

Inhibitory effect of oroxylin A on atopic dermatitis-like symptoms in mice

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Research Article

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Abstract

Scutellaria baicalensis has long been used in Asian traditional medicine to prevent and treat suppurative dermatitis, allergic diseases, inflammation, hyperlipemia, and arteriosclerosis. Oroxylin A is a flavone present in *Scutellaria baicalensis*. Because the root extracts of *Scutellaria baicalensis* have been shown to have anti-dermatitis effects, the authors investigated the effects of oroxylin A on chemically-induced atopic dermatitis (AD) in an *in vivo* AD model induced by 1-chloro-2,4-dinitrobenzene (CDNB) in BALB/c mice. CDNB-induced skin hypertrophy and accumulation of mast cells in the epidermis and dermis were significantly ameliorated by oroxylin A. Increased serum levels of immunoglobulin E, as well as pro-inflammatory chemokines and cytokines in the skin and lymph nodes were significantly ameliorated by oroxylin A. Suppression of immune responses in the skin and lymph nodes by oroxylin A decreased the symptoms of AD. Thus, these results proved that oroxylin A is an effective component of *Scutellaria baicalensis* for treating the symptoms of AD.

1. Introduction

Many flavonoids have been isolated from medicinal plants and studied for activities. Studies on structure-activity relationship have elucidated that a double bond at C2–3 and a ketone group at C4 of the C-ring of flavonoids are essential for their immunosuppressive activity along with a benzene ring at positions 2 or 3 of the C-ring [1]. Furthermore, two or three hydroxylations of the B-ring were found to exert more potent effects [1]. Oroxylin A, 5,7-dihydroxy-6-methoxy-2-phenyl-4H-1-benzopyran-4-one (also known as 5,7-dihydroxy-6-methoxyflavone), was first extracted from the root bark of *Oroxylum indicum* [2] and is one of the main active flavonoids in the dry root of *Scutellaria baicalensis* Georgi [3]. Oroxylin A reportedly possesses a broad spectrum of pharmacological effects, including anti-cancer, anti-coagulation, anti-inflammation, and neuroprotective effects *in vitro* and *in vivo* [4]. The anti-cancer activity of oroxylin A may result from the induction of apoptosis, inhibition of metastasis and invasion, and suppression of angiogenesis [4]. Oroxylin A is expected to have better immunosuppressive potency than other flavonoids, because it possesses a 2, 3-double bond and two hydroxyl groups (5 and 7) [4]. *Scutellariae radix* has been used in Chinese medicine as a remedy for suppurative dermatitis, allergic diseases, inflammation, hyperlipemia, and arteriosclerosis [3]. The anti-inflammatory and anti-allergic functions of oroxylin A have been recently reported [5–9]. However, its efficacy in atopic dermatitis (AD) has not been studied.

AD is a chronic inflammatory disease of the skin, and its pathogenesis is related to immune dysfunction and skin barrier abnormalities. Elevated serum immunoglobulin E (IgE) levels in patients with AD and T cell-mediated immune responses in murine models of AD have been reported [10–13]. Because oroxylin A inhibited mast cells degranulation [8], an important event in AD, the present study investigated the efficacy of oroxylin A in AD using a murine AD model induced by 1-chloro-2,4-dinitrobenzene (CDNB) in BALB/c mice.

2. Materials And Methods

2.1 Materials

Oroxylin A was purchased from Cayman. Olive oil and CDNB were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Animals and treatment

We purchased BALB/c mice from Daehan Biolink (Seoul, Korea) and provided food and water *ad libitum* in the laboratory animal facility at Kyung Hee University. The facility was maintained under controlled conditions (humidity: $60 \pm 5\%$; temperature: $22-24^{\circ}\text{C}$; and alternating 12 hours light/dark cycles). We fed the mice with standard laboratory chow and water. The animal protocol used in this study was approved by the Institutional Animal Care Committee of Kyung Hee University with respect to procedural ethics and scientific research (KHSASP-21-296).

Seven-week old male BALB/c mice were divided into three groups ($n = 5$): phosphate-buffered saline (PBS)-treated control group, CDNB-treated mice group, and CDNB + oroxylin A (5 mg/kg)-treated group. Mice were sensitized by smearing 1% CDNB (300 μL , acetone/olive oil (3:1)) to the dorsal skin on day 0. The mice were challenged by applying 0.3% CDNB (200 μL) on the ears every other day from day 7 to day 48. From day 19, oroxylin A was administered via intraperitoneal injection 30 min before the CDNB challenge. The mice were sacrificed on day 49.

