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Comparative Study of Pollen Extracts Allergenicity of *Chenopodium album L.* and *Chenopodium Botrys L.* an in Vivo Study

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Abstract— **Many pieces of evidence such as the synchraneity of seasonal variation in allergic symptoms with the rhythm of plant pollination suggest that pollen is one of the most probable causes of allergy. The aim of this study was the studying of pollens allergenicity in Chenopodium album and Chenopodium botrys. C. album and C. botrys grows commonly in different parts of the Iran. Pollens of these were collected from area of Tehran, Karaj city and around Kandovan. Pollens were extracted using phosphate-buffered saline, PH 7.4. Male guinea pigs were sensitized and treated with C.album and C. botrys pollen extracts and skin prick tests were performed on guinea pigs and quantified on the basis of wheal diameter. After treatment with pollen extracts, the guinea pigs blood was obtained directly from the heart and sera from samples were stored at-20ºc until analyzed. During the skin prick test, the allergenic sensitivity was observed for C.album pollen grains, with an average wheal diameter of about 4 cm and for C. botrys pollen grains, with an average wheal diameter of about 2/5 cm. Results of blood smears were seen that, the numbers of eosinophils, neutrophils and amount of IgE were increased in the animals treated with pollen extracts than in the control group.**

Keywords- Chenopodium album; Chenopodium botrys; allergenicity;

I. INTRODUCTION

Pollen grains are male gametophytes which carry the male cells. This major role is in sexual reproductive cycles in the plant world. Thus, allergies represent a health problem in the industrialized world. The immediate symptoms of type 1 allergy are caused by IgE recognition of environmental allergens[1].Pollen from weeds is one of the most potent and abundant allergen sources. The allergenic characteristics of pollen and their levels of expression may vary depending on the plant species, the degree of maturation and the influence of environmental factors such as climate and atmospheric pollution[2].Researchers have attributed this features to different substances particularly, organic materials located in the pollen and its well, the sporoderm[3].According to researchers, there are proteins as allergenic substances in Salsola kali, Artemisia, Amaranthus retroflexus and Achillea wilhelmsii pollens [4, 5]. Pollens from the Chenopodiaceae family have been reported as an important source of pollinosis in the Western United States, European countries and Asia. Furthermore, Chenopodiaceae sensitization is a severe problem in semidesertic countries such as Saudi Arabia, Iran and Kuwait [6]. Profilin and polcalcin are relevant panallergens in Chenopodium album pollen and candidates to be involved in IgE cross-reactivity with other pollen sources [7].Thus, allergenicity of pollen grains increased in polluted cities [8, 9].

Chenopodium album is one of the most important genera of the Chenopodiaceae family. This plant is widely found in different parts of Iran. C.album is a serious weed and does a salt-tolerant species inhabit semi-arid and light-saline environments in China [10]. Methanol extract of C.album leaves exhibited maximum antibreast cancer activity [11].Methanol inflorescence extract of C. album exhibited highest antifungal activity resulting in up to 96% reduction in fungal biomass production [12]. C.album has antioxidant capacity, total phenol flavonoid glycosides (quercetin, rutin, kaempferol), thus, should be considered as a nutraceutical food and an alternative source for nutrients and free radical scavenging compounds [13].

Chenopodium botrys is used in traditional medicine as an antispasmodic, onthelmintic and as a spice. These plants have monoterpenes, comprise: camphor, delta-3-carene, fenchone, linalool mentone, nerol, beta- pinene, pulegone, thujone, terpineol-4and sesquiterpenes, comprise: beta elemene, elemol, beta eudesmol [14]. C.botrys is rich in essential oil and the antimicrobial activity of the oil is also recorded [15].C. botrys can grow in some heavy metal contaminated soils and is a high accumulator plant species for Cu and moderately accumulator plant species for Fe, Mn, and Zn[16]. C.botrys has five flavonoids, comprise: hispidulin, salvigenin, 5-methylsalvigenin, 7-methyleupatulin and sinensetin [7.Essential oil, isolated from C. botrys aerial parts, expressed significant fungicidal activity [18].

The present investigation was undertaken to in vivo comparative studies the pollen extracts allergenicity of Chenopodium album L. and Chenopodium botrys L.

II. MATERIAL AND METHODS

Pollen grains were collected around of Tehran, Karaj city and around of Kandovan. Fresh pollen grains were purified by passage through mesh with 30-μm- diameter pores.

Male 4-6 week-old guinea pigs weighting 350-500 g were sensitized and treated with *Chenopodium album* and *Chenopodium botrys* pollen extracts. The animals were housed in an air-conditioned room at 25±2º c; they were fed a standard laboratory diet and given water with added vitamins. Animals were sensitized and treated by injection of 100μl pollen extract (containing 20μg protein in phosphate-

buffered saline) from pollen grains. Treatments were repeated five times, for 5 week, once each week [8, 9].

Pollen extracts were prepared from pollens by the method of Sheldon (1967), and skin prick tests were performed on guinea pigs. Each animal was tested with 100µl of the total extract diluted with 0.05M phosphate-buffered saline, PH 7.4, containing 50µg protein. The negative control was buffered saline and the positive control was histamine acid phosphate. Skin reactions were read 1, 4, 8, and 24h from the beginning of the test and quantified on the basis of wheal diameter.

Prior to each experiment, the guinea pigs were bled once. Blood samples were drawn directly from the heart. After treatment with pollen extracts, the guinea pigs blood was obtained directly from the heart and sera from samples were stored at-20ºc until analyzed .The sera from 10 *C.album*-allergic sensitized animals were tested for IgE reaction. In the next step, 10 guinea pigs were tested for *C. botrys* pollens and sera from 10 animals that were not sensitized to pollen were also tested as control sera. Total serum IgE was evaluated using the ELISA for blood. Total numbers of different kind of blood cells, especially eosinophils and neutrophils, were determined in all groups and then were statistically analyzed.

