
Occupational rhinoconjunctivitis and bronchial asthma due to *Acalypha wilkesiana* allergy

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Background: *Acalypha wilkesiana*, or copperleaf, is a plant of the Euphorbiaceae family. Although it is widely known as an outdoor ornamental plant, no cases of *A wilkesiana* allergy have been reported to date.

Objective: To describe a patient with occupational respiratory allergy to *A wilkesiana*.

Methods: Extracts from *A wilkesiana* leaves and flowers were used for skin prick testing, specific conjunctival and bronchial challenge tests, and in vitro studies. These studies range from *A wilkesiana* specific IgE determination to sodium dodecyl sulfate–polyacrylamide gel electrophoresis immunodetection of *A wilkesiana* protein bands in patient serum samples and immunoblot inhibition by preincubation with *Salsola kali* and *Chenopodium album* pollen extracts.

Results: Our patient had positive skin prick test reactions to *A wilkesiana* leaf and flower extracts; negative reactions were found in a control group of 20 atopic patients. On immunodetection of *A wilkesiana* extracts in patient serum samples, as many as 9 different IgE-binding proteins, with apparent molecular weights of 16 to 86 kDa, were revealed. Preincubation with *S kali* and *C album* pollen extracts completely inhibited IgE binding to the *A wilkesiana* extract. Specific bronchial challenge resulted in a spirometric 30% decline in forced expiratory volume in 1 second with respect to baseline 1 minute after 1:100 (vol/vol) *A wilkesiana* extract solution inhalation; 2 atopic controls had negative bronchial challenge test results.

Conclusion: *Acalypha wilkesiana* is a new etiologic agent for IgE-mediated occupational respiratory allergy.

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INTRODUCTION

Acalypha wilkesiana, or copperleaf, is a plant of the Euphorbiaceae family, native to New Hebrides. It is a robust branching shrub with ovate leaves, vastly extended as an ornamental plant. There are several *A wilkesiana* varieties, of which harlekim (green leaves) and macrophylla (red leaves) are widely grown. Occupational respiratory IgE-mediated allergy to ornamental plants is a well-known disorder, manifesting as rhinoconjunctivitis, bronchial asthma, or both.^{1,2} Early diagnosis of this condition is essential to avoid permanent airway damage. In the Euphorbiaceae family, *Hevea brasiliensis* latex (natural rubber latex [NRL]) has been shown to be an important source of occupational allergens.³ However, to our knowledge, cases of *A wilkesiana* allergy have not been previously described.

CASE REPORT

A 39-year-old man (nonsmoker) with no personal history of atopic disorders was studied in the Outpatient Allergy Service of the Dr. Negrín University Hospital, Gran Canaria. He had been working as a gardener for 7 years, and he referred a 5-year history of perennial rhinoconjunctivitis, followed by

dyspnea and wheezing, clearly worsening when he was near *A wilkesiana* plants (even in the absence of direct contact), especially when pruning its leaves. No cutaneous manifestations were noticed despite that he did not wear gloves while working. Symptoms usually persisted for less than 24 hours, and they completely disappeared during weekends and holidays. He might prune *A wilkesiana* plants during any season, whose flowering can occur throughout the year, and no seasonal symptom exacerbation was observed by the patient. Findings from physical examination were normal. White blood cell and eosinophil counts, findings from chest radiography, and basal spirometric measurements were all within their reference ranges. Because we suspected occupational rhinoconjunctivitis and bronchial asthma, an etiologic study was designed. Written informed consent was obtained from the patient.

METHODS

A wilkesiana Extracts and Skin Prick Tests

Leaves and flowers from *A wilkesiana* harlekim and leaves from *A wilkesiana* macrophylla were collected from various copperleaf shrubs. They were ground into small pieces and extracted by agitation in phosphate-buffered saline (PBS) (pH 7.5) for 4 hours. Then they were centrifuged for 30 minutes at 5,600g, dialyzed against water, filtered through a 0.22- μ m filter (Millex GS; Millipore Co, Bedford, MA), and freeze-dried. Extracts for skin prick tests (SPTs) were prepared by diluting them in PBS at 20 mg/mL (wt/vol), adding glycerine (50% vol/vol), and storing at 4°C until used.

