

ALLERGIC CONTACT DERMATITIS DUE TO PARTHENIUM HYSTEROPHORUS

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Summary

50 cases of allergic contact dermatitis from *Parthenium hysterophorus* have been studied in detail. Clinico-epidemiological features have been described. Patients present with any of the four observed patterns of dermatitis viz atopic, photosensitivity, seborrhoeic and miscellaneous. Patients were patch tested with 12 extracts of the plants in six solvents producing differing reactions. Limited chemical analysis of the plant extracts yielded various fractions of which three were found to be immunologically active. Chemical nature of parthenin which is a known allergen of this plant has been studied. Possibility of cross sensitivity with other plants is briefed.

Allergic contact dermatitis constitutes one of the largest and most important group of dermatoses. There appears to be an ever increasing awareness of contact allergens, and plants have occupied a status as one among them. Innumerable plants causing allergic contact dermatitis are encountered in nature with varying geographical distributions. *Parthenium hysterophorus*, a member of compositae family is a notorious example of such in our country. Although a foreigner, this visitor has come to stay and so deep seated are its roots that all efforts to uproot it from this country appear to be failing. French in 1930, reported from U. S. A. the first case of skin sensitivity to *parthenium hysterophorus*. Ranade² and Lonkar³ started observing cases of dermatitis on exposed surfaces in Pune during the last decade.

Parthenium hysterophorus Linn is a member of compositae family of plants. (Fig. 1) The course of this dermatitis is described in two phases, an acute phase



PARTHENIUM HYSTEROPHORUS HABITAT

Fig. 1 *Parthenium hysterophorus* plant with erythema, oedema, vesiculation and exudation involving the face, 'V' of the neck and chest, ante cubital and popliteal fossae and the chronic phase with extension of the areas of affection to upper parts of back, chest, hands,

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forearms, legs and feet; the cutaneous lesions being lichenification, pigmentation and thickening of involed skin^{2,4,5,6}.

Many authors have reported parthenin to be the responsible allergen in parthenium hysterophorus^{6,7,8,9,10}. Recently Lonkar et al¹⁰ have subjected parthenium hysterophorus to a detailed chemical analysis. Plant was extracted in petroleum ether, benzene, acetone and ethanol. Further fractionation of the extracts was done and many chemical substances were obtained. These substances were tested for their antigenicity and it was found that the fractions containing parthenin and compounds of similar polarity are the active allergens.

Material and Methods

70 patients suspected to be suffering from parthenium plant dermatitis on clinical grounds were selected at random from the out patient department of Dermato-Venereology of Armed Forces Medical College, Cantonment General Hospital, Sassoon Hospital and indoor patients of skin centre, Command Hospital, Pune-1. They all were subjected to patch tests with leaf and flower of parthenium hysterophorus and 50 patients showing positive reactions to both were finally chosen as material for the present study. These included 41 males and 9 females.

Studies were carried out as outlined below :-

- (I) Closed patch tests with leaf and flower of parthenium hysterophorus with a control were performed and reactions recorded.
- (II) Leaves and stems, flowers and pollens of the plant were separately extracted and standardised in six solvents viz. water, alcohol, acetone, ether, 2N hydrochloric acid and 10% sodium-hydroxide.
- (III) Patients were then submitted to patch tests using the above plant extracts and reactions recorded.
- (IV) Chemical analysis of the leaves-stems and flowers-pollens extracts of the plant was undertaken at department of Pharmacy of Armed Forces Medical College and National Chemical Laboratory, Pune.
- (V) 20 cases from this study were further patch tested with various compounds obtained from the chemical analysis.
- (VI) Photopatch tests using the plant and its pure chemical fractions were performed in 10 cases.

Recording of Patch Test Reactions

Following signs have been used in this work for recording degree of positivity of patch test reactions¹¹ :-

1. No reaction	0
2. Erythema	+
3. Erythema and papules	++
4. Erythema, papules and vesicles	+++
5. Marked oedema and vesicles	++++

Preparation of Extracts

Before subjecting the plants to extraction, it was duly and pharmacognostically identified by a qualified and competent botanist. The substance presumed to be responsible in causation of contact dermatitis in plants are reported to be the secondary products secreted by the resinous glands. Such oleoresins are present in all parts of the plant including the pollen grains in parthenium hysterophorus¹⁰. Hence for this study, leaves, stems, flowers and pollens were selected for extraction. They were obtained from parthenium hysterophorus collected from different localities of Pune.

