in 10% formalin, then cut them into 6-μm-thick sections. As previously described, these tissue sections were stained with H&E, toluidine blue, and immunohistochemical stains, as previously described.[22] Sections were examined under a light microscope (100–200× magnification) to count mast cells (toluidine blue) and eosinophils (H&E) in 10–15 high-power fields (HPFs). We evaluated COX-2 expression levels by staining sections with an anti-COX-2 antibody.

Measurements of serum IgE and inflammation-related cytokines

When the animals were sacrificed, we collected blood samples. Blood samples were centrifuged at 3000 ×g for 10 min at 4°C to isolate the serum. According to the manufacturer, we measured serum IgE, IgG1, and IgG2a concentrations with enzyme-linked immunosorbent assay (ELISA) kits (BD Biosciences, San Diego, CA, USA).

RNA isolation and quantitative real-time PCR analysis of skin inflammation-related cytokines

We extracted RNA from homogenized skin tissues with TRI reagent (Sigma). Then, we reverse-transcribed cDNA with the cDNA synthesis kit (Bio-Rad, San Francisco, CA, USA). To investigate specific gene expression levels, we performed a quantitative real-time polymerase chain reaction (qRT-PCR) with the SYBR Green kit (Roche Diagnostics Gmbh, Mannheim, Germany) on a spectrofluorometric thermal cycler (iCycler; Bio-Rad). Each sample was run in duplicate, and gene transcript levels were normalized to endogenous β-actin expression. **Table 1** shows the primer sequences.

Statistical analysis

Data are presented as the mean ± standard error (SEM). Data were analyzed with the one-way analysis of variance (ANOVA) and Tukey's test to evaluate statistical significance. *P*-values < 0.05 were considered statistically significant.

Results

Oleuropein attenuated epidermal and dermal swelling in DNCB-sensitized AD mice

DNCB induced AD-like skin lesions in all sensitized mice. Compared to normal mice, the AD-like skin lesions included ear swelling, scarring, and excoriation of the skin and ear. Topical administration of oleuropein (Ole) attenuated the severity of these DNCB-induced AD-like skin lesions (**Figure 1C**).

On day 30, due to DNCB-induced inflammation, ears were swollen in DCNB-sensitized mice (**Figure 1D**). The mean ear thickness was 0.64 ± 0.23 mm in mice treated with DNCB alone, but ear swelling was significantly reduced with Ole treatment (Ole-5: 0.392 ± 0.11 mm, $p < 0.01$; Ole-10: 0.30 ± 0.23 mm, $p < 0.01$; **Figure 1F**). H&E tissue section stains showed that, on day 30, both the epidermal and dermal thicknesses were swollen with the DNCB-challenge, but Ole treatment significantly attenuated the swelling (**Figure 1E**). Ear epidermal thicknesses were 24.48 ± 2.11 μm in the DNCB group, 19.47 ± 3.12 μm in the Ole-5 group ($p < 0.05$), and 15.94 ± 1.15 μm in the Ole-10 group ($p < 0.01$; **Figure 1G**). Ear dermal thicknesses were 72.34 ± 1.98 μm in the DNCB group, 52.26 ± 2.24 μm in the Ole-5 group $p < 0.05$), and 41.60 ± 1.18 μm in the Ole-10 group ($p < 0.01$, **Figure 1H**).

DNCB-sensitization significantly induced inflammation, swelling, and cracking in the dorsal skin of challenged mice. However, Ole effectively improved these dorsal skin symptoms (**Figure 1I**). H&E tissue-section stains showed that, on day 30, the dorsal skin epidermal and dermal thicknesses were significantly swollen with the DNCB-challenge (**Figure 1J**), but Ole significantly attenuated the swelling. The mean dorsal skin epidermis thicknesses were 67.17 ± 2.61 μm in the DNCB group, 32.59 ± 2.09 μm in the Ole-5 group ($p < 0.01$), and 22.49 ± 2.15 μm in the Ole-10 group ($p < 0.01$, **Figure 1K**). The mean dorsal skin dermal thicknesses were 145.61 ± 2.11 μm in the DNCB group, 127.31 ± 2.23 μm in the Ole-5 group ($p < 0.05$), and 91.40 ± 1.13 μm in the Ole-10 group ($p < 0.01$, **Figure 1L**).

