



Therapeutic effects of naringin in a guinea pig model of ovalbumin-induced cough-variant asthma



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ABSTRACT

Naringin, a well known component isolated from *Exocarpium Citri Grandis*, has significant antitussive effects. Recently, Naringin exhibited novel anti-inflammatory effect in chronic inflammatory diseases. In this work, we firstly evaluated the effects of naringin on enhanced cough, airway hyper-responsiveness (AHR), and airway inflammation in an ovalbumin-induced experimental cough-variant asthma (CVA) model in guinea pigs. We investigated the effect of naringin (18.4 mg/kg, per os, single dose or consecutively) on cough to inhaled capsaicin after challenge with an aerosolized antigen in actively sensitized guinea pigs. The effect of naringin on AHR to inhaled methacholine was evaluated 24 h after cough determination. Airway inflammation was assessed via bronchoalveolar lavage fluid (BALF) cytology and lung histopathology. Naringin, given consecutively, significantly reduced ovalbumin-induced enhanced cough and AHR, inhibited the increases in the leukocytes, interleukin-4 (IL-4), IL-5, and IL-13 in BALF compared with the model group. Moreover, the pathologic changes in lung tissues were clearly ameliorated by naringin treatment. These results suggest that naringin may be a beneficial agent for CVA treatment.

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1. Introduction

Chronic cough is a common and frustrating problem for patients and healthcare providers [1–3], which affects 9%–33% of the adult population [4]. Chronic cough in non-smoking adults that are not being treated with angiotensin-converting-enzyme (ACE)-inhibitors and with normal chest radiograms can be indicative of asthma, and can be sub-classified into cough-variant asthma (CVA), atopic cough, and eosinophilic bronchitis [5]. CVA is a type of asthma with the primary symptoms of a dry, nonproductive cough [6–8]. The fundamental features of CVA are prolonged nonproductive cough, no history of wheezing or dyspnea attack, no wheezing audible on auscultation, increased cough reflex sensitivity and increased bronchial responsiveness. The histological features of CVA are eosinophilic inflammation of central and

peripheral airways [9,10]. CVA is one of the major causes of chronic cough and affects the health of large numbers of people worldwide [5]. Thus, appropriate treatment of CVA is of great importance. Moreover, there is a vast unmet need for effective therapy agents.

Exocarpium Citri Grandis is the dried unripe or ripe fruit peel of *Citrus grandis* 'Tomentosa' or *Citrus grandis* Osbeck. It is a well-known traditional Chinese medicine with a long history of treatment for cough and asthma. Naringin extract was purified from *C. grandis* 'Tomentosa' and has a naringin content of greater than 98% [11]. Naringin is abundant in grapefruits, tomatoes, beans, cherries, cocoa and many other citrus fruits, and can be easily extracted at low cost. It has been reported that naringin possess many biological properties, such as being antitussive, anti-inflammatory, antioxidant, and anti-apoptotic. Recently, new research suggests that naringin exerts an anti-inflammatory effect in numerous chronic inflammatory diseases [12–15]. To the best of our knowledge, many respiratory inflammatory diseases are accompanied by cough. In a guinea pig model of chronic bronchitis induced by cigarette smoke, naringin was shown to be effective in inhibiting both airway neurogenic inflammation and coughs [16]. However, these models are mainly established using cigarette smoke. Allergic substances

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can also cause inflammation and cough, such as in allergic rhinitis and CVA. To date, no researchers have investigated the effects of naringin on enhanced cough, airway hyper-responsiveness (AHR), or airway inflammation in CVA.

In this novel study, we report the effects of naringin (18.4 mg/kg, per os, single dose or consecutively) on ovalbumin-induced experimental CVA in guinea pigs to assess the potential of naringin as a therapeutic for CVA. We investigated the effects of naringin on enhanced cough and AHR accompanied by airway inflammation in actively sensitized guinea pigs. We also examined the effects on the numbers of leukocytes in the bronchoalveolar lavage fluid (BALF), and levels of interleukin (IL)-4, IL-5, and IL-13 in the BALF, and inflammatory cell infiltration in lung tissues.