2.3 Histological examination

We prepared the ear skin of each mouse on day 49. We fixed the ears from different experimental groups with neutral-buffered formalin (10%), dehydrated in sucrose solution (30%), and embedded in OCT compound. The sections were stained with either toluidine blue O or hematoxylin and eosin (H&E). We counted the number of mast cells using the photographs of the toluidine blue O-stained samples.

2.4 Measurement of IgE, cytokines, and chemokines

We determined serum IgE levels by using an ELISA kit (eBioscience, San Diego, CA, USA). We also measured the cytokine levels of Th2 (IL-4 and IL-13), Th1 (IFN- γ)

and chemokine levels of CCL17 and CCL22 in the lymph nodes and ears by quantitative Real-Time PCR. Quantitative PCR was performed using Thunderbird Next SYBR qPCR Mix (Toyobo, Osaka, Japan) and CFX Connect Real-Time system (Bio-Rad, Hercules, CA, USA). Thermal-cycling conditions were as follows: one cycle at 95°C for 4 min, forty cycles at 95°C for 30 s and 57°C for 30 s, one cycle at 95°C for 30 s. The expression of individual genes was normalized to the levels of GAPDH.

2.5 Statistical analysis

The results are expressed as the mean $\pm$ standard error of the mean (SEM) of five measurements for the animal experiments. Analysis of variance (ANOVA) and Tukey's multiple comparison test was used to determine statistical significance of differences. Statistical significance was accepted for p values < 0.05 . Data were analyzed using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1 Oroxylin A ameliorated AD-like symptoms induced by CDNB in the ears

Based on the findings that *Scutellariae radix* has been used in Asian traditional medicine as a remedy for treating allergic diseases, dermatitis, and inflammation [3] and that oroxylin A has anti-inflammatory and anti-allergic functions [5–9], the effects of oroxylin A were investigated using an *in vivo* chemically induced AD model in mice (Fig. 1-A). The dorsal skin of BALB/c mice was sensitized using CDNB, and seven days later, both ears were challenged with CDNB every other day for 49 days (Fig. 1-A). Oroxylin A (5 mg/kg) was intraperitoneally injected 30 min prior to the CDNB challenge starting from day 19 to determine its therapeutic potential for AD-like symptoms (Fig. 1-A).

In a preliminary experiment using two doses of oroxylin A (1 and 5 mg/kg), we observed that 5 mg/kg of oroxylin A was effective. CDNB treatment of the ears elicited typical dermatitis-like symptoms, such as edema, dryness, erosion, cracking, and erythema, on day 49 in the skin of BALB/c mice. Administration of oroxylin A ameliorated the AD symptoms in the mice (Fig. 1-B).

The macroscopic effects of oroxylin A were further validated using hematoxylin and eosin (H&E) staining. CDNB treatment induced extensive hypertrophy (Fig. 2-A). The epidermis was thickened by CDNB treatment and suppressed by oroxylin A administration (Fig. 2-A). The ear thickness was quantitatively measured, as shown in Fig. 2-B. CDNB increased the thickness by 223%, whereas oroxylin A significantly suppressed it by 49%.

3.2 Oroxylin A decreased CDNB-induced mast cell accumulation in the skin lesions

Oroxylin A was previously shown to inhibit antigen-induced degranulation of RBL-2H3 mast cells [8]. Because mast cells play crucial roles in AD pathogenesis and symptoms, we assessed the mast cells in the skin by staining the tissue sections using toluidine blue O. Mast cells were increased by 557% in the skin tissues of CDNB-treated mice compared to that in the control mice (Fig. 3-A). Oroxylin A administration significantly suppressed the increase in the number of mast cells in the ears of the CDNB-treated group (Figs. 3-A and B).

3.3 Oroxylin A suppressed CDNB-induced increase of serum IgE levels

Because high serum IgE levels were detected in patients with AD, serum IgE levels were investigated as an immunologic response to CDNB. The serum IgE levels were significantly increased by 962% upon repeated topical challenges with CDNB (Fig. 4). In contrast, oroxylin A administration significantly suppressed serum IgE levels by 78% (Fig. 4).

3.4 Oroxylin A decreased CDNB-induced AD-like responses in the lymph nodes

Lymph nodes are small oval-shaped organs that contain immune cells and play a vital role in immunological responses. Because the cervical lymph nodes were the closest to the ears, their sizes were

measured. The lymph nodes were substantially enlarged in CDNB-treated mice compared to those in the control mice (Fig. 5-A and B). In contrast, oroxylin A administration significantly reduced the size of the lymph nodes in CDNB-treated mice by 50% (Fig. 5-A and B).

