



## Anti-inflammatory effects of ethanolic extract from *Lagerstroemia indica* on airway inflammation in mice

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### ABSTRACT

**Aim of the study:** In the present study, we investigated whether the *Lagerstroemia indica* Linn (LI) extract has an anti-inflammatory effect on lung inflammation in ovalbumin-induced asthmatic mice.

**Materials and methods:** The LI extract was obtained from dried and powdered whole plants of LI using 80% ethanol. ELISA was performed to evaluate cytokine concentration. BALB/c mice were used as a mouse model of asthma after asthmatic induction by ovalbumin sensitization and inhalation. We examined the effects of the LI extract on leukocyte infiltration and mucus secretion using cell count and histological stain.

**Results:** The amount of cytokines, such as interleukin (IL)-2, IL-4, IL-5, IL-13, and TNF- $\alpha$ , was increased in Jurkat cells using the extract from house dust mites. Increased cytokine concentrations were inhibited by the LI extract. The LI extract suppressed the increased expression of IL-6 after treatment with mite extract of EoL-1 cells and THP-1 cells. In an in vivo experiment using asthmatic mice, the LI extract significantly inhibited leukocytosis and eosinophilia in bronchoalveolar lavage (BAL) fluid and lung tissue samples. The LI extract inhibited the increase in mucus secretion by goblet cells, blocked the production of reactive oxygen species in BAL fluid cells, and blocked the protein expression of IL-5 in BAL fluid. The concentration of ovalbumin-specific IgE in BAL fluid was weakly inhibited by the LI extract.

**Conclusions:** These results suggest that the LI extract may be used as a valuable agent for treating allergic diseases such as asthma due to its anti-inflammatory property.

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## 1. Introduction

*Lagerstroemia indica* Linn (LI) belongs to the *Lythraceae* family and is distributed in Korea, China, and Australia. LI is known as an herb for treating asthma, hemostasis and detoxification in traditional oriental medicine. Although it has been reported that LI has anti-coagulation activity and contains biphenylquinolizidine alkaloids, however, the exact function of LI for the treatment of a disease is understood poorly (Chistokhodova et al., 2002; Kim et al., 2009).

Asthma is a chronic inflammatory disease of the lung. It begins in young children and persists in adulthood, resulting in high mortality and decreased quality of life (Braman, 2006). Allergic

inflammation shows an increase in eosinophil infiltration into the airways, mucus production, bronchoconstriction, and reduction of respiratory function. Allergic inflammation is caused by eosinophils which destroy lung tissues by producing several toxic granule proteins. T helper (Th) 2 cytokines, such as IL-4 and IL-5, induce elevation of IgE in serum and BAL fluid; increased IgE then aggravates the clinical features of the disease (Gould and Sutton, 2008).

We performed the present study to determine the anti-inflammatory effect of LI on asthma. First, we examined the inhibitory effect of LI on cytokine production in Jurkat cells, EoL-1 cells, and THP-1 cells. To test the effect of LI in vivo, we investigated the inhibition of inflammatory response, production of ROS, mucus production, and IgE elevation after the treatment with LI of ovalbumin-induced asthmatic mice.

## 2. Materials and methods

### 2.1. Preparation of the LI extract

Whole plants of LI were collected and standard extract have been deposited at the Herbarium of the Department of Herbal

**Abbreviations:** LI, *Lagerstroemia indica* Linn; i.p., intraperitoneal; BAL, bronchoalveolar lavage; Th, T helper; IgE, immunoglobulin E; ELISA, enzyme-linked immunosorbent assay; ROS, reactive oxygen species.

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Pharmaceutical Development, Korea Institute of Oriental Medicine, Daejeon, Korea and Division of Life Science, Daejeon University (TUT), Korea. The dried and powdered whole plants of LI (36 g) were extracted with 80% ethanol ( $3 \times 0.5$  l) for 2 days at room temperature. The combined liquid extracts (4.84 g) were concentrated under reduced pressure.

## 2.2. Cell culture

The human T cell line Jurkat cells and human monocytic cell line THP-1 cells were obtained from the American Type Culture Collection (Manassas, VA). The human eosinophilic leukemia cell line, EoL-1 cells were obtained from the Riken Cell Bank (Tsukuba, Japan). They were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 µg/ml).

## 2.3. MTT assay

MTT assay was performed to determine cell viability using the cell proliferation kit (Roche, Penzberg, Germany). Both THP-1 cells and EoL-1 cells in 100 µl of the culture medium were plated into a 96-well culture plate. The LI extract was added to each individual well as a final concentration of 10 µg/ml. The plate was then incubated for 24 h at 37 °C in a CO<sub>2</sub> incubator. 10 µl of MTT solution was added in each well. After incubation of the plate at 37 °C for 4 h, 100 µl of solubilization solution was added to each well. After 24 h incubation, the absorbance was measured using an ELISA reader (Bio-Tek Instruments, Winooski, VT) at 550 nm.

