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Inhibitory effect of clove methanolic extract and eugenol on dendritic cell functions

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ABSTRACT

Caryophyllata Flos or clove is widely used as a condiment and in Chinese medicine. It reportedly helps relieve asthma and allergies; however, the underlying mechanisms remain unclear. In particular, the mechanism affecting dendritic cells (DCs), which play a critical role in the immune response, remains unknown. In this study, we examine the effects of Caryophyllata Flos methanolic extract (CFME) and its major compound, eugenol, on DC functions. Our results showed that CFME and eugenol significantly inhibited DC activation and maturation. The IC50s of CFME and eugenol were approximately 25 μg/mL and 50 μM, respectively. CFME and eugenol halted T-cell proliferation. Contact hypersensitivity responses were inhibited in mice cosensitized with either CFME or eugenol. We demonstrated for the first time that clove and its major active ingredient, eugenol, exhibited a significant immunosuppressive effect on DC functions, revealing that clove is a functional food that can ameliorate chronic inflammation and autoimmunity.

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1. Introduction

Clove, the dried flower buds of *Eugenia caryophyllata* Thunb. (*Syzygium aromaticum* (L.) Merr. & L.M. Perry), belongs to the Myrtaceae family and is known as Caryophyllata Flos in traditional Chinese medicine. Caryophyllata Flos has been traditionally used in cooking and food processing as a flavouring agent as well as an antimicrobial ingredient. Caryophyllata Flos is one of the most commonly used materials in Chinese medicine, and it is reported to possess several therapeutic properties, such as antiseptic, analgesic, antiphlogistic, antivomiting, antispasmodic, anticarminative, and kidney reinforcement effects (Liu, Mao, & Hong, 1997; National Pharmacopoeia Committee, 2000). It has been observed that Caryophyllata Flos oil possesses antibacterial (Islam, Ferdous, Ahsan, & Faroque, 1990), insecticidal (Huang, Ho, Lee, & Yap, 2002), antifungal (Velluti, Sanchis, Ramos, & Marín, 2003), antioxidant (Lee & Shibamoto, 2001), asthma and allergy relief (Kim, Lee, & Hong, 1998), and human cytomegalovirus extracorporeal restraining properties (Liu et al., 1997). In addition, Caryophyllata Flos has been demonstrated to possess anti-inflammatory properties using murine macrophages *in vitro* (Bachiega, de Sousa, Bastos, & Sfrocin, 2012) and *in vivo* (Ozturk & Ozbek, 2005). Furthermore, the remarkable conflict between increased and inhibitory effects of the essential oil isolated from Caryophyllata Flos on humoral immunity and the hypersensitivity response, respectively, was explored (Halder, Mehta, Mediratta, & Sharma, 2011). Therefore, the underlying mechanisms of the immune response to Caryophyllata Flos remain to be elucidated. In particular, the effect of Caryophyllata Flos on the critical immune dendritic cells (DCs) of the immune system is still unknown.

Chemically, Caryophyllata Flos essential oil is composed of eugenol, eugenol acetate, β-caryophyllene, α-humulene, and α-copaene. Among these components, eugenol is the main active ingredient and is used as a marker compound to evaluate the quality of clove essential oil (Ozturk & Ozbek, 2005; Yun et al., 2010). In addition, eugenol is the major component of clove methanol (70%) extract detected by gas chromatography/mass spectrometry analyses (Bachiega et al., 2012). Moreover, eugenol is found in nutmeg (Bennett et al., 1988), *Cinnamomum tamala* Nees and Eberm (Dighe, Gursale, Sane, Menon, & Patel, 2005), *Ocimum basilicum* L. (Johnson, Kirby, Naxakis, & Pearson, 1999), *O. gratissimum* L. (Nakamura et al., 1999) and cinnamon (Kreydiyyeh, Usta, & Copti, 2000), and has been reported to possess a wide range of therapeutic properties (Kamatou, Vermaak, & Viljoen, 2012). However, the effect of eugenol on DCs is still unknown.

Numerous reports have demonstrated that DCs play a critical role in the regulation of innate and adaptive immunity (Banchereau & Steinman, 1998; Guermonprez, Valladeau, Zitvogel, Thery, & Amigorena, 2002; Rudulier, Kroeger, & Bretscher, 2012). Therefore, DCs represent a compelling new area of study to investigate materials that can modulate immune responses (Fu et al., 2013; Lin et al., 2011a; Lin et al., 2011b; Lin et al., 2015). This study examined the effect of Caryophyllata Flos methanolic extract (CFME) and its major component, eugenol, on DC activation and function as well as the hypersensitivity response in mice.

2. Materials and methods

2.1. Samples and preparation of the extract

Caryophyllata Flos was obtained from a traditional Chinese medicine store in Taichung, Taiwan. Caryophyllata Flos was ground into a fine powder and the powder was passed through a 50-mesh sieve. Twenty grams of the Caryophyllata Flos powder was extracted with 400 mL of methanol with sonication for 1 hour at 60 °C. The supernatant was separated by centrifugation at 2555 g for 15 minutes and was then collected. Afterwards, an additional 400 mL of methanol was added to the residue and the process was repeated. Both supernatants were then concentrated and dried with a rotary evaporator. The dried residue was dissolved into DMSO to prepare a solution with a concentration of 50 mg/mL for the bioassays. The dried residue was dissolved into methanol to prepare a solution with a concentration of 5 mg/mL for HPLC analysis. Eugenol was purchased from Sigma-Aldrich Corporation (Missouri, USA).

2.2. Preparation of mouse DCs

C57BL/6 mice obtained from National Laboratory Animal Center (NLAC, Taipei, Taiwan) were used in this study. All animals were maintained and handled according to standard protocols. The mouse bone marrow-derived DCs were prepared as described previously (Chu & Lowell, 2005; Lin et al., 2015). Briefly, bone marrow cells were collected and the red blood cells were removed. Next, the cells were seeded in 24-well plates. After 6 days, the DCs were harvested and ready for assays.

2.3. Measurement of cytokine and chemokine production

The protein amounts of cytokines (TNF-α, IL-6, and IL-12p70) and chemokines (RANTES, MIP-1β, and MCP-1) was determined by the enzyme-linked immunosorbent assay (ELISA; eBioscience), as described previously (Chu et al., 2008; Lin et al., 2015). DCs were treated with Caryophyllata Flos methanol extract (CFME) or eugenol, lipopolysaccharide (LPS; 100 ng/mL), or LPS + CFME or eugenol for 24 hours (6 hours for TNF-α). Triplicate treatments were performed for each sample in all experiments.

2.4. Assay for the cytotoxicity of CFME and eugenol

DCs were treated with CFME or eugenol (dissolved in DMSO; Sigma-Aldrich) at various concentrations for 24 hours. The cells were then measured for viability by the CCK-8 assay (Sigma), according to the manufacturer's instructions. Triplicate treatments were performed for each sample in all experiments.

2.5. Analysis of DC maturation

The maturation of DCs was examined by measuring up-regulation of MHC class II and two co-stimulatory molecule expression, as described previously (Huang et al., 2010; Lin et al., 2015). Briefly, DCs were treated with LPS, LPS + DMSO, LPS + CFME or eugenol for 16 hours. The cells were stained with the antibodies specific to mouse CD11c, CD40, CD80, and I-A^b,