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Suppression of dust mite extract and 2,4-dinitrochlorobenzene-induced atopic dermatitis by the water extract of *Lindera obtusiloba*

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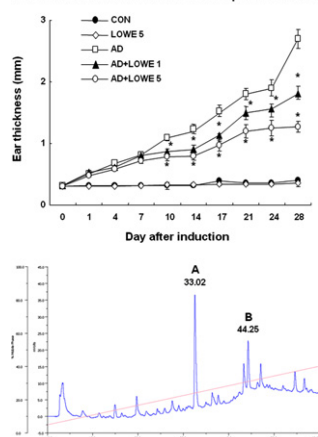
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GRAPHICAL ABSTRACT

Effect of *Lindera obtusiloba* on atopic dermatitis.



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ABSTRACT

Ethnopharmacological relevance: The *Lindera obtusiloba* has been used in traditional medicine for the treatment of inflammation and dermatitis.

Aim of the study: In this study, we investigated the effect of topical application of *Lindera obtusiloba* water extract (LOWE) on the house dust mite extract (*Dermatophagoides farinae* extract, DFE) and 2,4-dinitrochlorobenzene (DNCB)-induced atopic dermatitis (AD).

Materials and methods: We established AD model in BALB/c mice by repeated local exposure of DFE/DNCB to the ears. After a topical application of LOWE on the skin lesions, the epidermal thickness, mast cell infiltration, and serum immunoglobulin E (IgE) and histamine were measured. In addition, the gene expression of interleukin (IL)-4, IL-13, IL-31, and tumor necrosis factor (TNF)- α in the ears was assayed. **Results:** LOWE reduced AD symptoms based on ear thickness, histopathological analysis, and serum IgE levels. LOWE inhibited mast cell infiltration into the ear and elevation of serum histamine in AD model. Moreover, LOWE suppressed DFE/DNCB-induced expression of IL-4, IL-13, IL-31, and TNF- α in the ears. **Conclusions:** Our results showed that topical application of LOWE exerts beneficial effects in AD symptoms, suggesting that LOWE might be a candidate for the treatment of AD.

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Abbreviations: AD, atopic dermatitis; LOWE, *Lindera obtusiloba* water extract; DFE, *Dermatophagoides farinae* extract; DNCB, 2,4-dinitrochlorobenzene; IL, interleukin.

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1. Introduction

The traditional Korean medicine, *Lindera obtusiloba* is used for the treatment of inflammation and dermatitis, and herbal infusions of *Lindera obtusiloba* are applied to treat chronic liver disease (Ruehl et al., 2009). Extracts of *Lindera obtusiloba* contain lignans and butanolides, and were shown to exert anti-tumor, anti-allergy, and anti-inflammation activity (Kwon et al., 1999, 2000; Suh et al., 2011). However, the effect of *Lindera obtusiloba* on the atopic dermatitis (AD) was not elucidated.

The chronic and relapsing inflammatory dermatitis with immunologic disturbances are signature of AD. AD is a biphasic inflammatory skin disease, provoked by unbalance between Th1 and Th2 immune response (Jin et al., 2009). In particular, AD has been associated with the Th2 phenotype, showing the dominance of IL-4 and IL-13 secretion, and Th2 type cytokine-mediated IgE production (Grewé et al., 1998; Leung et al., 2003). Recently, association of IL-31 with AD-induced skin inflammation and pruritus was also reported (Kasraie et al., 2010). For human, house dust mites, such as *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, are the most common environmental allergens associated with AD (Matsuoka et al., 2003).

Many therapeutic trials have been performed to modulate AD with limited success. These include prevention of Th2 responses, enhancement of Th1 responses and decrease of IgE concentration. Topical glucocorticoids are important and effective remedies for treatment of AD. It is well known, however, that prolonged use of high dose of glucocorticoids causes a variety of side effect. Despite of several side effects, steroid has been used as a potent anti-AD treatment (Furue et al., 2003; Hanifin et al., 2004). Recently, natural herbs come into spotlight as an alternative therapeutics in immune disorders due to their proved safety with potent immunomodulatory effects. There are many reports about the use of natural herbs as a drug for treatment of AD and psoriasis (Koo and Arain, 1998; Kotani et al., 2000; Kang et al., 2008).

In this study, we examined the effect of *Lindera obtusiloba* water extract (LOWE) on AD lesions using BALB/c model. Assessment was made by measuring ear thickness, histopathological changes including mast cell count, cytokine expression in ear tissue, and serum IgE and histamine in each group.

