Chapter-5

CONCLUSION

5.1 Ultrastructral study of *Datura* sp

Datura being an entomophilous plant produces lesser amount of pollen than anemophilous ones. On this ground, although it is argued that the probability of anemophilous pollen to reach the human airways is always greater (Diethart et al., 2007), the accessibility of the allergenic proteins is another factor which determines the allergenicity to a significant degree. As reported earlier, less compact or hardly detectable endexine, presence of microchannels in ektexine and endexine and less abundant pollenkit are some of the characters found in allergenic pollen which facilitate the transit of allergen across the pollen grain's wall upon hydration and higher accessibility of these allergenic proteins (Diethart et al., 2007).

Among the three species of *Datura, Datura metel* and *Datura stramonium* shows a discontinuous almost absent endexine and a tectate or semitectate columellate ektexine and no such microchannels (laminated endexine) existed either in the entire endexine or in the apertural regions. In *Datura inoxia*, there is presence of endexine but it is thin and discontinuous at places. This characteristic feature of the exine which is largely seen in anemophilous allergenic pollen supports the allergenicity of *Datura* for the hydrophobic nature of endexine that restricts the exit of hydrophilic proteins through this layer of pollen or causes leaking rate to be lower in case of the hydrophilic proteins (Diethart et al., 2007). Hence there is every possibility that the allergenic proteins in *Datura* are located in the pollen cytoplasm or in the pollen wall.

Secondly the *Datura* pollen exhibits the presence of pollenkit in negligible amounts which is generally a feature of anemophilous pollen and more of allergenic pollen (Hesse, 1980). Since the electron dense pollenkit is composed largely of lipids, it not only helps in the clumping together of pollen grains but its hydrophobic nature also acts as a barrier for the water soluble proteins to pass out easily and thus sporophytic proteins remain inside the cavities of exine (Dickinson, 1973; Pacini and Franchi, 1996). However, this striking lack of pollenkit is clearly a distinguishable feature which results in the allergenicity of these pollen. Contrary, *Datura stramonium* has moderate amount of pollenkit than the other two species of its, which might result in its lower allergenicity.

Thus, the barrier function of these layers cannot be completely dismissed and further investigation needs to be done to come to any conclusions as to whether ultrastructure of pollen wall can modify the potentiality of a pollen grain in causing allergenic diseases or whether the allergenicity of pollen is largely determined by the water solubility nature of pollen and the amount of allergenic proteins which are expressed within pollen.

5.2 Protein concentration and characterization of the allergenic proteins

It was revealed that protein concentration of pollen was varied extensively depending on the prevailing season for both mature and immature one. During summer a steep increase of protein content was found for mature pollen of three species of *Datura* that have studied with the exception of immature pollen of the species of *Datura inoxia* which showed a steep increase in the quantity of protein content than mature one. However pollen protein content of *Datura stramonium* was the least among the three species studied. This could be imposed on the presence of moderate amount of hydrophobic pollenkit present on the ektexine which acted as a barrier for the extraction of the water soluble proteins. During summer a steep increase of protein content was found in case of mature pollen of three species of *Datura* that have studied with the exception of mature pollen of *Datura inoxia* which showed decreased amount of protein content than immature one. . Interestingly, inspite of presence of higher quantity of protein in mature pollen, it resulted in the less number of protein bands during SDS-PAGE pollen profile. It can be argued that these proteins in the immature pollen have certain contribution in the incident of pollen maturation and anthesis .

Immunodiffusion and ELISA with the several protein fractions obtained by gel filtration and the pooled blood serum of sensitive patients (patients who showed a positive response to the total antigenic extract of the pollen of *Datura metel*) helped in the identification of the allergenic protein fractions of the three species of *Datura.* The precipitation arcs for immunodiffusion study were obtained with 11 protein fractions of molecular weights of 205.80 kDa, 184.63 kDa, 172.7 kDa,, 107.79 kDa, 66.05 kDa, 60.06 kDa, 29.02 kDa, 21.61 kDa, 17.6 kDa, 16.6 kDa and another protein having molecular weight >205kDa. Of these the 205.80 kDa, 66.05 kDa and 60.06 kDa protein fractions were the major allergenic fractions found in all the 3 species.

5.3 Cross reactivity among the three species of *Datura sp*

Cross reactivity occurs when one antibody binds with several antigens. This is a common event when two antigens share similar three dimensional structures along with similar antigenic determinants i.e epitopes. Cross reactivity is robust among the same species and also among the related species. Sometimes it occurs among different antigens present in totally different species (Stephen, 2008).

As *Datura metel, Datura inoxia and Datura stramonium* belong to the same genus, cross reactivity was expected among the three species as they shared at least some common allergenic proteins. Precipitation arcs were obtained in immundiffusion reactions during the cross reactivity study of the total protein extract of the pollen of *Datura* sp. Later on ELISA was done to confirm the result.

From the study of cross reactivity reactions among the three species of *Datura* i.e. *Datura metel*, *Datura stramonium* and *Datura inoxia*, *Datura metel* and *Datura inoxia* shared 5 common allergenic proteins those were 205.80 kDa, 184.63 kDa, 107.79 kDa, 66.05 kDa and 60.06 kDa. ELISA and Immunodiffusion study showed the presence of 3 common allergenic proteins between *Datura metel* and *Datura stramonium* those were 205.80 kDa, 66.05 kDa and 60.06 kDa proved to be common allergen. On the other hand, *Datura stramonium* and *Datura inoxia* shared only 4 allergenic proteins of molecular weight 205.80kDa, 66.05 kDa, 60.06 kDa and 21.61 kDa which were revealed by immunodifffusion and ELISA.

