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Allergenic potential of two species of Poaceae: *Panicum maximum* Jacq and *Sacciolepis africana* C.E. Hubb pollen protein in albino mice

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Abstract

Pollen grains are the major cause of seasonal allergic rhinitis which affect millions of people worldwide. The presence of protein and glycoprotein content of their sporoderm and cytoplasm cause allergic sensitization in hypersensitive individuals. Immune system overreact by producing immunoglobulin E (IgE) antibodies which travel to cells that release allergic mediators causing allergic reactions. The objectives of this study were to; determine the concentration of pollen protein in two species of Poaceae: Panicum maximum and Sacciolepis africana, evaluate the level of immunoglobulin E (IgE) antibodies and immune cells of mice elicited by both pollen proteins. Pollen protein were extracted in 100 ml of 0.02 M of phosphate buffered saline (PBS) at pH 7.4 and protein content assayed. Albino mice were sensitized by two subcutaneous and one intranasal injections weekly for four weeks. Blood samples were obtained by retro-orbital bleedings, sera obtained were used for IgE evaluation by immuno assay. The result revealed a pollen protein contents of 420.27 µg/ml and 278.37 µg/ml in Panicum maximum and Sacciolepis africana respectively. Pollen protein of Panicum maximum induced a progressive change in the level of specific IgE in mice, after second sensitization. Both pollen proteins caused infiltration of basophil after first sensitization in albino mice. The result suggested that pollen which are dispersed from Panicum maximum and Sacciolepis africana are potential agents in inducing allergenic reaction. It also showed that Sacciolepis africana pollen protein are more potent than those of Panicum maximum. The research is the first study on pollen protein allergenicity in Nigeria.

Keywords: Allergy, Immunoglobulin E, Pollen protein, Immune cells, Mice.

Introduction

Pollen dispersal by wind is a natural event of great biological significance and an etiological factor in the genesis of allergic respiratory diseases[1]. Dispersed pollen find unscheduled detour into human noses, eyes, skin, respiratory tract where the allergen are leaked out causing allergenic diseases in hypersensitive individuals. Allergenic diseases are global health menace, with increasing prevalence of allergic rhinitis, dermatitis, conjunctivitis and asthma. Various sources around the world estimate allergies to affect around one in six children aged 6-7 years, one in ten children aged 13-14 years, 18% of those aged 15-34 years and 10% of older adults aged 35-54 years[2,3]. Typical complaints are those of a blocked nose and runny nose with mucus, itchy nose, sneezing and cough from post nasal drip. Allergic rhinitis may masquerade as continuous or recurrent respiratory infection, frequent sore throats and may be complicated by sinusitis or otitis media. Allergic conjunctivitis usually accompanies rhinitis with red and itchy eyes, sometimes complicated by infective conjunctivitis. Seasonal symptoms are most commonly triggered by pollen exposure, while perennial rhinoconjunctivitis is aggravated by exposure to fungal spores[4]. Allergen protein present in pollen are responsible for allergic reactions, they are released on hydration, by isotonic or hypotonic medium which enable them to diffuse on the mucosa surfaces such as the conjunctiva and the nose[5].

The increasing prevalence of allergic diseases over past decade is well established and accepted by most health authorities including the World Health Organization[6]. Major causes for the escalating trends in allergic diseases are increased exposure to sensitizing allergens and lesser stimulation of our immune system during the critical periods of its development[7]. Worldwide, the prevalence of asthma varies from population to population, and is influenced by factors like inbreeding, low altitude, damp climate, urbanization, and industrialization[7]. The importance of biochemical and immunological standardization of antigenic and allergenic component is being emphasized for effective diagnosis and management of allergic diseases all over the world[8]. From the clinical point of view it is important to determine the allergenic plant in our environment and the extent of their allergenicity, this will help in quality control of antigenic extracts and assist in proper clinical diagnosis, specific immunotherapy and prophylaxis.

