Journal of Life Science 2008 Vol. 18. No. 10. 1336~1341 ©JLS / ISSN 1225-9918

Amelioration of Asthmatic-Related Symptoms by an Aqueous Extract of *Angelica archangelica* L.

Jin-Chul Heo and Sang-Han Lee*

Food & Bio-Industry Research Institute, and Department of Food Science & Biotechnology Kyungpook National University, Daegu 702-701, Korea

Received September 2, 2008 / Accepted October 23, 2008

Inflammation through the respiratory tract is a crucial event in immune disorders, including asthma, and atopic rhinitis. To investigate whether an aqueous extract of *Angelica archangelica* L. (AaL) has a beneficial influence in terms of anti-asthmatic activity, its effects on an ovalbumin-induced asthmatic model were examined. Mice sensitized to ovalbumin were orally administered the AaL extract, and their lungs examined by Haematoxylin-Eosin staining to determine IL-4/13 cytokine expression. The AaL extract exerted strong anti-asthmatic effects by regulating each level in the $CD4^+$ cell number, IL-4/13, and other target markers in the lungs. Together, these results collectively indicate that the aqueous AaL extract ameliorates asthmatic symptoms effectively in a mouse ovalbumin-challenge model.

Key words : *Angelica archangelica* L., asthma, inflammation, aqueous extract, ovalbumin-challenge model

Introduction

It is well-documented that reactive oxygen species (ROS) play pivotal roles in triggering some degenerative diseases in cells [2]. To expedite the reduction of ROS, anti-oxidants are required for specific degenerative diseases, such as asthma and chronic obstructive pulmonary disorders. Chronic asthma-related disorders arise, in cases where the ROS attack bronchoalveolar cells [2,12]. Asthma is a classical disorder of airway hyper-responses, which is originated from a T cell imbalance leading to molecular inflammation [16]. In bronchial asthma, various mediators induce the infiltration of mast cells, eosinophils and Th2 lymphocytes into damage with downstream mediators, resulting in classical asthmatic symptoms, such as mucus over-production, airway hyper-responsiveness, and submucosal thickness [5].

Angelica archangelica L. (AaL) is a well-known table vegetable for its vitamin content. The alkaline vegetable contains vitamin C, B1, B2, and various minerals. Unsaturated linoleic acid, and chlorophylls are also important components of the vegetable [18]. The fresh leaves exhibit excellent vitality and have strong growth activity, therefore the vegetable is booming for the naturally functional food sources. Additionally, the vegetable has beneficial effects on hypertension, constipation, diuretic activity, anti-cholesterol activity, and moreover antitumor activity, due to its various kinds of flavonoids, saponin, coumarins content [14]. Recent report shows that some bioactive materials in *Angelica* sp. exerts anti-mutagenic and anti-tumor effects by removing radicals induced by smoking, and protecting their DNA in the lung tissues [11]. Other studies were carried out on the chemical composition of seed and root oils from AaL and revealed 58 compounds that were identified from the oils and a high content of β-phellandrene (74.7%) was found in *Angelica* seed oil, which contained and identified as 58 compounds [11]. The two types of essential oils including β-phellandrene from the fruits of AaL growing in Iceland were prepared by steam distillation and their constituent composition was investigated by GC/MS. The oils exhibited potent activities in reducing PANC-1 human pancreas cancer cell growth and Crl mouse breast cancer cells in a concentration-dependent manner [19]. It is well-known that AaL has been widely used in alternative medicines as a remedy against bowel disturbances, arthritic disease, immune-related symptoms etc [20]. However, there is still lack of scientific data about its capability against various degenerative disorders. It is also reported that total AaL has potential in reducing a chronic ethanol-induced in vivo experiment. This was confirmed by administering it to ICR mice with AaL (10, 25, and 50 mg/kg p.o.) after 2 weeks the samples ameliorated the ethanol-induced hepatotoxicity, suggesting that AaL hence indirectly protects the liver from oxidative stress such as radicals [23].