2. Materials and methods

2.1. Animals

Six weeks old female BALB/c J mice were purchased from SLC Inc. (Hamamatsu, Japan). The animals were housed 5–10 per cage in a laminar air flow room, maintained at a temperature of $22 \pm 2^\circ\text{C}$, with a relative humidity of $55 \pm 5\%$ throughout the study. The care and treatment of the mice were in accordance with the guidelines established by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

2.2. Preparation of LOWE and reagents

Lindera obtusiloba was collected in Daegu, South Korea and was identified by Prof. Bae (Chungnam University). *Lindera obtusiloba* was ground ($400 \times \text{g}$, 30 s) at room temperature using a Micro Hammer-Cutter Mill (Culatti Co., Zurich, Swiss). The particle size was 0.5–2 mm after grinding. The sample was extracted at 100°C for 4 h in purified water. The extract was first filtered through Whatman No. 1 filter paper, further filtered using a $0.45 \mu\text{m}$ syringe filter, and then freeze-dried. A freeze-dried crude *Dermatophagoides farinae* extract (DFE) (Greer Laboratories, Lenoir, NC, USA) was used as an antigen. All other reagents were purchased from Sigma (St.

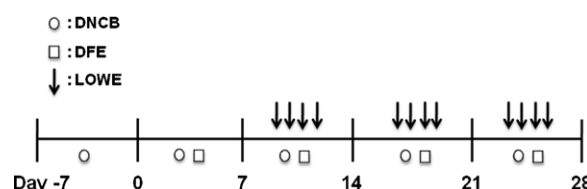


Fig. 1. Experimental schedule for the induction of AD lesion. After 7 days of first boosting with DNCB, DNCB and DFE were treated to the both ears once a week alternatively for 4 weeks. After 1 week of first induction, LOWE was painted to the ears with painting brush every day for 3 weeks.

Louis, MO, USA) unless otherwise stated. DFE was dissolved in phosphate buffered saline (PBS) containing 0.5% Tween 20. 2,4-Dinitrochlorobenzene (DNCB, 1%) was dissolved in acetone/olive oil (1:3) solution.

2.3. Induction of AD lesions in the ear

Induction of AD using DFE and DNCB was performed as previously described (Kwon et al., 2010). The schematic experimental procedure was described in Fig. 1. For the induction of AD, mice were divided into five groups and surface of both ear lobes were stripped five times with surgical tape (Nichiban, Tokyo, Japan). After stripping, $20 \mu\text{l}$ of DNCB (1%) was painted on each ear and then $20 \mu\text{l}$ of DFE (10 mg/ml) 4 days later. Treatment of DFE/DNCB was repeated once a week alternatively for 4 weeks. After two weeks later of 1st induction, tail bleeding was performed to check the serum IgE level. After confirming an atopic condition by IgE level, ears were treated with LOWE by painting brush until end of 4 weeks induction. Ear thickness was measured 24 h after DNCB or DFE application with a dial thickness gauge (Kori Seiki MFG Co., Japan).

At the days 14 and 28, blood samples were collected by tail bleeding. Plasmas were prepared from blood samples and stored at -70°C for further analysis. After blood collection, ears were removed and used for histopathological analysis. The serum IgE levels were measured using IgE ELISA kit (Bethyl Laboratories Inc., Montgomery, TX, USA) according to manufacturer's instruction.

2.4. Histological observation

Excised ears are fixed in 4% paraformaldehyde for 16 h and embedded in paraffin. Then, $6 \mu\text{m}$ sections were stained with hematoxylin and eosin. Infiltrated lymphocytes, thickening of the epidermis and fibrosis in the dermis were observed by microscope. For measurement of mast cell infiltration, skin sections were stained with toluidine blue and the number of mast cells in five sites chosen at random was counted.

2.5. Real-time polymerase chain reaction (PCR)

Quantitative real-time PCR was carried out using the Thermal Cycler Dice TP850 (Takarabio Inc., Shiga, Japan) according to the manufacturer's protocol. Total RNA was isolated from ear tissues of each group. The conditions for PCR were similar to ones previously described (Kim et al., 2007). Briefly, $2 \mu\text{l}$ of cDNA (100 ng), $1 \mu\text{l}$ of sense and antisense primer solution ($0.4 \mu\text{M}$), $12.5 \mu\text{l}$ of SYBR Pre-mix Ex Taq (Takarabio Inc.), and $9.5 \mu\text{l}$ of dH_2O were mixed together to obtain a final $25 \mu\text{l}$ reaction mixture in each reaction tube. The primers used for PCR were (IL-4 F 5'-ACAGGAGAAGGGACGCCAT, R 5'-GAAGCCGTACAGACGAGCTCA; IL-13 F 5'-GCAACATCAACAGG-CACAGA, R 5'-GTCAGGGAATCCAGGGCTAC; IL-31 F 5'-TCGGTCAT-CATAGCACATCTGGAG, R 5'-GCACAGTCCCTTTGGAGTTAAGTC; TNF- α F 5'-CCTGTAGCCACGTCGTAGC, R 5'-TTGACCTCAGCGCTGA-GTTG). The amplification conditions were 10 s at 95°C , 40 cycles