2. Materials and methods

2.1. Reagents

Capsaicin, ovalbumin, methacholine chloride, cyclophosphamide, and chlorphenamine maleate were purchased from J&K Scientific Ltd. (Beijing, People's Republic of China). Capsaicin was dissolved in 1 mL pure ethanol and 1 mL Tween 80 and then further dissolved in 8 mL physiological saline solution to yield a 0.01 mol/L stock solution [16]. The stock solution was then diluted with saline to produce a 50 μ mol/L working solution. Naringin was extracted by our laboratory from *C. grandis* 'Tomentosa' by hot water reflux, then separated and obtained after recrystallization procedures; purity was >98.3%, as determined by peak area normalization. Compound Codeine Phosphate Oral Solution (Cofetol) was purchased from Nanfang hospital as the positive control drug. Per mL, Cofetol contains codeine phosphate (1 mg), ephedrine hydrochloride (0.8 mg), chlorphenamine maleate (0.2 mg), and ammonium chloride (22 mg).

2.2. Animal models

Male Hartley strain guinea pigs (Guangdong Medical Laboratory Animal Center, Guangzhong, People's Republic of China), weighing 200–250 g, were used for all experiments. The animals were maintained under a 12-h light/dark cycle and housed at a constant temperature (20 °C–22 °C) in ordinary animal cages with free access to chow and water ad libitum. Animals were randomly divided into five groups: normal, model, Cofetol, naringin single-dose, and naringin multi-dose. All experimental procedures carried out during this study were approved by the Animal Care and Use Committee of the School of Life Sciences, Sun Yat-sen University, Guangzhou, People's Republic of China.

Guinea pigs were actively sensitized using the method reported by Muraki et al. [17]. The guinea pigs were sensitized intraperitoneally with a mixture of 2 mg ovalbumin and 100 mg aluminum hydroxide [$\text{Al}(\text{OH})_3$] 2 days after intraperitoneal injection of cyclophosphamide (30 mg/kg). Three weeks later, animals were boosted by an intraperitoneal injection of a mixture of 0.01 mg ovalbumin and 100 mg $\text{Al}(\text{OH})_3$. The normal group animals were intraperitoneally injected with saline for the above procedures. Three weeks later, animals were placed in a whole-body plethysmograph (Buxco Electronics, Sharon, CT, USA) and were challenged with 10 mg/mL ovalbumin aerosol for 90 s except the normal group animals which were challenged with saline. Fig. 1 shows the flow diagram of the procedure.

2.3. Drug administration

Every 24 h after the ovalbumin challenge, in the normal, model, Cofetol and naringin multi-dose groups, sensitized animals were

orally given saline, saline, Cofetol (2.3 mL/kg), and naringin solution (18.4 mg/kg) every day for consecutive 6 days (total: six times), respectively. For the naringin single-dose group, naringin was given orally only once in the last day (Fig. 1).

2.4. Cough measurement

The number of coughs and latency period of guinea pigs to inhaled capsaicin were determined after drug administration (Fig. 1). Each conscious guinea pig was placed in a whole-body plethysmography (Buxco Electronics) 60 min after the last drug administration. The pressure in the body chamber was recorded. Coughs were detected as a transient change in pressure (a rapid inspiration followed by rapid expiration). The capsaicin solution (50 μ mol/L) was inhaled for 2 min. The nebulizer output was 0.5 mL/min. The number of coughs were measured during the capsaicin inhalation (2 min) and the following 8 min. Coughs were recorded by a previously described method [18]. Briefly, coughs were recorded by both Biosystem XA software (Buxco Electronics) and a blinded observer. The blinded observer has been trained and can precisely manually accessed cough according the cough-like posture of guinea pig (splaying of the front feet and forward stretching of the neck, and opening of the mouth). The cough analyzer software of the Buxco system automatically recognized the cough basing on the analysis of the box flow during one cough like action. Compared to sneeze, cough has higher value of the area under the box flow curve during compression and longer time between 50% max of the compressive spike and 50% min of the expulsive spike. Therefore, the system can differentiate cough and sneeze [19].

2.5. AHR measurement

Twenty-four hours following capsaicin exposure, airway responsiveness was assessed by methacholine-induced airflow obstruction and through monitoring enhanced pause (Penh) in conscious guinea pigs using whole-body plethysmography (Buxco Electronics). The animals were first exposed to aerosolized saline then subsequently to increasing concentrations of aerosolized methacholine (e.g., 100, 200, 400, 800, 1600 mg/L in saline). Guinea pigs were challenged with each nebulization for 90 s and readings were taken for 3 min. The interval time for the next nebulization was 90 s. The Penh values measured during each exposure sequence were averaged and expressed as the percentage of Penh values at each administration of methacholine versus Penh value of saline control administration.