III. RESULTS

Results of the skin test in guinea pigs for different samples are summarized in (Table 1). Pollen extract had an allergenic effect. During the skin prick test , the maximum allergenic sensitivity was observed for *C.album* pollen grains , with an average wheal diameter of about 4 cm and for *C. botrys* pollen grains with an average wheal diameter of about 2/5 cm, thus, significant changes observed between *C.album* ,*C. botrys* pollen grains with control group ($P<0.01$).Results of blood smears from different groups are summarized in(Table 2).The numbers of eosinophils and neutrophils were increased in the animals treated with pollen extract. Data for determination of IgE in different groups are given in (Table 2).Animals serum of animals treated with *C.album* pollen extract contained more IgE than serum of animals treated with *C.botrys* pollen extract and thus, more IgE than serum of control animals. The amount of IgE in the serum of animals treated with *C.botrys* pollen extract was more than that in the control groups, but less than in *C.album* pollen extract. There was a significant relation ($P<0.05$) between allergic reactions and changes of blood factors.

IV. DISCUTION

In the past few decades the reported cases of the allergic rhinitis and other related diseases like asthma and urticaria have grown at tremendous rate as recorded from various epidemiological studies carried out all over the world. The pollen grains constitute one of the important components of air and are considered as major causative agent of respiratory disease, as is evident by the spurt of literature from across the world during last few decades [19].Since the industrial revolution, there has been continuous transformations in the vegetation of the localities at micro as well as macro levels, due to climatic changes and the anthropogenic activities.

Clinical and serological results emphasized a greater allergenicity for pollen. The presence of blood eosinophilia, an increase in neutrophilia number with the presence of the other factors which have been reported as allergic indicators proved the allergenicity of Chenopodium album and Chenopodium botrys pollen grains [20].The allergenic potential of C.album and C.botrys pollen grains was measured by wheal diameter, eosinophilia and neutrophilia number changes , and total blood IgE .Clinical and serological results indicated that number of eosinophils, average wheal diameter , and amount of IgE are greater in guinea pigs treated with extracts of fresh pollen than in the control group. Wurtzen et al.1995 were reported that Chenopodium album has three important allergens with molecular masses 14,35,42KD and Chenopodium botrys has one major allergen with molecular masse 42KD[4].These plants of Chenopodiaceae family are major weeds most areas of Iran. Our results indicate that the wheal diameter of skin prick tests with C.album pollen extract showed strong correlation with the results obtained by C.botrys pollen extract. Therefore, this difference may be because allergens number in C.album pollens were more than allergens number in C.botrys pollens.

Barderas et al.2004 have shown to profiling(Che a2) and polcalcin(Che a3) are relevant pan allergens in Chenopodium album pollens and good candidates to be involved in IgE cross-reactivity with other pollen sources, thus explaining the highly frequent polysensitization of patients allergic to chenopod[7]. Profilins are one of the families of cross-reactive plant allergens. These ubiquitous actin-binding proteins are involved in signal transduction and can be found as cross-reactive pan-allergens in almost all plant species and plant tissues[1].These proteins usually act as minor allergens [21] and are allergens in grass pollen and in a wide range of vegetable foods, like potato and celery. Within a grass-pollen-sensitive population, patients with IgE to vegetable foods have a high incidence of antibodies against profilins[22].

Furthermore, polcalcins are another of the families of cross-reactive plant allergens. Proteins belonging to this allergen family constitute a novel class of calcium-binding proteins that contain two EF-hand calcium-binding motifs that have been characterized in pollens of various plants (trees, bushes, grasses, weeds, and flowering plants) as extremely potent allergens and, due to their pollen-specific expression, are cross-reactive allergens for patients with pollen polysensitization[1].

In previous studies showed that the two allergens in Beta vulgaris should be named Beta v 1 and Beta v 2 according to the allergen nomenclature, in analogy with the related Chenopodium album allergens Che a 1 and Che a 2[21]. Thus, cross-reactivity was described between Salsola and Chenopodium spp. These results were showed extensive cross reactivity of S. kali with Chenopodium album and some degree with Amaranthus rtroflexus, Kochia scoparia and Artemisia douglasiana. It was noteworthy that the patients were most often sensitized to at least three allergenic pollen extract from different members of the Amaranthaceae/Chenopodiaceae family [6].

Moreover, the observations suggest that C.album pollen grains are more allergenic than C.botrys pollens. This means that a significant amount of protein allergens are produced in C.album pollen grains.

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TABLE I. RESULTS OF SKIN TEST FOR DIFFERENT POLLEN EXTRACTS.

Sample	Wheal diameter (cm)
C	1 ± 0/5
C.a	3/5 ± 0/7
C.b	2/1± 0/4

(C, control group treated with saline; C.a,group treated with C.album pollen extract ; C.b , group treated with C. botrys pollen extract)

TABLE II. RESULTS OF SEROLOGICAL STUDIES: DETERMINATION OF NUMBERS OF EOSINOPHILS AND NEUTROPHILS AND BLOOD IGE IN DIFFERENT EXPERIMENTAL.

	C	C.a	C.b
Eosinophils	3/1 ± 1/5 (x10 cells/ml blood)	20/5 ± 2/3	13/6 ± 3/9
Neutrophils	3/2 ± 1/3 (x10 cells/ml blood)	15/5 ± 1/3	10/3 ± 3/7
IgE(mg/ml)	8/1± 2/2	86/2 ± 3/4	49 ± 17/8

(C, control group treated with saline ; C.a,group treated with C.album pollen extract ; C.b , group treated with C. botrys pollen extract)