Oleuropein suppressed mast-cell and eosinophil infiltration into skin lesions, and modulated serum cytokines in DNCB-induced AD-like mice

Toluidine blue and H&E stains showed that topical Ole administrations inhibited the infiltrations of mast cells (**Figure 2A, B**) and eosinophils (**Figure 2E, F**) into the ear and skin lesions. The numbers of mast cells/HPF in ear tissues were 30.10 ± 6.1 in the DNCB-challenged group, 15.5 ± 3.7 in the Ole-5 group ($p < 0.05$), and 6.2 ± 1.8 in the Ole-10 group ($p < 0.01$, **Figure 2C**). The numbers of mast cells/HPF in skin tissues were 56.8 ± 2.2 in the DNCB-challenged group, 41.6 ± 1.7 in the Ole-5 group ($p < 0.05$), and 44.2 ± 1.6 in the Ole-10 group ($p < 0.05$, **Figure 2D**). The numbers of eosinophils/HPF in ear tissues were 230.4 ± 39.3 in the DNCB-challenged group, 81.3 ± 8.3 in the Ole-5 group ($p < 0.01$), and 72.5 ± 11.2 in the Ole-10 group ($p < 0.01$, **Figure 2G**). The numbers of eosinophils/HPF in skin tissues were 18,375.5 ± 225.8 in the DNCB-challenged group, 5050.5 ± 17.1 in the Ole-5 group ($p < 0.01$), and 2972.3 ± 10.4 in the Ole-10 group ($p < 0.01$, **Figure 2H**).