2.6. Bronchoalveolar lavage fluid

BALF was performed immediately after measuring airway responsiveness to methacholine. The trachea was cannulated after the left main bronchus near the bifurcation was ligated. The right lung was lavaged with 5 mL of ice cold saline three times via cannula. The total and differential leukocyte counts of BALF were carried out using a flow cytometric hematology system (XT-2000iV, Sysmex Corporation, Kobe, Japan) [20,21].

2.7. Determination of IL-4, IL-5, and IL-13 in the BALF

The levels of IL-4, IL-5, and IL-13 were determined in the BALF supernatant using an enzyme-linked immunosorbent assay for guinea pigs according to the manufacturer's instructions (Cusabio Biotech, Wuhan, People's Republic of China).

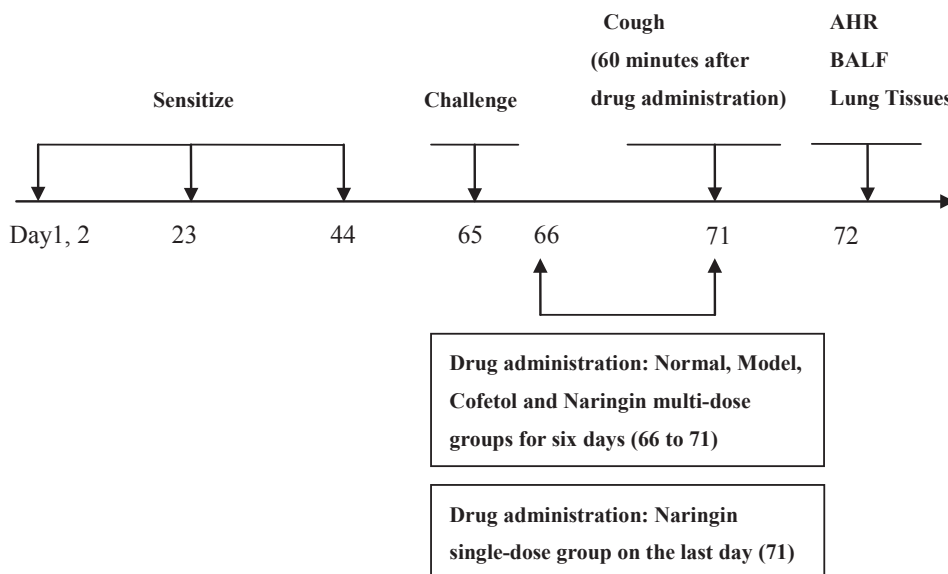


Fig. 1. The flow diagram of the experimental procedure.

2.8. Histologic evaluation

A segment of the non-lavaged left lung was removed and immersed in 10% neutral formalin overnight. The tissue was then incubated in xylene, embedded in paraffin, sectioned, and stained using a standard Hematoxylin and Eosin (H&E) staining method. Digital images were obtained from stained sections using a model DM 4000B camera (Leica DM 4000B, Solms, Germany) at an original magnification of $\times 20$. All samples were scored by semi-quantitative method to estimate lung inflammation. The scores were graded on three-points: interstitial inflammation, inflammatory cell infiltration in bronchus, and inflammatory cell infiltration in the vasculature. The change in each parameter was scored as 0, no change; 1, mild change; 2, moderate change; or 3, severe change. The total lung inflammation score was calculated by adding up the individual scores.

2.9. Statistical analysis

All data are presented as means $\pm$ standard error of the mean (SEM). Statistical differences between groups were analyzed by one-way analysis of variance (ANOVA) with subsequent Dunnett's multiple comparison test for all parametric data and Kruskal–Wallis test followed by Dunn's multiple comparison test for nonparametric using SPSS for windows 16.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of naringin on enhanced cough

Fig. 2 shows the effect of each treatment on the number of coughs and latency period elicited by aerosolized capsaicin in actively sensitized guinea pigs. The number of coughs induced by 50 $\mu\text{mol/L}$ of capsaicin was significantly ($P < 0.01$) increased in the model group compared with the normal group. The Cofetol group and naringin multi-dosed group significantly reduced the number of coughs and increased the latency cough period ($P < 0.01$) compared with the model group. The naringin single-dosed group also lowered the number of coughs and increased the latency cough period ($P < 0.05$) compared with the model group.

