Abstract

Food allergy is a raising health concern worldwide. A large number of food allergens, usually proteins capable of inducing allergic symptoms, including severe, even life-threatening reactions in predisposed individuals, have been identified and characterized. As most of these proteins are from our daily dietary intake, they are often difficult to avoid. However, the proteins that cause such immunoglobulin E (IgE)-mediated reactions can be assigned to only a limited number of protein families. Detailed knowledge about the characteristics of food allergens, their structures, biological activity, and stability, may be helpful in improving diagnosis of food allergy, avoiding unnecessary exclusion of diets, and assessing the risk of cross-reactive allergies to other food sources. Among several allergic foods, legumes play important role due to its high consumption throughout the world as one of the major vegetarian protein sources. The allergenicity of several legumes including peanut, soybean, lentil, green bean and red gram have been well studied. Red kidney bean or RKB (*Phaseolus vulgaris* L.), is a common legume, consumed worldwide. The delicacy of RKB is highly appreciable but, at the same time their toxicity and allergenicity have raised alarming concern.

In the Chapter 2, prevalence of RKB allergy was studied in human subjects suffering from nasobronchial asthma and allergic rhinitis. This study was carried
out in 350 human subjects by using a standard questionnaire, skin prick test (SPT), total IgE, specific IgE and IgG1 levels. Further, attempts were made to identify the responsible proteins of RKB by IgE immunoblotting. The proteolytic resistance of red kidney bean proteins was studied by simulated gastric and intestinal fluid (SGF and SIF) assays. The prevalence of RKB allergy was evident in 5.7% allergic patients. These patients showed clinical history of nasobronchial asthma, allergic rhinitis, dermatitis and urticaria. The levels of total IgE, specific IgE and IgG1 were found elevated when compared to that of the control subjects. The IgE immunoblotting using RKB allergic patient’s sera showed five IgE binding proteins in RKB of approx molecular weight 170, 100, 43-50, 34 and 20-25 kDa. The SGF and SIF assays showed five pepsin resistant proteins with approx molecular weight 170, 100, 43-50, 34 and 20-25 kDa. Finally, this work demonstrated that RKB consumption may induce allergic responses in human subjects, with identification of five clinically relevant IgE binding and pepsin resistant protein components.

As we cannot study exhaustively in human subjects, therefore in the Chapter 3 allergenicity of RKB was explored in BALB/c mice, splenocytes, bone marrow mast cells (BMMC), peritoneal cells derived mast cells (PCMC) and rat basophilic leukaemia (RBL-2H3) cells. This study was further extended to understand the role of phytohemagglutinin (PHA, mainly PHA-P) in RKB induced allergenicity. RKB-CPE treated mice showed enhanced levels of total and specific IgE, anaphylactic symptoms, histamine, and mouse mast cell
protease-1 (mMCPT-1) over control. An enhanced release of β-hexosaminidase release was observed in the passively sensitized RBL-2H3 cells exposed with RKB-CPE when compared to control. Further, it was also observed that PHA-P may augment RKB induced allergenicity. Taken together, oral exposure of RKB-CPE without and with adjuvant caused allergic symptoms in mice.

In the Chapter 2 and 3, prevalence of RKB allergy was described in human subjects as well as BALB/c mice. The prevalence of allergy induced by RKB led us to carry out identification and characterization of clinically relevant IgE binding proteins in RKB-CPE in the Chapter 4. Sera from 20 RKB allergic patients were used for one and two dimensional IgE immunoblotting. The IgE binding proteins were identified and characterized by IgE immunoblotting, liquid chromatography tandem mass spectrometry (LC-MS/MS) and bioinformatic approaches. One dimensional IgE-immunoblotting using individual patient’s serum demonstrated IgE binding proteins with approx molecular weight 170, 100, 43-50, 34 and 20-25 kDa in RKB-CPE. Twelve RKB-CPE proteins showed IgE binding capacity in two dimensional IgE-immunoblotting using pooled sera of RKB allergic patients. Further, five IgE-binding proteins were characterized using LC-MS/MS as legumin, phaseolin, IAA-protein conjugate, albumin-2 and phytohemagglutin. In conclusion, red kidney bean contains IgE binding proteins which may induce allergic responses in the susceptible subjects.

This study of the Chapter 5 was aimed at the purification and characterization and a thorough elucidation allergenic potential of phaseolin. Phaseolin was purified by (viv)
ammonium sulphate fractionation, anion exchange and gel exclusion chromatography and characterized by peptide mass fingerprinting (PMF) as a major IgE binding protein of RKB. Phaseolin treated mice showed enhanced levels of specific IgE and IgG1, monocyte chemtactic protein (MCP-1), anaphylaxis symptoms, histopathological changes, mRNA expressions of IL-4, IL-5, IL-13 and GATA-3 in the lung, spleen and intestine and IL-4, IL-5 and IL-13 in phaseolin exposed splenocytes culture supernatants over control. Taken together, phaseolin was found to possess characteristics of the potential allergen that may lead to hypersensitivity responses in the susceptible individuals.

In summary, consumption of RKB may leads to allergenic manifestations in the susceptible individuals due to the presence of its allergenic components including phaseolin a clinically relevant allergen.

**Keywords:** Allergy; Legumes; Allergens; Transcription factors; Allergic mediators; Proteomics; Bioinformatics