The release of pollen is seasonal and varies according to species and geographical location. The source strength for a particular species varies regionally due to differences in habitat and timing of flowering[9]. This can be seen by clear differences in the start of the grass pollen season throughout the UK, regional variations in grass pollen season in the UK and Spain or aerial pollen diversity in India[10-12].

Materials and methods

Extraction of pollen protein: Anthers of *Panicum maximum* and *Sacciolepis africana* flowers which have not undergone anthesis were procured from the University of Lagos environs. Anthers were dried at room temperature and defatted using diethyl ether for three times. They were extracted in 100 ml of 0.02M phosphate buffered saline (PBS) at pH 7.4. The mixtures were stirred for 3 hours at 4^{o}C, filtered with a muslin cloth, centrifuged and supernatant retained. The protein precipitation was carried out with 50 g of ammonium sulphate and dialysed against PBS overnight. Protein concentration was assayed according to Bradford procedures[13]. The crude pollen protein were stored at -80^{o}C for later use in inoculating into experimental animals (albino mice).

Inoculation of pollen protein extract: Albino mice which were 4-6 weeks and weigh between 21-32 g, were purchased from Nigerian Institute of Medical Research, maintained under 12 hours light-dark cycle with free access to water and standard laboratory food. Mice were fed ad libitum with a pelleted mouse diet and water. All experimental procedures conformed to international standard of animal welfare and ethical approval obtained from Nigerian Institute of Medical Research. Crude protein extract (100 µl) was inoculated into mice by two subcutaneous and one intranasal injections for four weeks. Blood samples were obtained by retro-orbital bleeding using heparinized capillary tubes and both pre and post sera obtained from the blood were stored at -80^{o}C for later use in detecting IgE levels using immuno assay.

Blood smears were obtained from the tail, the thin blood smears were fixed with methanol for 2-3 mins. One in three dilutions of Leishman stain and buffered water was prepared and covered the slides for 7-10 mins. The stain was washed off in a stream of buffered water. Distillled water was added on a slide and left for 2-3 minutes to differentiate the film. The slides were allowed to dry on a rack. Using light Olympus CH Trinocular microscope (LM) fitted with 650 IS Cannon Digital Camera, blood smears were examined and the immune cells were identified and quantified on differential count.

At the end of 4th week mice were sacrificed by cervical dislocation. The respiratory organ were obtained and processing of tissue samples for histological assessment followed established procedures[14].

Immuno assay for determination of IgE in mice sera: Elisa IgE kitt GWB 626057 was purchased from GenWay laboratory Technologies in United State of America and was employed for the immune assay. All manufacturers' instructions on reagent dilution with distilled water were strictly adhered to and prepared prior to use. Test samples (100µl) were measured into predesignated microtitre wells and incubated for 30minutes. After removal of unbound protein by washing with Elisa machine, the wells were blotted to remove residual buffer. Anti IgE antibodies conjugated with horseradish peroxidase (HRP) were added and incubated for 30minutes, this was followed by another washing. The enzyme bound to the immunosorbent was assayed by the use of chromogenic substrate 3,3,5,5-tetramethylbenzidine (TMB). Absorbance at 450 nm was determined which was a measure of the concentration of IgE in the test sample. The values of immunoglobulin E in the test samples were interpolated from the standard curve constructed from the standards and corrected for sample dilution.