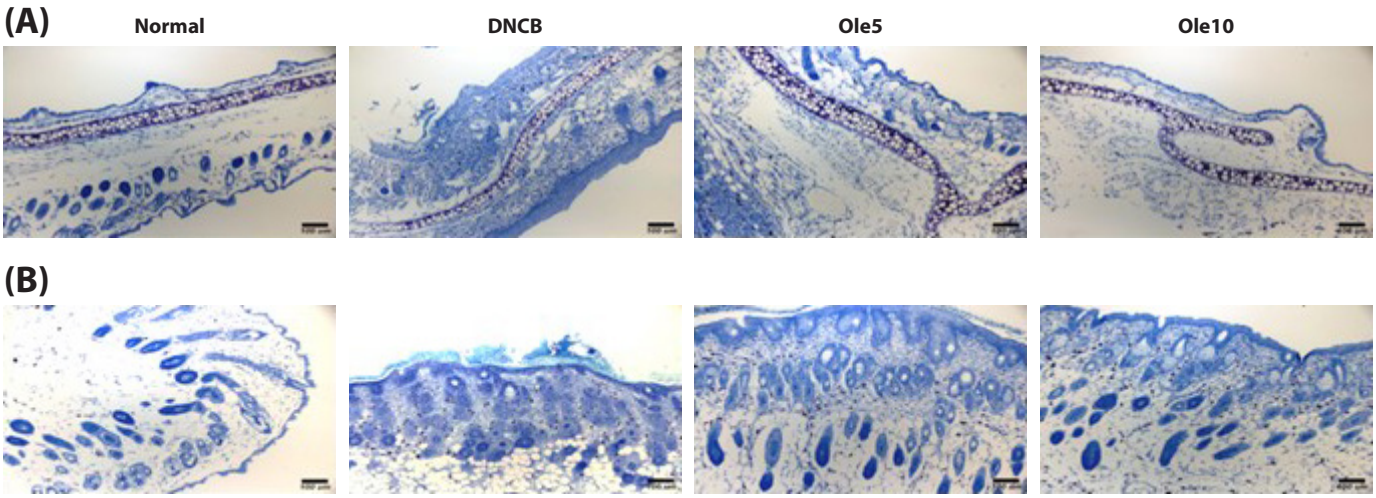

Figure 2. Oleuropein suppressed mast-cell and eosinophil infiltration into skin lesions, and modulated serum cytokines in DNCB-induced AD-like mice. Ole suppressed mast-cell infiltration into skin lesions in DNCB-induced AD-like mice. Light microscopic images show (A) ear and (B) skin tissues stained with toluidine blue to visualize mast cells; (C) the numbers of mast cells/high-power field (HPF) were evaluated in ear tissue sections and (D) skin tissue sections. Ole inhibited eosinophil infiltration into skin lesions in DNCB-induced AD-like mice. (E) light microscopic images show ear and (F) skin tissues stained with H&E to visualize eosinophils; (G) the numbers of eosinophils/high-power field (HPF) were evaluated in ear and (H) skin tissue sections. Ole modulated serum cytokines in DNCB-induced AD-like mice. (I) erum IgE levels; (J) serum IgG1 levels and (K) serum IgG2a levels. Normal (unsensitized and untreated); DNCB-sensitized mouse; Ole 5 (DNCB-sensitized mouse topically treated with 5 mg/kg Ole); Ole 10 (DNCB-sensitized mouse topically treated with 10 mg/kg Ole) measured in each mouse group (n = 6/group). Data are presented as the mean ± SEM (n = 6 mice/group). $^{\#\#}P < 0.001$ versus normal mice, and $^{**}P < 0.01$, $^{*}P < 0.05$ versus DNCB-sensitized mice.