Leaves-stems and flowers-pollens were macerated separately with 90% alcohol and extracted in Soxhlet apparatus for four hours. Thereafter the extracted material was filtered and the filtrate was heated in a water-bath to remove alcohol. A brownish, sticky, resinous material was obtained. Measured quantity of this material was then redissolved in alcohol to give a 2% solution. Similar method was used to prepare extracts using acetone, ether, water 10% sodium hydroxide and 2N hydrochloric acid separately. Thus 12 extracts were obtained, 6 from leaves and stems and 6 from flowers and pollens. The maximum yield of resinous matter was found in acetone and alcoholic extracts.

After these preliminary preparations, all extracts were tested (patch tests) on five normal healthy individuals to test for any primary irritant effect. Since extracts in sodium hydroxide and hydrochloric acid produced irritation as manifested by mild chemical burn in all five individuals, these were further diluted and tested till no irritation was produced. Dilution of 1 : 10 of alkaline and acidic extracts were finally considered fit for patch testing.

Chemical analysis

Chemical analysis of the plant extract was carried out at the department of pharmacy, Armed Forces Medical College and National Chemical Laboratory, Pune. All extracts were first subjected to thin layer chromatography and were found to be almost similar in composition in that all of them contained parthenin in varying amounts. Presence of parthenin was also confirmed by spectroscopic examination.

In view of the above facts petroleum ether, acetone and benzene extracts were freshly prepared and subjected to column chromatography. Silica gel grade II was used for column chromatography.

Six fractions were obtained from petroleum ether extracts of both leaves and flowers. Two fractions were polar in nature and were obtained from benzene extract and acetone extract. One substance in addition to parthenin was obtained from water extracts.

All such fractions were tested for their solubility in the six solvents used initially i.e. water, ether, acetone, alcohol, sodium hydroxide and hydrochloric acid. Only one substance was found to be soluble in all solvents while others had varying solubility in different solvents.

The chemical fractions thus obtained were coded and numbered for clinical patch testing. Initially 1% solutions were used in petroleum ether and ethanol. Depending upon the severity of reactions further dilutions of the substances were prepared. Decoding was done at the end of the study.

All solutions containing coded chemical fractions were stored in small glass bottles with a tight lid fitted with small plastic stirrer of the pattern used in Bencard allergy tests material. These bottles were properly labelled and stored under room temperature.

Patch Tests with Chemical Fractions

20 out of the 50 cases under this study who showed positive patch tests to all the extracts previously were selected for further clinical tests. For each case one normal control was used. Patch tests with 1% solutions in different dilutions of the chemical fractions were performed, method of patch testing and recording of results remained similar to that employed with extract testing in order to maintain uniformity.

Chemical Identification

Parthenin which is a known compound was further identified by spectroscopic method comparing the spectral data. For identification of other unknown

compounds (natural products) both chemical as well as spectroscopic methods were employed.

Results and Discussion

Sensitizing plants of Compositae family include *Chrysanthemum Helennium*, *Gaillardia*, *Helianthus*, *Tenacetum*, *Parthenium*, *Ambrosia*, *Iva* and *Xanthium*¹². Some plants of the parthenium species have been reported to be potential or proved sensitizers. Of these, five plants have been studied for their allergenicity. They are parthenium hysterochorus Linn., *P. fruticosum*, *P. incanum*, *P. confortum* and *P. intergrifolium* L.^{7,13}. Out of these parthenium hysterochorus has attracted much attention of different workers at home and abroad.

Allergic contact dermatitis is observed less frequently in younger age group though both allergic and irritant reactions occur during infancy and childhood¹⁴. In the present study no case under 20 years was detected. Maximum number of affected cases were in the age group of 30 to 39 years. Other workers have also reported similar observations^{6,10}.

Parthenium dermatitis affected more males than females in this study, the male-female ratio being 4.55:1. Females remain indoors most of the time irrespective of their status as housewives or working women. Hence the reduced risk of exposure to plant sensitization in them.

