



Chinese herbal medicine formula for acute asthma: A multi-center, randomized, double-blind, proof-of-concept trial

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ABSTRACT

Background: Despite advances in asthma management, exacerbations constitute a significant health economic burden.

Objective: To observe the efficacy and safety of Chinese herbal medicine formula entitled PingchuanYiqi (PCYQ) granule, on acute asthma and to explore its possible mechanism.

Materials and methods: This proof-of-concept study consisted of a randomized, double-blind, placebo-controlled trial in patients with acute asthma ($n = 300$). Participants with acute mild-to-moderate asthma recruited from seven centers in China were randomly assigned to receive PCYQ or placebo. The primary outcomes were PEF (L/min) and total asthma symptom scores. Furthermore, a panel of cytokines including serum IL-4, IL-5, IL-6, IL-8, IL-1 β , IL-17A, IFN- α , IFN- β , IFN- γ , CRP, CCL-5, IP-10, and PGD₂ levels was detected using ELISA.

Results: The PCYQ ($n = 139$) significantly improved the morning PEF on day 4 (349.73 ± 93.92 vs. 313.56 ± 92.91 L/min, $P = 0.004$) and day 7 (360.42 ± 94.39 vs. 329.52 ± 95.97 L/min, $P = 0.023$), and the evening PEF on day 4 (352.65 ± 95.47 vs. 320.58 ± 95.30 L/min, $P = 0.012$) and day 7 (360.42 ± 94.39 vs. 336.86 ± 95.59 L/min, $P = 0.029$) in comparison with the placebo ($n = 143$). The PCYQ also improved the clinical symptoms scores and reduced the puffs of short-acting β_2 -agonist (all $P < 0.05$). Furthermore, the PCYQ statistically reduced IL-5, IL-8, IL-1 β and PGD₂ in serum.

Conclusion: The PCYQ as the Chinese herbal medicine formula significantly improves lung function and symptoms of acute asthma, and reduces SABA dosage possibly via decrease of inflammatory biomarkers such as IL-5, IL-8, IL-1 β and PGD₂.

Trial registration: ISRCTN61674768 (<http://www.isrctn.com/>).

1. Introduction

Asthma is one of the most common chronic diseases worldwide affecting 1–18% of the population in different countries. The long-term

goals of asthma management are to achieve asthma control and to minimize future risk of exacerbations. Poor asthma control and exacerbations have significant implications for individuals and health care providers, and constitute a significant health economic burden [1].

Abbreviations: ASHMI, antiasthma simplified herbal medicine intervention; CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; ERS, European Respiratory Society; FEV₁, forced expiratory volume in 1 s; GINA, Global Initiative for Asthma; IFN, interferon; IL, interleukin; ITT, intention-to-treat; MMP, matrix metalloproteases; PCYQ, PingchuanYiqi granule; PEF, peak expiratory flow; PGD₂, prostaglandins D₂; SABA, short-acting β_2 -agonist; SD, standard deviation; TCM, traditional Chinese medicine; T_H2, T helper 2 cells; TNF, tumor necrosis factor

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Exacerbations of asthma are episodes which are characterized by a progressive increase in symptoms of breathlessness, cough, wheezing or chest tightness and progressive decrease in lung function, and represent a change from the patient's usual status that is sufficient to require a change in treatment [2]. Respiratory virus infections are usually the most common cause of asthma exacerbations [3]. Potent anti-inflammatory corticosteroids and β_2 -agonists can improve asthma symptoms and are recommended by Global Initiative for Asthma management (GINA) [4] and other guidelines. Systemic corticosteroids have broad immuno-suppressive effect and potential serious side-effects, and may further promote viral-induced acute exacerbations [5], although these adverse effects with inhaled corticosteroids are very low. Chronic use of these drugs can also cause disturbance of adrenal function, particularly long-term growth in children [6]. Traditional Chinese medicine (TCM) has been increasingly shown to be one of the complementary and alternative remedies in the treatment of asthma exacerbations.

Asthma has long been treated using herbs in multiple traditional medicine systems in Europe, the Middle East and China [7]. To achieve optimal asthma control, it has been reported [8] that TCM is effective in chronic persistent asthma, despite a lack of recommendations by the international guidelines. For example, A randomized, double-blind, placebo-controlled, and crossover study by Urata et al. [9] showed that TJ-96 as herbal medicine improved the inhaled methacholine-induced PC20, eosinophils and symptoms after 4-week treatment in chronic asthma. Chang et al. conducted a 6-month randomized, double-blind, placebo-controlled trial to explore effects of the TCM formula STA-1 in chronic mild-to-moderate asthma [10]. As a result, STA-1 has a statistically significant reduction of symptoms scores, systemic steroid dose, IgE levels, and improvement of FEV₁. Chan et al. found the Ding Chuan Tang as a Chinese herb decoction improved airway hyper-responsiveness, and symptom and medication scores in a 12-week randomized, double-blind, placebo-controlled trial [11]. A double-blind, randomized trial by Wen and his colleagues found anti-asthma herbal medicine entitled ASHMI that consisted of *Ganoderma lucidum*, *Sophora flavescens* and *Glycyrrhiza uralensis*, appeared to be a safe and effective alternative remedy for treating persistent moderate-severe asthma with no adverse effect on adrenal function [12] but had a homeostatic effect on T_H1 and T_H2 balance. Furthermore, recently published systematic review [13] including 29 randomized controlled trials indicated herbal medicines could improve lung function, use of rescue medication and asthma control, although it was of the low or moderate quality of the evidence assessed by GRADE approach.

Chinese herbal medicine formulas have also been shown to have antiviral and immunomodulatory effects [12,14–17], which would have potential implications for treatment of asthma exacerbations. The Chinese herbal medicine formula named Pingchuan Yiqi (PCYQ) in treatment of acute asthma has been widely and empirically used in Chinese population, although it indicated that PCYQ, composed of nine herbs including many chemical compounds, has multiple effects such as anti-allergic reaction, anti-inflammation, antitussive, and bronchial relaxation in vitro studies and animal models (supplementary materials). However, there are few well-controlled studies that support the efficacy of herbal remedies in the treatment and clinical improvement of patients with asthma exacerbations. In this study we conducted the multi-center, randomized, double-blind, placebo-controlled, proof-of-concept trial to observe the efficacy and safety of Chinese herbal medicine formula named PCYQ in asthma exacerbations, and to explore its possible mechanism of action.

2. Methods

2.1. Study design

This proof-of-concept study consisted of a multi-center, randomized, double-blind, placebo-controlled trial from July 2011 to December

2012 at seven sites in China. The study was in conformance with Good Clinical Practice standards and was approved by Institutional Review Board at West China Hospital, Sichuan University (Chengdu, China), with the ethics approval number No. 201004. All participants with acute asthma signed written informed consent forms before enrollments. This study was registered with No. ISRCTN61674768 at <https://doi.org/10.1186/ISRCTN61674768> [18]. In addition, this study was strictly reported according to recent CONSORT-CHM Formulas 2017 [19], which would improve the reporting quality of this randomized controlled trial for Chinese herbal medicine formula in treatment of acute asthma (Table E1).

