

Hypersensitivity to *Ailanthus altissima* (tree of heaven) pollen: identification of a major IgE-binding component

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Abstract *Ailanthus altissima* or *Ailanthus glandulosa* (Simaroubaceae) is known as tree of heaven. It is a dioecious plant with staminate and pistillate flowers that grow on separate trees. In recent years, *A. altissima* has been frequently planted in numerous areas, especially in arid and semiarid lands of Iran and also used as an ornamental tree in several Iran cities including Kerman. The aim of this research was to identify IgE-binding proteins responsible for type I hypersensitivities of *A. altissima* pollen extract. In this study, pollen's proteins were extracted and analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Total protein content of pollen extracts was measured by Bradford assay. Allergenicity of pollen extract was evaluated by skin test and immunoblotting. Results showed that total protein concentration of *A. altissima* pollen was found to be $1.34 \mu\text{g} \mu\text{l}^{-1}$. In *A. altissima* pollen extract, different protein fractions were identified by SDS-PAGE mostly at 42, 53.7, 63, 87.7, 100 and

120 kDa. Skin tests showed a delayed hypersensitivity. Immunoblotting of *A. altissima* pollen extract with pooled subject's sera detected a major IgE-binding component of 42 kDa. Moreover, these results will provide a platform for cloning cDNA encoding allergenic protein from *A. altissima* pollen.

Keywords *Ailanthus* · Allergenicity · Hypersensitivity · Immunoblotting · Pollen extract · Tree of heaven

1 Introduction

Ailanthus altissima or *Ailanthus glandulosa* (Simaroubaceae) known as tree of heaven or Chinese sumac is native to China and North Vietnam, which has become invasive in Europe and all other continents except Antarctica (Kowarik and Säumel 2007). It is a dioecious plant with staminate and pistillate flowers that grow on separate trees. The yellowish green inflorescences are arranged in a large double thyrsus at the end of new shoots (Fig. 1). Staminate inflorescences are larger and produce more flowers than those on a pistillate plant (Kowarik and Säumel 2007).

A. altissima was brought to Iran a very long time ago (Ghahreman 1993). In recent years, *A. altissima* has been frequently planted in numerous areas, especially in arid and semiarid lands of Iran including Kerman

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Fig. 1 *Ailanthus altissima* (tree of Heaven). **a** Staminate plant showing inflorescences at the end of new shoots (arrow) and inset in the figure is a staminate inflorescence. **b** Pistillate plant

and also used as an ornamental tree in several Iranian cities. It is both insect and wind pollinated (Aldrich et al. 2008), with a flowering period from April to June.

Pollen is the most important cause of allergic respiratory symptoms in Iran (Assarehzadegan et al. 2009). Previous studies have defined the allergenic potential of the pollen of *A. altissima*. Ballero et al. (2003) suggested an immunological cross-reactivity of its allergenicity. Using radioallergosorbent test (RAST) and skin test, they also stated the possible sensitization to *Ailanthus* pollen in patients suffering from allergic symptoms (rhinitis, asthma and conjunctivitis) during the pollination season, corresponding to the presence of several other types of pollen (grass, olive, *Parietaria*, plantain, *Amaranthus*). Maxia and Maxia (2003) have determined the airborne pollen component of *A. altissima* in the center of Cagliari in the 1997–2002 period. These investigators suggested that the clinical symptoms induced by inhalation of pollen grains of *A. altissima*, similar to those induced by the pollen grains of Poaceae or Oleaceae and even *Parietaria diffusa*, Chenopodiaceae and Plantaginaceae which pollinate at the same time and with a greater intensity than

Ailanthus, could have caused the clinical symptoms attributed to *A. altissima*.

The present study was therefore aimed at identifying IgE-binding proteins responsible for type I hypersensitivities of *A. altissima* pollen extract, a member of the Simaroubaceae family.