Since this work was mainly undertaken at a military hospital where more of servicemen are registered, 26% cases of parthenium dermatitis in this study were seen in defence personnel. Next in frequency was the group of farmers constituting 16% of the total. All these individuals regularly came in contact with the plant during their work or tenure of duty. Rest of the

patients (58%) belonged to different occupational status.

29 (58%) patients out of the 50 under this study belonged to Maharashtra and 20, (40% of the total) of these 29 belonged to Pune proper. 21 (42%) cases arrived at Pune from other parts of the country. This indicates that in areas like Pune where the plant grows in abundance, exposure risks are greater. Though the plant is reported to be growing in other parts of the country also^{15,16} allergic contact dermatitis cases reported from those areas are very few.

There was a wide variation in the period of existence of dermatitis in the range of 15 days to 15 years.

All previous workers have reported worsening of the dermatitis during growing season of the plant^{5,6,19}. In this series 14 patients (28%) had experienced aggravation of dermatitis during summer, 7 (14%) during monsoon season and 5 (10%) in winter indicating that acute symptoms can occur during any season. The maximum exacerbations were however during the summer months. It appears to be related to dry fragments and pollens of the plant becoming airborne and thus coming in contact with exposed skin surfaces.

48 (96%) patients experienced severe itching and at times exaggeration of dermatitic lesions after exposure to sunlight for variable periods. Sunlight as a source of nonspecific trauma may trigger pathological changes in predisposed subjects suffering from some other skin conditions. It is probable that the erythema produced by ultraviolet radiation lowers the itch threshold in such patients²⁰. Photosensitivity in relation to parthenium dermatitis has not been proved. No known photosensitizer compound has been found in this plant²¹. Photopatch

TABLE I
Showing comparative positivity of ordinary and photo patch tests

Serial No	Test Substance	Observation after 48 hours			Total No. of cases tested
		Ordinary patch	Photo patch	Photo test	
1.	Whole plant extract	+++	+++	0	10
2.	Parthenin	++++	++++	0	10
3.	Fraction '7'	+++	+++	0	10
4.	Fraction '8'	+++	+++	0	10

test observations in 10 cases in this study using plant extract, parthenin and two additional sesquiterpene lactones of the plant as test material have not shown any difference in the reactions in ordinary and irradiated patches. (Table I.)

It is further observed that those patients who obtained relief in their dermatitis after leaving weed infested areas do expose themselves to sunlight at new places and in doing so they do not get aggravation. In this context it is a well documented fact that contact sensitivity tends to persist, once acquired, and it is the degree of sensitivity which may vary from time to time²². These observations indicate that probably there is no role of photosensitization in parthenium dermatitis.

Four patterns of dermatitis were observed in patients under this study. The commonest was the one involving face, neck, 'V' of chest, antecubital and popliteal fossae. (Fig 2.) 24 (48%) cases presented with this pattern. The flexural affection was so typical of atopic distribution that one was easily led to suspect that an atopic diathesis has to do something with this dermatitis. Many other workers also found preponderance of this pattern in their cases⁶. Out of 24 (48%) cases presenting with atopic distribution and morphology of skin lesions, only 3 (6%) gave history of atopic manifestations in the past. As the irritable dermatitic skin is more susceptible to sensitiza-



Fig. 2 Shows involvement of face, neck, chest and antecubital fossae

tion of any nature^{6,23} it is reasonable inference, that individuals having atopic dermatitis are more likely to become sensitized to parthenium hysterophorus not because of atopy but due to their chronically irritated and damaged skin. 28% cases presented with photosensitivity type dermatitis. This aspect has been discussed earlier. (Fig 3.) 5 patients (10%) presented with itchy, dry, pigmented lesions involving nasolabial folds, upper lips, chin, cheeks, eye brows, retroauricular folds, chest and



Fig. 3 Shows photosensitivity type dermatitis involving exposed skin surface

upper reaches of back. This pattern was much closer to seborrhoeic type. These five patients had variable amounts of dandruff also. They all showed positive patch test reactions to the plant and its extracts. Thus it appears that contact dermatitis was superimposed on seborrhoeic dermatitis in their cases. The last group of 7 patients (14%), had dermatitic lesions which were not of any set pattern. Some had diffuse involvement and others had unilateral affection of antecubital and/or popliteal fossae.

