

Comparative Research of the Curative Effects of Pinelliae Rhizoma and Pinelliae Rhizoma Praeparatum cum Alumine on Ovalbumin-induced Allergic Asthma in Rats

Wei Peng, Da-Neng Wei, Yu-Jie Liu, Meng-Meng Zhang, Mei-Bian Hu, Chun-Jie Wu

Department of Chinese Medicinal Processing, School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, China

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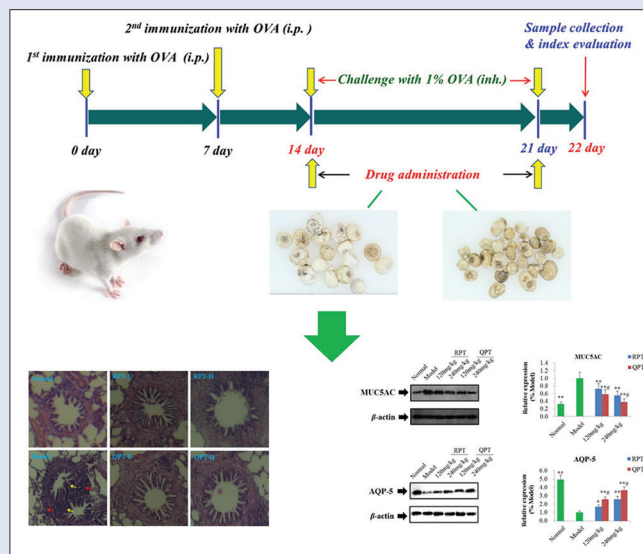
ABSTRACT

Aim: This study was designed to compare the curative effects of Pinelliae Rhizoma (RPT) and Pinelliae Rhizoma Praeparatum cum Alumine (QPT) on ovalbumin (OVA)-induced allergic asthma in rats. **Materials and Methods:** Asthmatic rats were prepared by intraperitoneal injection and inhalation of OVA, and RPT and QPT at the doses of 120 and 240 mg/kg/day were administered orally for consecutive 7 days. Then, histopathological changes of the lung tissues were examined; levels of interleukin (IL)-4 and interferon (IFN)- γ in the bronchoalveolar lavage fluids (BALFs) and immunoglobulin (Ig) E in the serum were measured by enzyme-linked immunosorbent assay; eosinophils (EOS) in BALFs were also determined. Furthermore, the expressions of mucin 5AC (MUC5AC) and AQP-5 in the lung tissues were determined by quantitative real-time fluorogenic polymerase chain reaction and western blot assays. Besides, high-performance liquid chromatography-LTQ-Orbitrap-Elite-MS analysis was applied to compare the constituent's difference between the RPT and QPT. **Results:** The present results revealed that both the RPT and QPT have the potential curative effects for treating OVA-induced asthma in rats via inhibiting inflammatory reactions (suppressing EOS influx, IgE secretion, and release of IL-4 whereas increasing the release of IFN- γ) and modulating the mRNA and protein expressions of MUC5AC and AQP-5. In addition, our results also evidenced that QPT exhibited a better curative effect on allergic asthmatic rats compared to RPT. **Conclusion:** Our results are beneficial for confirming the traditional Chinese medicinal theory of "QPT is good at removing dampness to reduce phlegm."

Key words: Allergic asthma, comparative research, Pinelliae Rhizoma, Pinelliae Rhizoma Praeparatum cum Alumine

SUMMARY

- Anti-asthmatic effects of Pinelliae Rhizoma Praeparatum cum Alumine (QPT) on allergic asthma in rats was first reported
- Anti-asthmatic effects of the QPT and Pinelliae Rhizoma (RPT) were compared
- Constituents between the QPT and RPT were compared
- The molecular mechanisms of the effects of QPT and RPT were studied.



Abbreviations used: BALF: Bronchoalveolar lavage fluid; ELISA: Enzyme-linked immunosorbent assay; EOS: Eosinophils; IFN: Interferon; IL: Interleukin; OVA: Ovalbumin; qRT-PCR: Quantitative real-time fluorogenic polymerase chain reaction; QPT: Rhizoma Pinelliae Praeparatum cum Alumine; RIPA: Radioimmunoprecipitation assay; RPT: Pinelliae Rhizoma; SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TCM: Traditional Chinese medicine.

Correspondence:

Dr. Chun-Jie Wu,
School of Pharmacy, Chengdu University of
Traditional Chinese Medicine, No. 1166 Liutai
Avenue, Chengdu 610075, China.
E-mail: wucjcdtcm@163.com
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INTRODUCTION

Pinellia ternata (Thunb.) Breit, belonging to the *Araceae* family, is a commonly used medicinal herbaceous plant mainly distributed in Eastern Asia.^[1] The tuber of *P. ternata* is a known Chinese herbal medicine with the functions of removing dampness-phlegm, and downbear counterflow, and check vomiting and is commonly used to treat cough, vomiting, infection, and inflammatory disorders in China for thousand years.^[2,3] Increasing phytochemical evidence has uncovered that the main chemical compositions of the tuber of *P. ternata* are alkaloids, cerebrosides, fatty acids, phenylpropanoids lectins, and

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polysaccharides.^[4-6] In addition, currently, pharmacological findings have revealed that this herbal medicine possesses various activities including antiemetic, anti-tussive, sedative, and anti-inflammatory and antitumor, antimicrobial, antifungal, antiviral, and anticonvulsant effect.^[3,7,8] Besides, nowadays, it is also reported that this herbal medicine also has some potential toxicities such as mucosal irritation, reproductive toxicity, and hepatotoxicity.^[9,10]

