

The Effects of Pollen on Serum Parameters, and Liver and Kidney Tissues of Rats

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SUMMARY

The objective of this study was to investigate any positive effects or possible side effects of the use of pollen. Mature male rats were fed pollen of three different plant sources (*Trifolium* spp., *Raphanus* spp. and *Cistus* spp.) at the rate of 60 mg/animal/day over a period of 30 days. After treatment, biochemical parameters and serum enzyme activities were analysed and weights of liver and kidney measured. Liver and kidney tissues of rats were examined by light microscope.

Serum cholesterol and HDL levels decreased in rats fed on pollen of *Trifolium* spp. and *Cistus* spp. Serum glucose levels increased in rats given pollen of *Trifolium* spp. and *Raphanus* spp. There was no change in serum enzyme levels in rats of any pollen group. While absolute liver weights of rats fed on pollen of *Trifolium* spp. and *Cistus* spp. increased, no change at all in absolute kidney weight and relative weight (organ weight/body weight) of liver and kidney of rats was found in any pollen group. Histopathological changes in the liver and kidney of rats given pollen were not observed.

Although serum cholesterol and HDL levels decreased, we cannot suggest that pollen caused either adverse or beneficial effects because of the short treatment period of 30 days.

Keywords: Pollen; Rat; Serum parameters; Histopathology

INTRODUCTION

Pollen is a food of perfectly balanced nutritional value. Pollen collected by honeybees normally contains 40% proteins, essential amino acids, low amounts of fat and high levels of minerals. Pollen, which is the male seed of flowers, is required for plant fertilization.

Bee pollen, used by many insect species as food, contains all the essential components of life. Besides proteins, it contains several vitamins, enzymes or coenzymes, carbohydrates and hormones. In addition, pollen has very low calorie value but is a rich source of minerals, including magnesium, calcium, copper, manganese, etc. Comparing it to agricultural crops, pol-

len contains more proteins, iron (Fe), thiamine, riboflavin and niacin than tomato, cabbage, apple, bread, beef or eggs of equal weight (Schmidt, 1997). Bee pollen is consumed as natural food in healthy human diet in many European and Asian countries.

There have been several studies about the effects of pollen on animals. Organ and body weights of rats fed on pollen for 90 days were reported to be higher than controls (Liebelt and Calcagineti, 1994). Some investigators also showed that female and male rats fed on pollen were healthier looking and more bright bristled than control animals.

When bee pollen is given to anemic patients, levels of hemoglobin increase considerably. It has been reported that bee pollen normalizes cholesterol and triglyceride levels in blood. A reduction in cholesterol and triglycerides has been observed after regular consumption of bee pollen. High-density lipoproteins (HDL) increase, while low-density lipoproteins (LDL) decrease (Mercola, 2003). It has also been suggested that pollen could be used for treatment of allergic reactions; when rats were fed 20 mg pollen during five days, IgG levels were increased but IgE was restrained in their blood (Palos et al., 1975). Eosinophil and lymphocyte levels in rats fed on pollen of *Trifolium* spp., *Raphanus* spp. and *Cistus* spp. at a rate of 60 mg/animal/day for 30 days were observed to increase. While neutrophil and monocyte levels decreased, different values were found in basophile leucocytes between the pollen groups. Different reductions in mesentery mast cell concentration, degranulation and cell localisation were found. Considering the three pollen groups, the rats fed on *Cistus* spp. pollen were observed to have higher blood lymphocyte, eosinophil, haemoglobin and hematocrit values than the other animals, as well as low mesentery mast cell concentration (Kolankaya et al., 2006).

Bee pollen contains nearly all nutrients that are present in plant sources and required by human organisms and it is used widely as food. Therefore, the present study was designed to evaluate the effects of three different pollens collected in Turkey on the functioning of liver and kidney of rats.

MATERIAL AND METHODS

Pollen

Pollen samples were collected from different sites in Bursa, Turkey. Three different plant species were used

as sources of pollen in this study. These are Fabaceae - *Trifolium* spp (A pollen); Brassicaceae - *Raphanus* spp (B pollen); Cistaceae - *Cistus* spp (C pollen).