## 2.4. Enzyme-linked immunosorbent assay (ELISA)

After pre-treatment with LI or dexamethasone for 30 min, Jurkat cells were treated with the extract of house dust mite, *Dermatophagoides pteronissinus* supplied by Dr. Tai-Soon Yong (Yonsei University College of Medicine, Seoul, Korea). The concentrations of IL-2, IL-4, IL-5, and TNF-α in the supernatant were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using OptEIA Set (BD Biosciences, San Diego, CA) and the concentration of IL-13 was measured with a DuoSet ELISA kit (R&D Systems, Minneapolis, MN), according to the manufacturer's instructions using an ELISA reader, BIO-TEK ELx808. Cytokine release of THP-1 cells and EoL-1 cells was measured using the method described by two reports (Lee et al., 2008, 2009). After pre-treatment with LI or dexamethasone for 30 min, the cells were treated with the extract of house dust mite, *Dermatophagoides pteronissinus*. The concentrations of IL-6, IL-8 and MCP-1 in the supernatant were measured by OptEIA Set (BD Biosciences). The cytokine concentrations were calculated using a linear-regression equation obtained from the standard absorbance values.

## 2.5. Asthma induction in BALB/c mice and drug administration

Six-week-old female BALB/c mice were purchased from Daehan Biolink Co. LTD (Seoul, Korea) and maintained in an air-conditioned room at room temperature. The mice were divided into six groups ( $n=10$ ), and airway inflammation was induced by ovalbumin (Grade III; Sigma–Aldrich, Korea) in five groups using the method described by Yuk et al. (2007). Each mouse was immunized by intraperitoneal (i.p.) injection of 20 µg of chicken ovalbumin (Grade III; Sigma–Aldrich, Korea) with 1 mg aluminum hydroxide (Sigma–Aldrich, Korea) on days 1 and 14. The mice were inhaled with 5% ovalbumin aerosolized using an ultrasonic nebulizer (ME-U12, Omrom, Tokyo, Japan) for 1 h per day from days 21 to 27 after the second sensitization. Four groups of asthma-induced mice were treated with oral injection of 50 mg/kg, 250 mg/kg

or 500 mg/kg of LI extract, or with i.p. injection of 1 mg/kg of dexamethasone (Sigma–Aldrich, Korea) between days 14 and 27, respectively. LI extract and dexamethasone are dissolved in ethanol or DMSO, respectively and then was diluted to less than 1/100 with PBS. The normal group was sensitized and challenged with PBS without drug treatment.

All animal experiments used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the Eulji University.

## 2.6. Collection of bronchoalveolar lavage (BAL) fluid and serum

BAL fluid was collected by lavaging the lung via the trachea with 1 ml of PBS. After five lavages, approximately 0.7 ml of BAL fluid was recovered. Blood was collected by heart puncture. BAL fluid and blood were centrifuged for 5 min at 4 °C. The supernatant was stored at –70 °C for the measurement of cytokines and IgE, respectively. The cells in the BAL fluid were resuspended in 100 µl of PBS for total cell and differential counts. After the measurement of total cell number using a Neubauer hemocytometer, the cells suspended in PBS were applied to a slide by cytospinning and stained with Wright stain solution. The percentage of each leukocyte was determined, and the absolute leukocyte count was calculated by multiplying each leukocyte percentage by the total cell count.

## 2.7. Measurement of intracellular ROS

The cells in the BAL fluid were washed and resuspended with PBS, and 3.3 µM of DCFDA was added to label the intracellular ROS. The cells were then incubated for 10 min at room temperature. Labeled cells were immediately observed using fluorescence-activated cell sorting (FACS) analysis (BD Biosciences).

## 2.8. Histological analysis

Lung tissues were fixed with Carnoy's solution overnight at 4 °C after isolation from the mice. The fixed tissues were embedded in paraffin and cut into 4-µm sections with a microtome (Leica, Nussloch, Germany). The sections were placed on slide glasses, deparaffinized, and stained with hematoxylin and eosin (Sigma–Korea) in order to examine the cells that had infiltrated into the peribronchial connective tissues or with periodic acid-Schiff stain (Sigma–Korea) to evaluate mucus production. A peribronchial cell count based on a five-point scoring system was performed as previously described (Duan et al., 2004). The scoring system was the following: 0 – no cells; 1 – a few cells; 2 – a ring of cells 1 cell layer deep; 3 – a ring of cells 2–4 cell layers deep; 4 – a ring of cells more than 4 cell layers deep. The extent of mucus production of goblet cells was evaluated using a five-point scoring system, as previously described (Kuperman et al., 2002). The scoring system was the following: 0 – no goblet cells; 1 – lower than 25%; 2 – from 25% to 50%; 3 – from 50% to 75%; 4 – higher than 75%. The scoring of the leukocytes and goblet cells was examined in three independent fields of lung section from each mouse.

