

Occupational respiratory allergic disease induced by *Passiflora alata* and *Rhamnus purshiana*

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Background: There has been an increase in the incidence of occupational asthma along with better understanding of its pathophysiologic mechanisms and etiologic factors. There are no reports of patients with asthma and rhinitis to *Passiflora alata* (passion flower) and *Rhamnus purshiana* (cascara sagrada).

Methods: We describe two substances of plant origin as causal agents of occupational allergic respiratory diseases in a patient who worked in a pharmacy devoted to the manual preparation of products.

Results: Skin testing and Western blot confirmed the sensitization of the patient to these plant extracts in vivo and in vitro, respectively. Bronchial challenge confirmed the cause-effect relationship between the allergen exposure and the diseases.

Conclusions: We conclude that *Passiflora* and cascara sagrada are two new etiologic agents of IgE-mediated occupational asthma and rhinitis. The present study also serves to alert physicians to the risks associated with work in pharmacies devoted to manual preparation of plant extracts, emphasizing the importance of the use of protective measures in these environments.

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INTRODUCTION

The association between asthma and occupational exposure was observed as far back as in the fourth century BC by Hippocrates, but was better characterized and described only in the 18th century by Bernardo Ramazzini.¹ With the industrialization of society, the introduction of new chemical products and improvement of diagnostic methods, there was an increase in the incidence of occupational asthma along with better understanding of its pathophysiologic mechanisms and etiologic factors.

The incidence and prevalence of occupational asthma have certainly been underestimated due to the great diagnostic difficulty and the uncertain reporting of this disease. The prevalence of occupational asthma has been estimated in several studies at 2% of the

general worker population, but it varies with the allergen exposure involved.^{2,3}

In the present study we describe two substances of plant origin, *Passiflora alata* (passion flower) and *Rhamnus purshiana* (cascara sagrada), as causal agents of asthma and rhinitis. We report a patient with allergic symptoms who was exposed to these substances in a pharmacy devoted to the manual preparation of products. The objective of the study was to determine the cause-effect relationship between these substances and the clinical manifestations of the patient.

CASE REPORT

The patient, a 30-year-old white man born in the state of Paraná and residing in São Paulo for the last 16 years, sought our service in June 1993.

The patient had been working for 2 years in a pharmacy devoted to the manual preparation of products, where he weighed and prepared chemical products eight hours each day, Monday through Saturday, without using a

mask, gloves, or other protective materials. In mid-1992, when he had been working at the pharmacy for 6 months, the patient started to experience episodes of sneezing, coryza, and nasal pruritus and congestion. He also reported ocular pruritus and hyperemia, tearing, and pruritus of the auditory canal and oropharynx. Three months after the original symptoms, he developed a dry cough, chest pain, sensations of chest tightening, dyspnea, and wheezing. The symptoms started to be continuous and were worse in the morning when he entered the pharmacy, and when he manipulated capsules containing cascara sagrada and passion flower preparations. The symptoms improved after the end of the working day, but on some occasions worsened again at night in his house. There was also relief of symptoms on Sundays. The patient had been using inhaled β_2 -adrenergic agents frequently. He had no history of atopy but had a smoking habit of 1 pack per day of 16 years' duration.

Physical examination revealed that the nasal turbinates were slightly hypertrophied and hyperemic. The patient had a respiratory rate of 20 rpm without using accessory respiratory muscles, with moderate wheezing heard diffusely on both sides of the chest upon auscultation. The peak expiratory flow rate was 480 L/minute (predicted: 622).

Laboratory tests showed 14% eosinophilia with 8,000 leukocytes per cubic min and total IgE levels of 1130 IU/mL. *Strongyloides stercoralis* was isolated from the feces. A radiograph of the paranasal sinuses showed reduced transparency of both maxillary sinuses. Prick tests for common inhalant allergens (*D. pteronyssinus*, *D.*

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farinae, cat, dog, cockroach, *Penicillium*, *Alternaria*, *Aspergillus*, and *Cladosporium*) were negative and the pulmonary function test suggested moderate bronchial obstruction with no significant reversibility after the use of appropriate medication (FVC: 71%; FEV₁: 68%; FEV/FCV ratio: 97%; FEF_{25–75%}: 47%; PFR: 73%).

The patient was advised to leave his job until he could be better studied. He began to use 1000 µg of beclomethasone per day.