3.5 Oroxylin A suppressed CDNB-induced increases in cytokine levels in the lymph nodes

The Th2/Th1 cytokine levels in the lymph nodes were investigated. Repeated topical challenges with CDNB significantly increased the mRNA levels of Th2/Th1 cytokines in the cervical lymph nodes (Fig. 6). However, oroxylin A administration significantly suppressed the levels of interleukin (IL)-4, but not IL-13 or interferon (INF)- γ (Fig. 6). In summary, oroxylin A administration suppressed the size of the lymph nodes as well as the levels of the inflammatory cytokines. Therefore, the immunological responses and histological features of CDNB-induced AD in the ears and lymph nodes were significantly suppressed by oroxylin A treatment.

3.6 Oroxylin A decreased CDNB-induced increases in cytokine levels in BALB/c mice

The expression patterns of cytokines and chemokines are distinct depending on the pathogenesis of AD in human patients [14]. Th2 subsets are dominant in the early stage, whereas Th1 subsets are dominant in the late stage. On day 49, a mixed population of Th2 and Th1 cells were detected [14, 15]. Thus, we investigated Th2/Th1 cytokine levels, that is, Th2 (IL-4 and IL-13) and Th1 (INF- γ) cytokines in the ears. The mRNA expression of the Th2 cytokines (IL-4 and IL-13) were significantly increased by 52653% and 840%, respectively, in the skin lesions of CDNB-treated mice compared to those in the control mice (Figs. 7-A and B). Oroxylin A administration significantly suppressed the levels of IL-4 and IL-13 by 96% and 72%, respectively, in the ear skin of CDNB-treated BALB/c mice (Figs. 7-A and B). Furthermore, the IFN- γ levels were significantly increased in the skin lesions of CDNB-treated mice by 365% compared to those in the control mice (Figs. 7-C). Oroxylin A administration did not suppress IFN- γ levels in the ear skin of CDNB-treated mice (Fig. 7-C).

The levels of CCL17 and CCL22 in the skin were elevated in patients with AD, and these levels were positively correlated with the severity of AD symptoms [16, 17]. In agreement with previous studies, CDNB treatment increased the mRNA expression levels of CCL17 and CCL22 in the ears by 281% and 904%, respectively. In contrast, oroxylin A treatment suppressed these expression levels in mice (Fig. 7-D-E). Oroxylin A-induced suppression of both chemokines may contribute to ameliorating AD-like symptoms in the skin because chemokines attract Th2 cells into the skin lesions [18].

Discussion

Oroxylin A had not been previously applied to inflammatory and allergic skin diseases, although the nanostructured lipid carrier gel of oroxylin A showed antioxidant properties against UV-induced oxidative damage [19]. Because the root extracts of *Scutellaria baicalensis* showed anti-dermatitis effects and oroxylin A is a *O*-methylated flavone in *Scutellaria baicalensis* [3], we studied the effect of oroxylin A on

chemically induced AD symptoms in a murine model. In the present study, we observed that oroxylin A suppressed CDNB-induced symptoms of AD through immunosuppressive actions.

Among the active components of *Scutellaria baicalensis*, baicalein, baicalin, wogonin, and chrysin have previously been reported to have suppressive effects on inflammatory skin disorders. Baicalein, 5,6,7-trihydroxyflavone, showed therapeutic efficacy against dust mite (*Dermatophagoides pteronyssinus*)-induced AD-like skin lesions in NC/Nga mice [20]. Baicalin, baicalein 7-*O*-glucuronide, suppressed CDNB-induced contact hypersensitivity and imiquimod-induced psoriasis in BALB/c mice [21, 22]. In addition, baicalin ameliorated CDNB-induced AD-like skin lesions in mice by suppressing skin inflammation via modulation of the nuclear factor-kappa B (NF- κ B) and Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways [23]. Wogonin, 5,7-dihydroxy-8-methoxyflavone, also inhibited proinflammatory gene expression in contact dermatitis as well as the edematous response caused by topical application in mice [24–26]. Chrysin ameliorated imiquimod-induced psoriasis-like skin inflammation by reducing CCL20 release [27]. Therefore, combined with the present experimental results of oroxylin A, the five main constituents of *Scutellaria baicalensis* have been confirmed to have anti-dermatitis and anti-inflammatory effects through immunosuppression.