The raw tuber of *P. ternata* is named Pinelliae Rhizoma (RPT) in Chinese Pharmacopoeia.^[2] Furthermore, the RPT is commonly processed with adjuvant materials to decreasing toxicity and increasing efficiency before clinical application, and Rhizoma Pinelliae Praeparatum cum Alumine (QPT) is one of the processed products of RPT with alumen.^[2,11,12] Previous researches indicated that the RPT possesses good curative effects against ovalbumin (OVA)-induced allergic asthma in rats;^[7,13] however, so far, experimental evidence for curative effect of QPT on allergic asthma is still lacking. Importantly, in traditional Chinese medicinal (TCM) theory, allergic asthma is a common typical damp-phlegm syndrome,^[14] and the QPT is commonly considered to be “good at removing dampness to reduce phlegm,” compared to other processed products of RPT.^[15] Consequently, in the present study, we aimed to compare the curative effect of RPT and QPT on OVA-induced allergic asthma in rats.

MATERIALS AND METHODS

Plant materials and extracts preparation

The RPT and QPT were purchased from the Neautus Chinese Herbal Pieces Ltd. Co. (Chengdu, China) and were identified by Prof. Min Li (Department of Pharmacognosy, School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, China). The voucher specimen of the RPT and QPT was deposited at our laboratory (No. 20160813-1# and 20160813-2#, respectively).

The RPT and QPT were powdered and subsequently extracted by decocting with water. Then, the extracts were filtered and concentrated *in vacuo* under 60°C, and the dried residues were treated as the extracts of RPT and QPT, respectively, and the extraction yields of RPT and QPT were all calculated as approximately 20%–22.4%.

Animals

Experimental groups consisted of SD rats (200 ± 20 g), which were purchased from the Dashuo Experimental Animal Center (Chengdu, China). They were housed at 21°C ± 1°C under a 12-h light/dark cycle and had free access to standard pellet diet (Purina chow) and tap water. All animal treatments were performed strictly in accordance with the International Ethical Guidelines and the National Institutes of Health Guide concerning the Care and Use of Laboratory Animals. The experiments were carried out with the approval of the Animal Experimentation Ethics Committee of Chengdu University of Traditional Chinese Medicine.

Chemicals

OVA and aluminum hydroxide were obtained from the Sigma-Aldrich (St. Louis, MO, USA). Commercially enzyme-linked immunosorbent assay (ELISA) kits for immunoglobulin (Ig) E, interleukin (IL)-4, and interferon (IFN)-γ were purchased from ExCell Bio Co. (Shanghai, China). PVDF membrane was purchased from Millipore Biotech (MA, USA). The primary antibodies for mucin 5AC (MUC5AC), AQP-5, and β-actin were purchased from GeneTex Inc. (Irvine, CA, USA). Radioimmunoprecipitation assay (RIPA) lysis buffer, BCA protein assay kit, QuickBlock™ blocking buffer, Beyo ECL Star, and HRP-conjugated anti-rabbit/mouse secondary antibody were purchased

from the Beyotime Institute of Biotechnology (Haimen, China). Trizol reagents were purchased from the Invitrogen Co. (Carlsbad, CA, USA). SYBR Green PCR kits and reverse transcription kit were purchased from the Thermo Fisher Scientific (Shanghai, China). All other chemicals used in this study were of analytical reagent grade.

Experimental protocol

A total of 60 SD rats were randomly divided into six groups ($n = 10$), including normal group, model group (allergic asthma model rats), and two RPT and two QPT treatment groups. The experimental protocol was performed according to the previous reports with some modifications,^[16] and the experimental schematic diagram was shown in Figure 1. Briefly, rats in model, RPT, and QPT groups were immunized by intraperitoneal injection with the mixture of 50 mg OVA and 1 mg aluminum hydroxide on the 1st and 7th day. Subsequently, from the 14th to 21st day, rats in model, RPT, and QPT groups were challenged with 1% OVA normal saline solution for 30 min using an ultrasonic nebulizer (OMRON Co., Tokyo, Japan). Doses of the RPT and QPT were determined based on the common used clinical doses and water extraction yields of the RPT and QPT (the commonly used clinical doses of RPT and QPT are ranging from 6.0 to 15.0 g/person/day, crude herb medicine equivalent,^[17] and the water extraction yields of RPT and QPT are approximately 20%–22.4%). Consequently, the doses of 120 and 240 mg/kg/day for RPT and QPT were selected, and RPT and QPT were administered orally from the 14th to 21st days. After 24 h of the final drug administration, rats were sacrificed, and the blood samples, bronchoalveolar lavage fluids (BALFs), and lung tissues were also collected and stored at –70°C for further analysis.

Eosinophils cell counting in bronchoalveolar lavage fluids

BALFs were centrifuged at 4°C for 5 min with a speed of 1500 rpm, and subsequently, the supernatants of the BALFs were collected and stored at –80°C. Furthermore, the cell precipitate smears were prepared and then fixed with formalin, and Wright-Giemsa staining was subsequently performed, and the eosinophils (EOS) counting was performed under an optical microscope (Nikon TS2, Tokyo, Japan).

Histopathological examinations

Histopathological examinations were carried out referring to the previous reported methods with some modifications.^[18] Briefly, lung tissues were fixed in 10% paraformaldehyde for 24 h, which were subsequently embedded in paraffin, sectioned at 5 μm, deparaffinized, and stained with hematoxylin-eosin. Finally, histopathological changes of lung tissues were examined under an optical microscope (Nikon TS2, Tokyo, Japan).

