



Ethanol extracts of *Saururus chinensis* suppress ovalbumin-sensitization airway inflammation

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ABSTRACT

Aim of the study: The aerial part of *Saururus chinensis* has been used in folk medicine to treat several inflammatory diseases in China and Korea. Previously, our group reported that anti-asthmatic activity of an ethanol extract of *Saururus chinensis* (ESC) might occur, in part, via the inhibition of prostaglandin D₂ (PGD₂) and leukotriene C₄ (LTC₄) production, and degranulation reaction *in vitro*, as well as through the down-regulation of interleukin (IL)-4 and eotaxin mRNA expression in an *in vivo* ovalbumin-sensitization animal model. However, the effects of *Saururus chinensis* on eicosanoid generation, as well as Th2 cytokines and eotaxin production in an *in vivo* asthma model, have not been fully investigated. Moreover, it has not been determined whether ESC can ameliorate airway inflammation *in vivo*. In the present study, we investigated the therapeutic activity of *Saururus chinensis* on ovalbumin (OVA)-sensitized airway inflammation and its major phytochemical compositions.

Materials and methods: Asthma was induced in BALB/c mice by ovalbumin-sensitization and inhalation. ESC (10–100 mg/kg) or dexamethasone (5 mg/kg), a positive control, was administered 7 times orally every 12 h from one day before the first challenge to 1 h before the second challenge. The recruitment of inflammatory cells and hyperplasia of goblet cells were evaluated by H&E and PAS staining. Levels of Th2 cytokines, eotaxin, PGD₂ and LTC₄ were measured to evaluate the anti-inflammatory activity of ESC in OVA-sensitized mice. Contents of major components were analyzed by HPLC using a reversed-phase C18 column.

Results: ESC (10 mg/kg) suppressed allergic airway inflammation by inhibition of the production of IL-4 ($P < 0.001$), IL-5 ($P < 0.05$), IL-13 ($P < 0.001$), eotaxin ($P < 0.001$), PGE₂ ($P < 0.001$), LTC₄ ($P < 0.001$) in lung extract and IgE level ($P < 0.001$) in the serum. In addition, ESC (50 mg/kg) reduced the infiltration of inflammatory cells and hyperplasia of goblet cells in the lung tissues. The anti-inflammatory effect of ESC was comparable to that of the positive control drug, dexamethasone. Its major phytochemical composition includes manassantin A, B and sauchinone.

Conclusions: These results suggest that ESC decreased inflammation and mucus secretion in the OVA-induced bronchial asthma model, and its anti-asthmatic activity may occur in part via the inhibition of Th2 cytokines and eotaxin protein expression, as well as through prostaglandin E₂ (PGE₂) and leukotriene C₄ (LTC₄) generation. This effects may be attributed particularly to the presence of manassantin A, B and sauchinone major component evidenced by a HPLC analysis.

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Abbreviations: ESC, ethanol extract of *Saururus chinensis*; OVA, ovalbumin; AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; *p.o.*, per oral; *i.p.*, intraperitoneal; Th2 cytokine, T helper 2 cytokine; COX-2, cyclooxygenases-2; 5-LOX, 5-lipoxygenases; PBS, phosphate buffered saline; PGD₂, prostaglandin D₂; PGE₂, prostaglandin E₂; LTC₄, leukotriene C₄; IL-4, interleukin-4; IL-5, interleukin-5; IL-13, interleukin-13; IgE, immunoglobulin E; DEX, dexamethasone; ELISA, enzyme-linked immunosorbent assay; H&E staining, hematoxylin and eosin staining; PAS staining, periodic acid-Schiff staining; HPLC, high performance liquid chromatography.