***Corresponding author**
Tel : +82-53-950-7754, Fax : +82-53-950-6772
E-mail : sang@knu.ac.kr

Here, for the first time, the potential of AaL in alleviating asthmatic symptoms *in vivo* are demonstrated. The major finding of this study is that AaL exhibits anti-oxidant and anti-asthmatic activities *in vivo*, as confirmed with data from the evaluation of IL-4, IL-13, IgE, and $CD4^+$ levels using ELISAs, and immunohistochemistry.

Materials and Methods

Antioxidant activity

DPPH and FRAP assays were carried out as described elsewhere [6].

Animal experiment

Mice weighing 25.5±2.5 g (Balb/c, male, Dae Han Biolink, Eumsoung, Korea) were utilized. Each group was singly housed in cages, and five mice were used for *in vivo* experiments. Animal care was performed as described previously, with slight modifications [8]. All procedures were performed in compliance with the Guiding Principles for the Care and Use of Animals (National Research Council, 1996), the Animal Welfare Committee of Kyungpook National University, and KRIBB (NPRC) Committee for Animal Care. Animals received tap water and food *ad libitum*, and were maintained in a room under standard laboratory conditions (23±1°C and 50±5% humidity) with a 12 hr dark/light cycle. Rules for animal experiments, including ethical care, were strictly observed under guidance of the Committee.

Preparation and fractionation of samples

Angelica archangelica L. was obtained from a Farm in Yuseong, Korea. The leaf was incubated in an oven at 60°C for 12 hr, and the dried leaf was extracted with water (1:1; v/v) for 18 hr, and centrifuged at 2,000 g for 10 min to collect supernatant fractions before storage at -70°C. The supernatant fractions obtained were freeze-dried and solubilized in distilled water for *in vivo* assays (data not shown). The plant was collected between September and November, 2005, and identified by a senior of Kyungpook National University, Daegu, Korea. Voucher specimens of the plant have been deposited in the Enzyme Biotechnology Lab, KNU.

In vivo model and immunohistochemistry

Mice underwent ovalbumin (OVA) sensitization and challenge using an earlier protocol with slight modifications [3,10,22]. OVA (100 μg/ml in saline, sterile filtered) was mixed with an equal volume of 0.1% (w/v) aluminum potassium phosphate (alum; Sigma), and the pH adjusted to 6.5 with 10 N NaOH. For the histopathology and immunohistochemistry, tissues were embedded in paraffin, cut into 4-6 μm sections, and set overnight on a slide warmer at 37°C as described previously [6]. Hematoxylin and Eosin (HE) staining was performed as described previously [9,15]. For each mouse, five randomly-selected airways of the left lung were analyzed. For immunohistochemical analysis, lung tissues were fixed for 24 hr in a 10% neutral buffered formalin solution, and routinely processed as described previously [6]. Paraffin section slides were subsequently incubated overnight at 4°C with rabbit CD4 antibody (SantaCruz Biotechnology; 1:200). Periodic Acid Schiff (PAS) staining was carried out, as described previously [22].

Statistical analysis

All data was expressed as means±standard deviation. Statistical significance was determined with the Student-Newman-Keuls method for independent means, using the Microsoft Excel program [4]. The critical level for significance was set at $P<0.05$.

Results and Discussion

Oxidative stress levels are increased in the respiratory tracts of patients with asthma symptoms, including atopic rhinitis. Since oxidative attack of the bronchial pathways is an initial step that triggers asthmatic symptoms [7], it is important to evaluate the molecular events underlying the inflammatory process and oxidative stress [1].

Antioxidant activity

It was first speculated that, the antioxidant activity is correlated with molecular inflammation, resulting in inhibition of asthmatic features. As shown in Fig. 1, the anti-oxidant activity was examined by an aqueous extract of *Angelica archangelica* L. (AaL). Three solvents were used to fractions to test the activity. As a result, an aqueous extract of AaL exhibited potent antioxidant activity by DPPH (Fig. 1A) and FRAP assay (Fig. 1B) in a concentration-dependent manner. The other absolute ethanol, 50% ethanolic and methanolic extracts did not show any significant activity (Fig. 1A, B, and data not shown), compared to the aqueous extract.