2 Materials and methods

2.1 Pollen samples

Pollen grains of *A. altissima* were collected from Kerman city during pollination period of 2010. Pollen samples were stored at 4 °C for later extraction. Kerman province is located in hyper-arid and arid regions of Iran. Major plant species of Kerman province include *Calligonum comosum*, *Haloxylon ammodendron*, *Haloxylon persicum*, *Haloxylon ammodendron*, *Prosopis juliflora*, *Prosopis spicigera* and *Tamarix stricta* (Amiraslani and Dragovich 2011). *A. altissima* is an invasive and fast-growing species in Kerman city; however, no precaution has been undertaken in order to reduce its uncontrolled distribution in the city of Kerman.

2.2 Preparation of pollen extracts

The pollen grains were extracted at 1.5:10 (W/V) in 0.2 M phosphate-buffered saline (pH = 7.2) by continuous string at 4 °C overnight. The supernatant was separated by centrifugation at 5,000g for 30 min at 4 °C and stored at −20 °C until further use.

2.3 Pollen protein content

The total protein content of *A. altissima* pollen extract was estimated using Bradford (1976) method.

2.4 SDS-PAGE

SDS-PAGE was carried out according to discontinuous method described by Laemmli (1970) in 10 % polyacrylamide gel, with parallel application of a reference standard of proteins with known molecular masses. Pollen extract was boiled for 5 min at 100 °C in twofold concentration of sample buffer containing B-mercaptoethanol. Fifteen microgram proteins, according to Bradford (1976), were applied per lane. The proteins were electrophoresed at a constant voltage of 80 V and bands were detected by staining with 0.2 % Coomassie Brilliant Blue R-250.

2.5 SDS-PAGE immunoblotting

Protein bands separated on SDS-PAGE were electrophoretically transferred to nitrocellulose (NC) membrane with Bio-Rad MiniTransblot apparatus at 4 °C. The non-specific sites on NC were blocked with 2.5 % defatted milk in TBST (Tris-buffered saline and 0.1 % Tween 20, PH = 7.4) for 1 h at room temperature (RT). The membrane was washed with TBST 3 times for 10 min each, and the blots were incubated overnight at 4 °C with pooled subject's sera (diluted 1:20 in 2.5 % defatted milk in TBST). After several washing with TBST, horseradish peroxidase labeled antiRat-IgE peroxidase (Koma, Korea, 1:10,000v/v in 2.5 % defatted milk in TBST) was added to the blotted membrane and incubated for 1 h at RT. The allergens were visualized on chemiluminescence's film using ECL plus western blotting detection reagents (Amersham kit).

2.6 Animal model

Ten male Wistar rats, 12 weeks of age, weighting 180–210g were purchased from animal laboratory of Shahid Bahonar University and maintained under control light (12:12 light:dark) and temperature (24 ± 1) conditions. They were fed with a standard laboratory diet and water ad libitum.

2.7 Immunization of Wistar rats

Ten male rats were randomly divided into 2 groups: in the immunization group, rats were sensitized by intraperitoneal injection of 55 µl pollen extract containing about 75 µg protein + 75 mg Al (OH)₃ diluted in PBS (phosphate-buffered saline, pH = 7.2) twice a week for 4 weeks. Control (sham) group received PBS. Five animals were used for each treatment.

2.8 Skin tests

On the 7th day after the last sensitization, each animal was tested by subcutaneous (S.C) injection of 55 µl pollen extract (containing 75 µg protein with 75 mg Al (OH)₃ diluted in PBS, pH = 7.2). The negative control was buffered saline. Reactions were read after 48–72 h, and the result was considered to be positive when the induration's size was 3 mm greater than the negative control. Data of skin test were analyzed using the MSTAT-C program. MSTAT-C is statistical and data management package. It consists of an executable program that aids in experimental design, and managing, transforming and analyzing data. (MSTAT-C 1990).

2.9 Blood sampling

One week after the skin tests, rats were killed with decapitation and trunk blood was collected into tubes, sera were obtained by centrifugation of blood at 3,000g (20 min). Samples were immediately stored in aliquots at −20 °C until use.

3 Results

3.1 Protein concentration

Protein content in the pollen extract of *A. altissima* was found to be $1.34 \mu\text{g } \mu\text{l}^{-1}$.