2.2. Subjects

Subjects aged 15–75 years who presented within 72 h after onset of mild to moderate asthma exacerbations were enrolled. Asthma was diagnosed according to GINA [4] based on current (within the past 12 months) episodic symptoms, physician diagnosis, and confirmed evidence of airway hyperresponsiveness or a 12% or greater improvement in forced expiratory volume in 1 s (FEV₁) after bronchodilator. The severity of asthma exacerbations was defined by an official ATS/ERS statement [2] and GINA 2006 (Table E2). The controller therapies for at least one month before this study need unchanged for eligible subjects during this acute asthma episode. If the subjects received any more additional maintenance therapies such as theophylline, corticosteroids, or leukotriene modifiers during this acute asthma episode before entry, they were excluded. Further, they also were excluded if were pregnant or breastfeeding, had uncontrolled co-morbidities, or if they have ever experienced invasive mechanical ventilation or ICU stay for asthma. Equal allocation randomization into either Chinese herbal medicine formula or placebo interventions, stratified by centers, was performed using computerized by PROCPLAN of the analysis system of SAS (Version 6.12 for Windows) and stratified by the seven centers. Allocation sequence was placed in a sealed envelope and study investigators and patients were blinded to treatment allocation.

2.3. Interventions

The formula of PCYQ granule (Lot number: YBZ09112005; Validity: 2 years) form used in this study was composed of nine herbs including Mahuang, Hongshen, Chuanshanlong, Kuxingren, Houpo, Suyue, Chaihu, Chenpi, Gancao (Table 1), which was supplied by Hangzhou East China Pharmaceutical Company (Hangzhou, China) [20–38]. The standardized quality control for the Chinese herbal medicine formula was in accordance with the 2005 Chinese pharmacopoeia [39] and approved by China Food and Drug Administration (CFDA) (No.Z20050258). PCYQ was standardized to 3.0 g per bag and the color appearance, dissolved color, taste and smell for the placebo were almost similar to the PCYQ granule as the intervention. The participants with acute asthma received 6.0 g of PCYQ or placebo 3 times daily via oral administration for 7 days. During this study period, all participants were asked to maintain their controller medications and receive inhaled short-acting β_2 -agonist (SABA) (100 μ g/puff, Ventolin, GSK, Spain) as needed in self-management. If the symptoms deteriorated after entry, the subjects were required to contact with investigators or physicians at any time for initiating further management according to GINA and China Asthma guideline.

2.4. Primary and secondary outcomes

The predetermined primary outcome measures were peak expiratory flow rate (PEF) and asthma symptom scores. When the participants were enrolled, the investigators trained them how to test PEF until they were qualified. Then, all patients had a measure of PEF using a PEF meter (Keka®, Shanghai, China) on admission. Participants conducted PEF monitoring at morning and evening by themselves

Table 1
Standard formula of Chinese herbal medicine formula entitled PingchuanYiqi granule.

Chinese name	Pharmaceutical name	Source	Place of production	Weights (%)	The possible compounds	Possible mechanism of action
Mahuang	<i>Ephedrasinicaatpf</i>	Stems, leaves	Gansu province	12.19	Ephedrine, pseudoephedrine, methylephedrine, ephedra et al.	Inhibit the release of allergic neurotransmitters; reduce hemolysis, anti-complement effect, antiasthmatic effect [20,21]
Hongshen	<i>Red ginseng</i>	Roots	Jilin province	12.19	Ginsenosides, ginseng polysaccharides, volatile oil, azoxystrobin et al.	Antioxidant properties, antiproliferative effects [22,23], anti-allergic reaction, anti-inflammation, improve immune function, inhibit allergic reactions to food by preventing reductions in the ratio of IFN- γ to IL-4 and in IL-12 production induced by dietary antigens in spleen cells [24], enhance antioxidant enzyme activity [25]
Chuanshanlong	<i>Rhizome Dioscoreae nipponicae</i>	Stems, Roots	Heilongjiang province	24.39	Dioscin, gracillin, asperin, 25-D-spirosta-3,5-diene, piscidic acid et al.	Anti-inflammatory, antitussive effect, induce apoptosis [26], promote blood circulation and suppress cough [27], anti-oxidant properties [28].
Kuxingren	<i>Semen armeniacae amarae</i>	Seeds	Neimenggu province	7.32	Amygdalin, fatty oil, bitter amylases et al.	Anti-inflammatory, relieve cough and dyspnea [29]
Houpo	<i>Mangolia officinalis</i>	Branches, Leaves	Sichuan province	7.32	Magnolol, isomagnolol, tetrahydromagnolol, honokiol et al.	Antibacterial effect, skeletal muscle relaxation, antifungal properties [30], honokiol have promise as a novel class of anti-inflammatory agents in the treatment of chronic asthma which symptoms and pathology of asthma can be alleviated even in the presence of increased Th2 cytokines [31], Magnolol inhibits TNF- α -induced ICAM-1 expression via suppressing NF- κ B and MAPK signaling pathways in human lung epithelial cells [32].
Suye	<i>Beef-steak plant leaf</i>	Stems, Leaves	Jiangsu province	7.32	Perilla frutescens, L-citric acid, α -pinene et al.	Antibacterial effect, sedative effect, itching effect, antitussive, and bronchial relaxation [33]
Chaihu	<i>Radix bupleuri</i>	Roots	Shanxi province	9.74	saikosapoinins a, b, c, d, sterol, saponins et al.	Anti-inflammatory, enhance phagocytosis, enhance natural killer cell function, anti-virus effect, antibacterial endotoxin [34]
Chenpi	<i>Dried tangerine</i>	Peel	Guangdong province	7.32	Hesperidin, amygdalin et al.	Antiviral and antibacterial effects, anti-photoaging effect [35], anti-oxidative stress and anti-apoptosis [36]
Gancao	Licorice	Stems, Roots	Shaanxi province	12.19	Triterpenoid saponins, glycyrrhizin, licoricesaponin, flavonoids, alkaloids, polysaccharides et al.	Inhibit the proliferation of airway smooth muscle cells, antitussive and expectorant effect [37], inhibit production of IL-4 and IL-5 by murine memory Th2 cells and eosinophil production [38]

Abbreviations: TNF- α , tumor necrosis factor- α ; ICAM-1, intercellular adhesion molecule-1.

