The Eurasia Proceedings of
Educational & Social Sciences (EPESS)
ISSN: 2587-1730

The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 2020

Volume 10, Pages 71-73

ICVALS 2020: International Conference on Veterinary, Agriculture and Life Sciences

Allergenic Proteins of Tilia Cordata

Delal TIVSIZ
Gaziantep University

Basak SIMITCIOGLU
Gaziantep University

Isik Didem KARAGOZ
Gaziantep University

Mehmet OZASLAN
Gaziantep University

Abstract: There are a lot of environmental allergenic factors; pollens, fungal spores, house dust mites, some fooods and drinks. These factors are harmless for many people in despite of making hypersensitivity any people. This sensitivity is calling "allergy''. Allergens generally has a protein structure and when Charles Blakey's discovered of pollens making allergenic diseases in 1873 after that pollens have determined a pivotal role to allergenic diseases. The pollens allergenic qualifications have known orginated layers of exine and intine including of glycoproteins, lipoproteins, polysaccaride and proteins. In this study, we investigated that allergenic proteins of *Tilia cordata* collected from Gaziantep. *T. cordata* pollen samples were collected during dissemination period and pollen extract prepared. We used Wodehouse method and prepared slides for determination of pollen morfology and were taken photos under light microscopy and measured morphological parameters. Total concentration of pollen proteins was determined using by BCA method. The amount of *T.cordata* allergen proteins was calculated as standard graph obtained from BSA. The average diameter of the *T. cordata* pollens diameter was measured as 88,83 µm and allergenic proteins from *T. cordata* was determined as significantly high. 1259.28 µg/ml concentration of *T. cordata* protein was measured. We suggested that this study provides important data to the literature by determining the amount of allergenic protein of *T. cordata* in this region because of differences about the content of allergenic proteins among regions.

Keywords: Allergy, Allergen, Pollen, T. cordata

Introduction

The term "allergy" was introduced in 1906 by von Pirquet, who recognized that in both protective immunity and hypersensitivity reactions, antigens had induced changes in reactivity. There are a lot of environmental allergenic factors; pollens, fungal spores, house dust mites, some fooods and drinks. These factors are harmless for many people in despite of making hypersensitivity any people. This sensitivity is calling "allergy''. Allergic reaction can be cell or antibody mediated. Gell and Combs categorized allergic reaction to 4 groups (Type I, Type II, Type III, TypeIV). The Ige ısotype is responsible for allergic reactions in many patients and it's calling IgE mediated allergy. The term "atopy" is often used to describe IgE-mediated diseases. Person with atopy is calling "atopic''. Persons with atopy, by contrast, have an exaggerated response characterized by the production of allergen-specific IgE antibodies; they have elevated serum levels of IgE antibodies and positive reactions to extracts of common aeroallergens on skin-prick tests. T cells from their blood respond to allergens in vitro by inducing cytokines produced by type 2 helper T (Th2) cells. In the nose allergens are processed by antigen-presenting cells (dendritic cells expressing CD1a and CD11c and macrophages) in the nasal epithelial mucosa, with subsequent presentation of allergenic peptides by MHC class II molecules to T-cell receptors on resting CD41 T lymphocytes in regional lymph nodes. With costimulatory signals, allergen-stimulated T cells