## 2.9. Statistical analysis

The statistical significance of any difference was determined by one-way ANOVA followed by LSD and Turkey. The data are expressed as the means ± S.E.M. The SPSS statistical software package (Version 10.0, Chicago, IL) was used for the statistical analysis. A *p* value of less than 0.05 was considered statistically significant.

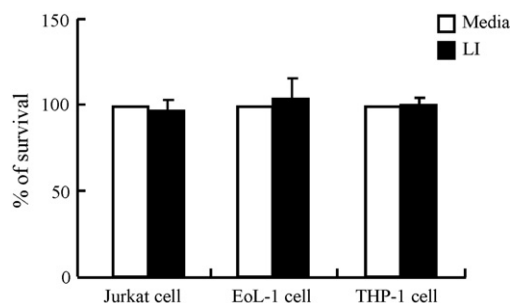


**Fig. 1.** The LI extract has no cytotoxic effect on Jurkat cells, THP-1 cells and EoL-1 cells. Jurkat cells, THP-1 cells and EoL-1 cells were incubated with the LI extract at a concentration of 10  $\mu\text{g/ml}$  for 24 h. The survival rate was measured by performing MTT assay, as described in Section 2. The data are expressed as the relative ratio to the absorbance of the untreated cells, which was set at 100%. The data are expressed as the means  $\pm$  S.E.M. of three independent experiments.

### 3. Results

#### 3.1. The LI extract has a suppressive effect on cytokine production of Jurkat cells, EoL-1 cells, and THP-1 cells

We first investigated whether the LI extract affected the cell survival of Jurkat cells, EoL-1 cells, and THP-1 cells. The LI extract had no cytotoxic effect on these cells because cell viability was not altered after the treatment with LI extract at a high concentration of 10  $\mu\text{g/ml}$  for 24 h (Fig. 1). To investigate the anti-inflammatory effect of the LI extract, we evaluated the inhibition by the LI extract on cytokine expression which had been increased by mite extract. As shown in Fig. 2, the LI extract strongly inhibited the protein release of IL-2, IL-4, IL-5, IL-13, and TNF- $\alpha$  in Jurkat cells, with different degrees of inhibition depending on the type of cytokine. The increase in IL-6 production in EoL-1 cells and THP-1 cells was also inhibited by the LI extract (Fig. 3). The LI extract, even at a low concentration (0.1  $\mu\text{g/ml}$ ), suppressed the increase in cytokines.

#### 3.2. The LI extract blocks the infiltration of total leukocytes and eosinophils into the airways

To examine the inhibitory effect of the LI extract in asthma, we induced lung inflammation in BALB/c mice using ovalbumin and evaluated leukocyte infiltration into the airways by counting the total leukocytes and eosinophils in the BAL fluid. As shown in

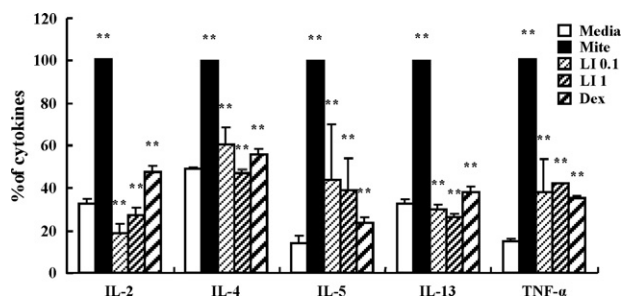


**Fig. 2.** The LI extract inhibits the increased production of IL-2, IL-4, IL-5, IL-13, and TNF- $\alpha$  after treatment with mite extract in Jurkat cells. Jurkat cells were pre-treated in the absence (Mite) or the presence of the LI extract at the concentration of 0.1  $\mu\text{g/ml}$  (LI 0.1) and 1  $\mu\text{g/ml}$  (LI 1), or dexamethasone (Dex) at a concentration of 1  $\mu\text{g/ml}$  for 24 h. And then the cells were treated with 1  $\mu\text{g/ml}$  mite extract (Mite) for 24 h. The supernatant was collected and analyzed by ELISA. The data are expressed as the relative ratio of the cytokine concentration of the mite-treated group, which was set at 100%. The data are presented as the means  $\pm$  S.E.M. of three independent experiments. \*\* $p < 0.01$  was considered a significant difference between the untreated group and the mite group or between the mite group and the LI-treated group.