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1. Introduction

Allergic asthma is a chronic pulmonary disease that is characterized by bronchoconstriction and airway hyperresponsiveness (AHR) associated with an elevated serum IgE level and bronchial inflammation. Primary inflammatory lesions of asthma consist of accumulation of T helper type 2 (Th2) cells and high levels of eosinophils in the airway mucosa. Th2 cells regulate asthmatic inflammation through the secretion of Th2 cytokines in the form of interleukin IL-4, IL-5, IL-13, and IL-9 (Renauld, 2001). In addition to its role in the activation of mature eosinophils, IL-5 is the principal Th2 cytokine involved in terminal differentiation and proliferation of eosinophil precursors (Sanderson, 1992; Yamaguchi et al., 1988). Further exposure to allergens leads to the activation of mast cells and the release of histamine and lipid mediators such as prostaglandin D₂ (PGD₂) and leukotriene C₄ (LTC₄), which are important in the inflammatory response (Wells et al., 1986; Baker et al., 1993). Eicosanoids are lipid inflammatory mediators that are biosynthesized by cyclooxygenases (COX) and lipoxygenases (LOX) in many cell types. They are strongly associated with inflammatory disorders, acute as well as chronic. The inhibition of eicosanoid production is one of the important therapeutic strategies used to treat various inflammatory diseases. Among the eicosanoid-generating enzymes, COX-2 (inducible form)-dependent generation of PGs was found to be essential to various inflammatory responses (O'Banion et al., 1992). In addition, LTC₄ produced by 5-LOX also has biological properties that would be expected for molecules that participate in the pathogenesis of bronchial asthma (Tsunoda et al., 1991; Henderson et al., 1996). It has been reported that COX-2 expression in alveolar macrophages from asthmatic subjects may contribute to enhance eosinophil survival through increased PGE₂ production (Profita et al., 2003). Therefore, the dual inhibition of COX-2/5-LOX is believed to be the ideal treatment for allergic diseases and asthma (Fiorucci et al., 2001).

Saururus chinensis (Saururaceae) is a perennial herb that is distributed throughout China and southern Korea, and the aerial portion of *Saururus chinensis* (*Saururus chinensis*) has been used in folk medicine for the treatment of edema, jaundice, gonorrhea, and several inflammatory diseases in Korea (Chung and Shin, 1990). Extracts of *Saururus chinensis* have demonstrated anti-inflammatory and anti-oxidant activities (Kim et al., 2003; Cho et al., 2005), anti-asthmatic (Lee et al., 2006), antihypertensive (Ryu et al., 2008), anti-angiogenic (Yoo et al., 2008), and therapeutic activities for atopic dermatitis (Choi et al., 2008). Among the bioactive compounds isolated from *Saururus chinensis*, lignans such as sauchinone, manassantin A and B, saucerenol D have various biological activities, including anti-inflammatory activity (Lee et al., 2003; Hwang et al., 2003; Rho et al., 2003; Kwon et al., 2005; Moon et al., 2008a,b; Seo et al., 2008; Min et al., 2009), cardiovascular effects (Oh et al., 2008), hypercholesterolemic activities (Lee et al., 2004), and bone destruction- and osteoclast formation-inhibiting activities (Han et al., 2007). Recently, we reported that the anti-asthmatic activity of *Saururus chinensis* might occur via the inhibition of eicosanoid generation and degranulation *in vitro*, as well as the down-regulation of IL-4 and eotaxin mRNA expression in an OVA-sensitized murine asthma model (Lee et al., 2006). However, the effect of extracts of *Saururus chinensis* (ESC) on Th2 cytokines and eotaxin production, as well as the generation of PGE₂, LTC₄, IgE level in the serum and contents of major components of ESC has not been reported to date. In the present study, we investigated ESC would have protective effects on allergic airway inflammation through the regulation of various cytokine, IgE level and the generation of eicosanoids in an OVA-sensitized mouse airway inflammation model as well as its major phytochemical compositions.

2. Materials and methods

2.1. Reagents and instrument

Commercially available enzyme-linked immunosorbent assay antibodies were used to measure PGE₂, LTC₄ (Cayman Chemical, Ann Arbor, MI, USA), IL-4, IL-13 (Biosource, Carlsbad, CA, USA), IL-5, IgE (BD Biosciences, Franklin Lakes, NJ, USA), and eotaxin (R&D System, Minneapolis, MN, USA) at the lung tissue level according to the manufacturer's instructions. Dexamethasone was purchased from Cayman Chemical (Ann Arbor, MI, USA). The HPLC system consisted of a Shimadzu (Shimadzu Co., Japan) LC-20AD pump, SPD-20A UV/VIS detector, and SIL-20A autosampler injector. Column temperature was set at 40 °C using a column heater, CBM-20A (Shimadzu Co., Japan). Ultra-sonicator was Branson 8510 (Branson, Danbury, CT, USA). Sauchinone (SC), manassantin A (MA) and manassantin B (MB) were purified by Prof. J.K. Son at Yeungnam University, Korea and identified their purities by HPLC (purity ≥95%) (Seo et al., 2008). An internal standard, pyrene was purchased from Aldrich (St. Louis, MO, USA, purity ≥98.0%), HPLC grade reagents, ethanol and methanol were purchased from Fisher Scientific Co. (Fairlawn, NJ, USA).

