



# Skullcapflavone II attenuates ovalbumin-induced allergic rhinitis through the blocking of Th2 cytokine production and mast cell histamine release

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## ABSTRACT

Allergic rhinitis is a common heterogeneous chronic upper airway disorder and is an IgE-mediated inflammation characterized by one or more nasal symptoms such as sneezing, itching, nasal discharge, rhinorrhea, post nasal drainage and nasal blockage. In the present study, the effects of skullcapflavone II (SCFII) on upper airway inflammation, Th2 cytokines, and NF- $\kappa$ B signaling in an ovalbumin (OVA)-induced allergic rhinitis (AR) murine model *in vivo* were investigated. OVA-induced AR mice increased nasal symptoms, eosinophils and mast cells infiltration into nasal cavity, OVA-specific IgE/IgG1 and histamine in serum, Th2 cytokines including IL-13 and GATA3, and NF- $\kappa$ B signaling in NALF and lung homogenate. Interestingly, treatment of SCFII reduced the levels of OVA-specific IgE/IgG1 and histamine in serum, of Th2 cytokines and of NF- $\kappa$ B signaling in the NALF and the lung homogenate, and histopathological changes in the nasal tissue and the lung. Also, dexamethasone suppressed such increases. The results of this study suggested that SCFII may ameliorate allergic inflammation of upper airway in AR mice model by blocking the Th2 cytokine production, the NF- $\kappa$ B signal pathway and the mast cell histamine release. Taken together, we suggest that SCFII may be used as a therapeutic agent for patients with Th2-mediated or mast cell-mediated allergic diseases.

## 1. Introduction

Allergic rhinitis (AR) is a common heterogeneous chronic upper airway disorder and is an IgE-mediated inflammation characterized by one or more nasal symptoms such as sneezing, itching, nasal discharge, rhinorrhea, post nasal drainage and nasal blockage [1,2]. AR is triggered after allergen-specific IgE and Th2 cells recognize inhalant allergen in the environment, and its inflammatory process involves many different inflammatory cells including eosinophils and mast cells, cytokines, and other regulatory molecules [3]. Among immune cells, mast cells and B cells play pivot roles in the pathogenesis of allergic reaction in AR. In allergic reactions, mast cells activate when an allergen interacts with IgE [4]. After antigen-IgE stimulation, mast cells release allergic mediators including histamine, cytokines, chemokines and arachidonic acid derivatives, mediating acute and chronic inflammation [5]. These steps are associated to induce the recruitment of inflammatory cells and eosinophils into affected site.

Even though AR is currently regulated with steroidal and non-steroidal anti-inflammatory agent, the rates of side effects and recurrence have been increasing [6]. Since these agents are still not perfect,

development of new better drugs is need. Herbal medicines have been used for the traditional treatment of various allergic diseases with their potential safety and efficacy. There has been growing interest in natural product-based anti-inflammatory agents, which have high safety and less side-effect.

*Scutellaria baicalensis* Georgi is one of the most popular and multi-purpose herbal medicines or medicinal plants used in oriental countries including China, Japan, and Korea to treat inflammation, allergy, and bacterial and viral infections [7–10]. Recently, investigations have shown that *S. baicalensis* has beneficial properties such as anti-oxidative effect [11], and inhibits anti-dinitrophenyl IgE-mediated anaphylactic reactions and compound 48/80-induced histamine release mast cells [12]. Among flavonoids isolated from the dry roots of *S. baicalensis*, Skullcapflavone II (SCFII; 5,2'-dihydroxy-6,7,8,6'-tetramethoxy-flavone) has been known a wide range of biological activities such as anti-inflammatory, antibacterial, and antioxidant effects [12–16]. However, there is no report regarding the effects of SCFII on AR in a mouse model. Here, we investigated the effects of SCFII on upper airway inflammation, Th2 cytokine production, histamine secretion and NF- $\kappa$ B signal pathway in an ovalbumin (OVA)-induced AR murine model.

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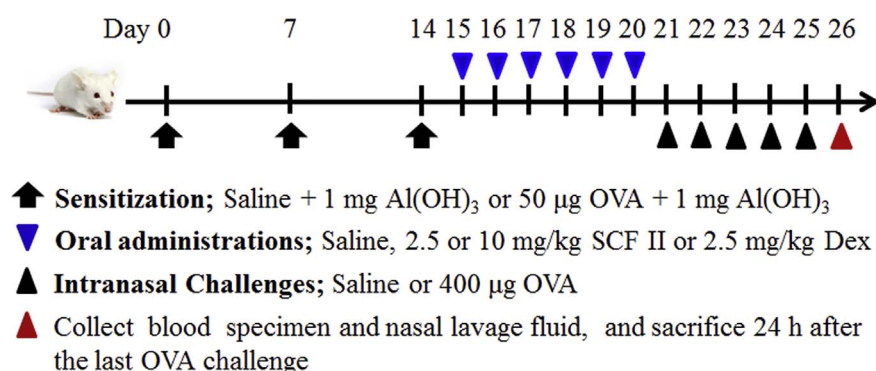


**Fig. 1.** Sensitization, challenge, and oral administration protocol for allergic rhinitis mouse model. Mice were sensitized on days 0, 7 and 14 and challenged on days 21–25 by ovalbumin (OVA). Mice in control group was sensitized and challenged by saline. Mice in the Skullcapflavone II (SCFII) or Dexamethasone (Dex) treatment groups were sensitized and challenged by OVA and administered orally once a day at 2.5, 10 mg/kg SCFII or 2.5 mg/kg Dex for 6 days.