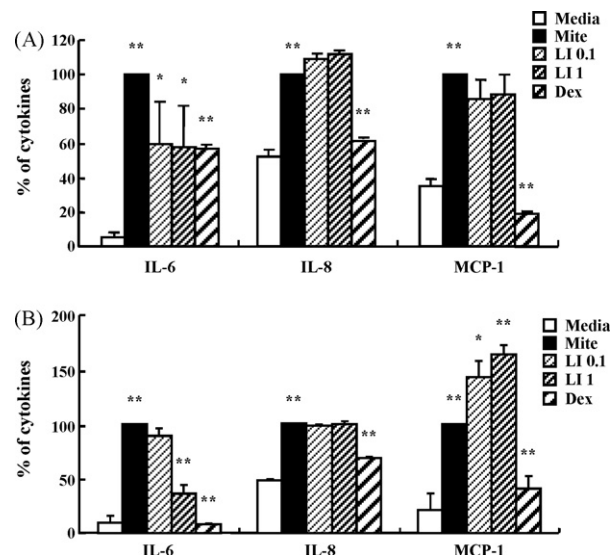


**Fig. 3.** The LI extract inhibits the increased production of IL-6 after treatment with mite extract in EoL-1 cells and THP-1 cells. EoL-1 cells (A) and THP-1 cells (B) were pre-treated in the absence (Mite) or the presence of the LI extract at the concentration of 0.1  $\mu\text{g/ml}$  (LI 0.1) and 1  $\mu\text{g/ml}$  (LI 1), or dexamethasone (Dex) at a concentration of 1  $\mu\text{g/ml}$  for 24 h. And then the cells were treated with 1  $\mu\text{g/ml}$  the mite extract (Mite) for 24 h. The supernatant was collected and analyzed by ELISA. The data are expressed as the relative ratio of the cytokine concentration of the mite-treated group, which was set at 100%. The data are presented as the means  $\pm$  S.E.M. of three independent experiments. \* $p < 0.05$  and \*\* $p < 0.01$  were considered a significant difference between the untreated group and the mite group or between the mite group and the LI-treated group.

Fig. 4A, the LI extract inhibited inflammatory leukocytes with no significant difference. The LI extract also decreased the number of eosinophils as compared to the control group ( $p < 0.05$ ) (Fig. 4B). Cytokine measurement showed that IL-5 protein expression in the lungs increased following the induction of asthma and was then strongly inhibited by the LI extract (Fig. 4C). ROS production in BAL fluid cells was also blocked by the LI extract (Fig. 4D).

#### 3.3. The LI extract suppresses the degree of histopathological alteration of lung tissues in a mouse model of asthma

The LI extract reduced the inflammatory cells in BAL fluid, we examined the alteration of the histological features of asthma after treatment of lung tissues with the LI extract using a histochemical method. The lung tissue in ovalbumin-induced asthmatic mice showed severe peribronchial inflammation by excessive leukocyte infiltration as compared to the normal tissue (Fig. 5). Mucus production by goblet cells increased after asthma induction. Both inflammation and mucus hypersecretion resulted in the narrowing of the bronchi. The LI extract inhibited the pathological characterization of asthma such as leukocyte infiltration and mucus production. Data from histological tests indicate that the LI extract may aggravate the degree of clinical phenotype of asthma, and these results are in agreement with the data shown in Fig. 4.

#### 3.4. The LI extract down-regulates ovalbumin-specific IgE levels in BAL fluid and serum

Since total IgE and allergen-specific IgE play essential factors in the pathogenesis of allergic asthma, we investigated the concentrations of ovalbumin-specific IgE and total IgE in BAL fluid and serum. The LI extract suppressed the ovalbumin-specific IgE level in BAL fluid and serum without significance (Fig. 6A), although the LI extract had no effect on the total IgE level in BAL fluid and rather increased the total IgE level in the serum (Fig. 6B).