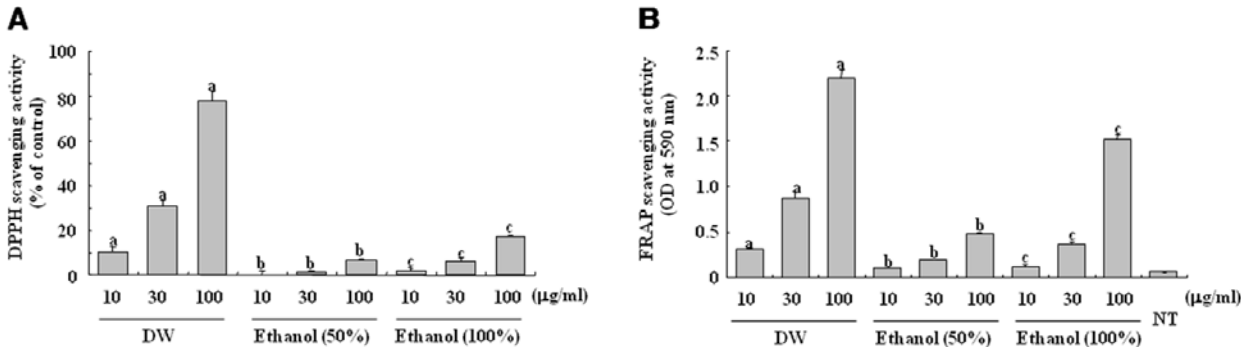

Fig. 1. Comparison of antioxidant activity by *in vitro* DPPH and FRAP scavenging assays. An aqueous extract of *Angelica archangelica* L. (AaL) enhanced DPPH (A) and FRAP (B) scavenging activities. [a]Significant differences from NT, $p<0.05$. [b]Significant differences from aqueous extract at each concentration, $p<0.05$. [c]Significant differences from ethanol (50%) extract at each concentration, $p<0.05$.

Anti-asthmatic activity by an *in vivo* asthma model

Whether the aqueous extracts of AaL had anti-asthmatic effects *in vivo* was investigated next using an ovalbumin-induced mouse model. HE staining exposed clean tissue in the control (Fig. 2a), which is not treated with ovalbumin, but revealed many immune positive cells around small parts of the lung in ovalbumin-treated lung tissue (Fig. 2d). Periodic Acid Schiff (PAS) staining showed that mucus was detected in the OVA exposed lung tissue (Fig. 2b and e). This result strongly suggests that OVA treatment causes asthmatic symptoms in the animals, resulting in strong mucus production potential (Fig. 2e, arrows and data not shown). The administration of aqueous extracts of AaL to animals, caused many immune-positive cells to disappear (Fig. 2g), and indirectly confirmed that the mucus production was significantly reduced in the lung tissues (Fig. 2h, head-arrow). In contrast, the number of total immune cells from the group treated with AaL was significantly lower than that in the ovalbumin-challenged lung tissues, as shown in Figs. 2e and h.

Immunohistochemical analysis

In asthma, the status of Th1 and Th2 cell populations play an important role in immune cell differentiation by controlling the balance of the cells [17]. Critically speaking, Th1 cell populations have more numbers of $CD4^+$ cells than $CD8^+$ cells, whereas Th2 cell populations have not. Therefore, the comparison of the cell populations by some materials or components such as food fractions is a hallmark of the homeostasis of the human body. Therefore whether $CD4^+$ cells were expressed additionally in immune cells around the lung tissues was assessed by immunohistochemistry using

