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Allergenicity assessment of *Buchanania lanzan* protein extract in Balb/c mice

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ABSTRACT

Tree nuts are among "Big Eight" and have been reported globally for causing allergy. *Buchanania lanzan* (Bl) is one of the major tree nuts consumed by Indian population. However, very little is known about *B. lanzan*'s induced allergic manifestation. Therefore, evaluation of it's allergenic potential was undertaken. Bl-crude protein extract sensitized BALB/c mice sera were used to identify the allergic proteins by it's IgE binding capability. The major IgE binding proteins found with molecular weight of 11, 20, 23, 25, 48, 54, and 65 kDa. Specific IgE, specific IgG1, MCPT-1, PGD_2 and histamine were assessed in mice sera. Enormous amount of mast cell infiltration was noted in different organs. The levels of Th1/Th2 transcription factors GATA-3, SOCS3 and STAT-6 were found upregulated, whereas T-bet was downregulated. Furthermore, elevated Th1/Th2 cytokine responses were observed in mice sera. All together, these reactions developed systemic anaphylaxis upon Bl-CPE challenge in sensitized BALB/c mice. In order to confirm the evidences obtained from the studies carried out in BALB/c, the investigation was extended to human subjects as well. Control subjects and allergic patients were subjected to skin prick test (SPT). Later sera collected from those positive to SPT along with controls were used for IgE immunoblotting. The study evaluated the allergic manifestation associated with Bl, and identified it's proteins attributing Bl-mediated allergy. This work may help in managing tree nuts mediated allergies especially due to *Buchanania lanzan* sensitization.

1. Introduction

Food allergy is a widespread health concern all over the world with an increasing prevalence. The increased prevalence can be attributed to many factors like genetic, environmental and processing of food [1]. In India, the prevalence of food allergy has not been systematically studied and not much awareness about food hypersensitive reactions in Indian sub-continent is there. Food allergy is simply a reproducible hypersensitive reaction of the immune system following ingestion of food or its components, and this abnormal response can either be IgE or non-IgE mediated in nature [2].Various food components have been linked with fatal IgE mediated immune response, but major focus is given to mainly four food items that are cow's milk, hen's egg, peanut/tree nuts, and fish/shellfish [3]. Out of these peanut and tree nut together correspond to 70–90% of recorded anaphylactic reactions and tree nut alone summed up to 18–40% with almost every tree nut capable of inducing fatal allergic response [4]. Tree nuts that account for maximum reported allergic responses are walnut with 34%, cashew with 20%, almond with 15%, and pistachio with 7% [5]. Major tree nut derived food allergens from cashew nut (Ana o 1, Ana o 2, Ana o 3) and pistachios (Pis v1, Pis v 2) are well identified and characterized [5, 6]. Cashew nut and pistachios, both belong to Anacardiacecae family and exhibit cross reactivity to each other. *Buchanania lanzan* (Bl) is another tree nut from this family that is enriched with the 22% protein, 59% lipid, 12% carbohydrate and 4% fibres [7]. Bl is native to India, Australia, Thailand, Burma, Nepal and Pacific islands [8–10]. Bl is also called Chironji, or Charoli in India, Priyala in ayurveda (branch of Medicine practised in India) and Almodette in English [11]. It is used as a key ingredient in many Indian traditional cuisines, sweet dishes, for thickening of various savoury sauces, to flavour batters, to sprinkle fruit salads, soups, meaty kormas and in stewed rice. The Bl seed is also used as a substitute for almonds and the oil extracted from it is used in the

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place of both almond/olive oil [12]. It's high protein content (~22%) and widespread use in foods has raised concerns that it may cause IgE-mediated allergic reactions in sensitive individuals. Also, Bl has been reported to accelerate cell-mediated immunity and humoral immunity in BALB/c mice along with increased macrophage migration index (MMI), hemagglutination antibody (HA) titres and plaque-forming cell count [13]. Thus, it is perfect case to assess the allergenic potential of Bl.

Food allergy varies according to the food habits of different region/country. Indian population being major consumer of Bl (as flavoring agent) increases the probability of prevalence of allergy to this dry fruit and therefore this may be a cause of concern. Barely, any mechanistic studies have been carried out on allergens from Bl. Therefore, evaluation of allergenic potential of Bl followed by exploration of involved mechanistic pathways, are required in order to develop appropriate strategies to ameliorate its allergenicity. As mostly protein components of foods cause sensitization and result in allergy as well as severe anaphylaxis therefore, the aim of the current study was to explore the allergenicity potential of Bl and find out the culprit proteins that are responsible for its allergenicity. Female BALB/c mice were selected due to their immune responsiveness and were administered with Bl crude protein extract to mimic different parameters of allergenicity on humans as well. Present investigation is an effort towards assessment of allergenic potential of tree nuts, which would be an important contribution in the direction to develop specific immunotherapy as therapeutic approach to the problem.