Above all cross reactivity study among three species of *Datura* i.e. *Datura metel*, *Datura stramonium* and *Datura inoxia* revealed the presence of 3 common allergens among them of molecular weight 205.80 kDa, 66.05 kDa and 60.06 kDa.

5.4 Epitope mapping

Cross reactivity is generally used to reduce the allergic load and to detect the common epitopes so that a least no of antigens can be used in the immunotherapy. So, epitope mapping of the allergen is the most successful modern technique to combat allergic rhinitis. MALDI was employed to map the epitopes in *Datura* allergens. Total 12 allergenic proteins were isolated from *Datura metel*, *Datura stramonium* and *Datura*

inoxia with molecular weights 16.6 kDa, 19.3 kDa, 21.61 kDa, 29.02 kDa, 60.1 kDa, 66.05 kDa, 70.78 kDa, 82.9 kDa, 97.2 kDa, 107.8 kDa, 184.6 kDa and 205.80 kDa.

From the sequence analysis study it was found that proteins' length ranged from 149-2043 amino acids with PI values 4.6-9.6. The total peptides present in the proteins were 1-5 in number. Among 12 proteins 8 were uncharacterized. Rest four belongs to the family of tumor protein homolog, apoplastic invertase, cullin and heat shock cognate.

5.5 Future direction of the study

About a quarter of the world's population is afflicted with a wide spectrum of allergic diseases, including asthma, hayfever, eczema and potentially fatal food allergy. The prevalence of these diseases, at all ages, have shown an increasing trend in both technologically developed and developing countries, possibly related to out-of-door and indoor environmental pollution and changes in life style. Unlike other aeroallergens, the global distribution of pollen from a wide variety of grasses, trees and weeds preclude any realistic possibility for pollen-allergic individuals to avoid these aeroallergens.

- The present study which was aimed to detect the sensitization of the pollen of three species of *Datura*, establishes the capability of the pollen of these plants in inducing hypersensitivity and thus the necessity of their pollen to be considered seriously as potential allergenic sources. Secondly *Datura* is of typical entomophilous nature and proved to be potential allergens which shows the necessity to evaluate the nature of entomophilous pollen in proper perspective as they too contribute significantly to the air-spora as they release appreciable amount of pollen which subsequently become airborne especially close to the source and may be a matter of great concern to a sensitive individual who might show pronounced allergenic reactions.
- Although pollen allergy has been known for more than a century, it is only during the last two decades or so that the need for standardization of allergenic extracts to have an International Reference standard has been recognized and several *in vitro* method like IEF, CRIE, SDS-PAGE, immunoprint and immunoblot have been adopted by scientists round the world for achieving

standardization of pollen extracts. Unfortunately Allergen (AL) - specific immunotherapy (ASIT) as practised today has not only proved to be cumbersome and expensive but is also associated with the risk of fatal side reactions including systemic anaphylaxis, often showing no improvement in the patient's condition. This is because the commercial antigenic extracts used for immunotherapy consists of heterogeneous (crude) extracts of the pollen and may contain the relevant allergens in minute, irreproducible amounts leading up to 100x variation in allergenic potency. Each extract of pollen may contain several antigenic proteins of which only a limited number of these may be allergenic. Pollen antigens generally comprise 0.5 to 1.0% of the total extractable pollen proteins. Further only limited areas in the antigen take part in forming immune complexes. These sites are known as antigenic determinants or epitopes. Small molecules (haptens) may become immunogenic when bound to a bigger molecule. Therefore, the contaminating constituents unrelated to the few allergens to which a patient is actually allergenic, can lead to the induction of IgE antibodies with new specificities and lead to untoward effects, including anaphylaxis. In man, systemic anaphylaxis may occur after administration of heterologous proteins in the form of antisera, hormones, enzymes, polysaccharides, etc. The severity of the disorder varies with the level of sensitization. However, the shock dose of antigen may be exceedingly small, e.g. the tiny amounts used in ordinary skin testing for various forms of allergies. Within minutes after exposure, itching, hives and skin erythema appear, followed shortly thereafter by a striking contraction of respiratory bronchioles and the appearance of respiratory distress. Laryngeal edema will result in hoarseness. Vomiting, abdominal cramps, diarrhoea, and laryngeal obstruction follow, and the patient may go into shock and even die within an hour. Thus, during the last two decades scientists, particularly allergologists, have concentrated more on the efficacy and safety of ASIT and have succeeded to a certain extent by purifying the relevant allergens by conventional physicochemical and immunochemical methods and produce these allergens, which may be present in minute amounts in pollen, in sufficient quantities by the application of the principles of recombinant DNA technologies for the characterization of allergens and

molecular cloning of an impressive array of allergenic proteins from a variety of pollen from diverse plants.

- With the present day knowledge of epitope mapping and the fast developing method of molecular cloning, the present study will not only enable us in designing standardized immunotherapic vaccines but will also help in the production of unlimited quantities of defined allergens for allergen immunotherapy (AIT). Once diagnosis of atopy is confirmed and specific antigens identified, the mainstay of treatment would be to avoid the allergen wherever possible followed by general awareness by pollen control ordinances through media and instead of the symptomatic treatment using tropical and antiallergic drugs which shows troublesome side reactions in certain patients, one should adopt treatment with properly standardized antigenic extracts in AIT.