## 2. Materials and methods

### 2.1. Animals

BALB/c male mice, aged 5–6 weeks, were purchased from Damool Science (Daejeon, Korea). These animals were housed in an air-conditioned room with a 12 h light/dark cycle and had unrestricted access to OVA-free food and water. All animal experiments were performed in accordance with the Guidelines on Animal Care and Use and were approved by the Institutional Animal Care and Use Committee of Chonbuk National University Laboratory Animal Center (CBN 2016-37).

### 2.2. Induction of AR murine model

Mice were divided into 5 groups according to treatment: (1) control group, (2) OVA group, (3) 2.5 mg/kg/day SCFII, (4) 10 mg/kg/day SCFII, and (5) 2.5 mg/kg/day dexamethasone (Dex) as reference drug for positive control in OVA-induced AR mice. The experimental procedure for the mouse model of AR is summarized in Fig. 1. Independent experiments of each group were repeated twice. Mice of groups (2), (3), (4) and (5) were immunized by intraperitoneal injection of 50 µg OVA (Sigma, St. Louis, MO, USA) with 1 mg Imject Alum (Pierce, Rockford, IL, USA) in a total volume of 200 µl on days 0, 7, and 14 while control group was injected with alum in saline on the same schedule. Next, on 5 consecutive days from 21 to 25 after the beginning of the sensitization period, these mice were challenged with 400 µg OVA (10 mg/ml, 20 µl/nose) through intranasal instillation of both nostrils. On days 15 to 20, the treatment groups were also orally treated once daily with SCFII (Sigma, St. Louis, MO, USA) or Dex (Innovative Research of America, Toledo, Ohio, USA). OVA group was orally received saline instead of SCFII or Dex. Animals were sacrificed 24 h after the last challenge on day 26 to investigate the inhibitory effect of SCFII on OVA-induced AR.

### 2.3. Clinical symptoms

Nasal symptoms were evaluated by counting the frequencies of nasal rubbing and sneezing. After 400 µg of OVA intranasal challenge on days 21 to 25, the numbers of sneezing and nasal rubbing behaviors were recorded during a 20-minute period and were counted by blinded observers. And then, mice were sacrificed 24 h after the last OVA challenge, and head, lung, and nasal lavage fluid (NALF) were obtained for analysis.

### 2.4. Collection of blood sample

Twenty-four hours after the final OVA challenge, blood specimens were collected from retro orbital plexus and serum was obtained by centrifugation 1000 g for 10 min at 4 °C (Centrifuge 5403; Eppendorf, Hamburg, Germany). Samples were stored at –20 °C until use.

### 2.5. Collection and analysis of NALF

Twenty-four hours after the last OVA challenge, NALF was collected by cannulating the upper part of the trachea into the nasal cavity direction and lavaging. The total number of viable cells in NALF was determined by trypan blue exclusion using a hemocytometer. Differential cell counts were determined with cytopsin (Centrifuge 5403; Eppendorf, Hamburg, Germany) preparation, followed by Diff Quik staining (Sysmex Co., Kobe, Japan).

### 2.6. Histopathologic analysis

Heads of mice were excised and fixed in 10% neutral formalin for 2 days at room temperature. After fixation, the heads were decalcified in histological decalcifying agent containing hydrochloric acid and EDTA (National diagnostics, Atlanta, GA) for 6 days and then embedded in paraffin. Histopathologic analysis of lung was performed according routine procedures. Samples were cut into 4 µm thickness sections and sections were stained with either hematoxylin-eosin for overall inflammation, periodic acid-Schiff (PAS) for goblet cells and mucus, and Giemsa for eosinophils and mast cells. After Giemsa staining, the mast cells under high magnification (×1000) were classified as follows: (1) severely degranulated (> 50% of the cytoplasmic granules exhibiting fusion, staining alteration, and extrusion from the cells), (2) slight to moderately degranulated (10–50% of the granules exhibiting fusion or discharge), or (3) normal.

### 2.7. Measurements of Th1 and Th2 cytokines, OVA-specific IgE, IgG1, and IgG2a and NF-κB, IkB

The levels of Th1 cytokines such as IFN-γ, Th2-related cytokines such as TNF-α, IL-4, IL-6, IL-13 and GATA3 in the NALF and lung homogenates from each mouse using the appropriate ELISA (BioSource International, Camarillo, CA, USA) were measured. OVA-specific IgE, IgG1, IgG2a in serum were quantified by using ELISA kits (R & D Systems Inc., USA) according to the manufacturer's instructions. Also, NF-κB and IkB in NALF and lung homogenate were examined by using ELISA kits (eBioscience Inc. USA) according to the protocols from the manufacturer.

### 2.8. Measurement of histamine in serum and NALF

The concentrations of histamine in serum and NALF from each mouse using the appropriate ELISA (MyBioSource, USA) were measured according to the manufacturer's instructions.

