

Extracts of *Anacardium occidentale* (cashew) pollen in patients with allergic bronchial asthma

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SUMMARY

Allergy to pollens of the trees is among the most prevalent allergic sensitivities. The cashew tree grows in abundance in the northeast region of the Brazil, mainly, Fortaleza city, state of the Ceará - Brazil. The cashew tree flowering once a year between August and October. For the first time, a study was conducted to establish the possible role of the cashew pollen extract causing skin test reactivity in patients with allergic asthma. A stock solution of pollen extract was prepared with the standard weight/volume method for intradermal skin tests and for the protein content of the extract, estimated with the use of Folin phenol reagent and a espectrophotometer. Ten non allergic volunteers and 80 subjects with allergic asthma, as documented by previous positive skin test reaction to various pollens, were studied. Of the 80 patients, all 80 patients (100%) had positive test reactions. All the patients had grade III and grade IV reactions. None of the control subjects (n = 10) had positive responses to intradermal tests. This study provided us with knowledge of an additional pollen extract of the *Anacardium occidentale*, which could provoke skin test reactivities in asthmatic individuals from the area of Northeastern of Brazil. This results of the high positivity in intradermal test of the cashew pollen extract can suggest a relation between the period of flowering and the incresead number of allergic asthma cases.

Key words: Cashew, allergic asthma, extract, *Anacardium occidentale*, pollen

INTRODUCTION

Pollen from trees, shrubs, grasses and weeds can be inhaled, and cause allergy and asthma symptoms. Infectious coryza and asthma are particularly common during the pollination of the flowers. Experimental data from Calhoun et al. (1991) indicate that pollen may act synergistically with viral respiratory infections, which are particularly prevalent in winter¹.

Demonstration that a pollen is a cause of allergic symptoms has traditionally been governed by Thommen's postulates, which are mainly concerned with the habitat of the plant and the production of windborne pollen in large inhalable amounts².

This study applies for evaluation of the allergenicity of pollen of an abundantly growing tree, *Anacardium occidentale* (cashew) (figure 01). The cashew tree is a native plant of the tropical belt of Northeast Brazil and naturalized itself to various ecosystems in other parts of the world. It is of tremendous industrial value and hence has been grown extensively in public orchards, as in private courtyards and gardens. The flowers are small and yellowish pink with one celled ovaries (figure 01). Flowering is seasonal from August to October³.

All years, there is a significant increase of allergy cases and asthma symptoms during this period. The Clinicals of the allergology are always full in the Fortaleza city of Northeast of Brazil. From there our interest in show a relation among the flowering of the *Anacardium occidentale* (cashew) as a possible cause of the allergic bronchial asthma.

In view of its widespread existence, a study was conducted to establish that Cashew pollen extracts provoke skin test reactivities in asthmatic patients from the area of Fortaleza city of Brazil.



Figure 01: Cashew tree (*Anacardium occidentale*) with flowers

MATERIAL AND METHODS

Air sample method⁴

The cashew tree was a native plant of the tropical belt to Fortaleza – city, Ceará State - Brazil.

Cashew pollen identification and counting were done by a palynologist using Durham's apparatus (gravitational method)⁴, which was kept at a distance of 5m from the trees. The pollen count was a measure of the number of pollen per cubic metre of air sampled, averaged over 24 hours.

Preparation of pollen extract^{5,6}

The cashew pollen allergen was prepared with the standard weight/volume method (10mg per 100ml). Which included grinding,

defatting, extraction in buffered saline solution, filtration/clarification, and sterility testing.

The pollen was obtained from 30 day of harvest. All pollen was measure in cubic metre of air sampled and storage in Petri dish. The extract was obtained by adding 5,0ml saline solution pH 8,0 in 0,5mg of the pollens of the cashew, and left overnight at 4°C with constant agitation. The mixture was filtered in filter paper. Then the extract was centrifuged at 1.500 rpm for 10 min and sterilited for Milipore filtration.

Protein content⁷

The protein content of the allergenic extract was determined by Lowry method with the use of Folin phenol reagent and a spectrophotometer.

Study patients^{8,9}

Eighty patients wich allergic bronchial asthma who earlier showed varying degrees of positive skin test reactivity to various allergens, ranging in age from 10 to 65 years (Table 01). All patients studied had grad 1 or grad 2 asthma according to the GINA guidelines. And 10 non allergic volunteers, who acted as the control group were studied after their informed written consent was obtained.