2.2. Plant material and extraction

The aerial parts of *Saururus chinensis* were collected and prepared as described previously (Lee et al., 2006). A voucher specimen has been deposited at the herbarium of the College of Pharmacy, Yeungnam University (YNU 6024), Republic of Korea.

2.3. PLC analysis of marker compounds

HPLC analysis of ESC was achieved on a reversed-phase C18 column (Intersil® ODS-3, 250 mm × 4.6 mm, particle size, 5 μm, GL Sciences Inc., Japan) with a guard column of C18 cartridge (4.0 mm × 3.0 mm, I.D.). An isocratic mobile phase of H₂O and MeOH (30:70) was employed with column temperature at 40 °C. The flow-rate of the mobile phase was 1.0 mL/min, where the column pressure was 120 kg/cm. The chromatogram was monitored at a wavelength of 280 nm throughout the experiments. The injection volume was 10 μL. To obtain calibration curve, stock solutions of sauchinone (1000 μg/mL), manassantin A (1000 μg/mL), manassantin B (1000 μg/mL) and pyrene as an internal standard (300 μg/mL) were prepared in methanol and kept below 4 °C. Standard solutions were prepared by serial dilution of the stock solutions to working ranges of these substances with mobile phase. Calibration graphs were prepared from peak area-ratio of analytes to I.S. in the concentration range, sauchinone; 0.20–100.00 μg/mL, 0.10–50.00 μg/mL, manassantin A; 0.20–100.0 μg/mL and manassantin B; 0.20–100.0 μg/mL, respectively and each compound showed good linearity with correlation coefficient (*r*) of more than 0.9992.

2.4. Animals

Female BALB/c mice (16–20 g) were obtained from Hyochang Science, Daegu, Republic of Korea, and were fed with laboratory chow (Purina Korea) and water *ad libitum*. Animals were acclimatized in a specific pathogen-free animal facility under the conditions of 20–22 °C, 40–60% relative humidity, and 12 h/12 h (light/dark) cycle for at least 7 days. They had free access to food and water except during the time of experiments. Groups of 6 animals were used in all tests. The study was approved by the Institutional Animal Care and Use Committee of Yeungnam University, and all

procedures were conducted in accordance with the U.S. National Institutes of Health guidelines.

2.5. Protocol for allergen sensitization/challenge and ESC treatment

Seven-week-old female BALB/c were sensitized on days 0, 7, and 14 by intraperitoneal (i.p.) injection with 10 µg of OVA in PBS mixed with equal volumes of alum (1 mg) as an adjuvant in a total volume of 200 µL. On days 22 and 24, mice were exposed to aerosolized OVA (1% OVA in PBS) or PBS for 1 h. ESC or dexamethasone (DEX) was administered 7 times orally every 12 h from one day before the first challenge to 1 h before the second challenge.

2.6. Lung histology

At 24 h after the last OVA challenge, mice were sacrificed and the lungs were fixed in 10% neutral buffered formalin. After fixation, 5-µm paraffin sections were stained with haematoxylin and eosin (H&E) for the evaluation of inflammatory cell infiltration, or with periodic acid-Schiff (PAS) for the identification of goblet cells. The slides were examined by a treatment-blind pathologist. The inflammatory score was graded on the following scale: 0, none; 1, minimal; 2, mild; 3, moderate; 4, severe. Hyperplasia of the goblet cells was quantified according to the percentage of PAS-positive among all epithelial cells: 0, none; 1, <25%; 2, 25–50%; 3, 50–75%; 4, >75%.

2.7. Lung homogenates

Mice lungs were removed 24 h after the last OVA-sensitization, weighed, homogenized (IKA T10 basic Homogenizer Workcenter, Germany) in 1.0 mL PBS, and centrifuged (10,000 × g, for 10 min at 4 °C). The supernatants were collected and stored at –70 °C for subsequent cytokine, eotaxin, and eicosanoid determination. Cytokines, eotaxin, and eicosanoid levels were determined per mg of tissue using ELISA.

