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Enzyme-Assisted Extraction of Narirutin from Citri Reticulatae Pericarpium and Anti-allergic Asthma Activity

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ABSTRACT

Background: Asthma is a heterogeneous disorder of the airways related to inflammation; it affects millions of people worldwide. Due to the side effects of inhaled corticosteroids, researchers focused on the therapeutic effects of compounds derived from natural products.

Objective: To investigate the therapeutic benefits of Narirutin a valuable flavonoid in *Citri Reticulatae* Pericarpium for asthma.

Methods: Narirutin was extracted using the enzyme-assisted method with the L_9 (3^4) orthogonal array to optimize the temperatures, pH, and reaction time. The mechanism of action of Narirutin was investigated via ELISA, flow cytometry, and Western blot analysis *in vivo*.

Results: Narirutin suppressed inflammatory cell infiltration in the lung tissue and decreased IgE and IgG1 levels in serum *in vivo*. It can also alleviate interleukin (IL)-4, IL-5, and interferon-g concentrations in bronchoalveolar lavage fluid in mice. Moreover, it increased the ratio of $CD4^+/CD8^+$ T cells. Additionally, Narirutin significantly suppressed p-ERK1/2 and p-JNK expression in the MAPK signaling pathway.

Conclusion: Narirutin affects the Th1/Th2 imbalance through the p-ERK and p-JNK suppression in the MAPK signaling pathway.

Keywords: Allergy, *Citri Reticulatae* Pericarpium, Immunology, Inflammation, Narirutin

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INTRODUCTION

Nearly 300 million people are affected by asthma worldwide [1, 2]. It may be caused by an allergic disorder of the airways related to inflammation [3]. Increasing evidence indicates that inflammation is related to the response of T cells, especially to the balance of Th1/Th2 [4, 5] and Th2 cytokines releasing, such as interleukin 4 (IL-4), interleukin 5 (IL-5) and interleukin 13 (IL-13), which are important in the asthmatic response [6, 7]. In recent years, researchers have focused on compounds derived from natural products and their potential therapeutic application in allergic asthma, as these natural compounds have fewer side effects [8, 9].

Citri Reticulatae Pericarpium, known as Chenpi, is commonly used in China. Flavonoids are the major components in *Citri Reticulatae* Pericarpium and have several functional benefits [10, 11]. It has been seen that flavonoids have anti-tumor, anti-inflammatory, and antimicrobial activities. Narirutin, the main flavonoid from *Citri Reticulatae* Pericarpium, is claimed to attenuate inflammation [11]. The only published work on narirutin inhibitory effects on airway inflammation was performed by Norihiko Fungaguchi in 2007 [12]. However, the exact mechanism underlying this effect remains unclear. Much more, as the biggest citrus production country, each year, China processes tons of citrus peels into *Citri Reticulatae* Pericarpium. The extraction and study of the anti-asthma mechanism of narirutin are important for the expanded application of citrus peels.

Thus, the target of this work is to optimize the extraction conditions of narirutin by enzyme-assisted extraction method and to identify the mechanism underlying the anti-allergic asthma activity of narirutin *in vivo*. Results obtained strongly support the application of enzyme-assisted extraction as an effective way to extract narirutin in *Citri Reticulatae* Pericarpium. Furthermore, it also clarifies that the exact mechanism of narirutin on anti-allergic asthma was related to p-ERK and p-JNK suppression in the MAPK signaling pathway.

MATERIALS AND METHODS

Chemicals and Materials

Citri Reticulatae Pericarpium was purchased from Changchun, China. HPLC-grade acetonitrile and methanol were purchased from Merk. Milli-Q water purification system was applied to purify water for HPLC analysis. All other analytical grade chemicals and reagents were obtained from Beijing Chemicals (China). Narirutin was obtained from Chengdu Must Bio-Technology Co., Ltd (Chengdu, China). The standards were dissolved in methanol for stock solution (concentrations of 0.3125, 0.625, 1.25, 2.50, and 5.0 mg/mL). All solutions were stored at 4°C. The standards were brought to room temperature before use. 0.22 μm nylon membranes (Millipore, MA) were used to filter all the standard solutions and samples for Liquid chromatography (LC) injection. The OVA and aluminum hydroxide gel were purchased from Sigma Co. (St. Louis, MO, USA). ELISA kits for IgE, IL-4, IL-5, and INF-γ detection were obtained from eBioscience (USA). Nylon wool fiber was provided by Polysciences and pretreated according to the instructions, Inc. Rat anti-mouse CD3-PerCP-Cy5.5, CD4-FITC, and CD8-PE were provided by BD (USA).

