Chapter-2

REVIEW OF LITERATURE

2. Review of literature

The history of onset of allergic diseases is almost as old as the history of mankind. The modern perspective with regard to allergy started in early 19th century. Today more than 20-30% of the global population is widely known to experience with various allergic disorders like allergic rhinitis or hay fever, asthma, eczema and urticaria etc (Charpin et al., 2000; Chhabra et al., 1998; Schafer and Ring, 1997; Schmid, 1998;). Bostock (1819) had wrongly attributed the root of hay fever to the heat of summer (Ramachandran and Aronson et al., 2011). Blackely (1857) being a sufferer himself noted that hay fever was more prevalent in educated town dwellers and uncommon in farm workers. He also noted that some army officers in India were affected by hay fever. Later, he provoked himself with 35 types of pollen and concluded that grass and cereal pollen grains are important factors for allergic disorders. Risk factors of allergic rhinitis were clearly identified during 19th century when the origin of hay fever was attributed to pollens (Blackley, 1873; Pheobus, 1859).

Different studies in Australia contributed significantly to the development of aeropalynological knowledge with discovering the significant role of air borne pollen in the diagnosis as well as the treatment of allergic ailments. Pollen count was investigated by Meier (1941) in atmosphere of Adelaide (Australia) and recorded Asteraceae, Casuarina, Cupressus, Myrtaceae, Grasses, Pinus, Plantago, Populus, Quercus, Ulmus, and some other pollen types (Derrick,1965, Moss 1965).

Scanning electron microscopy has reached very high levels of refinements and is virtually an everyday tool for study of microcharacters of surface features. It was in late 1960s that the first scanning electron microscopes became commercially available. From a barely attainable resolution of 50nm (500 Å) on the first instruments, a resolution of down to 2 nm (20 Å) is guaranteed on modern machines. Hundreds of thousands of SE micrographs are produced each year and several annual conferences are held and specialized bibliographies issued. As a consequence has been that the study of microcharacters is now an important field. The impact of the SEM on the study of surface features- seeds, spores, fruits, pollen grains, leaf surfaces was immediate and by 1971, the scanning electron microscope had become a routine tool in biological research (Heywood, 1984).

Siemens produced a transmission electron microscope (TEM) in 1939. Transmission electron microscopes have remarkably higher resolution than light microscopes due to the use of electrons as illuminating agent. The smaller de Brogile wave length of electrons helps in the analysis of minute details of the objects. Due to this reason, Transmission Electron Microscope is able to capture the fine details of an image as smaller as a single beam of electrons which is thousand times smaller than the resolution obtained under a light microscope. Thus TEM becomes the major analytical tool in the field of chemical, physical and biological sciences. The application of TEMs is not only restricted to material science, cancer research and virology but also to pollution, nanotechnology and semiconductor research. The higher resolution and contrast of images of TEM at lower magnification is due to differential absorption of electrons brought about by variations in the thickness and composition of the material. However at higher magnification, analysis of images requires an expert observation as complex wave interactions in this case modulates the intensity of the overall image. TEM can also be alternately used to observe and study modulation in orientation of crystals, identity of chemicals, and shift in electron phase induced by sample, electronic structure as well as imaging based on regular absorption by using alternate modes of this technique.

Max Knoll and Ernst Ruska were the first to develop a Transmission Electron Microscope in 1931 which had a resolution much higher than that of light microscope. More sophisticated TEM with greater resolution came up in 1933 and the first ever commercial TEM was available in 1939.Ruska achieved Nobel Prize in 1986 for the invention of Transmission Electron Microscope in physics. Albert Prebus and James Hillier at the University of Toronto were the first to construct TEMs in North America in 1935 and 1938, respectively, with advancing TEM design.

In identification of plant taxa pollen morphology performs a great role. Deficiency of enough information in the field of palynology leads to wrong identification of plant taxa which subsequently results in wrong interpretation or diagnosis of symptoms of diseases cause by the airspora. The morphological characteristics of pollen grains are embodied in the exine. The pollen grains are double walled- outer exine and inner intine with exoaperture and endoaperture containing colpi, costae and fastigia (Kosonko et al., 1999; Larsen et al., 2000). Exine ornamentation often performs as an important character in identification of plant taxa.