Oroxylin A inhibited antigen-induced degranulation in RBL-2H3 mast cells, as well as ovalbumin (OVA)-induced allergic asthma in animal models [8, 9]. Oroxylin A inhibited the inductions of both Th2 and Th1 cytokines in an OVA-induced mouse model of asthma [8] and reduced airway hypersensitivity, probably via NF- κ B inhibition [9]. Furthermore, oroxylin A showed anti-histaminic effects in the guinea pig ileum and inhibited histamine-induced scratching behavior in mice [28]. In THP-1 cells, oroxylin A inhibited lipopolysaccharide (LPS)-stimulated inflammatory IL-6 production by activating the nuclear respiratory factor 1 (Nrf1) signaling pathway [7]. Moreover, oroxylin A, concentration-dependently, suppressed LPS-induced expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) by inhibiting NF- κ B activation in RAW264.7 macrophages [6]. Combining the previously reported anti-allergic and anti-inflammatory effects of oroxylin A with the present observations of immunosuppression, such as reduced lymph node size and inflammatory cytokine levels, strongly suggests that oroxylin A suppression of AD symptoms resulted from its immunosuppressive actions on inflammatory cells and mast cell degranulation.

Declarations

Author Declarations:

Ethics approval and consent to participate: not applicable

Consent for publication:

Availability of data and materials: not applicable

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Statement of contribution: YJ Lee and DS Im designed the experiments. YJ Lee performed the experiments and analyzed the data. YJ Lee and DS Im wrote the manuscript.

Conflict of interest: The authors declare that there is no conflict of interest.

References

1. Kim C-J, Cho SK. 1991. Pharmacological activities of flavonoids (III) structure-activity relationships of flavonoids in immunosuppression. *Archives of Pharmacal Research* 14:147–159.
2. Shah R, Mehta C, Wheeler T. 1936. 131. The constitution of oroxylin-A, a yellow colouring matter from the root-bark of *Oroxylum indicum*, vent. *Journal of the Chemical Society (Resumed)*:591–593.
3. Li H-B, Jiang Y, Chen F. 2004. Separation methods used for *Scutellaria baicalensis* active components. *Journal of chromatography B* 812:277–290.
4. Lu L, Guo Q, Zhao L. 2016. Overview of oroxylin A: a promising flavonoid compound. *Phytotherapy Research* 30:1765–1774.
5. Yang X, Zhang F, Wang Y, Cai M, Wang Q, Guo Q, et al. 2013. Oroxylin A inhibits colitis-associated carcinogenesis through modulating the IL-6/STAT3 signaling pathway. *Inflammatory bowel diseases* 19:1990–2000.
6. Chen Y-C, Yang L-L, Lee TJ. 2000. Oroxylin A inhibition of lipopolysaccharide-induced iNOS and COX-2 gene expression via suppression of nuclear factor- κ B activation. *Biochemical pharmacology* 59:1445–1457.
7. Ye M, Wang Q, Zhang W, Li Z, Wang Y, Hu R. 2014. Oroxylin A exerts anti-inflammatory activity on lipopolysaccharide-induced mouse macrophage via Nrf2/ARE activation. *Biochemistry and Cell Biology* 92:337–348.
8. Lee A-Y, Kang S, Park S-J, Huang J, Im D-S. 2016. Anti-Allergic Effect of Oroxylin A from *Oroxylum indicum* Using in vivo and in vitro Experiments. *Biomolecules & Therapeutics* 24:283.
9. Zhou D-G, Diao B-Z, Zhou W, Feng J-L. 2016. Oroxylin A inhibits allergic airway inflammation in ovalbumin (OVA)-induced asthma murine model. *Inflammation* 39:867–872.
10. Kang J, Im DS. 2020. FFA2 Activation Ameliorates 2,4-Dinitrochlorobenzene-Induced Atopic Dermatitis in Mice. *Biomol Ther (Seoul)* 28:267–271.
11. Kang J, Lee JH, Im DS. 2020. Topical Application of S1P(2) Antagonist JTE-013 Attenuates 2,4-Dinitrochlorobenzene-Induced Atopic Dermatitis in Mice. *Biomol Ther (Seoul)* 28:537–541.
12. Park SJ, Im DS. 2020. Blockage of sphingosine-1-phosphate receptor 2 attenuates 2,4-dinitrochlorobenzene-induced atopic dermatitis in mice. *Acta Pharmacol Sin* 41:1487–1496.
13. Son SE, Park SJ, Koh JM, Im DS. 2020. Free fatty acid receptor 4 (FFA4) activation ameliorates 2,4-dinitrochlorobenzene-induced atopic dermatitis by increasing regulatory T cells in mice. *Acta*