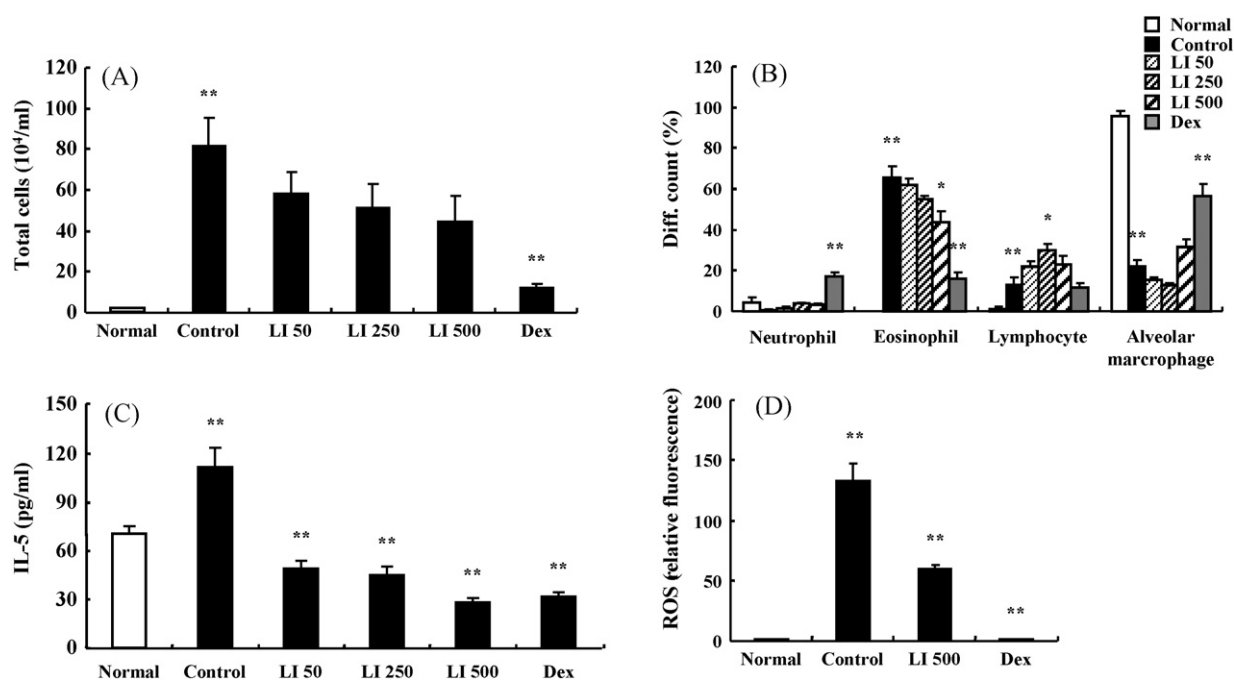


**Fig. 4.** The LI extract decreases the inflammatory cells and IL-5 expression in BAL fluid, and ROS production in BAL fluid cells in a mouse model of asthma. BAL fluid was collected from ovalbumin-induced mice untreated (Control) or treated with 50 mg/kg (LI 50), 250 mg/kg (LI 250) and 500 mg/kg (LI 500), or 1 mg/kg dexamethasone (Dex) between the sensitization and inhalation period. The negative control group (Normal) was sensitized and challenged with PBS without drug administration. The number of cells in the fluid was totally and differentially counted (A and B) and IL-5 expression in BAL fluid was analyzed by ELISA (C). After the centrifugation of the BAL fluid, the cells were incubated with PBS containing 3.3  $\mu$ M DCFDA and were then immediately observed by fluorescence-activated cell sorting analysis (D). The data were expressed as the means  $\pm$  S.E.M. \* $p < 0.05$  and \*\* $p < 0.01$  were considered a significant difference between the normal group and asthma control group or between the asthma control group and drug-treated group.

#### 4. Discussion and conclusion

In the present study, we demonstrated that (1) the increased release of IL-2, IL-4, IL-5, IL-13, and TNF- $\alpha$  after mite extract treatment of Jurkat cells is inhibited by the LI extract; (2) the LI extract suppresses the increased expression of IL-6 in THP-1 cells and EoL-1 cells; (3) in asthmatic mice, the LI extract significantly suppresses total leukocytosis and eosinophilia in BAL fluid; (4) the LI extract decreases protein expression of IL-5 in BAL fluid and ROS production of BAL fluid cells; (5) it reduces both inflammation and mucus hypersecretion in lung tissues; and (6) it weakly diminishes the level of ovalbumin-specific IgE in BAL fluid but not the level of total IgE in BAL fluid.

Asthma is an inflammatory disease of the respiratory system, and its key pathogenesis is associated with infiltrated leukocytes from the blood into the lungs along with mucus production (Busse and Rosenwasser, 2003). Both inflammation and mucus production induce narrowing of the airway passage and finally result in poor respiratory function. Although the therapeutic effects of LI extract on allergic diseases including asthma are not fully understood, we demonstrated its possible therapeutic efficacy on asthma using an in vitro system associated with measurement of the released mediators as shown in Figs. 2 and 3. The LI extract inhibited the increase of cytokines such as IL-2, IL-4, IL-5, IL-13, and TNF- $\alpha$  after mite extract treatment of Jurkat cells. Also, it decreased IL-6 secretion in THP-1 and EoL-1 cells. In animal experiments, we evaluated the expression of IL-4, IL-5, and eotaxin in BAL fluid samples from asthmatic mice. The LI extract decreased IL-5 expression (Fig. 4C), but not IL-4 and eotaxin (data not shown). The results from an animal model partly agree with data that LI has inhibitory effects on several cytokines in in vitro cell lines. Decrease in cytokine production is important in the recovery of asthma, where the most important feature in its clinical cure is decreased inflammation and mucus production in lung tissues (Woodruff and Fahy, 2002; Elias et al.,