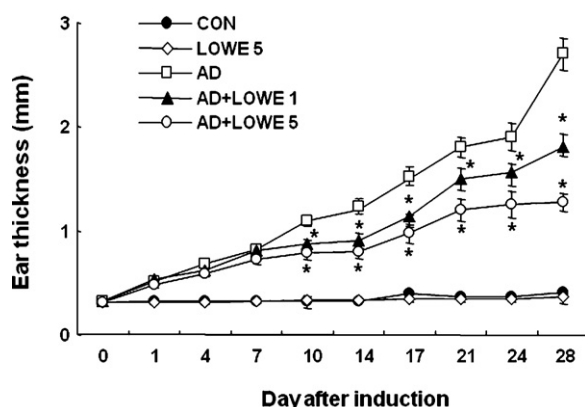


Fig. 2. Effect of LOWE on ear thickness. Detailed experimental procedure is described in Section 2. (A) Vehicle (acetone/olive oil) and LOWE (1 or 5 μ g/ear) were pretreated for 2 h before 1% DNCB (20 μ l/ear) or DFE treatment (10 mg/ml, 20 μ l/ear). Ear thickness was measured 24 h after DNCB or DFE application with a dial thickness gauge. Data are presented as mean $\pm$ SD of six determinations. *Significant difference from the DFE/DNCB-treated value at $P < 0.05$. AD: DFE and DNCB treatment.

of 5 s at 95 $^{\circ}$ C and 30 s at 60 $^{\circ}$ C, 15 s at 95 $^{\circ}$ C, 30 s at 60 $^{\circ}$ C, and 15 s at 95 $^{\circ}$ C. The normalization and quantification of mRNA expression was performed using the TP850 software supplied by the manufacturer.

2.6. Histamine assay

The histamine content was measured by the *o*-phthalaldehyde spectrofluorometric procedure as previously described (Kim et al., 2006b). The serum was separated from the released histamine by centrifugation at 400 $\times$ g for 5 min at 4 $^{\circ}$ C. The fluorescent intensity was measured at an excitation 355 nm and emission 450 nm using a Perkin-Elmer Fluorescence Spectrometer LS-50B (Norwalk, CT, USA).

2.7. Statistical analysis

Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC, USA). Treatment effects were analyzed using ANOVA followed by Dunnett's multiple range tests. Significance was set at $P < 0.05$.

3. Results

3.1. Clinical symptoms at the inflamed site

To investigate the effect of LOWE on AD, a BALB/c AD model was established by alternate painting of DFE and DNCB for 4 weeks on both ear lobes (Kwon et al., 2010). As shown in Fig. 2, repeated topical application of DFE/DNCB significantly increased ear thickness in mice. However, treatment of LOWE (1 and 5 μ g/ear) significantly suppressed DFE/DNCB-induced ear thickness. DFE/DNCB induced remarkable AD lesions, such as hemorrhage, edema, excoriation, and scaling. These AD lesions were reduced by LOWE treatment.

3.2. Histopathological observation and serum IgE

To analyze the effect of LOWE on the skin hypertrophy and granulocyte infiltration, sections of ear were stained and observed under an optical microscope. Repeated DFE/DNCB exposure caused potent inflammatory changes, such as thickening of the epidermis, fibrosis in the dermis and accumulation of inflammatory cells such as lymphocytes, eosinophils and neutrophils in the ear tissue of the atopic

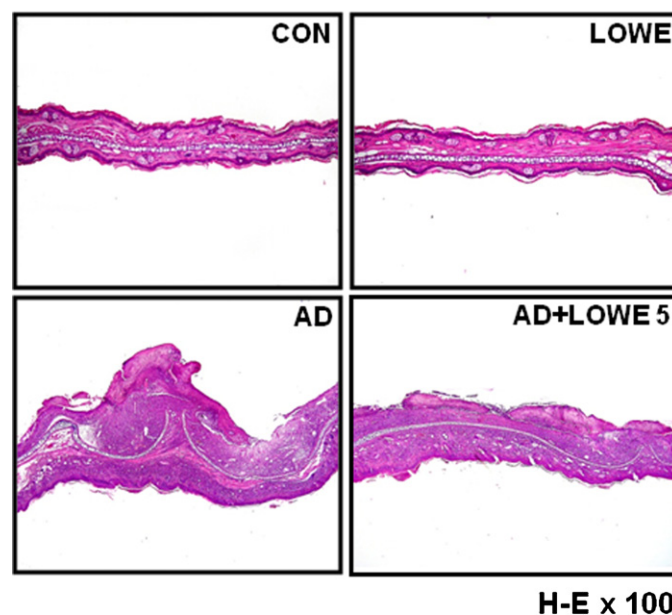


Fig. 3. Histopathological analysis. The ears of groups of vehicle, LOWE (5 μ g/ear), DFE/DNCB plus vehicle, and DFE/DNCB plus LOWE (5 μ g/ear) were excised, fixed with 10% formaldehyde, embedded in paraffin and then sections were made. The skin sections were stained with hematoxylin and eosin. AD: DFE and DNCB treatment.

dermatitis (Fig. 3). The inhibitory effect was observed by LOWE treatment. Hyper-production of IgE is a major characteristic of AD. Repeated application of DFE/DNCB caused an apparent elevation of serum total IgE (Fig. 4A) and DFE mite specific IgE (Fig. 4B). LOWE