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Skin prick tests with the *A wilkesiana* extracts and with standard batteries of common inhalants (mites, fungi, pollens, and animal dander) and foods (milk, egg, fruits, nuts, fish, and shellfish) (Bial Aristegui, Bilbao, Spain) were performed according to standard procedures.⁴ Histamine phosphate (10 mg/mL) and isotonic sodium chloride solution were used as positive and negative controls, respectively. A mean wheal diameter of 3 mm or greater 15 minutes after puncture compared with the negative control was considered a positive response. The SPTs with the *A wilkesiana* extracts were also performed in a control group of 20 atopic subjects.

Total and Specific IgE Measurements

Total IgE was determined by using the CAP-FEIA System (Pharmacia, Uppsala, Sweden), following the manufacturer's instructions. Specific IgE to *A wilkesiana* extracts was measured using the enzyme allergosorbent test technique. The *A wilkesiana* extracts (10 mg/mL [wt/vol]) were coupled to cyanogen-activated paper disks, as described by Ceska and Lunqvist.⁵ The enzyme allergosorbent test was then performed, and the results are expressed in accordance with the manufacturer's instructions (Hytec-specific IgE EIA; Hycor Biomedical Ltd, Edinburgh, Scotland). Specific IgE levels to protein extracts of other Euphorbiaceae species were also determined, including pollens from *Salsola kali* (saltwort), *Chenopodium album* (fat hen), *Mercurialis annua* (annual mercury), and *Ricinus communis* (castor oil plant); leaves from *Ficus benjamina* (weeping fig); fruit from *Ficus carica* (fig); and NRL.

Specific IgE Immunodetection and Immunoblot Inhibition Assays

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed according to the method of Laemmli⁶ using 12.5% and 4% acrylamide as separating and stacking gels, respectively. Twenty micrograms of protein was applied per lane. Separated protein bands were electrophoretically transferred to polyvinylidene difluoride, essentially as described by Towbin et al.⁷ Membranes were incubated overnight at 4°C with patient serum samples, followed by anti-human IgE–horseradish peroxidase conjugate incubation, and detected by a chemiluminescence method (ECL-Plus; Amersham Pharmacia Biotech, Uppsala) as recommended by the manufacturer. Immunoblot inhibition tests of IgE binding to the *A wilkesiana* macrophylla extract were performed by preincubating patient serum samples with the inhibitor phases (5 mg/mL [wt/vol]) overnight at 4°C before exposure to the membranes. A pool of serum samples from nonallergic subjects was used as a negative control for in vitro tests.

Conjunctival and Bronchial Challenge Tests

A specific conjunctival challenge test (CCT) was performed with the *A wilkesiana* macrophylla extract. For that purpose, SPTs were performed with 7 progressive 1/10 (vol/vol) extract dilutions in PBS, and CCT began with the dilution before that inducing a wheal at least 3 mm greater than the saline control, following standard procedures.⁸

A nonspecific bronchial challenge test (BCT) with methacholine was performed before the specific challenge tests following the method described by Cockcroft et al.⁹ A specific BCT was also performed with the *A wilkesiana* macrophylla extract following the procedure proposed by Chai et al,¹⁰ with modifications. Aerosolized particles generated by a continuously pressurized nebulizer (DeVilbiss 646; DeVilbiss Co, Somerset, PA) were inhaled for 2 minutes at normal breathing volume, starting with the control PBS solution and followed, at 10-minute intervals, by progressive concentrations of the *A wilkesiana* macrophylla extract. Pulmonary function tests were performed 30 and 60 seconds after each dose. A positive response was defined as a greater than 20% decline from the basal forced expiratory volume in 1 second (FEV₁). After specific BCT, hourly peak expiratory flow measurements were recorded for 9 hours.