2003). Since the LI extract inhibited both total leukocytes, including eosinophils, and mucus hypersecretion in lung tissues, it may be useful in the treatment of asthma (Fig. 5). The anti-asthmatic effect of the LI extract on lung tissues is similar to that of other herbs such as *Duchesnea chrysantha* and *Viola mandshurica* W. Becker as described in prior reports (Yang et al., 2008; Lee et al., 2010). To further understand the mechanism of inflammatory cell influx and mucus production, we investigated ROS production in BAL fluid cells. The LI extract strongly decreased intracellular ROS production of BAL fluid cells (Fig. 4D). ROS has also been known as an important regulator of myocyte proliferation, function of epithelial cilia, and release of inflammatory mediators (Henricks and Nijkamp, 2001).

Th2 lymphocytes and B lymphocytes play essential roles in the initiation and development of asthma by releasing Th2 cytokines and IgE production (Brightling et al., 2002; Ngoc et al., 2005; Owen, 2007). IL-5 is especially a core cytokine in the differentiation and activation of eosinophils (Sanderson, 1988). The elevation of the total IgE and allergen-specific IgE levels in early asthmatic response induces the degranulation of mast cells by cross-linkage of allergen-specific IgE. The LI extract strongly inhibited the elevated protein expression of IL-5 to a level not greater than that observed in the normal group (Fig. 4C). It also decreased the ovalbumin-specific IgE in BAL fluid after induction of asthma, while the total IgE was not blocked by the LI extract (Fig. 6). Based on these results, the LI extract may regulate the specific mechanisms for expressing allergen-specific IgE, but is not associated with mechanisms for increasing IgE quantity. The clear mechanism for this activity can be further studied. In conclusion, we focused on the possibility of the LI extract as an additional therapeutic drug for asthma therapy. Our results showed that it can be an effective anti-asthmatic agent using in vitro cell lines and ovalbumin-challenged asthmatic mice. It may also be a promising candidate in herbal medicine for the treatment of asthma.