Enzyme-linked immunosorbent assay assays

Serum samples were prepared by centrifuging for 15 min at 5000 rpm. Subsequently, the serum samples and BALF supernatants mentioned

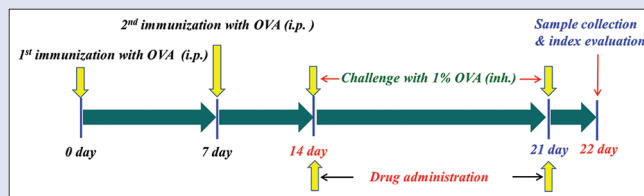


Figure 1: Schematic diagram for preparation of OVA-induced allergic asthma model rats. i.p.: Intraperitoneal injection; inh.: Inhalation; OVA: Ovalbumin

above were used to carry out the ELISA assays, and the levels of IL-4 and IFN- γ in BALFs and IgE in the serum were determined by commercial ELISA assay kits following the corresponding manufacturer's instructions. In addition, the serum was prepared from the collected blood by centrifugation at 4°C for 10 min (5000 rpm), and subsequently, the serum samples were used to determine the IgE levels using ELISA assays as mentioned above.

Real-time fluorogenic polymerase chain reaction assays

Total RNA of the lung tissues was extracted by using Trizol reagents and then was used for cDNA synthesis of MUC5AC, AQP-5, and GAPDH by reverse transcription with quantitative real-time fluorogenic polymerase chain reaction (qRT-PCR, ABI-7300, USA). All mRNA primers were designed by Premier 5.0 and synthesized by Sangon Biotech (Shanghai, China). Primers used for the real-time PCR are shown in Table 1.

Western blot assay

Lung tissues were homogenated with RIPA lysis buffer using a glass homogenizer, and subsequently, the homogenates were centrifuged at 4°C for 5 min with a speed of 10,000 rpm. Then, the supernatants were collected to afford the total proteins of the lung tissues. After determination of the protein concentration by BCA protein assay kits,

and total proteins (35 μ g) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to PVDF membrane. Subsequently, the protein blotted on PVDF membrane was probed with primary antibodies of MUC5AC and AQP-5, respectively. After that, the PVDF membrane was incubated with HRP-conjugated secondary antibodies. Finally, the target protein bands were visualized by chemiluminescence method, and β -actin was used as the internal reference to normalize for protein loading.

High-performance liquid chromatography-LTQ-Orbitrap-Elite-MS analysis

An UltiMate 3000 system equipped with LTQ Orbitrap Elite mass spectrometer (Thermo Technologies, USA) was used for the separation and identification of constituents in RPT and QPT. The separation was performed on a CAPCELL PAK MG II S5 C₁₈ chromatographic column (5 μ m, 250 mm $\times$ 4.6 mm, i.d.) with the column temperature set at 30°C. A linear gradient elution of A (acetonitrile) and B (pure water) was used with the gradient procedure as follows: 0–15 min, 99.9% B (v/v); 15–20 min, 99.9%–95% B (v/v); 20–30 min, 95% B (v/v); 30–50 min, 95%–85% B (v/v); 50–60 min, 85%–75% B (v/v). The flow rate was 1.0 mL/min, and the injection volume was 10 μ L. DAD was on, and the target wavelength was simultaneously set at 265 nm. For the mass analysis, the acquisition parameters for positive ion mode were: ion spray voltage 3.5 kV, capillary temperature 400°C, mass scan range, m/z 100–1000. All the data were analyzed by Chemstation software.

Statistical analysis

Data are presented as mean $\pm$ standard deviations. Statistical comparisons were made by one-way analysis of variance using SPSS software version 18.0 (IBM Corporation, Chicago, IL, USA) followed by Dunnett multiple comparison test. $P < 0.05$ was considered as the significance level.

Table 1: Primers used for the real-time polymerase chain reaction

Gene name	Primer sequence	Size
MUC5AC		
F	5'-ATCGTCTGTGTCCATCTC-3'	106 bp
R	5'-GCTGCCATCTATCCAATC-3'	
AQP-5F	5'-CGCTGAACAACAACACAAC-3'	144 bp
R	5'-CAGACAAGCCAATGGATAAGG-3'	
GAPDHF	5'-GTCGGTGTGAACGGATTG-3'	181 bp
R	5'-TCCCATTCTCAGCCTTGAC-3'	