Results and discussion

Immunoglobulin E antibodies were low in all animals before inoculation (w0) of pollen protein. Mean values of 2.87 ng/ml was recorded in control mice whereas 2.87 ng/ml and 0.125 ng/ml were detected in group of mice before inoculation of *Panicum maximum* and *Sacciolepis africana* pollen protein respectively. At week one (w1), mice inoculated with *Panicum maximum* pollen protein presented a lowered level of IgE (1.625ng/ml) than the level before inoculation. IgE changed progressively from week two (3.92ng/ml) through week three (5.13 ng/ml) to week four (7.19 ng/ml). Mice which received *Sacciolepis africana* pollen protein had a tenfold increase in IgE level after the first sensitization, IgE was lowered at the 2nd week (0.750 ng/ml), increased further at third week (4.43 ng/ml) and had an abrupt increase at 4th week (13.13 ng/ml). Conversely, the IgE of the control mice were same for initial and first week and a little decrease at the end of the 4th week. *Sacciolepis africana* pollen yielded pollen protein (278.37 µg/ml) less than that of *Panicum maximum* (420.27 µg/ml) and its protein elicited a higher IgE level in albino mice (Figure-1).

Lymphocyte, basophil, monocyte and eosinophil were the dominant immune cells present in inoculated mice. Among them lymphocyte dominated, they increased over the weeks as the mice received pollen protein, this was depicted by the number of fields that gave hundred percent immune cells. In week one to week two, twenty (20) to fifty (50) fields gave hundred percent immune cells whereas in 3rd and 4th weeks less than twenty fields gave hundred percent immune cell. Basophil were higher after first sensitization by both pollen protein and decreases over the weeks (Figure-2).

Eosinophil were elicited higher at 4th week in groups of mice inoculated with *Panicum maximum*. Total Immunoglobulin E were elicited higher in mice inoculated with *Sacciolepis africana*. In control mice, which received phosphate buffered saline, lymphocyte dominated throughout the weeks than other leucocytes in more than fifty fields.

Histopathology result showed a normal trachea in group which received PBS and *Panicum maximum*. The mice group which received *Sacciolepis africana* showed proliferation of sub epithelial mucous gland in the trachea (Plate-1).

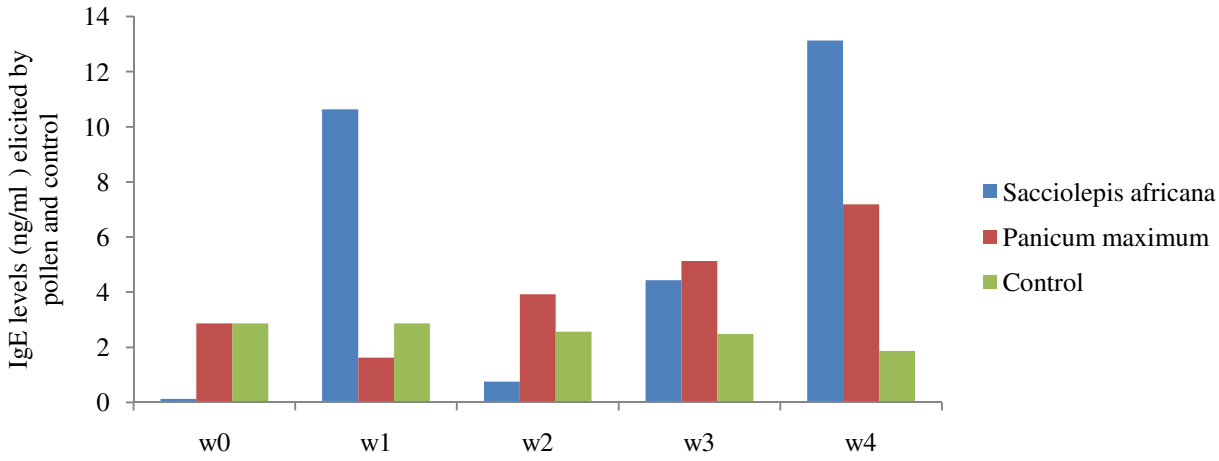

Figure-1: Levels of IgE (ng/ml) elicited by *Sacciolepis africana*, *Panicum maximum* and control (PBS) in albino mice.