Skin tests^{10,11,12}

All the routine precautions were taken before skin testing was performed. Was made intradermal test because, the Clinical of allergology where was made the study, use only this test like routine. Extract (0,02 ml of 1:100 concentration) and a control buffered saline solution were injected intradermally, and readings were taken after 20 minutes according to the

criteria of Shivpuri¹³ (Table 02) in both patients and control subjects. The concentration of protein used in the test was 0,56 mg/0,02ml.

TABLE 01: Demographics of patients in the study (n= 80)

		Total
N ^o of patients		80
Control subjects		10
Age range (yr)	10 - 25	28
	26 - 45	40
	46 - 65	12
Type of asthma		
Perennial		72
Seasonal		8

TABLE 02: Skin test positivity according to Shivpuri's criteria¹³

Grade	Criteria
0	Nearly same as control
1+	W: Twice the size of control E: ≤ 15mm
2+	W: 3 times control E: > 15mm
3+	W: 3.5 to 4 times control E: ≤ 30mm
4+	W: 4 to 6 times control E: > 30mm [*]

W, Wheal; E, erythema.

* Or any size with pseudopods

RESULTS

The palynologic portion of the study showed a total cashew pollen count of 505mm³/24hours approximate from August through October. Other pollens counted during the same period were *Graminae*, *Compositae*, *Urticaceae*, *Palmae* and *Caesalpineaceae*. All the other pollens isolated were discarded, only interest pollen of the cashew.

The protein content of the extract was approximate 27,96 mg/ml. The concentration of protein used in the intradermal test was 0,56 mg/0,02ml.

Of the 80 asthmatic patients evaluated, all had a positive response to *Anacardium occidentale* extract as determined by intradermal skin testing. Of the 80 patients all lived in urban areas and living near the plantations, 72 of them had perennial asthma of symptoms, and only 08 had a seasonal asthma. Detailed history did not reveal any mixed type of symptoms. Twenty of the 80 patients were involved in harvesting or processing the fruits of the cashew tree, 25% of the study subjects were occupationally involved having contact with cashew trees. All (100%) of patients had grade III and grade IV reactions (Table 02). 50 patients had erytoma approximate of 30mm and 30 patients had erytoma bigger than 30mm, skin test positivity according to Shivpuri¹³. None of the control subjects (n = 10) had positive responses to intradermal tests.

After careful study in that was taken the relation among patients with allergic bronchial asthma and the sensitivity the this patients at the extracts of *Anacardium occidentale* (cashew), correlation could be derived during the

flowering season because most of the subjects had a perennial type of asthma and also showed multiple pollen allergy reactions.

DISCUSSION

In addition to the cashew pollen count of 505mm³/24hours, pollen counts of *Graminae*, *Compositae*, *Urticaceae*, *Palmae* and *Caesalpineaceae*, were 446, 245, 215, 197 and 140mm³/24hours, respectively, during the same period. Pollen may travel many miles. Therefore, pollens of trees, grasses and weeds in your typical area can be the cause allergy and asthma symptoms. A high grass pollen count pertains to the fact that grass pollination is purely anemophilous and *Graminae* species are abundant throughout the hills and plains of this area. *Palmae* species, which are also abundantly grow and have an entomophilous type of pollination. *Compositae*, *Urticaceae* and *Caesalpineaceae* species, though pollinated anemophilously, had comparatively lower counts during this period. This could be attributed to the lesser density of these plants¹⁴. A seasonal pollen from an entomophilous tree may explain the increase of consultations of subjects with perennial or chronic asthma in the Clinicals of the allergology in the Fortaleza city of Nordeste Brazil, in this period.

There are very little studies in the literature of cashew pollen count and on the antigens and allergens of *Anacardium occidentale* (cashew) pollen. Previous work with skin testing has demonstrated sensitivity to cashew pollen in asthmatic patients in Goa city, India¹.

In our study, were 80 asthmatic patients evaluated, had grad 1 or grad 2 asthma according to the GINA guidelines, all had a positive response to *Anacardium occidentale* extract determined by intradermal skin testing. Of the

80 patients all lived in urban areas, 72 of them had perennial asthma of symptoms, and only 08 had a seasonal asthma. Detailed history did not reveal any mixed type of symptoms. Twenty of the 80 patients were involved in harvesting or processing the fruits of the cashew tree, were occupationally involved with Cashew trees.