F: Forward primer; R: Reverse primer; MUC5AC: Mucin 5AC

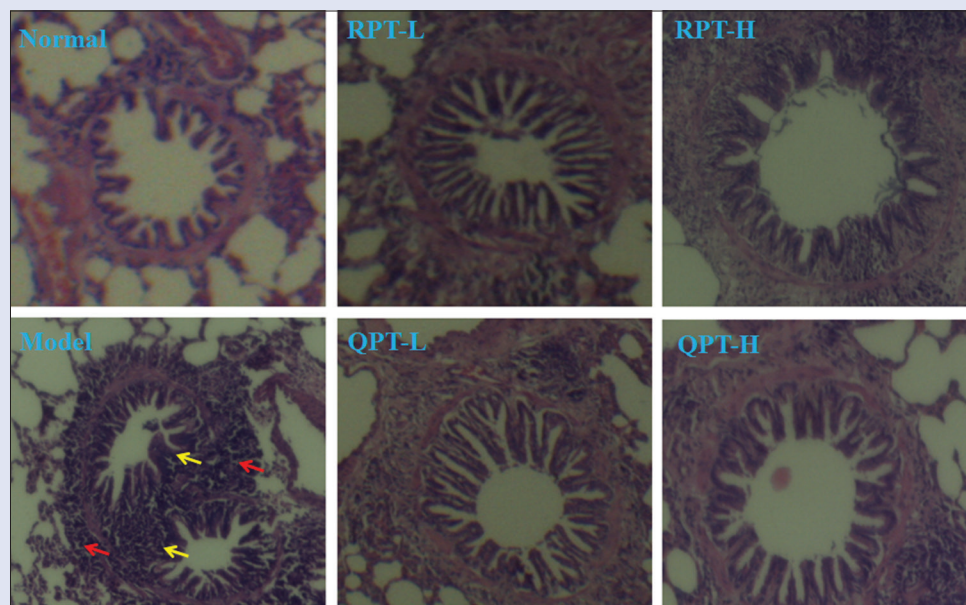


Figure 2: Results of the histochemical examination of lung tissues ($\times 200$). RPT-L and RPT-H means the low and high doses of Pinelliae Rhizoma (120 and 240 mg/kg/day); RQPT-L and QPT-H means the low and high doses of Rhizoma Pinelliae Praeparatum cum Alumine (120 and 240 mg/kg/day). Red arrows indicate the inflammatory cell infiltration; yellow arrows indicate the bronchial wall

RESULTS

Results of the lung histopathological examinations in asthmatic rats

Results of the lung histopathological examinations are shown in Figure 2. For normal rats, no obvious pathological alterations were observed, and the bronchial wall tissues possess structural integrity with moderate airway wall thickness and no obvious evidence of inflammatory cell infiltration. After being induced by OVA, obvious pathological changes were found, and the bronchial walls were thickened and damaged, leading to a distorted and narrow bronchial airway. Besides, lots of inflammatory cells and obvious inflammatory cell infiltration were observed around the bronchial. Interestingly, compared to model rats, the aforementioned abnormal alterations were markedly alleviated by treatment with both the RPT (120 and 240 mg/kg/day) and QPT (120 and 240 mg/kg/day). Importantly, the QPT seems to have the better ameliorative effects on the inflammatory cell infiltration and lung histopathological changes in asthmatic rats than that of RPT at both the low (120 mg/kg) and high (240 mg/kg) dosage.

Results of the eosinophils counting and enzyme-linked immunosorbent assay assays

As can be seen from Figure 3, results of the EOS counting revealed that after being challenged with OVA, the EOS count in BALFs was sharply increased compared to normal rats ($P < 0.05$). However, both RPT (120 and 240 mg/kg/day, $P < 0.05$ and $P < 0.01$, respectively) and QPT (120 and 240 mg/kg/day, $P < 0.01$ and $P < 0.01$, respectively) treatments could effectively reduce the EOS count in BALFs compared to the model rats. Interestingly, our results also indicated that the QPT has better inhibitory effects against the EOS increase in BALFs of asthmatic rats than that of RPT at all the same testing doses in the present experiment ($P < 0.05$).

Furthermore, the present results exhibited the levels of IgE in serum and IL-4 in BALFs [Figure 3] of asthmatic rats. Similar with the results

of EOS counting, after being induced by OVA, the levels of IgE and IL-4 of the asthmatic rats were markedly increased compared to the normal rats ($P < 0.01$ and $P < 0.01$, respectively). Furthermore, both the RPT (120 and 240 mg/kg, $P < 0.01$ and $P < 0.01$, respectively) and QPT (120 mg/kg and 240 mg/kg, $P < 0.01$ and $P < 0.01$, respectively) treatments significantly attenuated the levels of IgE and IL-4 of the asthmatic rats compared to the model rats. Importantly, the QPT also showed the better suppressive effects on the levels of IgE and IL-4 in asthmatic rats than that of RPT at all the same testing doses in the present experiment ($P < 0.05$ and $P < 0.01$, respectively).

Besides, the ELISA results of IFN- γ in BALFs showed that in allergic asthmatic rats, the IFN- γ level was obviously decreased by OVA challenge compared to the normal rats ($P < 0.01$). However, treatments of RPT (120 and 240 mg/kg, $P < 0.05$ and $P < 0.01$, respectively) and QPT (120 and 240 mg/kg, $P < 0.01$, respectively) significantly increased the levels of IFN- γ level in BALFs of the asthmatic rats compared to the model rats. Importantly, the QPT showed better-enhanced effects on the IFN- γ level in BALFs in asthmatic rats than that of RPT at all the same testing doses in the present experiment ($P < 0.05$).

Results of the expressions of mucin 5AC and AQP-5 in lung tissues

As shown in Figure 4, the results of mRNA expressions of MUC5AC and AQP-5 by qRT-PCR were shown. Our present results showed that after being challenged by OVA, the mRNA expressions of MUC5AC were significantly upregulated ($P < 0.01$) whereas the mRNA expressions of AQP-5 were markedly downregulated ($P < 0.01$) in the lung tissues of asthmatic rats compared to the normal rats. On the contrary, RPT (120 and 240 mg/kg/day, $P < 0.05$ and $P < 0.01$, respectively) and QPT (120 and 240 mg/kg/day, $P < 0.01$ and $P < 0.01$, respectively) treatments significantly decreased the mRNA expressions of MUC5AC whereas increased the mRNA expressions of AQP-5 in lung tissues of the asthmatic rats compared to the model rats. Importantly and