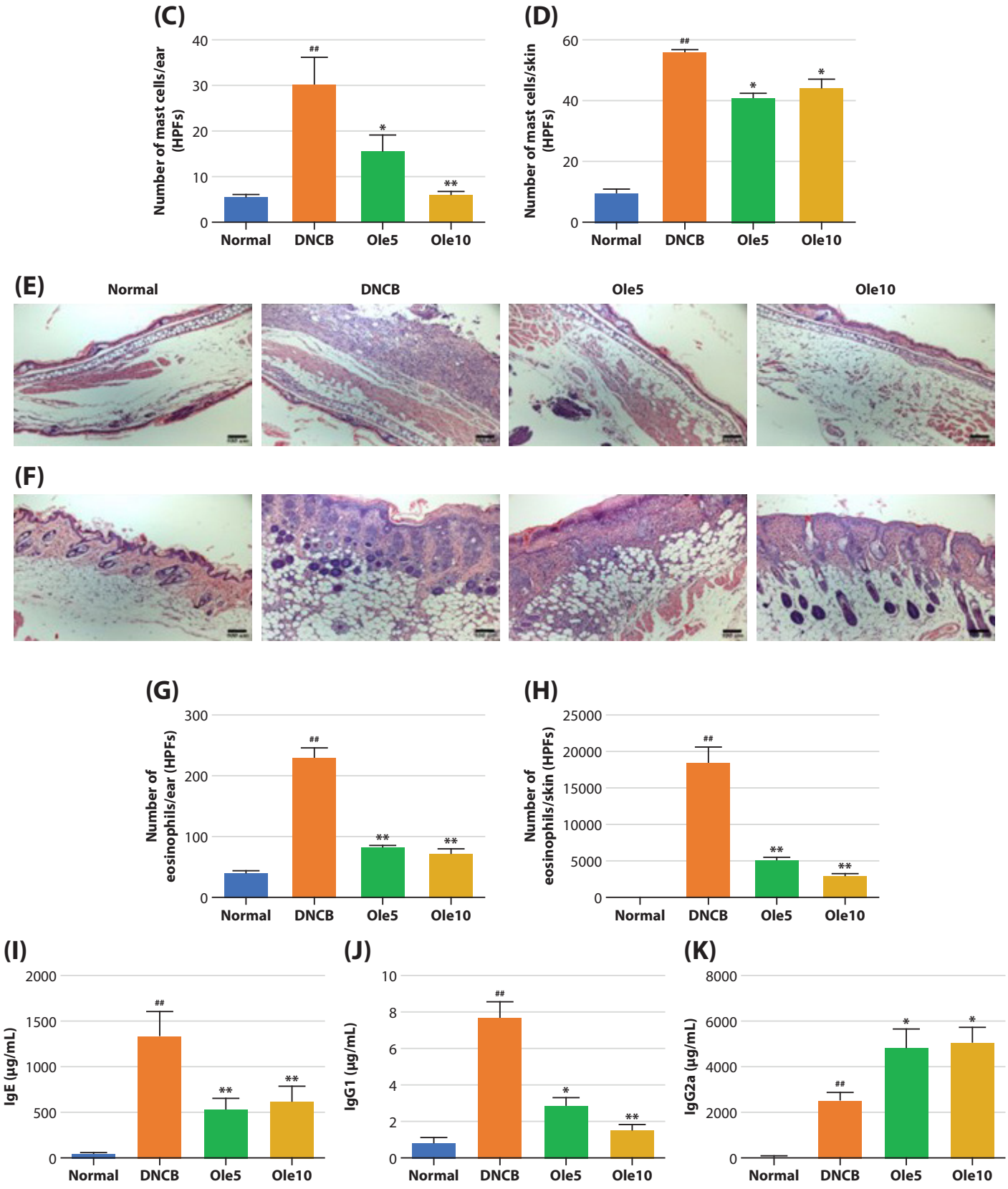

Figure 2. (Continued)

We measured serum IgE antibody levels to explore whether Ole could modulate the allergic response in serum. Our results showed that IgE and IgG1 levels were elevated in mice challenged with DNCB, unlike in the control group ($p < 0.001$). The topical Ole administration suppressed serum IgE and IgG1 levels compared to the levels observed with DNCB treatment alone ($p < 0.05$, **Figure 2I, J**). However, Ole treatment significantly increased serum IgG2a levels compared to those observed with DNCB treatment alone (**Figure 2K**). These findings suggested that, in addition to suppressing mast cell and eosinophil infiltrations, Ole treatment could modulate the immune responses.

Oleuropein reduced ear and skin COX-2, Th2-related cytokines, and ICMA-1 expression in DNCB-induced AD-like mice

Previous studies indicated that a COX-2 inhibitor increased eosinophil infiltration and enhanced antigen-specific IgE and IgG1 antibody responses, which promoted systemic and cutaneous Th2 responses in ovalbumin-sensitized mice.[9,25] We explored whether Ole might inhibit COX-2 expression and thus, regulate Th2-related cytokine levels. We performed histopathology and PCR analyses and found that topical Ole administration significantly attenuated COX-2 expression in the ear and dorsal skin compared to DNCB treatment alone (**Figure 3A-F**). Importantly, PCR analyses of ear and dorsal skin samples showed that topical Ole administration significantly inhibited the expression of genes that encoded Th2-related cytokines (IL-4 and IL-5) and inflammatory cytokines, IL-6 and ICAM-1, compared to DNCB treatment alone in ear (**Figure 3G-J**) and in the skin (**Figure 3K-N**). Hence, Ole improved DNCB-induced AD-like lesions by inhibiting inflammatory responses and regulating Th2-related cytokine gene expression.

