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PII: S0378-8741(19)30289-2

DOI: <https://doi.org/10.1016/j.jep.2019.112083>

Article Number: 112083

Reference: JEP 112083

To appear in: *Journal of Ethnopharmacology*

Received Date: 21 January 2019

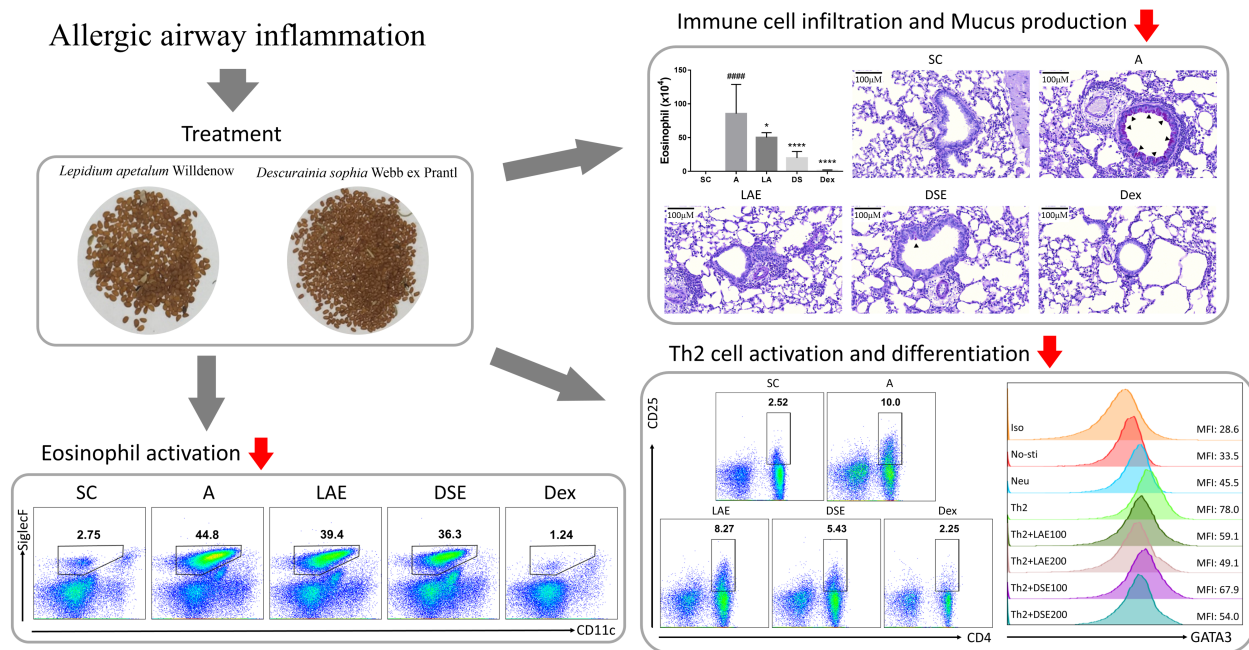
Revised Date: 25 June 2019

Accepted Date: 11 July 2019

Please cite this article as: Kim, S.-B., Seo, Y.-S., Kim, H.S., Lee, A.Y., Chun, J.M., Moon, B.C., Kwon, B.-I., Anti-asthmatic effects of *lepidii seu Descurainiae Semen* plant species in ovalbumin-induced asthmatic mice, *Journal of Ethnopharmacology* (2019), doi: <https://doi.org/10.1016/j.jep.2019.112083>.

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**Anti-asthmatic effects of Lepidii seu Descurainiae Semen plant species in
ovalbumin-induced asthmatic mice**

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Conflicts of interest: The authors declared no conflict of interest.

ABSTRACT

Ethnopharmacological evidence: *Lepidii seu Descurainiae Semen* (LDS) is used as a traditional herbal medicine in northeast Asia, mainly in Korea, Japan, and China to treat lung disorders including coughs and phlegm caused by acute and chronic airway inflammation.

Aim of the study: Recently, interest regarding health problems incurred by air pollution has rapidly grown. Herbal medicines are being considered as alternative agents to treat various diseases. In the present study, we evaluated and compared the anti-inflammatory effects of LDS, which is derived from *Lepidium apetalum* Willd. extracts (LAE) and *Descurainia sophia* (L.) Webb ex Prantl extracts (DSE), on allergic airway inflammation.

Materials and methods: We established an ovalbumin-induced asthmatic mouse model to evaluate the impact of LDS extracts. We performed histological examination and measured relevant inflammatory mediators and cells in bronchoalveolar lavage fluid and lung. Furthermore, we conducted an in vitro T helper 2 (Th2) polarization assay, flow cytometry, and western blot analysis.

Results: Asthmatic phenotypes were attenuated by LDS extract treatments. LDS extract administration significantly reduced mucus production, inflammatory cell infiltration into airways, and eosinophil activation. Furthermore, LDS extracts reduced the expression of type 2 cytokines and inhibited differentiation and activation of Th2 cells.

Conclusion: LDS alleviated eosinophilic inflammation by inhibiting Th2 cell differentiation, and DSE was more effective in attenuating allergic lung inflammation than LAE.

Keywords: *Lepidium apetalum* Willd.; *Descurainia sophia* (L.) Webb ex Prantl; eosinophilic asthma; Th2 cell activation; Type 2 cytokines

1. Introduction

Interest in health problems incurred by fine, ultrafine, and yellow dust have increased in recent years in Korea. Concerns about yellow dust have been ongoing for decades. However, public awareness of fine and ultrafine dust has rapidly grown since 2013 (Kang and Kim, 2014). In Asian countries, especially Korea and China, interest in air pollution has increased because of health threats due to fine and ultrafine dust. Inhaled fine dust induces lung tissue changes, oxidative stress, and immune response (Kim et al., 2012). Increased numbers of immune cells, such as eosinophils, neutrophils, and T cells, in peribronchiolar and perivascular lesions are a characteristic feature of asthma. Eosinophils are considered an especially significant pathological component of airway inflammation and reflector of asthma severity (Walford and Doherty, 2014). Activation of eosinophils accompanies production of cytokines, chemokines, and other mediators and up-regulators of surface markers, such as MHC II and CD11c (Kay, 2001a, b; Rose et al., 2010). Additionally, some studies report that eosinophils not only act as antigen-presenting cells (APCs) themselves, but also control dendritic cells to initiate T helper 2 (Th2) immune response (Chu et al., 2014). As a result, reduction of eosinophils in airways has been a major target for asthma therapy and clinical trials for blocking interleukin (IL)-5, which is a key player in eosinophil activation, have been performed (Lim and Nair, 2015).