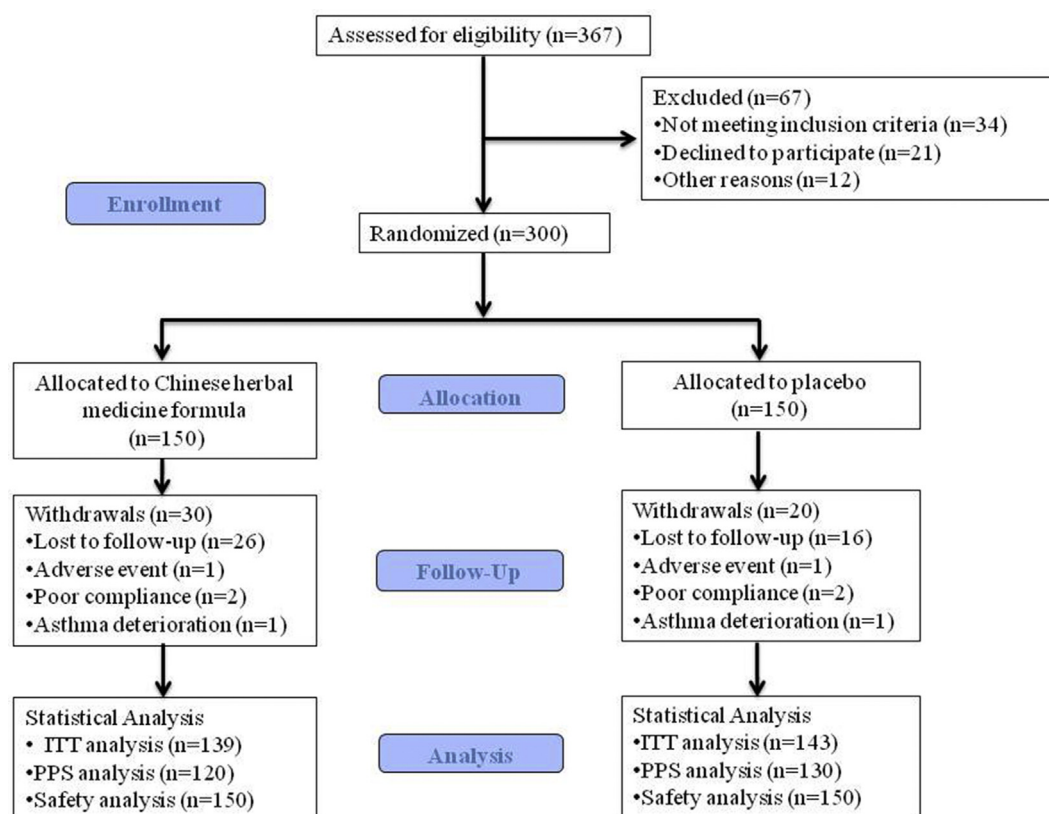


Fig. 1. Study flow diagram.

throughout the study period, and recorded the PEF data before as-needed inhalation of SABA in the daily asthma diary. All measurements of PEF were determined according to European Respiratory Society (ERS) [40]. Asthma symptom scores were assessed using symptoms-based scale by investigators at entry and on days 4 and 8. Assessment of shortness of breath, and wheeze ranged on a scale from 0, 3, 6, and 9 scores for no, mild, moderate, severe symptoms, respectively, while measurements of chest tightness and cough ranged on a scale from 0, 1, 2, 3 for no, mild, moderate and severe symptoms. The secondary outcomes included dosage of SABA as needed during the study period, absolute and percentage of eosinophils in peripheral venous blood, the number of patients requiring systemic glucocorticoids or hospitalizations.

2.5. Cytokine assays

To explore the possible mechanisms if PCYQ improved clinical features of acute asthma, inflammatory mediators in serum were detected at entry and after treatment. Serum samples were collected from the participants ($n = 60$) at West China Hospital, Sichuan University (Chengdu, China), which were obtained from peripheral venous blood by centrifugation at 3000 rpm, 4 °C for 10 min (H2050R, cence®, Changsha, China), and then were frozen immediately at -80 °C for further detection. A panel of cytokines including interleukin (IL) -4 , IL-5, IL-6, IL-8, IL-1 β , IL-17A, interferon (IFN)- α , IFN- β , IFN- γ , chemokine ligand (CCL)-5, IFN- γ -induced protein (IP)-10, PGD $_2$ was determined using commercially available enzyme-linked immunosorbent assays (ELISAs) (VeriKine ELISA Kit, Pestka Biomedical Laboratories, Inc., USA) following the manufacturer's instructions. The sensitivities of IL-1 β , IL-4, IL-5, IL-6, IL-8, IP-10 and PGD $_2$ were < 1.0 pg/mL, < 5.0 pg/mL for IL-17A, 1.0 pg/mL for IFN- α and IFN- γ , 0.1 ng/mL for IFN- β , 1.0 ng/mL for CCL-5. All of the cytokines do not cross-react with other soluble structural analogues.

2.6. Safety

All participants were required to record any unexpected signs and symptoms during the study period. Safety evaluations included adverse event reporting, physical examinations, electrocardiograms and clinical laboratory testing.

2.7. Statistical analysis

The targeted sample size of 296 randomized subjects would need to be enrolled, to allow for 20% withdrawals, in order to provide 80% power to detect a difference of 40 L/min in PEF at a one-sided 0.05 level, according to our previous study [41], thus we intended to finally recruit 300 participants. The primary and secondary efficacy measures were evaluated by using the intention-to-treat (ITT) population analysis. Safety analyses were performed on those populations who received at least one dose of medication and who had at least one follow-up for safety, whether or not they were withdrawn prematurely. Means (SDs) or medians (Q1, Q3) were calculated to summarize continuous variables if no indications were provided. For categorical variables, the proportion of patients in each category was calculated. Measurement data showing a non-normalized distribution were described as median and analyzed by the Wilcoxon rank-sum test. Frequency data were analyzed using the chi-squared (χ^2) or Fisher's tests. Multi-center continuous data were analyzed using CMH method. $P \leq 0.05$ for one-tailed tests was considered to be significant. All analyses were conducted using STATA 11.0 (College Station, Texas, USA).

3. Results

3.1. Participant characteristics

Of the 367 subjects who were enrolled, 67 were excluded. Thus, 300 patients underwent randomization at seven sites, of whom 282

Table 2
Demographic and clinical characteristics of patients at baseline.

Variables	Chinese herbal medicine formula (n = 139)	Placebo (n = 143)	P value
Demographic details			
Men, n (%)	56 (40.29)	62 (43.36)	0.601
Ages(years)	45.3 ± 14.4	45.7 ± 13.6	0.808
BMI (kg/m ²)	23.28 ± 2.90	22.92 ± 2.67	0.354
Current smoker, n (%)	11(7.91)	12(8.39)	0.581
Asthma features			
Asthma duration (years)	3.00 (1.50–5.00)	3.00 (1.98–6.00)	0.205
Duration of asthma exacerbations (hours)	24.00 (15.50–40.00)	24.00 (12.00–36.00)	0.130
Spirometry			
PEF (L/min)	308.6 ± 101.9	302.6 ± 101.8	0.625
PEF(% predicted)	72.80 ± 22.66	71.24 ± 24.38	0.582
Maintenance treatment			
Use of maintenance therapy, n (%)	37(26.62)	35(24.48)	0.680
Theophylline, n (%)	5(3.60)	7(4.90)	0.568
Leukotriene modifiers, n (%)	8(5.76)	4(2.80)	0.378
Long-acting β_2 -receptor agonists, n (%)	4(2.88)	3(2.10)	0.731
Anticholinergics, n (%)	0(0.00)	1(0.70)	0.310
ICS, n (%)	1(0.70)	0(0.00)	0.324
Rescue therapy drugs			
SABA, n (%)	40(28.78)	41(28.67)	0.794
Co-morbid diseases			
Allergic rhinitis, n (%)	22(15.83)	28(19.58)	0.295
Atopic dermatitis, n (%)	4(2.88)	1(0.70)	0.371
Other diseases, n (%)	2(1.44)	4(2.80)	0.442

Data are n, n/N (%) or mean ± standard deviation (SD) or median (Q1, Q3). BMI = body mass index. PEF = Peak expiratory flow rate. ICS = inhaled corticosteroids. SABA = Short-acting β_2 -receptor agonists.

provided sufficient data in the asthma diaries and entered into the analysis of the ITT population (Fig. 1). The rate of adherence to study medication was 98.56% for Chinese herbal medicine formula and 99.31% for placebo. The mean age was 45.5 years (SD, 14.0), and 41.8% of participants were male. All of included patients were of mild or moderate severity of acute asthma. Table 2 presents baseline demographic and clinical characteristics between the two groups. Subjects in both groups were comparable with respect to baseline demographic, asthma characteristics, baseline PEF and co-morbid diseases.