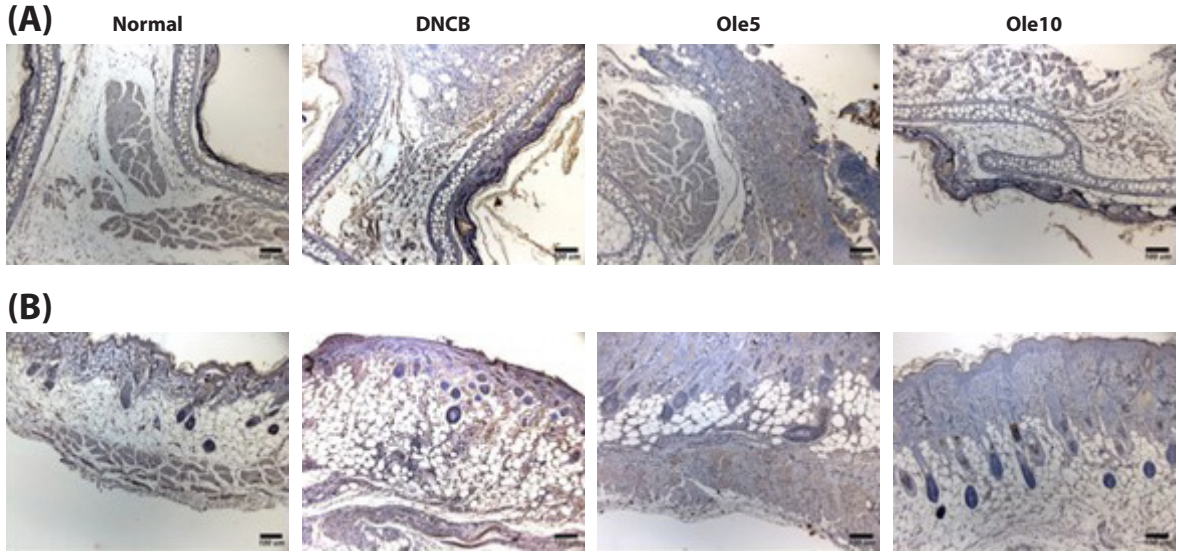

Figure 3. Oleuropein reduced ear and skin COX-2, Th2-related cytokines, and ICMA-1 expression in DNCB-induced AD-like mice. (A) light microscopic images show ear and (B) skin tissues stained with antibodies to visualize COX-2; (C) the number of COX-2-positive cells/high-power field (HPF) were evaluated in ear and (D) skin; (E) COX-2 gene expression measured with PCR in RNA extracted from ear and (F) skin. PCR analysis results show Ole suppressed (G) IL-4; (H) IL-5; (I) IL-6 and (J) ICAM-1 gene expression in ear tissues. PCR analysis results show Ole suppressed dorsal skin (K) IL-4; (L) IL-5; (N) IL-6 and (N) ICAM-1 gene expression. Normal (unsensitized and untreated); DNCB-sensitized mouse; Ole 5 (DNCB-sensitized mouse topically treated with 5 mg/kg Ole); Ole 10 (DNCB-sensitized mouse topically treated with 10 mg/kg Ole) measured in each mouse group (n = 6/group). Data are presented as the mean ± SEM (n = 6 mice/group). $^{\#\#}P < 0.001$ versus normal mice, and $^{**}P < 0.01$, $^{*}P < 0.05$ versus DNCB-sensitized mice.