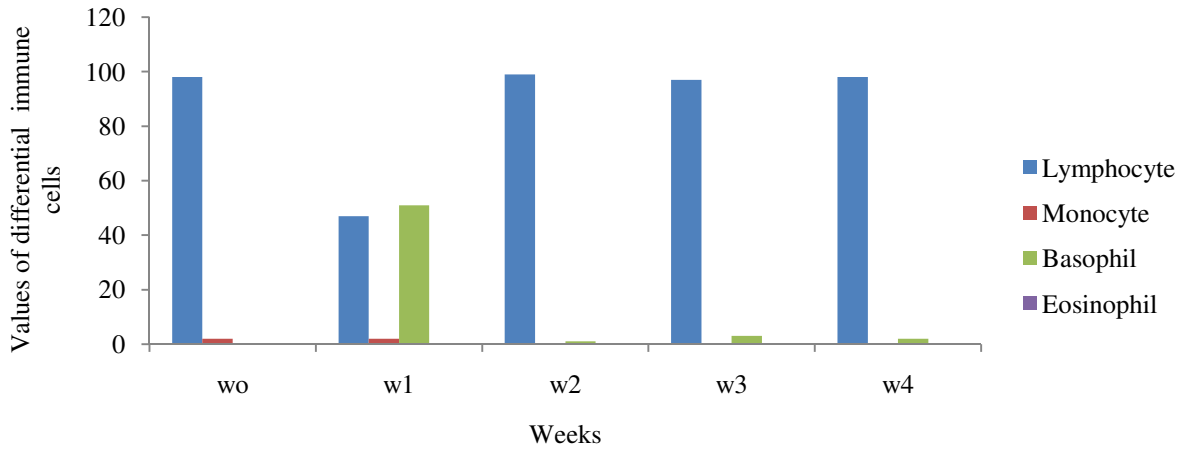

Figure-2: Values of differential immune cells elicited by *Sacciolepis africana* pollen protein in albino mice.

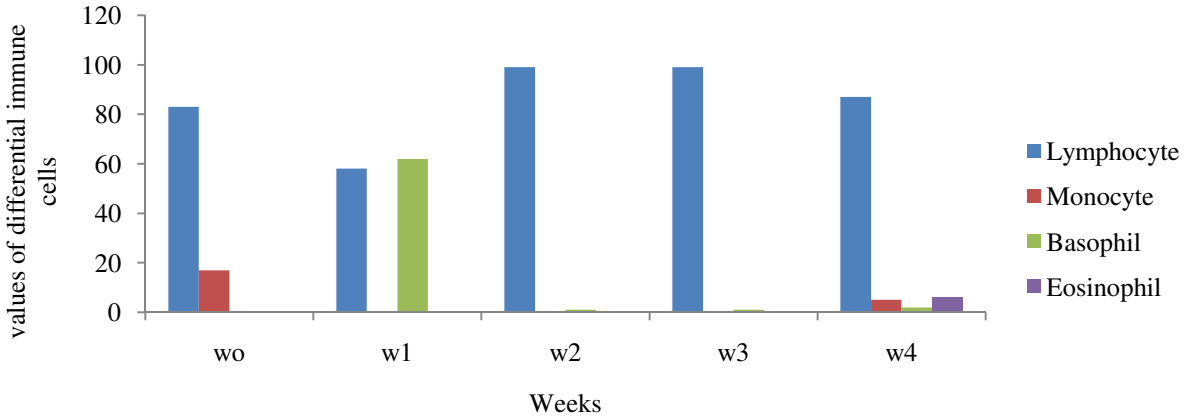

Figure-3: Values of differential immune cells elicited by *Panicum maximum* pollen protein in albino mice.