proliferate into TH2-biased cells that release IL-3, IL-4, IL-5, IL-13, and other cytokines. Inhalation of allergen in sensitized subjects, deposited allergens are recognized by IgE antibody bound to mast cells and basophils, causing degranulation and release of preformed mediators, such as histamine and tryptase, and the rapid de novo generation of mediators, including cysteinyl leukotrienes (leukotrienes C4, D4, and E4) and prostaglandin D2 (Dykewicz ve Hamilos, 2010). Rhinitis and sinusitis are among the most common medical conditions and are frequently associated. In Western societies an estimated 10% to 25% of the population have allergic rhinitis, with 30 to 60 million persons being affected annually in the United States. Pollens are cause of type I allergenic disease around the world and they are called aeroallergens most important facts of allergy (Boral and at al., 2004; Kaneko and at al., 2005). A lots of trees, grasses and weeds are during their pollination, the air pollen consantration is going higher and thats make hypersensitive pepople rhinit, alergic rhinit, asthma and bronchial asthma (Rauder ve Breiteneder, 2006). Pollen allergy is a seasonal disease, typically due to the individual's sensitivity to pollen during thebloom period of plants. However hypersensitive allergic people can be observed allergic rhinits sypmtoms all year around (Weerd and at al., 2002). Pollens are the most common of all outdoor allergens and part of the life cycle. Pollen is a natural, biologically active substance composed of the male reproductive cells of many plants. Because it is used as a source material to manufacture allergen extracts used to diagnose and treat allergic diseases, it is considered an active pharmaceutical ingredient. A pollen grain contains vegetative and generative nucleus, endoplasmic reticulum, mitocondria and intine, exine (Knox, 1984). The intine wall creates a barrier to contain the pollen components, while the openings in the exine allow the formation of the germination-dependent tube and the movement of water and proteins due to hydration (Singh and at al., 1991). Pollen proteins that don't interact with the immune system are harmless. In other words, protein that doesn't interact with IgE antibodies in the immune system is not considered an allergen. Linden is a large tree of variable form. In woodland up to 30 m (rarely to 37 m) high, with a cylindrical trunk up to about 1 m in diameter at breast height, tapering gradually and unbranched to two-thirds of its height. Lower branches of the first-order horizontal and arching; branches of the second-order horizontal, ascending or vertical: upper branches ascending or vertica. This description applies to the species in the strict sense but *T. cordata* may also be regarded as a collective species, which extends from western Europe to eastern Asia and includes at least seven species or subspecies (d.piggot) Variation in *T. cordata* in central and western Europe can also arise as a consequence of hybridization with *T. platyphyllos* (Pigott 1969). Analysis of hybrid populations shows that the majority of morphological characters, which normally separate the two species, can exist in all combinations and all degrees of intermediacy. The small-leaved linden – *T. cordata* - is found throughout Europe and most parts of North America. The dried leaves are used as herbal tea. *T.cordata's* height can reach up to 30m. Its leaves are half-heart-shaped, dark green, 4-8cm long. Pollen dissemination continues from June to July. Sometimes it can continue until August.

Method

In this study, we collected *T.cordata* pollens in Gaziantep University at first step. Collected pollen for extraction had to be fresh as stated in the literature (Aytuğ and Peremeci, 1987). The most suitable period for this is the phase immediately after the anthers are opened. Considering these, pollens were collected by appropriate methods during the dissemination period of the plant to be used. We dried the our sample in dry and sterile laboratory. Pollen was poured onto a clean blotter by hitting the dried flowers. The spilled pollen was placed in dark glass bottles and kept in a desiccator for 24 hours to dry. After drying, the flowers were separated from their pollen by sieving with 3 different pore diameters (180, 90, 63 μm). Then, washing with acetone was performed to separate the pollen from foreign materials such as plant parts. The pollens were then dried in a climate cabinet at 20-37 ℃. Then the drying process was continued in the vacuum desiccator, thus preventing mold growth. Finally, the pollens were placed in dark glass bottles, capped with paraffin and put in the refrigerator, so the pollens became suitable for study.

Preparing Pollen Extract

For extraction of *T.cordata* pollens procured from Gaziantep University campus. It was mixed in a 1:12 (weight: volume) 125mM NH_4HCO_3 (ammonium bicarbonate) solution at + 4 ° C for 12 hours in a low speed magnetic stirrer. Then the pollen residues will be removed by settling in a centrifuge (13000xg, + 4 ° C, 1 hour). The upper liquid phase was first passed through the 125mm thick whatman paper and then through the filtration system. The filtrate obtained was transferred to the dialysis tube. Dialysis was performed at + 4 ° C for 48 hours in a shaker against pure water.

Determination of Protein Concentration In Pollen Extracts

The protein concentration in the pollen extracts was determined according to the bicinchoninic acid (BCA) method by BCA Protein Assay Macro Kit (Serva, Germany). This method is based on the processing of proteins in alkaline solution with biurea reagent, reduction of Cu (II) ions to Cu (I) ions and spectrophotometric measurement of the complex formed by Cu (I) ions with BCA. First of all, the bovine serum albumin (BSA) was dissolved in water and diluted in appropriate proportions, and standards were prepared in certain concentrations. The BCA indicator was obtained by mixing the reagent solutions in the kit in a certain ratio. 200µL reagent was added onto the protein samples, standards and blank. Then it was incubated for 30 minutes in a 37°C incubator. After the incubation process, absorbance values at 562 nm were measured in the spectrophotometer. The protein concentrations were determined by placing the absorbance values of the protein values into the line equation.