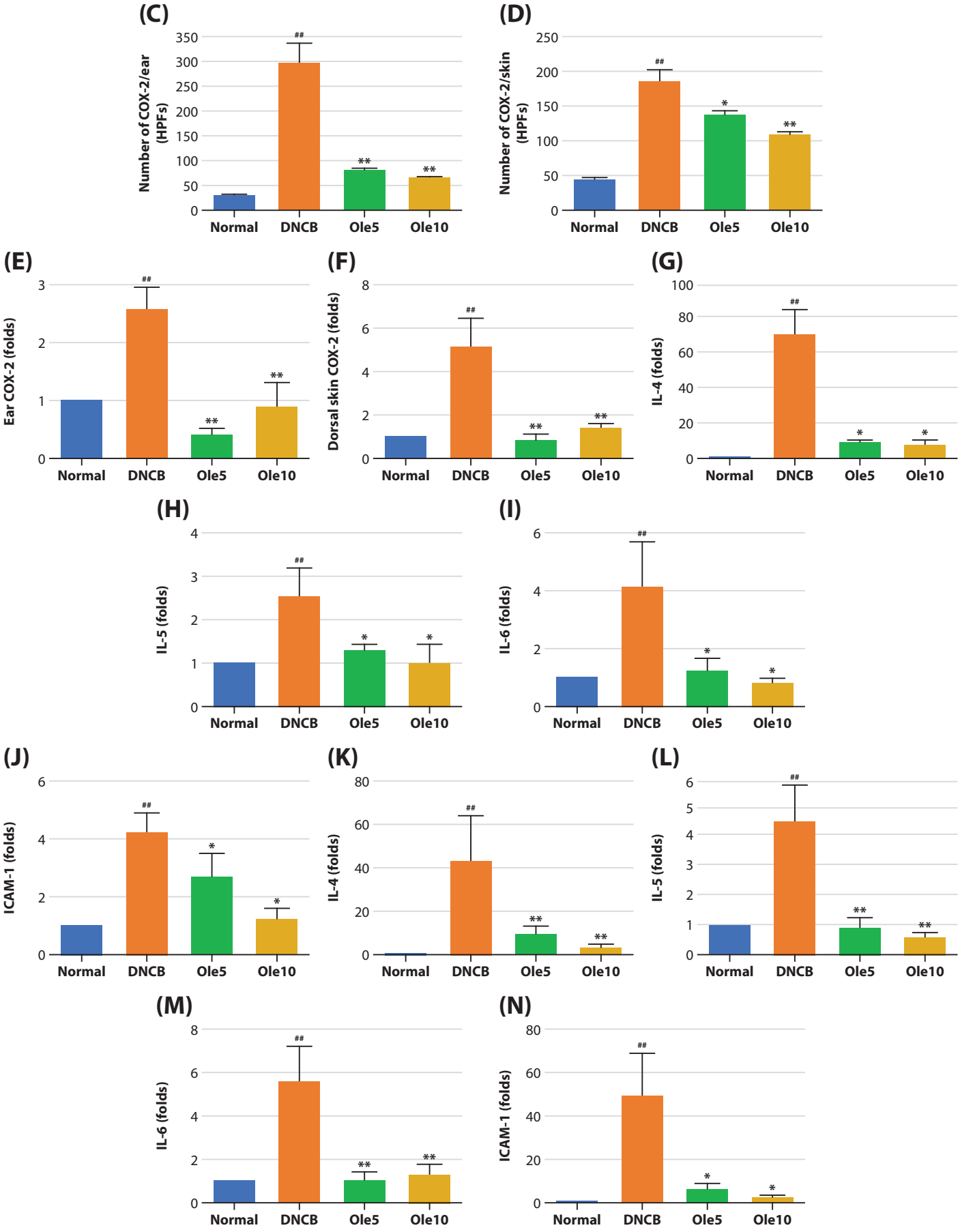

Figure 3. (Continued)

Discussion

AD is a chronic inflammatory skin disease characterized by an impaired immune response and skin barrier dysfunction. DNCB covalently interacts with proteins to form an immunogenic compound, activating innate immune cells, including dendritic cells. DNCB initiates an antigen-specific immune response during the initial phase of sensitization.[24] AD induces systemic and partial type 2 inflammation, leading to increases in IgE production and the release of Th2-related cytokines. Thus, patients with AD have elevated IL-4 and IL-5 compared to healthy individuals.[25] In the present study, we observed that a DNCB challenge caused epidermal and dermal thickening, enhanced serum IgE levels, and promoted mast-cell and eosinophil infiltration into AD-like lesions in a mouse model. In the past decade, studies have shown that AD is an allergic disease characterized by impaired immune responses. Therefore, we explored the mechanisms of Ole treatment in AD-like mice. When applied during a DNCB challenge, Ole profoundly mitigated skin inflammation and regulated Th2-related cytokine expression.

In AD pathogenesis, type-2 cytokines, IL-4 and IL-5, contribute to inflammation and epidermal barrier impairments.[26] Previous studies found that IL-4 down-regulated filaggrin expression in keratinocytes exacerbated epidermal barrier dysfunction.[27] Moreover, studies showed that overexpressing epidermal IL-4 in transgenic mice induced epidermal thickening, lesion formation, and elevated IgE levels. IL-4 enhanced IgE-mediated mast-cell responses and IL-4 was necessary for allergy development.[28] Indeed, IL-4 is critical for directing Th2-cell differentiation and B-cell class-switching to produce allergenic IgE antibodies, essential in allergy pathogenesis.[29] Additionally, more IgE is produced with continuous allergen stimulation, and tissues become hypersensitive.[30] Our results indicated that Ole treatment reduced serum levels of IgE and reduced IL-4 expression in ear and skin lesions (**Figures 2I, 3G and 3K**).

IgE mediates mast-cell activation in allergic reactions, and B cells bind with IgM to the native protein antigen and active allergic-inflammatory signals in the allergen area. Then, B cells are triggered by local IL-4 to switch to IgE- and IgG1-producing cells.[28-30] In the present study, we found that Ole reduced serum IgE and IgG1 levels and attenuated IgE-mediated mast-cell infiltration into AD-skin lesions (**Figures 2I, 2J and 2A-D**).

