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Inhibitory effects of cultured *Dendrobium tosaense* on atopic dermatitis murine model

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ABSTRACT

Dendrobium tosaense is one of the most valuable Chinese medicines and well developed health food. Atopic dermatitis (AD) is a chronic skin disease that occurs mainly in childhood. The pathogenesis of atopic dermatitis had been studied in BALB/c mice modeling by skin-inoculated ovalbumin (OVA) with 2,4,6-trinitro-1-chrolobenzene (TNCB). These mice exhibit features of chronic dermatitis, including skin rash, mast cells infiltration, and elevated serum anti-OVA specific IgE and cytokines modulation. In this study, a standardized ethyl acetate extract of *D. tosaense* (DtE) was used to protect these mice from the OVA/TNCB-induced skin lesions of atopic dermatitis. The results indicated an increased population of natural T regulatory cell was accompanied by immunosuppression in cytokine profiles and anti-OVA IgE level to significantly reduce Th2 polarization. Finally, toluidine blue staining indicated mast cell infiltration and degranulation was reduced in skin lesion. Our results were shed light on the usage of *D. tosaense* in AD.

1. Introduction

According to the World Health Organization survey, about 70–80% of the world's population depends on alternative medicine for their primary healthcare. (Cordeiro and Oliveira, 2005; Rolim et al., 2006) The herbal extractions contain anti-inflammatory, antioxidant, antiallergic and anti-diabetic activities (Ribnicky et al., 2009). *Dendrobium* species are one of the leading cut and potted orchids grown in the tropics. The genus *Dendrobium* (Orchidaceae) includes about 1600 species (Zhou, 1993), 15 of which are found in Taiwan (Su, 1978). Dried or fresh stems of several *Dendrobium* species are using to prepare "Shi-hu" (*Dendrobium* spp. or Herba Dendrobii), which is widely used in both traditional Chinese and folk remedies for antipyretic, ophthalmic, and tonic purposes (Li et al., 2008). *Dendrobium* species are known to contain a number of substances, such as flavonoids (Chang et al., 2010; Yang et al., 2006), phenanthrenes (Honda and Yamaki, 2000; Lin et al., 2001), bibenzyls (Majumder et al., 1999; Zhang et al., 2006a, 2007), fluorenones and sesquiterpenes (Yamaki and Honda, 1996; Zhang et al., 2007), alkaloids (Wang et al., 2010), and polysaccharides (Chen et al., 2012), which are responsible for their wide variety of medicinal properties. In recent years several reports have published on these compounds possess anti-inflammatory (Li et al., 2009a), antioxidant (Miyazawa et al., 1999), anti-cancer (Tsai et al., 2010), anti-aging, anti-diabetic properties (Chang et al., 2010) and antiallergic activities (Majumder et al., 1999). The species *Dendrobium tosaense* MAKINO used as one of important ingredient of "Shi-hu" and also consumed as functional heath food in Taiwan. In the help of the Council of Agriculture, farmers used tissue culture propagation and large-scale cultivation of *Dendrobium tosaense* in green house. It is domestically cultivated and has free radical scavenging activity

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due to its quercetin content (Lo et al., 2004a). Quercetin, 3,3′,4′,5,7-pentahydroxylflavone, is a typical flavonoid ubiquitously present in vegetables and fruits, and its antioxidant effect is implied to be helpful for human health. It occurs mainly in leaves and in the outer parts of the plants as aglycones and glycosides, in which one or more sugar groups is bound to phenolic groups by glycosidic bond (Casagrande et al., 2007; Montenegro et al., 2007). Recently, many research has been focused on the application use of flavonoids to treat skin disease (Gregoris et al., 2011).

Atopic dermatitis (AD) is an allergic disease of unknown origin that usually starts in early infancy; it is characterized by pruritus, eczematous lesions, xerosis, and lichenification. AD was associated with other atopic diseases such as asthma, allergic rhinitis, urticaria, acute allergic reactions to foods and is a pervasive health problem woldwide (Li et al., 2009b; Pople and Singh, 2010). The incidence of AD is increasing, and about 10–20% of children are affected. Though the pathogenesis of this allergic disease is still being researched, its primary causes are associated with genetic factors, environmental interactions, skin barrier disorders or immunological reactions (Abramovits, 2005; Wuthrich et al., 2007). Mast cells are key effector cells in IgE-mediated allergic disorders and are activated by cross-linking of a high affinity IgE receptor (FcεRI) on their surface. Mast cells undergo degranulation on activation and release variety of biologically active substances including platelet activating factor (PAF), histamine, leukotriene C4, leukotriene D4 and prostaglandin E2 (PGE2), they play important role in allergic reactions (Zhang et al., 2007). Vascular endothelial growth factor (VEGF) is a potent agent that causes hyperpermeability of blood vessels and endothelial cell proliferation. The production of VEGF is enhanced in AD, and might be involved in the persisting erythema and edema (Jung et al., 2010; Zhang et al., 2006b). AD is commonly associated with allergen (dust mite, ovalbumin, sea food or fungus) related IgE mechanisms, and the vast majority of patients with AD exhibit over-expression of cytokines and increase IgE production. These cytokines like interleukin-4 (IL-4), IL-5 and IL-13, are produced by type 2 T helper cells (Th2). In addition to increased Th2 cells and type-2 cytokines, most AD patients have decreased levels of interferon gamma (IFN-γ) and IL-12, cytokines that produce a type 1 environment that is capable of counteracting Th2 responses. It is well established that a Th1/Th2 imbalance skewed toward Th2 plays an important role in the development of AD (Choi et al., 2008; Kang et al., 2010; Lee et al., 2006).

