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Letter to the editor

Effects of essential oil from *Chamaecyparis obtusa* on the development of atopic dermatitis-like skin lesions and the suppression of Th cytokines

Atopic dermatitis (AD) is a chronic inflammatory skin disease that has a prevalence of 10–20% in children which has a complex etiology that encompasses immunologic responses, susceptibility genes, environmental triggers, and compromised skin-barrier function [1]. The pathogenesis of AD is still not clearly understood, but an elevated level of serum IgE is the most characteristic feature in extrinsic or allergic AD [2]. Increased level of serum IgE is known to be closely related to the production of Th2 cytokines [3], i.e., IL-4, IL-5, and IL-13. DNCB (2,4-dinitrochlorobenzene)-induced contact hypersensitivity of the skin in mice is a commonly used model for studying the pathogenesis of allergic contact dermatitis [4]. Mechanistically, DNCB, an immunogen, can be internalized by local antigen presenting cells, processed and presented to T cells in the lymph nodes for activation [5]. The prevailing explanation of the mechanism of this model is that the pathogenesis of DNCB-induced contact dermatitis is predominantly the result of T cell-mediated immune responses [5].

Chamaecyparis obtusa (*C. obtusa*) is a tropical tree species found in Japan and the southern region of South Korea. Essential oil extracted from *C. obtusa* contains several types of terpenes, including sabinene, limonene, bornyl acetate, borneol + α -terpineol and elemol, and those in the fruit oil are myrcene, γ -terpinene, p-cymene, borneol + α -terpineol and β -caryophyllene [6]. Although the biological activities of essential oil from *C. obtusa* are not yet fully understood, essential oil or β -thujaplicin (hinokitiol), a tropolone-related compound, has been shown to have antibacterial activities [6]. Thus, in the present study, we further examined the anti-atopic properties of the essential oil from *C. obtusa* to determine if they would be amenable for use as a pharmaceutical candidate.

BALB/c mice (7-week old) were employed in this study, and all procedures were conducted in accordance with the Animal Protocol. DNCB (Sigma, MO, USA) dissolved in acetone was used to induce dermatitis in BALB/c mice as described in a previous study with some modifications [7]. The essential oil from freshly cut needles of each species was obtained by steam distillation using a manufactured apparatus with a condenser as we previously performed [6,8].

In Fig. 1A, the fully developed AD-like skin lesions in DNCB-treated mice (a positive control, *b*) were observed compared to those of normal mice (*a*), whereas 2, 5 or 10% CO oil-treated mice were remarkably recovered (*d*, *e* and *f*). Higher concentrations of CO oil (10%) were expected to have more potential to recover AD-like skin compared to the therapeutic control, bufixamac ointment (*c*). The distribution of mast cells beneath the dermis and hypodermis of the AD-like mice was evaluated

by staining selected tissues with toluidine blue solution after formalin fixation (Fig. 1B). The number of mast cells were counted under a light microscope and expressed as the number of cells per square millimeter of dorsal skin (Fig. 1C). Infiltration of mast cells in DNCB-treated mice (a positive control) increased when treated with only DNCB (*b*), while infiltration was significantly decreased when treated with 5 and 10% CO oil (*e* and *f*). Serum IgE levels were significantly decreased when treated with CO oil (2 and 5%) for 6 weeks, compared to the short-term treatment for 2 weeks, indicating that CO oil may prevent the increase in IgE levels induced by DNCB after several weeks (*data not shown*).

CO oil was not cytotoxic at concentrations less than $10^{-3}\%$ in RBL-2H3 cells (*data not shown*). The release of β -hexosaminidase, an indicator of mast cell degranulation, was measured in supernatants after treatment with DNP-HSA. CO oil significantly inhibited the release of β -hexosaminidase from RBL-2H3 at concentrations of $10^{-4}\%$ and $10^{-6}\%$ (*data not shown*). A treatment with CO oil alone at varying concentrations, without antigen stimulation, did not affect β -hexosaminidase secretion (*data not shown*). In the analysis of Th1 and Th2 cytokine mRNAs from the lymph nodes in DNCB-treated mice, it is believed that 5% CO oil effectively inhibited the expression of IFN- γ and IL-5 mRNA (*data not shown*). The results suggests that CO oil may play an important role in suppressing T cell activation in lymph nodes, where T cells first meet with dendritic cells and are then activated.

To investigate the cytotoxicity of CO oil on splenocytes, mouse splenocytes were incubated with various concentrations of CO oil ($\sim 10^{-8}$). CO oil was not cytotoxic up to 24 h at concentrations ranging from 10^{-8} to $10^{-4}\%$, but a significant increase in LDH release from cells was observed at higher concentrations ($>10^{-4}\%$), indicating that these higher concentrations were cytotoxic (Fig. 2A). In a gene level, CO oil inhibits the expression of T cell-mediated Th1/Th2 cytokines in splenocytes activated with ConA. The mRNA levels of the selected Th1/Th2 cytokines were assessed using quantitative real-time PCR. As shown in Fig. 2B, C, and D, T cells were highly activated by ConA. However, the mRNA expression levels of IL-2, IFN- γ , and IL-5 were significantly inhibited after treatment with $10^{-4}\%$ CO oil. Interestingly, CO oil was particularly effective in inhibiting the expressions of IL-5 mRNAs at all concentrations.