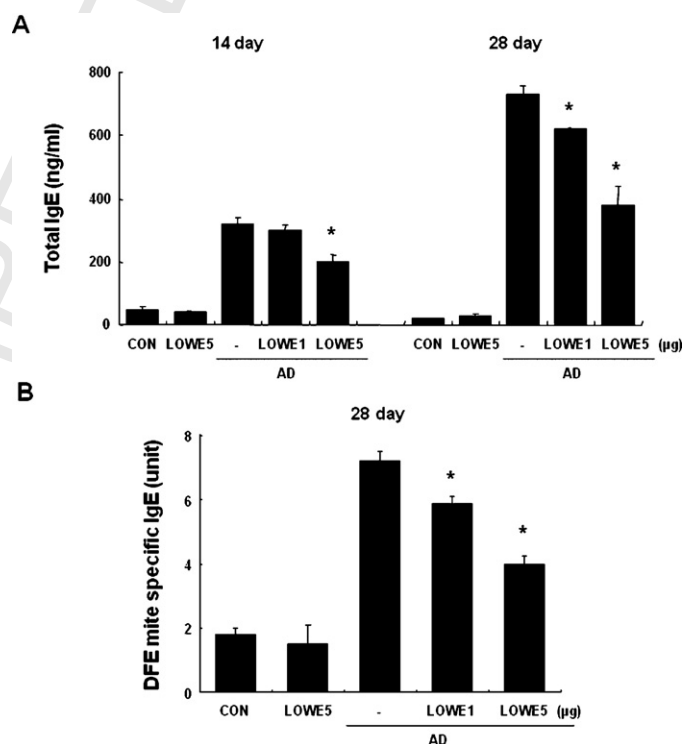


Fig. 4. Effect of LOWE on serum total and mite specific IgE. (A) The blood samples of groups of vehicle, LOWE (5 μ g/ear), DFE/DNCB plus vehicle, and DFE/DNCB plus LOWE (1 or 5 μ g/ear) were collected at 14 and 28 day, and plasma level of total IgE was quantified by ELISA. (B) The DFE mite specific IgE level was measured at 28 day. Data are presented as mean $\pm$ SD of triplicate determinations. *Significant difference from the DFE/DNCB-treated value at $P < 0.05$. AD: DFE and DNCB treatment.

significantly reduced DFE/DNCB-induced serum total and DFE mite specific IgE levels.

3.3. Effect of LOWE on the histamine release and mast cell infiltration

Next, to understand the inhibitory mechanism of LOWE, we measured a representative symptom-inducing substance such as histamine (Hammerberg et al., 2001) to test whether LOWE suppresses the release of histamine. Interestingly, LOWE inhibited the enhanced level of histamine induced by DFE/DNCB (Fig. 5A). To further investigate, we assessed the effects of LOWE on the infiltration of mast cells, an important effector cell and source of histamine involved in AD, into the ears. As shown in Fig. 5B and C, the infiltration of mast cells induced by DFE/DNCB was suppressed by LOWE treatment.

3.4. Effect of LOWE on expression of cytokines in the ear

We examined the expression levels of cytokines in the ear by real-time PCR. It is known that Th2 cytokine such as IL-4, IL-13, and IL-31 are highly expressed in acute stage of AD. Repeated application of DFE/DNCB increased the expression of IL-4, IL-13, IL-31 (Fig. 6). LOWE significantly reduced the expression of these Th2 cytokines. In addition, TNF- α , the important pro-inflammatory cytokine, was markedly increased by DFE/DNCB. The elevated TNF- α was also reduced by LOWE.

4. Discussion

In this present study, we investigated the effect of LOWE on DFE/DNCB-induced AD in BALB/c mice model. Among the several AD models, we used DFE/DNCB-induced AD model based on the reproducibility and repeatability from our previous study (Kwon et al., 2010). In this report, we clearly showed that typical AD symptoms were observed by the repeated alternative application of DFE/DNCB. Ear swelling and eczematous skin lesion were observed, and serum total and mite specific IgE were increased. Histopathological analysis also revealed the infiltration of leukocytes, especially mast cells, into the AD region. Topical application of LOWE significantly inhibited these AD symptoms suggesting the beneficial effect LOWE on AD.

Mast cells are known as key effector cells in IgE-mediated allergic disorders and activated by cross-linking of a high affinity IgE receptor. Upon activation, mast cells undergo degranulation and release a variety of biologically active substances, which play an important role in host defense and allergic reactions including AD. Among the immune mediators released from the mast cells, histamine is one of the best characterized and most potent mediators (Galli et al., 2005, 2008). In patients with AD, increases of histamine levels have been observed (Yanagisawa et al., 2009). Infiltration of mast cells into the dermis is a necessary factor to define an appropriate animal model for AD (Matsuoka et al., 2003). In the present study, we demonstrated that repeated treatment of DFE/DNCB resulted in mast cell infiltration into the dermis in the ear, suggesting that the animal model of AD used in this study satisfy AD conditions. Topical application of LOWE significantly suppressed the infiltration of mast cells in the skin lesions of the AD mice and the serum histamine release, suggesting that the activation and migration of mast cells may be a target of LOWE in AD.