14. Su C, Yang T, Wu Z, Zhong J, Huang Y, Huang T, et al. 2017. Differentiation of T-helper cells in distinct phases of atopic dermatitis involves Th1/Th2 and Th17/Treg. *European Journal of Inflammation* 15:46–52.
15. Lee J-H, Im D-S. 2022. Honokiol suppresses 2, 6-dinitrochlorobenzene-induced atopic dermatitis in mice. *Journal of Ethnopharmacology*:115023.
16. Kezic S, McAleer MA, Jakasa I, Goorden SM, Ghauharali-van der Vlugt K, Beers-Stet FS, et al. 2022. Children with atopic dermatitis show increased activity of β -glucocerebrosidase and stratum corneum levels of glucosylcholesterol that are strongly related to local cytokine milieu. *British Journal of Dermatology*.
17. Montero-Vilchez T, Cuenca-Barrales C, Rodriguez-Pozo J-A, Diaz-Calvillo P, Tercedor-Sanchez J, Martinez-Lopez A, et al. 2022. Epidermal Barrier Function and Skin Homeostasis in Atopic Dermatitis: The Impact of Age. *Life* 12:132.
18. Salimian J, Salehi Z, Ahmadi A, Emamvirdizadeh A, Davoudi SM, Karimi M, et al. 2022. Atopic dermatitis: molecular, cellular, and clinical aspects. *Molecular Biology Reports*:1–16.
19. Zhao Z, Qin W, Liu T, Yang Y, Wang Z, Ma H, et al. 2022. The Nanostructured lipid carrier gel of Oroxylin A reduced UV-induced skin oxidative stress damage. *Colloids and Surfaces B: Biointerfaces*:112578.
20. Yun M-Y, Yang J-H, Kim D-K, Cheong K-J, Song H-H, Kim D-H, et al. 2010. Therapeutic effects of Baicalein on atopic dermatitis-like skin lesions of NC/Nga mice induced by dermatophagoides pteronyssinus. *International Immunopharmacology* 10:1142–1148.
21. Hung C-H, Wang C-N, Cheng H-H, Liao J-W, Chen Y-T, Chao Y-W, et al. 2018. Baicalin ameliorates imiquimod-induced psoriasis-like inflammation in mice. *Planta medica* 84:1110–1117.
22. Wu J, Li H, Li M. 2015. Effects of baicalin cream in two mouse models: 2, 4-dinitrofluorobenzene-induced contact hypersensitivity and mouse tail test for psoriasis. *International journal of clinical and experimental medicine* 8:2128.
23. Wang L, Xian Y-F, Loo SKF, Ip SP, Yang W, Chan WY, et al. 2022. Baicalin ameliorates 2, 4-dinitrochlorobenzene-induced atopic dermatitis-like skin lesions in mice through modulating skin barrier function, gut microbiota and JAK/STAT pathway. *Bioorganic Chemistry* 119:105538.
24. Lim H, Park H, Kim HP. 2004. Inhibition of contact dermatitis in animal models and suppression of proinflammatory gene expression by topically applied flavonoid, wogonin. *Archives of Pharmacal Research* 27:442–448.
25. Park BK, Heo MY, Park H, Kim HP. 2001. Inhibition of TPA-induced cyclooxygenase-2 expression and skin inflammation in mice by wogonin, a plant flavone from *Scutellaria radix*. *European Journal of Pharmacology* 425:153–157.
26. Chi YS, Lim H, Park H, Kim HP. 2003. Effects of wogonin, a plant flavone from *Scutellaria radix*, on skin inflammation: in vivo regulation of inflammation-associated gene expression. *Biochemical pharmacology* 66:1271–1278.

27. Li H-J, Wu N-L, Pu C-M, Hsiao C-Y, Chang D-C, Hung C-F. 2020. Chrysin alleviates imiquimod-induced psoriasis-like skin inflammation and reduces the release of CCL20 and antimicrobial peptides. *Scientific Reports* 10:1–13.

28. Trinh H-t, Joh E-h, Kwak H-y, Baek N-i, Kim D-h. 2010. Anti-pruritic effect of baicalin and its metabolites, baicalein and oroxylin A, in mice. *Acta Pharmacologica Sinica* 31:718–724.