METHODS

Skin Tests

A prick test was performed using the substances associated with the symptoms of the patient diluted in physiologic saline at the concentrations of 0.01%, 0.1%, 1% and 10%. All tests were carried out in duplicate, using histamine (10 mg/mL) as positive control and saline as negative control. For control, skin tests were performed on 100 volunteers with asthma and/or rhinitis. A wheal 3 mm in diameter was considered a positive test.⁴ The patient and the controls gave written consent for these tests and the bronchial challenges done.

Bronchial Challenge

Specific and nonspecific bronchial challenge tests were performed with the patient hospitalized and with emergency equipment at hand. The tests included five bronchial challenges done on five consecutive days: with saline (day 1 = control), with histamine (day 2), with passion flower (day 3), with cascara sagrada (day 4), and another time with histamine (day 5).

For the nonspecific bronchial challenge tests, a 10-mg/mL histamine solution was used at different dilutions (1:100, 1:80, 1:40, 1:20, 1:10, 1:8, 1:4, 1:2, and 1:1). The test was discontinued when a 20% fall of the FEV₁ occurred compared with basal values. Using the logarithmic dose-response curve, we calculated in a noncumulative manner the “challenge concentration” that caused a 20% fall in FEV₁ (PC₂₀).

For the specific bronchial challenge tests, a 1% solution of each extract was used at different dilutions, starting with the concentration 2-fold smaller than the concentration that gave a positive prick test with a wheal of 2 mm in the patient (1:8192, 1:4096, 1:2048, 1:1024, 1:512, 1:256, 1:128, 1:64, 1:32, 1:16, 1:8, 1:4, 1:2, and 1:1). The test was discontinued when a 15% fall of the FEV₁ occurred compared with basal values. Using the logarithmic dose-response curve, we calculated in a cumulative manner the “challenge dose” that caused a 15% fall in FEV₁ (PD₁₅). We preferred this procedure instead of bronchial challenges with the patient manipulating the substances in a chamber because we think this would be a more accurate, more controlled and safer method.

Five healthy volunteers and five patients with asthma were the controls for the challenges.

Electrophoresis

Cascara sagrada and passion flower were analyzed by electrophoresis on 12% polymerized polyacrylamide gel (wt/vol) in the presence of 0.1% (wt/vol) sodium dodecyl sulfate (SDS). The plant extracts were diluted to 25% in deionized water and applied to the wells together with previously standardized sample buffer. The protein standards of known molecular weight used were phosphorylase b = 94 kD, albumin = 67 kD, ovalbumin = 43 kD, carbonic anhydrase = 30 kD, trypsin inhibitor = 20.1 kD, and α-lactalbumin = 14.1 kD (M.W.-Pharmacia).

Western Blot

The gel was immersed in transfer buffer containing 48 mM Tris-base, glycine, 0.1% SDS (wt/vol) and 20% methanol (wt/vol), pH 7, for two hours at room temperature. The protein bands were transferred to a 0.45-µm nitrocellulose membrane in the BIO-RAD system using a current of 1.2 A and 100 V, for one hour at 4 °C. This step was checked by the visualization of protein bands in Ponceau for 30 minutes followed by band washing with deionized water.

The nitrocellulose paper was cut into strips that were blocked with PBS Tween 20 containing 1% BSA, for one hour at room temperature. After blockade, the strips were washed three times with PBS Tween 20 for five minutes, with shaking. Serum from a normal person (control) and patient serum diluted 1:50 in PBS Tween 20 were left to stand with the strips overnight at room temperature. The strips were then washed three times for five minutes with shaking. Alkaline phosphatase-conjugated anti-IgE and anti-IgG antibodies (Sigma, USA) were added to the assay at respective dilutions of 1:250 and 1:500 and left to stand overnight at room temperature.

After two more washes with PBS Tween 20 and one wash with buffer, tris and magnesium chloride, the substrates nitrobluetetrazolium (NBT) and 5-bromo-4-chloro-3-indole phosphate (BCIP) (Sigma, USA) were added to the strips.

RESULTS

Prick Test

The prick test was positive for cascara sagrada and passion flower at all dilutions tested (Fig 1). All patients used as controls had negative prick tests for 10% solutions of passion flower and cascara sagrada.

Bronchial Challenge

The three 0.9% NaCl inhalations performed during the challenge with saline did not change the FEV₁ value. When serial FEV₁ measurements were taken on the first day of challenge (control day), FEV₁ values were found to fall at night, although the patient did not report symptoms or present signs suggesting bronchospasm upon auscultation.