Mast cell activation and histamine release are tightly regulated by IgE from B cells, and serum IgE levels are elevated in proportion to the development of AD (Matsuoka et al., 2003; Kim et al., 2006b; Kang et al., 2008). As shown in Fig. 4, DFE/DNCB-induced serum total and mite specific IgE levels were reduced by LOWE. From these results, we assume that LOWE alleviates AD lesions through the

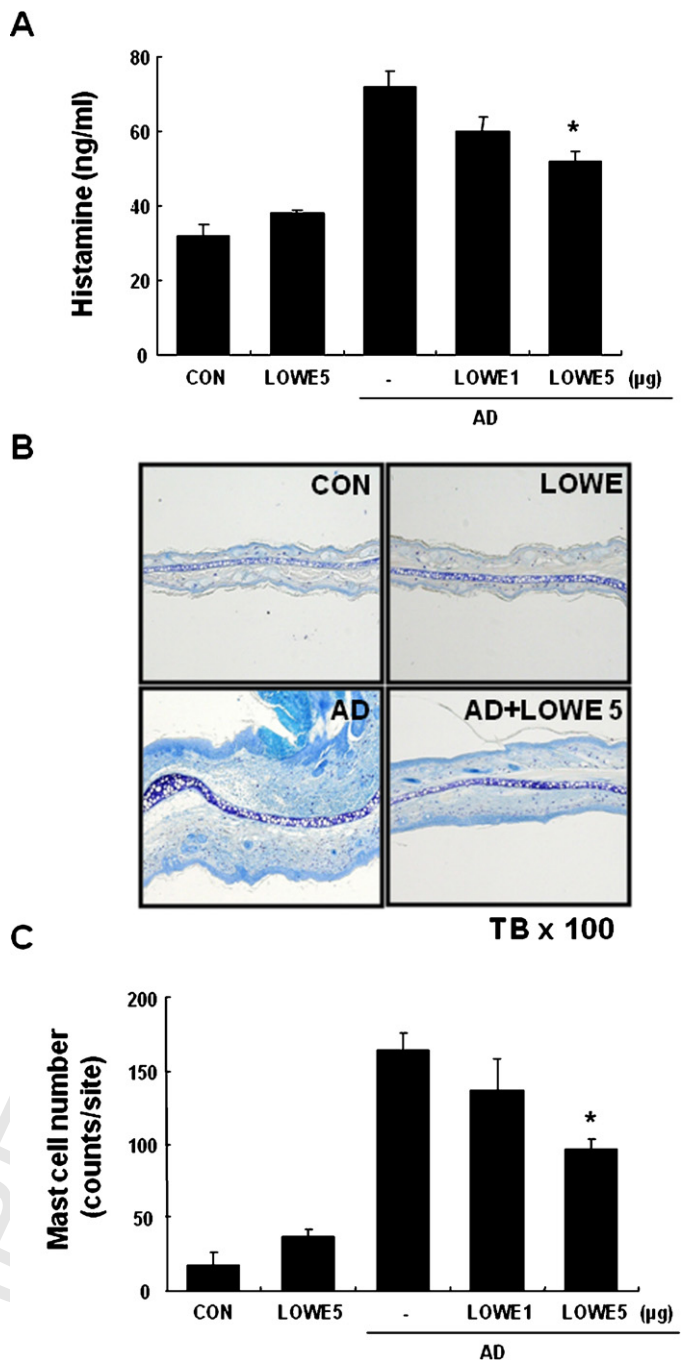


Fig. 5. Effect of LOWE on serum histamine and mast cell infiltration. (A) The blood was obtained from each mouse, and histamine content was measured using the spectrofluorometric procedure. (B) The ear sections were stained with toluidine blue. (C) The number of mast cells in five sites chosen at random was counted. Data are presented as mean $\pm$ SD of triplicate determinations. *Significant difference from the DFE/DNCB-treated value at $P < 0.05$. AD: DFE and DNCB treatment. TB: toluidine blue.

inhibition of IgE production. IgE synthesis by B cells is regulated by Th2 cytokines, especially IL-4 and IL-13. In human, overproduction of IL-4 is a critical factor in AD development (Yamazaki et al., 2002). Sensitization to an allergen reflects the allergen's ability to elicit a Th2 cell response, in which IL-4 and IL-13 drive IgE production by promoting class-switch recombination in B cells (Galli et al., 2008). In our results, LOWE decreased IL-4 and IL-13 which play an important role on isotype switching into IgE. These results imply that LOWE can reduce the serum IgE by suppressing the Th2 response

