

Allergens of *Salmaaliala Malabarica* (Eng. silk cotton) Tree Pollen and Seed Fibres

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Salmaaliala malabarica (SM) pollen and seed fiber (SF) allergens were investigated for their role in type-I allergic disorders. Intradermal tests with SM pollen extracts showed markedly positive skin reactivity (2+ & above) in 5.6% of nasobronchial allergy patients tested. Skin sensitivity to old and new SF extracts was observed in 12.4% and 6.6% patients, respectively. SDS-PAGE resolved SM pollen extracts into 22 Coomassie brilliant blue stained bands (MW 14 — 100 kDa). Immunoblotting with pooled patient's sera demonstrated 15 allergenic proteins in pollen extracts. Proteins with molecular weights 50, 42, 35, 30, 20 and 14 kDa were detected as major IgE binding bands using individual patient sera. Based on the data, we recommend inclusion of SM pollen and its SF extracts in kits routinely used for diagnosis of allergies in India.

Key words *Salmaaliala malabarica*, tree pollen, seed fiber, Allergens, Immunoblot, IgE binding proteins.

INTROUCTION

Airborne pollen from several tree species are known to induce nasobronchial allergy in pre-disposed individuals (1-3). *Salmaaliala malabarica*, (syn. *Bombax malabaricum*) family Bombacaceae is a tall tree, naturally occurring in forests and planted widely in cities/towns of Northern and Penninsular region of India. The tree bears large bright red flowers from December to March (4). Aerobiological surveys showed low incidence of its pollen during Jan-April which may be due to entomophilous/bird pollinated nature of flowers. The floss surrounding the seed is airborne when dry (March-May) and used for stuffing, pillows and mattresses in the country. In an earlier

report, a number of allergy cases showed sensitivity to *Bombax ceiba* pollen, seed fiber and cotton dust allergens/extracts (5). In the present study, investigations on *Salmaaliala malabarica* pollen and seed fiber extracts have been carried out using standard in vivo and in vitro methods.

MATERIAL AND METHODS

Preparation of extracts: Mature floral buds were collected from locally growing plants of *Salmaaliala malabarica* (SM). Anthers were separated, shade dried, pulverized and passed through different mesh sieves. After sieving, the pollen material was subjected to purity checking by microscopic analysis and defatted with peroxide free diethyl ether. The samples with >99% pollen and <1% anther/parts of the same species were subjected to antigen/allergen extraction, 1:20 w/v in 0.05M ammonium bicarbonate containing phenyl methyl sulfonyl fluoride (100 mM).

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The extracts were also prepared from newly collected floss (silk cotton), old used "silk cotton" and cotton dust as above. The supernatant was collected after centrifugation at 15000 rpm, 30 minutes at 4°C. The protein was precipitated with 90% ammonium sulfate. The pellet was dissolved in distilled water, dialyzed, freeze-dried and stored at -20°C (3). Protein content of each extract was estimated by modified Lowry's method (6-7). Bovine serum albumin was taken as standard.

Intradermal tests (ID) and sera collection: Skin tests (ID) were carried out with these extracts on 105 randomly selected nasobronchial allergy patients aged 17-45 years attending Allergy Clinic, VP chest Institute, Delhi. For ID tests, a bleb of 2 mm was made by injecting 0.02 ml of each extract into the forearm of the patient. Simultaneously phosphate buffered saline (PBS) was injected on a separate site as a negative control. Histamine diphosphate was also injected as a positive control to assess the skin reactivity. ID test reactions were graded after 15 minutes in comparison to the negative control (3). The sera were collected from patients showing markedly positive skin reactions (> 12 mm wheal) to the extracts tested. Normal human sera were collected from non-allergic subjects and used as a negative control. The informed consent of the subjects was obtained prior to skin testing and sera collection.

Enzyme Linked Immunosorbent Assay (ELISA): ELISA was performed to measure the specific IgE levels to different extracts by the method of Voller et al, 1976 (8). Briefly 5 µg in 100 µl of each extract diluted in carbonate buffer (0.1M, pH 9.6) was coated per well of the microtitre plate. The plate was washed three times with PBS-Tween 20 (0.1 M phosphate buffered saline pH 7.4 with 0.05% Tween 20). Uncoated binding sites were blocked with 3% BSA. After blocking, the plate was washed with PBS-Tween 20. For specific IgE estimation, individual patient's sera (1: 10 v/v diluted in PBS) were added in each well and the plate incubated for 16 hours at 4°C. Normal human serum was used as a negative control. After washing with PBS-Tween 20, the plate was incubated with 100 µl of anti human IgE peroxidase labeled conjugate (epsilon specific, Sigma chemical Co, USA) for 3 hours at room temperature. The color was developed with orthophenylene diamine

and hydrogen peroxide. The absorbance was measured at 490 nm on an ELISA reader (NuncTM, Denmark).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot: Pollen extract of SM (150 µg protein per lane) was electrophoresed in a 12% polyacrylamide gel containing 0.1% sodium dodecyl sulfate, as described by Laemmli, 1970 (9). The resolved proteins were either stained with Coomassie brilliant blue R-250 or used for immunoblot.

For immunoblot, the separated proteins of SM extracts were transferred onto a nitrocellulose membrane as described (10) by Towbin et al. (1979) using semi-dry blotting apparatus (LKB). The membrane was used for the detection of allergenic proteins using individual patient's sera as described earlier (3).