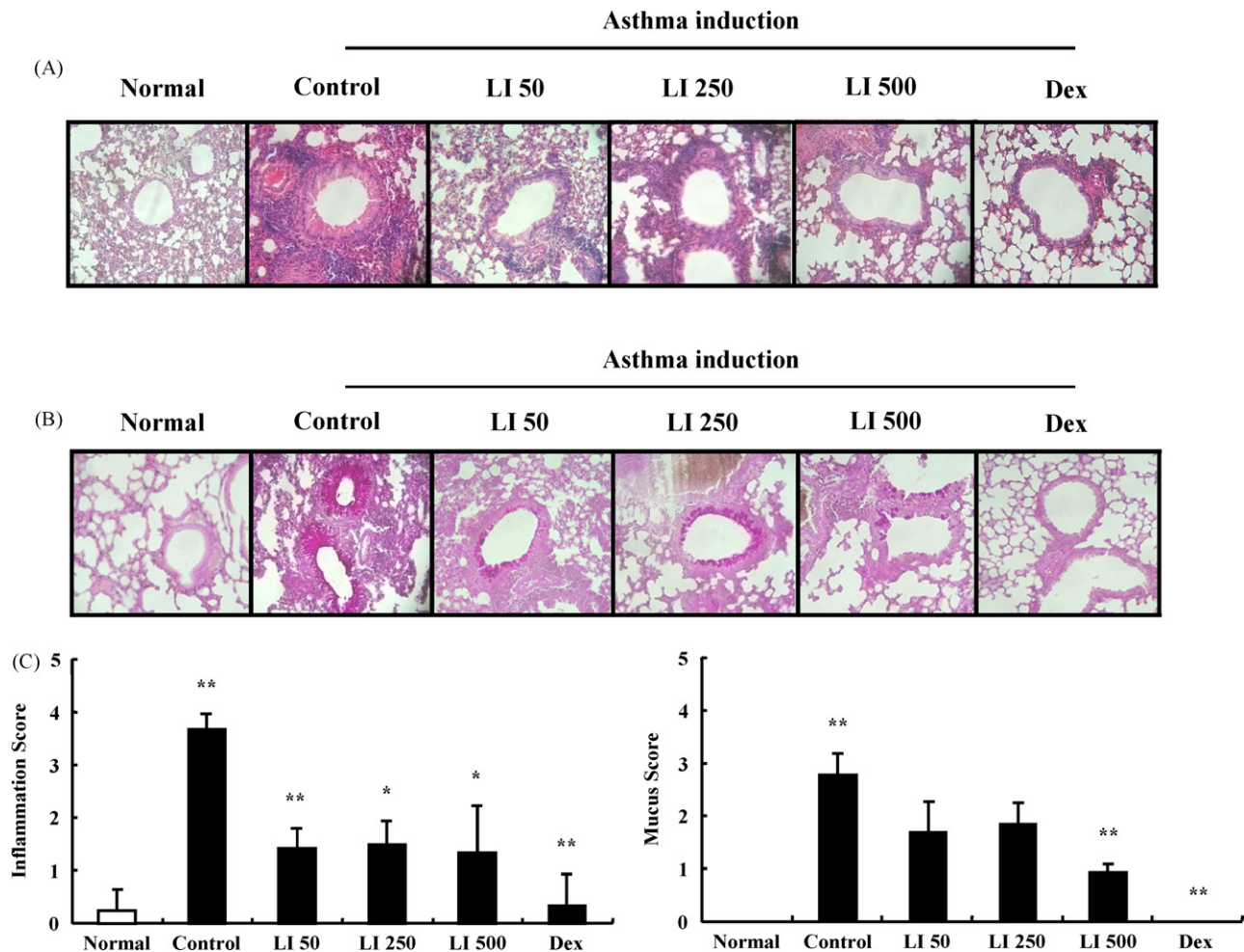


**Fig. 5.** The LI extract inhibits airway inflammation and mucus hypersecretion in lung tissues. Hematoxylin and eosin staining (A) and periodic acid-Schiff staining (B) *t* was performed to examine the pathologic alteration in lung tissues. Magnification 200 $\times$ . The inflammation and mucus production in lung tissues were scored as described in Section 2 (C).

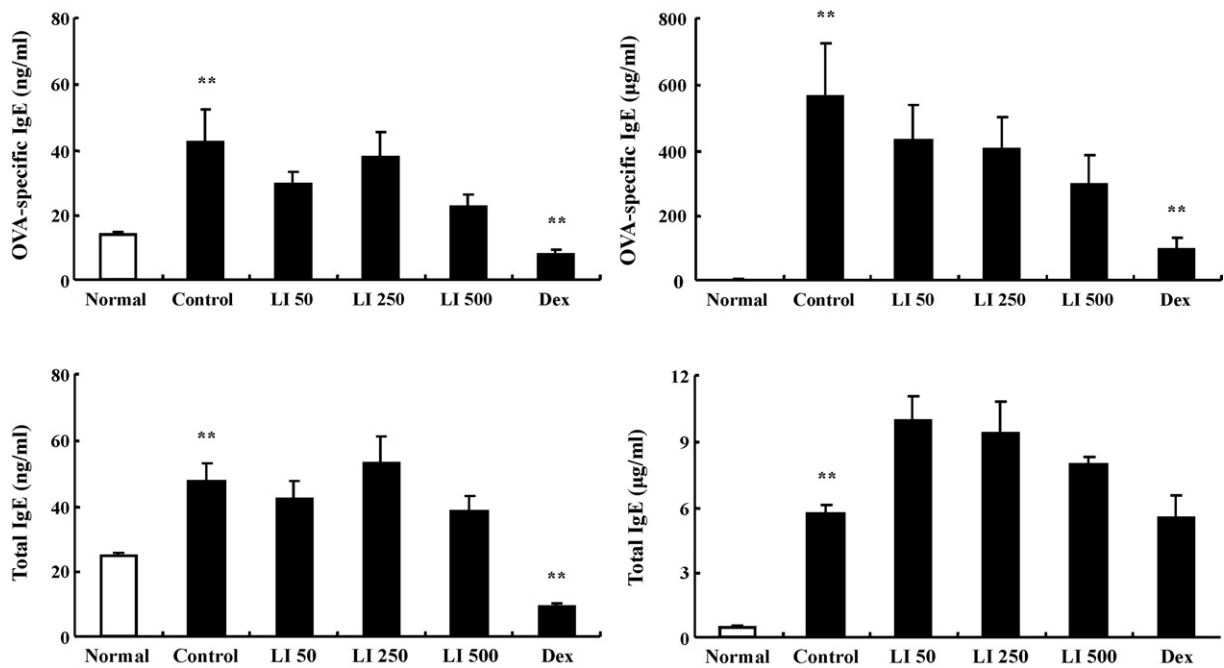


**Fig. 6.** The LI extract inhibits on ovalbumin-specific IgE level in BAL fluid and serum. The ovalbumin (OVA)-specific IgE (A) and total IgE (B) in BAL fluid (left panel) and serum (right panel) were analyzed by ELISA, as described in Section 2. The data are expressed as the means  $\pm$  S.E.M. \*\**p* < 0.01 were considered to be indicative of significant differences between the normal group and asthma control group or between the asthma control group and drug-treated group.