3.2. Efficacy

The morning PEF in the PCYQ group statistically improved on day 4 (349.73 ± 93.92 vs. 313.56 ± 92.91 L/min, $P = 0.004$) and day 7 (360.42 ± 94.39 vs. 329.52 ± 95.97 L/min, $P = 0.023$) compared with the placebo group (Fig. 2A), and the corresponding evening PEF in the PCYQ group was much better than that in the placebo group on day

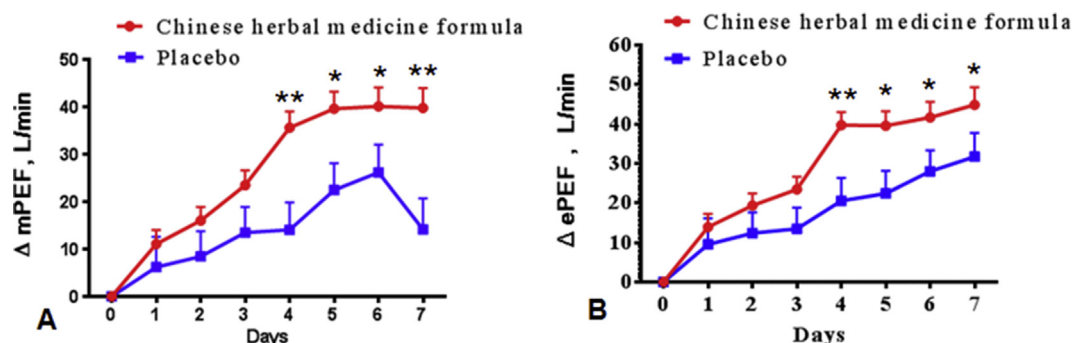


Fig. 2. The improvements of morning (A) and evening (B) PEF after Chinese herbal medicine formula and placebo intervention during the 7-day treatment period. mPEF, morning PEF; ePEF, evening PEF. Bar indicated as standard error. * $P < 0.05$; ** $P < 0.001$.

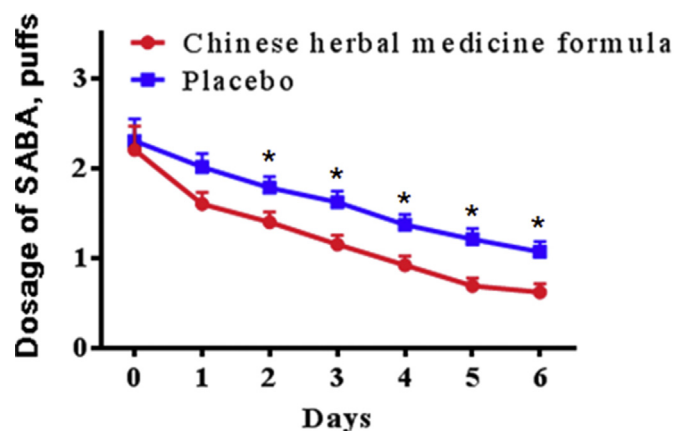


Fig. 3. Dosage of as-needed salbutamol use (puffs/day) after Chinese herbal medicine formula and placebo intervention during 7-day treatment period. Bar indicated as standard error. * $P < 0.05$.

4 (352.65 ± 95.47 vs. 320.58 ± 95.30 L/min, $P = 0.012$) and day 7 (360.42 ± 94.39 vs. 336.86 ± 95.59 L/min, $P = 0.029$) (Fig. 2B). The largest mean difference was achieved between the two groups (35.3 ± 38.23 vs. 15.0 ± 66.91 L/min, $P < 0.001$) in evening on day 4.

The puffs of salbutamol in the PCYQ group were less than those in the placebo group (1.60 ± 1.52 vs. 2.01 ± 1.74 puffs, $P = 0.058$; 1.40 ± 1.29 vs. 1.78 ± 1.48 puffs, $P = 0.031$; 1.15 ± 1.22 vs. 1.62 ± 1.46 puffs, $P = 0.010$; 0.92 ± 1.19 vs. 1.37 ± 1.37 puffs, $P = 0.005$; 0.69 ± 1.01 vs. 1.21 ± 1.37 puffs, $P = 0.001$; and 0.62 ± 1.10 vs. 1.07 ± 1.34 puffs, $P = 0.003$) from days 1–6 (Fig. 3). The area of under cure (AUC) analysis for SABA showed no statistical differences for the total puffs of SABA within one week of the treatment between the two groups (10 (4, 16) vs. 8 (3, 12) puffs/week, $P = 0.101$). Accordingly, the difference of PEF between intervention and placebo was not attributed to the usage of SABA.

Chinese herbal medicine formula significantly improved the asthma symptoms (shortness of breath, wheeze, chest tightness, and cough) compared with placebo after 4-day (-3.93 ± 6.8 vs. -2.58 ± 2.82 , $P = 0.002$) and 7-day treatment (-5.55 ± 3.95 vs. -3.83 ± 3.24 , $P = 0.001$). But there were no differences in numbers of eosinophils in blood (0.206 ± 0.153 vs. $0.195 \pm 0.156 \times 10^9/L$, $P = 0.2753$) or percentage of eosinophils (3.09 ± 2.31 vs. $3.02 \pm 2.35\%$, $P = 0.4005$) between the two groups at the end of the treatment. During the study period, no patients required systemic glucocorticoids or hospitalizations due to asthma deterioration, although the patients were asked to contact physicians if asthma symptoms worsened.

