The Effect of *Trifolium*, *Raphanus*, and *Cistus* Pollen Grains on Some Blood Parameters and Mesentery Mast Cells

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Three kinds of pollen taxa belonging to 3 families (Fabaceae - *Trifolium* spp., Brassicaceae - *Raphanus* spp. and Cistaceae - *Cistus* spp.) and commonly collected by honeybees were fed to mature male rats separately, in the form of 60 mg/animal/day for a 30-day period. The objective of this study was to investigate any positive effects or possible side effects of the use of pollen on the immune system. This was achieved through blood analysis and cell count on blood, hemoglobin, erythrocyte and immune system cells. The cell concentration of mast cells, degranulization and cell localization were investigated in prepared mesentery tissue samples. Histological investigations of the stomach and duedenum sections of pollen-fed rats were carried out to learn the reason for eosinophil gastroenteritis in the alimentary canal.

The eosinophil and lymphocyte levels of rats fed with pollen of *Trifolium* spp., *Raphanus* spp., and *Cistus* spp. were observed to have increased blood cell counts, while neutrophil and monocyte levels decreased; different values were found in basophil leucocytes between the pollen groups. Differing reductions in mesentery mast cell concentration, degranulization and cell localization were found. Within the three separate pollens, the rats having been fed with *Cistus* spp. pollen were observed to have higher blood lymphocyte, eosinophil, hemoglobin and hematocrit values than those fed with the others, as well as low mesentery mast cell concentration. Hemoglobin values were determined to increase at a proportion of between 10.0–11.3%. No difference was found in other blood parameters.

The fat proportion of the male rats fed with the three taxa was between 4.03–8.75%, while that for protein proportion was between 16.11–24.25%. Male rats receiving these taxa did not experience allergic reactions and it is possible to argue that the low protein and fat content of these pollens have a strengthening effect on the immune systems by the increase in lymphocyte content and the amount of hemoglobin leads to an increase of oxygen transport capacity in the tissues.

Key words: Pollen, Cell Blood Count, Mast Cell

Introduction

The pollen collected from *Apis mellifera* L. contains generally 40% of protein, essential amino acids, low amounts of fat, and high levels of minerals (D'Albore, 1997; Sahinler, 2000). Bees collect the pollen for their own nutrition and for use in the development of larvae. The most important side effect of wind-borne plant pollination is the allergic reaction to pollen experienced in the respiratory system (Nilsson *et al.,* 1983; Mateescu *et al.,* 1997). This may be the reason why pollen gathered from plants by honeybees may also cause allergies in people (Pehlivan, 1995; Bauer *et al.,* 1996) and eosinophil gastroenteritis (Castells, 1997).

Pollen containing protein, free amino acids and minerals, along with antioxidants, particularly by means of flavonoids, when taken daily together with food in suitable doses may be used to strengthen the immune system, reduce the effect of radiation (Green, 1993) and retard the aging process (Geyman, 1994). It may also be used for the goal of aiding the regression of some degenerative illnesses (Liebelt and Calcaginetti, 1999). Moreover, it has an androgenic and muscle strengthening action (Faegri and Iversen, 1989) and is for this reason added to the feed of racehorses (Xie *et al.*, 1994). Many studies have established the obstacle that pollen offers to benign prostrate growths (BPH). Furthermore, some

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studies have reported the use of pollen as an aid in protection from liver toxins.

Mast cells are generally found close to the mesentery connective tissue, blood vessels and epithelial, and the cells are important in the speedy response to antigens. In allergic and inflammatory illnesses, it is the cells that supply immune mediators which are known as sitocyne in granules. IgE receptors, found on the surface, regulate allergens in relation to immunity and secrete histamine and other mediatories from the commencement of the allergy. This is the reason for the swift response of antigens entering the body through the blood path. The anti-allergic hindering effect of histamine oscillation has been shown to occur from mast cells through the antioxidant quercetin molecules found in the content of some pollen grains (Schmidt, 1997).

The histamine which is stored in granules and produced by mast cells occurs with the existence of foreign antigens. The histamine and seratonine found in the granules give rise to blood level muscle contraction, blood vessel dilation/expansion and an increase in blood circulation which is the reason for local inflammation and redness in tissues together with loss of liquid. The congestion experienced is a result of stimulation of mucus on the epithelial in the respiratory path, due to the release of histamine in response to antigens entering throuh the airway. On the other hand, the onset of stimulation of the lymphocytes functions causes an increase in the secretion of cytokine. IgE's production directly activates B lymphocytes.