RESULTS

SPT and IgE Measurements

The SPT responses to all the *A wilkesiana* extracts were positive, showing 4-, 6-, and 7-mm mean wheal diameters to the *A wilkesiana* macrophylla, *A wilkesiana* harlekim leaf, and *A wilkesiana* harlekim flower extracts, respectively. Results of SPT were also positive to *C album* (7 mm) and *S kali* (6 mm) pollens. Other aeroallergen and all food SPTs yielded negative results. The SPTs to *A wilkesiana* extracts showed negative responses in control subjects. The total IgE concentration was 73.2 IU/mL, which is within the reference range. Specific IgE levels to all *A wilkesiana* extracts were nondetectable. Specific IgE levels to *S kali* and *M annua* pollens were 3.3 and 0.9 kU/L, respectively. Specific IgE levels to the other Euphorbiaceae tested were undetectable.

Specific IgE Immunodetection and Immunoblot Inhibition Assays

When our patient's serum sample was used on immunodetection of *A wilkesiana* macrophylla and *A wilkesiana* harlekim leaf extracts, several IgE-binding proteins with apparent molecular weights of 54, 37, 27, 24, 18, and 16 kDa were shown. When the *A wilkesiana* harlekim flower extract was immunodetected, additional bands of 86, 44, and 32 kDa were also revealed (Fig 1A). On immunoblot inhibition, preincubation of our patient's serum sample with *S kali* and *C album* pollen extracts completely inhibited IgE binding to the *A wilkesiana* macrophylla extract (Fig 1B).

Challenge Tests

The SPT showed a 3-mm mean wheal diameter with the *A wilkesiana* macrophylla extract at the 2 mg/mL (wt/vol) concentration. The CCT result was positive with this 1:10 dilution. Five minutes after administration, ocular and nasal itching, watery eyes, sneezing, and conjunctival erythema were observed. The CCT performed in 5 atopic control subjects yielded negative results. Nonspecific methacholine BCT also showed a positive result, with a provocation concentration

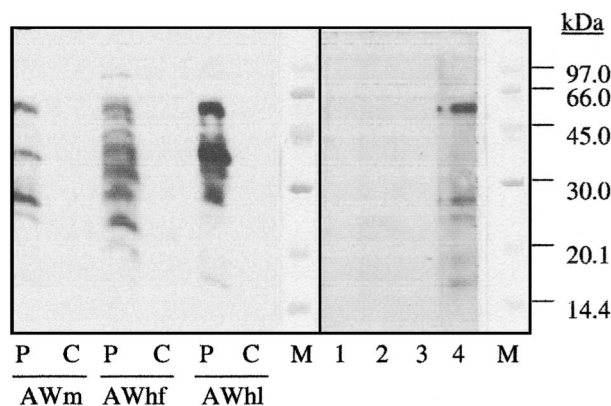


Figure 1. Left, Sodium dodecyl sulfate–polyacrylamide gel electrophoresis immunodetection of *Acalypha wilkesiana* macrophylla (AWm), *A wilkesiana* harlekim flower (AWhf), and *A wilkesiana* harlekim leaf (AWhl) extracts with our patient's serum sample (P) and a pool of control serum samples (C). Right, Immunoblot inhibition assays. Our patient's serum sample was preincubated with AWm (lane 1), *Chenopodium album* pollen (lane 2), *Salsola kali* pollen (lane 3), or lamb (lane 4) extract before immunodetecting the AWm extract. M indicates molecular weight markers.

that caused a decrease in FEV₁ of 20% of 1.65 mol, thus revealing mild bronchial hyperresponsiveness.