2.8. Determination of PGE₂, LTC₄, eotaxin, and Th2 cytokines

Commercially available enzyme-linked immunosorbent assay antibodies were used to measure IL-4, IL-5, IL-13, eotaxin, PGE₂, and LTC₄ in lung extract according to the manufacturers' instructions.

2.9. Measurement of serum IgE level

Blood was collected from the mice while under ether anesthesia. Serum samples were obtained by centrifugation, and were stored at –80 °C until assay. Serum IgE level was measured by an enzyme-linked immunosorbent assay (BD Biosciences, Franklin Lakes, NJ, USA).

2.10. Statistical analysis

All values were represented as an arithmetic mean ± S.D. One-way ANOVA was used for comparison between the PBS-sensitized control and ESC or DEX treatment groups (SPSS version 17.0).

3. Results and discussion

Recently, we reported on the anti-asthmatic activity of ethanol extracts of *Saururus chinensis* (ESC) in an OVA-sensitized murine asthma model (Lee et al., 2006) and anti-inflammatory compounds from *Saururus chinensis* such as meso-dihydroguaiaretic acid (Moon et al., 2008a,b), saucerneol D (Moon et al., 2008a,b),

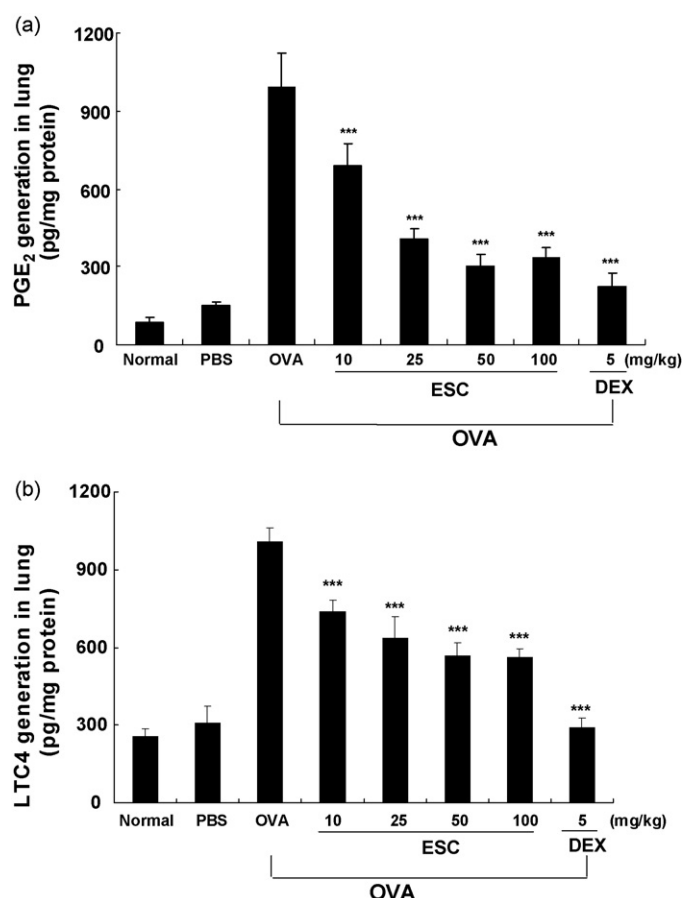


Fig. 1. ESC reduced PGE₂ and LTC₄ generation in the lung tissues. Lung homogenates were prepared as described in Section 2. The levels of PGE₂ (a) and LTC₄ (b) were measured by ELISA, and are expressed as pg/mg of total protein contents. ****P* < 0.001 was considered significantly different from the PBS-sensitized control using a one-way ANOVA test.

and various lignans (Seo et al., 2008). We previously suggested that the anti-asthmatic activity of ESC might occur through the inhibition of both PGD₂ and LTC₄ generation in mast cells, and the reduction of eosinophil infiltration through the down-regulation of eotaxin and IL-4 mRNA transcription (Lee et al., 2006). However, the effects of ESC on the Th2 cytokine and eotaxin protein expression, as well as on the generation of eicosanoids, have not been sufficiently elucidated. In the present study, we investigated whether ESC would be capable of reducing these bioactive compounds in a mouse model of OVA-sensitized asthma.

