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Topical application of an ethanol extract prepared from *Illicium verum* suppresses atopic dermatitis in NC/Nga mice

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ABSTRACT

Ethnopharmacological relevance: *Illicium verum* is a traditional herbal medicine with anti-inflammatory properties used in Asia. However, its usefulness in the treatment of allergic diseases remains unclear. This study evaluated the anti-inflammatory and antiallergic effects of *I. verum* extract (IVE) in a mouse model of atopic dermatitis.

Materials and methods: We investigated the effects of IVE on compound 48/80-induced histamine release, and phorbol 12-myristate13-acetate and calcium ionophore A23187-stimulated cytokines secretion in MC/9 mast cells. Atopic dermatitis was induced in NC/Nga mice by exposure to extract of house dust mite (*Dermatophagoides farinae*). After a topical application of IVE on ear and skin lesions, we evaluated the severity of skin symptoms, ear thickness, inflammatory cell infiltration, and serum levels of immunoglobulin E (IgE), histamine, interleukin (IL)-6, and intercellular adhesion molecule (ICAM)-1. In addition, we determined the expression of IL-4, IL-6, tumor necrosis factor (TNF)-α, interferon (IFN)-γ thymus- and activation-regulated chemokine (TARC), regulated on activation, normal T cell expressed and secreted (RANTES), ICAM-1, and vascular cell adhesion molecule (VCAM)-1 in ear tissues.

Results: IVE inhibited secretion of histamine, IL-4, IL-6, and TNF-α from mast cells in a dose-dependent manner. Topical application of IVE significantly reduced dermatitis scores, ear thickness, and serum levels of IgE, histamine, IL-6, and ICAM-1. Histopathological analysis demonstrated decreased epidermal thickening and dermal infiltration by inflammatory cells. In the ear lesions, IVE treatment reduced expression of IL-4, IL-6, TNF-α, TARC, RANTES, ICAM-1, and VCAM-1, but not IFN-γ.

Conclusions: These results indicate that IVE inhibits atopic dermatitis-like skin lesions by suppressing the expression of cytokines, chemokines, and adhesion molecules. These results suggest that IVE may be a potential therapeutic candidate for atopic dermatitis.

1. Introduction

Illicium verum Hook. f. (Illiciaceae), an aromatic evergreen tree commonly called star anise, is distributed primarily in eastern Asia. In addition to its culinary use, the fruit of *I. verum* is used in traditional Chinese medicine to treat stomachache, colic, vomiting, insomnia, skin inflammation, and rheumatic pain (Chouksey et al., 2010; Editorial Committee of Chinese Pharmacopoeia, 2010). It is also useful in dyspepsia, facial paralysis, asthma, and bronchitis (Warrier et al., 1996; Nadkarni, 2002). The fruits contain tannins and essential oil that consists of anethole, α-pinene, limonene, β-phellandrene, α-terpineol, anisaldehyde, farnesol, shikimic acid, and safrole (Chouksey et al., 2010; Huang et al., 2010). The essential oil of *I. verum* is traditionally used as a topical antiseptic and rheumatism treatment (Verghese, 1988), and previous studies have suggested that the essential oil also exhibits insecticidal, antioxidant, and antimicrobial properties (De et al., 2002; Padmashree et al., 2007). The major chemical constituent of *I. verum*, *trans*-anethole, exerts anti-inflammatory and anticarcinogenic actions by inhibiting tumor necrosis factor (TNF)-induced cellular responses (Chainy et al., 2000). Another essential oil component, shikimic acid, is a primary ingredient of the antiviral drug Tamiflu, a remedy for the H5N1 avian influenza virus (Wang et al., 2011). In addition, anethole and limonene have been reported to exert anti-inflammatory effects by suppressing

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nitric oxide, prostaglandin E_2, and cytokines in lipopolysaccharide-stimulated RAW 264.7 macrophages (Conforti et al., 2010; Yoon et al., 2010). However, it has not yet been determined whether *I. verum* can suppress the development of allergic inflammation.

Atopic dermatitis (AD) is a chronic inflammatory skin disease that occurs most often in infants and children and is increasing in prevalence (Spergel and Paller, 2003). AD is characterized by elevated serum immunoglobulin E (IgE) levels, peripheral eosinophilia, and pruritic and relapsing eczematous skin lesions infiltrated by inflammatory cells such as T lymphocytes, monocytes/macrophages, eosinophils, and mast cells (Soter, 1989; Leung and Bieber, 2003). In response to antigen cross-linking of the IgE receptor or direct activation, mast cells release inflammatory mediators including histamine, proteases, chemokines, and cytokines (Galli et al., 1991). In the acute phase of AD, T helper 2 (Th2) cells secrete interleukin (IL)-4, IL-5, and IL-13, which are important in the onset and development of AD. Th1 cells secrete interferon (IFN)-γ, which contributes to pathogenesis during the chronic phase (Leung et al., 2004).