In a 3-year study carried out by Conway (2005) on patients with allergies, patients were given a low dose of pollen with their diet. Pollen consumed orally has no side effects, and patients were determined to have been completely released of 94% of their allergic symptoms (Bauer *et al.,* 1996; Conway, 2005).

Material and Methods

Collection of materials

Pollen samples were collected from Cumalıkazık, Narlıdere, Akçalar, İkizce, Çekrice and Baraklı regions of Bursa, where beekeeping is widespread, during May, June, July, August and September 2001.

Three taxa belonging to three families which were commonly preferred by honeybees were chosen. These were Brassicaceae - *Raphanus* spp.; Cistaceae - *Cistus* spp.; Fabaceae - *Trifolium* spp.

Preparing of pollen samples

The investigation followed the method of Wodehouse (1935) for preparing the pollen samples.

Microscopic studies of pollen samples

Pollen samples were researched with a Nikon Eclipse E400 microscope, and an immersion objective ($\times 100$) was used for the description of pollen grains. In the research the whole area of 18×18 mm^2 was checked. Relevant sources consulted in the diagnosis of the pollens were from Erdtman (1969), Kapp *et al.* (2000), Markgraf and D'Antoni (1978), Nilsson *et al.* (1983), Iwanami *et al.* (1988), Faegri and Iversen (1989), Moore *et al.* (1991), Pehlivan (1995), D'Albore (1997) as well as prepared reference samples.

Kjeldahl protein analysis in pollen samples

In pollen samples total nitrogen was determined by using the Kjeldahl method adapted for the Kjeltec system digestion and distillation units (Leco Corporation FP-528, St. Joseph, Michigan).

Protein amount was measured both for pollen samples dried in a pollen drying machine at 45 °C for 6 h and for wet pollen samples. Total protein was calculated by multiplying the pollen nitrogen content by 5.6.

Total fat analysis in pollen grains with Soxhlet extraction method

The Soxhlet extraction method is based on total fat extraction from a solid sample in an organic solvent. Generally this method is used to calculate the total fat (lipid) content in food substances (Swaile, 2001).

10 g of pulverized pollen was extracted with petroleum ether (40–60 °C) for 4 h using a Soxhlet apparatus. This procedure was done for every pollen sample.

Animal experiments

2- to 3-month-old Wistar albino male rats were obtained from Hacettepe University's Experimental Animal Laboratory for use in the study. Four groups of rats were formed, with each group comprising eight rats. Each cage housed two rats.

The first group was selected as a control group and the other three ones were selected as experimental groups. Three different pollen taxa have been applied to the experimental groups. Pollen A

(Fabaceae - *Trifolium* spp.) was applied to the second group rats, pollen B (Brassicaceae - *Raphanus* spp.) was applied to the third group rats and pollen C (Cistaceae - *Cistus* spp.) was applied to the fourth group rats. The pollen amount administered was based on that for and it was given 60 mg/rat/d mixed with bait for 30 d. Experimental and control groups rats were fed with standard rat bait and tap water during 30 d. Daily bait and water consumption were registered. Also rats' weights were followed weekly during the experiment.

Blood and serum analysis

Throughout the study, blood samples were taken from the hearts of the rats in order to perform hematological, serum and hormone analysis, after first performing dissection through cervical dislocation. In order to perform a partial hematological analysis of the blood samples, blood was placed in EDTA test tubes, while serum was extracted for other partial biochemical analyses and hormone analyses by centrifuging for 30 min at 3200 rpm. Hematological analyis was conducted using a Beckman Coulter Brand Max-M cell counter.

Histological investigation

For the purpose of histopathological investigations, tissue was removed from the mesentery, stomach, and large and small intestine; organ weights were recorded and organ/body weight proportions were calculated. In order to perform histopathological investigations, the mesentery was immediately extracted and fixed in Bouin solution and 70% alcohol. After first preparing the samples for the routine histopathological procedures, pictures for each sample were obtained through a light microscope.