Result and Discussion

In this study,we used BCA method at the first step, prepared the BCA according to the method suggested by Walker (2002). After we calculated the protein concentration as 4.193,32 µg / mL in the measurements we made at 562nm. In addition, as a result of morphological examinations, it was determined that the diameter of pollen was approximately 83 micrometers.

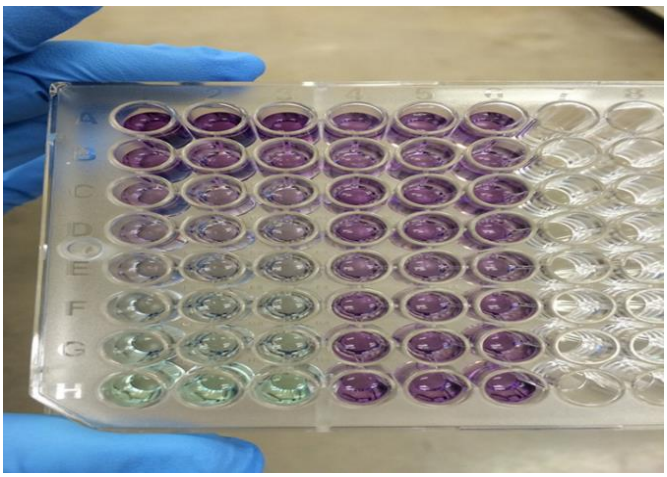

Figure 1. BCA method for calculate protein concentration.

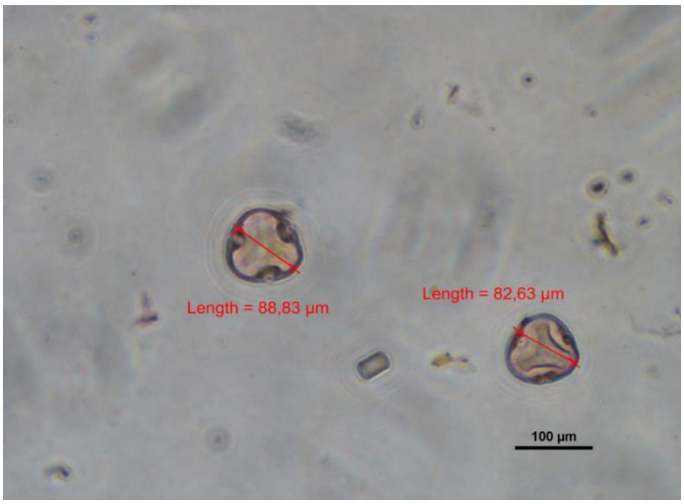

Figure 2. Pollen images of T.cordata under microscope

A lot of studies show that allergy is important health problem in recent years. Pollens are the one of the important sour of allergen and allergenic disease. Pollens are aeroallergenic allergens and they enter the body

through respiration, causing allergic rhinitis and asthma. Allergenicty of proteins varies depending on size of molecule, affect mucosal barriers and solubility.

We could not find extensive research on *T.cordata* among the studies conducted so far. Against *T. cordata* in a small number of publications have been described allergic rhinitis, rhinoconjunctivitis, cough and allergic contact dermatitis. Mur et al. in their 2001 study, they reported allergic reactions to *T.cordata* in a 21-year-old woman. In laboratory examinations, the total Ig E level is high and the skin against *T.cordata* pollen extract They detected that the prick test (5% w / v) as positive. Several bands were identified by Ig E immune detection after SDS-PAGE on *T. cordata* extract. Non-specific ones at the molecular weight of ~ 20 kDa and ~ 21 kDa and essentially a specific allergen protein band at the ~ 50 kDa level were detected. This observation show that *T.cordata* has diferrent allergenic pollens. In the future, pollen allergies can be treated with specific immunotherapies using vaccines containing recombinant allergens to replace the ordinary simple pollen extracts used today. Therefore, in our further studies, we intend to characterize the tilia cordata pollen proteins in our region and observe the level of ig E in humans.

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Author Information

Delal TİVSİZ
Gaziantep University
Department of Biology, University of Gaziantep,
Şehitkamil/Gaziantep, Turkey
delaltvsz@outlook.com.tr

Işık Didem KARAGÖZ
Gaziantep University
Department of Biology, University of Gaziantep,
Şehitkamil/Gaziantep, Turkey

Başak SİMİTÇİOĞLU
Gaziantep University
Department of Biology, University of Gaziantep,
Şehitkamil/Gaziantep, Turkey

Mehmet ÖZASLAN
Gaziantep University
Depertmant of Biology,University of Gaziantep
Şehitkamil/Gaziantep, Turkey