### 2.9. Statistical analysis

Results were expressed as mean ± standard deviation for the number of experiments. Student's *t*-test and ANOVA with Dunnett's test were used for statistical comparison among the groups. Results with

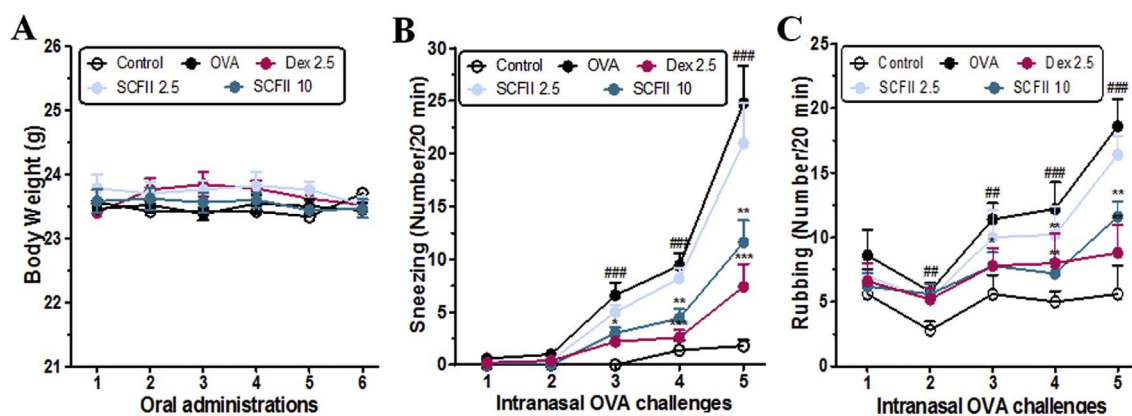


Fig. 2. Effects of Skullcapflavone II (SCFII) on (A) body weight, (B) sneezing, and (C) rubbing of ovalbumin (OVA)-induced allergic rhinitis mice. Values are presented as the mean  $\pm$  SD ( $n = 6$  per group).  $^{*}P < 0.01$ ,  $^{***}P < 0.001$ , significantly different from the value of control group.  $^{*}P < 0.05$ ,  $^{**}P < 0.01$ ,  $^{***}P < 0.001$ , significantly different from the value of OVA group. Dex; Dexamethasone.

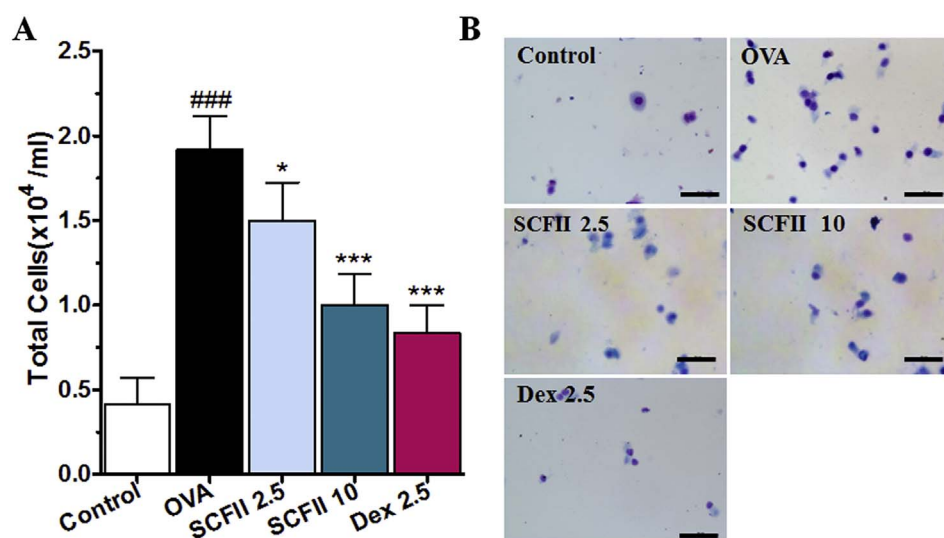


Fig. 3. Effects of Skullcapflavone II (SCFII) on (A) total cells and (B) differential cellular components in NALF of ovalbumin (OVA)-induced allergic rhinitis mice. Bar = 50  $\mu$ m. Values are presented as the mean  $\pm$  SD ( $n = 6$  per group).  $^{***}P < 0.001$ , significantly different from the value of control group.  $^{*}P < 0.05$ ,  $^{***}P < 0.001$ , significantly different from the value of OVA group. Dex; Dexamethasone.

$P < 0.05$  were considered statistically significant.

### 3. Results

#### 3.1. Body weight and food intake

Body weight and food intake were monitored during the study. Even though 2.5 mg/kg SCFII group exhibited little bit higher weight as compared to other groups, there was no significant difference between these groups (Fig. 2A). SCFII did not show any toxicity on keeping body weight (Fig. 2A).

#### 3.2. Effects of SCFII on nasal symptoms in OVA-induced AR mice

The main symptoms of AR are sneezing, runny nose, itching, rhinorrhea, and/or nasal congestion [17]. To investigate the effect of SCFII on nasal symptoms, after 400  $\mu$ g of OVA intranasal challenge on days 21 to 25, the numbers of sneezing and nasal rubbing behaviors were recorded during a 20-minute period and were counted by blinded observers. OVA-induced AR mice showed a significantly increase in sneezing (Fig. 2B) and nasal rubbing (Fig. 2C) as compared to control mice. At dose of 10 mg/kg/day, SCFII markedly decreased the number of sneezing. Similar results were obtained with nasal rubbing. SCFII showed anti-inflammatory effect as demonstrated by significantly prevention of OVA-induced nasal symptoms as compared with saline group of AR mice (Fig. 2B–C).