Results and Discussion

The protein and fat values supplied by pollen collected by bees are 23.7% and 4%, respectively. Of the whole 1000 kcal of energy, 96.3 g are protein and 19.5 g are fat. The percentage protein and percentage fat contents for the pollen of the plants Brassicaceae (*Raphanus* spp.), Cistacea (*Cistus* spp.) and Fabaceae (*Trifolium* spp.) are presented in Table I. The protein and fat measurements for each plant are as follows: *Trifolium* spp., protein 24.25%, fat 4.03%; *Cistus* spp., protein 16.11% and fat 5.25%; and *Raphanus* spp., 24.01% protein, 8.75%. In terms of fat percentage values *Raphanus*

Table I. Protein and total fat contents of Brassicaceae - *Raphanus* spp., Cistaceae - *Cistus* spp. and Fabaceae - *Trifolium* spp. pollen grains.

	Raphanus spp.	*Cistus* spp.	*Trifolium* spp.
Protein (%)	24.01	16.11	24.25
Fat (%)	8.75	5.25	4.03

Table II. Hematologic analysis results of control and experimental rats.

Parameter	Control	Pollen A	Pollen B	Pollen C
Erythrocyte [$\times 10^{12}$/L]	8.14 ± 0.13	7.75 ± 0.11	8.15 ± 0.15	8.35 ± 0.09
Hemoglobin [g/dL]	13.61 ± 1.21	14.67 ± 0.19	15.02 ± 0.20	15.44 ± 0.16
Hematocrit (%)	43.6 ± 0.38	42.8 ± 0.55	44.2 ± 0.68	46.0 ± 0.71
Total leucocyte [$\times 10^9$/L]	4.65 ± 0.38	4.65 ± 0.27	4.90 ± 0.45	5.27 ± 0.49
Lymphocyte (%)	66.32 ± 3.35	78.21 ± 3.31	81.20 ± 2.26	80.37 ± 1.54
Monocyte (%)	2.42 ± 0.16	1.75 ± 0.34	2.41 ± 0.20	1.57 ± 0.30
Neutrophil (%)	24.55 ± 1.92	18.20 ± 1.68	15.41 ± 1.46	16.00 ± 1.32
Eosinophil (%)	0.94 ± 0.23	0.70 ± 0.13	1.60 ± 0.08	2.55 ± 0.19
Basophil (%)	0.18 ± 0.12	0.14 ± 0.14	0.33 ± 0.23	0.14 ± 0.14
MCH [pg]	18.45 ± 0.14	18.95 ± 0.22	18.50 ± 0.29	18.47 ± 0.24
MCHC [g/dL]	33.98 ± 0.07	34.28 ± 0.22	33.97 ± 0.15	33.54 ± 0.47
MCV [fL]	54.28 ± 0.53	55.30 ± 0.60	54.37 ± 0.76	55.07 ± 0.84
RDW (%)	12.70 ± 1.25	12.57 ± 0.45	13.72 ± 0.56	13.48 ± 0.42
Thrombocyte [$\times 10^9$/L]	914.2 ± 23.5	827.5 ± 37.3	881.3 ± 43.9	879.1 ± 41.8
PCT	0.47 ± 0.01	0.43 ± 0.02	0.44 ± 0.01	0.45 ± 0.008
PDW	16.02 ± 0.12	15.98 ± 0.32	16.30 ± 0.15	15.77 ± 0.15

MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RDW, red cell distribution width; PCT, plateletcrit; PDW, platelet distribution width.

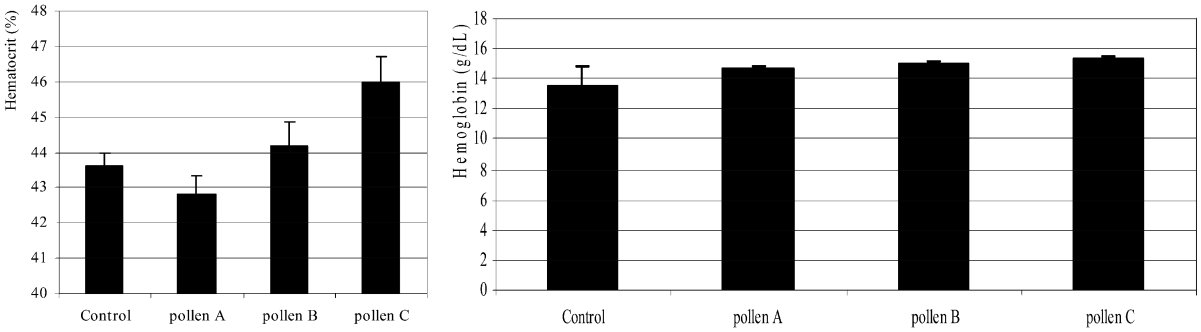

Fig. 1. Hematocrit and hemoglobin values of rats which were fed with pollen A, B and C for 30 experimental days.

spp. had the highest, in terms of protein percentages, *Cistus* spp. had the lowest value.