In our previous study, we found that cuminal, eucarvone, and calamenene were common components in the experimentally active fractions [8]. These components have been shown to possess antibacterial, antifungal, antioxidant, and anti-carcinogenic activities and enhance permeation into human skin [9,10], suggesting that the essential oil from *C. obtusa* may have potent pharmacological activities for the treatment of skin disorders, such as AD. These results revealed that CO oil suppresses the development of DNCB-induced AD-like lesions in BALB/c

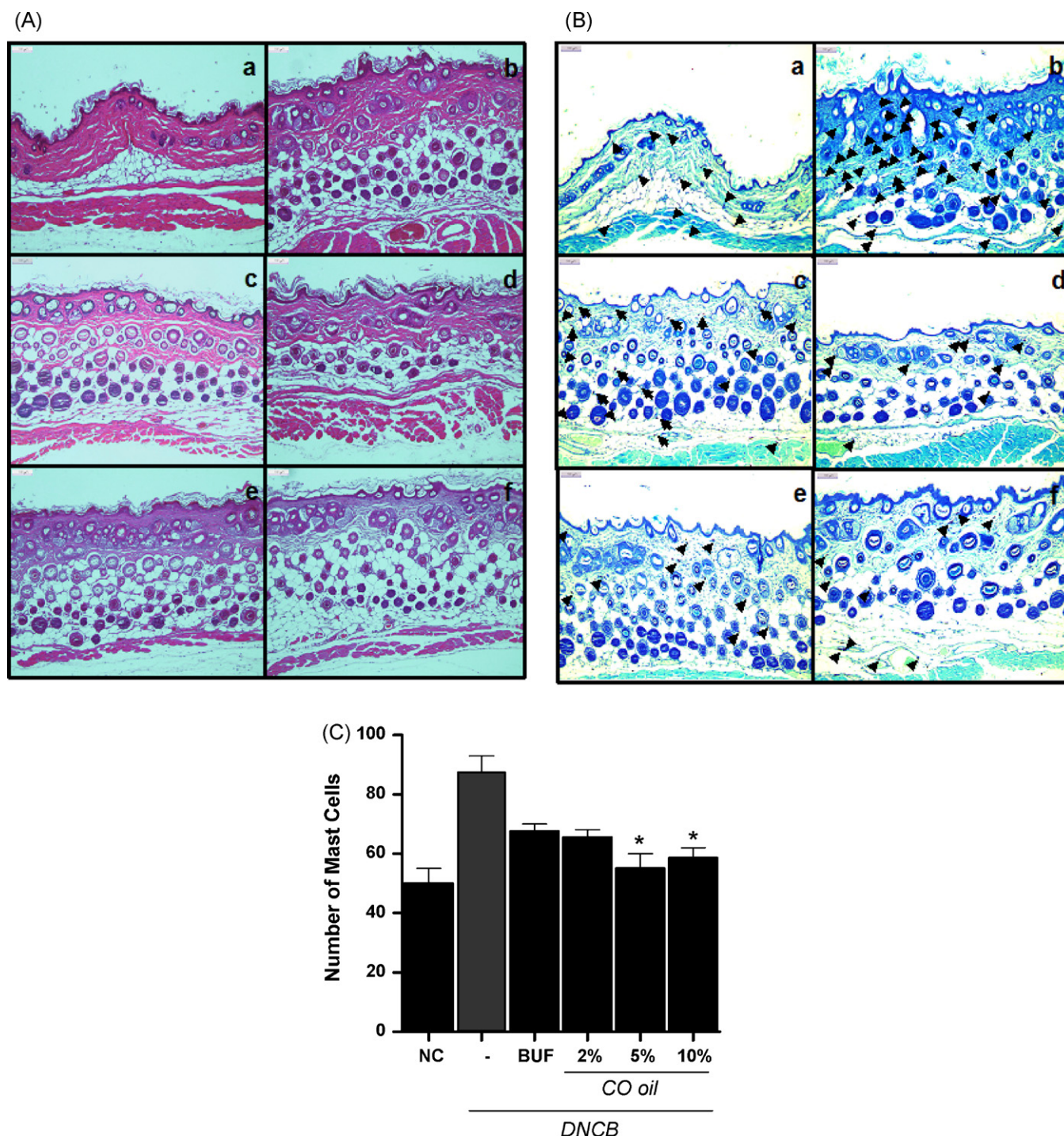


Fig. 1. Histological recovery of the dorsal skin lesion and mast cell infiltration beneath the dermis and hypodermis in DNCB or/and CO oil-treated mice after 4 weeks of treatment. Tissues were fixed with formaldehyde, embedded in paraffin, and stained with the hematoxylin and eosin staining solution (A) and with the toluidine blue staining solution (B) as described in Materials and Methods. a, untreated; b, DNCB-treated; c, bufexamac (therapeutic control); d, 2% CO oil; e, 5% CO oil; f, 10% CO oil. Arrows indicate the mast cells. Scale bar, 100 μ m for all pictures. Number of infiltrated mast cells was calculated under a light microscopy (400 $\times$) and expressed as the number of cells per square millimeter of dorsal skin. NC, normal control; BUF, bufexamac. *P < 0.05 vs. DNCB-treated group.