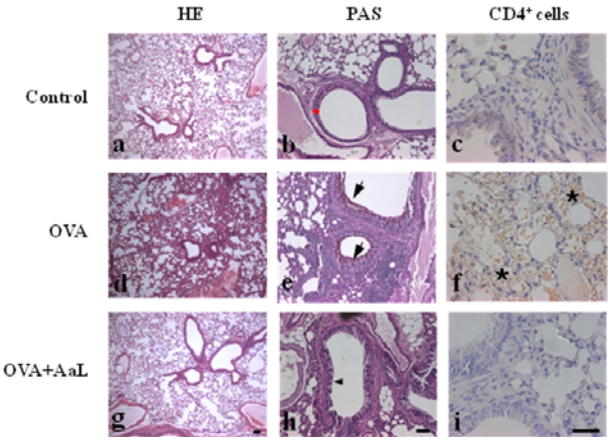

Fig. 2. Immunological comparison of alleviation of asthmatic symptoms using an aqueous extract of *Angelica archangelica* L. Immunohistochemical analysis of an OVA-induced asthmatic mouse model clearly demonstrated that the *Angelica archangelica* L. extract ameliorates $CD4^+$ cells by controlling immune cell proliferation. $CD4^+$ cells (f, and i), HE-stained (d, and g), and PAS-responsive (e, and h) cells with or without AaL in lung tissue of the asthma-induced model. A part of the immune response (arrowhead, scale bar=50 μm) is shown to present the exact distribution of asthma-related immune cells. OVA: ovalbumin-treated (100 μg/ml), AaL: 25 μg/ml of the AaL extract. Asterisks denote $CD4^+$-responsive cells, such as eosinophils.

a CD4 monoclonal antibody. As a result, the total $CD4^+$ cell numbers of control were at a very low level, whereas that of OVA-induced sample showed significant expression in the tissues (asterisks in Fig. 2f). In contrast to the OVA-induced samples, AaL treatment dramatically reduced the expression of $CD4^+$ cell numbers (Figs. 2f and i), which was similar in pattern to that estimated using HE (Figs. 2d, and g) and PAS staining (Figs. 2e, and h). Next, we confirmed

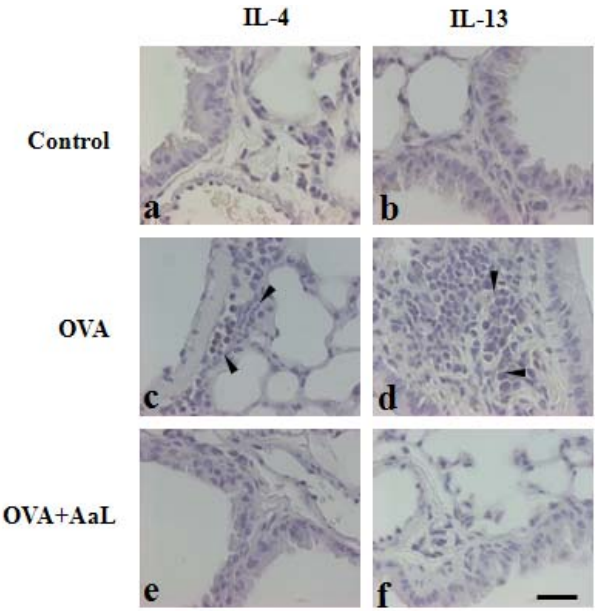

Fig. 3. Alleviation of IL-4 and IL-13 expression by an aqueous extract of *Angelica archangelica* L. Immunohistochemical analysis of an OVA-induced asthmatic mouse model definately demonstrated that the AaL extract decreases IL-4 and IL-13 expression by controlling immune cell proliferation. Control (a, and b), OVA-treated (c, and d), or OVA-treated with AaL extract (e, and f) was treated and immunohistochemically analyzed with IL-4 or IL-13 antibody in lung tissue of the asthma-induced model. A part of the immune response (arrowhead, scale bar=50 μm) is shown to present the exact distribution of asthma-related immune cells. OVA: ovalbumin-treated (100 μg/ml), AaL: 25 μg/ml of the *Angelica archangelica* L. extract. Asterisks denote $CD4^+$-expressing cells, such as eosinophils.

whether the AaL extract show the inhibitory activity by decreasing IL-4 and Il-13 expression, which is a pivotal clinically asthma symptom. In Fig. 3, OVA-treated tissue samples showed lots of IL-4 (Fig. 3c) and IL-13 (Fig. 3d) positive cells (arrowheads), whereas OVA plus AaL treated tissue samples significantly reduced the numbers (Fig. 3 e and f). These results suggest that the extract exhibit some potent anti-asthmatic activity in vivo.