Studies in human and mouse have shown that asthma is a consequence of Th2-based responses to allergens. IL-5 produced by Th2 cells is the most important pathogenic factor for mediating eosinophil development, and an anti-IL-5 mouse antibody (mAb) blockade or IL-5 deficiency abrogates airway hyperresponsiveness (AHR) in a mouse strain-dependent manner (Walsh et al., 2008). In C57BL/6 mice, anti-IL-5 mAb treatment or eosinophil deficiency results in the abrogation of allergen-induced AHR (Foster et al., 1996; Ikutani et al., 2012). In BALB/c mice, AHR induction was unaffected by IL-5 deficiency. However, IL-5/eotaxin dual deficiencies abolished AHR induction and significantly reduced CD4⁺ T-cell production of IL-13 in BALB/c (Mattes et al., 2002). Both AHR induction and IL-13 production were

recovered by airway instillation of eotaxin or eosinophils, indicating that eotaxin is critical for T-cell IL-13 production and AHR induction.

Previous studies have shown that Th2 cells are critical in the development of eosinophils and asthma (Schatz and Rosenwasser, 2014). Inhaled antigens such as air pollution and house dust are processed by APCs triggering Th2 cell activation and Th2 cytokine release. Released Th2 cytokines, such as IL-4, -5 and -13, induce mucus production, bronchoconstriction, and isotype switching of B cells (Hansbro et al., 2013). Especially, IL-5 mediates eosinophil activation, survival, and recruitment.

Lepidii seu Descurainiae Semen (LDS) is traditionally used in north-east Asian countries for treatment of lung disorders such as dyspnea, cough, abundant expectoration, and symptoms that the lung is blocked due to phlegm retention (Yang et al., 2016; Zhiyi et al., 2014). In the Korean Pharmacopoeia, LDS has been demonstrated as adulterated seed of *Lepidium apetalum* Willd. (LA) or *Descurainia sophia* Webb ex Prantl (DS). In China both species, named *Lepidii Semen* and *Descurainiae Semen*, equivalent to LA and DS respectively, were used for treatment of lung disorders. However, there are only a few studies concerning the effects of LDS on lung inflammation (Baek et al., 2018; Gong et al., 2015; Lee et al., 2013; Zhou et al., 2014). Furthermore, Although there have been many efforts to demonstrate the anti-asthmatic effects of botanical drugs, our knowledge remains limited for several reasons. Firstly, most studied simply conducted experiments for discovering or developing new drugs, regardless of traditional clinical uses based on Korean medicine theory. Secondly, there have not been examined and compared of related species in the same genus. With these limitations in mind, we attempt to determine and compare the anti-asthmatic properties of LAE and DSE in the allergic airway inflammation model. In other words, different species traded under similar herbal names were used against similar airway diseases, but confirmation of action against airway inflammation and comparison between the two species were not performed.

In this study, we investigated the effects of LDS extracts on eosinophilic asthma and type 2 immune response and made a comparison between the effects of the LA extract (LAE) and the DS extract (DSE) on allergic inflammation.

2. Methods and Materials

2.1. Preparation of ethanolic extracts from *Lepidii seu Descurainiae* Semen

LA was imported from Jilin, China, and was purchased from Korean herbal markets, and DS (Geumsan, Chungnam, South Korea) was collected by Drs. Sungyu Yang and Byeong Cheol Moon from the Korea Institute of Oriental Medicine (KIOM). Morphological and genetic identifications were authenticated by Drs. Jun-ho Song and Byeong Cheol Moon, respectively. The taxonomic origin of these samples was determined at the species level using microscopic analysis as described previously (Song et al., 2018) and also based on comparative analysis of *matK* DNA barcode sequences as described previously (Moon et al., 2018). Furthermore, chemical analysis of LDS was performed by UHPLC system (Kim et al., 2019). The voucher specimen of seeds was deposited in the Korean Herbarium of Standard Herbal Resources (Index Herbarium code KIOM, specimen no. 2–18–0136, 2–18–0137) at KIOM. Dried LDS seeds (LA – 293 g, DS – 760 g) were prepared using reflux, then extracted twice in 70% ethanol (v/v) for 3 h and then concentrated under reduced pressure (yield: LAE – 8.07%, DSE – 7.44%).

2.2. Animals and experimental study design

Female Balb/c mice, seven weeks of age, were purchased from Daehan Biolink Co., Ltd. (Chungcheongbuk-do, Korea). Mice were maintained under the specific pathogen-free conditions supplied and provided standard food and tap water ad libitum. The mice were divided into five groups (n = 6 per group); saline control group, OVA group (vehicle treatment), OVA + LAE (100 mg/kg) treatment group, OVA + DSE (100 mg/kg) treatment group, and OVA + dexamethasone (DEX) (5 mg/kg) group. Mice, except the saline control group, were intraperitoneally injected with 50 mg of OVA (chicken egg ovalbumin; Sigma-

Aldrich, St. Louis, MO, USA) in 200 μ L aluminum sulfate (Alum; InvivoGen, San Diego, CA, USA) on days 0 and 7. Beginning on day 13, the mice were challenged with intranasal inhalations of 25 μ g of OVA in 50 μ L PBS once a day for four consecutive days with anesthesia using 2% isoflurane (Piramal Critical Care Inc., Bethlehem, PA, USA) delivered by VevoTM Compact Anesthesia System (FUJIFILM VisualSonics, Toronto, Canada) as previously described (Chun et al., 2018; Kwon et al., 2017). The four allergic lung inflammation groups were treated orally with vehicle, LAE, DSE, or DEX for the last seven days (Supplementary Figure S1). The dose of current study was chosen based on USFDA and previous study with similar design (Lee et al., 2017; Seo et al., 2019). Briefly, based on traditional dosage (averagely 7g/day), we calculated the dose for mice according to USFDA guideline and yield (almost 8%), 0.5g/60kg (8.3mg/kg) for human is equivalent to that of dose of 100mg/kg for mouse (Nair and Jacob, 2016; Chinese pharmacopocia Commission, 2015). All animal care and experimental procedures were performed with the approval of the Animal Care committee of the Korean Institute of Oriental Medicine (17-047).

2.3. Histological analysis

Euthanasia was performed using an overdose injection of pentobarbital sodium (a short acting barbiturate) in accordance with IACUC guidelines. To examine histological changes, mice lung tissues were excised and fixed in 10% formalin. The tissue was then embedded in paraffin and cut into 4 μ m sections. The sections were deparaffinized and stained with hematoxylin and eosin (H&E) for analysis of inflammatory changes and periodic acid–Schiff (PAS) for analysis of mucus production.