Diethart et al. in 2007 examined the pollen wall ultrastructure of allergenic as well as non allergenic pollens of the most central European region by employing Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). He specially focused on pollen architecture and orientation of the exine of the pollens to show the relationship of pollen wall in allergenic discharge and also in protein storage and allergy induction. The pollen morphology of *Datura* along with the other plants of family Solanaceae was examined in detail by Parveen et al., in 2007. Weber et al., in 2010 focused on the Transmission electron micrographs of endexine of pollen outer wall and their different staining properties.

Sometimes, limitations and taxonomic relationships become very controversial among similar species. In case of pollen, among all other characters, two distinguishable characters i.e. pollen wall ultra structure and exine ornamentation (sculpture) are very important for identification of similar species. The pollen grain morphology, the structure of pollen wall and the ornamentation on sculpturing of the outer wall i.e. the exine have proved to be valuable characters for taxonomic studies particularly for distinguishing between closely related species as a lot of controversy and discrepancy still prevails regarding the taxonomic relationships, circumscription and systemic position of some very closely related species. Several weeks have been done in this context. The diversity in pollen morphology amidst the same species was shown by Rosenfeldt et al., in 2006 in *Oxalis* pollen grains. He studied the pollen grains of some closely related species of *Oxalis* and commented on the minute variations in the pollen morphology.

So from the context of ultrastructural, taxonomical and palynological studies the analysis of ultrastractures of pollen by SEM and TEM is a prerequisite for further studies.

In India, it has been estimated that 20% of the total population suffers from one or other form of allergic disorders. Due to the great diversity of vegetation and varied meteorological factors the aeroallergens vary from place to place. Cunningham (1873) was the first who carried out systematic aerobiological work in India. His work was quite comprehensive and was published as a Book entitled 'Microscopic Examination of Air' and included studies over presidency jails, (Kolkata). He observed a large number of spores and other vegetable

cells in the atmosphere but he was not successful in showing a relationship between the numbers, the types of airborne molecules which were airborne and the prevalence of Zymotic diseases.

Banik (1987) carried out detailed investigations on airborne pollen in Calcutta with special implication to respiratory allergy. During study 33255 pollen grains were recorded by using Burkard Volumetric Trap and Vertical Cylinder Trap at the same height and the same place. The total recorded pollen count (4124) indicated greater efficiency of the volumetric trap. Ronmark et al. (2009) reported in his study about the patients of asthma, rhinitis and eczema were sensitive to different air borne allergens. A cross study was performed in northern Sweden among younger children between the age of 7-8 years. In the study, 3431 (97%) patients participated of which two-thirds of the children had undergone through a skin prick test with 10 common airborne allergens.

Allergen specific immunotherapy is the most successful treatment for allergy. It reduces the severity of symptoms. Allergen-specific immunotherapy was first reported by Leonard Noon in 1911 in his experiments with grass pollen. The earlier work conducted by Willium Dunbar was quoted in his article, where it had been shown that anti-sera raised against pollen extract can also neutralize allergen-induced conjunctival inflammation (Noon 1911; Dunbar 1903). Allergy is a hypersensitivity disease mediated by immunoglobulin E affecting more than 25% of the population (Floristrup et al., 2006). A recent study revealed the molecular activity of IgE activity profiles which profoundly changed the knowledge of vaccines designed for allergy among the allergologists (Sastre et al., 2012). Dunbar (1978) showed by his earlier study that allergen specific blocking antibodies induced a series of reactions through which AIT worked. Before 80 years , in 1935, when the IgE antibodies were not identified, Cooke et al. (1935) had demonstrated by a series of sophisticated experiments that IgG induced by AIT can inhibit allergen specific skin inflammation. Since then, the importance of allergen specific IgG antibodies competing with IgE to bind with allergen had been studied and demonstrated to understand the mechanism of action of AIT (Larche et al., 2006). So, AIT and the traditional form of it, preferably known as subcutaneous AIT might be considered as therapeutic vaccines of allergy.