RESULTS AND DISCUSSION

Salvia malsabarica (local name-Semul) is cultivated in parks, gardens and roadsides as ornamental tree with beautiful Crimson colored flowers. Each flower has 66 anthers and produces approximately 42,000 grains per anther (11). Pollen production per flower is very high, however low incidence of pollen has been reported (< 1%) from Delhi, Lucknow, Meerut and Bombay. Despite this, the atopic subjects having the tree in their surroundings may be sufficiently exposed to *Salvia* pollen allergens. In the present study, SM pollen extract showed intradermal sensitivity in 6 (5.6%) patients out of total 105 tested. Agnihotri et al, 1981 (5) reported skin sensitivity to SM pollen extract in 20.7% atopics and 30.7% non atopics, respectively. In a multi-center study (12) at four allergy clinics in India, skin sensitivity to *Cedrus deodara* pollen extract was reported in the range of 4.8 - 9.3%. The variation in the skin sensitivity may be due to different set of patients tested or the severity of exposure to the allergen concerned.

A large number of nasobronchial allergy patients usually report winter (Nov-Feb) /change of season (March-April) as worst season. *Salvia* blooms during this period(s) and the seed fiber (silk cotton) becomes airborne from March onwards. Therefore, the patients were skin tested with seed fiber (SF)

extract and also cotton dust extracts used frequently in pillows, quilts during winter months. We have observed markedly positive skin reactions in 13 (12.4%) patients to old "silk cotton" extract. However, the new "silk cotton" extract elicited positive skin response in only 7 (6.6%) patients. The silk cotton turns into fine powder due to repeated use in pillows and beddings and hence can be easily inhaled by the user. It has been observed that the regular use of such fibers by the patients can aggravate their symptoms. But, the increase in virulence i.e., skin sensitivity of old "silk cotton" extract may be due to associated organic dust containing insect parts, mites, fungi, etc with fibers over the period of time. Previous study (5) at Lucknow, India showed skin sensitivity to old SF extracts in 42.3% patients. Skin sensitivity to cotton dust extract was reported to be higher (28.3%) in the earlier study than observed in the present study (7.6%). Studies conducted elsewhere (13) have identified allergenic proteins from plant materials such as seeds, fruits, etc other than pollen.

SDS-PAGE resolved SM extract in 22 Coomassie brilliant blue protein bands with molecular weight (MW) 14-100 kDa (Fig 1). Out of twelve sera tested (ID positive) by ELISA, 8 showed elevated specific IgE titer (OD's 0.8-1.2) for *Salmalia* allergens. The sera with high titer were used for Immunoblotting. SM extract showed 15 allergenic proteins on immunoblot with pooled patient's sera sensitive to SM pollen allergens. The major allergenic proteins were MW 50, 42, 35, 20 and 14 kDa as recognized by most

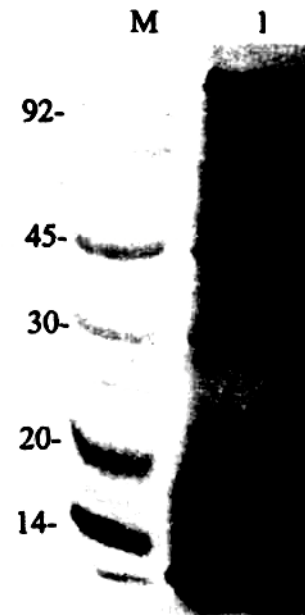


Fig. 1. SDS-PAGE profile of *Salmalia* pollen extract, (M)- molecular weight markers.

of the individual patient's sera (Fig.2). Previous studies with tree pollens reported 10-20 CBB stained bands on SDS-PAGE in MW 14-100 kDa (3,14). Immunoblotting studies with other tree pollen extracts such as *Cupressus arizoizica*, *C sempervirens*, *Holoptelea integrifolia*, *Phoenix dactylifera*, *Putranjiva roxburghii* and *Morus alba* demonstrated 2-16 allergenic proteins in MW range of 10-100 kDa. IgE binding proteins of similar molecular weights as in SM were reported from *C deodara*, *H. integrifolia*, *P. roxburghii* and tree pollen belonging to Cupressaceae (3,15-18).

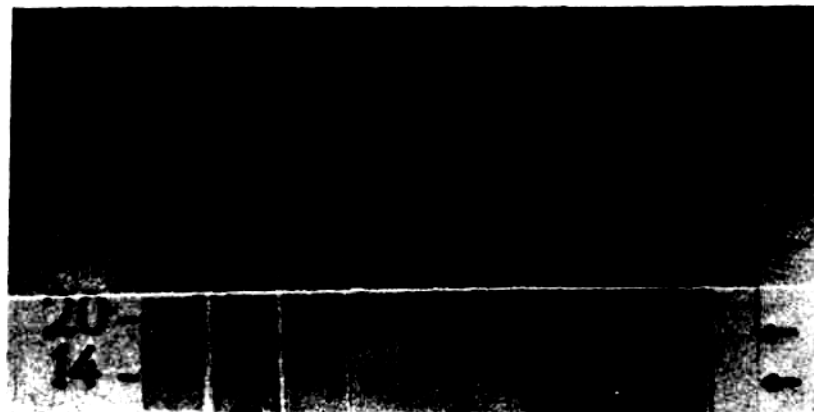


Fig. 2. Immunoblot of *Salmalia* pollen extract with individual patients sera (1-8).

In conclusion, immunobiochemical characterization of SM pollen extract has been completed for IgE binding proteins. Based on the clinical data, we recommend the inclusion of SM pollen and silk cotton extracts in testing kit for patients having allergy symptoms during Dec-April. Patients are also advised not to use silk cotton in their bedding, quilts, pillows, etc as preventive measures.

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