3.2 SDS-PAGE

The SDS-PAGE of the protein extract of the pollen of *A. altissima* revealed six distinct bands in the 42–120 KDa range. Protein fractions of 42, 53.7 and 63 KDa were most prominent and were stained strongly by coomassie brilliant blue. However, bands at 87.7, 100 and 120 KDa were weak and poorly stained but were distinguishable on gel (Fig. 2).

3.3 Immunoblot

Immunoblotting of *A. altissima* pollen extract with pooled subject's sera detected a major IgE-binding component of 42 KDa (Fig. 3). No other

protein band in our SDS-PAGE gel reacted with the IgE.

3.4 Skin tests

Results of subcutaneous injection in immunization group showed induration without erythema at the injection site 48–72 h post-immunization. Induration diameter in the immunization group was recorded as more than 3 mm. The maximum diameter of induration was measured at 72 h post-injection. Animals in the sham group did not show any induration or erythema. Analysis of variance of skin test data showed significant differences in induration's size between the immunization and sham groups at the 1 % probability level (Table 1).

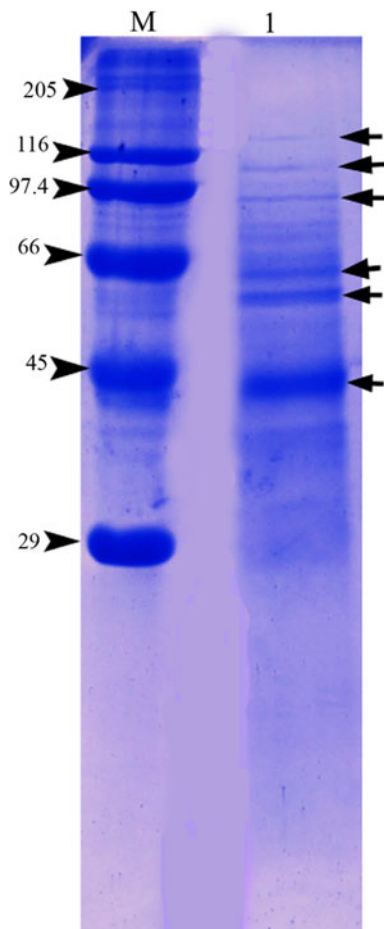


Fig. 2 SDS-PAGE profile of pollen extract of *Ailanthus altissima* collected from Kerman city. Lane M: molecular weight markers Lane 1: *A. altissima* pollen extract. The arrows show the protein bands

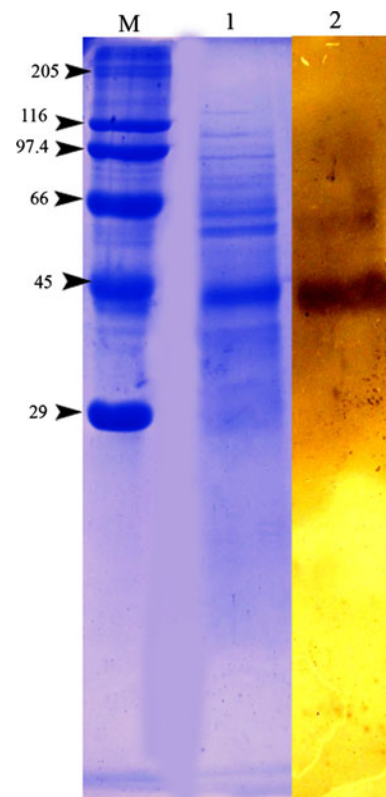


Fig. 3 SDS-PAGE profile of pollen extract of *Ailanthus altissima* collected from Kerman city and SDS-PAGE immunoblotting using from subjects sensitized with the *A. altissima* pollen extract. Lane M: molecular weight markers, Lane 1: *A. altissima* pollen extract and Lane 2: a major IgE-binding protein of *A. altissima* pollen extract

Table 1 Simple variance analysis of skin test in rats

SOV	df	SS	MS	F
Replication	4	9.4	2.35	1
Treatment	1	129.6	129.6	55.148 ^a
Error	4	9.4	2.35	