It has been reported that repeated challenges with allergens induce changes in the COX-2 protein level, and cause a corresponding increase in the PGE₂ level in BALF in murine lungs. Among the eicosanoid-generating enzymes, COX-2 is thought to be essential for the production of PGE₂ in various inflammatory sites (Profita et al., 2003; Park et al., 2007; Chen et al., 2008), and the inhibitory activity of ESC on the generation of PGE₂ in the lung tissues has thus been examined. In this study, the PGE₂ level in the lung tissues obtained from the PBS-sensitized group was 152.4 ± 11.2 pg/mg. Conversely, the PGE₂ level of the OVA-sensitized lung tissues was significantly higher compared with the PBS-sensitized group (987.0 ± 131.7 pg/mg). When 10–100 mg/kg of ESC was administered orally 7 times to the mice, the level of PGE₂ was significantly reduced in a dose-dependent manner (Fig. 1a). This result suggests that the reduced level of PGE₂ in the lung tissues may be due to the inhibition of COX-2 protein expression in inflammatory cells. Under

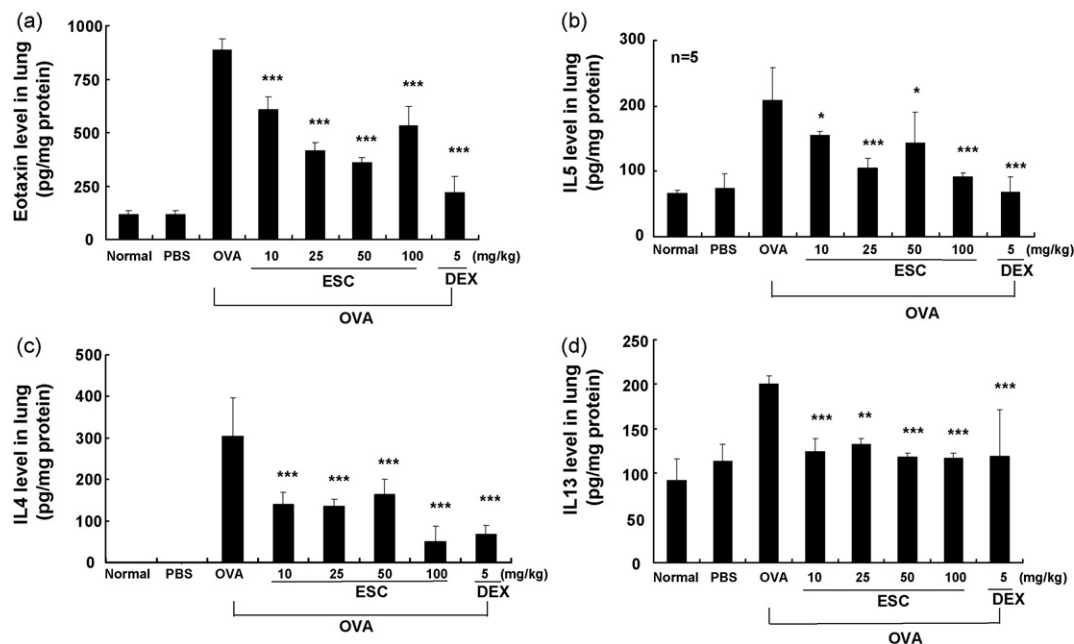


Fig. 2. ESC reduced eotaxin and various Th2 cytokines in the lung tissues. Lung homogenates were prepared and stored as described in Section 2. The eotaxin (a), IL-5 (b), IL-4 (c) and IL-13 levels (d) were measured by ELISA, and are expressed as pg/mg of total protein contents. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ were considered significantly different from the PBS-sensitized control using a one-way ANOVA test.

the same conditions, DEX (5 mg/kg) inhibited PGE₂ generation by about 80%.

Arachidonic acid can also be converted to the cysteinyl leukotrienes (LTC₄, LTD₄, and LTE₄) that are characteristic of asthma, and induce conditions such as potent bronchoconstriction, increased endothelial membrane permeability leading to airway edema, and enhanced secretion of thick, viscous mucus (Wenzel, 2003). The antagonists and inhibitors of 5-LOX may provide a new approach to the pharmacologic treatment of asthma (Israel, 1994). Given the significant role of the inflammatory process in asthma, the 5-LOX inhibitor, zileuton, has been developed for the treatment of airway inflammation (Hui et al., 1991). Therefore, the inhibitory activity of ESC on the generation of LTC₄ in the lung tissues was examined. Fig. 1b shows that the LTC₄ level in the lung tissues obtained from the PBS-sensitized group was 74.3 ± 21.5 pg/mg, whereas the LTC₄ level in the OVA-challenged lung tissues was about threefold higher than that of the PBS-sensitized group. When 10–100 mg/kg of ESC was administered orally 7 times to the mice, LTC₄ generation was inhibited in a dose-dependent manner, from about 25% (10 mg/kg) to 56% (100 mg/kg). This clearly demonstrates that ESC has COX-2/5-LOX dual inhibitory activity both *in vivo* and *in vitro* (Lee et al., 2006).