Both bronchial challenges with histamine were positive, demonstrating airway hyperreactivity. The tests were interrupted after the inhalation of histamine solution at the concentrations of 0.25 mg/mL and 0.125 mg/mL during the examinations carried out the day before (day 2) and the day after (day 5) the bronchial challenges with allergens (day 3 and 4), respectively.

CONTROL		HISTAMINE (10 mg/ml)	
			
CONCENTRATION ¹	PASSIONFLOWER ²	CASCARA SAGRADA ³	
0.01%			
0.1%			
1%			
10%			

Figure 1. Prick test with the substances under investigation. 1: concentration of the substances involved, 2: sizes of the wheals observed in the prick tests with passion flower, and 3: sizes of the wheals observed in the prick tests with cascara sagrada.

Using the dose-response curve, a PC_{20} of 0.150 mg/mL was calculated for the test performed before the specific challenge and a PC_{20} of 0.115 mg/mL was calculated for the test performed after challenge. The patient reported respiratory difficulty, and wheezing of mild intensity was auscultated after the last inhalation in both tests.

Bronchial challenge with passion flower was positive, with a PD_{15} of 0.0024 mg calculated from the dose-response curve (Fig 2). With inhalation of the allergen at the concentration of 0.01953 mg/mL there was an 18.75% fall in basal FEV_1 , the patient started to report dyspnea and chest pain, and moderate wheezing could be auscultated diffusely on both sides of the chest. After spontaneous recovery from the bronchospasm without the use of any medication, the patient again presented symptoms (dyspnea and wheezing) seven hours after the end of the challenge. The occurrence of these delayed symptoms was associated with a 43.75% fall in FEV_1 compared with basal FEV_1 and of 18.18% compared with the FEV_1 measured at the same time of day on the day of the bronchial challenge tests with physiological saline. Bronchial challenge

with cascara sagrada gave a PD_{15} of 0.0079 mg as indicated in the dose-response curve (Fig 3). Upon inhalation of 0.01953 mg/mL of the allergen the patient experienced bronchospasm, with dyspnea, moderate wheezing upon auscultation, and a 24.56% fall in FEV_1 compared with basal values. As in the previous test, there was spontaneous recovery from the symptoms triggered by the challenge and the patient had delayed symptoms (dyspnea) four hours after the challenge. On this occasion, FEV_1 was 26.31% lower than basal FEV_1 and 27.59% lower than the FEV_1 measured at the same

time of day on the day of bronchial challenge with saline (control day). None of the control volunteers challenged with cascara sagrada and with passion flower presented bronchial symptoms.

Electrophoresis

The passion flower extract submitted to electrophoresis was separated into several protein bands of molecular weight ranging from 20.1 to 94 kD (Fig 4). Cascara sagrada was also separated into several bands but of lower molecular weight, ranging from 14.1 to 67 kD (Fig 4).

Western Blot

The Western blot technique revealed IgE and IgG antibodies in the patient's serum against a protein of approximately 20-kD molecular weight present in the passion flower extract (Fig 5). On the tape with control serum there was no IgE antibody binding, but IgG antibody binding to a protein band of approximately 35 kD did occur.

In the test with cascara sagrada, we observed patient IgE and IgG antibody binding to a protein of molecular weight between 20 and 30 kD (Fig 5). There was no binding of IgE or IgG antibodies from the control individual.

DISCUSSION

The plant family Passifloraceae was defined by Jussieu in 1805 and is currently subdivided into 650 species and 16 genera. Predominantly occurring in America and tropical Africa, it was probably discovered by Monardes, a

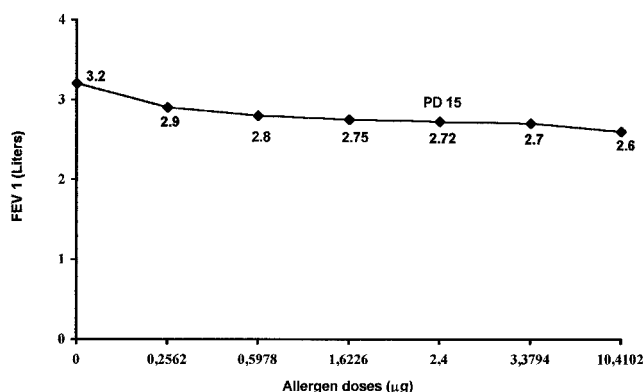


Figure 2. FEV_1 measured after the inhalation by the patient of different doses of passion flower.