Enzyme-assisted Extraction Method

Citri Reticulatae Pericarpium was powdered and then extracted. Briefly, 0.75 g of powder was mixed with the enzymes. Enzyme-assisted extraction was performed at different temperatures, pH levels, and enzyme hydrolysis times. Finally, 6 mL of 60% ethanol solution was applied to extract narirutin; this process was performed in triplicate. After evaporation to dryness, the extracts were topped up to a constant volume of 10 mL using methanol for HPLC detection.

The HPLC conditions were consistent with our previous work [13]. The orthogonal experiment of L 9 (3^4) was applied to optimize the extraction conditions.

Animal Protocol

BABL/c mice (Female, six-weeks-old) were provided by Changchun Institute of Biological Products Co, Ltd. The Certificate No. was SCXK (Ji) 2011-0003, China. The mice were kept in a clean environment at room temperature 24±2 ℃ and used after one week of acclimatization. All the experiments were performed under the direction of the Animal Welfare and Research Ethics Committee of Jilin University. Five groups were gathered: the control, the OVA, DXMS, and narirutin with two concentrations (25 mg/kg and 50 mg/kg). The experimental protocol was as follows: all groups except the control one were sensitized with the OVA on days 1, 7, and 14 by intraperitoneal (i.p.) injection. The control group was sensitized with saline. All groups except the control one were treated with the OVA on days 21, 22, and 23 by nasal excitation. The control group was excited with saline administered nasally. The DXMS and narirutin groups received i.p. injections on days 21, 22, and 23, two hours before nasal excitation (Figure 1). The animals were sacrificed 24 hours after the last challenge (day 23).