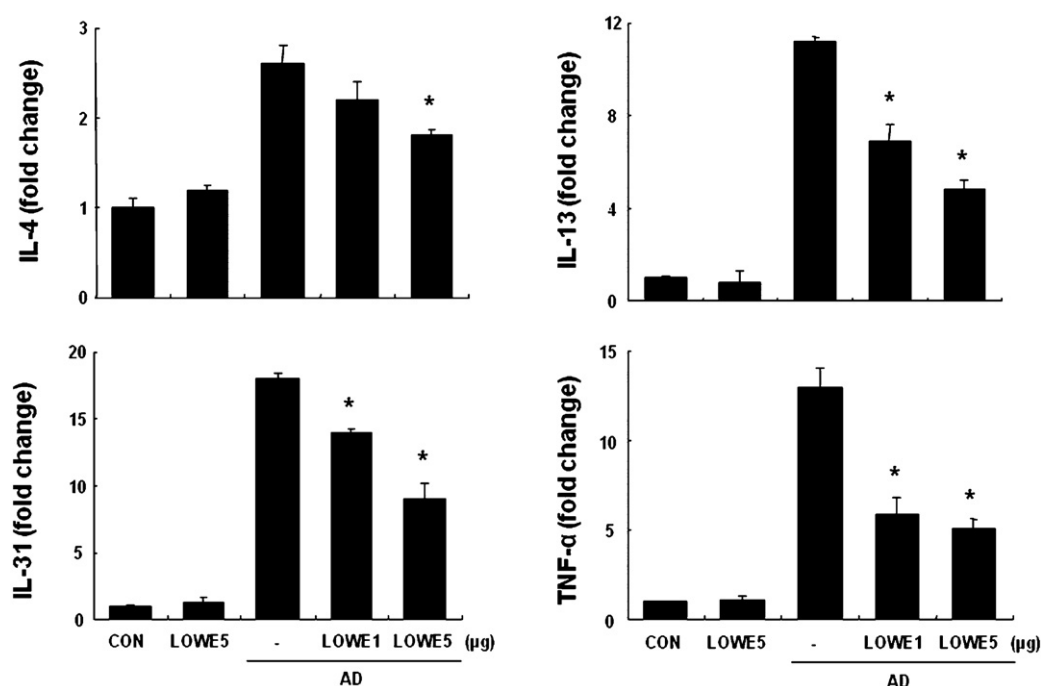


Fig. 6. Effect of LOWE on the expression of cytokines in ear. The ears were excised and total RNA was isolated. Quantitative real-time PCR was performed as described in Section 2. Data are presented as mean $\pm$ SD of triplicate determinations. *Significant difference from the DFE/DNCB-treated value at $P < 0.05$. AD: DFE and DNCB treatment.

especially IL-4 and IL-13 production. IL-31 is a newly characterized Th2 cytokine, which is also involved in AD. A major function that has been attributed to IL-31 is the induction of pruritus in the skin and allergic diseases (Dillon et al., 2004). Our results showing the suppression of DFE/DNCB-induced IL-31 by LOWE suggest that IL-31 is also a target of LOWE in AD.

The production of pro-inflammatory cytokines, especially TNF- α , by epidermal cells is known as one of the key events on the initiation of AD (Homey et al., 2006). TNF- α produced at the initiation stage of AD induces the production of various chemokines/adhesion molecules which causes the recruitment and proliferation of leukocytes within the skin. LOWE is known to exert anti-inflammatory activity (Kim et al., 2006a, 2008). We

previously showed that the production of TNF- α was inhibited by LOWE in activated human mast cells (Suh et al., 2011). Therefore, we speculate that the inhibitory effect of LOWE on leukocyte infiltration in our AD model might be mediated by the blocking of TNF- α and downstream chemokines/adhesion molecules.

We previously reported the anti-allergic inflammatory effect of LOWE through the inhibition of systemic and local allergic reaction. LOWE reduced histamine release by the attenuation of intracellular calcium, and expression of pro-inflammatory cytokines by the attenuation of NF- κ B (Suh et al., 2011). The intracellular calcium and cAMP are the most important signaling pathways to the degranulation of mast cells and downstream histamine release (Kim et al., 2006b). The effect of LOWE on the

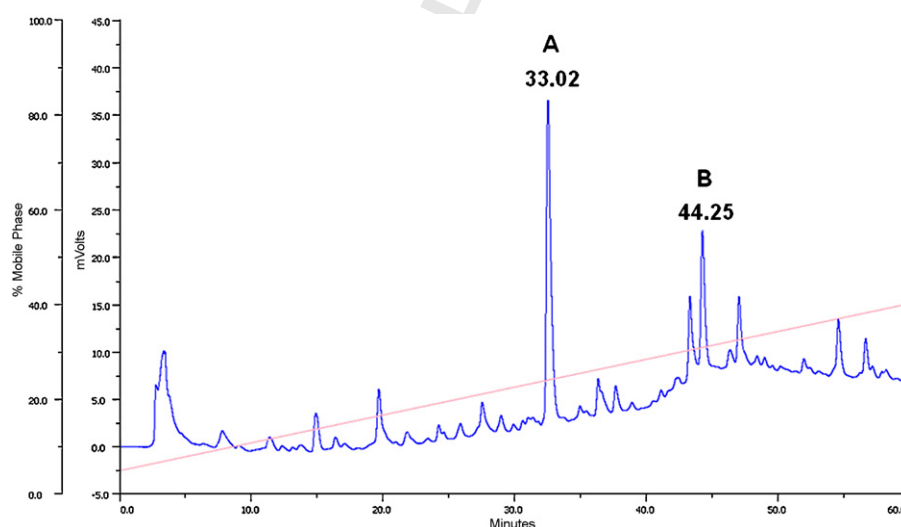


Fig. 7. HPLC chromatogram of crude water extract of *Lindera obtusiloba*. The analysis condition was flow rate: 1 ml/min, detection wavelength: 210 nm, solvent gradient: 60 min (MeOH, H₂O) 5–40%, Column-Shim-pack CLS-ODS (M). The peaks were confirmed by the addition of standard (+)-catechin and (–)-epicatechin in LOWE. (A) (+)-catechin. (B) (–)-epicatechin.

cAMP, and other transcription factors regulating the expression of cytokines in our AD model will be our future study to identify the molecular target of LOWE.