Animals

Male Wistar albino rats, aged 2-3 months obtained from the experimental Animal Production Center of Hacettepe University, Ankara, Turkey, were used. The rats were randomly divided into four groups of eight animals each. Each cage housed two rats. The first group was the control group and the other three were experimental groups. Three different pollen taxa were applied to the experimental groups. A pollen was applied to the second group of rats, B pollen to the third group, and C pollen to the fourth group. Pollen in the amount of 60 mg/rat/day was given with mixed standard laboratory diet for a 30-day period. The animals were kept in polyethylene cages (two animals in each) with food and water available *ad libitum*.

Body weight, organ weight, food and water consumption

Water and food consumption was recorded daily and individual animal body weight weekly throughout the experiment. Liver and kidneys were removed and weighted after the 30-day period. Relative organ weight (organ weight/body weight) of each animal were calculated.

Biochemical analysis

At the end of the experiment, blood samples were taken from the heart of each rat for biochemical analysis after cervical dislocation. After centrifugation at 3200 rpm for 30 min, serum was separated. Serum enzyme activities (AST = Aspartate Aminotransferase; ALT = Alanine Transaminase; ALP = Alkaline Phosphatase; acid phosphatase) and biochemical parameters (HDL, albumin, glucose, creatinine, cholesterol, urea, triglyceride, total bilirubin, total protein) were measured by spectrophotometer using an autoanalyzer. Analyses were carried out in the Duzen Laboratories Group. Zinc was analyzed by atomic absorption spectrophotometer (Perkin Elmer Analyst 100) and the other biochemical and electrolyte parameters (sodium, potassi-

um, chlorine, calcium, phosphorus) using a Hitachi 912 autoanalyzer.

Histopathological investigation

Liver and kidneys of each animal were immediately removed. For histopathological examination, liver and kidneys were fixed in Bouin solution and embedded in paraffin. After routine processing, paraffin sections of each tissue were cut into 5-6 μm thickness and stained with haematoxylin and eosin (Gurr, 1972). The sections were examined under light microscope.

Statistical Analysis

Data for biochemical blood analyses, organ weights and relative organ weights (organ weight/body weight) were analyzed using the SPSS software package, version 9.0. The results were presented as the mean \pm standard deviation (SD) for each parameter. Comparisons were made between the control and experimental groups. Values of $P=0.05$ were regarded as statistically significant.

RESULTS AND DISCUSSION

Data on the biochemical parameters, enzyme activities and serum electrolyte levels in control rats and those given pollen **A**, **B** or **C** are presented in Tables 1-3. Acid phosphate activity in rats given pollen **A** and **C**, ALP levels in rats given pollen **B** and AST levels in rats fed pollen **C** were significantly decreased compared to the control group (Table 1). No statistically significant differences were found in the ALT activity of treated

animals. An increase in AST, ALP and ALT activities indicates liver injury. Since there was no significant increase in enzyme activities, we can say that pollen did not cause any toxic effect on liver and kidney functioning. Wojcicki et al. (2004) investigated the hepatoprotective effect of pollen extracts (Cernitins) in male Wistar rats, and reported a positive effect of Cernitins against liver cell injury. As regards our histological examination, no pollen caused histological changes in the liver and kidney tissues of rats.

Statistically significant differences, compared with control, were found in glucose, bilirubin, cholesterol and HDL levels (Table 2). Glucose level increased in all treated groups, but it was not statistically significant in the pollen group **C**. Carbohydrate contents showed significant differences (ranging 7.5-40%), depending on plant sources (http://www.garynull.com/Documents/Arthritis/bee_pollen.htm). Increase in glucose level may be attributed to the regular pollen consumption for 30 days. The cholesterol levels in rats given pollen **A** and **C** decreased significantly compared with the control group. The decrease in HDL levels of rats given pollen **C** was also statistically significant. We found that total cholesterol and triglycerides decreased, which is the most beneficial effect on the lipid profile. Mercola (2003) found that high-density lipoproteins (HDL) increased while low-density lipoproteins (LDL) decreased. It has been reported that bee pollen in a diet normalizes cholesterol and triglyceride levels in blood. After regular consumption of bee pollen, reports have been made of reduced cholesterol and triglycerides, HDL increase and LDL decrease (<http://www.florahealth.com/flora/home/canada/products/r1510.asp>).

