

Table of Contents

Page No.

Acknowledgement	i-ii
1. Introduction	1
1.1 Role of protein in allergenic rhinitis	1
1.2 Symptoms	3
1.3 Allergenic reactions	4
1.4 Types of allergic rhinitis	5
1.5 Diagnosis.....	6
1.6 Treatment of allergic rhinitis	6
1.7 Undesirable side effects	7
1.8 Cross reactivity in allergic rhinitis.....	8
1.9 Significance of the study.....	8
1.9.1 Pollen of <i>Datura</i> sp.....	8
1.9.2 Role of the pollen of <i>Datura</i> in allergenic rhinitis.....	9
1.9.3 Pollen morphology.....	10
1.9.4 Importance of epitope mapping in Immunotherapy.....	12
1.10 Aims and objectives of the study	13
2. Review of the literature	14
3. Materials and methods	24
3.1 Pollen collection.....	24
3.2 Plant Identification.....	25
3.3 Ultrastuctural analysis of pollen of <i>Datura</i> sp.....	27
3.3.1 Light Microscope Study	27
3.3.2 SEM study.....	28
3.3.3 TEM study	28
3.4 Protein extraction, isolation and characterization.....	29
3.4.1 Protein extraction	29
3.4.2 Estimation of proteins	29
3.4.3 Gel electrophoresis.....	30

3.4.4	Isolation of individual protein fractions by gel filtration.....	30
3.4.5	Analysis of protein fractions by PAGE	31
3.5	Identification of antigenic fractions and sequencing	31
3.5.1	Preparation of antigenic extracts from total protein	31
3.5.2	Preparation of antiserum	32
3.5.3	Ouchterlony- Double immunodiffusion.....	32
3.5.4	Production of species specific antibody in rat	32
3.5.5	Collection of blood serum from rat.....	33
3.5.6	Identify the allergenic protein fractions and measure the total and specific immunoglobulin in serum by ELISA- inhibition.....	33
3.5.7	Study the cross reactivities.....	34
3.5.8	Epitope mapping	35
4.	Results and discussions.....	37
4.1	Diagnostic Characters	37
	Ultrastructural study of the pollen of <i>Datura metel</i> , <i>Datura</i> <i>stramonium</i> and <i>Datura inoxia</i>	46
4.2	Study of ultrastructure of pollen	47
4.2.1	Ultrastructure study by SEM	47
4.2.2	Ultrastructure study by TEM	63
4.2.3	Analysis of TEM study	68
	<i>Datura metel</i>	69
4.3	Protein Extraction, isolation and characterization of <i>Datura metel</i>	70
4.3.1	Protein concentration of the pollen of <i>Datura metel</i>	70
4.3.2	Analysis of SDS-PAGE protein profile study of <i>Datura</i> <i>metel</i>	71
4.3.3	Isolation of individual protein fractions by gel filtration.....	76
4.3.4	Analysis of protein fractions isolated by gel filtration.....	76
4.3.5	Identification of antigenic fractions by immunodiffusion	78
	<i>Datura stramonium</i>	81
4.4	Protein Extraction, Isolation and Characterization of <i>Datura</i> <i>stramonium</i>	82
4.4.1	Protein concentration of the pollen of <i>Datura stramonium</i>	82

4.4.2	Analysis of SDS-PAGE protein profile of <i>Datura stramonium</i>	83
4.4.3	Analysis of protein fractions isolated by gel filtration.....	87
4.4.4	Identification of antigenic fractions by immunodiffusion	89
	<i>Datura inoxia</i>	91
4.5	Protein Extraction, isolation and Characterization of <i>Datura inoxia</i>	92
4.5.1	Protein concentration of the pollen of <i>Datura inoxia</i>	92
4.5.2	Analysis of SDS-PAGE protein profile of <i>Datura inoxia</i>	93
4.5.3	Analysis of protein fractions isolated by gel filtration.....	97
4.5.4	Identification of antigenic fractions by immunodiffusion	99
	Study of cross reactivity.....	101
4.6	Study of cross reactivity.....	102
4.6.1	Cross reactivity between total extract of pollen.....	102
4.6.2	Cross reactivity between different protein fractions of the three species of pollen.....	104
4.6.2.1	Antiserum of <i>Datura metel</i> and protein fraction of <i>Datura inoxia</i>	104
4.6.2.2	Antiserum of <i>Datura inoxia</i> and protein fractions of <i>Datura metel</i>	105
4.6.2.3	Analysis of cross reactivity reaction between <i>Datura metel</i> and <i>Datura inoxia</i>	106
4.6.2.4	Antiserum of <i>Datura metel</i> and protein fractions of <i>Datura stramonium</i>	107
4.6.2.5	Antiserum of <i>Datura stramonium</i> and protein fractions of <i>Datura metel</i>	108
4.6.2.6	Analysis of cross reactivity reaction between <i>Datura metel</i> and <i>Datura stramonium</i>	109
4.6.2.7	Antiserum of <i>Datura stramonium</i> and protein fractions of <i>Datura inoxia</i>	110
4.6.2.8	Antiserum of <i>Datura inoxia</i> and protein fractions of <i>Datura stramonium</i>	111
4.6.2.9	Analysis of cross reactivity reaction between <i>Datura stramonium</i> and <i>Datura inoxia</i>	112

A comparative study of the protein profile of the pollen of <i>D. metel</i> , <i>D. inoxia</i> and <i>D. stramonium</i> and degree of allergenicity	113
4.7 A comparative study of the protein profile of the pollen of <i>D. metel</i> , <i>D. inoxia</i> and <i>D. stramonium</i> along with the degree of allergenicity of the pollen proteins	114
4.7.1 Study of pollen protein concentration of the three species of <i>Datura</i>	114
4.7.2 A comparative account of the SDS-PAGE protein profile of the pollen of <i>D. metel</i> , <i>D. stramonium</i> and <i>D. inoxia</i>	115
4.7.3 Comparative study of cross reactivity showing common allergenic proteins among the pollen of three species of <i>Datura</i>	118
Epitope mapping	119
4.8 Epitope mapping	120
4.8.1 Allergenic protein ID	120
4.8.2 The length, PI value, Molecular weight, No. of peptide and coverage of the allergenic pollen proteins	120
4.8.3 Description of the allergenic proteins	121
4.8.4 Protein sequence of the allergenic proteins	122
5. Conclusion	129
5.1 Ultrastructural study of <i>Datura</i> sp	129
5.2 Protein concentration and characterization of the allergenic proteins.....	130
5.3 Cross reactivity among the three species of <i>Datura</i> sp.....	131
5.4 Epitope mapping	131
5.5 Future direction of the study	132
6. Bibliography	135
7. List of publications.....	150