In this study, we tested the ability of ethyl acetate extract of *D. tosaense* (DtE) to prevent the IgE-related inflammatory response seen in AD. We simulated AD by using the ovalbumin (OVA) with 2,4,6-trinitrochlorobenzene (TNCB)-sensitized dermatitis murine model and studied the *in vivo* effects of DtE on the production of specific anti-OVA antibodies, Th1/Th2 cytokines, Treg modulation, and mast cell degranulation.

2. Materials and methods

2.1. Preparation of D. tosaense extract

Cultured *D. tosaense* (Dt) plants were propagation by tissue culture and grown in the green house of Chaoyang University of Technology to one year (Lo et al., 2004b,c). This plant was identified by the Institute of Chinese Pharmaceutical Sciences, China Medical University (plant specimen number: CMC DT 0303) and genetic identification by internal transcribed spacer (GenBank accession number: HM590367). Dried stems of Dt was grinded into fine powder, about 30 g of powdered Dt was dissolved in 300 ml of methanol and sonicated at an amplitude of 15 for 10 min. The methanolic extract was partitioned for three times with n-hexane followed by ethyl acetate (EA), chloroform and finally with water. All the fractions were filtered and evaporated under reduced pressure to yield a crude residue. The quercetin content was confirmed by LC/MS (data not shown) and maximum quercetin content was found in EA fraction (DtE), hence EA fraction was further used for this study. The average quercetin content in EA fraction of DtE was 650 μg/g dry weight of the sample.

2.2. Animals and 2,4,6-trinitrochlorobenzene (TNCB) sensitization and challenge

Female BALB/c mice weighing 18–22 g each obtained from National Laboratory Animal Center (Taiwan) and maintained for 2 weeks to acclimatized laboratory conditions before the start of the experiments. They were 8 weeks old at the time of experiments. The animals were housed in plastic cages and maintained at 21 °C to 24 °C with a 12/12 h dark/light cycle. The animals were supplemented with standard sterile rodent chow diet (Test-Diet 5010, Ralston-Purina, St. Louis, MO) and distilled water *ad libitum*. The experimental protocol adhered to the rules of the Animal Protection Act of Taiwan and was approved by the Laboratory Animal Care and Use Committee of the Tunghai University.

Allergic modeling mice were modified by Dai et al., and Kigasawa et al. (Dai et al., 2002; Kigasawa et al., 2010) briefly, mice were sensitized to produce allergic skin lesions by applying 7% TNCB dissolved in acetone:olive oil (4:1) and 25 μg ovalbumin (OVA, grade V, Sigma–Aldrich, San Diego, USA) on topically to the dorsal skin (100 μl) for 7 days. After 7 days modeling, DtE at a dose of 30, 100 and 300 mg/kg or quercetin (1.6 mg/kg) dissolved in CMC (carboxymethyl cellulose) gel and orally administered *via* gastrogavage throughout the experimental period (Senthilkumar et al., 2008). Sterilized CMC gel (10 ml/kg) was orally administered after OVA/TNCB treatment as control group, and without OVA/TNCB or drug treatment was given as "naïve" group. After the 7 days of DtE administration period, blood sampled from axillary plexus of mouse was used for immunologic or hematologic analysis, and skin tissue was sampled and fixed with 10% neutral buffered formalin solution for histopathological examinations, splenocyte was harvest and stimulated by mitogen (ConA, 5 μg/ml) for 24–72 h and the supernatant was used to analyzed cytokines (IL-4, IL-6 and IFN-γ) by ELISA.

2.3. Ovalbumin-specific antibodies in serum

Ovalbumin-specific antibodies were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) method, as previously described (Hsieh et al., 2010). In brief, different dilutions of serum 1:20-1:100 were added in duplicate into ELISA plates coated with OVA (20 μg/ml in 0.05 M carbonated buffer, pH 9.5) and incubated overnight at 4 °C. The plates were washed and incubated with HRP-conjugated goat anti-mouse polyclonal IgE and IgG1 (Bethyl Laboratories, Inc. Montgomery, TX, USA) for 1 h, followed by washing and developed with SureBlue Reserve TMB Microwell Peroxidase Substrate (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD, USA). The colorimetric reaction was measured at 450 nm and level of OVA-specific IgE and IgG1 were calculated with high-titer of reference serum by our previously study (Hsieh et al., 2010). The calculated equation is list below:

$$\text{ELISA units, EU} = \frac{(A_{\text{sample}} - A_{\text{blank}})}{(A_{\text{reference}} - A_{\text{blank}})}$$