Figures

Fig 1

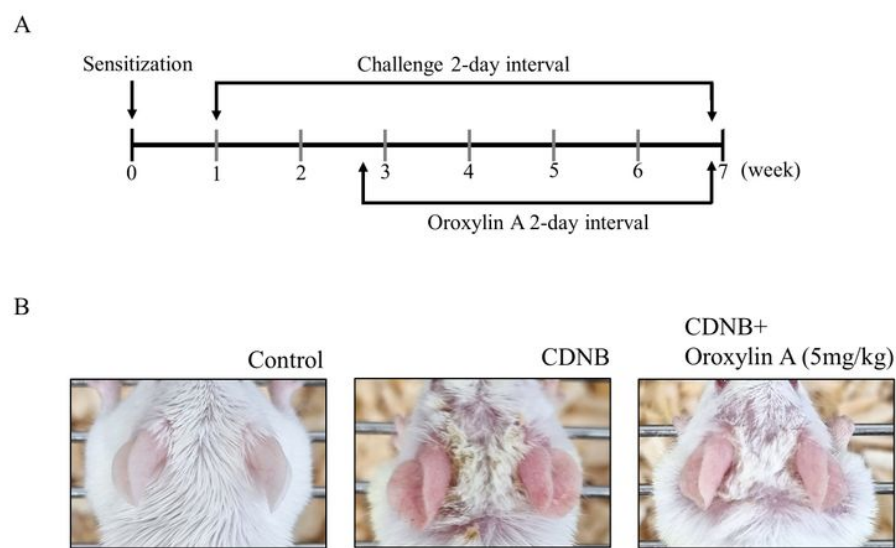


Figure 1

AD induction protocol and effect of oroxylin A on CDNB-induced AD-like symptoms in ears (A)

Experimental timeline. Cutaneous sensitization with CDNB was performed on day 0. Repeated CDNB challenge was followed on days 7 - 48 to induce AD-like phenotypes. BALB/c mice were treated topically with a vehicle or CDNB, whereas oroxylin A was administered by intraperitoneal injection 30 min before the CDNB challenge. (B) Cutaneous manifestations on the ears. CDNB, 1-chloro-2,4-dinitrobenzene.

Fig 2

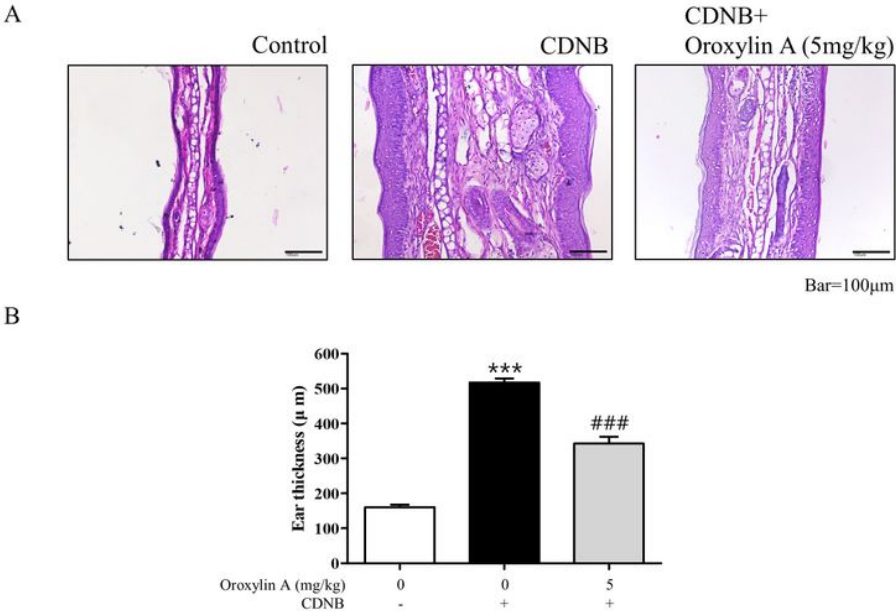


Figure 2

Effect of oroxylin A on cutaneous histopathologic observations

(A) Representative histologic findings of cutaneous tissue sections on day 49. Ear tissue sections were stained with H&E. (B) Ear thickness was quantified and compared among the groups. H&E; hematoxylin and eosin. Data represent the mean ± SEM (n=5). ****p* < 0.001 vs. the control group, ###*p* < 0.001 vs. the CDNB-treated group.