#### 3.3. Effects of SCFII on histopathological changes, eosinophil infiltration and mast cell activation of nasal mucosal tissues in OVA-induced AR mice

As shown in Fig. 3, the numbers of total cells and inflammatory cells in NALF from OVA-induced AR mice were significantly elevated compared to those of control mice (Fig. 3A–B). Oral administration of 10 mg/kg/day SCFII or 2.5 mg/kg/day Dex significantly reduced the increase of eosinophils and showed inflammatory cells similar to those of the mice treated with saline alone (Fig. 3A–B).

Because SCFII suppressed eosinophil infiltration into the NALF, the anti-allergic effects of the SCFII were next examined. Histopathological examinations showed epithelial disruption, mucosa edema, goblet cell hyperplasia in the mucosa and submucosa of nasal cavity and in the perivascular and peribronchial area of lung tissue from OVA-induced AR mice (Fig. 4A). The subepithelial layer of nasal cavity in OVA-induced AR mice also exhibited thicker than that of control mice (Fig. 4A). In addition, significant eosinophil infiltration, goblet cell hyperplasia (PAS + cells), mast cell infiltration and degranulation were observed in the mucosal and the submucosal layer of nasal tissues from OVA-induced AR mice (Fig. 4B–E). However, SCFII at doses of 2.5 mg/kg and 10 mg/kg protected nasal mucosa and submucosa layers against allergic responses and greatly reduced mucosa edema, eosinophil infiltration, goblet cell hyperplasia, mast cell infiltration and degranulation, especially the SCFII at dose of 10 mg/kg (Fig. 4).