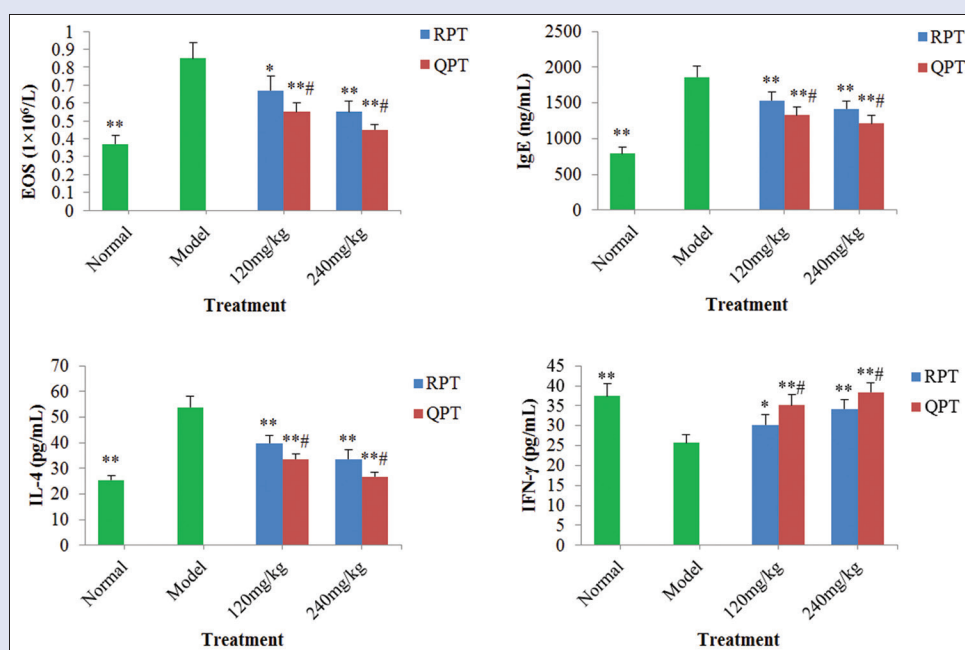


Figure 3: Results of the EOS counting and ELISA assays of IgE, IL-4 and IFN- γ . Data were expressed as mean $\pm$ standard deviation ($n = 10$), * $P < 0.05$, ** $P < 0.01$, vs. model; # $P < 0.05$, versus RPT. RPT: Pinelliae Rhizoma; EOS: Eosinophils; IFN: Interferon; IL: Interleukin; Ig: Immunoglobulin; Enzyme-linked immunosorbent assay

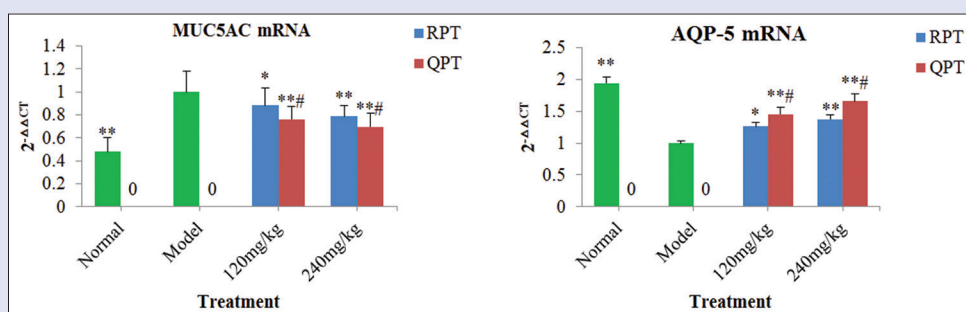


Figure 4: Results of the qRT-PCR assays. Data were expressed as mean $\pm$ standard deviation ($n = 10$), * $P < 0.05$, ** $P < 0.01$, vs. model; # $P < 0.05$, versus RPT. RPT: Pinelliae Rhizoma; qRT-PCR: Quantitative real-time fluorogenic polymerase chain reaction

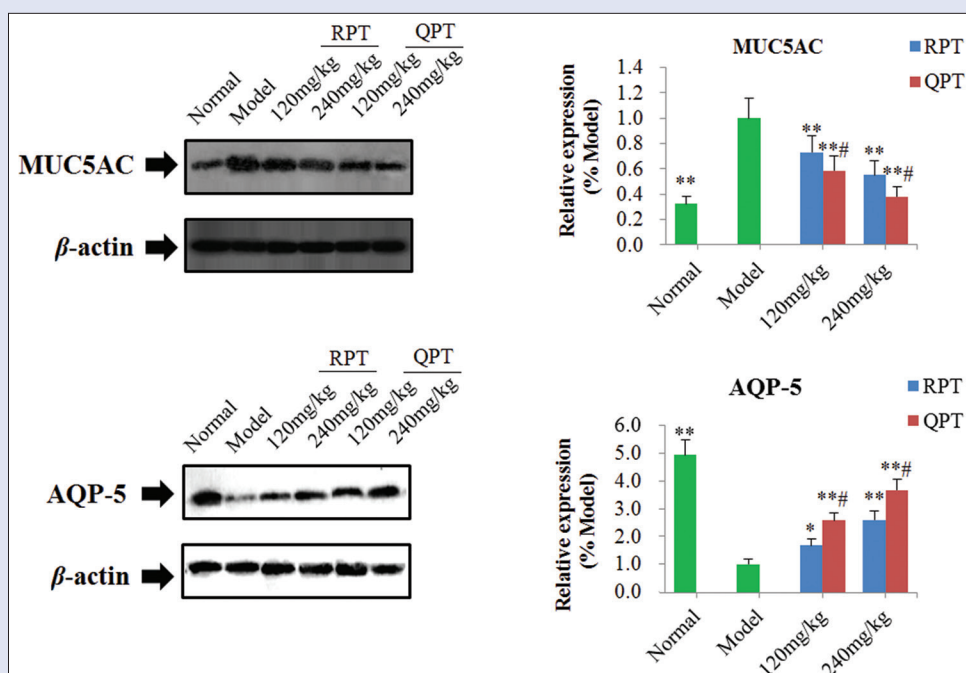


Figure 5: Results of the western blot assays. Data were expressed as mean $\pm$ standard deviation ($n = 10$), * $P < 0.05$, ** $P < 0.01$, vs. model; # $P < 0.05$, versus RPT. RPT: Pinelliae Rhizoma