A

Control CDNB CDNB+ Oroxylin A (5mg/kg)

Bar=100μm

B

Group	Oroxylin A (mg/kg)	CDNB	Number of mast cells (approx.)	Significance
Control	0	-	18	
CDNB	0	+	105	***
CDNB+ Oroxylin A	5	+	60	###

Effect of oroxylin A on cutaneous mast cell accumulation in the ears

Page 11/15

Fig 4

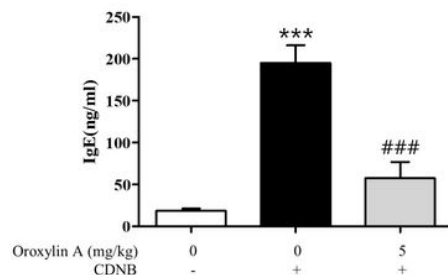


Figure 4

Effect of oroxylin A on elevated serum immunoglobulin E levels

The serum was collected on day 49 of the experiment. Serum IgE levels were measured using an enzyme-linked immunosorbent assay. IgE, immunoglobulin E. *** $p < 0.001$ vs. the control group, ### $p < 0.001$ vs. the CDNB-treated group.

Fig 5

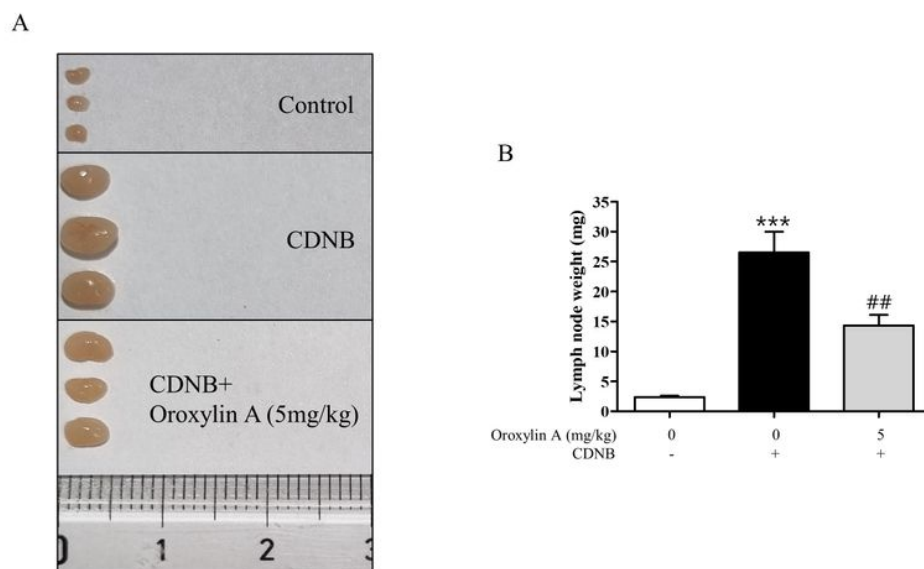


Figure 5

Effect of oroxylin A on sizes of lymph nodes

(A) The lymph nodes were photographed to measure morphological changes. (B) The weight of the lymph nodes were also measured. Results are presented as mean $\pm$ SEM (n = 5). CDNB, 1-chloro-2,4-dinitrobenzene; SEM, standard error of the mean. *** p < 0.001 vs. the control group, ## p < 0.01 vs. the CDNB-treated group.