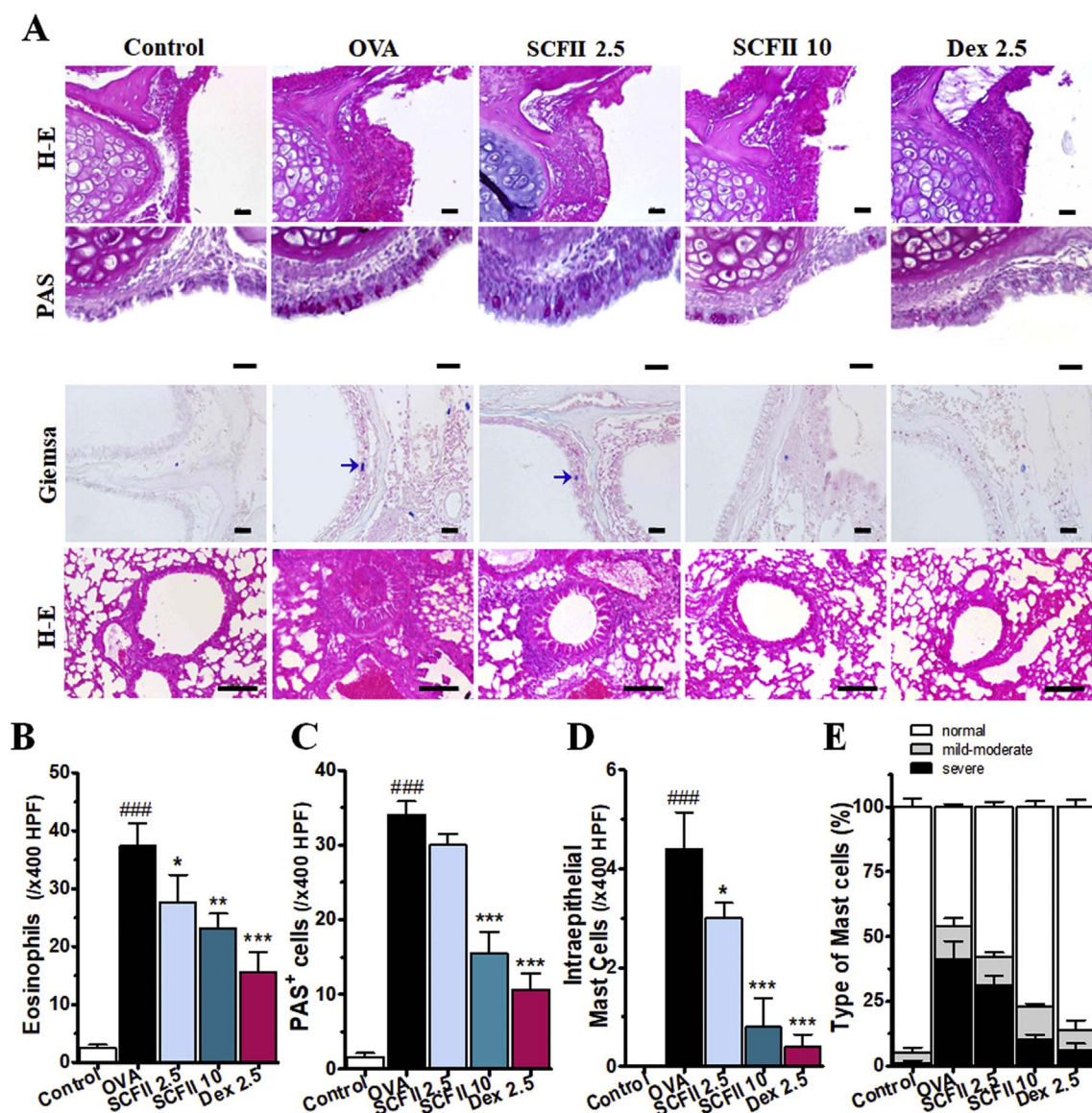


**Fig. 4.** Effects of Skullcapflavone II (SCFII) on (A) histopathological changes of the nasal tissues and the lung, (B) eosinophil infiltration, (C) PAS<sup>+</sup> cells, (D) mast cell infiltration, and (E) type of mast cells in the nasal tissues of ovalbumin (OVA)-induced allergic rhinitis mice. Nasal tissues from each group were stained with hematoxylin-eosin (H-E) for general inflammation, periodic acid-Schiff (PAS) for goblet cell hyperplasia, and Giemsa for mast cells (blue arrows). Also lung tissues from each group were stained with H-E. Only a representative picture is shown for each group. Bar = 25  $\mu$ m in the nasal tissues and Bar = 100  $\mu$ m in the lungs. <sup>###</sup> $P < 0.001$ , significantly different from the value of control group. <sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$ , <sup>\*\*\*</sup> $P < 0.001$ , significantly different from the value of OVA group. Dex; Dexamethasone. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 3.4. Effects of SCFII on the production of OVA-specific IgE, OVA-specific IgG1, and OVA-specific IgG2a in serum of OVA-induced AR mice

Allergic response is mainly based on the production and effects of IgE antibodies and other immunoglobulin classes such as IgG1 and IgG2a have been focused in allergy research [18]. To investigate the underlying anti-allergic mechanism of SCFII on the secretion of Th2- and Th1-related cytokines in NALF of AR mice induced by OVA, we detected the serum levels of Th2-related immunoglobulin such as OVA-specific IgE and IgG1 and Th1-related immunoglobulin such as OVA-specific IgG2a. As shown in Fig. 5, the levels of OVA-specific IgE and OVA-specific IgG1 were markedly increased in serum of OVA-induced AR mice compared with control mice. However, both 10 mg/kg SCFII and Dex significantly suppressed the levels of both the OVA-specific IgE and IgG1 in serum (Fig. 5A–B). In addition, SCFII tended to increase the OVA-specific IgG2a serum levels compared with OVA-induced AR mice (Fig. 5C), there were no significant differences between AR group and

SCFII treated group. These results showed that SCFII ameliorate AR symptoms through inhibiting production of OVA-specific IgE and OVA-specific IgG1.

### 3.5. Effects of SCFII on the production of Th1/Th2 cytokines in the NALF and the lung homogenate of OVA-induced AR mice

Next, Th2-related cytokines including TNF- $\alpha$ , IL-4, IL-5, IL-10, IL-13, GATA3 and Th1-related cytokines including IFN- $\gamma$  and IL-12 have been known to regulate IgE-mediated allergy and asthma [19,20] were also investigated in the NALF of each group mice using ELISA. As shown in Fig. 6, the levels of TNF- $\alpha$ , IL-4, IL-5, IL-13 and GATA3 not IL-10 in the NALF (Fig. 6A) and the lung homogenate (Fig. 6B) of OVA-induced AR mice were significantly increased compared with that of control mice. The level of IL-12 was reduced, though IFN- $\gamma$  level did not changed in both the NALF (Fig. 6A) and the lung homogenate (Fig. 6B) of OVA-induced AR mice. However, 10 mg/kg SCFII treatments

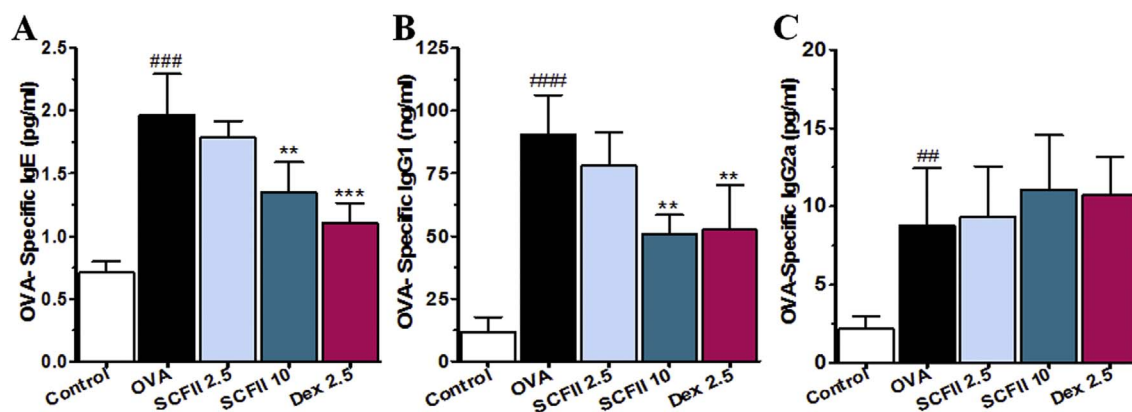


Fig. 5. Effects of Skullcapflavone II (SCFII) on the levels of (A) ovalbumin (OVA)-specific IgE, (B) OVA-specific IgG1 and (C) OVA-specific IgG2 in serum of OVA-induced allergic rhinitis mice. Values are presented as the mean  $\pm$  SD ( $n = 6$  per group). ## $P < 0.01$ , ### $P < 0.001$ , significantly different from the value of control group. \*\*  $< 0.01$ , \*\*\* $P < 0.001$ , significantly different from the value of OVA group. Dex; Dexamethasone.

attenuated the increases in TNF- $\alpha$ , IL-4, IL-5, IL-13 and GATA3, and improved the decrease of IL-12 in the NALF and the lung homogenate of AR mice induced by OVA (Fig. 6). These data indicated that SCFII might effectively regulate Th2-immune response on the OVA-induced AR model.