Expressions of cytokines

To assess again whether the asthma-related cellular markers decrease during AaL treatment, cytokines (IL-4, IL-13), and IgE levels were measured using ELISA [6,21]. The serum IL-4 content was 91.5±17.8 pg/ml in the control, and 235.8±108.9 pg/ml in the OVA-challenged group. This increase in the IL-4 level (up to 2.5 times) may be associated with the OVA-induced responses of immune cells. Treatment with *Angelica archangelica* L. extracts decreased the IL-4 level to approximately 44% (Fig. 4a). A 77% decrease in IL-13 expression was clearly observed in the group treated with OVA plus AaL (Fig. 4b). ELISA data, confirmed by IgE expression in cells (Fig. 5), showed that the levels of asthma-related molecular markers were decreased by AaL in an OVA-challenged mouse model (approximately 80%). The numbers of bronchoalveolar lavage (BAL) eosinophils and neutrophils, and moreover IL-25 and IL-31 [17] were additionally confirmed by examining the cells under a phase contrast microscope and additional ELISA (data not shown). The expression patterns of matrix

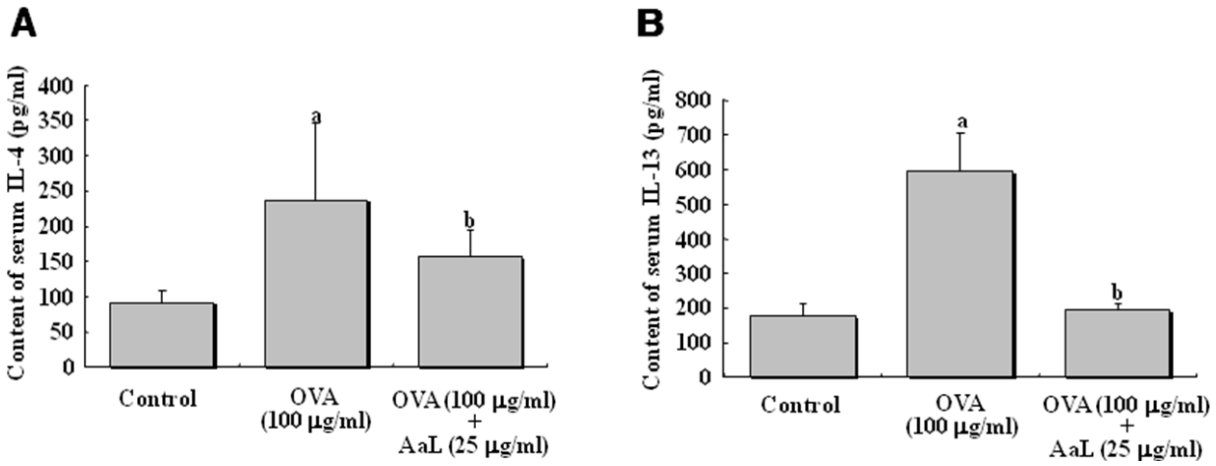

Fig. 4. Inhibitory effects of the aqueous extract of *Angelica archangelica* L. on an OVA-challenge asthmatic mouse model. Serum IL-4 (A) and IL-13 (B) levels were determined using ELISA, as described previously [6]. OVA: ovalbumin-treated (100 μg/ml), AaL: 25 μg/ml of AaL extract. [a]Significant differences from control, $p<0.05$. [b]Significant differences from OVA, $p<0.05$.

