

Comparison of patch test contact sensitivity to acetone and aqueous extracts of *Parthenium hysterophorus* in patients with airborne contact dermatitis

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Our aim was to compare the degree of patch test positivity to acetone and aqueous extracts of *Parthenium hysterophorus* in patients with airborne contact dermatitis. We performed patch testing with the Indian standard series (which includes aqueous extracts of parthenium, xanthium and chrysanthemum), and with 1:100 and 1:200 dilutions of an acetone extract of parthenium in 72 patients with airborne contact dermatitis. All patients showed contact sensitivity to the 1:100 dilution and 67 patients had positive allergic reactions to the 1:200 dilution of the acetone extract, whereas only 45 patients showed a positive reaction to the aqueous extract of *P. hysterophorus*. Our results confirm that parthenium allergens are more soluble in acetone than in water, and that the acetone extract is significantly better in detecting contact sensitivity to parthenium in patients with suspected plant dermatitis. Hence, the acetone extract is recommended for routine patch testing.

Key words: acetone extract; airborne contact dermatitis; aqueous extract; *Parthenium hysterophorus*; patch test. © Blackwell Munksgaard, 2004.

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Airborne contact dermatitis due to *Parthenium hysterophorus* is a major dermatological problem in India (1). The first report of contact dermatitis due to *P. hysterophorus* was by French (2) in 1930 from the USA, and Lonkar and Jog (3) were the first to report parthenium dermatitis in field workers and agriculturists at Pune, India. Since then, it has been widely reported across the Indian continent and in virtually every state (2, 4–7). Parthenium dermatitis in its classical form known as airborne contact dermatitis primarily affects exposed areas of skin like the face, especially the eyelids, neck, V of the chest, cubital and popliteal fossae (1). Other clinical patterns reported are the seborrhoeic pattern (1), widespread dermatitis (1), photosensitive lichenoid eruptions (6) and prurigo nodularis-like lesions (8).

P. hysterophorus is a member of Compositae family of plants. The allergens responsible for the allergic contact dermatitis have been characterized as sesquiterpene lactones. These are found

in the oleoresin fraction of the leaves, stems and flowers, the highest concentration being seen in the small glandular hairs known as trichomes present on the undersurface of the leaves and on the stem (9). Parthenin is the major allergen in *P. hysterophorus* and belongs to pseudoguaianolide class of sesquiterpene lactones. The other allergens are coronopilin and tetraeurin A and some other closely related pseudoguaianolides (9).

These allergens can be extracted in various solvents such as acetone, alcohol, ether, acid, alkali, water, etc. and then used for patch testing (1). It has been found that the solubility of the allergens may vary in various solvents and so also the concentration, which in turn may influence the degree of patch test positivity. Such allergens are probably more soluble in acetone, alcohol or ether than in water, alkali or acid (1). Patch testing with a different extract of plants can elicit a positive allergic reaction. However, some differences in the degree of positive reaction may be seen between the different extracts.

We aimed at comparing the degree of contact sensitivity to acetone and aqueous extracts of *P. hystrophorus* in patients with airborne contact dermatitis.

Materials and Methods

72 patients with airborne contact dermatitis were patch tested with the Indian standard series, which contains aqueous extracts of plant allergens (parthenium, xanthium and chrysanthemum). Patients were also tested with 1:100 and 1:200 dilutions of an acetone extract of parthenium. An acetone extract has been routinely used for patch testing in our department for the last 5 years. It has been tested in 20 controls with hand eczema and footwear dermatitis and showed no irritant reactions.

Preparation of the aqueous extract of P. hystrophorus (7)

Fully grown and healthy-looking leaves of parthenium plants were picked, washed with tap water to remove contaminants and then air-dried at room temperature. Once the leaves were completely dehydrated and brittle, they were powdered in a blender to obtain dry leaf powder. 50 g of leaf powder was mixed with 300 ml of distilled water and kept at room temperature for 24 h, with frequent shaking during this time. At the end of this period, the extract was filtered through Whatman no.1 filter paper (Whatman International Ltd, Maidstone, UK), and the filtrate thus obtained was stored in an air-tight glass container at 4°C.

Antigen-impregnated discs were prepared by soaking a sheet of Whatman no.3 filter paper in an excess quantity of the plant extract for 30 min and then drying this sheet in the air at room temperature for 24 h. The margins of this sheet were cut-off and discarded while the central portion of the filter paper sheet (which had soaked up the plant extract uniformly) was cut into 1 cm² pieces and stored in plastic containers at room temperature. Antigen-impregnated discs so prepared were applied with the help of forceps on a 2.5-cm wide Micropore strip (3M Health Care, St Paul, MN, USA) keeping the distance between adjacent discs (xanthium and chrysanthemum) to 4 cm. These were then protected with a transparent polythene sheet of a slightly larger size. These units were then stored at room temperature.