The hematological analysis results obtained for the control and experimental groups were investigated for comparison and then prepared as graphic representations. 10.07–11.34% increase was seen in the hemoglobin values of the pollen-fed rats. In the blood cell count of the group of rats eating the pollen of *Trifolium* spp., *Raphanus* spp., and *Cistus* spp., the eosinophil and lymphocyte proportions increased as compared with the control group, while neutrophil and monocyte proportions decreased, and different values were found for the basophil leukocyte values in the pollen groups (Table II and Figs. 1–3).

The hematocrit measurements of the rats fed with pollens B and C were seen to have increased as compared with the control, while the group fed with pollen A revealed no significant decrease. The biggest increase was again observed in the pollen of *Cistus* spp. An increase was shown during investigation of the hemoglobin levels of the pollen-fed rats (10.07%, 11.0% and 11.34%, respectively). These informations are presented in Fig. 1. The largest increase of 11.7% was seen in the pollen of *Cistus* spp. (pollen C). Of the three different pollens fed to the rats, those rats having eaten *Cistus* spp. were found to have higher values for lymphocytes, eosinophil, hemoglobin and hematocrit than the others, as well as lower concentrations of mesenteric mast cells. Differences in the pollens' chemical contents were determined for a relationship with the different effects seen between the basophil leukocyte values of the pollen-fed groups. In the same way, the greatest decrease in monocyte was established with the *Trifolium* spp. (pollen A) and *Cistus* spp. (pollen C) pollens, and the greatest decrease was seen in neutrophils in *Raphanus* spp. (pollen B) and *Cistus* spp. (pollen C) (Fig. 2).

The biggest change observed upon investigation of the rats' blood cells in the pollen-fed group during the 30-day period was in lymphocytes and eosinophil. The greatest increase in percentage lymphocyte and eosinophil values was shown in pollen C during research (Figs. 2 and 3). No difference was found in other blood values during testing.

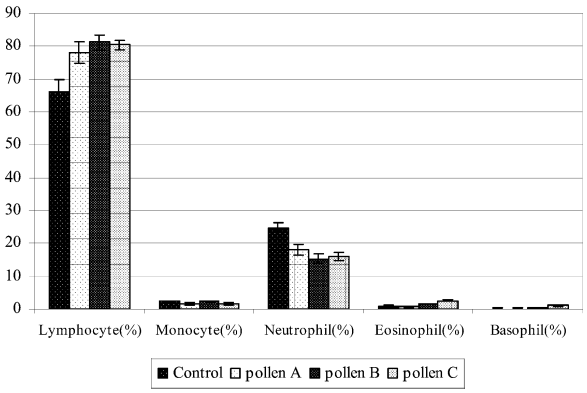

Fig. 2. Percentage of white blood cells and of changing values of blood cell numbers of rats which were fed with pollen A, B and C for 30 experimental days.

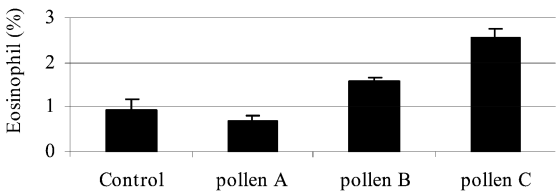

Fig. 3. Percentage of eosinophil leucocytes of rats which were fed with pollen A, B and C for 30 experimental days.