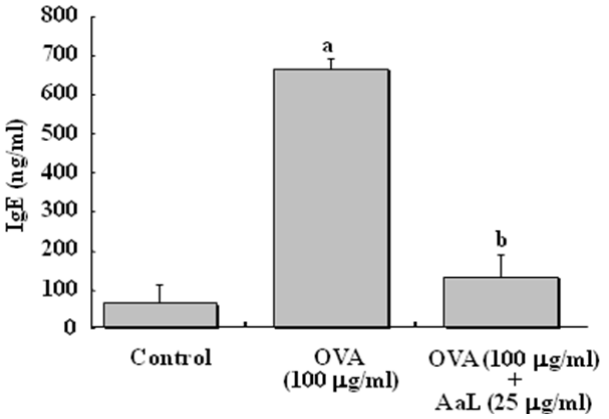

Fig. 5. Decrease of IgE level by the aqueous extract of *Angelica archangelica* L. on an OVA-challenge asthmatic mouse model. Serum IgE level was determined using ELISA, as described previously [6]. OVA: ovalbumin-treated (100 μg/ml), AaL: 25 μg/ml of AaL extract. [a]Significant differences from control, $p<0.05$. [b]Significant differences from OVA, $p<0.05$.

metalloproteinases (MMPs) were also examined to compare the basal levels of asthmatic molecular markers under anti-asthmatic conditions, which resulted in the decrease of the activity by the AaL treatment (data not shown).

In summary, it was confirmed that the aqueous AaL extract exerts anti-asthmatic activity *in vitro* and *in vivo* by alleviating asthma-related cytokine activity, as confirmed by cell staining, cytokine ELISA, and IgE excretion activity. This study cannot rule out the possibility on the precise atopic dermatitis mechanisms by manipulating anti-asthmatic activity via amelioration of asthma-related molecular signal(s). Future investigations should focus on the collection of potential anti-asthmatic component(s) , such as polyphenols from AaL extracts, and subsequent purification on a large scale, if it exists.

Acknowledgment

This work was supported by the Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea (S.-H. L).

References

1. Aruoma, O. I. 2003. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. *Mutat. Res.* **523-524**, 9-20.
2. Bast, A., G. R. Haenen and C. J. Doelman. 1991. Oxidants and antioxidants: state of the art. *Am. J. Med.* **91**, 2S-13S.
3. Choi, J. H., S. W. Oh, M. S. Kang, H. J. Kwon, G. T. Oh and D. K. Kim. 2005. Trichostatin A attenuates airway inflammation in mouse asthma model. *Clin. Exp. Allergy* **35**, 89-96.
4. Falkeholm, L., C. A. Grant, A. Magnusson and E. Moller. 2001. Xylene-free method for histological preparation: a multicentre evaluation. *Lab. Invest* **81**, 1213-1221.
5. Greene, L. S. 1995. Asthma and oxidant stress: nutritional, environmental, and genetic risk factors. *J. Am. Coll. Nutr.* 14, 317-324.
6. Heo, J. C., S. W. Woo, M. A. Kweon, J. Y. Park, H. K. Lee, M. Son, J. R. Rho and S. H. Lee. 2008. Aqueous extract of the Helianthus annuus seed alleviates asthmatic symptoms *in vivo*. *Int. J. Mol. Med.* **21**, 57-61.
7. Huang, D., B. Ou and R. L. Prior. 2005. The chemistry behind antioxidant capacity assays. *J. Agr. Food Chem.* **53**, 1841-1856.
8. Hwang, Y. K., J. S. Chun, P. D. Yoo, J. Y. Ma, B. H. Hyun, S. U. Kim, K. T. Chang and S. H. Lee. 2004. Occlusal reduction of unilateral molars influences change of stress-related hormones in rats. *Scand. J. Lab. Anim. Sci.* **31**, 73-77.
9. Kim, D. Y., S. Y. Ryu, J. E. Lim, Y. S. Lee and J. Y. Ro. 2007. Anti-inflammatory mechanism of simvastatin in mouse allergic asthma model. *Eur. J. Pharmacol.* **557**, 76-86.
10. Lee, E., K. Ha, J. M. Yook, M. H. Jin, C. S. Seo, K. H. Son, H. P. Kim, K. H. Bae, S. S. Kang, J. H. Son and H. W. Chang. 2006. Anti-asthmatic activity of an ethanol extract from Saururus chinensis. *Biol. Pharm. Bull.* **29**, 211-215.
11. Lopes, D., H. Strobl and P. Kolodziejczyk. 2004. 14-Methylpentadecano-15-lactone (muscolide): a new macrocyclic lactone from the oil of Angelica archangelica L. *Chem. Biodivers.* **1**, 1880-1887.
12. Mak, J. C. and M. M. Chan-Yeung. 2006. Reactive oxidant species in asthma. *Curr. Opin. Pulm. Med.* **12**, 7-11.
13. Matsubara, K., H. Matsumoto, M. Iushinal, M. Mori, N. Nakajima, M. Fuchigami, H. Yoshida and H. Hada. 2005. Inhibitory effect of glycolipids from spinach on *in vitro* and *ex vivo* angiogenesis. *Oncol. Rep.* **14**, 157-160.
14. Piersen, C. E. 2003. Phytoestrogens in botanical dietary supplements: implications for cancer. *Integr. Cancer Ther.* **2**, 120-138.
15. Ponikau, J. U., D. A. Sherris, G. M. Kephart, E. B. Kern, T. A. Gaffey, J. E. Tarara and H. Kita. 2003. Features of airway remodeling and eosinophilic inflammation in chronic rhinosinusitis: is the histopathology similar to asthma? *J. Allergy Clin. Immunol.* **112**, 877-882.
16. Rahman, I., S. K. Biswas and A. Kode. 2006. Oxidant and antioxidant balance in the airways and airway diseases. *Eur. J. Pharmacol.* **533**, 222-239.
17. Riedl, M. A. and A. E. Nel. 2008. Importance of oxidative stress in the pathogenesis and treatment of asthma. *Curr. Opin. Allergy Clin. Immunol.* **8**, 49-56.
18. Sarker, S. D. and L. Nahar. 2004. Natural medicine: the genus Angelica. *Curr. Med. Chem.* **11**, 1479-500.
19. Sigurdsson, S., H. M. Ogmundsdottir and S. Gudbjarnason. 2005. Antitumour activity of Angelica archangelica leaf