The late symptoms of allergy, (e.g. atopic dermatitis and chronic asthma) can result from IgE-mediated allergen presentation and leading to the activation of T cells with the subsequent release of pro inflammatory cytokines (Bieber et al., 1996; Stingl, 1997). Unfortunately, in immunotherapy crude extracts of natural resources directly prepared by extraction, are used as reagents and contain additional, ill-defined components and for which it is very difficult to (if not impossible) to set a standardize dose of allergen. Despite of these limitations, immunotherapy till date is considered to be the most viable therapeutic option. Recent developments in molecular cloning and the sequencing of amino acid of major allergens have facilitated the design and manufacture of novel therapeutic vaccines which have made it possible to design novel therapeutic approaches for safer and improved forms of allergen immunotherapy (Singh et al., 2003).

Another very effective test i.e. skin prick test (SPT) is used as the major diagnostic process to detect type I allergic reactions which includes allergic rhinitis, acute utricaria, atopic asthma, food allergy etc. So, SPTs are done to identify the cause of allergy sensitivity in a patient and applied to express immunotherapy as a therapeutic treatment (Rasool et al., 2013).

Skin prick tests have been performed in various laboratories and clinics around the world. Tilak et al (1989) performed intra dermal skin prick tests on 160 patients. Out of 160 patients 58.75% of the patients were male and 41.25% were female. Among the total tested patients, 142 showed positive response to one or more antigens, while 18 showed negative response to all the antigens. 95 patients showed markedly positive reactions i.e.2+ to 3+ reactions. From these results it was evident that 2+ and 3+ reactions were commonly found in allergic patients sensitive to pollen antigens. An extreme of 4+ reaction was observed in allergic patients sensitive to *Dodonea* and *Panhenim* pollen antigens. Batabyal et al. (1985) performed RAST and PRIST for the determination of IgE level in the atopic and non-atopic allergy caused by *Chenopodium album* pollen.

Banik (1987) performed skin sensitizing tests (SPT) using a sterile antigenic extract of 22 pollen types. The selection of specific types was based on the respective case history symptoms and data of the patients' residential surroundings. Out of the 2224 patients tested, 54.64% were male and 42.36% were female. Significant positive

reaction were observed applying grass pollen antigen (73.92%) followed by *Saccharum* sp, (also a grass species 66.66%) and *Azadirachta indica* (49%).

Mahadik et al. in 2005 studied *Borassus flabellifer* to explore its pollen allergy by the skin prick test and nasal provocation test in Mumbai. They also identified major allergens by immunoblast analysis. The allergic potential of the pollen was studied on 333 patients by the skin prick tests (SPT) and nasal provocation test with pollen extract from *Borassus flabellifer* on SPT positive tests. SPT on 333 respiratory allergic patients with whole pollen extracts exhibited severe (4+) response in 22 patients (6.9%).

To determine and to monitor the IgE reactivity profiles of allergic patients, protein microarray is used and it offers additional advantages of fast and reliable diagnosis of the disease causing molecule(s), using a very small quantity of subject's serum (Hiller et al., 2002). The potential side-effect of injecting allergen into any specifically IgE-sensitized individual is anaphylaxis (Larsen et al., 2016). Later depending on the structure of recombinant allergens, design of epitopes and peptides, invitro and invivo diagnostic tests have also been developed which allow to properly diagnose the allergens and monitor the immune responses precisely during treatment (Valenta et al., 2010). Most significantly, it has been possible to develop effective and sophisticated allergy vaccines for the availability of the sequences and structures of particular allergens which after passing through successful clinical evaluation herald the emergence of more useful, safe and convenient allergy vaccines in near future and it even expects that development of prophylactic vaccination is at doorstep (Valenta,2010). SITs can induce allergen specific immune responses (Linhart et. al., 2012). Linhart et. al. later on proved the involvement of T-cell epitope which do not induce IgG responses in SIT.