3.2. Effect of naringin on AHR

Airway responsiveness to inhaled methacholine and the effect of naringin in actively sensitized guinea pigs are shown in Fig. 3. Aerosolized methacholine elicited an increase in airway responsiveness in all groups. Guinea pigs in the model group had significantly greater ($P < 0.01$) airway responsiveness with each concentration of methacholine compared with the normal group. Guinea pigs in the Cofetol, naringin multi-dose, and naringin single-dose groups had significantly less ($P < 0.01$) airway responsiveness than the model group at each methacholine concentration. However, there were no statistically differences between the naringin single-dose group and the normal group, but the naringin single-dose group showed a trend toward higher airway responsiveness than the normal group. Guinea pigs in the Cofetol and naringin multi-dose groups showed a trend of less airway responsiveness than the normal group.

3.3. Effect of naringin on ovalbumin-induced airway leukocyte infiltration

To evaluate the pulmonary inflammatory response to naringin, BALF cells were recovered and the total, differential BALF leukocyte counts were compared (Table 1). The numbers of total leukocytes, neutrophils, lymphocytes, and eosinophils increased significantly ($P < 0.01$) in the model group compared with the normal group. The Cofetol and naringin multi-dose groups had a significantly decreased ($P < 0.01$) number of lymphocytes compared with the model group. The number of total cells, neutrophils, and eosinophils were also decreased ($P < 0.05$) in the Cofetol and naringin multi-dose groups, but they were still significantly higher ($P < 0.01$) than normal group. Moreover, the naringin single-dose group did not have a significant change in the total or differential BALF leukocyte counts compared with the model group.

3.4. Effect of naringin on the concentrations of IL-4, IL-5, and IL-13 in the BALF

The concentration of IL-4 in the BALF increased greatly in the model group compared with the normal group ($P < 0.01$; Fig. 4). The increase in IL-4 levels was significantly inhibited in the Cofetol

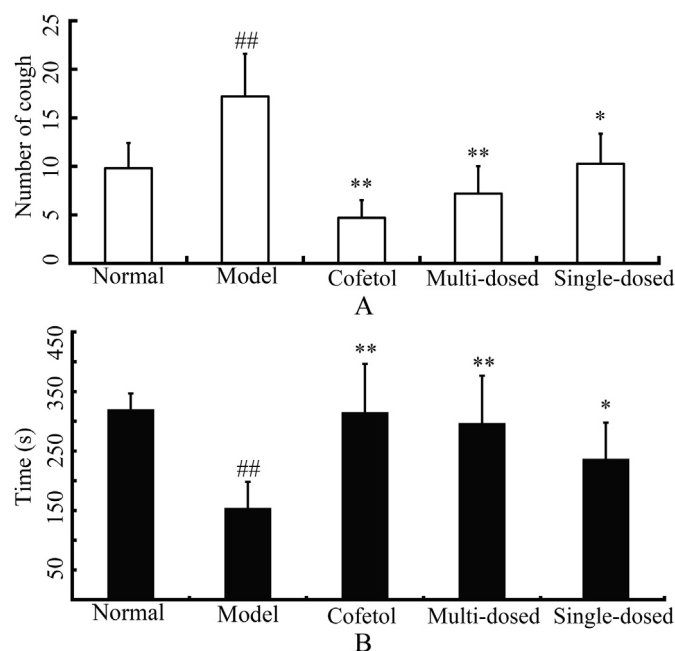


Fig. 2. Effect of naringin on capsaicin-induced the number of coughs in actively sensitized animals (Normal, Model, Cofetol, naringin multi-dose, naringin single-dose) (A), and latency cough period (B). Each column represents means $\pm$ standard error of the mean ($n = 8$ for each group). * $P < 0.05$, ** $P < 0.01$, compared with the model group; ## $P < 0.01$, compared with the normal group.

($P < 0.05$) and naringin multi-dose ($P < 0.01$) groups compared with the model group.

IL-5 levels also increased significantly in the BALF of the model

group compared with the normal group ($P < 0.01$; Fig. 4), while Cofetol ($P < 0.01$) and multi-dose naringin ($P < 0.05$) inhibited the ovalbumin-induced IL-5 increase compared with the model group.