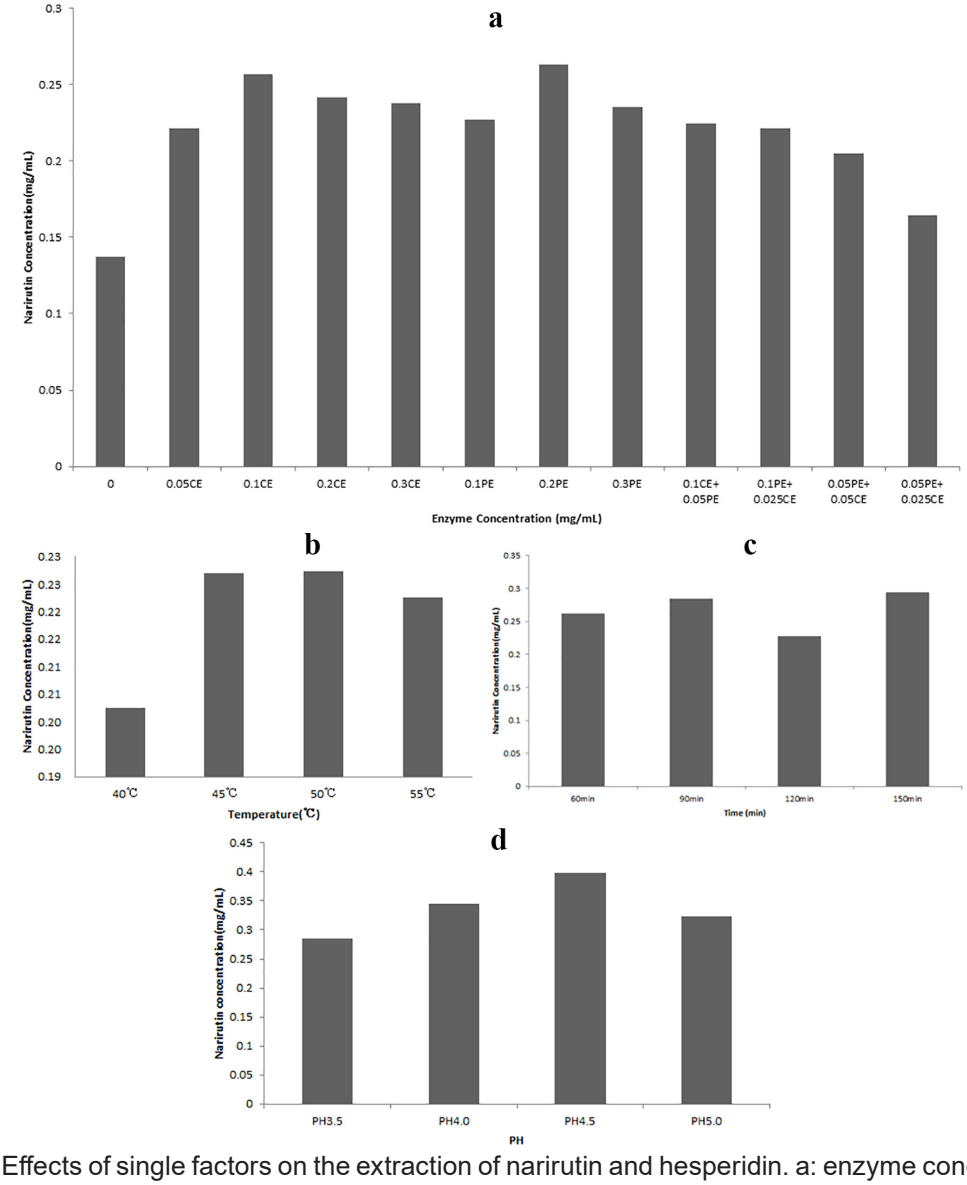

Figure 1. Effects of single factors on the extraction of narirutin and hesperidin. a: enzyme concentration; b: extraction temperature; c: enzyme hydrolysis time; d: pH.

Histological Assessment

Lung tissues were obtained after being fixed with 10% formalin: tissues were embedded in paraffin wax. The tissues from the left lung were used for analysis. Briefly, tissues were rinsed in PBS and dehydrated with ethanol. After being embedded in wax, tissues were sliced into sections. Hematoxylin and eosin (H&E) was used to stain the fixed tissues. Then, they were observed by an inverted microscope (OLYMPUS, TH4-200). H&E staining is mainly used to distinguish various cells through the morphology and size of cells and nuclei. When treated with alkaline hematoxylin dye, the chromatin in the nucleus and ribosome in the cytoplasm presented purple-blue; when treated with acidic Eosin dye, the components in the cytoplasm and extracellular matrix presented red. In this work, the accumulation of eosinophils around the bronchioles, airway lumen, and smooth muscle thickness were used to evaluate the effects of narirutin on lung tissues.

Determination of IgE and IgG1

Blood samples were collected 24 hours after the last challenge and were centrifuged (10000 r/min, 4 ℃). Total blood plasma IgE and IgG1 levels were determined by ELISA. Broncho alveolar lavage fluid (BALF) was obtained and centrifuged (2500 r, 15 min, 4 ℃), then the supernatant was collected for cytokine assay and was stored at -80 ℃. Levels of IL-4, IL-5, and INF-γ in BALF were determined by ELISA.

$CD4^+$ and $CD8^+$ T Cell Populations in Splenocytes

After being sacrificed, the spleens of mice in the different groups were obtained. Ice-cold PBS (5 mL) was used to collect the splenocytes; this was performed three times. The total volume obtained was 15 mL. After centrifuging 1000 rpm/min, 5 min, 4 ℃, splenocytes were obtained. After being treated with lysis buffer, the cells were cultured overnight at 37 ℃ with 5% CO_2 and were purified with a nylon wool-fiber column. Rat anti-mouse CD3-PerCP-Cy5.5, CD4-FITC, and CD8-PE were applied for flow cytometry (FCM) analysis. FACSCalibur and Cell Quest software (BD Biosciences) were used to obtain the FCM results.

Western Blot Analysis

Lung tissues were obtained from mice after being sacrificed and then lysed in RIPA buffer. A BCA protein assay kit was used to detect the protein concentrations. Total protein (80 μg) was used for western blot analysis. The protocols were as follows, proteins were fractioned by SDS, and transferred to membranes, which would be blocked for 4 hours with skim milk. At last, the membranes were incubated overnight with the appropriate primary antibodies. The concentration of ERK, p-ERK, JNK, and p-JNK (Beyotime Biotechnology, China) were 1:1000. After incubation with secondary antibodies, the immune complexes were detected with a bioanalytical imaging system (Azurebiosystems, Inc).