2.4. Collection of bronchoalveolar lavage fluid and differential cell counting

BALF was collected 18 h after the last airway challenge with 1 mL dPBS (Sciencell Research Laboratories, Carlsbad, CA, USA) for differential cell counting and measurement of cytokines. Total cell number in the BALF was counted. Subsequently, the cell suspension was applied to a slide by cyto-spinning, and then stained with hema 3 solution (Fisher HealthCare,

Pittsburgh, PA, USA). After evaluating the ratio of each leukocyte, the number of each cell type was calculated by multiplying their proportions by the total cell count. After centrifugation (1250 rpm, 4°C, 5 min), BALF supernatants were stored at -80°C until assayed for cytokines.

2.5. Measurement of cytokines in bronchoalveolar lavage fluid and lung cell cultured media

The levels of IL-4, -5, and -13 were measured in BALF and cultured media using ELISA as previously described (Kim et al., 2018). Briefly, antibodies 11B11, TRFK5, and eBio13A were used for the capture of cytokines IL-4, -5, and -13, respectively, and biotinylated antibodies BVD6-24G2, TRFK4, and eBio1316H were used for their detection. Antibodies were purchased from BD Biosciences (San Diego, CA, USA), except for eBio13A and eBio1316H, which were purchased from eBioscience (San Diego, CA, USA).

2.6. Flow cytometry

Single cell suspension from lung tissues was prepared and used for flow cytometry as previously described (Kwon et al., 2013; Lee et al., 2017). In brief, the lung tissues were digested, and a single-cell suspension was obtained using Lung Dissociation Kit (Miltenyi Biotec, Bergisch-Gladbach, Germany). Subsequently, cells were stained with fluorescence-labeled antibodies specific for fluorescein isothiocyanate (FITC)-conjugated anti-CD4 (GK1.5), phycoerythrin (PE)-conjugated anti-CD25 (PC61), PE-conjugated anti-GATA3 (16E10A23), peridinin chlorophyll (Percp)-conjugated anti-CD3 (145-2C11), FITC-conjugated anti-CD11b, PE-conjugated anti-CD11c, V500-conjugated anti-CD45, PE-CF594-conjugated anti-Ly-6G and APC-Cy7-conjugated anti-Siglec F (all from Biolegend [San Diego, CA, USA], except for anti-CD3, anti-CD45, anti-Ly-6G, and anti-Siglec F which were from BD Biosciences). Data were collected using the LSRFortessa X-20 instrument (BD Biosciences) and analysed using the FlowJo software (FlowJo LLC, Ashland, OR, USA).

2.7. Western blot analysis

Total proteins from lung tissues were extracted using RIPA buffer (Cell signaling, Billerica, MA, USA). Equal amounts of each sample (20 µg) were electrophoresed on a 10% SDS-PAGE gel and transferred onto nitrocellulose membranes. Membranes were blocked with 5% BSA and incubated at 4°C overnight with primary antibodies against GATA3 (0.5 µg/mL, BD Biosciences). After washing, membranes were incubated with secondary antibodies (Santa Cruz Biotechnology, Dallas, TX, USA). Signals were developed with ECL select (GE Healthcare, Buckinghamshire, UK), detected using ChemiDoc (Bio-RAD, Hercules, CA, USA), and quantified by band intensities using ImageJ software (National Institute of Health, Bethesda, MD, USA).

2.8. *In vitro* T helper 2 polarization assay

To isolate CD4⁺ T cells from splenocytes, CD4⁺ T cell isolation kit (Miltenyi Biotec) was used according to the manufacturer's protocol. Purified cells were stimulated with or without anti-CD3/28 pre-coated particles (neutral condition) and IL-2, IL-4, and anti-IFNγ Ab (Th2 polarization condition) for five days. During T cell differentiation, LDS extracts were added to cells in the Th2 polarization condition. After cultivation, Th2 polarization was analyzed by measuring type 2 cytokines such as IL-5 and IL-13 using ELISA, and GATA3 expression using flow cytometry. For intracellular staining, stimulated cells were fixed and permeabilized with the Foxp3/Transcription factor staining buffer set according to the manufacturer's instructions (eBioscience) and stained with antibodies (FITC-conjugated anti-CD4, Percp-conjugated anti-CD3, PE-conjugated anti-GATA3).

2.9. Statistics

Data are presented as means ± standard error of means. Differences were assessed using ANOVA, and significance was assigned at $P < 0.05$. The analyses were carried out using GraphPad Prism Software version 6.0 for Windows (GraphPad Software, La Jolla, CA, USA).

3. Results

3.1. *Lepidii seu Descurainiae Semen extracts attenuated histological changes*

To investigate the pharmacological effects of LDS extracts on airway inflammation, H&E staining and PAS staining were performed on lung tissues from experimental mice. After induction of allergic inflammation with OVA, infiltration of immune cells was increased around peribronchiolar and perivascular lesions. Secretion of mucus also increased compared with the saline control (SC) group. Treatment with LAE and DSE reduced immune cell infiltration compared with the asthma group (Fig. 1). In addition, mucus production was reduced after treatment with LDS extracts (Fig. 1).

3.2. *Lepidii seu Descurainiae Semen extracts reduced the number of inflammatory cells in bronchoalveolar lavage fluid*

To confirm the inhibitory effects of LDS extracts against immune cell infiltration, total cells and differentiated cells in BALF were counted after staining with hema 3 solution. Inflammatory cell infiltrations were significantly increased in OVA triggered compared with SC groups (Fig. 2). In addition, the number of eosinophils significantly increased. After treatment with LDS extracts, the number of eosinophils was decreased in both groups (Fig. 2). However, only DSE significantly reduced total cell number. Treatment with DSE showed higher efficiency for reducing infiltration of inflammatory cells, especially eosinophils.

3.3. *Lepidii seu Descurainiae Semen extracts reduced eosinophil activation*

To investigate the effects of LDS extracts on eosinophil activation, the expression levels of CD11c were measured using flow cytometry. In the asthma group, Eosinophil proportion and CD11c expression levels were significantly increased compared with the SC group (Fig. 3). After treatment with DSE, eosinophil proportion and activated marker expression were significantly reduced compared to the asthma group (Fig. 3). LAE

administration also reduced CD11c expression but not significantly. DSE was relatively effective compared with LAE for treatment of eosinophilic lung inflammation.