IL-13 levels also increased significantly in the BALF of the model group compared with the normal group ($P < 0.01$; Fig. 4). Similarly, Cofetol and multi-dose naringin significantly inhibited antigen-induced IL-13 increase compared with the model group ($P < 0.05$).

The naringin single-dose treatment did not significantly affect the concentration of IL-4, IL-5, and IL-13 in the BALF compared with the model group.

3.5. Effect of naringin on ovalbumin-induced lung inflammation

Semi-quantitative scoring of lung tissues revealed that there were normal histological structures and none or minimal inflammation in the normal group (Fig. 5A). The model group (Fig. 5B) showed an increase in inflammatory cell infiltration in the sub-mucosal area. Most of the inflammatory cells were lymphocytes with a small proportion of eosinophils and neutrophils. The Cofetol group (Fig. 5C) significantly inhibited inflammatory cell infiltration compared with the model group, as did the naringin multi-dose group (Fig. 5D). There was no significant difference in inflammatory cell infiltration between the naringin single-dose group (Fig. 5E) and the model group.

4. Discussion

Naringin, as an effective antitussive agent, is being investigated by a clinical Phase I study approved by the China Food and Drug Administration. Our group has established a few models to evaluate the antitussive effect of naringin. Citric acid and capsaicin are important agents for studies on cough. In our laboratory, citric acid and capsaicin have been used for naive guinea pigs to evoke coughs that could be inhibited by naringin (data not yet published). Pre-treatment with naringin (18.4 mg/kg) substantially inhibited both enhanced cough and airway neurogenic inflammation in cigarette smoke-exposed guinea pigs [16,18]. Gao et al. [22] observed an antitussive effect of naringin at 30 mg/kg in guinea pigs and pointed out that naringin was not a central antitussive drug. Moreover, naringin did not exert its peripheral antitussive effect through either the sensory neuropeptides system or the modulation of ATP-sensitive K^{+} channels. To characterize its peripheral antitussive mechanism, further studies are needed to investigate whether naringin has an inhibitory effect on the responses of rapidly adapting receptors (RARs) or C-fibers. Our group has researched the effect of naringin on coughs induced by mechanical stimulation of the trachea in capsaicin-desensitized guinea pigs. The number of coughs in the naringin group was significantly different compared with the model group ($P < 0.05$). Our group has also observed the effect of naringin on the histamine-induced discharge of RARs. Naringin significantly inhibited this discharge from 23.19% to 34.4% ($P < 0.01$). The results of the two experiments demonstrated that naringin could possibly work on RARs to inhibit cough (data not yet published). Naringin also significantly alleviated LPS-induced acute lung injury in mice via suppression of myeloperoxidase, inducible nitric oxide synthase (iNOS) activity, tumor necrosis factor (TNF)- α secretion, and nuclear factor (NF)- κ B activation [15]. However, to date, there are no reports investigating the effects of naringin in allergic airway diseases, such as CVA. Therefore, our group established an ovalbumin-induced experimental CVA model in guinea pigs to investigate the effects of naringin on enhanced cough, AHR, and airway inflammation.

In preclinical studies of naringin, oral administration doses of naringin were 9.2, 18.4, and 36.8 mg/kg in guinea pigs [16]. In pharmacokinetic studies, naringin was administered orally to rats