mice by suppressing overproduction of serum IgE and Th1/Th2 cytokines in both spleen and lymph node. Moreover, CO oil effectively inhibited the infiltration of mast cells beneath the dermis and hypodermis. Taken together, CO oil may be a potent therapeutic compound for the treatment of AD, and therapeutic

advantages of active ingredients contained in CO oil would allow for the development of clinically relevant therapeutic agents for AD. [Supported by National Research Foundation of Korea (NRF) grant funded by Korea government (MEST; No. 2010-0011433)]

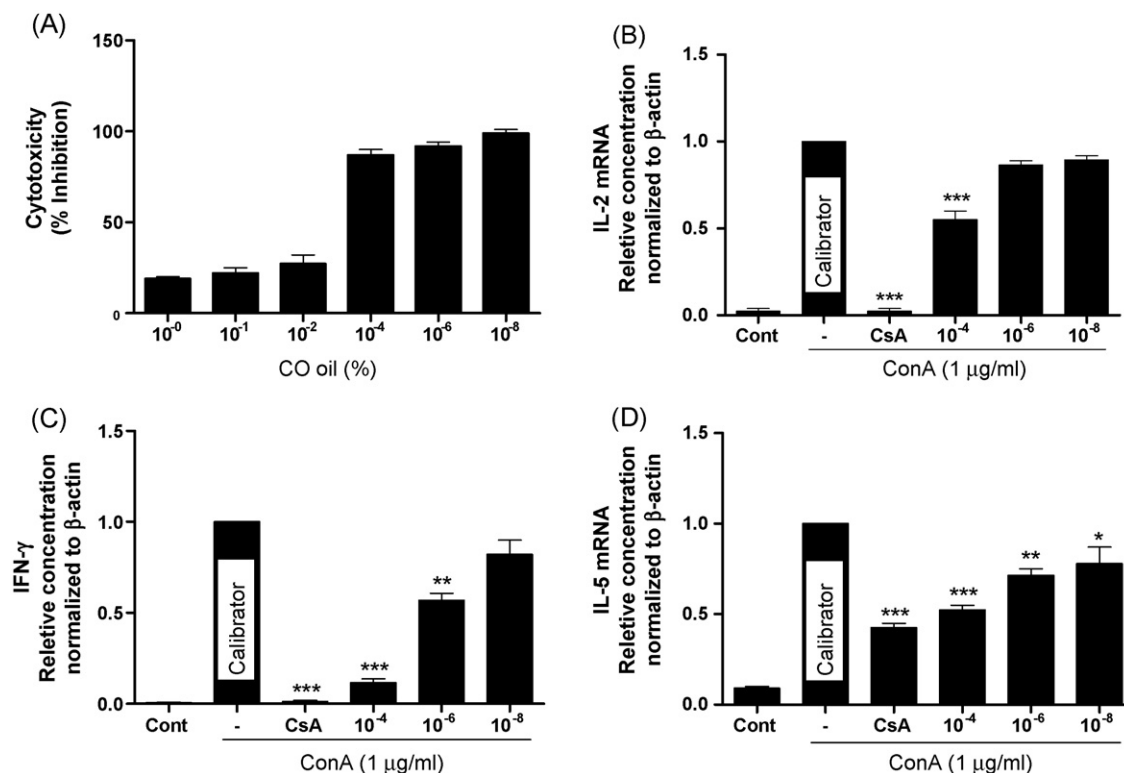


Fig. 2. Th1/Th2 cytokine mRNA expression and cytotoxicity in splenocytes. Cells were cultured for 24 h as described in the Methods and Materials, and CsA (cyclosporine A) was used as a positive control for mRNA analysis. (A) Splenocytes were exposed to CO oil (10^{-0} to $10^{-8}\%$) for the indicated times. Concentration dependent cytotoxicity was measured as %LDH released into culture supernatant. (B) Isolated mRNA was analyzed by real-time RT-PCR for IL-2, IFN- γ , IL-5, and β -actin. Results were internally confirmed by comparative cycle count (Ct, cycle number threshold) against β -actin as the standard gene. 1 μ g/ml ConA (concanavalin A-activated group) was used as a calibrator for a relative comparison of each group. Results are mean $\pm$ S.D. ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. calibrator.

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