The inbred strain NC/Nga mouse was established in 1957 from Japanese fancy mice (Nishiki-Nezumi). When placed in conventional surroundings, these mice spontaneously develop AD, which is accompanied by frequent scratching, elevated IgE levels, and inflammatory cell infiltration into the skin lesions (Matsuda et al., 1997; Vestergaard et al., 1999). However, this mouse model does not show AD-like symptoms under specific pathogen-free conditions and thus requires exposure to stimulus for developing a cutaneous hypersensitivity reaction. Patients with AD are highly sensitized to house dust mite allergens. Topical application of an extract of *Dermatophagoides farinae*, a major species of house dust mite, induces AD-like skin lesions in NC/Nga mice (Matsuoka et al., 2003; Gao et al., 2004; Yamamoto et al., 2007). Thus, we investigated the therapeutic effects of *I. verum* extract (IVE) on AD in NC/Nga mice exposed to the dust mite allergen.

2. Materials and methods

2.1. Preparation of IVE

Dried fruits of *I. verum* were purchased from Omniherb Co. (Yeoungcheon, Korea) and authenticated based on its macroscopic characteristics by the Classification and Identification Committee of the Korea Institute of Oriental Medicine (KIOM). A voucher specimen (HRA-60) was deposited in the herbarium of the Department of Herbal Resources Research at KIOM. The dried herb (300 g) was extracted twice with 70% ethanol (with a 2-h reflux), and the extract was concentrated under reduced pressure. The decoction was filtered, lyophilized, and stored at 4 °C. The yield of dried extract from starting crude materials was approximately 15.73% (wt/wt).

2.2. Reagents and cell culture

Compound 48/80, phorbol 12-myristate 13-acetate (PMA), calcium ionophore A23187, L-glutamine, and 2-mercaptoethanol

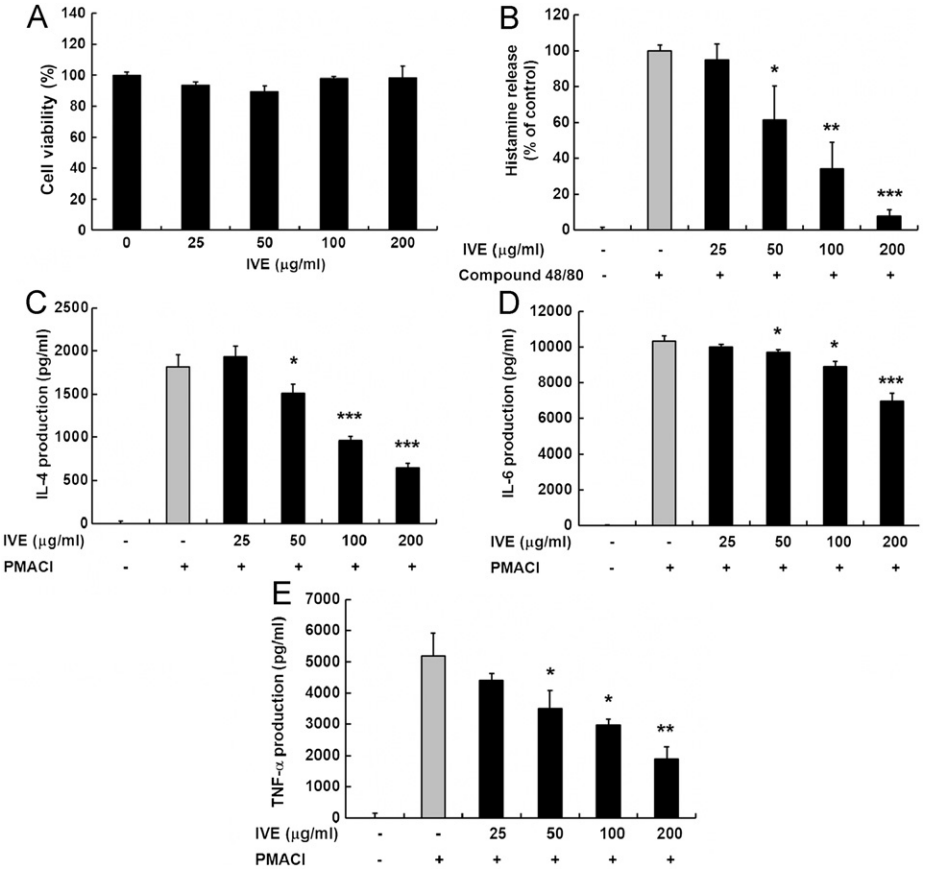

Fig. 1. Effects of *Illicium verum* extract (IVE) on compound 48/80-induced histamine release or PMACI-induced inflammatory cytokines production in mast cells: (A) cell viability. Cells were cultured in 96-well plates and stimulated with or without the extracts (0–200 μg/ml). After 24 h, cell viability was measured using the MTT assay. Production of (B) histamine, (C) IL-4, (D) IL-6, and (E) TNF-α in MC/9 cells was measured using an ELISA kit. MC/9 cells (2×10^5 cells/ml) were pretreated with IVE at 37 °C for 30 min prior to compound 48/80 (25 μg/ml) or PMA (20 μM) and A23187 (1 μM) stimulation. Values represent the means ± SD of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. compound 48/80- or PMACI-treated. PMA, phorbol 12-myristate 13-acetate; CI, calcium ionophore A23187.