3.3. Cytokines

After 7-day oral administration of PCYQ, the levels of IL-5 (−10.50 [−31.25, 5.63] vs. 3 [−14.50, 30.75] pg/mL, $P = 0.0487$), IL-8 (17.50 [−37.50, 33.50] vs. 2.06 [−5.36, 6.07] pg/mL, $P = 0.0483$), IL-1 β (−2.57 [−21.88, 10.03] vs. 25.00 [1.25, 52.25] pg/mL, $P = 0.027$), PGD₂ (0.00 [−109.84, 27.09] vs. 1.50 [−12.04, 43.26] pg/mL, $P = 0.022$) were statistically reduced compared with those in the placebo group. These differences remained statistically adjusted for SABA dosage, baseline asthma symptom scores and cytokine levels. However, there was no difference in levels of IL-4 (−16.00 [−34.62, 19.00] vs. 0.00 [−20.25, 14.50] pg/mL, $P = 0.842$), IL-6 (7.75 [−9.50, 21.50] vs. 13.00 [1.00, 21.75] pg/mL, $P = 0.536$), IFN- α (−0.50 [−3.88, 5.12] vs. 0.00 [−3.25, 6.25] pg/mL, $P = 0.870$), IFN- β (1.00 [−2.33, 3.38] vs. 0.00 [−3.25, 4.00] pg/mL, $P = 0.555$), IFN- γ (−21.25 [−104.75, 62.50] vs. −8.50 [−143.50, 164.50] pg/mL, $P = 0.916$), CCL-5 (−0.50 [−34.13, 51.88] vs. 22.00 [−21.50, 66.00] pg/mL, $P = 0.443$), IP-10 (9.75 [−19.88, 20.88] vs. 3.00 [−17.25, 32.25] pg/mL, $P = 0.771$), and IL-17A (0.25 [−4.13, 3.00] vs. 0.00 [−2.50, 5.50] pg/mL, $P = 0.265$) between the two groups.

3.4. Safety

During the treatment period, Chinese herbal medicine formula was generally well tolerated compared with placebo, with no increase in withdrawal rates because of adverse events. There were 22 adverse events, half of which were in the PCYQ group, but no serious adverse events were found. Manifestations of adverse events in patients who received Chinese herbal medicine formula were palpitations ($n = 2$), nausea ($n = 4$), dry mouth ($n = 1$), epigastric discomfort ($n = 2$), insomnia ($n = 1$) and rash ($n = 1$).

4. Discussion

To our knowledge, this is the first registered randomized, placebo-controlled trial to investigate the efficacy and safety of Chinese herbal medicine formula in treatment of acute asthma. We found that Chinese herbal medicine formula significantly improved lung function, decreased the dose of SABA, and improved the clinical symptoms (shortness of breath, wheezing, chest tightness, cough) potentially via reducing systemic inflammatory biomarkers such as IL-5, IL-8, IL-1 β and PGD₂, with no serious adverse events.

Asthma exacerbation is an exaggerated lower airway response to an environmental exposure. Airway inflammation is a key part of the lower airway response in asthma exacerbations, and occurs together with airflow obstruction and increases airway responsiveness. Respiratory virus infections, especially human rhinovirus, is the most common environmental exposure to cause an asthma exacerbation [3]. However, there are no effective treatment against rhinoviruses [42]. Chinese herbs (*Herba Ephedra*, *Semen Armeniacae Amarum*, *Radix Glycyrrhizae*) were effective in treatment of influenza virus and rhinovirus infection in adults [15].

According to a study by Santanello et al., the minimal important changes for asthma measures in a clinical trial are 18.79 L/min for PEF and −0.81 puffs/day for inhaled SABA use [43]. Our study found that the Chinese herbal medicine formula significantly improved the morning and evening PEF, with difference between the two groups reached the minimal important clinical changes that ranged from 23.6 to 35 L/min. This indicates that the patients with mild to moderate asthma exacerbation could obtain a minimal important clinical improvement of lung function in PEF after a 7-day treatment of PCYQ. Therefore, PCYQ granule could improve the lung function in acute asthma. Furthermore, although there was a significantly statistical difference in the puffs of salbutamol from days 1–6 between intervention and placebo, the reduced dosage of salbutamol after treatment of PCYQ did not reach the minimal important clinical changes. This is

likely due to the included participants who were mild to moderate exacerbations and the heterogeneity of asthma. Reddel HK et al. [2] reported that minimal important differences have rarely been reported for symptom scores, with most papers merely reporting statistically significant changes in mean scores. Therefore, Chinese herbal medicine formula significantly improved lung function, decreased the dose of SABA, and improved the clinical symptoms, with no serious adverse events.

The mechanisms of Chinese herbal medicines in the treatment of acute asthma is complex. Jayaprakasam et al. discovered individual *ganoderma lucidum*, *flavescens*, licorice extracts and ASHMI (the combination of individual extracts) inhibited production of IL-4 and IL-5 by murine memory T_H2 cells [5]. Yang et al. demonstrated that three flavonoids of *Glycyrrhizauralensis* inhibited memory T_H2 cells proliferation in vitro and reduced production of IL-4 and IL-5 [44]. Shih et al. discovered hesperetin which is present in the TCM ‘Tangerine peel’ significantly suppressed elevations of total inflammatory cells, macrophages, lymphocytes, neutrophils, and eosinophils, and levels of cytokines, including IL-2, IL-4, IL-5, IFN- γ , and tumor necrosis factor (TNF)- α in bronchoalveolarlavage fluid (BALF) [45]. Wen et al. found anti-asthma herbal medicine intervention appears to be a safe and effective alternative medicine for treating asthma in humans. In contrast with prednisone, ASHMI had no adverse effect on adrenal function and had a beneficial effect on T_H1 and T_H2 balance [12]. In this study, PCYQ granule and its compounds were effective in treatment of asthma exacerbations possibly involving in the mechanism of actions including inhibiting the release of allergic neurotransmitters, anti-complement effect, anti-inflammatory, antitussive effect, antiviral, antibacterial effects, enhanced antioxidant enzyme activity, corticosteroid-like effects and inhibiting TNF- α -induced ICAM-1 expression in human lung epithelial cells [46].

IL-1 β is a subtype of IL-1 which is mainly derived from monocytes and macrophages which is a potent pyrogenic cytokine that can further increase the production of pro-inflammatory cytokines. IL-1 β increased the thickness of conducting airways, enhanced mucin production, and lymphocytic aggregates in the airways. IL-1 β increases the production of the neutrophil attractant CXC chemokines KC (CXCL1) and MIP-2 (CXCL2), matrix metalloproteases (MMP-9) and −12 [47]. Our study demonstrated that PCYQ granule as Chinese herbal medicine formula, reduced the systemic IL-1 β level, which indicated that this Chinese herbal medicine formula could improve the exacerbation-induced airway remodeling and reduce the production of the neutrophil attractant CXC chemokines, thereby reduce the production of pro-inflammatory cytokines. PGD₂, a lipid mediator released by mast cells, is known to participate in asthma inflammation. Lipid peroxidation, assessed as isoprostane levels, is found to be markedly increased in acute asthma, and falls with resolution of the exacerbation [48]. Srivastava et al. found the anti-asthma herbal medicine ASHMI inhibited airway smooth muscle contraction via prostaglandin E₂ activation of EP2/EP4 receptors [49]. PGD₂ can accelerate T_H2 type (IL-4 and IL-5) inflammation by induction of macrophage-derived chemokines [50]. As Chinese herbal medicine formula, *Huo Luo Xiao Ling Dan* [51] and *Nujiangexanthone A* [52] were shown to prevent mast cell-related immediate and delayed allergic diseases via inhibiting PGD₂ release in mast cell dependent passive cutaneous anaphylaxis animal model. We speculated that PCYQ could reduce IL-5 release through inhibiting PGD₂ production. One component of the PCYQ granule, licorice could inhibit production of IL-4 and IL-5 by murine memory T_H2 cells and eotaxin-1 production [41]. More importantly, PCYQ did not affect the production of antiviral cytokines (IFN- α /- β), this indicates that PCYQ may provide a more precise inhibition of cytokines involved in inflammation and fibrosis, without affecting antiviral immunity that is observed in corticosteroids [53].