Regarding serum electrolytes analysis, statistically significant decreases were found in sodium (Na), chlorine (Cl), phosphorus and zinc (Zn) levels in rats

Table 1. Serum enzyme activity in control and treated rats

Tabela 1. Aktivnost serumskih enzima kod kontrolnih i tretiranih pacova

Parameter Parametar	Group - Grupa*			
	Control Kontrola	Pollen A Polen A	Pollen B Polen B	Pollen C Polen C
AST (U/L)	146.7 \pm 14.3	122.7 \pm 6.1	127.5 \pm 10.7	114.0 \pm 9.8**
ALT (U/L)	46.2 \pm 2.92	43.7 \pm 3.73	47.7 \pm 2.80	42.1 \pm 2.09
ALP (U/L)	189.1 \pm 9.5	169.7 \pm 10.4	154.0 \pm 11.4**	184.1 \pm 41.0
Acid phosphatase (U/L) Kisela fosfataza (U/L)	32.0 \pm 1.33	22.2 \pm 0.80**	26.9 \pm 1.73	22.3 \pm 2.16*

* Values are given as the mean \pm SD; * Srednja vrednost \pm SD

** Significantly different from control ($P<0.05$); ** Razlika je statistički značajna ($P<0.05$)

Table 2. Biochemical parameters in control and treated rats
Tabela 2. Biohemijski parametri kod kontrolnih i tretiranih pacova

Parameter Parametar	Group – Grupa*			
	Control Kontrola	Pollen A Polen A	Pollen B Polen B	Pollen C Polen C
Glucose (mg/dL) Glukoza (mg/dL)	116.7±12.41	150.2±5.19**	139.8±4.44**	133.8±7.71
Creatinine (mg/dL) Kreatinin (mg/dL)	0.51±0.018	0.51±0.010	0.54±0.017	0.48±0.010
Urea (mg/dL) Urea (mg/dL)	19.57±0.77	17.90±0.70	17.02±1.10	19.57±1.00
Total bilirubin (mg/dL) Ukupni bilirubin (mg/dL)	0.13±0.008	0.09±0.006**	0.11±0.014	0.14±0.011
Total protein (g/dL) Ukupni proteini (g/dL)	7.00±0.08	6.71±0.12	7.03±0.13	7.04±0.09
Albumin (g/dL) Albumin (g/dL)	3.52±0.03	3.60±0.04	3.53±0.05	3.48±0.05
Cholesterol (mg/dL) Holesterol (mg/dL)	57.7±1.71	52.2±2.03**	55.7±2.01	49.4±1.13**
Triglyceride (mg/dL) Trigliceridi (mg/dL)	31.71±2.24	40.0±4.77	40.37±4.79	37.42±6.86
HDL (mg/dL)	42.14±1.58	40.28±1.71	40.75±1.81	35.28±1.52**

* Values are given as the mean ± SD; * Srednja vrednost ± SD;

** Significantly different from control (P<0.05); ** Razlika je statistički značajna (P<0.05)

Table 3. Serum electrolyte levels in control and treated rats
Tabela 3. Nivo serumskih elektrolita kod kontrolnih i tretiranih pacova

Parameter Parametar	Group – Grupa*			
	Control Kontrola	Pollen A Polen A	Pollen B Polen B	Pollen C Polen C
Sodium (mEq/L) Natrijum (mEq/L)	149.1±0.59	151.7±0.56**	147.2±0.61**	148.8±0.40
Potassium (mEq/L) Kalijum (mEq/L)	5.90±0.17	5.77±0.16	5.98±0.28	5.32±0.34
Chlorine (mEq/L) Hlor (mEq/L)	106.2±0.47	108.5±0.42**	101.7±0.75**	102.1±0.59**
Calcium (mg/dL) Kalcijum (mg/dL)	9.60±0.77	10.71±0.12	10.57±0.23	10.42±0.19
Phosphorus (mg/dL) Forfor (mg/dL)	10.02±0.34	9.95±0.30	8.88±0.18**	8.40±0.48**
Zinc (µg/L) Cink (µg/L)	182.8±3.91	179.1±2.97	171.8±4.99	167.7±7.21**

* Values are given as the mean ± SD; * Srednja vrednost ± SD;

** Significantly different from control (P<0.05); ** Razlika je statistički značajna (P<0.05)

in the pollen groups **B** and **C**, compared with control animals. However, Na and Cl levels in rats fed pollen **A** were increased and the increase was statistically significant (Table 3). Those changes may be of biological importance, but we cannot say that the difference in electrolyte levels resulted from pollen treatment.