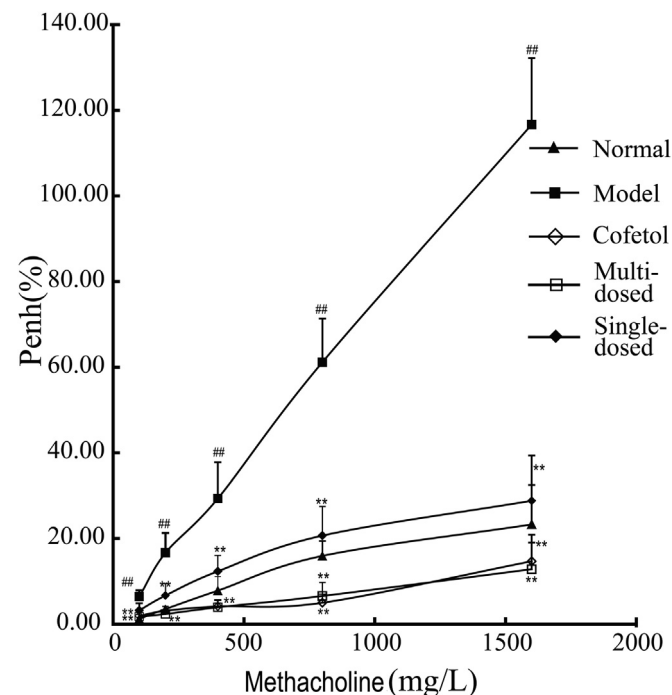


Fig. 3. Effect of naringin on airway responsiveness in actively sensitized animals (Normal, Model, Cofetol, naringin multi-dose, naringin single-dose). Data are presented as means $\pm$ standard error of the mean ($n = 8$ for each group). Penh (%) expressed as the Penh values at each administration of methacholine versus Penh value of saline control administration. ** $P < 0.01$, compared with the model group; ## $P < 0.01$, compared with the normal group.

Table 1
Effect of naringin on ovalbumin-induced leukocyte infiltration in the BALF.

Group	Total cells ($10^7/\text{mL}$)	Neutrophils ($10^7/\text{mL}$)	Lymphocytes ($10^7/\text{mL}$)	Monocytes ($10^7/\text{mL}$)	Eosinophils ($10^7/\text{mL}$)	Basophils ($10^7/\text{mL}$)
Normal	51.8 ± 11.92	3.63 ± 3.00	3.29 ± 2.72	39.8 ± 10.8	3.84 ± 0.97	1.24 ± 0.33
Model	161.29 ± 44.24 ^{##}	43.14 ± 15.19 ^{##}	29.14 ± 9.06 ^{##}	40.86 ± 9.96	45.29 ± 18.10 ^{##}	1.29 ± 0.95
Cofetol	106.33 ± 20.40 ^{###}	32.16 ± 7.96 ^{###}	4.83 ± 1.83 ^{**}	46.33 ± 13.87	22.33 ± 6.65 ^{###}	0.67 ± 0.82
naringin multi-dosed	97.14 ± 21.59 ^{###}	25.86 ± 16.13 ^{###}	5.57 ± 4.79 ^{**}	34.43 ± 6.02	29.57 ± 8.42 ^{###}	0.71 ± 0.49
naringin single-dosed	150.51 ± 35.68 ^{##}	40.23 ± 12.03 ^{##}	27.03 ± 6.06 ^{##}	37.89 ± 5.21	44.28 ± 17.59 ^{##}	1.08 ± 0.87

Data are expressed as the means ± standard error of the mean (* $P < 0.05$, ** $P < 0.01$, compared with the model group; ^{##} $P < 0.01$ compared with the normal group).

at a dose of 42 mg/kg, which was converted from 36.8 mg/kg in guinea pigs [23,24]. Thus, the middle dose (18.4 mg/kg) was chosen in the present study based on pharmacodynamic and pharmacokinetic studies. The dose/response curves study has been researched in previous antitussive effect studies of naringin in our laboratory [16]. Therefore, in the present study, we only investigated whether naringin has a therapeutic effect on CVA, but did not include the dose/response study. The T_{max} of naringin was approximately an hour in rats [25] and beagle dogs [26]. Therefore, the effects of naringin on enhanced cough were evaluated 60 min after the last drug administration. Cofetol was adopted as the positive control drug. The clinical dose of Cofetol was converted to a dose for guinea pigs and used in present study.

The model was first established by Muraki et al., in 1994 [17] and has been used by many other researchers since [27–31]. In this model, cough response to capsaicin and bronchial responsiveness of guinea pigs is increased after induction by ovalbumin. The histological feature of this model is primarily eosinophilia inflammation of lung, which was observed in our study.

Ovalbumin-induced enhanced cough may be associated with airway allergy and airway inflammation. Liu et al. [32] demonstrated that antigen-induced increases in cough response resulted from the increased cough reflex sensitivity. Airway allergy accompanied with airway eosinophilia induced increases in cough response, which was also supported by other studies [33,34]. Liu et al. [32] also found that the increased cough sensitivity may result in part from inactivation of neutral endopeptidase and thromboxane A₂ (TXA₂), inflammatory mediators. Furthermore, some studies have supported the hypothesis that various inflammatory mediators play a role in exciting airway afferent nerves by direct stimulation, by increasing their excitability, or indirectly by smooth muscle or vasculature-mediated effects [35].