There are some strengths in our study. First, this is the first well-designed randomized controlled trial to explore the efficacy and safety of Chinese herbal medicine formula PCYQ in asthma exacerbations.

Second, we explored the possible mechanism of PCYQ as a treatment in asthma exacerbations. However, our study also has some limitations. First, we did not measure FEV₁ and FVC which are quite critical in assessing airway obstruction of asthma. We used PEF because it is convenient for participants and widely used in everyday life at home, and which can be easily accessible to the participants. PEF as an indicator in lung function, has also been applied as the primary outcome in many studies [54]. Second, the time of treatment with 7 days is relatively short, but for asthma exacerbations it is enough to determine the efficacy, and further we did not include follow-up after treatment. Third, almost all subjects included were on GINA steps 1 and 2 strategies in stable stage before entry, and about 20% of them were using diverse controller drugs. However, the diverse controller drugs are evenly distributed between Chinese herbal medicine formula and placebo groups, and the fixed-dose maintenance for controlled drugs could reduce their effects on the judgment of efficacy. Fourth, our study only restricted to patients with acute mild to moderate asthma that led to no patient required systemic glucocorticoids or hospitalizations due to asthma deterioration, because this is only a proof of concept study in design. Fifth, although the statistical difference in asthma symptoms scores between the intervention and the placebo was found, the asthma symptom scoring system had not been validated before.

In conclusion, Chinese herbal medicine formula entitled PCYQ, significantly improved lung function and the clinical symptoms of acute mild-to-moderate asthma, reduced the dose of SABA, and decreased systemic inflammatory biomarkers such as IL-5, IL-8, IL-1 β , and PGD₂ without affecting antiviral immune responses. Furthermore, PCYQ was generally well tolerated, and its safety profile was comparable to that of the placebo group. Chinese herbal medicine formula as an important supplementary alternative medicine, could improve the shared-decision treatment for asthma patients, but it needed to be validated in patients with acute moderate to severe asthma in further studies.

Declarations of interest

None.

Funding

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.rmed.2018.05.014>.

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Table E1. **Items Checklist for CONSORT Extension for Chinese Herbal Medicine Formulas 2017**

(Cheng C, et al. CONSORT Extension for Chinese Herbal Medicine Formulas 2017: Recommendations, Explanation, and Elaboration. Annals of Internal Medicine. 2017; Jun 27. Doi: 10.7326/M16-2977)

Section/Topic	Item Number	Standard CONSORT Checklist Item	Extension for CHM Formulas	Reported on Page Number
Title, abstract, and keywords	1a	Identification as a randomized trial in the title	<i>Statement of whether the trial targets a TCM Pattern, a Western medicine–defined disease, or a Western medicine–defined disease with a specific TCM Pattern, if applicable</i>	1, 4
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance, see CONSORT for abstracts)	<i>Illustration of the name and form of the formula used, and the TCM Pattern applied, if applicable</i>	4
	1c		<i>Determination of appropriate keywords, including “Chinese herbal medicine formula” and “randomized controlled trial”</i>	4
Introduction				
Background and objectives	2a	Scientific background and explanation of rationale	<i>Statement with biomedical science approaches and/or TCM approaches</i>	6, 7
	2b	Specific objectives or hypotheses	<i>Statement of whether the formula targets a Western medicine–defined disease, a TCM Pattern, or a Western medicine–defined disease with a specific TCM Pattern</i>	6, 7
Methods				
Trial design	3a	Description of trial design (such as parallel, factorial), including allocation		8

		ratio		
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons		8
Participants	4a	Eligibility criteria for participants	<i>Statement of whether participants with a specific TCM Pattern were recruited, in terms of 1) diagnostic criteria and 2) inclusion and exclusion criteria. All criteria used should be universally recognized, or reference given to where detailed explanation can be found.</i>	8
	4b	Settings and locations where the data were collected		8
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	<i>Description(s) for different types of formulas should include the following: 5a. For fixed CHM formulas 1. Name, source, and dosage form (e.g., decoctions, granules, powders) 2. Name, source, processing method, and dosage of each medical substance. Names of substances should be presented in at least 2 languages: Chinese (Pinyin), Latin, or English. Names of the parts of the substances used should be specified. 3. Authentication method of each ingredient and how, when, where, and by whom it was conducted; statement of whether any voucher specimen was retained, and if so, where they were kept and whether they are accessible 4. Principles, rationale, and interpretation of forming the formula 5. Reference(s) as to the efficacy of the formula, if any 6. Pharmacologic study results of the formula, if any 7. Production method of the formula, if any 8. Quality control of each ingredient and of the product of the formula, if any. This would include any quantitative and/or qualitative testing method(s); when, where, how, and by whom these tests were conducted; whether the original data and samples were kept, and, if so, whether they are accessible. 9. Safety assessment of the formula, including tests for heavy metals and toxic</i>	9

			elements, pesticide residues, microbial limit, and acute/chronic toxicity, if any. If yes, it should be stated when, where, how, and by whom these tests were conducted; if the original data and samples were kept; and, if so, whether they are accessible. 10. Dosage of the formula, and how the dosage was determined 11. Administration route (e.g., oral, external) 5b. For individualized CHM formulas 1. See recommendations 5a 1–11 2. Additional information: how, when, and by whom the formula was modified 5c. For patent proprietary CHM formulas 1. Reference to publicly available materials, such as pharmacopeia, for the details about the composition, dosage, efficacy, safety, and quality control of the formula 2. Illustration of the details of the formula, namely 1) the proprietary product name (i.e., brand name), 2) name of manufacturer, 3) lot number, 4) production date and expiry date, 5) name and percentage of added materials, and 6) whether any additional quality control measures were conducted 3. Statement of whether the patent proprietary formula used in the trial is for a condition that is identical to the publicly available reference 5d. Control groups Placebo control 1. Name and amount of each ingredient 2. Description of the similarity of placebo with the intervention (e.g., color, smell, taste, appearance, packaging) 3. Quality control and safety assessment, if any 4. Administration route, regimen, and dosage 5. Production information: where, when, how, and by whom the placebo was produced Active control 1. If a CHM formula was used, see recommendations 5a–5c 2. If a chemical drug was used, see item 5 of the CONSORT Statement	
Outcomes	6a	Completely defined, prespecified primary and secondary outcome measures, including how and when they were assessed	Illustration of outcome measures with Pattern in detail	9,10