Statistical Analysis

The results are expressed as mean±SE. The experimental results accorded with the normal distribution and were suitable for one-way ANOVA analysis. Statistically significant differences between the groups were determined with one-way ANOVA tests. Statistical significance was defined as $P<0.05$.

RESULTS

Extraction of narirutin

The $L_9(3^4)$ orthogonal array was applied to optimize the extraction conditions by a calibration curve prepared by using standard solutions. y=25484716.04 x+1019569.6 (R^2=0.9916) was the regression equation to calculate the extraction fields of the narirutin. The precision and repeatability of the tests were excellent. Before the $L_9(3^4)$ orthogonal array experiments, single-factor experiments were performed. The effects

of the enzymes and their concentrations on the extraction fields are presented in Figure 1-a. It can be seen that the PE was the best enzyme, with a concentration of 0.1-0.3 mg/mL, in comparison with the CE alone or a mixture of the PE and the CE. When the extraction temperature was set to 40-45 ℃, the extraction fields were better, compared with the other temperatures (Figure 1-b). In addition, a reaction time of 60-120 min best (Figure 1-c) and a pH of 3.5-4.5 was optimal (Figure 1-d). Based on the above analysis, the L_9 (3^4) orthogonal array method with four control factors and three levels was applied to design the experiments in Table 1. According to the results shown in Table 2, there were no significant differences in the effects of enzyme concentration, temperature, reaction time, or pH on the extraction field. After careful analysis, the trend in the effects was as follows: concentration>pH>reaction time>temperature.

When the concentration of PE was 0.3 mg/mL, the temperature was 45 ℃, the reaction time was 90 min and the pH was 5.0, the extraction field of narirutin was highest, at 5.8320 mg/g.

Effects of Narirutin on the Spleen Index and Thymus Index

Generally, the spleen and thymus are important immune organs and are implicated in immune cell generation. The spleen and thymus index results are shown in Table 3. Compared with the control (0.56±0.02%), there was a significant increase to 0.74±0.02%

Table 1. The orthogonal test

Factors Levels	Concentration (mg/mL) A	Temperature (℃) B	Time (min) C	pH D
1	0.1	40	90	4.0
2	0.2	45	120	4.5
3	0.3	50	150	5.0

Table 2. L_9 (3^4) orthogonal array results

Factors	A	B	C	D	Narirutin
Number	Con (mg/mL)	Tem (℃)	Time (min)	pH	(mg/g)
1	1	1	1	1	4.602
2	1	2	2	2	5.309
3	1	3	3	3	4.808
4	2	1	2	3	4.65
5	2	2	3	1	4.068
6	2	3	1	2	4.369
7	3	1	3	2	4.395
8	3	2	1	3	5.776
9	3	3	2	1	4.912

Table 3. Effects of DXMS and narirutin on spleen index and thymus index in mice sensitized with the OVA.

Groups	Spleenindex	P	Thymus index	P	INFγ/IL-4 Ratio	P
Control	0.56±0.02%		0.32±0.02%		2.77±0.34	
OVA	0.74±0.02%^{**a}	0.000	0.47±0.03%^{**a}	0.000	1.02±0.15^{*a}	0.049
DXMS	0.52±0.03%^{**b}	0.000	0.27±0.02%^{**b}	0.000	3.98±0.92^{**b}	0.002
Narirutin (25mg/mL)	0.58±0.03%^{**b}	0.000	0.31±0.02%^{**b}	0.000	3.55±0.57^{**b}	0.007
Narirutin (50mg/mL)	0.61±0.03%^{**b}	0.001	0.38±0.02%^{**b}	0.007	2.96±0.49^{*b}	0.041

OVA: Ovalbumin; [a]Significant difference from the control group; [b]A significant different from the OVA group, **P<0.01; *P<0.05

of spleen index in the OVA group. Compared with the OVA group, the spleen index significantly decreased to 0.52±0.03%, 0.58±0.03%, and 0.61±0.03% in DXMS and narirutin groups (25 mg/mL and 50 mg/mL). Moreover, the spleen index values in these groups were similar to the control group. There were no significant differences between the DXMS and the narirutin groups. There was a significant increase to 0.47±0.03% of thymus index in the OVA group compared with the control. It decreased to 0.27±0.02%, 0.31±0.02%, and 0.38±0.02% in the DXMS and the narirutin groups, respectively compared with the OVA group; there were also no significant differences between the DXMS and the narirutin groups in different concentrations (*P<0.05, **P<0.01).