Spanish doctor who described the plant in Peru in 1569. The word *passiflora* is from the Latin and means "passion flower." In Brazil, these plant family is known as "maracuja," a word from the Tupi-Guarani' language.

Its major use is an edible fruit, but its extracts are used in various medications. In Brazil, only the use of the leaves of *Passiflora alata* of the variety dryander is permitted for the preparation of medications. It is known, however, that extracts are made also with other parts of the plant, such as the stalk, and with other varieties.

The major pharmacologic property of *passiflora* is depression of the central nervous system, causing sedation. This effect was described by Phares in 1867, who used this substance for patients with insomnia and irritability. Freitas, who reviewed the literature, has reported other effects: hypotension, contraction of intestinal smooth muscle, bradycardia, hypothermia, skeletal muscle relaxation, and anti-spasmodic and anti-inflammatory effects.⁵

As a plant extract, passion flower extract contains many simple substances. Some active principles have been isolated from the plant using thin-layer chromatography. Harman, harmine, and harmol have been extracted from the alkaloid phase, and vitexin, isovitexin, orientin, iso-orientin, and saponarin from the flavonoid phase.⁵

Cascara sagrada, also called *Rhamnus purshiana*, is prepared from the bark of the tree *Rhamnus purshiana* deCandolle. This tree is indigenous to the Pacific coast of North America. Cascara sagrada has been reported to contain two types of anthracene compounds, O-glycosides and C-glycosides.⁶ This drug is used for its laxative effect and also as a restorer of colon tonus in cases of constipation.

In a Brazilian dictionary of pharmaceutical specialties⁷ there are 20 medications containing passion flower and seven containing cascara sagrada. The patient reported here manipulated a medication prescribed for weight loss that contained passion flower and cas-

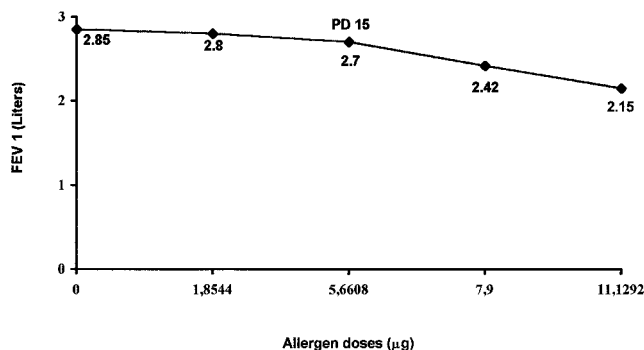


Figure 3. FEV₁ measured after the inhalation by the patient of different doses of cascara sagrada.

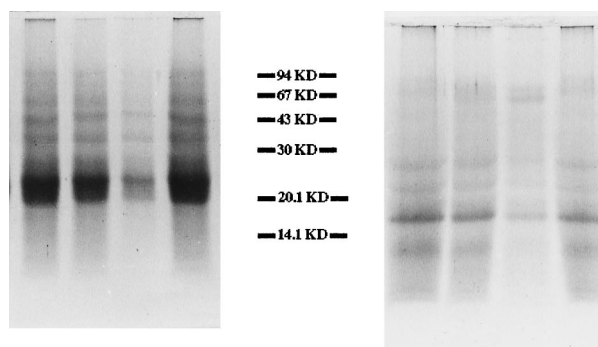


Figure 4. Electrophoresis of passion flower (left) and cascara sagrada (right).

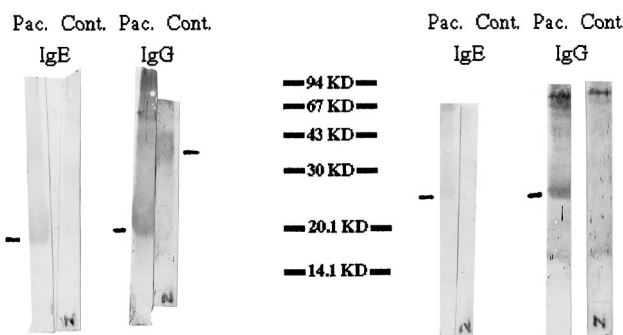


Figure 5. Western blot of passion flower (left) and cascara sagrada (right). Pac. = patient and Cont. = control.

cara sagrada in combination in addition to other substances. A review of the literature did not show reports of rhinitis or asthma related to *passiflora* or cascara sagrada.