	6b	Any changes to trial outcomes after the trial commenced, with reasons		Not applicable
Sample size	7a	How sample size was determined		10,11
	7b	When applicable, explanation of any interim analyses and stopping guidelines		10,11
Randomization Sequence generation	8a	Method used to generate the random allocation sequence		9
	8b	Type of randomization; details of any restriction (such as blocking and block size)		9
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned		9
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions		9
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how		9
	11b	If relevant, description of the		9

		similarity of interventions		
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes		10, 11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses		10, 11
Results				
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome		12
	13b	For each group, losses and exclusions after randomization, together with reasons		12
Recruitment	14a	Dates defining the periods of recruitment and follow-up		12
	14b	Why the trial ended or was stopped		Not applicable
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group		12, 28, 29
Numbers analyzed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was		12, 28, 29

		by original assigned groups		
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)		12, 13
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended		12, 13
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing prespecified from exploratory		12, 13
Harms	19	All important harms or unintended effects in each group (for specific guidance, see CONSORT for harms)	<i>(There is no extension for this item)</i>	13
Discussion				
Limitations	20	Trial limitations; addressing sources of potential bias; imprecision; and, if relevant, multiplicity of analyses		16,17
Generalizability	21	Generalizability (external validity, applicability) of the trial findings	<i>Discussion of how the formula works on different TCM Patterns or diseases</i>	14, 15, 16
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	<i>Interpretation with TCM theory</i>	14, 15, 16

Other information				
Registration	23	Registration number and name of trial registry		8
Protocol	24	Where the full trial protocol can be accessed, if available		8
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders		18

CHM = Chinese herbal medicine; CONSORT = Consolidated Standards of Reporting Trials; TCM = traditional Chinese medicine. * The original CONSORT items are provided; elaborations for CHM formulas are in italicized text. We strongly recommend reading this checklist in conjunction with the CONSORT 2010 Explanation and Elaboration (29) for important clarifications on all original items of CONSORT Statement.

Supplementary materials

Chinese herbal medicine formula for acute asthma: a multi-center, randomized, double-blind, proof-of-concept trial

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This formula entitled “PCYQ” has been previously assessed in animal models, but these data were not published before. Now we added this in the supplementary materials.

1. Anti-allergic effects

1) 2.5, 5.0 and 10.0 g / kg (inject gavage, ig) of PCYQ granule in rats inhibited passive cutaneous anaphylaxis which showed dose response.

2) 2.5-10.0 g / kg (ig) of PCYQ granule had a strong inhibitory effect on skin capillary permeability in histamine-induced rat.

3) 5.0-10.0 g / kg (ig) of PCYQ granule had a significant inhibitory effect on skin capillary permeability in serotonin-induced rat.

4) 5.0-10.0 g / kg (ig) of PCYQ granule had a protective effect on lung function reduction which was induced by conscious sensitized guinea pig antigens.

2. Anti-inflammatory effect

1) PCYQ granule had a dose-response dependent anti-inflammatory effect at 2.5-10.0 g / kg (ig) which included decreasing the number of inflammatory cells in bronchoalveolar lavage fluid (BALF) after challenge with sensitized guinea pig antigen.

2) PCYQ granule had an anti-inflammatory effect at 2.5-10.0 g / kg (ig) which inhibited carrageenan-induced rat pleurisy, and the amount of inflammatory exudate and white blood cells significantly reduced.

3. Bronchial relaxation effect

1) PCYQ granule had a significant protective effect in histamine and acetylcholine-induced guinea pig of asthma.

2) PCYQ granule had a dose-response dependent bronchial relaxation effect at 1.0-3.0mg / ml of the tracheal smooth muscle in guinea pig.

4. Antitussive effect

PCYQ granule with 10.0 g / kg (ig) can significantly reduce citric acid-induced guinea pig cough frequency, but the dose of 2.5-5.0 g / kg (ig) had no obvious antitussive effect.

5. Expectorant effect

PCYQ granule had an expectorant effect at 5.0 g / kg (ig) which promotes airway phenol red excretion in mice.

6. Enhance the immune function

1) PCYQ granule at 2.5-10.0 g / kg (ig) once a day, continuous administration for 8 days, had a significant role in promoting antibody-secreting cells of the mouse.

2) PCYQ granule at 2.5-10.0 g / kg (ig) once a day, continuous administration for 10 days, can enhance the function of spleen T-lymphocyte transformation in immunosuppressed mice induced by cyclophosphamide in a dose-dependent manner.

3) PCYQ granule at 2.5-10.0 g / kg (ig) once a day, continuous administration for 8 days, had no significant effect on the phagocytosis of mouse peritoneal macrophages.

The data of the animal models were presented in detail as following.

1. Inhibit passive cutaneous anaphylaxis

Compared with the saline group, PCYQ granule at 5.0 g / kg (ig) and 10.0 g / kg (ig) inhibited rat passive skin allergy test on a dose-response ($P<0.05$ and $P<0.01$) (Table E2).

Table E2. Rat passive skin allergy test (mean $\pm$ SD) (* $P<0.05$; ** $P<0.01$.)

Group	Dose (g/kg $\times$ d)	n	Evans blue exudates (ug / point)	Inhibition rate (%)
Saline group	10ml $\times$ 3	9	3.34 $\pm$.34	-
PCYQ group	2.5 $\times$ 3	8	2.12 $\pm$ 0.59	36.5
	5.0 $\times$ 3	9	1.42 $\pm$ 0.44*	57.5
	10.0 $\times$ 3	7	1.23 $\pm$ 0.51**	63.2

2. Inhibitory effect on skin capillary permeability in histamine-induced rat

Compared with the saline group, 2.5-10.0 g / kg (ig) of PCYQ granule on skin capillary permeability in histamine-induced rat had a strong inhibitory effect (Table E3).

Table E3. Inhibitory effect on skin capillary permeability in histamine-induced rat (mean $\pm$ SD) (* $P<0.05$; ** $P<0.01$.)

Group	Dose (g/kg $\times$ d)	n	Evans blue exudates(ug / point)
Saline group	10ml $\times$ 3	8	1.13 $\pm$ 0.15
PCYQ group	2.5 $\times$ 3	8	0.31 $\pm$ 0.16
	5.0 $\times$ 3	7	0.21 $\pm$ 0.10*

	10.0 × 3	7	0.20±0.10 ^{**}
Chlorpheniramine group	5.0mg/kg × 1	7	0.57±0.14 [*]

3. Effect on the lung function

Compared with the saline group, 5.0-10.0 g / kg (ig) of PCYQ granule had a protective effect on lung function reduction which is induced by conscious sensitized guinea pig antigens. In the saline group, 5/10 guinea pigs showed respiratory arrest after antigen challenge, tidal volume (TV) and air flow velocity (AFV) and respiration rate disappeared or significantly decreased, and 3/10 guinea pigs showed decreasing of TV, AFV and respiration rate. Compared with the saline group, 5.0 g / kg (ig) of PCYQ granule had a protective effect on lung function reduction which is induced by conscious sensitized guinea pig antigens. 10.0g / kg (ig) of PCYQ granule showed complete protective effect which was similar to the 2 mg / kg ketamine positive control group.

4. Anti-inflammatory effect

Compared with the blank group, the total number of leukocytes, eosinophils and monocytes in the saline group were significantly increased after challenge with 1% ovalbumin. PCYQ granule had a dose-response dependent anti-inflammatory effect at 2.5-10.0 g / kg (ig) which included decreasing the number of inflammatory cells in bronchoalveolar lavage fluid after challenged with sensitized guinea pig antigen and reducing the total number of leukocytes in pleurisy rats (Table E4).

Table E4. The number of inflammatory cells in bronchoalveolar lavage fluid (BALF) after challenged with guinea pig antigen challenge (mean ±SD) (* $P<0.05$; ** $P<0.01$.)