Previous reports on the interaction between eotaxin and IL-5 have indicated that eotaxin causes substantial airway eosinophilia and a marked increase in eosinophils in conjunction with IL-5 (Collins et al., 1995; Mould et al., 2000). IL-5 produced by Th2 cells is a key regulator in the development, migration, and activation of eosinophils (Hogan et al., 1998). We previously reported that ESC reduced the number of eosinophils that infiltrated the airway (Lee et al., 2006). Therefore, the effect of ESC on the protein level of eotaxin and IL-5 was examined. The eotaxin level (Fig. 2a) in the lung tissues obtained from the PBS-sensitized group was 117.2 ± 18.5 pg/mg. In contrast, the eotaxin level of the OVA-sensitized mice lung tissues was significantly higher than that of the PBS-sensitized group (887.2 ± 51.3 pg/mL). ESC inhibited the levels of eotaxin by about 31% at a dose of 10 mg/kg, and more gradually inhibited the eotaxin level at doses up to 50 mg/kg. Similarly, the level of IL-5 was significantly reduced at dose of 10 mg/kg

ESC (Fig. 2b). As expected, DEX (5 mg/kg) strongly inhibited both eotaxin (about 75%) and IL-5 levels (about 67%) in the lung tissues. This suggests that the reduction of eosinophils infiltrating the airway by ESC (Lee et al., 2006) may occur in part via the inhibition of eotaxin and IL-5 protein expression.

Clinical and experimental investigations have reported a strong correlation between the presence of Th2 cells and disease severity, suggesting an important role of these cells in asthmatic diseases (Finotto et al., 2000; Renauld, 2001). Among the Th2 cytokines, high levels of IL-4 and IL-13 were produced in asthmatic lungs, and are believed to be the key regulators of allergic disease (Finotto et al., 2000; Renauld, 2001; Romagnani, 2002). Therefore, we measured the IL-4 and IL-13 levels in the lung tissues. As shown in Fig. 2c and d, IL-4 and IL-13 levels in the lung tissues obtained from the PBS-sensitized group were 3.5 ± 0.4 and 114.0 ± 18.4 pg/mg, respectively, while IL-4 and IL-13 levels in the OVA-sensitized lung tissues were 305.5 ± 91.5 and 200.1 ± 9.6 g/mg, respectively. However, at a dose of 10 mg/kg ESC, increased levels of IL-4 and IL-13 were significantly reduced by about 50% and about 37%, respectively.

Next, we examined the effect of total serum immunoglobulin (Ig)E level in an experimental animal model. In general, IgE synthesis by B cells is primarily regulated by Th2 cytokines such as IL-4 and IL-13, which play a key role in the hyperproduction of IgE. Elevated levels of IgE are associated with both the immediate allergic response and the late-phase or chronic inflammatory response in the skin and lungs (Platts-Mills, 2001). Thus, we attempted to determine the effects of ESC on total serum IgE levels, as shown in Fig. 3. The total IgE levels in the serum obtained from the PBS-sensitized and OVA-sensitized groups were 381 ± 172.7 ng/mg and 2629.6 ± 226.1 ng/mg, respectively. The level of IgE was decreased about 60% following treatment with 10 mg/kg of ESC, and the inhibition gradually increased up to 50 mg/kg. It has been reported that enhanced production of IL-4 and IL-13 by allergen-specific T cells is responsible for increased IgE synthesis and the development of allergic diseases in certain individuals (de Vries, 1994). ESC inhibited this rise in serum total IgE level, probably as a result of the inhibition of IL-4 and IL-13 generation.