The diagnosis of asthma and rhinitis is confirmed on the basis of clinical history, physical examination, and laboratory tests. The histories also suggest that the conditions diagnosed are allergic, IgE mediated, and occupational.

Work in a pharmacy for the manual preparation of medications, a quite common activity in Brazil, with inhalation of various substances of high molecular weight, may be considered a condition extremely favorable to the development of diseases of the airways due to hypersensitivity.

The present patient reported a direct relationship between symptoms and work environment, especially when he

directly manipulated chemical products, with symptoms becoming milder at home. The fact that improvement is not total and at times does not occur is understandable and compatible with descriptions in the literature that report the absence of improvement of some individuals with occupational asthma who left their jobs as much as 4 years or more earlier.⁸ The existence of a "period of latency of exposure", ie, a period of 6 months from the beginning of exposure to the drugs to the onset of symptoms, suggests an IgE-mediated disease. Another sign of allergic reaction to passiflora and cascara sagrada was the occurrence of delayed symptoms in the patient.

Smoking⁹ and atopy¹⁰ are risk factors for the development of occupational asthma. The present patient is a cigarette smoker.

The eosinophilia and increased IgE levels detected in the patient are compatible with IgE-mediated allergic respiratory disease but may also be explained by his parasitic infestation.

The strongly positive prick test is indicative of sensitization to the allergens under study. Allergen concentrations of 0.01% produced papules larger than those obtained with the 10-mg/mL histamine solution. Since several atopic patients seen routinely had negative tests to the allergens under study, despite the use of 10% solutions, we think the pathophysiologic mechanism involved in the positive reactions is type I hypersensitivity and not a process of direct histamine release or of primary irritation.

The clinical, occupational, and environmental histories strongly suggest that this sensitization to the allergens demonstrated by the prick test was the cause of the respiratory disease of the patient studied. The cause-effect relationship can be confirmed by bronchial challenge.

The first challenge with histamine indicated bronchial hyperreactivity, with a PC₂₀ of 0.150 mg/mL, although the patient had left his working environment 9 months earlier and was using beclomethasone by inhalation.

Many investigators who study pulmonary reactivity consider that normal individuals usually have a PC₂₀ > 8 mg/mL.^{11,12} The observation of a variation in FEV₁ on the day of challenge with physiologic saline is compatible with the known fact that the higher the hyperreactivity of the bronchial tree, the greater the variation of daily pulmonary function.¹³

Specific bronchial challenge demonstrated that the patient experienced acute asthma after allergen inhalation, with a significant reduction of FEV₁ compared with basal values, ie, an 18.75% reduction with the inhalation of 0.0024 mg passion flower and a 24.56% reduction with the inhalation of 0.0079 mg cascara sagrada. There are three clues indicating that the bronchoconstriction triggered by the plant extracts was due to a mechanism of type I hypersensitivity. First, and most important, control individuals, including asthmatic patients with bronchial hyperreactivity, did not have positive tests even when they inhaled a 1% allergen solution. Second, the occurrence of delayed symptoms, with a 43.75% fall in basal FEV₁ upon challenge with passion flower and a 26.31% fall upon challenge with cascara sagrada, suggests an IgE-mediated allergic response. Last, the second challenge with histamine, performed after the specific challenge, showed that the airway was more constricted, more reactive, and possibly more inflamed due to allergen exposure than on the occasion of the first examination carried out before challenge with the allergens. Only small amounts of the allergens were needed for a positive test, with a PD₁₅ of 0.0024 mg for passion flower and 0.0079 mg for cascara sagrada.

Electrophoresis of the plant extracts permitted the identification of at least four protein bands for each. Western blotting confirmed in vitro the sensitization of the patient to a protein of approximately 20 kD of passion flower and a protein of approximately 25 kD of cascara sagrada.

We conclude that passiflora and cas-

cara sagrada are two new etiologic agents for IgE-mediated occupational asthma and rhinitis. The description of these professional diseases permits new studies, such as the determination of the incidence and prevalence of sensitization and of diseases among employees dealing with these substances. The present study also serves to alert physicians to the risks associated with work in pharmacies devoted to manual preparation of plant extracts, emphasizing the importance of the use of protective measures in these environments.

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