Group	Dose (g/kg ×d)	n	The total number of leukocytes (× 10 ⁶ /ml)	The number of eosinophils (× 10 ⁶ /ml)	The number of neutrophils (× 10 ⁶ /ml)	The number of monocytes (× 10 ⁶ /ml)
Blank control group	-	9	0.62±0.27	0.05±0.05	0.08±0.07	0.45±0.14
Saline group	10ml	7	7.25±1.45	1.05±0.46	2.01±0.82	4.15±1.14
PCYQ group	2.5	6	4.06±1.37 [*]	0.53±0.18 [*]	1.41±0.54 [*]	2.03±0.81 [*]

	5.0	8	3.92±1.80 [*]	0.48±0.29 [*]	1.35±0.59 [*]	2.10±0.72 [*]
	10.0	6	2.58±0.50 ^{**}	0.23±0.13 ^{**}	0.96±0.30 ^{**}	1.40±0.27 ^{**}

5. Effect of PCYQ granule on pleurisy in rats

PCYQ granule had an anti-inflammatory effect at 2.5-10.0 g / kg (ig) and aspirin group at 0.5g / kg (ig) which significantly inhibited carrageenan-induced rat pleurisy, the amount of inflammatory exudate and white blood cells significantly reduced (table E5).

Table E5. Effect of PCYQ granule on pleurisy in rats (mean ±SD) (n=10) (compared with saline group, ^{*}P<0.05; ^{**}P<0.01.)

Group	Dose (g/kg × d)	Exudate (ml)	The total number of white blood cells (×10 ⁷ /ml)
Saline group	5ml × 3	4.22±1.59	11.9±6.30
PCYQ group	2.5 × 3	4.12±1.51	8.0±3.2
	5.0 × 3	3.6±1.34	5.0±2.60 ^{**}
	10.0 × 3	2.68±1.05 [*]	3.7±1.6 ^{**}
Aspirin group	0.5 × 3	2.33±0.83 [*]	2.1±0.9 ^{**}

6. The effect of PCYQ granule in bronchial relaxation

PCYQ granules had a significant protective effect for histamine and acetylcholine-induced asthma in guinea pigs (Table E6).

Table E6. The effect of PCYQ granule in bronchial relaxation (mean ±SD) (compared with saline group, ^{*}P<0.05; ^{**}P<0.01.)

Group	Dose (g/kg × d)	n	Wheezing latency (sec)	Extension%
Saline group	5ml × 3	10	60.50±18.04	-
PCYQ group	2.5 × 3	11	75.72±14.41	25.15
	5.0 × 3	9	80.22±12.34 [*]	32.60
	10.0 × 3	10	92.9±16.74 ^{**}	53.55
Salbutamol group	4mg × 3	10	120.0±0.0 ^{**}	>100

7. The effect of PCYQ granule on bronchial smooth muscle

PCYQ granule had a dose-dependent bronchial relaxation effect at 1.0-3.0mg / ml of the tracheal smooth muscle in guinea pigs.

8. Antitussive effect

PCYQ granule 10.0 g / kg (ig) can significantly reduce citric acid-induced guinea pig cough frequency, 2.5-5.0 g / kg (ig) had no obvious antitussive effect (Table E7).

Table E7. The effect of PCYQ granule in airway phenol red excretion in mice (mean $\pm$ SD (compared with saline group, ** $P<0.01$.)

Group	Dose (g/kg $\times$ d)	n	Cough times
Saline group	10ml $\times$ 3	10	17.6 $\pm$ 1.4
PCYQ group	2.5 $\times$ 3	11	14.7 $\pm$ 2.6
	5.0 $\times$ 3	9	13.8 $\pm$ 1.3
	10.0 $\times$ 3	10	7.6 $\pm$ 2.0**

9. Expectorant effect

PCYQ granule had an expectorant effect at 5.0 g / kg (ig) which promoted airway phenol red excretion in mice, not in 2.5 g / kg (ig) and 10.0 g / kg (ig) (Table E8).

Table E8. The effect of PCYQ granule in citric acid-induced cough frequency in guinea pigs (mean $\pm$ SD (compared with saline group, * $P<0.05$.)

Group	Dose (g/kg $\times$ d)	n	Phenol red excretion (OD)
Saline group	10ml $\times$ 3	15	0.302 $\pm$ 0.06
PCYQ group	2.5 $\times$ 3	15	0.288 $\pm$ 0.08
	5.0 $\times$ 3	15	0.435 $\pm$ 0.15*
	10.0 $\times$ 3	15	0.327 $\pm$ 0.16*

10. The effect of PCYQ granule on antibody-secreting cells of the mouse

Using SRBC quantitative hemolysis assay (QHS), PCYQ granule at 2.5-10.0 g / kg (ig) once a day, continuous administration for 8 days, had a significant role in promoting antibody-secreting cells of the mouse (Table E9).

Table E9. The effect of PCYQ granule on antibody-secreting cells of the mouse (mean $\pm$ SD (compared with saline group, * $P<0.05$)

Group	Dose (g/kg ×d)	n	QHS OD	Enhancement%
Saline group	10ml ×8	13	0.650±0.309	-
PCYQ group	2.5 ×8	14	0.943±0.184 [*]	45.0
	5.0 ×8	14	0.830±0.197 [*]	27.7
	10.0 ×8	13	0.958±0.200 [*]	47.4

11. The effect of PCYQ granule on function of spleen T-lymphocyte transformation

The number of pulses per minute of the spleen T-lymphocyte was measured by a liquid scintillator. PCYQ granule at 2.5-10.0 g / kg (ig) once a day, continuous administration for 10 days, can enhance the function of spleen T-lymphocyte transformation in immunosuppressed mice induced by cyclophosphamide on a dose-response (Table E10).

Table E10. The effect of PCYQ granule on the function of spleen T-lymphocyte transformation (mean ±SD (compared with saline group, ^{*}*P*<0.05, ^{**}*P*<0.01.)

Group	Dose (g/kg × d)	n	CPM	Enhancement% (vs. saline group)
Normal control group	-	10	32253±22527	-
Saline group	10ml × 8	10	3472±1574	
PCYQ group	2.5 × 8	10	5027±1446 [*]	44.9
	5.0 × 8	10	5862±2154 [*]	68.84
	10.0× 8	9	6123±2787 ^{**}	76.35

12. The effect of PCYQ granule on phagocytosis of mouse peritoneal macrophages

PCYQ granule at 2.5-10.0 g / kg (ig) once a day, continuous administration for 8 days, had no significant effect on the phagocytosis of mouse peritoneal macrophages (Table E11).

Table E11. The effect of PCYQ granule on phagocytosis of mouse peritoneal macrophages

(mean ±SD) (compared with saline group, ^{*}*P*<0.05, ^{**}*P*<0.01.)

Group	Dose (g/kg ×d)	n	Phagocytosis function (%)
Saline group	10ml × 8	13	17.00±7.82

PCYQ group	2.5×8	12	15.97 ± 6.30
	5.0×8	14	19.54 ± 11.99
	10.0×8	14	17.68 ± 8.87