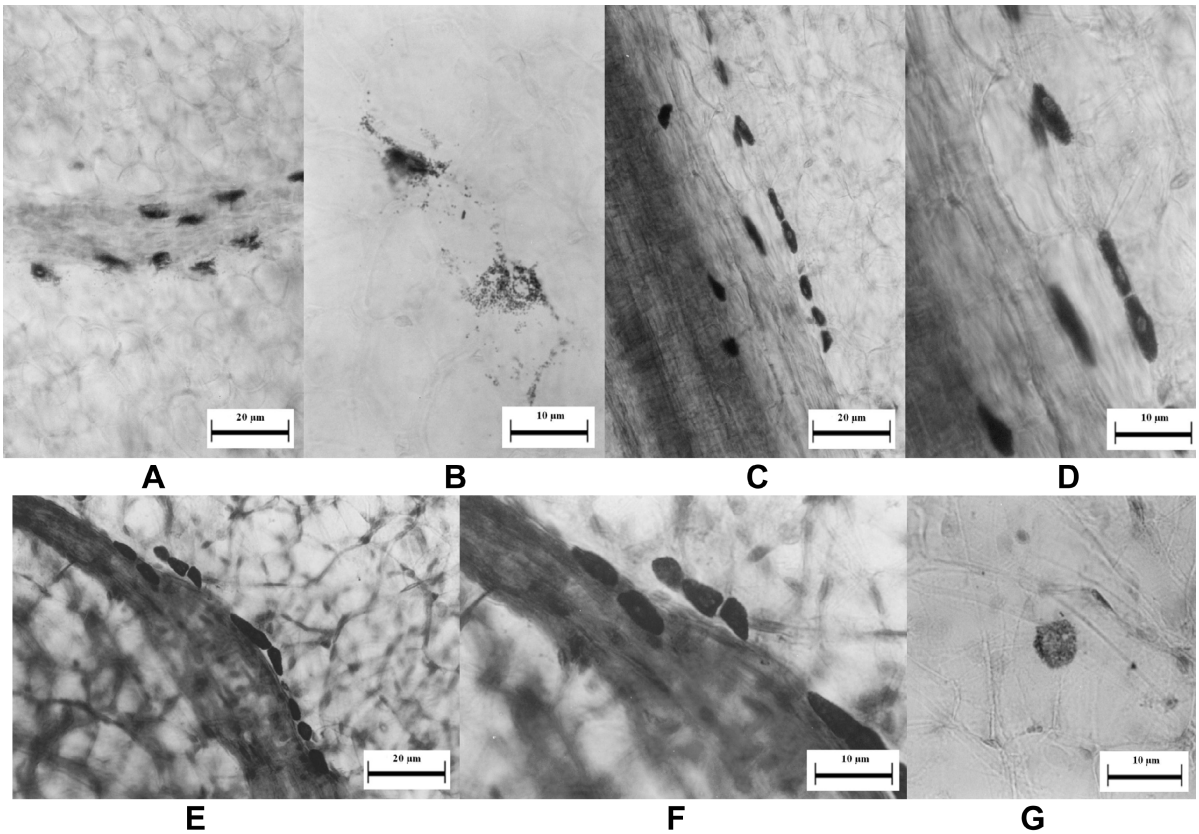

Fig. 4. Light microscope appearance of normal mesentery (A) and degranule mast cells (B) (control), A pollen (C, D), C pollen (E, F) and non-degranule mast cells (G). Enlargement for A, C, E: X 80; for B, D, F, G: X 160.

Despite the increase in eosinophil in the blood, no differences were found between the control and test groups in terms of low eosinophil count and concentration upon examination of mucus taken from the stomach and small intestine. For this reason pollen taken orally is said not to be the reason for the occurrence of allergic eosinophil gastroenteritis.

The increase seen in % lymphocyte and % eosinophil values during examination in rats fed pollen and % neutrophil values demonstrated different reductions between the pollen groups. On the side of immunity cellulisation, the responsible cells cause an increase in the lymphocytes; pollen has been seen to play an important role in the strengthening of immunity (Fig. 2). A similar study was conducted at the Romanian Faculty of Zootechnics of the Institute of Agriculture. This study is alike in that it also reports an increase in the quantity of blood lymphocytes, as well as in protein and gamma globin level (Palos *et al.,* 2005).

Upon examination of the mesentery tissue preparations taken, different degrees of reduction in low mast cell concentration, the state of degranulization and cell localization were observed. Concerning the examination and comparison of the mast cell count and state of granulization in the rats receiving pollen A, the rats were found to have a lower cell count in comparison with the other groups (Fig. 4). The mucus and epithelium of the rats' alimentary canals were examined. No eosinophils were found in the normal control and test groups in results given from mucus samples for allergic eosinophil gastroenteritis.

This study established an important difference previously not seen and the existence of an allergic reaction during the 30-day study period in mesentery mast cell count, degranulization and eosinophil proportion. Taken into consideration with the results of previous studies, the pollen of *Cistus* spp. in particular can be said to have a strengthening effect on the immune system through an increase

in the ratio of lymphocytes accommodated. The fact that the rats fed with *Cistus* spp. pollen in particular show a very small increase in eosinophils supports this idea. The pollen content changes according to various plant taxa. So, various pollen grains caused different increase in hemoglobin amount in blood.

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