Mast cells release a large variety of vasoactive substances, which induce the influx of fluids into the tissue from the blood, resulting in tissue swelling.[31] We found that Ole treatment improved ear swelling (**Figure 1D, F**). In addition, activated mammalian mast cells release proteases (e.g., fibronectin), which actively cleave components and contribute to tissue remodeling. Mast cells also release matrix metalloproteases, which degrade collagen in connective tissues.[30,31] Our results showed that Ole improved epidermal and dermal thicknesses in AD-skin lesions (**Figure 1D-L**).

Activated mast cells also lead to the recruitment of eosinophils, a process partly dependent on locally produced IL-5, a Th2 cytokine. IL-5 is related to eosinophil differentiation, activation, and proliferation. In the skin lesions of patients with AD, elevated IL-5 levels were correlated with increased serum IgE levels.[31] Studies have indicated that eosinophils contributed to dermal tissue damage in allergic skin diseases.[32] These findings suggested that IL-5 plays a central role in eosinophilic infiltration and inflammation. Healthy individuals have low levels of circulating eosinophils generated from bone marrow. However, the numbers of blood and tissue eosinophils are high densities, the recruitment of eosinophils is thought antigen-presenting cells, mast cells, T cells, B cells, and their released cytokines as immune signals in inflammatory and allergic responses. Tissue eosinophil levels tend to increase markedly during allergic inflammation.

Furthermore, eosinophil-derived cytokines and chemokines contribute to the propagation of immune responses. Eosinophil recruitment depends on the Th2-related cytokines, IL-4 and IL-5, the inflammatory cytokine, IL-6, and intercellular adhesion molecule 1 (ICAM-1). It was previously demonstrated that these cytokines were expressed at sites of allergic inflammation.[33] IL-6 is a crucial cytokine in host defense, and it can function as a pro-inflammatory cytokine. A previous study showed that serum IL-6 levels were significantly elevated in patients with AD compared to healthy individuals.[34] Those findings indicated that serum IL-6 levels might be a suitable target for controlling AD.

Moreover, ICAM-1 mediates leukocyte attachment to endothelial cells, controlling their retention and migration through the skin. Studies have shown that ICAM-1 expression is up-regulated in the skin of patients with AD.[35] In the present study, we found that Ole significantly reduced IL-5 expression (**Figure 3H, 3L**) and suppressed eosinophil infiltration into skin lesions (**Figure 2E-H**). Furthermore, Ole significantly suppressed IL-6 and ICAM-1 expression in the ear (**Figure 3I, J**) and skin (**Figure 3M, N**). Ole inhibited COX-2 expression (**Figure 3e and 3f**) in-ear and skin lesions. These data indicated that Ole suppressed inflammatory cytokine production in DNCB-induced AD-like allergic inflammation.

Experimental studies have shown that phenolic compounds have antioxidant and anti-inflammatory properties that can attenuate DNCB-induced allergic contact dermatitis and AD in mice.[36] Evidence has shown that AD is an IgE-mediated disease, and its pathogenesis is associated with T-cell activation, particularly Th2 polarization in the acute phase. In the late phase, Th2 cells recruit eosinophils, mast cells, and dendritic cells, contributing to skin-lesion thickening and elevated collagen accumulation in patients with AD.[37] In particular, Th2 cytokine (IL-4 and IL-5) levels were positively correlated with the AD scoring index, known as SCORAD, which evaluates extrinsic and intrinsic AD disease severity.[38]