Whatever the pattern, parthenium plant dermatitis is basically a chronic lichenified eczematous process with post inflammatory hyperpigmentation punctuated by acute and sub-acute stages. In all such stages pruritus is the chief symptom. At times itching

had been intense enough to create suicidal tendency in two (4) cases of this series. Sudden increased itching, erythema, oedema, vesiculation and oozing marks an acute exacerbation.

Some enzymatic studies were also carried out in all cases which included SGOT, SGPT and alkaline phosphatase estimations. No abnormality in any cases was detected. Serum protein estimations were also within normal ranges. So also, routine blood, urine and stool examinations. Distribution of ABO gene frequency was also studied in the 50 cases. Incidence of ABO group is shown in Table II.

TABLE 2
Incidence of ABO group in 50 cases of Parthenium Dermatitis

ABO Group	No. of cases	Percentage
A	11	22
B	9	18
AB	12	24
O	18	36
Total	50	100

The 50 cases studied comprised of a grossly heterogenous group of individuals belonging to different castes, sub-castes, states of origin, tribes, occupations, families etc. The only factor that they had in common was the disease itself. Any generalisation therefore, should appear inadequate for correlation of the ABO gene frequency and parthenium dermatitis at present.

All 50 cases included in this study were subjected to a battery of patch tests. Positive reactions to leaf and flower of the plant were observed in all cases in varying degrees of severity. To begin with, 2% extracts of leaves and flowers were used so that weak reactors could also be included. All extracts of leaf and flower produced positive patch test reactions. However some differences in degrees of positivity were discernible in case of each extract

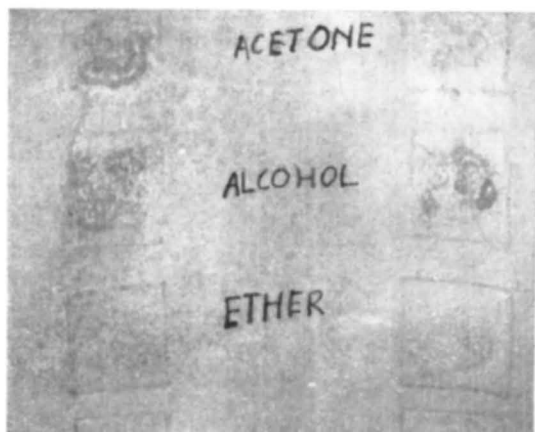


Fig. 4 Patch test reactions to acetone, alcohol and ether extracts of *Parthenium hysterophorus*

in different patients. No other previous study of this type is available for comparison. Acetone, ether and alcoholic extracts produced positive patch test reactions in all cases (100%). (Fig 4). Watery extracts of leaf and flower gave positive reactions in 43 (86%) cases only. Alkaline extracts of leaves produced 32 (64%) reactions while that of flower gave 29 (58%) positive patch tests. 39 (78%) and 34 (68%) cases were found to be positive to acidic extracts of leaves and flowers respectively. Visually positive reactions to acetone and alcoholic extracts were more intense as compared to ether extracts though in the same grade of degree. These differences in patch test reactions lead to some important conclusions. Since all extracts produce positive patch test reactions it is reasonable to conclude that either there is only one allergen in the plant which is soluble in all the solvents used or there are different allergens with differing solubility in various solvents. If it is a single allergic compound, then the difference in degree of positivity to the extracts used can be explained by its degree of solubility. Probably the allergen/allergens are more soluble in alcohol, acetone and ether than in water, alkali

and acid. Parthenin, the active substance is reported to be soluble in all solvents used in this study²¹. These facts were confirmed later in the study after doing chemical analysis. All solvents were also used along with extracts for patch testing and no positive reactions to solvents were detected.

Limited chemical analysis of the plant extracts used for patch testing yielded eight substances. Fractions one to eight including the sixth which was found to be parthenin, were the chromatographic fractions with increasing polarity successively.