3.4. *Lepidii seu Descurainiae Semen extracts inhibited expression of type2 cytokines*

Th2 cytokines mediate the development of allergic inflammation including immune cell infiltration. To investigate the anti-allergic effects of LDS extracts, Th2 cytokines, including IL-4, -5, and -13, were measured in BALF and media collected from spontaneously cultured lung cells isolated from experimental mice. Both in BALF and cultured media, the expression levels of Th2 cytokines were significantly decreased in LDS treatment groups compared with asthmatic mice (Fig. 4). Treatment with LDS extracts attenuated the inflammatory reaction by down-regulating Th2 cytokines which are known as a key factor in allergic asthma.

3.5. *Lepidii seu Descurainiae Semen extracts reduced CD4⁺ T cell activation in asthmatic mice*

To investigate the relationship between Th2 cytokine expression and T cell activation, the population of activated T cells (CD25⁺, CD4⁺) was determined using flow cytometry. In the asthmatic group, populations of activated CD4⁺ T cells were significantly increased (Fig. 5A and B). After treatment with DSE, T cell activation significantly decreased compared with the asthma group (Fig. 5A and B). However, no significant difference was observed in the LAE treated group. To confirm the effects of LDS on Th2 cell activation, the expression levels of GATA3, key marker of asthma and Th2 cells, were measured using western blot. Increased expression of GATA3 in the asthma group was decreased after treatment with both LDS extracts (Fig. 5C and D). Administration of LDS extracts alleviated eosinophilic asthma by inhibiting Th2 cell activation.

3.6. *Lepidii seu Descurainiae Semen extracts inhibited T helper 2 cell differentiation in vitro*

We found that LDS treatment ameliorated asthmatic lung inflammation via inhibiting immune cell infiltration and mucus production by regulating Th2 cell activation and cytokine production. Therefore, we next tried to verify whether LDS extracts directly inhibit the differentiation and activation of Th2 cells. Purified CD4⁺ T cells were triggered with or without CD3 and CD28, anti-INF γ , and recombinant IL-4. After treatment with LDS extracts, GATA3 expression was significantly reduced, evaluated by mean fluorescence intensity of GATA3 (Fig. 6A). Furthermore, LDS extracts significantly reduced production of IL-5 and IL-13 in a dose dependent manner (Fig. 6B and C). These results indicate that LDS extracts directly inhibited the differentiation of CD4⁺ T cells towards the Th2 phenotype and activation.

4. Discussion

In Korean medicine theory, immune responses in lungs are explained by coldness and damp. These two factors trigger various abnormal reactions which include an allergic-similar reaction. Coldness is a way of understanding the immune response caused by respiratory infections or cold temperatures in Korean medicine theory (Peate, 2008). Damp is a way of understanding body fluid circulation disorder including overproduction of phlegm and production of inflammatory mediators in Korean medicine theory (Smith, 2009). Based on Korean medicine theory, LDS were used for attenuation of symptoms occurred by coldness and damp. On this basis, LDS has been used for treatment of lung disorders involving coughs and phlegm, caused by acute and chronic airway inflammation in north-east Asian countries.

In this study, we demonstrated that treatment with LDS extracts attenuated allergic inflammation by reducing immune cell infiltration, especially eosinophils, and mucus production in the lungs. Furthermore, LDS treatment reduced cytokine secretion and Th2 cell polarization, which are known as major mediators of eosinophilic inflammation.

In allergic airway inflammation, infiltration of immune cells is a major marker of severity. The OVA-induced asthmatic mouse model is well-established for the study of immune cell recruitment, especially eosinophils (Kandhare et al., 2013; Kim et al., 2018). We investigated

the pharmacological effects of LDS extracts using this model and we observed that LDS treatment reduced the number of eosinophils in BALF, which suggests inhibitory effects of LDS on eosinophil recruitment.

To further understand the anti-allergic effects of LDS, we measured the activation of eosinophils. Histologically, eosinophils are considered as a part of the downstream responses mediated by Th2 activation (Leckie et al., 2000). However, numerous recent studies have shown that eosinophils play far more complex roles than being exclusively end-stage destructive cells (Bossley et al., 2012; Uhm et al., 2012). These studies report that eosinophils are involved in remodeling/repair of tissue, T cell proliferation, and effector T cell recruitment. In the homeostatic state, eosinophils show very low levels of MHC II and CD11c expression. However, after activation, expression levels of these two markers are increased and support the adaptive immune system (Chu et al., 2014; Jacobsen et al., 2011; Kambayashi and Laufer, 2014). Expression levels of CD11c in eosinophils was significantly increased in lung tissue of the asthma group compared with the saline group (Fig. 3). After treatment with DSE, CD11c expression levels and the number of activated eosinophils significantly decreased. Treatment with LAE also reduced CD11c expression but not significantly. These results indicate that treatment with DSE attenuated allergic asthma by suppressing recruitment and activation of eosinophils.

In the development of asthma, complicated interactions between Th2 cells and innate immune cells, such as eosinophils, occur. Th2 cells were considered as regulators which control the eosinophils (Seo et al., 2019; Spencer and Weller, 2010). IL-5, known as Th2 cytokine, is the principal eosinophilopoietic cytokine in humans (Clutterbuck et al., 1989; Simon et al., 2004). IL-5 promotes the maturation, chemotaxis, survival, and activation of eosinophils, which then secrete chemical mediators and generate cytotoxicity (Assa'ad et al., 2011; Takatsu and Nakajima, 2008). Treatment with LDS extracts significantly reduced secretion of Th2 cytokines, such as IL-4, -5, and -13 (Figs. 4 and 6). Cytokine levels in BALF could represent the airway condition during inflammation and the levels of cytokines in lung cell culture media could represent the activation degree of lung cells at acquisition time

(Zhang et al., 2012). Results from BALF and media indicated that LDS extract treatments suppressed Th2 cell activation, and that DSE had more powerful effects on the allergic reaction. These results were confirmed using flow cytometry. Treatment with DSE significantly reduced the proportion of activated CD4⁺ T cells, however, treatment with LAE did not affect the proportion of activated CD4⁺ cells.

Interestingly, differences existed between in vivo experiments and in vitro results from the Th2 cell polarization assay. Both LDS extracts similarly suppressed Th2 cell polarization and secretion of Th2 cytokines in vitro (Fig. 6). Based on in vitro results, it could be expected that both LDS extracts directly suppress Th2 cell development and attenuate allergic inflammation. However, DSE had stronger effects on allergic asthma by almost all measurements in vivo. We expect that these differences were caused by a change in the effector compound of LAE during digestion or in the liver. It is clear that oral administration of DSE suppressed eosinophilic inflammation by blocking Th2 cell development and activation, but further studies, such as metabolite analysis produced by gut microbiome or Pharmacokinetic studies, are needed on LAE to explain difference between in vitro and in vivo assays.