Histologic Analyses

To validate the effects of narirutin on the lungs, histologic analyses were performed. As shown in Figure 2, representative H&E staining of lung tissue in the control, the OVA, DXMS, and narirutin groups were achieved. The OVA group (Figure 2-b) presented a dense accumulation of eosinophils around the bronchioles compared with the control group (Figure 2-a). This indicates eosinophil infiltration in the peribronchial regions. Furthermore, the airway lumen was narrow and the smooth muscle was thickened. In the DXMS (Figure 2-c) and narirutin groups (Figure 2-d of 25 mg/kg group, and Figure 2-e of 50 mg/kg group), the results of lung tissue showed only a small amount of inflammatory cell infiltration, no obvious thickening of smooth muscle, and eosinophil accumulation was less compared with the OVA group.

Effects of Narirutin on Total IgE and IgG1 Production in the OVA-sensitized Allergic Mice

To clarify the effects of narirutin on serum antibodies, ELISA was used to determine the total IgE and IgG1 concentrations. As shown in Figure 3-a, total IgE and IgG1 levels significantly increased in the OVA group compared with the control. The results show no significant difference in IgE between the OVA and the DXMS groups, nor between the two narirutin groups. However, there was a significant difference in IgG1 in the DXMS and the narirutin groups compared with the OVA group; though, obviously, the DXMS and the narirutin groups of different concentrations had no significant differences (*P<0.05, **P<0.01).

Effect of Narirutin on Cytokine Levels in BALF

To evaluate the effect of the narirutin on cytokine release in the OVA-sensitized mice, the levels of IL-4, IL-5, and INF-γ in BALF were detected [14]. The results for IL-4 are shown in Figure 3-b. There was a significant increase in the OVA group (129.42±33.36 pg/mL) compared with the control (41.86±11.62 pg/mL). The level of IL-4 decreased to 26.02±0.88 pg/mL in the DXMS group. IL-4 production was significantly suppressed by the narirutin to 72.18±15.92 and 53.92±11.37 pg/mL,

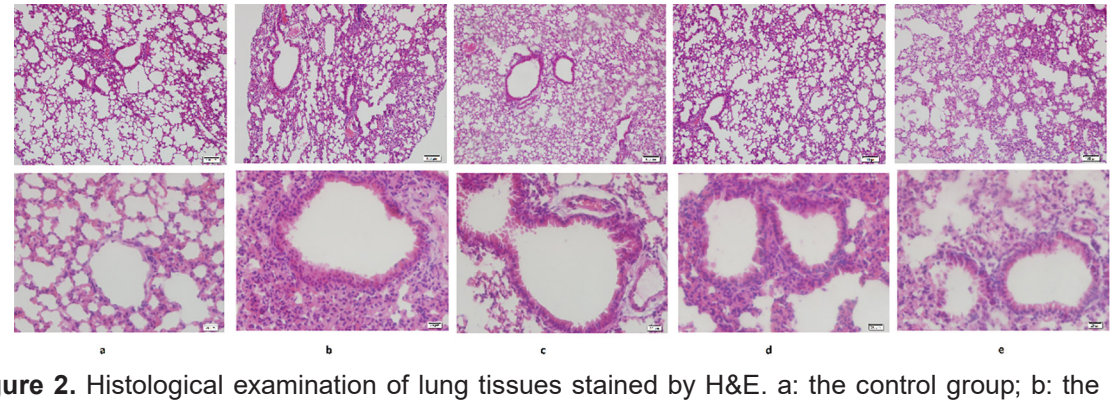

Figure 2. Histological examination of lung tissues stained by H&E. a: the control group; b: the OVA group; c: DXMS group; d: narirutin 25 mg/kg; e: narirutin 50 mg/kg