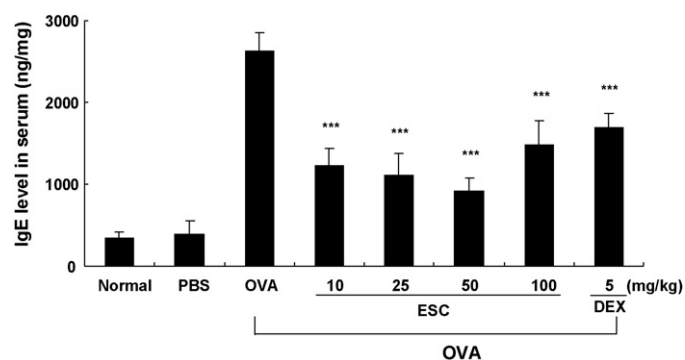


Fig. 3. ESC reduced the serum total IgE level. Sera were prepared and stored as described in Section 2. The IgE level was measured by ELISA, and is expressed as pg/mg of total protein contents. *** $P < 0.001$ was considered significantly different from the PBS-sensitized control using a one-way ANOVA test.

It has been suggested that Th2 cytokines such as IL-4, IL-5, and IL-13 have important functions in the processes of airway inflammation and airway remodeling characterized by the infiltration of inflammatory cells and hyperplasia of goblet cells/mucus hypersecretion (Hoshino et al., 2005). We therefore performed a

Table 1

Effects of ESC on infiltration of inflammatory cells and hyperplasia of goblet cells in lung tissues of OVA-challenged mice.

Group	Inflammatory cells (H&E)	Goblet cell hyperplasia (PAS)
Normal	0.0 ± 0.0	0.0 ± 0.0
PBS	0.0 ± 0.0	0.0 ± 0.0
OVA	3.8 ± 0.3 ^a	3.5 ± 0.5 ^a
ESC (50 mg/kg)	2.8 ± 0.2 ^b	1.4 ± 0.2 ^c
DEX (5 mg/kg)	0.6 ± 0.2 ^d	0.0 ± 0.0 ^d

Inflammatory cells: 0, none; 1, minimal; 2, mild; 3, moderate; 4, severe.

Goblet cells: 0, none; 1, <25%; 2, 25–50%; 3, 50–75%; 4, >75%.

Values represent the mean ± SE of 4–5 mice in each group. DEX, dexamethasone.

^a $P < 0.001$ (vs. PBS group).

^b $P < 0.05$ (vs. OVA group).

^c $P < 0.01$ (vs. OVA group).

^d $P < 0.001$ (vs. OVA group).

histological examination. The tissue sections were stained with H&E and PAS in order to evaluate inflammatory cell infiltration and goblet cell hyperplasia, respectively. The infiltration of inflammatory cells was remarkably increased in the lungs of OVA-sensitized mice as compared with that of normal or PBS-sensitized mice. This increase was significantly reduced by the treatment of mice with ESC (50 mg/kg, data not shown). Similarly, there was a