The most modern, effective and versatile technique to determine partial structures of soluble antigens which bind with antibodies in solution is mass spectrometric epitope mapping. Generally the length of an antigen is 100 amino acids while the length of an epitope is decreased to 10-15 amino acids. The early 1990s showed a boom in the development of more and more sophisticated techniques and successful approaches have been developed towards the field of epitope mapping with immobilized, partially immobilized and soluble antibodies. Currently monoclonal antibodies are mostly used

in epitope mapping although the the use of polyclonal antibodies in this field has also been proven. Two very important methods in case of epitope mapping viz- epitope excision and epitope extraction are also developed based on the resistance of antibody towards enzymatic proteolysis. Sample consumption for both antigen and antibody has been minimized to low picomole for fruitful epitope mapping. Currently mass spectrometry rapidly outperforms all other alternative techniques in the epitope mapping. Thus its use has been reached at a state of maturity. After the constant and steady improvement during, the application of it in case of clinical sample such as patient characterization and stratification has been evolved as a great approach in scientific field.

To understand the proper cellular mechanisms related with tolerance in healthy individuals and immunopathology in atopic patients, characterization of immunodominant epitopes is critical in case of major allergens (Stoecklinger et. al, 2012). Increasing evidences are there that the T-cell recognition by major allergens is attributed to the binding capacity of discrete regions of overlapping epitopes with Human Leukocyte Antigens (HLA) (de Lalla et al, 1999; Friedl-Hajek et al, 1999; Taxier et al, 2002,). Therapeutic applications of immunotherapy are based on the practical importance of identification of such regions (Ali et al., 2005, Campbell et. al, 2009) or the application of hypoallergenic allergen derivatives, where conservation of immunodominant T cell epitopes has to be warranted (Campana et al, 2010; Saarne et al, 2005). During *in vitro* assay of peptides recombinant proteins simplifying the identification of the restimulation of allergen specific CD4+ T cell restimulation. In this process no uptake and processing by antigen presenting cells is required. Additionally, MHC (major histocompatibility complex) class II peptide tetramer technology is used to detect and characterize allergen specific CD4+ T cell response with knowledge of proper structure and function of T cell epitopes.

The diagnosis of hypersensitivity is attained through vigilant case history and thorough physical check up and a prior reasoning concerning the clinical features of the particular allergen and judicious selection and interpretation of tests including skin test RAST, ELISA etc (Aalberse, 2000; Sampson, 2005; Sicherer, 1999,). These tests are limited to the specific monoclonal IgE antibody. Compounding the clinical challenges of identification of particular allergen present in various plants and animal

proteins is a matter of phenomenon of cross reactivity (Sicherer, 2001). Pollen allergens are small proteins or glycoproteins in nature, stable in structure within molecular weight of 5-80 kDa (Grote et al., 2000, 2005; Knox & Suphioglu, 1996). From sequence comparison it was found that they correspond to only 29 protein families of seed plants which include expansins, calcium binding proteins, pathogen related proteins and profilins (Chapman et al., 2007; Radauer & Breiteneder, 2006). As a result there may be sequence similarities between two or more proteins generating the incident of cross reactivity.

Cross- reactivity results in allergic reactions without exposure to a previous source of allergens due to sequence similarity of IgE epitopes of proteins. The common example of it is oral allergy which is happened for the similarity between food proteins and pollen allergens. The same incident is common for brich pollen for the similarity between allergens of Bet V1 protein family and profilin (Palacin et al., 2006; Salcedo et al., 2004; and Valenta et al., 1996). Grass pollen gr 1 allergens show extensive cross reactivity reactions among grass species as for 50% sequence homology (Chapman et al., 2007). Teuber et al. in 1999 showed the cross reactivity reactions among IgE binding protein from coconut with other nuts like peanut, almond and walnut. Subiza et al. in 1989 revealed the cross reactivity reaction of Artemisia pollen with the pollen of *Matricaria*.