Our results indicate that DSE is relatively effective at attenuating allergic lung inflammation compared with LAE. However, this study was performed using one specific model of allergic asthma, and further studies must be performed using another cause such as virus, smoke and ultrafine particles to compare the pharmacological effects of the two species from LDS on lung disorders. Furthermore, based on the suppressive effects of DSE on Th2 cell activation and eosinophils, DSE has potential as a therapeutic for not only lung disorder, as mentioned in traditional books, but also other eosinophilic allergic disorders which occur in the skin or intestines.

5. Conclusion

Although LDS is widely used in north-east Asian countries to treat lung disorders, there is a lack of pharmacological evidence and comparison between the two species that make up LDS. In the current study, both extracts of LDS attenuated eosinophilic asthma caused by Th2 cell

activation. Both LDS treatments directly blocked Th2 cell differentiation in an in vitro assay but DSE had stronger effects on allergic asthma in vivo. These results indicate that DSE is relatively effective to treat eosinophilic airway inflammation mediated by Th2 cells.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

Funding: This work was supported by grants for Evaluation of effectiveness of alternative Herbal Medicine Resources (K17402) from KIOM and Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through the High Value-added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MARFA) (315-064-3) and the National Research Foundation of Korea (NRF) grant funded by the Korea government (Ministry of Science and ICT) (NRF-2018R1C1B5086478).

Author contributions

SB Kim and YS Seo performed the experiments, analysed data, and wrote the manuscript; HS Kim prepared plant materials; AY Lee and JM Chun performed the in vivo experiments; BC Moon verified the origin of the plant materials using comparative analysis of *matK* DNA barcode sequences; B-I Kwon designed the experiments, analysed the data, and wrote the manuscript.

Figure legends

Figure 1. Effect of *Lepidii seu Descurainiae* Semen extract on histopathological change in lung. Sections of lung tissue were stained with hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS). Arrowheads indicate mucus production. OVA, ovalbumin; SC, saline

control group; A, asthma group; LAE, *Lepidium apetalum* Willd. extract; DSE, *Descurainia sophia* Webb ex Prantl extract; LAE, A + LAE 100 mg/kg; DSE, A + DSE 100 mg/kg; Dex, A + dexamethasone 5 mg/kg

Figure 2. Effect of Lepidii seu Descurainiae Semen extract on infiltration of

inflammatory cells in BALF. Total cells (A) and differentiated cells (B, C) were counted.

SC, saline control group; A, asthma group; LAE, *Lepidium apetalum* Willd. extract; DSE, *Descurainia sophia* Webb ex Prantl extract; LAE, A + LAE 100 mg/kg; DSE, A + DSE 100 mg/kg; Dex, A + dexamethasone 5 mg/kg. $^{####}P < 0.001$ compared with the SC group. $^{*}P < 0.05$ and $^{****}P < 0.001$ compared with the A group.

Figure 3. Effect of Lepidii seu Descurainiae Semen extract on recruitment (A) and

activation (B, C) of eosinophils in lung. Recruitment and activation were measured using

flow cytometry. OVA, ovalbumin; SC, saline control group; A, asthma group; LAE, *Lepidium apetalum* Willd. extract; DSE, *Descurainia sophia* Webb ex Prantl extract; MFI, mean fluorescence intensity; LAE, A + LAE 100 mg/kg; DSE, A + DSE 100 mg/kg; Dex, A + dexamethasone 5 mg/kg. $^{##}P < 0.01$ compared with the SC group. $^{*}P < 0.05$ and $^{****}P < 0.001$ compared with the A group.

Figure 4. Effect of Lepidii seu Descurainiae Semen extract on expression of type2

cytokines in BALF (A) and cultured media (B). BALF was collected 18 h after the last

OVA challenge. Cultured media was collected after culture of total lung cells for four days.

OVA, ovalbumin; SC, saline control group; A, asthma group; LAE, *Lepidium apetalum* Willd. extract; DSE, *Descurainia sophia* Webb ex Prantl extract; LAE, A + LAE 100 mg/kg; DSE, A + DSE 100 mg/kg; Dex, A + dexamethasone 5 mg/kg. $^{##}P < 0.01$ and $^{####}P < 0.001$ compared with the SC group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, and $^{****}P < 0.001$ compared with the A group.

Figure 5. Effect of *Lepidii* seu *Descurainiae* Semen extract on activation of CD4⁺ T cells in lung. Activation was measured using flow cytometry (A, B). GATA3 expression was measured using western blot (C) and normalized using β -actin (D). OVA, ovalbumin; SC, saline control group; A, asthma group; LAE, *Lepidium apetalum* Willd. extract; DSE, *Descurainia sophia* Webb ex Prantl extract; LAE, A + LAE 100 mg/kg; DSE, A + DSE 100 mg/kg; Dex, A + dexamethasone 5 mg/kg. $^{####}P < 0.001$ compared with the SC group. $^{**}P < 0.01$ and $^{****}P < 0.001$ compared with the A group.

Figure 6. Effects of *Lepidii* seu *Descurainiae* Semen extracts on Th2 cell differentiation and activation in vitro. Mean fluorescence intensity (MFI) of GATA3 in CD4⁺ T cells were analyzed. Representative data (A) is shown. IL-5 and IL-13 were measured by ELISA (B, C). no-sti, without stimulation; neutral, stimulated with CD3 and CD28; Th2, stimulated with neu + IL-2, IL-4 and anti-IFN γ ; LAE, *Lepidium apetalum* Willd. extract; DSE, *Descurainia sophia* Webb ex Prantl extract; LAE100, Th2 + LAE 100 μ g/ml; LAE200, Th2 + LAE 200 μ g/ml. DSE100, Th2 + DSE 100 μ g/ml; DSE200, Th2 + DSE 200 μ g/ml. $^{**}P < 0.01$ and $^{****}P < 0.001$ compared with the Th2 group.