extract. *In Vivo* **19**, 191-194.
20. Sigurdsson, S., H. M. Ogmundsdottir and S. Gudbjarnason. 2005. The cytotoxic effect of two chemotypes of essential oils from the fruits of *Angelica archangelica* L. *Anticancer Res.* **25**, 1877-1880.
21. Striz, I., T. Mio, Y. Adachi, R. A. Robbins, D. J. Romberger and S. I. Rennard. 1999. IL-4 and IL-13 stimulate human bronchial epithelial cells to release IL-8. *Inflammation* **23**, 545-555.
22. Wu, A. Y., S. C. Chik, A. W. Chan, Z. Li, K. W. Tsang and W. Li. 2003. Anti-inflammatory effects of high-dose montelukast in an animal model of an acute asthma. *Clin. Exp. Allergy* **33**, 359-366.
23. Yeh, M. L., C. F. Liu, C. L. Huang and T. C. Huang. 2003. Hepatoprotective effect of *Angelica archangelica* in chronically ethanol-treated mice. *Pharmacology* **68**, 70-73.

초록 : 신선초의 물 추출물에 의한 천식 증상의 감소

허진철 · 이상한*
(경북대학교 식품생물산업연구소 및 식품공학과)

기관지를 통하여 발병하는 염증은 대표적인 면역질환의 현상인데 천식이나 아토피 피부염 등에 나타난다. 신선초의 물 추출물이 항천식 활성을 나타내는지를 알아보기 위하여 ovalbumin으로 유도시킨 동물모델을 사용하였다. 마우스에 경구투여하여 폐의 조직을 Haematoxylin-Eosin 염색과 면역조직화학을 이용하여 인터루킨-4 및 -13의 발현을 측정한 결과, 신선초의 물 추출물은 $CD4^+$ 세포의 수 및, 인터루킨-4 및 -13의 발현을 억제하는 것으로 나타났다. 이의 결과는 신선초의 물 추출물이 ovalbumin 으로 유도시킨 마우스의 천식 증상을 경감시키는 것으로 이의 활용이 기대된다.