### 3.6. Effects of SCFII on the level of histamine in the serum and the NALF of OVA-induced AR mice

Mast cells play main roles in the pathogenesis of allergic reaction to AR [4,21]. After antigen-IgE stimulation, activated mast cells release allergic mediators including histamine, pro-inflammatory cytokines and chemokines that are associated to induce the recruitment of inflammatory cells and eosinophils [5]. To underlay the anti-allergic mechanism of SCFII on the secretion of Th2-related cytokines in NALF of AR mice induced by OVA, we assessed the levels of histamine in serum and NALF of each group mice. The level of histamine was markedly increased in serum and NALF of OVA-induced AR mice compared with control mice (Fig. 7). However, both SCFII and Dex inhibited histamine release of AR mice induced by OVA (Fig. 7). These data indicated that SCFII might improve Th2-mediated AR symptoms via blocking the histamine release.

### 3.7. Effects of SCFII on the production of NF- $\kappa$ B signaling in the NALF and the lung homogenate of OVA-induced AR mice

NF- $\kappa$ B, an important transcription factor, is well-known to play an important role in the progression of asthma and has been reported to regulate Th2 cell differentiation and cytokines such as TNF- $\alpha$ , IL-4, and IL-5 expression [22–24]. To investigate the underlying anti-allergic mechanism of SCFII on the secretion of Th2-related cytokines in NALF of AR mice induced by OVA, we assessed the levels of NF- $\kappa$ B activation and I $\kappa$ B degradation in NALF and lung homogenate of each group mice. The level of NF- $\kappa$ B was markedly increased and the level of I $\kappa$ B decreased in NALF and lung homogenate of OVA-induced AR mice compared with control mice (Fig. 8). However, both 10 mg/kg SCFII and Dex inhibited NF- $\kappa$ B activation and I $\kappa$ B degradation of AR mice induced by OVA (Fig. 8). These data indicated that SCFII might improve Th2-mediated AR symptoms via blocking the NF- $\kappa$ B signal activation.

## 4. Discussion

In this study, we investigated the effect of SCFII, one of major flavonoids from *S. baicalensis*, on allergic responses in OVA-induced AR mice and its therapeutic efficacy in AR. AR of mice induced by OVA show similar nasal allergic symptoms as observed in humans [25]. OVA-induced AR mice exhibited the increase of nasal symptoms such as

sneezing and rubbing and increased the infiltration of eosinophils and mast cells into nasal cavity. Interestingly, SCFII suppressed such increases. Histopathological examinations showed SCFII improved epithelial disruption, mucosa edema, goblet cell hyperplasia in the mucosa and submucosa of nasal cavity induced by OVA. Moreover, induction of various cytokines due to AR was also diminished by SCFII in OVA-induced AR mice.

Jang's study using an OVA-induced asthma mouse model [13] showed that SKFII inhibited OVA-induced airway inflammation, airway hyperresponsiveness, and early stage airway remodeling via decreased production of TGF- $\beta$ 1/Smad signaling pathway. It also focused on bradykinin antagonist functions of SKFII by comparing to montelukast. Although here were used the same Th2 inflammatory cytokines, the same SKFII dose and the same mice background, our study used the AR mice models induced by OVA and concentrated upon the effects of SKFII on the histamine and the mast cell functions as well as the relationship among Th2 cytokines especially IL-13, GATA3 as a modulator of Th2 cytokines, and NF- $\kappa$ B signal pathway.

Allergic response is mainly based on the production and effects of IgE antibodies and other immunoglobulin classes such as IgG1 and IgG2a have been focused in allergy research [18]. Specially, AR is triggered after allergen-specific IgE and Th2 cells recognize inhalant allergen in the environment, and its inflammatory process involves many different inflammatory cells including eosinophils and mast cells, cytokines, and other regulatory molecules [3,21]. OVA-induced AR mice showed highly expressed OVA-specific IgE, IgG1 and IgG2a in serum. Treatment of SCFII reduced the levels of OVA-specific IgE and OVA-specific IgG1, which are hall markers of the Th2 immune response. These findings imply that SCFII improves AR symptoms by reducing the levels of OVA-specific IgE and OVA-specific IgG1.

Mast cells play main roles in the pathogenesis of allergic reaction to AR [4,21]. After antigen-IgE stimulation, activated mast cells release allergic mediators including histamine, pro-inflammatory cytokines and chemokines that are associated to induce the recruitment of inflammatory cells and eosinophils [5]. A proinflammatory cytokine TNF- $\alpha$  secret from mast cells promotes inflammation, leukocyte infiltration, tissue fibrosis and is thought to be an initiator of cytokine-related inflammatory states via stimulation of cytokine production [26,27]. Histamine from mast cells exerts many effects related to allergic inflammations such as tissue edema, contraction of bronchial and smooth muscle, and mucus overproduction [28]. It has been reported that treatments of antihistamine drugs and mast cell stabilizers suppress allergic symptoms of allergic diseases such as allergic conjunctivitis and ocular allergy [29,30] and blockade of TNF- $\alpha$  reduce pathological allergic inflammation in the nasal and lung tissues of AR guinea pigs [31,32]. Even though, OVA-induced AR mice significantly increased the level of TNF- $\alpha$  in NALF and histamine in serum, SCFII administration