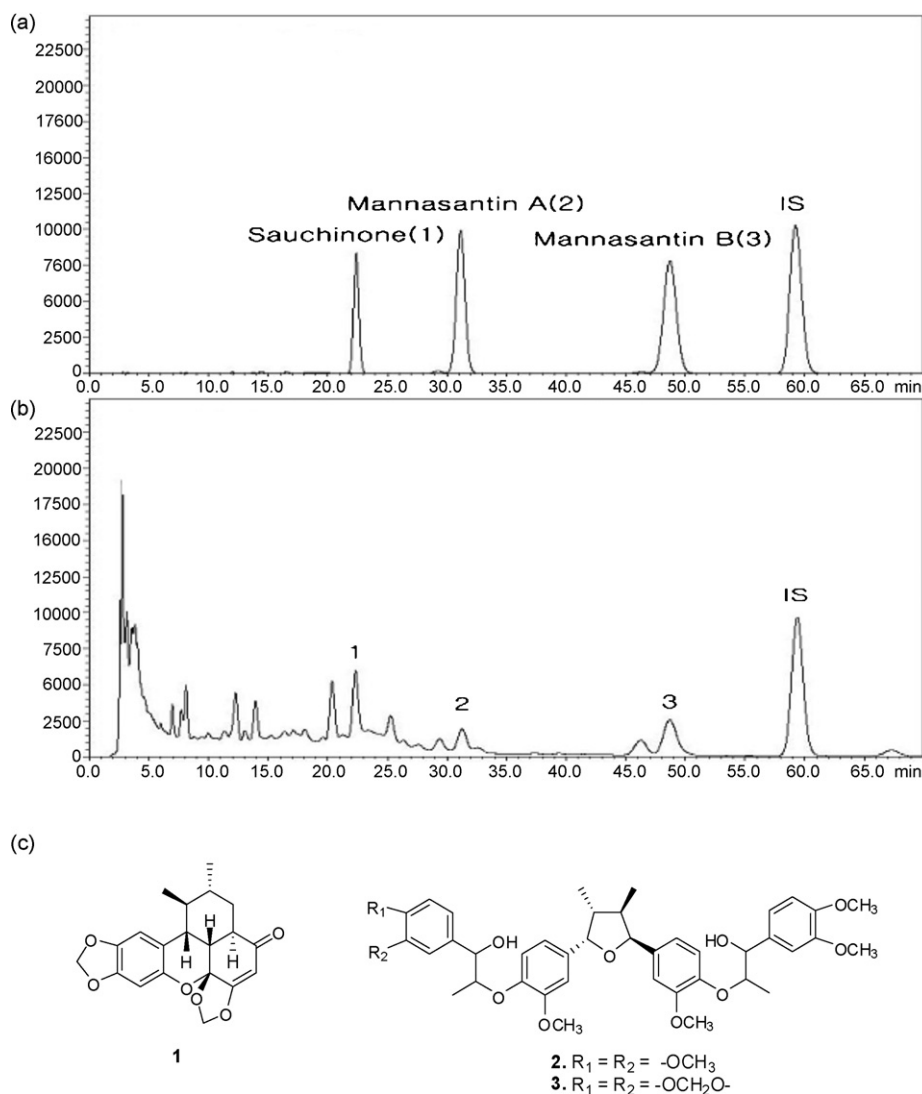


Fig. 4. HPLC chromatograms of standard mixture (A) and ethanol extract (B) of the aerial part of *Saururus chinensis*, and structures (C) of **1**; sauchinone, **2**; mannasantin A, **3**; mannasantin B, **IS**; internal standard, pyrene.

marked increase in the hyperplasia of goblet cells in the lungs of OVA-sensitized mice. The treatment of mice with ESC (50 mg/kg) significantly decreased the number of PAS-positive cells. The infiltration of inflammatory cells and PAS-positive cells was quantified, and the scores are shown in Table 1. DEX (5 mg/kg) was used as a positive control to treat asthmatic lung disease, and almost completely blocked the increases in the inflammatory cell and goblet cells. These results indicate that ESC decreased the airway inflammation and mucus hypersecretion.

In order to determine the acute toxicity of ESC, mice were given up to 800 mg/kg of ESC, and no apparent toxicity (including mortality and changes in body weight) was observed during the 14-day observation period (data not shown). Thus, oral administration of ESC might be nontoxic, and may be used safely in humans at moderate doses.

Quantitative HPLC analysis of those compounds from ESC was accomplished on a reversed-phase C18 column. The ESC contained 3.5% (w/w) of sauchinone, 0.86% (w/w) of manassantin A and 1.83% (w/w) of manassantin B (Fig. 4). Quite recently, it was reported that sauchinone isolated from *Saururus chinensis* inhibited allergen-induced airway inflammation through the repressing GATA-3 activity for Th2 cell development *in vivo* (Min et al., 2009). It has been also reported that anti-inflammatory activity of manassantin A and B (Lee et al., 2003; Kwon et al., 2005; Seo et al., 2008). Our recent results showed that the inhibitory activity of sauchinone on the generation of eicosanoids and degranulation reaction in bone marrow-derived mast cells is similar to that of manassantin A and B (manuscript in preparation). Therefore, even though sauchinone showed airway anti-inflammatory activity *in vivo*, the possibility that suppression of airway inflammation by manassantin A and B are not completely excluded. Further experiment will be needed to clarify this possibility near the future.

In conclusion, in experimental asthma, treatment with ESC effectively inhibits allergic airway inflammation via the down-regulation of both eotaxin and Th2 cytokines generation, and additional inhibition of both eicosanoid generation in the lung tissues and suppression of IgE level in the serum. Therefore, ESC plays multiple roles as an anti-inflammatory and anti-asthmatic activity via the regulation of various bioactive mediators. Although the mechanism of action of ESC in the murine asthma model appears to be similar to that of DEX, further studies will be needed to delineate the inhibitory mechanism of ESC. The results of this study provide an additional rationale for using *Saururus chinensis* to treat various inflammation-related disorders.

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