Several findings have suggested that the capsaicin-induced cough is mediated mainly by the activation of the transient receptor potential vanilloid type channel-1 (TRPV1), a capsaicin

receptor on the terminal of the nonmyelinated C-fibers and thin myelinated A δ -fibers [30]. Several studies have also reported that immunosensitization may facilitate the release of tachykinins from C-fiber endings by the stimulation of capsaicin. Tachykinins then stimulated RARs, which causes a strong cough response [36–38]. Therefore, the cough response of guinea pigs to capsaicin was increased after they were sensitized by ovalbumin. The anti-inflammatory effect of naringin [16,39] could contribute to its antitussive effect. In this model, naringin inhibited the ovalbumin-induced enhanced cough, which may have resulted from both its inhibitory effect on RARs (A δ -fibers) and its anti-inflammatory effect.

The effect of naringin on AHR has been observed in chronic cigarette smoke exposure-induced chronic bronchitis in guinea pigs [16]. The AHR in ovalbumin-induced CVA model has been observed in several studies [17,28,29]. The airway responsiveness to methacholine in conscious, spontaneously breathing animals was successfully measured by Penh units obtained from WBP (Buxco Electronics) in previous studies [40]. Penh is an arbitrary dimensionless value that has been shown to correlate closely with airway resistance [40–45]. Bergren [46] has determined that Penh and sRaw were correlative measurements of airway reactivity. In our study, we observed the AHR in CVA model group to methacholine in conscious, spontaneously breathing animals; naringin could significantly inhibit the AHR.

A few studies have established that Th2 cells play a central role in the pathogenesis of asthma. The inflammatory process is dominated by Th2 cells that produce IL-4, IL-5, and IL-13 [47]. IL-4 can induce isotype switching in B cells, from IgG to IgE production [48]. The predominant inflammatory cell recruited into asthmatic lung tissues is the eosinophil, which is associated with the production of IL-5 [49]. IL-5 is most specific to eosinophils, and is the major hematopoietin responsible for eosinophils growth, differentiation, mobilization, recruitment, activation, and survival [50]. IL-13 directly induces important features of bronchial asthma, such

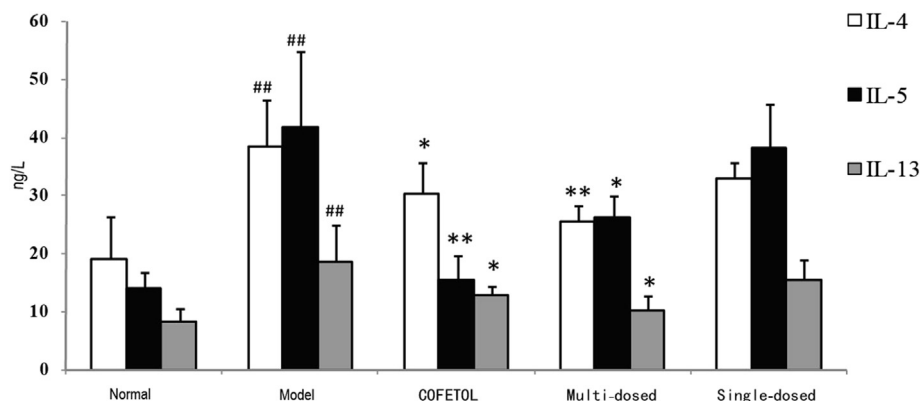


Fig. 4. Effect of naringin on IL-4, IL-5, and IL-13 concentration in the BALF (Normal, Model, Cofetol, naringin multi-dose, naringin single-dose). Each column represents mean ± standard error of the mean ($n = 8$ for each group). * $P < 0.05$, ** $P < 0.01$, compared with the model group; ^{##} $P < 0.01$, compared with the normal group.