Specific BCT with the *A wilkesiana* macrophylla extract was started with the 1:10,000 dilution, for safety reasons. One minute after 1:100 (vol/vol) solution inhalation, the patient showed cough and wheezing, with a spirometric 30% decline in FEV₁ from baseline (Fig 2). Symptom relief 30 minutes after salbutamol inhalation was accompanied by a spirometric improvement to reference values. Peak expiratory flow monitoring showed no decrease in FEV₁ for the next 9 hours. Specific BCT results were negative in the 2 atopic control subjects tested.

DISCUSSION

Euphorbiaceae comprises a huge family of vegetables, of which some are of great economic importance, eg, *H brasil-*

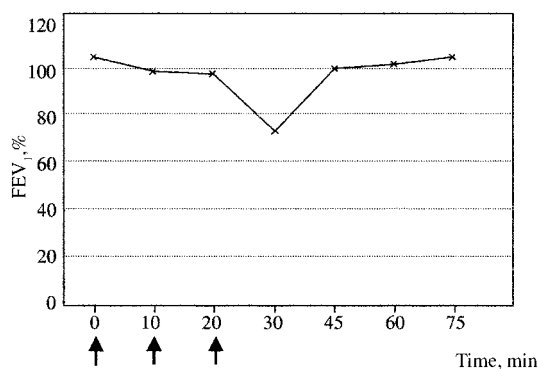


Figure 2. Specific bronchial challenge test performed with the *Acalypha wilkesiana* macrophylla extract. Arrows represent antigen inhalations, which were progressive at 1:10 (vol/vol) concentrations, starting with the 1:10,000 dilution in phosphate-buffered saline of the pure extract at 20 mg/mL (wt/vol). FEV₁ indicates forced expiratory volume in 1 second.

ensis, the source of NRL. Allergy to NRL is a worldwide problem. Other Euphorbiaceae species, such as *M annua* and *R communis*, have also been related to allergy for the past few decades.^{11,12}

Despite its wide distribution and its detection in pollen counters,¹³ *A wilkesiana* had never been described as a cause of allergic disease. However, our patient had a clinical history consistent with an occupational respiratory allergy to this plant. Positive SPT results to *A wilkesiana* extracts, together with IgE binding to several *A wilkesiana* protein bands in immunoblotting assays, confirmed IgE-mediated hypersensitivity to *A wilkesiana*. In our opinion, until commercial methods are available, diagnosis of *A wilkesiana* allergy can be based on clinical history, complemented with SPT with an *A wilkesiana* extract. Because our patient referred symptoms even in the absence of direct contact with the plant, and *A wilkesiana* pollen is clearly detected in pollen counters, *A wilkesiana* allergens are probably airborne and are potentially allergenic for others.

Immunoblot inhibition assays showed cross-reactivity between *A wilkesiana* allergens and those from *S kali* and *C album* pollens, 2 related species to which our patient was also sensitized but not occupationally exposed. Further studies are needed to discern whether patients allergic to these pollens could have respiratory symptoms when exposed to *A wilkesiana*. Interestingly, *A wilkesiana* flowering can occur throughout the year, *A wilkesiana* plants can be pruned at any season, and *A wilkesiana* allergens are present not only in flowers but also in leaves. For these reasons, *A wilkesiana* induced perennial respiratory symptoms in our patient. Thus, *A wilkesiana* could be an etiologic agent for perennial respiratory allergy, as already reported for the Euphorbiaceae *F benjamina*.¹⁴ Cross-reactivities between Euphorbiaceae plants and certain food allergies have been described, eg, the latex-fruit syndrome.¹⁵ However, our patients did not show associated food sensitizations, suggesting a lack of *A wilkesiana*–food cross-reactivity.

In conclusion, we described a patient with *A wilkesiana* IgE-mediated occupational respiratory allergy, confirmed by specific BCT, and described the molecular weights of the possible allergens involved. Once such a disorder is known to occur, it would be interesting to study the prevalence of *A wilkesiana* allergy not only in gardeners exposed but also in pollen-allergic patients or even the general population.

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