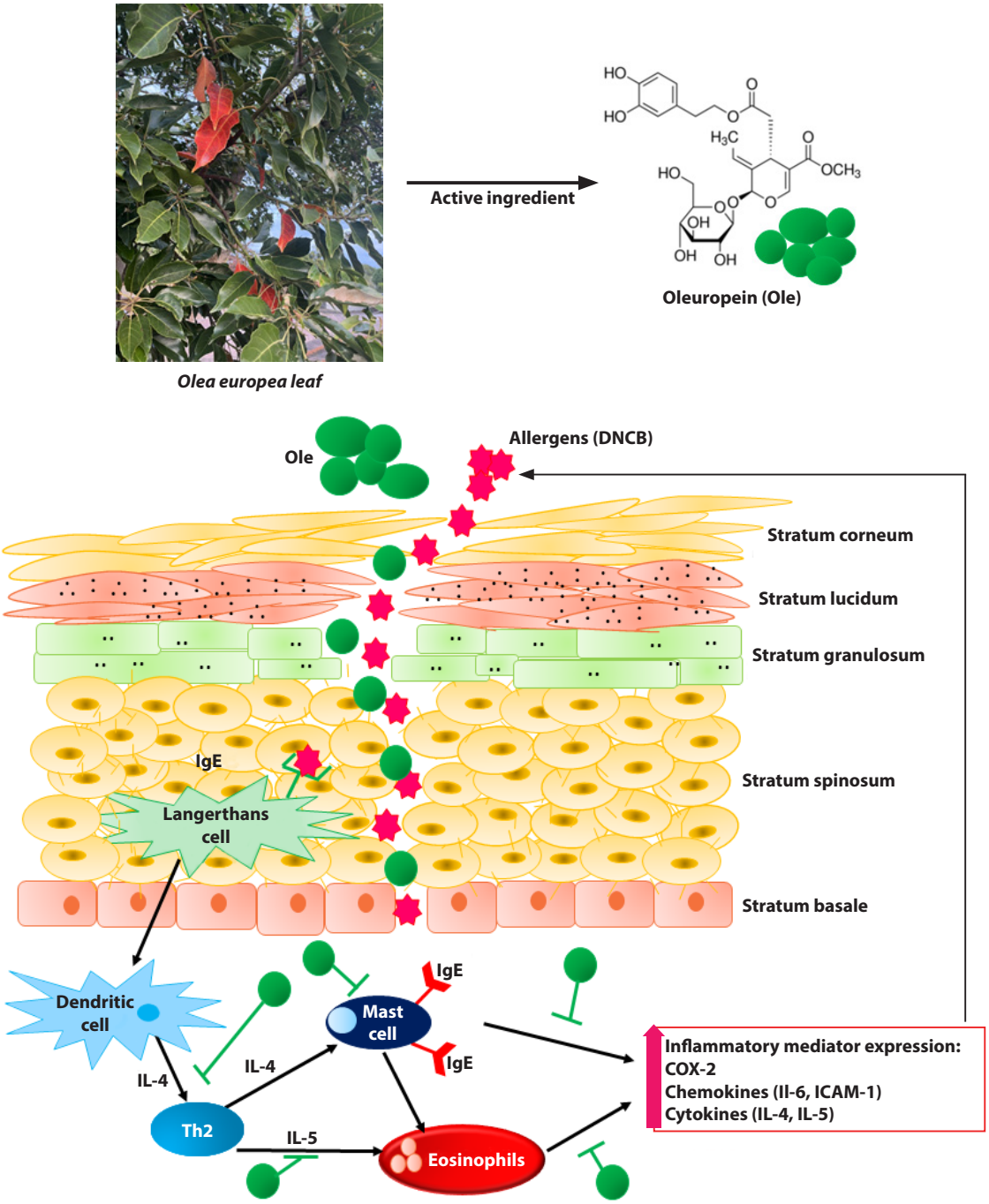

Figure 4. Ole might as an effective phenolic treatment for allergic inflammation via modulating Th2-related axis and inflammatory responses.

Conclusions

This study suggested that Ole could suppress the Th2-related inflammatory axis and alleviate allergic inflammation in DNCB-induced AD-like mice. Our results demonstrated that Ole reduced IL-4 and IL-5 levels to alleviate mast cells affinity with IgE and eosinophils infiltrated into skin lesions. Hence, Ole mediated the Th2-related axis to suppress inflammatory mediators COX-2, IL-6, and ICAM-1 expression (described in **Figure 4**). These findings suggested that topical Ole might be an effective phenolic treatment for allergic inflammation.

Acknowledgements

This study was supported, in part, by grants from the Chang Gung Memorial Hospital (CMRPF1K0082, CMRPF1L 0011 and CMRPF1M0031), Ministry of Science and Technology (109-2320-B-255-006-MY3), and Chang Gung University of Science and Technology (ZRRPF3L0091) of Taiwan.

Conflict of interest statement

The authors declare no conflict of interest.

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