Fig 6

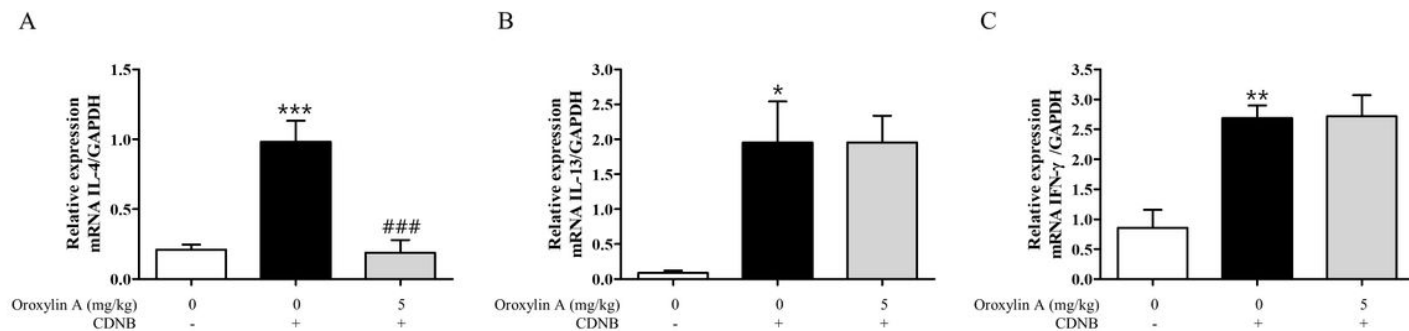


Figure 6

Effect of oroxylin A on mRNA expression of cytokines in lymph nodes

(A–C) qRT-PCR analyses for Th2 cytokines, IL-4 (A) and IL-13 (B), and Th1 cytokine, IFN- γ (C) were performed using mRNA isolated from mouse lymph node tissues. In addition, mRNA levels were normalized to that of GAPDH. The results are presented as the mean $\pm$ SEM ($n = 5$). Th, T helper; IL, interleukin; INF, interferon; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; SEM, standard error of the mean. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. the control group, ### $p < 0.001$ vs. the CDNB-treated group.

Fig 7

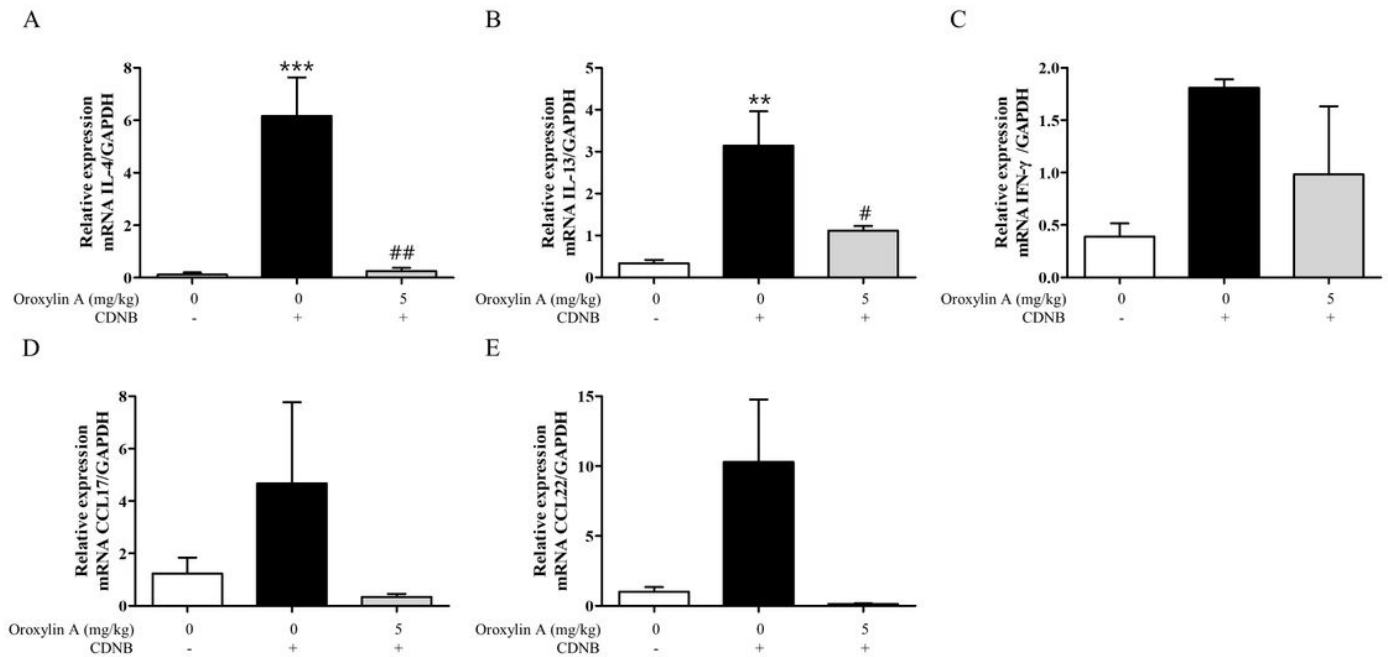


Figure 7

Effect of oroxylin A on mRNA expression of cytokines and chemokines in the ears

(A–E) qRT-PCR analyses for Th2 cytokines, IL-4 (A) and IL-13 (B), Th1 cytokine, INF-g (C), and chemokines CCL17 (D) and CCL22 (E) were performed using mRNA isolated from mouse ear tissues. In addition, mRNA levels were normalized to that of GAPDH. The results are presented as the mean $\pm$ SEM ($n = 5$). Th, T helper; IL, interleukin; INF, interferon; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; SEM, standard error of the mean. *** $p < 0.001$ vs. the control group, # $p < 0.05$, ## $p < 0.01$ vs. the CDNB-treated group.