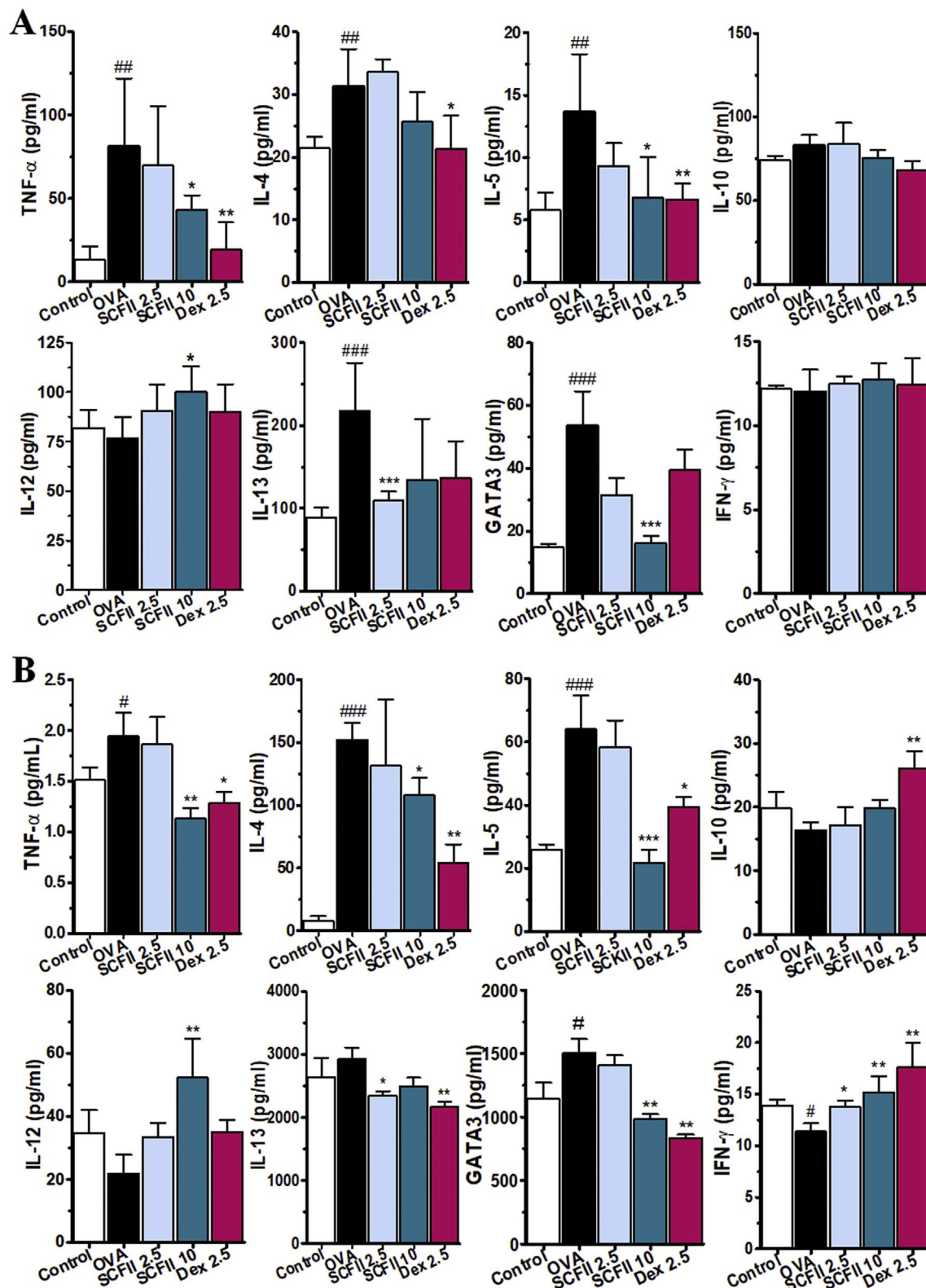


Fig. 6. Effects of Skullcapflavone II (SCFII) on the levels of TNF-α, IL-4, IL-5, IL-10, IL-12, IL-13, GATA3 and INF-γ in the NALF (A) and the lung homogenates (B) of ovalbumin (OVA)-induced allergic rhinitis mice. Values are presented as the mean  $\pm$  SD ( $n = 6$  per group). <sup>#</sup> $P < 0.05$ , <sup>##</sup> $P < 0.01$  and <sup>###</sup> $P < 0.001$  significantly different from the value of control group. <sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$  and <sup>\*\*\*</sup> $P < 0.001$  significantly different from the value of OVA group. Dex; Dexamethasone.

reduced the level of TNF-α in NALF and histamine in serum induced by OVA. Consistent with the reduction of TNF-α and histamine levels, the infiltrations of mast cell into epithelial tissues of nasal cavity were decreased after SCFII treatment.

Eosinophilia in the blood and tissues is one of main characteristics of human allergic inflammation [33] and recruitment of eosinophils during human allergen-induced rhinitis is associated with cells expressing mRNA for IL-4, IL-5, and granulocyte [34,35]. Also, it has been