Presence of parthenin was confirmed by the spectral data, thin layer chromatography and by the melting point 163–166°C. Ultraviolet spectral data for parthenin was λ_{max} 215 and 340nm and infrared wave lengths were 3450, 1755, 1718, 1725, 1592 cm^{-1} with specific rotation +7.02. All the 20 selected patients were finally tested with these eight fractions. No reaction was seen in any case with fractions 1 to 5. Fractions 6, 7 and 8 produced positive patch tests. Fraction 6 was found to be parthenin as mentioned earlier. Logically the fractions adjoining to fractions 6 were expected to be compounds of the same class as parthenin i.e. sesquiterpene lactones. This was confirmed by isolating the pure compounds and by study of the spectral data. Spectroscopically compounds 7 and 8 were found to be sesquiterpene lactones also possessing alpha methylene group exocyclic to gamma lactone as in parthenin (Fig 5). This group is the necessary prerequisite for allergenicity of such compounds^{7,10}.

Thus three fractions of parthenium hysterophorus have been found to be immunologically active and all the three belong to sesquiterpene lactones. These

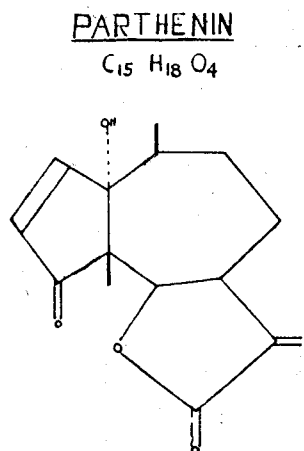


FIG. 5

Fig. 5 Chemical structure of Parthenin

three fractions are present both in leaves and flowers of the plant and also in pollens and stems in varying concentrations.

Parthenin is the major allergenically active compound in parthenium hysterophorus. It belongs to pseudoguaionolide class of sesquiterpene lactone. Its molecular weight is only 262 but it is capable of combining with large protein molecules containing NH and SH groups forming effective hapten-protein conjugates. Parthenin appears to be a strong sensitizer in susceptible individuals. An antigenic determinant of fairly large size is required to induce allergic contact sensitivity. Most of the compounds which are good sensitizers are small enough to cross the stratum corneum, but also have the ability to combine with protein within the skin to produce a new molecule or complex which is large enough to be antigenic, the sensitizing molecule in this situation being the hapten protein. There are many other sesquiterpene lactones which have low molecular weight as parthenin and yet they are also reported to be sensitizers. It is possible that strong sensitizer like parthenin must combine with a number of proteins of epidermal, dermal and vascular origin but it has

not been possible to find out the exact hapten-protein combination that may induce contact sensitivity. However probability of a specific, sensitizer like parthenin or other two sesquiterpene lactones detected from parthenium hysterophorus, reacting with a specific protein at different locations cannot be denied at present.

Among the large numbers of other plants possessing the antigenic determinant of parthenin; and the other two active compounds of parthenium hysterophorus parthenium bipinnatifidum and Inula have been reported^{6,10}. Parthenium bipinnatifidum contains ambrosin and Inula, alantoeactone. Other compounds having alpha methylene group exocyclic to gamma lactone i.e. the immunochemical pre requisite are coronolipin, parthenolide, constunolide, xanthumin and iso-alantolactone to name a few only. Fig 6 gives the structures and plant sources of these compounds. Thus it is obvious that common Indian plants like Champa (*Michelia champaca*), Kuth (*Saussurea lappa*), Gokhru (*Xanthium Strumarium*) and poshkar (*Inula race mosa*) may produce cross sensitivity with parthenium hysterophorum and vice versa.

COMPOUNDS HAVING ALPHA METHYLENE GROUP EXOCYCLIC TO GAMMA-LACTONE

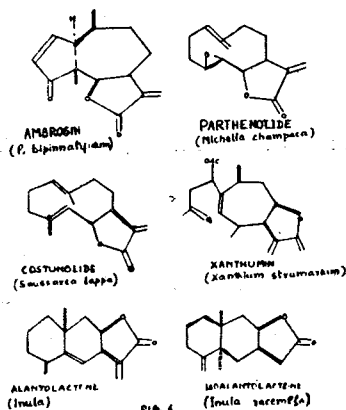


Fig. 6 Chemical structures of compounds having alpha-methylens group exocyclic to gammalactone

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