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

Eun-Ju Choi[a], Soyoung Lee[b], Hui-Hun Kim[b], Thoudam S.K. Singh[b], Jin Kyeong Choi[b], Hyun Gyu Choi[c], Won Mo Suh[c], Seung-Ho Lee[c], Sang-Hyun Kim[b,*]

[a] *Division of Sport Science, College of Natural Sciences, Konkuk University, Chungbuk 380-702, Republic of Korea*
[b] *Laboratory of Immunotoxicology, Department of Pharmacology, School of Medicine, Kyungpook National University, Daegu 700-422, Republic of Korea*
[c] *College of Pharmacy, Youngnam University, Kyungsan 712-749, South Korea*

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ABSTRACT

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

1. Introduction

The traditional Korean medicine, *Lindera obtusiloba* is used for the treatment of inflammation and dermatits, 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 (Grewe 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.

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

* Corresponding author. Tel.: +82 53 420 4838; fax: +82 53 423 4838.
E-mail address: shkim72@knu.ac.kr (S.-H. Kim).

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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 ± 2 °C, with a relative humidity of 55 ± 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 × *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 μm 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. 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 μl of DNCB (1%) was painted on each ear and then 20 μl 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 μm 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.

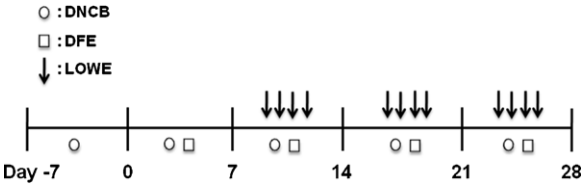

Fig. 1. Experimental schedule for the induction of AD lesion. After 7 days of first boosting with DNCB, DNCB and DFE were treated to the both ears once a week alternatively for 4 weeks. After 1 week of first induction, LOWE was painted to the ears with painting brush every day for 3 weeks.

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 μl of cDNA (100 ng), 1 μl of sense and antisense primer solution (0.4 μM), 12.5 μl of SYBR Premix Ex Taq (Takarabio Inc.), and 9.5 μl of dH_2O were mixed together to obtain a final 25 μl reaction mixture in each reaction tube. The primers used for PCR were (IL-4 F 5′-ACAGGAGAAGGGACGCCAT, R 5′-GAAGCCGTACAGACGAGCTCA; IL-13 F 5′-GCAACATCAACAGGACCAGA, R 5′-GTCAGGGAATCCAGGGCTAC; IL-31 F 5′-TCGGTCATCATAGCACATCTGGAG, R 5′-GCACAGTCCCTTTGGAGTTAAGTC; TNF-α F 5′-CCTGTAGCCCACGTCGTAGC, R 5′-TTGACCTCAGCGCTGAGTTG). The amplification conditions were 10 s at 95 °C, 40 cycles of 5 s at 95 °C and 30 s at 60 °C, 15 s at 95 °C, 30 s at 60 °C, and 15 s at 95 °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*-phthaldialdehyde spectrofluorometric procedure as previously described (Kim et al., 2006b). The serum was separated from the released histamine by centrifugation at 400 × *g* for 5 min at 4 °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 μg/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.