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## References

- Braman, S.S., 2006. The global burden of asthma. *Chest* 130, 4S–12S.
- Brightling, C.E., Symon, F.A., Birring, S.S., Bradding, P., Pavord, I.D., Wardlaw, A.J., 2002. Th2 cytokine expression in bronchoalveolar lavage fluid T lymphocytes and bronchial submucosa is a feature of asthma and eosinophilic bronchitis. *The Journal of Allergy and Clinical Immunology* 110, 899–905.
- Busse, W.W., Rosenwasser, L.J., 2003. Mechanisms of asthma. *The Journal of Allergy and Clinical Immunology* 111, S799–804.
- Chistokhodova, N., Nguyen, C., Calvinom, T., Kachirskaja, I., Cunningham, G., Howard Miles, D., 2002. Antithrombin activity of medicinal plants from central Florida. *Journal of Ethnopharmacology* 81, 277–280.
- Duan, W., Chan, J.H., Wong, C.H., Leung, B.P., Wong, W.S., 2004. Anti-inflammatory effects of mitogen-activated protein kinase inhibitor U0126 in an asthma mouse model. *The Journal of Immunology* 172, 7053–7059.
- Elias, J.A., Lee, C.G., Zheng, T., Ma, B., Homer, R.J., Zhu, Z., 2003. New insights into the 354 pathogenesis of asthma. *The Journal of Clinical Investigation* 111, 291–297.
- Gould, H.J., Sutton, B.J., 2008. IgE in allergy and asthma today. *Nature Review Immunology* 8, 205–217.
- Henricks, P.A., Nijkamp, F.P., 2001. Reactive oxygen species as mediators in asthma. *Pulmonary Pharmacology and Therapeutics* 14, 409–420.
- Kim, H.J., Lee, I.S., Youn, U., Chen, Q.C., Ngoc, T.M., Ha do, T., Liu, H., Min, B.S., Lee, J.Y., Seong, R.S., Bae, K., 2009. Biphenylquinolizidine alkaloids from *Lagerstroemia indica*. *Journal of Natural Products* 72, 749–752.
- Kuperman, D.A., Huang, X.L., Koth, L., Chang, G.H., Dolganov, G.M., Zhu, Z., Elias, J.A., Sheppard, D., Erle, D.J., 2002. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nature Medicine* 8, 885–889.
- Lee, J.S., Kim, I.S., Ryu, J.S., Yun, C.Y., 2008. House dust mite, *Dermatophagoides pteronissinus* increases expression of MCP-1 IL-6, and IL-8 in human monocytic THP-1 cells. *Cytokine* 42, 365–371.
- Lee, J.S., Kim, I.S., Ryu, J.S., Yun, C.Y., 2009. House dust mite, *Dermatophagoides pteronissinus* increases expression of MCP-1 IL-6, and IL-8 in human eosinophilic leukemia EoL-1 cells. *Animal Cells and Systems* 13, 391–397.
- Lee, M.Y., Yuk, J.E., Kwon, O.K., Kim, H.S., Oh, S.R., Lee, H.K., Ahn, K.S., 2010. Anti-inflammatory and anti-asthmatic effects of *Viola mandshurica* W. Becker (VM) ethanolic (EtOH) extract on airway inflammation in a mouse model of allergic asthma. *Journal of Ethnopharmacology* 127, 159–164.
- Ngoc, L.P., Gold, D.R., Tzianabos, A.O., Weiss, S.T., Celedon, J.C., 2005. Cytokines, allergy, and asthma. *Current Opinion in Allergy and Clinical Immunology* 5, 161–166.
- Owen, C.E., 2007. Immunoglobulin E: role in asthma and allergic disease: lessons from the clinic. *Pharmacology Therapy* 113, 121–133.
- Sanderson, C.J., 1988. Interleukin-5: an eosinophil growth and activation factor. *Developments in Biological Standardization* 69, 23–29.
- Woodruff, P.G., Fahy, J.V., 2002. Airway remodeling in asthma. *Seminars in Respiratory and Clinical Care Medicine* 23, 361–367.
- Yang, E.J., Lee, J.S., Yun, C.Y., Kim, J.H., Kim, J.S., Kim, D.H., Kim, I.S., 2008. Inhibitory effects of *Duchesnea chrysantha* extract on ovalbumin-induced lung inflammation in a mouse model of asthma. *Journal of Ethnopharmacology* 118, 102–107.
- Yuk, J.E., Woo, J.S., Yun, C.Y., Lee, J.S., Kim, J.H., Song, G.Y., Yang, E.J., Hur, I.K., Kim, I.S., 2007. Effects of lactose-beta-sitosterol and beta-sitosterol on ovalbumin-induced lung inflammation in actively sensitized mice. *International Immunopharmacology* 7, 1517–1527.