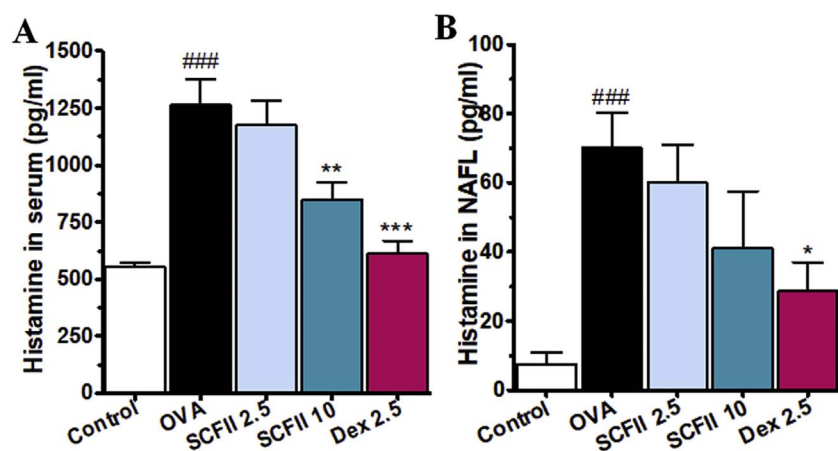


Fig. 7. Effects of Skullcapflavone II (SCFII) on the levels of histamine in the serum (A) and in the NALF (B) of ovalbumin (OVA)-induced allergic rhinitis mice. Values are presented as the mean  $\pm$  SD ( $n = 6$  per group). ### $P < 0.001$ , significantly different from the value of control group. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ , significantly different from the value of OVA group. Results are presented as the mean  $\pm$  SD ( $n = 6$  per group). Dex; Dexamethasone.

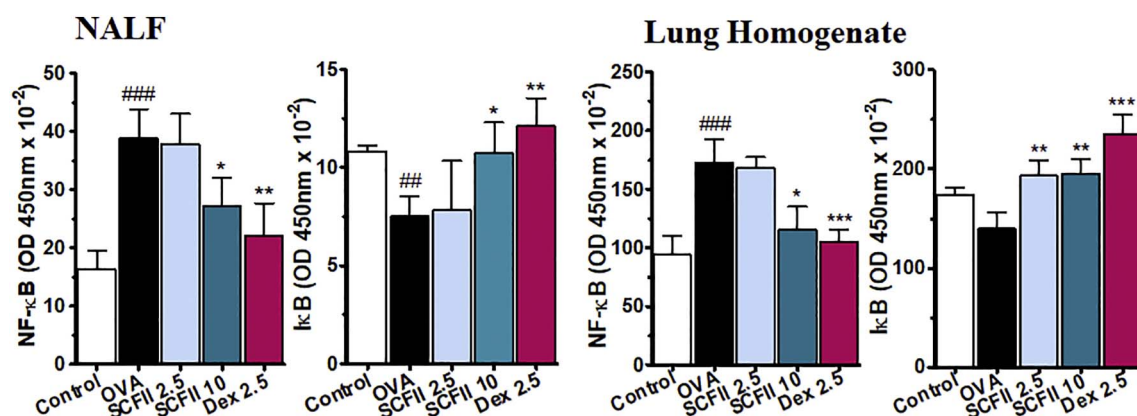


Fig. 8. Effects of Skullcapflavone II (SCFII) on the levels of NF-κB and IκB in NALF and lung homogenate of ovalbumin (OVA)-induced allergic rhinitis mice. Values are presented as the mean  $\pm$  SD ( $n = 6$  per group). ### $P < 0.01$ , ### $P < 0.001$ , significantly different from the value of control group. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ , significantly different from the value of OVA group. Results are presented as the mean  $\pm$  SD ( $n = 6$  per group). Dex; Dexamethasone.

well known that there is the association between clinical severity and Th2 cell-mediated immune imbalance [36,37]. Specially, IL-4 and IL-13 are related to Th2 cells differentiation and regulate IgE synthesis, and IL-5 is associated to eosinophilic inflammation and infiltration into airway. Moreover, IL-4 and IL-13 induce mucus hypersecretion and contribute to the increase in airway hyperreactivity in allergic asthma [38]. Th2 cells produce IL-4, IL-5 as a result of GATA3 actions and Th2 responses are completely dependent on the transcription factor GATA3 [39–40] that is essential for the differentiation of Th2 cells and also relatively abundantly expresses in eosinophils [41]. Oral administration of SCFII alleviated the increased levels of Th2 cytokines and GATA3 in NALF and lung homogenates, suggesting that it may exert its anti-allergic effect by inhibiting Th2 cytokine activity. These results are also supported by the histopathologic findings. Consistent with the reduction of these cytokines levels, the infiltration of inflammatory cells especially of eosinophils and mast cells was decreased in the NALF and in the nasal tissue after oral administration of SCFII. The suppression of Th2 cytokine activity may contribute to the anti-allergic effects of SCFII.

NF-κB is an important transcription factor and is well-known to play an important role in the progression of airway inflammation [22–24]. Activated NF-κB has been mediated increasing the expression of Th2 cytokines such as IL-4 and IL-5 and the activation of NF-κB signal pathway may play a critical role in the pathogenesis of AR [42]. Therefore, local inhibition of NF-κB could have therapeutic effect on the treatment of AR [43]. SCFII pretreatment relieved the elevation of NF-κB level and the degradation of IκB in NALF and lung homogenate, suggesting that it may have its anti-allergic effects by inhibiting the activation of NF-κB signal pathway. Th2 cytokine analysis supported

these results. Consistent with blocking NF-κB signal pathway, the levels of IL-4, IL-5, IL-13 and GATA3 were decreased in the NALF by SCFII. These findings imply that SCFII may have therapeutic potential for AR through the blocking of NF-κB signal pathway.

In conclusion, we distinctly demonstrates that SCFII ameliorates allergic inflammation of AR mouse model by blocking the Th2 cytokine productions via the inhibition of histamine release and NF-κB signal pathway. These findings suggest that SCFII could be useful agent for the treatment of AR as well as mast cell-mediated allergic diseases.

#### Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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