The intradermal skin test results showed a good correlation with the grade of cutaneous sensitivity, 100% positivity in grade III (50 patients) and grade IV (30 patients) reactions. Similar study were made by Fernandes and Mesquita, 1995, with cashew pollens, they showed that 40% had positive skin test reactions in various grade, but the majority of patients had grade III and grade IV reactions. They studied 65 patients in Goa, India¹.

In a similar study by Garcia – Ortega at al., 1992, in which the pollen antigen of *Mercurialis annua* was used, skin sensitivity to the pollen extract was detected in 85% of the 152 patients¹⁵.

In summary, cashew pollen appears to be capable of inducing a significant triggering allergic bronchial asthma, in patients, this because of the clinical correlation, during the flowering season of the cashew happens a significant increase of allergic bronchial asthma in the Fortaleza city. This patients demonstrated high positive skin test reactions to cashew pollen extract. However, the significance of this study requires further clinical correlation; but certainly believe that cashew pollen could be a significant triggering factor of allergic asthma mainly in patients working with the plants during the pollination period.

This study provided a cashew pollen extract does provoke skin test reactivities in asthmatic subjects from Fortaleza city area of Northeast region of Brazil.

REFERENCES

1. Fernandes, L. & Mesquita, A. M. – *Anacardium occidentale* (cashew) pollen allergy in patients with allergic bronchial asthma. *J. Allergy Clin. Immunol.*; 1995; 95(2):501-4.
2. Norman P. S. – Discovering allergens. *J. Allergy Clin. Immunol.*, 1992; 89:923-4.
3. Matos, F. J. A. – *Farmácia viva* – Ed. UFC, 3^a edição, Fortaleza, 1998 pág. 87.
4. Underdown, B. J.; Goodfriend, L – Isolation and characterization of na allergen from short ragweed pollen. *Biochemistry* 8: 980, 1969
5. Menezes, E. A.; Gambale, W.; Macedo, M. S.; Castro, F.; Paula, C. R.; Croce, J. – Characterization of allergenic fractions from *Drechslera monoceras*. *J. Invest. Allergol. Clin. Immunol.*; 1998; 8(4):214-18.
6. Menezes, E. A.; Gambale, W.; Croce, J.; Macedo, M. S.; Abdalla, D. S. P.; Paula, C. R. – Biochemical antigenic and allergenic characterization of crude extracts of *Drechslera* (Helminthosporium) *monoceras*. *Mycopathologia*, 1995; 131:75-81
7. Lowry. O. H.; Rosenbrough, N. J.; Farr, A. L; Randall, K. J. – Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, 1951; 193:265-75.
8. Speelburg, B. Egidius, A. H.; Panios, M. D. – Late asthmatic responses after exercise challenge are reproducible. *J. Allergy Clin. Immunol.*, 1991; 87:1128-37

9. Kelly, L. A.; Gibson, G. J. – Relation between FEV₁ and peak expiratory flow in patients with chronic air flow obstruction. *Thorax*, 1998; 43:335-6
10. Croce, J.; Gambale, W.; Paula, C. R.; Zuppi, L. J. – Provocation of asthma by non-sporulating molds. In: *Advantages in Asthmologia*. Kobayashi, K.; Bellant, J. A. (Eds.). Excerpta Medica, Amsterdam, 1990; 401-4.
11. Mohovic, J.; Gambale, W.; Croce, J. – Cutaneous positivity in patients with respiratory allergens to 42 allergenic extracts of airborne fungi isolated in São Paulo, Brazil. *Allergol. Immunopathol.*; 1988; 16: 397-402
12. Bryant, D. J.; Burne, M. W./ Lazarus, L. – The correlation between skin test, common allergens in patients with asthma. *Clin. Allergy*, 1995; 5:145-7.
13. Shivpuri, D. N. – Comparative evaluation of sensitivity of common methods of diagnostic antigen tests in patients of respiratory allergy. *Ind. J. Chest. Dis.*, 1962; 4:102.
14. Niphadker, P. V.; Narvekar, A. K. – Role of BPT in diagnosis of nasobronchial allergy, *Lung India*; 1985; 3:21-4
15. Garcia Ortega, P.; Martinez, A.; Palacios, R.; Belmonte, J.; Richart, C. – *Mercurialis annua*: a new source of allergic sensitization and respiratory disease. *J. allergy Clin. Immunol.*; 1992; 89:987-93

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