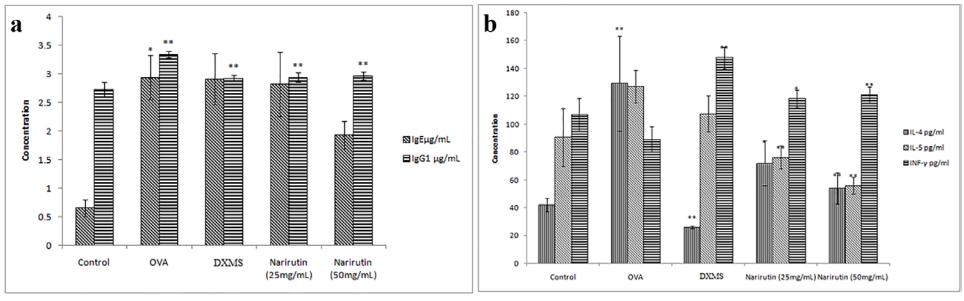

Figure 3. Effects of DXMS and narirutin on the production of antibodies and cytokines. a: IgE and IgG1 in serum (IgE: p_{OVA}=0.034; IgG1: p_{OVA}=0.000, p_{DXMS}=0.002, $p_{narirutin\ 25\ mg/kg}$=0.003, $p_{narirutin\ 25\ mg/kg}$=0.004); b: IL-4 (p_{OVA}=0.002, p_{DXMS}=0.000, $p_{narirutin\ 25\ mg/kg}$=0.031, $p_{narirutin\ 25\ mg/kg}$=0.006); IL-5 (p_{OVA}=0.055, p_{DXMS}=0.031, $p_{narirutin\ 25\ mg/kg}$=0.008, $p_{narirutin\ 25\ mg/kg}$=0.001); and IFN-γ (p_{DXMS}=0.000, $p_{narirutin\ 25\ mg/kg}$=0.012, $p_{narirutin\ 25\ mg/kg}$=0.007); Production in BALF. Values are the mean±SE for six mice. **Significant difference from the OVA-sensitized mice (control), P<0.01; *Significant difference from the OVA-sensitized mice (the control), P<0.05. OVA: Ovalbumin

respectively, in the two narirutin groups. There were no significant differences compared with the DXMS group. The production of IL-5 in the OVA group was a little higher than in the control. Compared with the control group of 90.67±11.61 pg/mL, the value of the OVA group was 127.17±28.72 pg/mL. However, IL-5 in the DXMS group decreased to 107.5±12.99 pg/mL compared with the OVA group, while in the narirutin-treated groups, it significantly decreased to 76.33±8.42 and 55.94±5.96 pg/mL, respectively. IL-5 production was significantly lower in the narirutin group (50 mg/kg) than in the DXMS group. The INF-γ concentration of the OVA group (89.21±8.98pg/mL) was a little lower than in the control (106.95±11.87 pg/mL). However, the DXMS group (147.82±7.82 pg/mL) was significantly higher than the narirutin-treated groups (118.46±6.32 pg/mL and 121.20±5.62 pg/mL). There were no significant differences between the DXMS and the narirutin groups (*P<0.05, **P<0.01). To evaluate Th1/Th2 imbalance, the ratio of IFN-γ/IL-4 was calculated. The results are shown in Table 3. The OVA group was less than the control group. The ratio of the DXMS and narirutin-treated groups were significantly higher than that in the OVA group. We did not find any significant differences between the DXMS and the narirutin groups (*P<0.05, **P<0.01).

Effects of Narirutin on Splenic Immune Functions in the OVA-sensitized Mice

To evaluate the effects of narirutin on splenic immune function, T cells in the spleen were detected by FCM. In Figure 4, the content of $CD4^+$ T cells in the control was 78.95±4.29%; in the OVA group, it increased to 82.23±4.53%, though this was not statistically significant. The percentage decreased to 80.02±3.92% in the DXMS group compared with the OVA group, while the narirutin treatment at doses of 25 and 50 mg/kg significantly decreased the percentage of $CD4^+$ T cells to 78.63±3.92% and 72.19±2.11%, respectively (*P<0.05, **P<0.01).

