Epitope mapping of the allergenic proteins of the pollen of *Datura* sp. and its implications for immunotherapy

Allergic rhinitis, also known as hay fever is the most common of all allergic diseases affecting approximately 400 million people worldwide. It is triggered by allergens present in our immediate environment such as pollen, mould, fungal spores, dust, mites, pet hair, etc. The role of pollen in the etiology of nasobronchial allergy is now very well established. There are several tests to reveal the specific allergens to which an individual is sensitive. Among these skin prick test, intradermal test, intradermal or intracutaneous injection inhalation, nasal or bronchial mucosa-provocation tests or the histamine release test are some commonly employed to detect allergens (Stanley and Linskens, 1974). There are also certain immunoassay methods which include the radio-allergo-sorbent-test (RAST) developed by Wide et al. (1967) and a cheaper and easier method - enzyme linked immuno sorbent assay (ELISA) developed by Engvall and Pearlman (1971).

One of the most successful treatment for allergic rhinitis is immunotherapy, also called hypo-sensitization. Identification of the actual allergenic components of pollen, their characterization and epitope mapping of these allergenic fractions is very essential for preparation of vaccines for successful immunotherapy.

The present study was undertaken to identify the allergenic protein fractions of the pollen of three related species of *Datura* (*Datura metel, Datura inoxia* and *Datura stramonium*) and Epitope mapping of the allergenic proteins of the pollen of *Datura* sp. in order to identify and characterize the binding sites of antibodies which can aid in the development of new therapeutic vaccines for successful immunotherapy. Thus the overall study involved the following:

- Ultrastructure study of the pollen by Light microscopy, SEM and TEM for proper identification of the airborne pollen to which a patient is exposed to in his or her immediate vicinity and to study any specific

features of pollen morphology as well as the ultrastructure that might have the potential to influence its allergenicity.

- Extract, quantify and study the soluble protein profile of the pollen of the three related species of *Datura* using standard methods and gel electrophoresis and partial characterization of the proteins (determining the molecular weights).
- Study the variation in protein profile with maturity of pollen (before and after anthesis).
- Isolation of the individual protein fractions by gel filtration.
- Identification of the allergenic protein fractions by performing Ouchterlony Immunodiffusion and ELISA with blood plasma of *Datura* sensitive patients.
- Development of antibodies in Male LOBUND –Wistar rats and study cross reactivity among the 3 species of *Datura* by Ouchterlony Immunodiffusion and ELISA to identify the common proteins fractions.
- Crosslinking coupled Mass Spectrometry for epitope mapping. Detection and identification of antigen-antibody binding location with high mass MALDI detection (high resolution mass spectrometry or MS/MS techniques) and amino acid sequencing of the epitopes.

The pollens from the three species of *Datura* i.e. *Datura metel, Datura inoxia* and *Datura stramonium* were collected and then processed by applying different methodologies to evaluate the comparative protein concentrations of the pollen on seasonal basis, SDS-PAGE protein profiles of the pollen of the three species of *Datura* were also performed. Isolation of individual protein fractions by gel filtrations, analysis of protein fractions by PAGE were also carried out to obtain the individual proteins from the pollen. Later on, identification of allergenic protein fractions was completed by employing Immunodiffusion, ELISA and many other techniques. Cross reactivity among these three species of *Datura* was also performed. Finally epitope mapping was conducted to identify antigenic determinants of the allergenic protein fractions

of the pollen of three species of *Datura* i.e. *Datura metel, Datura* inoxia and *Datura stramonium* by MALDI/TOF.

An additional study on ultrastructure of pollen byy SEM and TEM was also carried out to analyze the relativity of pollen structure with its allergenicity.