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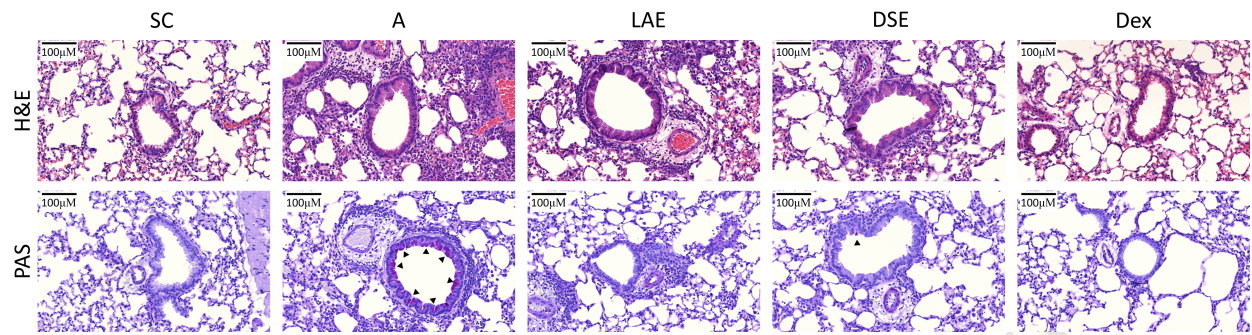
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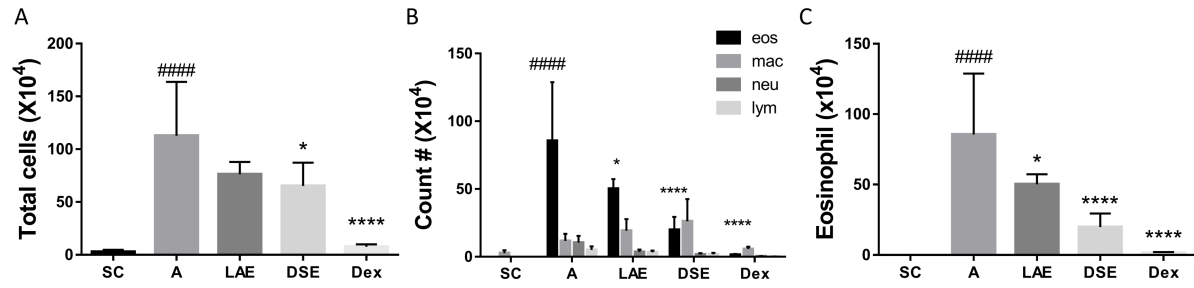
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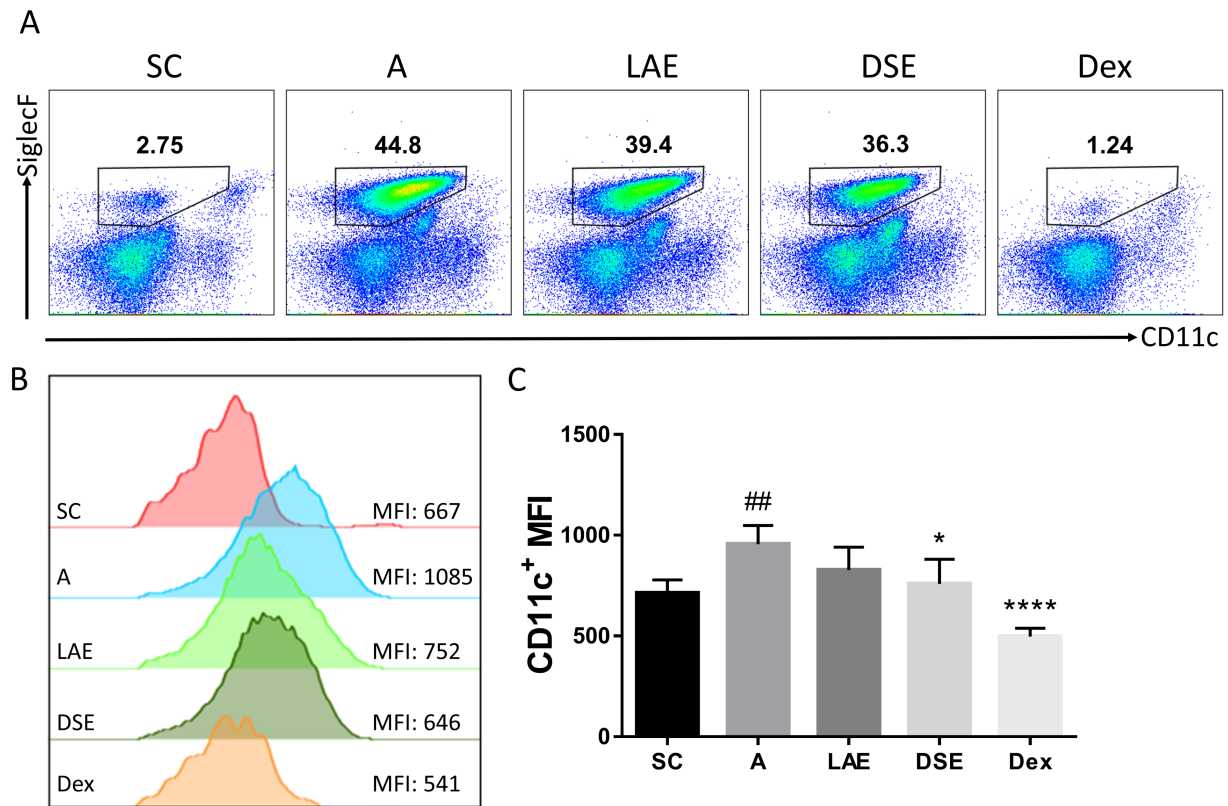
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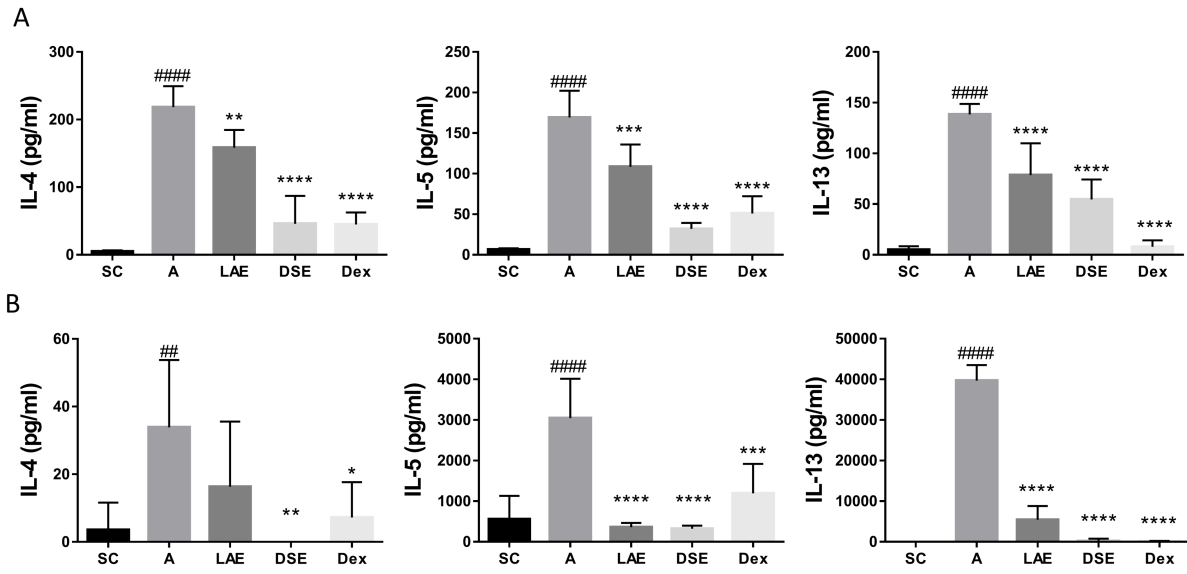
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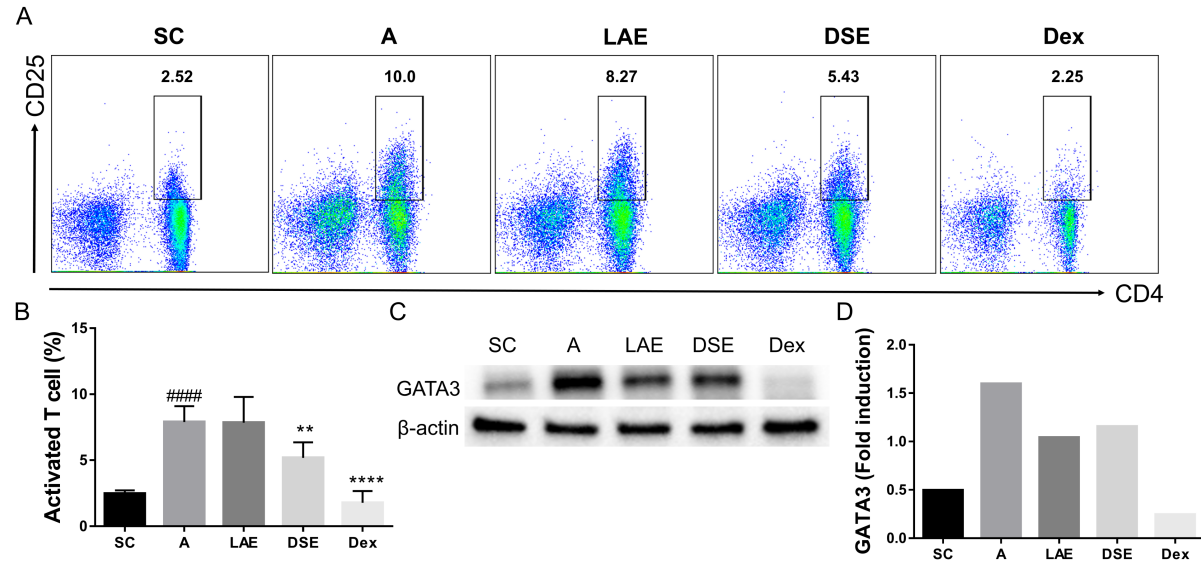
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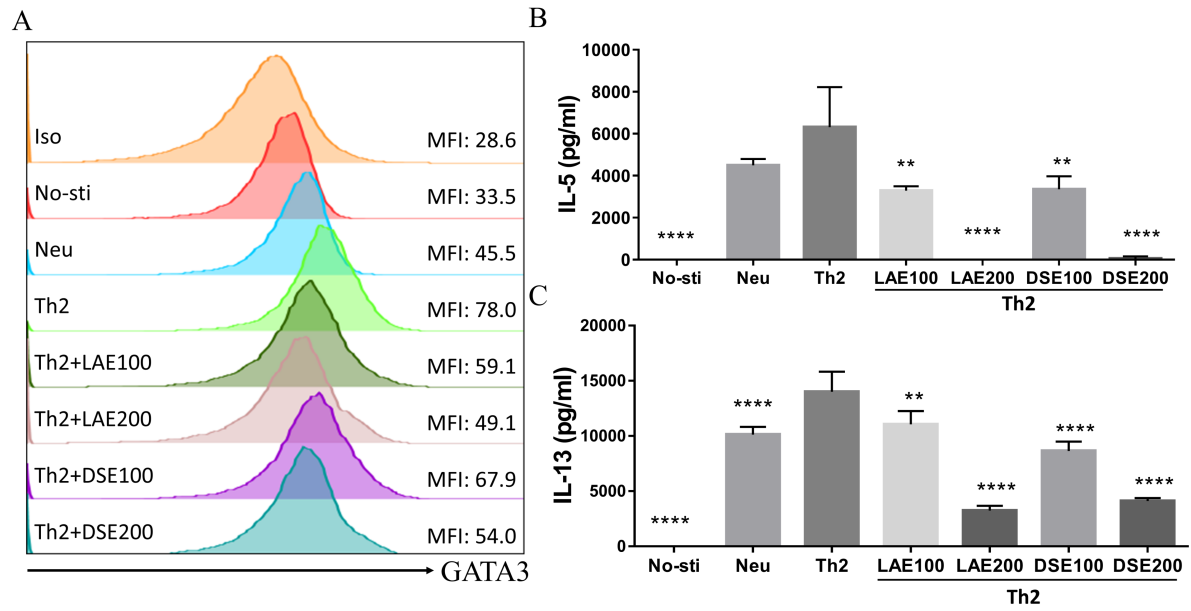