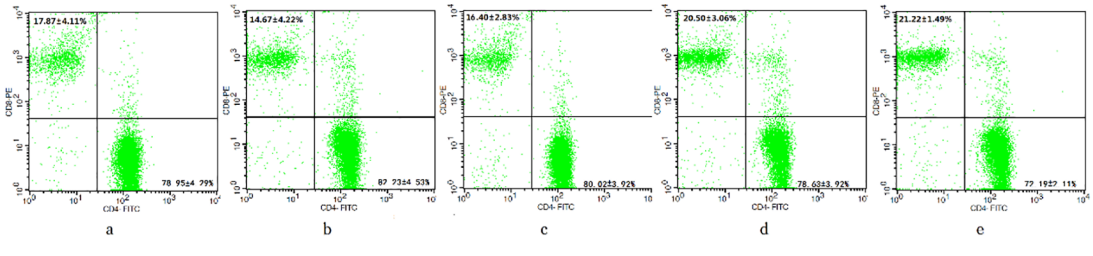

Figure 4. Effects of the DXMS and narirutin on $CD4^+$ T cells and $CD8^+$ T cells in mice sensitized with the OVA. a: the control group; b: the OVA group; c: DXMS group; d: narirutin 25 mg/kg (P=0.038); e: narirutin 50 mg/kg(P=0.017). * a significant difference from the OVA-sensitized mice (the control), P<0.05.

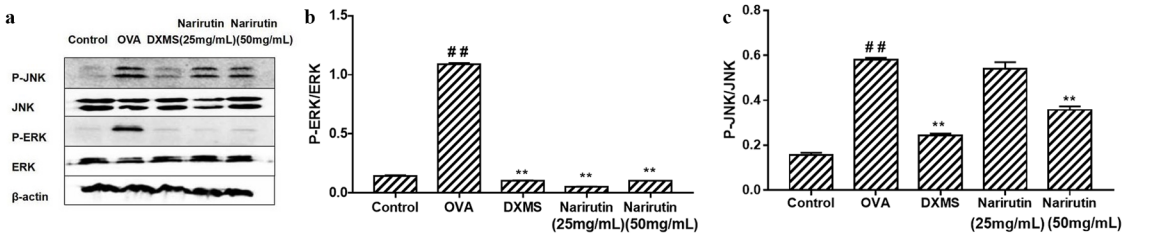

Figure 5. Effects of the DXMS and narirutin on JNK and ERK pathway activation in the lungs of the asthma mouse model. a: WB results; b: P-ERK/ERK results (the OVA group, P=0.001; DXMS, P=0.0073; Narirutin 25 mg/kg, P=0.000; Narirutin 50 mg/kg, P=0.0085); c: P-JNK/JNK results (OVA group, P=0.0054; DXMS, P=0.0082; Narirutin 25 mg/kg, P=0.37; Narirutin 50 mg/kg, P=0.005). **a significant difference from the OVA-sensitized mice (the control), P<0.01; *a significant difference from the OVA-sensitized mice (the control), P<0.05. OVA: Ovalbumin

Furthermore, the percentage of $CD8^+$ significantly increased after treatment with the DXMS and the narirutin.

Effect of Narirutin on the MAPK Pathway

To determine the mechanism underlying the effects of narirutin on airway inflammation, the phosphorylation status of JNK and ERK was investigated. The results are presented in Figure 5. As can be seen in Figure 5-a, there was no change in the basal levels of JNK and ERK, but significant changes in the phosphorylation of JNK and ERK were observed. In Figure 5-b, the p-ERK/ERK value of the OVA group was higher than in the control. Furthermore, the value significantly reduced in the DXMS and the narirutin groups. The results for p-JNK are shown in Figure 5-c, with trends similar to those observed for p-ERK/ERK.