Body weight, organ weight (liver and kidneys) and relative organ weight of control and pollen-fed rats

are presented in Table 4. Body weight gain was significantly higher in treated than in control animals. There were increases in liver and kidney weights of rats in pollen **A** and pollen **C** groups. Those increases were only statistically significant for liver weight. There were no statistically significant changes in the relative organ weight of liver and kidneys.

Table 4. Body weight, body weight gain, absolute and relative organ weight in control and treated rats ($\times 10^3$)
Tabela 4. Telesna masa, prirast telesne mase, apsolutna i relativna masa organa kontrolnih i tretiranih pacova ($\times 10^3$)

Parameter Parametar	Group – Grupa*			
	Control Kontrola	Pollen A Polen A	Pollen B Polen B	Pollen C Polen C
Initial weight (g) Početna masa (g)	146.14±7.15	148.14±13.28	140.37±6.65	145.42±10.81
Final weight (g) Konačna masa (g)	195.92±6.79	219.28±11.31	204.00±10.15	214.21±7.07
Weight gain (g) Prirast telesne mase (g)	49.78	71.14	63.63	68.79
Absolute organ weight (g): Apsolutna masa organa (g):				
• Liver (jetra)	5.51±0.27	6.83±0.27**	6.16±0.36	6.44±0.19**
• Kidneys (bubrezi)	0.78±0.04	0.82±0.04	0.76±0.03	0.84±0.03
Relative organ weight (g): Relativna masa organa (g):				
• Liver (jetra)	28.0±0.63	31.5±1.72	30.2±0.86	30.1±0.92
• Kidneys (bubrezi)	3.99±0.13	3.78±0.22	3.71±0.05	3.93±0.07

* Values are given as the mean \pm SD; * Srednja vrednost \pm SD

** Significantly different from control ($P < 0.05$); ** Razlika je statistički značajna ($P < 0.05$)

In histopathological examination, we did not observe histological changes in liver and kidney tissues of rats in any pollen group compared to control. Histological appearance of the liver and kidneys of rats given pollen resembled that of control rats.

In the present study, when all results were evaluated, we observed no statistically significant toxic effect either in the biochemical analysis or in histopathological investigation of liver and kidneys of rats given three different pollens. Finally, the results of our investigation suggest that the pollens caused no negative effect on liver and kidney functions in rats. There are good grounds for saying that some pollen, such as pollen C, has positive effect. However, in order to be able to draw reliable conclusions in that respect we suggest that the investigation period be extended beyond 30 days.

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Uticaj polena na parametre seruma i tkiva jetre i bubrega kod pacova

REZIME

Polen je hrana sa savršeno uravnoteženim vrednostima hranljivih materija. Polen koji prikupljaju pčele uglavnom ima 40% sadržaja proteina, esencijalnih aminokiselina, malo masti i visok sadržaj minerala. Predmet ovog istraživanja bilo je ispitivanje pozitivnog delovanja ili mogućeg sporednog delovanja polena. Odrasli mužjaci pacova su u ovom istraživanju dobijali ishranom polen tri vrste biljaka (*Trifolium* spp., *Raphanus* spp. i *Cistus* spp.) i to u koncentraciji od 60 mg po životinji dnevno tokom 30 dana. Na kraju tretmana, analizirani su biohemijski parametri i aktivnost serumskih enzima i zabeležena masa jetre i bubrega. Tkiva jetre i bubrega su takođe ispitivana pod svetlosnim mikroskopom.

Kod pacova koji su hranjeni polenom *Trifolium* spp. i *Cistus* spp. došlo je do smanjenja nivoa holesterola i HDL u serumu. Nivo glukoze u serumu je povećan kod pacova koji su primali polen *Trifolium* spp. i *Raphanus* spp. Nije bilo promene nivoa enzima kod pacova koji su primali različite vrste polena. Dok se apsolutna masa jetre kod pacova koji su dobijali polen *Trifolium* spp. i *Cistus* spp. povećala, nije zapažena nikakva promena apsolutne mase bubrega, niti relativne mase jetre i bubrega kod pacova koji su dobijali različite vrste polena. Tkivo jetre i bubrega pacova koji su dobijali polen bilo je slično onom kod kontrolne grupe pacova.

Prema podacima dobijenim u ovom istraživanju, iako su se nivoi holesterola i HDL u serumu smanjili, ne možemo sa sigurnošću utvrditi eventualne negativne ili pozitivne efekte zbog trajanja tretmana polenom od samo 30 dana.

Ključne reči: Polen; pacov; parametri u serumu; histopatologija