At the time of testing, the polythene sheet covering the ready-to-use patch test units was removed and discarded. The antigen-impregnated discs on the Micropore tape thus exposed were wetted with a drop of water, and these strips were

then applied on the upper back of the patient. Each of these discs was pressed onto the skin firmly to obtain good occlusion.

Preparation of the acetone extract of P. hystrophorus

50 g of the leaf powder was mixed with 500 ml of acetone in a glass container covered with black paper or aluminium foil and kept at room temperature for 24 h, with frequent shaking. The supernatant was filtered through Whatman no.1 filter paper, and the filtrate thus obtained was allowed to air dry at room temperature. 1:100 and 1:200 dilutions were prepared in acetone. 1 microdrop of each dilution was used for patch testing and was added to the plain Whatman no.3 disc papers in the chambers, and then applied to the patient's back.

Patch test readings were taken on day 2 and day 4. The grading of the patch test reactions was done as per the ICDRG recommendation (10). The results of contact sensitivity to aqueous and acetone extracts of *P. hystrophorus* were compared. McNemar's chi-square test was used to assess the differences in the positivity between the acetone extracts (1:100, 1:200 dilutions) and the undiluted aqueous extract, and also between the two concentrations of acetone extract. A *P*-value of <0.05 was taken as an indication of significance.

Results

Of the 72 patients, there were 29 females and 43 males. The mean age of the patients was 41.5 (±14.05) years. The duration of illness varied from 2 months to 20 years, with a mean of 5.25 (±4.76) years. 7 patients had features suggestive of chronic actinic dermatitis, and the remaining 65 patients had airborne contact dermatitis.

The frequency of contact sensitivity to the 1:100 dilution of acetone extract of *P. hystrophorus* was as follows: + in 58 (80.6%), ++ (Fig. 1) in 10 (13.9%) and +++ in 4 (5.6%) patients. At 1:200 dilution, + was seen in 60 (83.3%) patients, ++ in 4 (5.6%), +++ in 3 (4.2%), negative in 4 (5.6%) and doubtful (?) in 1 (1.4%). The frequency of contact sensitivity to the aqueous extract was as follows: + in 42 (58.3%) patients, ++ in 3 (4.2%) patients, none had +++, negative in 25 (34.7%) patients and doubtful (?) in 2 (2.8%) patients.

The differences in the positivity (+, ++ or +++) between the acetone extract 1:100 dilution and the aqueous extract (100 versus 62.5%, *P* < 0.001), and also between the acetone extract 1:200 dilution and the aqueous extract (93.0

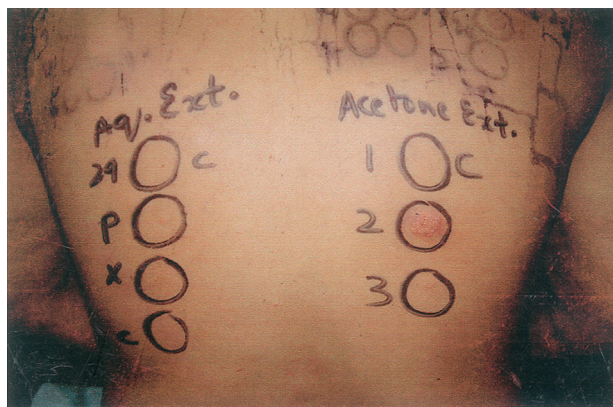


Fig. 1. Showing ++ reaction to 1:100 dilution and + reaction to 1:200 dilution of acetone extract of *Parthenium hysterophorus* and the negative reaction to aqueous extract of the same. (Aqueous extract C=control; P=parthenium; X=xanthium; C=chrysanthemum. Acetone extract 1=control; 2=acetone extract 1:100 dilution; 3=acetone extract 1:200 dilution.)

versus 62.5%, $P < 0.001$) were significant. However, the difference in positivity between 1:100 and 1:200 dilutions of the acetone extract (100 versus 93.0%, $P = 0.08$) was not significant.

Discussion

Our comparative analysis of the degree of contact sensitivity to acetone extracts (1:100 and 1:200 dilution) and the undiluted aqueous extract of parthenium in 72 patients showed that the acetone extract of parthenium produced a very highly significant and strong positivity compared to that of the aqueous extract. In a study conducted by Tiwari et al. (2) in 50 cases of allergic contact dermatitis due to *P. hysterophorus*, acetone extracts produced positive patch test reactions in all cases (100%), while aqueous extracts of leaf and flower gave positive reactions in only 43 (86%) cases. Our results confirm that parthenium allergens are more soluble in acetone than in water. Hence, we recommend the acetone extract

of parthenium for routine patch testing in patients with suspected plant dermatitis.

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