The active components responsible for the anti-AD effect of LOWE and the molecular targets of this effect are not elucidated yet. The HPLC profile of LOWE indicated that (+)-catechin and (–)-epicatechin are the major components of LOWE (Fig. 7). It was reported that epigallocatechin-3-gallate (EGCG) improves *Dermatophagoides pteronissinus*-induced AD symptoms (Noh et al., 2008). Because of the similarity of EGCG with (+)-catechin and (–)-epicatechin on structure and biological effects such as anti-inflammation, anti-allergy, and anti-cancer, we assume that (+)-catechin and (–)-epicatechin might responsible for the anti-AD effect of LOWE. However more detailed study should be addressed.

In conclusion, the present study demonstrates that topical application of LOWE inhibits the development of DFE/DNCB-induced AD symptoms in BALB/c mice. The inhibitory effect of LOWE was mediated by the histamine release, the production of IgE, and the expression of IL-4, IL-13, IL-31 and TNF- α . Our results suggest that LOWE could be a potential therapeutic candidate for AD.

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References

Dillon, S.R., Sprecher, C., Hammond, A., Bilsborough, J., Rosenfeld-Franklin, M., Presnell, S.R., Haugen, H.S., Maurer, M., Harder, B., Johnston, J., Bort, S., Mudri, S., Kuijper, J.L., Bukowski, T., Shea, P., Dong, D.L., Dasovich, M., Grant, F.J., Lockwood, L., Levin, S.D., LeCiel, C., Waggle, K., Day, H., Topouzis, S., Kramer, J., Kuestner, R., Chen, Z., Foster, D., Parrish-Novak, J., Gross, J.A., 2004. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. *Nature Immunology* 5, 752–760.

Furue, M., Terao, H., Rikihisa, W., Urabe, K., Kinukawa, N., Nose, Y., Koga, T., 2003. Clinical dose and adverse effects of topical steroids in daily management of atopic dermatitis. *British Journal of Dermatology* 148, 128–133.

Galli, S.J., Nakae, S., Tsai, M., 2005. Mast cells in the development of adaptive immune responses. *Nature Immunology* 6, 135–142.

Galli, S.J., Tsai, M., Piliponsky, A.M., 2008. The development of allergic inflammation. *Nature* 454, 445–454.

Grewe, M., Bruijnzeel-Koomen, C.A., Schopf, E., Thepen, T., Langeveld-Wildschut, A.G., Ruzicka, T., Krutmann, J., 1998. A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. *Immunology Today* 19, 359–361.

Hammerberg, B., Olivry, T., Orton, S.M., 2001. Skin mast cell histamine release following stem cell factor and high-affinity immunoglobulin E receptor cross-linking in dogs with atopic dermatitis. *Veterinary Dermatology* 12, 339–346.

Hanifin, J.M., Cooper, K.D., Ho, V.C., Kang, S., Krafchik, B.R., Margolis, D.J., Schachner, L.A., Sidbury, R., Whitmore, S.E., Sieck, C.K., Van Voorhees, A.S., 2004. Guidelines of care for atopic dermatitis, developed in accordance with the American Academy of Dermatology (AAD)/American Academy of Dermatology Association "Administrative Regulations for Evidence-Based Clinical Practice Guidelines". *Journal of the American Academy of Dermatology* 50, 391–404.

Homey, B., Steinhoff, M., Ruzicka, T., Leung, D.Y., 2006. Cytokines and chemokines orchestrate atopic skin inflammation. *Journal of Allergy and Clinical Immunology* 118, 178–189.

Jin, H., He, R., Oyoshi, M., Geha, R.S., 2009. Animal models of atopic dermatitis. *Journal of Investigative Dermatology* 129, 31–40.

Kang, J.S., Yoon, W.K., Han, M.H., Lee, H., Lee, C.W., Lee, K.H., Han, S.B., Lee, K., Yang, K.H., Park, S.K., Kim, H.M., 2008. Inhibition of atopic dermatitis by topical application of silymarin in NC/Nga mice. *International Immunopharmacology* 8, 1475–1480.

Kasraie, S., Niebuhr, M., Werfel, T., 2010. Interleukin (IL)-31 induces pro-inflammatory cytokines in human monocytes and macrophages following stimulation with staphylococcal exotoxins. *Allergy* 65, 712–721.