interestingly, the QPT showed the better regulative effects on the mRNA expressions of MUC5AC and AQP-5 in lung tissues of asthmatic rats than that of RPT at all the same testing doses in the present experiment ($P < 0.05$). Furthermore, the mRNA expressions of the two genes were evidenced and confirmed by the similar results of western blot assays which described in Figure 5.

Results of the high-performance liquid chromatography-LTQ-Orbitrap-Elite-MS analysis

To compare the chemical compositions difference between the RPT and QPT, high-performance liquid chromatography(HPLC)-LTQ-Orbitrap-Elite-MS analysis was performed in our present study. From our present results [Figure 6 and Table 2], over 20 peaks were detected within 60 min in the HPLC chromatogram of RPT and QPT [Figure 6], among which 10 compounds were identified or deduced based on their positive ion mass spectra according to their precursor ions ($[M + H]^+$) data [Table 2] after comparison with literature data.^[3,19,20] The 10 compounds were uridine (peak 2), tyrosine (peak 3), cyclo-(leucine-tyrosine) (peak 5), uracil (peak 6), adenine (peak 7),

Table 2: Chemical constituent's analysis of Rhizoma Pinelliae Praeparatum cum Alumine and Pinelliae Rhizoma by high-performance liquid chromatography-LTQ Orbitrap Elite-MS

n	tR (min)	[M+H] ⁺ /(m/z)	Molecular formula	Identification
1	6.39	217.1290	Unknown	Unknown
2	7.05	245.0761	C ₉ H ₁₂ N ₂ O ₆	Uridine
3	8.87	182.0806	C ₉ H ₁₁ NO ₃	Tyrosine
4	11.22	175.1184	Unknown	Unknown
5	13.33	276.1434	C ₁₅ H ₂₀ O ₃ N ₂	Cyclo-(leucine-tyrosine)
6	15.05	113.0341	C ₄ H ₄ N ₂ O ₂	Uracil
7	23.82	136.0614	C ₆ H ₇ N ₅	Adenine
8	24.77	269.0874	C ₁₀ H ₁₂ N ₄ O ₅	Inosine
9	25.32	284.0982	C ₁₀ H ₁₃ N ₅ O ₅	Vernine
10	26.49	127.0385	C ₆ H ₆ O ₃	5-hydroxymethylfurfural
11	31.53	205.0945	C ₁₁ H ₁₂ N ₂ O ₂	Tryptophan
12	34.22	268.1034	C ₁₀ H ₁₃ N ₅ O ₄	Adenosine
13	58.58	565.1532	Unknown	Unknown

inosine (peak 8), vernine (peak 9), 5-hydroxymethylfurfural (peak 10), tryptophan (peak 11), and adenosine (peak 12). In addition, as shown in

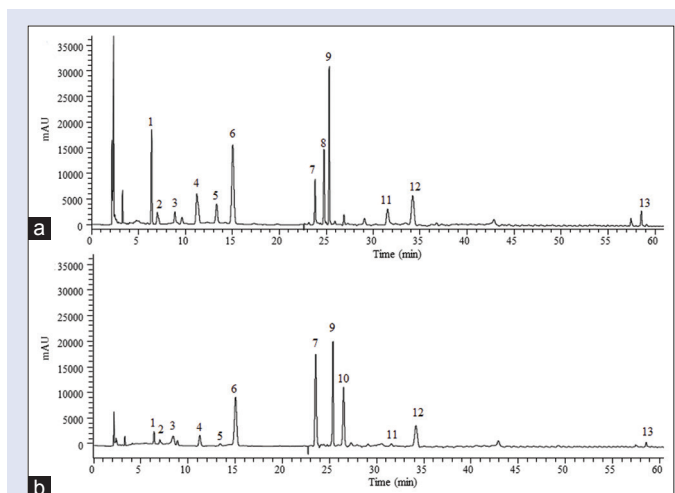


Figure 6: Constituent's analysis of RPT (a) and QPT (b) by HPLC-LTQ Orbitrap Elite-MS. QPT: Rhizoma Pinelliae Praeparatum cum Alumine; RPT: Pinelliae Rhizoma; HPLC: High-performance liquid chromatography

our present results, compared to the HPLC chromatogram of RPT, most of the constituents' contents of QPT obvious reduced, such as the peak 1 (unknown), peak 2 (uridine), peak 3 (tyrosine), peak 4 (unknown), peak 5 (cyclo-(leucine-tyrosine)), peak 6 (uracil), peak 8 (adenine), peak 9 (vernine), peak 11 (tryptophan), peak 12 (adenosine), and peak 13 (unknown); in particular, peak 8 (adenine) cannot be detected in the HPLC chromatogram of QPT. On the contrary, the peak 7 (Vernine) contents in HPLC chromatogram of QPT increased compared to the RPT, and peak 10 (5-hydroxymethylfurfural) in HPLC chromatogram of QPT is a new processed constituent compared to RPT.