2. Material and methods

2.1. Buchanania lanzan (Bl), cashew, pistachio and peanut crude protein extraction

Seeds of all the nuts were obtained from supermarket (Big Bazaar) (Bl- Batch no. RAI 1958; cashew-0F/117/05; pistachio-B/0418; peanut-B102016), and the same lot of seeds was used in the entire study. The crude protein extract was prepared as described in the previous method [14]. Precisely seeds were milled in a food processor and the powder so produced was defatted using of n-hexane (Merck, Worli, Mumbai, India), after which it was softened by PBS (phosphate buffer saline, 20 mM Na2HPO4, 2 mM KH2PO4, 5.4 mMKCl, 0.5 M NaCl, pH 7.0). The mixture formed was flustered overnight at 4 °C followed by centrifugation at 10,000 × *g* for 30 min. After that the resulting supernatant was collected and filtered via a 0.45 μm filter and stored (in aliquots) in a −80 °C freezer till the time of use.

2.2. Ethical approval

Skin prick test (SPT) and blood collection were carried out with patient's consent and the study protocol was approved by the human ethics committee of King George's Medical University, Lucknow, India (Ref. no.-2931/Ethics/R.Cell-18). Animal study was accomplished after the approval of Institutional Animal Ethical Committee of CSIR-IITR, Lucknow, India (IITR/IAEC/56/17).

2.3. Animal treatment protocol

Female BALB/c mice of 6–8 weeks old, weighing 22 ± 3 g were received from the animal breeding colony of Indian Institute of Toxicology Research, Lucknow. These mice were put in standard laboratory conditions in animal care facility of CSIR-IITR, M.G. Road campus, Lucknow and treated according to the method mentioned earlier [15, 16]. Briefly, mice were sensitized with either PBS, or Bl-CPE (*Buchanania lanzan*-crude protein extract) or Pn-CPE (Peanut-crude protein extract), where Pn-CPE was taken as positive control for the study. BALB/c mice (n = 15/group) were divided into three above mentioned subgroups and were administered with 100 μg protein intraperitoneally once in a week. Blood samples were collected via retro-orbital sinus to measure serum IgE and IgG1 antibodies on day 59. On day 60, the experimental mice were challenged i.p. with 1 ml of 5 mg/ml of Bl-CPE intraperitoneally. Tissue and blood samples were collected for further experiments.

2.4. Screening of Buchanania lanzan sensitive patients

Skin prick test (SPT) were executed with the allergen kit (All cure Pharma Pvt. Ltd. New Delhi, India) on allergic patients (n = 100) and normal, healthy individual (n = 10) at the Department of Pulmonary and Critical Care Medicine, King George's Medical University (KGMU), Lucknow, India. Briefly, glycerol histamine acid phosphate and glycerinated buffer saline (1:100) were used as positive and negative, respectively. Comparison was done with reference to the weal diameter and scoring was done as: smaller than histamine weal then +1, equal +2, larger +3 and for bigger with pseudopodia then +4. Blood was collected from Bl SPT positive patients and normal healthy volunteers. Patients showing no weals against Bl were assigned as Bl SPT negative patients and they were omitted from the study. Serum was separated from whole blood and stored at −80 °C for further analysis [17].

2.5. IgE immunoblots analysis

To identify IgE specific protein in Bl crude protein extract, IgE immunoblotting was accomplished by the method described earlier [18, 19].

2.6. Measurement of anaphylaxis score and rectal temperature

The systemic anaphylaxis index was measured in Bl treated mice as defined formerly on the scale of 0–5, where 0 stands for no symptoms, 1 for scratching and rubbing around the nose and head, 2 for puffiness around the eyes and mouth, diarrhoea, and reduced activity or standing still with an increased respiratory rate, 3 for wheezing, labored respiration, and cyanosis around the mouth, in score 4 symptoms are same as in No. 3 with loss of consciousness, tremor, and/or convulsion and 5 for mortality (death) [20]. Rectal thermometer (Bioseb, France) was used to record rectal temperature in Bl protein administered mice and their respective controls 20 min prior and subsequent to challenge.

2.7. Detection of cross-reactive proteins with cashew, pistachio and peanut using Bl-sensitized mice sera

Proteins extracts of cashew, pistachio and peanut were separated by SDS-PAGE and electroblotted to polyvinylidene difluoride (PVDF) membrane. A serum pool containing IgE specific to the major allergens of Bl was used to incubate the immunoblots. Detection of IgE was carried out by the method discussed earlier [18, 19].

2.8. Buchanania lanzan protein specific IgE and IgG1 measurement in mice sera

Assessment of specific IgE and IgG1 levels in control and Bl protein treated mice sera was conducted using previously illustrated method [15].

2.9. Estimation of MCPT-1 and TSLP

Blood was collected from the all the experimental groups after 30 mins of challenge and serum was collected to be used for the assay. The serum levels of mouse mast cell protease-1 (MCPT-1) and thymic stromal lymphopoietin (TSLP) were assessed in Bl protein treated and control mice using ELISA (for MCPT-1: Gen Asia Biotech Co. Ltd. Shanghai China and for TSLP: Wuhan Fine Biotech Co., Ltd., Wuhan, China) according to the manufacturer's instructions. The results so