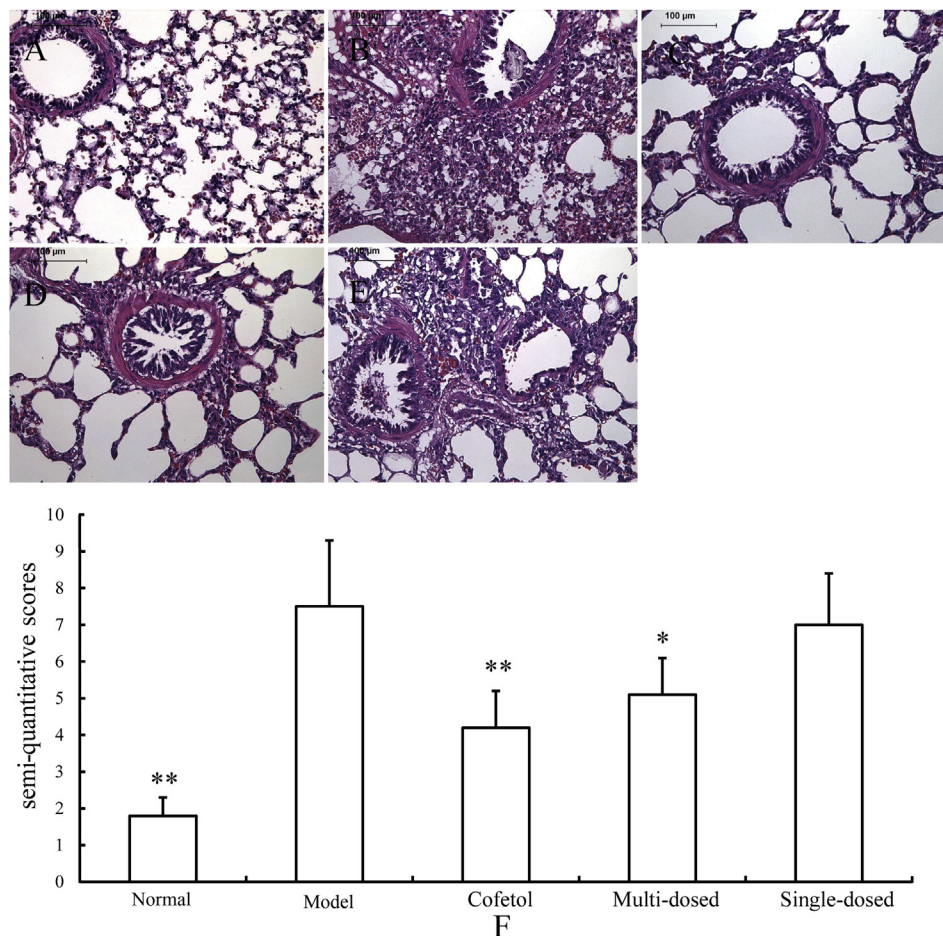


Fig. 5. Effects of naringin on ovalbumin-induced lung inflammation. Representative micrographs of lung tissues (stained with HE, $\times 40$). (A) Normal group, (B) Model group, (C) Cofetol group, (D) naringin multi-dose group, (E) naringin single-dose group, and (F) Histological semi-quantitative scores of lung inflammation. Each bar represents the mean $\pm$ standard error of the mean ($n = 5$). * $P < 0.05$, ** $P < 0.01$, compared with the model group.

as eosinophilic infiltration, mucus hypersecretion, and AHR or goblet cell hyperplasia [51,52]. BALF levels of IL-4, IL-5, and IL-13 were used as parameters of airway inflammation. Therefore, we determined the levels of IL-4, IL-5, and IL-13 in the BALF.

In our research, multi-dose and single-dose groups of naringin reduced ovalbumin-induced cough. The single-dose group exhibited no anti-inflammatory effect, while the multi-dose group showed better cough inhibition due to the obvious anti-inflammatory effect. The multi-dose group could decrease the levels of IL-4, IL-5, and IL-13 in the BALF. The total numbers of leukocytes, lymphocytes, eosinophils, and neutrophils in the BALF were significantly decreased in the multi-dose group compared to the model group. Moreover, the pathologic changes in lung tissues were obviously ameliorated following consecutive naringin treatment. The results verified the correlation between airway inflammation and cough. Furthermore, the therapeutic effects of naringin on CVA resulted from its antitussive and anti-inflammatory effects.

In this study, Cofetol also reduced lung inflammation, as well as naringin. To date, no study has established definitively that codeine phosphate has an anti-inflammatory effect. Some studies have demonstrated that the histamine H1 receptor antagonist has an anti-inflammatory effect [53]. Therefore, the specific ingredient of cofetol that mediates the anti-inflammatory effect requires further study.

5. Conclusions

In conclusion, our study demonstrated that the effects of naringin in a guinea pig model of ovalbumin-induced CVA. Naringin can inhibit the cough, AHR, and airway inflammation in sensitized animals. We believe that naringin may provide a therapeutic benefit in patients with allergic airway disorders.

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