DISCUSSION

Similar with other allergic diseases, asthma, a complex chronic airway inflammatory disorder, markedly affects life quality of patient and may result in substantial medical care expenditures.^[21] Furthermore, asthma is a common disease affecting millions of children and adults with an increasing prevalence in the world, and therefore, allergic asthma has become a public health issue worldwide.^[16,21] Nowadays, although great improvements have been achieved in diagnosis and treatment of asthma, it remains approximate 15% asthma patients which cannot be satisfactory treated by currently available drugs, such as glucocorticoid inhalation.^[22] Thus, it is currently urgent required for finding more new alternative therapies for asthma treatment. Plants are the primary sources of food and drugs, and it is currently reported that over 50% of the currently drugs are closely correlated to the natural plant-derived compounds/extracts.^[23-25] In addition, in Chinese folk medicine, herbal medicines have been applied for centuries to cure various difficult miscellaneous diseases, including allergic disorders.^[25,26] Consequently, herbal medicine is one of the feasible approaches to treat allergic asthma. In the present study, our results revealed that both the RPT and QPT have potential curative effects on OVA-induced allergic asthma in rats.

The allergic asthma is a chronic airway inflammatory disease and is featured by EOS infiltration and structural remodeling in airway, and previous findings have suggested that inhibition of the airway inflammatory reactions could be beneficial for ameliorating the symptoms of allergic asthma.^[7,27] In addition, increasing evidence has also revealed that EOS is a main effector cell of allergic asthma which is commonly considered as a major biomarker for allergic asthma in

clinical.^[28] It is interesting our present results showed that both the RPT and QPT treatments could alleviate the inflammatory reactions in lung tissues and decrease the EOS in BALFs of asthmatic rats. The unbalance of type-1 T-helper (Th1)/type-2 T-helper (Th2) cell and overreleased Th2 cells are commonly considered as the important reason for the development of asthma.^[16,29] Our present results showed that both the RPT and QPT treatments could decrease Th2 cytokine of IL-4 whereas increased Th1 cytokine of IFN- γ , suggesting that both RPT and QPT treatments could modulate the balance of Th1/Th2 cells. IgE is generally considered to be a crucial factor for the pathogenesis of allergic disorders including asthma and allergic rhinitis, and it is also recognized as an important target for treating allergic asthma.^[7,26] The present results revealed both the RPT and QPT treatments could decrease IgE in serum of allergic asthmatic rats. In recent years, increasing studies have revealed that the abnormal expressions of MUC5AC and aquaporins in lung tissues have closely related to the airway hypersecretion diseases, such as allergic asthma; in addition, there is commonly a negative correlation between the two genes/proteins.^[1,7,30] In our present work, we found that treatments of RPT and QPT significantly decreased the mRNA and protein expressions of MUC5AC whereas increased the mRNA and protein expressions of AQP-5 in lung tissues of the asthmatic rats.

In the traditional medicinal system of China, the QPT, a known and frequently-used TCM, is one of the main components in many decoctions for treating dampness-phlegm symptoms (including cough and asthma), such as *Er-chen* decoction, *Xiao-qing-long* decoction, and *Ding-chuan* decoction.^[3,4] Interestingly, the QPT is generally considered to be good at removing dampness-phlegm compared to RPT.^[29,31] However, no definite available animal evidence has confirmed this issue mentioned above. Importantly, in the present work, our results demonstrated that the QPT exhibited the better curative effects on OVA-induced allergic asthma in rats at all the same testing dosage. Thus, our present result is a potential evidence for demonstrating the issue that the QPT is good at removing dampness-phlegm compared to RPT, which would be beneficial for illumination of the processing mechanism of RPT.

CONCLUSION

Both the RPT and QPT have potential curative effects for treating OVA-induced asthma in rats via inhibition of inflammatory reactions and modulation of MUC5AC and AQP-5. Furthermore, our results also evidenced that QPT exhibited the better curative effects on allergic asthmatic rats compared to the RPT, which could be beneficial for confirming the TCM theory of QPT is good at removing dampness to reduce phlegm."

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Chen Z, Wang X, Gao L, Bai L, Zhu R, Bai C, *et al.* Regulation of MUC5AC mucin secretion by depletion of AQP5 in SPC-A1 cells. *Biochem Biophys Res Commun* 2006;342:775-81.
- Editorial Committee of Chinese Pharmacopoeia. *Chinese Pharmacopoeia* 2015th ed., Vol. 2. Beijing: China Medical Science and Technology Press; 2015. p. 119-20.
- Ji X, Huang B, Wang G, Zhang C. The ethnobotanical, phytochemical and pharmacological profile of the genus *Pinellia*. *Fitoterapia* 2014;93:1-7.