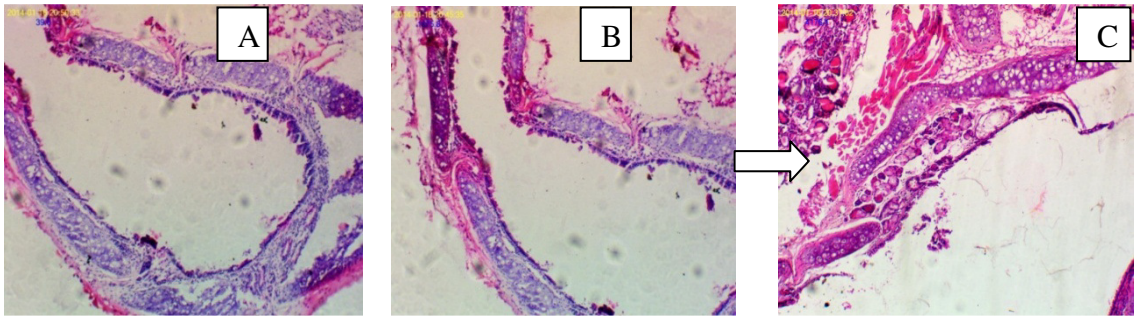

Plate-1: A and B, trachea of mice inoculated with PBS (control) and *Panicum maximum* respectively, showing normal trachea. Plate C: trachea of mice inoculated with *Sacciolepis africana* pollen protein, showing proliferation of sub epithelial mucous gland.

Discussion: *Panicum maximum* and *Sacciolepis africana* are wild perennial Poaceae plant which flowers almost throughout the year, their pollen protein contents and immune system modulation were different. This shows that protein content and pollen allergenicity could vary even among plants of the same family and could be dependent on genetic background. *Sacciolepis africana* with a lower protein content than *Panicum maximum* elicited a higher IgE and histopathological changes in trachea of mice. It implies that pollen releasing from *Sacciolepis africana* are intrinsically more allergenic and more potent than pollen from *Panicum maximum.* Nevertheless previous research has shown that *Panicum maximum* pollen protein elicited a high percentage of allergenic positivity rates among allergic rhinitis patients[15]. According to National Institute of Health (NIH) rating scales of Immunoglobulin E antibodies, both pollen allergens are within the class of "high level allergen specific IgE''. *Panicum maximum* and *Sacciolepis africana* are anemophilous and copious pollen producers. Their pollen protein induced an alteration in immune system which resulted in increased immunoglobulin E, basophil and lymphocyte proliferative response in mice. IgE induced by *Panicum maximum* were fewer but more progressive than those of *Sacciolepis africana*. The reduction in IgE values in week three in group which received *Sacciolepis africana* could be due to immune tolerance of mice. Research has shown that Immunoglobulin E play a very important role in type 1 hypersensitivity which manifest various allergic diseases. High IgE level could lead to production of free oxygen radicals (FORs), which leads to tissue damages, impairs the membrane permeability and fluidity and results in functional and structural disorders and even cell death[16].

Both pollen protein induced infiltration of basophil**,** after first sensitization. Basophil is a type of leukocyte characterized histologically by its ability to be stained by basic dyes and functionally by its role in mediating hypersensitivity reactions of the immune system[17]. Basophils, along with eosinophils and neutrophils, constitute a group of leucocyte known as granulocytes and it is less than 1% of leucocyte in healthy animals[17]. The basophil elicited by *Sacciolepis africana* in mice could be responsible for inflammation and proliferation of sub-epithelial mucous gland. Previous research showed that high level of basophil expression could also predict severe chronic urticaria[18]. Eosinophil were also elicited in mice which received both pollen proteins than in control mice. Association between eosinophil and allergic diseases has been known for many years. The role of eosinophil to allergic symptoms of the airways has also received increasing attention recently.

Conclusion

Pollen protein of *Panicum maximum* and *Sacciolepis africana* induced alteration in immune system which resulted in proliferative response of IgE, lymphocyte, eosinophil and basophil. *Sacciolepis africana* triggered histological changes. The research revealed that *Panicum maximum* and *Sacciolepis africana* pollen possess allergen protein and could trigger allergies in hypersensitive individuals.

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