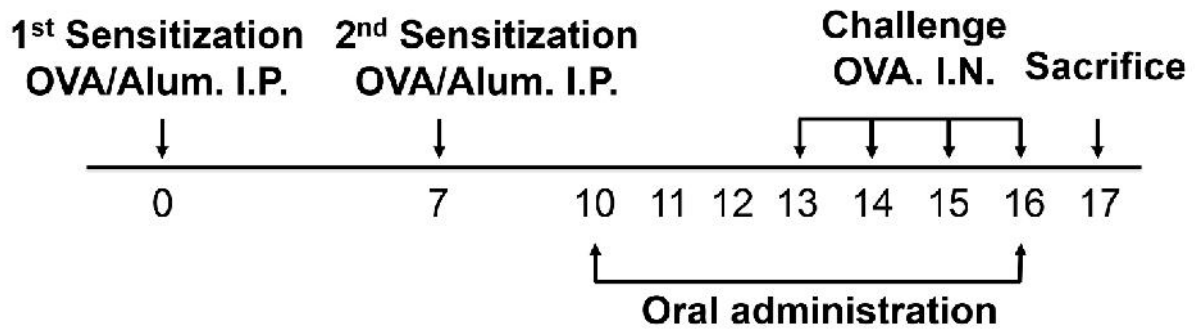












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D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