Kim, H.S., Kundu, J.K., Lee, J.S., Oh, T.Y., Na, H.K., Surh, Y.J., 2008. Chemopreventive effects of the standardized extract (DA-9601) of *Artemisia asiatica* on azoxymethane-initiated and dextran sulfate sodium-promoted mouse colon carcinogenesis. *Nutrition Cancer* 60 (Suppl. 1), 90–97.

Kim, J.Y., Kim, D.Y., Lee, Y.S., Lee, B.K., Lee, K.H., Ro, J.Y., 2006a. DA-9601 *Artemisia asiatica* herbal extract, ameliorates airway inflammation of allergic asthma in mice. *Molecular and Cells* 22, 104–112.

Kim, S.H., Jun, C.D., Suk, K., Choi, B.J., Lim, H., Park, S., Lee, S.H., Shin, H.Y., Kim, D.K., Shin, T.Y., 2006b. Gallic acid inhibits histamine release and pro-inflammatory cytokine production in mast cells. *Toxicological Science* 91, 123–131.

Kim, S.H., Lee, S., Suk, K., Bark, H., Jun, C.D., Kim, D.K., Choi, C.H., Yoshimura, T., 2007. Discoidin domain receptor 1 mediates collagen-induced nitric oxide production in J774A.1 murine macrophages. *Free Radical Biology and Medicine* 42, 343–352.

Koo, J., Arain, S., 1998. Traditional Chinese medicine for the treatment of dermatologic disorders. *Archives of Dermatology* 134, 1388–1393.

Kotani, M., Matsumoto, M., Fujita, A., Higa, S., Wang, W., Suemura, M., Kishimoto, T., Tanaka, T., 2000. Persimmon leaf extract and astragaloside inhibit development of dermatitis and IgE elevation in NC/Nga mice. *Journal of Allergy and Clinical Immunology* 106, 159–166.

Kwon, H.C., Choi, S.U., Lee, J.O., Bae, K.H., Zee, O.P., Lee, K.R., 1999. Two new lignans from *Lindera obtusiloba* blume. *Archives of Pharmacological Research* 22, 417–422.

Kwon, H.J., Kang, M.J., Kim, H.J., Choi, J.S., Paik, K.J., Chung, H.Y., 2000. Inhibition of NF κ B by methyl chlorogenate from *Eriobotrya japonica*. *Molecular and Cells* 10, 241–246.

Kwon, H.K., Lee, C.G., So, J.S., Chae, C.S., Hwang, J.S., Sahoo, A., Nam, J.H., Rhee, J.H., Hwang, K.C., Im, S.H., 2010. Generation of regulatory dendritic cells and CD4⁺Foxp3⁺T cells by probiotics administration suppresses immune disorders. *Proceedings of the National Academy of Sciences of the United States of America* 107, 2159–2164.

Leung, D.Y., Jain, N., Leo, H.L., 2003. New concepts in the pathogenesis of atopic dermatitis. *Current Opinion Immunology* 15, 634–638.

Matsuoka, H., Maki, N., Yoshida, A., Arai, M., Wang, J., Oikawa, Y., Ikeda, T., Hirota, N., Nakagawa, H., Ishii, A., 2003. A mouse model of the atopic eczema/dermatitis syndrome by repeated application of a crude extract of house-dust mite *Dermatophagoides farinae*. *Allergy* 58, 139–145.

Noh, S.U., Cho, E.A., Kim, H.O., Park, Y.M., 2008. Epigallocatechin-3-gallate improves *Dermatophagoides pteronissinus* extract-induced atopic dermatitis-like skin lesions in NC/Nga mice by suppressing macrophage migration inhibitory factor. *International Immunopharmacology* 8, 1172–1182.

Ruehl, M., Erben, U., Kim, K., Freise, C., Dagdelen, T., Eisele, S., Trowitzsch-Kienast, W., Zeitz, M., Jia, J., Stickel, F., Somasundaram, R., 2009. Extracts of *Lindera obtusiloba* induce antifibrotic effects in hepatic stellate cells via suppression of a TGF- β -mediated profibrotic gene expression pattern. *Journal of Nutrition Biochemistry* 20, 597–606.

Suh, W.M., Park, S.B., Lee, S., Kim, H.H., Suk, K., Son, J.H., Kwon, T.K., Choi, H.G., Lee, S.H., Kim, S.H., 2011. Suppression of mast-cell-mediated allergic inflammation by *Lindera obtusiloba*. *Experimental Biology and Medicine* (Maywood) 236, 240–246.

Yamazaki, F., Aragane, Y., Maeda, A., Matsushita, K., Ueno, K., Yudate, T., Kawada, A., Tezuka, T., 2002. Overactivation of IL-4-induced activator protein-1 in atopic dermatitis. *Journal of Dermatological Science* 28, 227–233.

Yanagisawa, R., Takano, H., Inoue, K., Koike, E., Kamachi, T., Sadakane, K., Ichinose, T., 2009. Titanium dioxide nanoparticles aggravate atopic dermatitis-like skin lesions in NC/Nga mice. *Experimental Biology and Medicine* (Maywood) 234, 314–322.