4. Chen JH, Cui GY, Liu JY, Tan RX. Pinelloside, an antimicrobial cerebroside from *Pinellia ternata*. *Phytochemistry* 2003;64:903-6.
5. Liu YJ, Mo XL, Tang XZ, Li JH, Hu MB, Yan D, *et al.* Extraction optimization, characterization, and bioactivities of polysaccharides from *Pinelliae* rhizoma praeparatum cum alumine employing ultrasound-assisted extraction. *Molecules* 2017;22. pii: E965.
6. Wu YY, Huang XX, Zhang MY, Zhou L, Li DQ, Cheng ZY, *et al.* Chemical constituents from the tubers of *Pinellia ternate* (Araceae) and their chemotaxonomic interest. *Biochem Syst Ecol* 2015;62:236-40.
7. Lee MY, Shin IS, Jeon WY, Lim HS, Kim JH, Ha H, *et al.* *Pinellia ternata* Breitenbach attenuates ovalbumin-induced allergic airway inflammation and mucus secretion in a murine model of asthma. *Immunopharmacol Immunotoxicol* 2013;35:410-8.
8. Xu JY, Dai C, Shan JJ, Xie T, Xie HH, Wang MM, *et al.* Determination of the effect of *Pinellia ternata* (Thunb.) Breit. On nervous system development by proteomics. *J Ethnopharmacol* 2018;213:221-9.
9. Zhang ZH, Zhao YY, Cheng XL, Dai Z, Zhou C, Bai X, *et al.* General toxicity of *Pinellia ternata* (Thunb.) Berit. In rat: A metabonomic method for profiling of serum metabolic changes. *J Ethnopharmacol* 2013;149:303-10.
10. Zhong LY, Wu H, Zhang KW, Wang QR. Study on irritation of calcium oxalate crystal in raw *Pinellia ternata*. *Zhongguo Zhong Yao Za Zhi* 2006;31:1706-10.
11. Xiu YF, Hong XK, Wang ZH. Progress of studies on preparation of Rhizoma Pinelliae. *Chin Trad Patent Med* 2004;26:38-40.
12. Yuan HJ, Jia XB, Yin WJ, Wang H, Wang HJ, Li W, *et al.* Effects of processing on toxic components of *Pinellia rhizoma* and its detoxification mechanism. *Zhongguo Zhong Yao Za Zhi* 2016;41:4462-8.
13. Shin IS, Lee MY, Jeon WY, Shin NR, Seo CS, Ha H, *et al.* EBM84 attenuates airway inflammation and mucus hypersecretion in an ovalbumin-induced murine model of asthma. *Int J Mol Med* 2013;31:982-8.
14. Wang J. Phlegm of traditional Chinese medicine and mucin. *Chin Arch Trad Chin Med* 2011;29:1600-1.
15. Zhou JL, Guo ZH, Zhang P. Airway mucus hyper-secretion in rats is inhibited by the extracts of *Pinellia*. *Chin J Respir Crit Care Med* 2009;8:479-81.
16. Xue Z, Zhang XG, Wu J, Xu WC, Li LQ, Liu F, *et al.* Effect of treatment with geraniol on ovalbumin-induced allergic asthma in mice. *Ann Allergy Asthma Immunol* 2016;116:506-13.
17. Peng W, Qiu XQ, Shu ZH, Liu QC, Hu MB, Han T, *et al.* Hepatoprotective activity of total iridoid glycosides isolated from *Paederia scandens* (lour.) merr. var. *Tomentosa*. *J Ethnopharmacol* 2015;174:317-21.
18. Zhang SY, Tao CH, Zhou ZX, Liu SL, Jiang YW, Fan X, *et al.* Study on dose of *Pinellia* based on herb clinical use of modern Chinese medicine masters. *Chin Arch Trad Chin Med* 2013;31:1007-9.
19. Xu WY, Liu CS, Feng MX, Ma KY, Gao SS, Hou WZ, *et al.* Study on the chemical composition of traditional Chinese medicine *Pinellia ternate* by C18-mixed-stationary phase column LC-MS. *Northwest Pharm J* 2015;30:354-7.
20. Yang BY, Li M, Lu DH, Jing Y, Huang X. Research on the HPLC characteristic fingerprints of rhizoma *Pinelliae* and processed products. *Chin Pharm J* 2014;49:955-62.
21. Holt PG, Sly PD. Viral infections and atopy in asthma pathogenesis: New rationales for asthma prevention and treatment. *Nat Med* 2012;18:726-35.
22. Olin JT, Wechsler ME. Asthma: Pathogenesis and novel drugs for treatment. *BMJ* 2014;349:g5517.
23. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. *J Nat Prod* 2016;79:629-61.
24. Peng W, Shen H, Lin B, Han P, Li CH, Zhang QY, Ye BZ, Rahman K, Xin HL, Qin LP, Han T. Docking study and antiosteoporosis effects of a dibenzylbutane lignan isolated from *Litsea cubeba* targeting Cathepsin K and MEK1. *Med Chem Res.* 2018; 27:2062-2070.
25. Wu WY, Hou JJ, Long HL, Yang WZ, Liang J, Guo DA, *et al.* TCM-based new drug discovery and development in China. *Chin J Nat Med* 2014;12:241-50.
26. Peng W, Ming QL, Han P, Zhang QY, Jiang YP, Zheng CJ, *et al.* Anti-allergic rhinitis effect of caffeoylxanthiazonoxide isolated from fruits of *Xanthium strumarium* L. In rodent animals. *Phytomedicine* 2014;21:824-9.
27. Yoon H, Kim W. Assessment of eosinophils and eosinophil cationic protein in induced sputum in childhood asthma. *J Aller Clin Immunol* 2003;111:s306.
28. Dong F, Wang C, Duan J, Zhang W, Xiang D, Li M, *et al.* Puerarin attenuates ovalbumin-induced lung inflammation and hemostatic unbalance in rat asthma model. *Evid Based Complement Alternat Med* 2014;2014:726740.
29. Yeh WJ, Harrington LE. Regulation of effector CD4⁺T cell functions by T-bet. *J Immunol* 2009;182:48.
30. Zhao GC, Liu JN. Analysis of the processing and clinical application of *Pinellia ternate*. *Shanxi TCM* 2009;30:601-2.
31. Cai BC. *Science of Processing Chinese Materia Medica*. Beijing: China Press of Traditional Chinese Medicine; 2008. p. 283-6.