DISCUSSION

The present study optimized the extraction of the narirutin from *Citri Reticulatae* Pericarpium using the enzyme-assisted method and evaluated the possible mechanism underlying the effects of narirutin on the allergic murine model of airway inflammation. The results indicated that the effects of narirutin on airway inflammation may be related to the MAPK signaling pathway. This study is the first, to our knowledge, to apply enzyme-assisted extraction to extract narirutin from *Citri Reticulata*e Pericarpium. These findings are significant for the wider therapeutic application of *Citri Reticulatae* Pericarpium. The typical symptoms of airway inflammation are an increased total IgE concentration in serum, an increased IL-4 and IL-5 concentration in BALF, and lung tissue inflammation. In the current study, the concentration of IL-4 and IL-5 in BALF downregulated in the narirutin-treated groups. Moreover, the total IgE in serum was also suppressed by the narirutin. These results are in good agreement with the study by Funaguchi (2007). These results demonstrate that the narirutin has inhibitory effects on the OVA-induced airway inflammation and the mechanism is likely associated with IL-4 and IgE. Furthermore, allergic diseases are closely related to the immune system. The spleen and thymus are important immune organs that are capable of generating immune cells. Thus, the examination of the spleen and thymus indices can provide insight into the state of the immune system. As the references have shown, immune-competence (immune functions) is often assessed by ascertaining spleen size [15]. As spleen is the largest secondary lymphoid organ in the body, it is involved in the capture and destruction of pathogens and the induction of immune responses. The results indicated that the narirutin can affect the immune system. Considering the mechanism of airway inflammation, the T cell response is important. The narirutin significantly inhibited the increase in $CD4^+$ T cells in splenocytes induced by the OVA sensitization,

which suggests that the narirutin affects the T cell response.

$CD4^+$ T can be induced by IFN-γ and differentiated into Th1 cells, or induced by IL-4 and differentiated into Th2 cells. In airway inflammation mice, firstly, dendritic cells primed basophils or naïve $CD4^+$ T lymphocytes, which have primary secretion of IL-4 , then T helper lymphocytes differentiation toward Th2 cells was induced, which in turn secrete more IL-4 that would increase Th 2-differentiation [16, 17]. Besides, the activated Th2 cells could overproduce Th2 cytokines, such as pro-inflammatory cytokines IL-4 and IL-5, induces an inflammatory response and airway remodeling [18]. To add, IgE secretion of B cells could increase by activated Th2 cells. IgE sensitizes mast cells by adsorbing on their surface connected with their high-affinity receptors, then binds with allergen and causes mast cellactivation [19]. After activation, mast cells could degranulate. At the same time, various biological mediators were released such as β-HEX, histamine, and IL-4. On the contrary, the antibody IgE is promoted by the secretion of IL-4 and IL-5 from Th2 cells but inhibited by the secretion of IFN-γ from Th1 cells [20]. IgG1 is also related to the Th2 response, for it also can be promoted by Th2 cells and Th2 cytokines [21]. In the present work, we investigated the effects of the narirutin on the secretion of pro-inflammatory IFN-γ from Th1 cells and cytokines IL-4 and IL-5 from Th2 cells in the OVA-challenged mice. We confirmed that IL-4 and IL-5 levels decreased and IFN-γ increased in BALF by being treated with the narirutin. The levels of IFN-γ increasing indicated an enhanced Th1-dominant response [22], Much more, the concentration of IL-4 and IL-5 decreasing indicated that Th2-dominant response was suppressed. Thus, careful analysis of the above results suggests that the narirutin inhibits airway inflammation which might be related to the Th1/Th2 cell response.

MAPK signaling pathways are classical pathways related to many aspects of cell viability, such as degranulation and migration of various immune cells [23]. The subfamilies of MAPKs are proposed to be involved in the pathogenesis of asthma [24]. The most important members of the MAPK pathway are ERK and JNK, which are responsible for signal transduction. When MAPK is activated, various inflammatory mediators are produced [25]. The results of this study indicated that the narirutin suppressed the activation of the ERK and JNK pathways induced by the OVA. Expressions of p-ERK and p-JNK decreased after treatment with the narirutin.

CONCLUSION

The present study used the enzyme-assisted method to extract the narirutin from *Citri Reticulatae* Pericarpium. Then, an asthma murine model was applied to study the anti-allergic asthma effects of narirutin *in vivo*. Based on the observed suppression of antibodies in serum, decreased IL-4 and IL-5, and increased IFN-γ increase in BALF, as well as the FCM and Western blot results in the OVA-challenged mice, so it can be concluded that the narirutin affects the Th1/Th2 imbalance induced by the OVA allergic asthmatic model. The mechanism underlying these effects appears to be related to p-ERK and p-JNK suppression of the MAPK signaling pathway. Our study indicated that the enzyme-assisted method was an effective way to extract narirutin from *Citri Reticulatae* Pericarpium, and narirutin could be used as an effective potential candidate for the treatment of allergic asthma.

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Conflict of Interest: None declared.

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