230 240 250 260 270 280 290 300 310 320 330 340 350 360 370 380 390 400 410 420 430 440

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

470 480 490 500 510 520 530 540 550 560 570 580 590 600 610 620 630 640 650 660 670

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

690 700 710 720 730 740 750 760 770 780 790 800 810 820 830 840 850 860 870 880 890

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

910 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080 1090 1100 1110

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

1130 1140 1150 1160 1170 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320 1330

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

1350 1360 1370 1380 1390 1400 1410 1420 1430 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690 1700 1710 1720 1730 1740 1750 1760

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

1780 1790 1800 1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920 1930 1940 1950 1960 1970 1980

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

2000 2010 2020 2030 2040 2050 2060 2070 2080 2090 2100 2110 2120 2130 2140 2150 2160 2170 2180 2190 2200

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

2220 2230 2240 2250 2260 2270 2280 2290 2300 2310 2320 2330 2340 2350 2360 2370 2380 2390 2400 2410 2420

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

2440 2450 2460 2470 2480 2490 2500 2510 2520 2530 2540 2550 2560 2570 2580 2590 2600 2610 2620 2630 2640

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

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D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

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D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

3100 3110 3120 3130 3140 3150 3160 3170 3180 3190 3200 3210 3220 3230 3240 3250 3260 3270 3280 3290 3300

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

3320 3330 3340 3350 3360 3370 3380 3390 3400 3410 3420 3430 3440 3450 3460 3470 3480 3490 3500 3510 3520

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

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D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

3760 3770 3780 3790 3800 3810 3820 3830 3840 3850 3860 3870 3880 3890 3900 3910 3920 3930 3940 3950 3960

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

3980 3990 4000 4010 4020 4030 4040 4050 4060 4070 4080 4090 4100 4110 4120 4130 4140 4150 4160 4170 4180

D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

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D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

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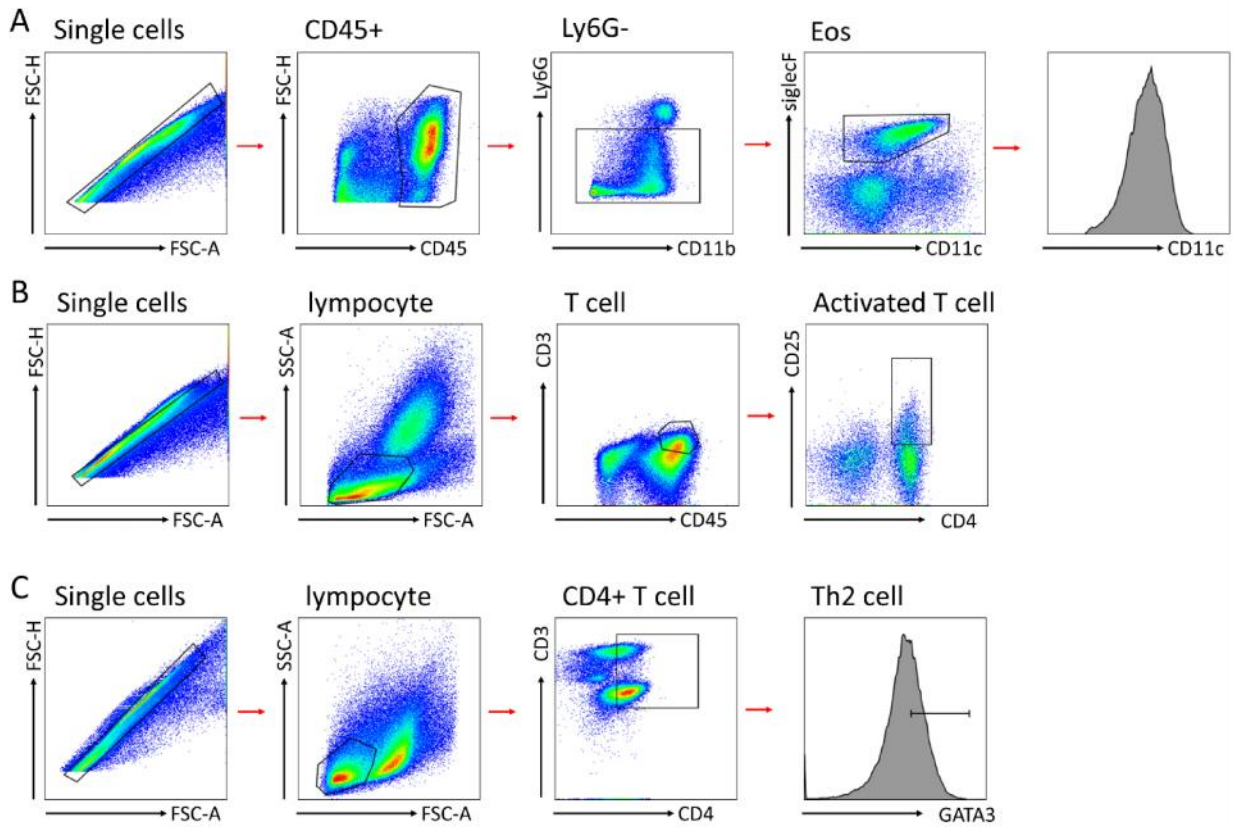
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Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

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D. eugenia
Sample D8(2-18-0137)
L. apetalum
Sample LA(2-18-0136)
L. latifolium
R. cheiranthoides
R. aciculateum
D. nemorosa

4860 4870 4880 4890 4900 4910 4920 4930 4940 4950 4960 497

Supplementary Fig. 2. Authentication of botanical origin of Lepidii Seu Descurainiae Semen samples by the comparative analysis of *matK* sequences (*D. sophia*, KIOM-CNB2016-028; *L. apetalum*, KIOM-MBC2016-0881; *L. latifolium*, KIOM-MBC2016023; *E. cheiranthoides*, KIOM-MBC2016-2281; *E. macilentum*, KIOM-MBC2016-0401; *D. nemorosa*, KIOM-YSG2016-010; Sample DS, 2-18-0137; Sample LA, 2-18-0136). Dots (·) represent the identical nucleotide sequence to *D. Sophia* sequence. Dashes (-) represent gaps to maximized alignment. The yellow and blue boxes represent the samples revealed identical nucleotide sequences, respectively.



Supplementary Fig. 3. Flow cytometric gating strategy for analysis of eosinophil activation (A), CD4⁺ T cell activation (B) and Th2 cell polarization (C).