Cross-reactivity occurs when two proteins from two different sources resemble each other. As the consequences, immune system can't recognize them differentially. Tremont et. al. (1974) in his study with different strains of *Neisseria gonorrhoae* showed atigenic differences and similarities in serum bacterial reactions against antisera of hyperimmune rabbit to *Neisseria gonorrhoae*. Thus cross reactivity is the modern area of research to evaluate the same characters of anitigens present in different species.

Datura is a herbacious perennial plant of the family Solanaceae grown in the tropical and temperate region of the globe. A number of species especially jimsonweed or thornapple (*Datura stramonium*) toloache (*Datura inoxia*) and *Datura metel* had been used by Mexiacan Indian people for various medicinal purposes from ancient era. Leaves are dark green in colour, simple, alternate, broadly ovate, shallowly lobed and glabrous. Flowers are large, solitary, and trumpet-shaped. It is a pleasantly

scented flower usually emitted its fragrance in early mornings and evenings. It has a variety of range of colors from white to dark and light purple and yellow. The flowers are bisexual or hermaphrodite and entomophilous (insect pollinated) in nature and fruits are in form of capsule containing spines (Khaton et al., 2012). The medical value of *Datura* was proved from ancient era (Parveen et. al., 2006). *Datura* can withstand average range of soil with rich and moist even alkaline soil. It can't survive under shade. The compatible warm temperature is necessary for its growth and hence mainly found in warmer regions (Drake et al., 1996). It is mainly American in origin and available in tropical and subtropical regions for its medicinal use and beautiful flowers (Glotter et al., 1973).

Datura metel is largely grown in East Asia and India and also being used as traditional medicines in Bangladesh. In Chinese traditional medicine it is often used with the name of Baimantuoluo to treat skin inflammation and Psoriasis (Wang et al., 2008). In Indian Ayurveda the seeds are used in the treatment of Ulcer, Bronchitis, skin rashes, Jaundice and Diabetes. Presently, different species of *Datura* also yield secondary metabolites (Agharkar et al., 1991). In Brazil, tea is made from dried seeds which serves as sedative and flowers are dried and crushed to smoke like cigerettes (Agra et al., 2007).

D. inoxia is an Old World plant closely related with *D. metel* and similar effect was found in case of it described by Avicenna in eleventh century Persia. The closely related *Datura stramonium* has smaller flowers like *Datura stramonium* with toothed edged leaves. *D. inoxia* differs from other two species of *Datura* in having 7-10 secondary veins on either side of midribs which anastomose at arches at 1-3mm top from the margin.

It has been proved by scientific studies that the pollen of *Datura* creates a risk of allergenic diseases in sensitive patients (Parui and Mandal, 1998). In spite of this, it is not being considered as serious allergenic hazards due to its entomophilous nature of taxa. But the presence of *Datura* pollen in air spora have been reported by several surveys. (Agashe et al., 1983; Agashe, 1989; Atluri et al., 1992; Singh and Babu, 1982; Singh and Devi, 1992; etc.). Tilak (1980) has critically reviewed the air borne entomophilous pollen disproving the earlier concept that entomophilous pollen is not airborne. During routine aeropalynological work pollen of *Aradirachta indica*,

Acalypha, *Cantata* sp., *Bougainvillea* sp., *Brassica campestris, Mangifera indica* , *Nerium* sp., *Hibiscus rosasinensis*, *Polygonum* sp., *Melia azedarach* were entrapped. Singh et al. (1980) made a survey of airborne pollen and spores of Delhi. They made a pollen calendar to render identification of airborne polynomorphs. Singh et al., (1981) investigated the concentration of pollen allergens at human height. The atmosphere of Delhi was reported dominated by the pollen of *Acer, Cupressus. Fraxinus, Morus, Pinus, Populus. Salix* and *Ulmus* abundantly in the air spora of Delhi.

Thus, epitope mapping of the allergenic protein of the pollen of different species of *Datura*, its implications for immunotherapy and ultrastructural studies of the allergenic pollen by electron micrographs still remain a subject for further study.