^a Significant at the 1 % probability level

4 Discussion

In the present study, allergenicity of the pollen extract of *A. altissima* was evaluated by skin test and western blotting. Skin tests were performed with the 15 % pollen extract and indurations at the injection site were observed 48–72 h later. These reactions are termed “type IV delayed hypersensitivity” in which sensitized T cells, usually a subpopulation of Th1 cells play the major role in its manifestations, antibody and complement are not directly involved (Stevens 2010). Type I immediate hypersensitivity is mediated by IgE that the term “allergy” is often used for it, and its effects are apparent within 8 h of exposure to an immunogen (Ahmed et al. 2007). Skin test results are consistent with the signs and symptoms of type IV hypersensitivity. These observations can be attributed to high-dose alum (Al (OH)₃) application in the present study. A part from the immunoenhancing effects, certain adjuvants can also influence the quality of an immune response (Herz et al. 2004). There is evidence that alum-adsorbed allergens can be effective in reducing allergic symptoms in appropriately diagnosed and treated individuals (Wheeler and Woroniecki 2001). It has been demonstrated that adjuvants such as alum biases the immunological response to either Th1 (type IV hypersensitivity) or Th2 (type I hypersensitivity) type responses (Marciani 2003). McNeel et al. (1999) showed in a rodent model that use of granulocyte-macrophage colony-stimulating factor (GM-CSF) as a vaccine adjuvant, mixed with a soluble antigen, led to induce both an antibody and T cell antigen-specific immune response. Skin test results are consistent with the role of alum in the stimulation of Th1 type immune response.

Immunoblot profile of *A. altissima* revealed an IgE reactive protein of 42 kDa. These results indicated the production of IgE and a mechanism of type I hypersensitivity. Shahali et al. (2007) also detected an IgE-reacting protein in their Western analysis of two

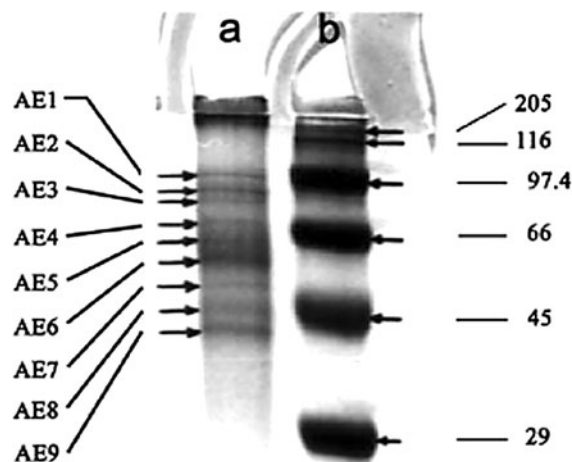


Fig. 4 SDS-PAGE profile of pollen extract of *A. excelsa* obtained by Mondal et al. (2007) showing nine prominent bands which were designed AE1 to AE9. AE1: 97.4, AE2: 92.6, AE3: 85.3, AE4: 73.3, AE5: 64.8, AE6: 59.7, AE7: 52.4, AE8: 45, AE9: 42.7

varieties of *Cupressus arizonica* protein extracts using the serum of allergic subjects.

The protein profile of the pollen of *A. excelsa*, another species of the Simaroubaceae family, has been reported earlier (Mondal et al. 2007) in which nine bands were obtained (AE1 to AE9), the molecular weights of which ranged between 42.7 and 97.4 kDa (Fig. 4). Among them, AE6 (59.7 kDa) and AE8 (45 kDa) proved to be the major allergenic determinants. Protein content in the pollen extract of *A. excelsa* was found to be 1.46 $\mu\text{g } \mu\text{l}^{-1}$. In addition, the protein profile as well as the protein content of *A. altissima* appears to be almost similar to that of *A. excelsa*.

In conclusion, *A. altissima* pollen with a major IgE-binding component may be an important allergen source in arid and semiarid regions of Iran. Thus, the 42 kDa protein has potential to be used as diagnostic and therapeutic reagent for patients allergic to *A. altissima*. Finally, these findings will provide a platform for cloning cDNA encoding allergenic protein from *A. altissima* pollen.

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