

Evaluation of the effectiveness of beehive air and pollen treatments in rats

Sıttıka Delen^{1a}, Elif Yılmaz Şahin^{1b}, Vahdettin Altunok^{1c}, Meral Kekeçoğlu^{2d},
Beyza Suvarıklı Alan^{1e}, Tuğçe Çaprazlı^{3f}, Abdullah Sivrikaya^{4g}

¹Selçuk University, Faculty of Veterinary, Department of Biochemistry, Konya, Türkiye

²Düzce University, Faculty of Arts and Science, Department of Biology, Düzce, Türkiye

³Düzce University, Düzce Vocational School, Plant and Animal Production

Department, Beekeeping Program, Düzce, Türkiye

⁴Selçuk University, Faculty of Medicine, Department of Biochemistry, Konya, Türkiye

^a0000-0001-6540-5904, ^b0000-0001-7730-393X, ^c0000-0002-4076-5492, ^d0000-0002-2564-8343,
^e0000-0003-4698-9291, ^f0000-0001-9109-0969, ^g0000-0003-2956-5681

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Abstract

In recent years, beehive air (apiair), which is used as a supportive treatment for respiratory disorders like chronic obstructive pulmonary disease (COPD), asthma, and bronchitis, has attracted significant interest. The aim of this study was to investigate changes in selected serum biochemical parameters immunoglobulines, and antioxidants in rats using combinations of beehive air, pollen, and beehive air + pollen. Furthermore, the effect of apiair treatment on asthma and COPD patients was evaluated. Rats were divided into the five groups: (1) Control, (2) sham, (3) beehive air, (4) pollen, and (5) beehive air + pollen. They were treated for 20 days and fed *ad libitum*. Inorganic phosphate (Pi), zinc (Zn) and iron (Fe) concentrations were higher in the beehive air and the beehive air + pollen groups ($P < 0.05$), and blood urea nitrogen, immunoglobulin G and M concentrations were higher in the pollen group ($P < 0.05$) compared to control. The elevation in blood albumin, Fe, Zn, and Pi concentrations following beehive air treatment ($P < 0.05$) and the elevation in blood Fe, Zn, Pi, and calcium concentrations following beehive air + pollen treatment ($P < 0.05$) indicated that beehive air alone or in combination with pollen may be an effective supportive treatment option in cases of Zn deficiency disorders, iron deficiency anaemia, hypoalbuminaemia, and hypocalcaemia. Furthermore, the finding of high Zn and Fe concentrations in beehive air ($P < 0.05$) may explain the effect of beehive air treatment on pulmonary disorders reported in literature such as asthma and COPD with low blood Zn and Fe concentrations.

Apiair, biochemical parameters, immunoglobulines, antioxidants

Apitherapy can be defined as the use of bee products such as honey, beeswax, pollen, propolis, apilarnil, larvae of queen bees, royal jelly, and bee venom for medicinal purposes (Topal et al. 2021). Another novelty was added to the bee products used in apitherapy studies with the ‘bee air or hive air (apiair)’ treatment practised for the first time in the world by the beekeeper Hans Musch in Germany (Musch 2014). Today, beehive air and all other bee products have become the subject of studies due to their beneficial effects. It has been reported that relaxation, uninterrupted and quality sleep, expansion of lung capacity, and therefore comfortable breathing are achieved in individuals who breathe beehive air or sleep in the hive for therapeutic purposes (Lovrić 2015; Topal et al. 2021).

Beehive air (apiair) is administered as a supportive treatment for respiratory disorders such as chronic obstructive pulmonary disease (COPD), asthma, bronchitis, allergies, emphysema, immune system deficiencies, as well as pulmonary fibrosis (Bengsch 2014; Gosar 2015; Ünal et al. 2015; McKenzie and Schmiedgen 2021). However,

Address for correspondence:

Beyza Suvarıklı Alan
Department of Biochemistry
Faculty of Veterinary
Selçuk University, Konya, Türkiye

E-mail: beyza.alan@selcuk.edu.tr
<http://actavet.vfu.cz/>

the effectiveness of beehive air treatments in lung diseases, particularly COPD and asthma, as well as immune system deficiencies, remains unclear. It is stated that zinc (Zn) concentrations are low in COPD and asthma patients (Karadağ et al. 2004; Sarıbal 2006; Kırkıl et al. 2008) and that including Zn in the treatment would be beneficial. Al-Fartusie et al. (2021) reported that Zn concentrations were significantly ($P < 0.01$) lowered in asthmatic patients compared to healthy subjects and stated that low Zn concentrations were among the factors that might contribute to allergic diseases due to their role in the synthesis of some antioxidants or their effects on the immune system. It was reported that serum Zn concentrations of patients with allergic asthma were lower than in the healthy control group. Low concentrations of trace elements (especially Zn) may play a role in the pathogenesis of allergic asthma, and supplementation of these elements would be beneficial for treatment (Ariaee et al. 2016).

Germany, Russia, Ukraine, Hungary, Slovenia, and Austria are among the countries that widely use beehive air. However, it has been reported that scientific data related to the beehive air treatment and the evaluation of its outcomes are insufficient; no scientific studies have been found on living organisms, and therefore, further research in this field is required (Maennle et al. 2020; Topal et al. 2021; Guardia 2021).

The aim of this study was to investigate possible changes in some serum biochemical parameters, immunoglobulins, antioxidants, bronchoalveolar lavage (BAL) fluid parameters, and live weight gain in rats exposed to beehive air, pollen, and beehive air + pollen combinations.

Materials and Methods

In this study, 40 male Wistar Albino rats aged 12 weeks and raised at the Experimental Animal Research Centre (DÜDAM) of Düzce University were used. The study was conducted with the approval (dated 17/12/2019 and No. 2019/12/1) from the Animal Experiments Local Ethics Committee (HADYEK) of Düzce University. The rats were kept at a temperature of 21 ± 2 °C with a relative humidity of 54–56% under a constant 12/12-h light-dark cycle.

Experimental design

Before the beginning of the study, the procedure of beehive air treatment was established by running preliminary trials for the beehive air treatments to be applied on rats. Then, the rats used in the study underwent an adaptation period of one week. After adaptation, all rats were weighed, and their live weights were recorded. The rats to be used in the study were divided into five groups evenly, with no significant (Kolmogorov Smirnov and Shapiro Wilk tests) difference between their live weights. The study was conducted on five groups: (1) Control group; (2) sham group; (3) beehive air group; (4) pollen group; and (5) beehive air + pollen group. The rats assigned to the groups were fed tap water and standard rat chow *ad libitum*. Treatments were carried out in groups 1, 2, 3, 4, and 5 for 20 days.

The control group ($n = 7$) was housed in cages under the same experimental conditions. The sham group of rats ($n = 8$) were administered 0.5 ml of distilled water by gavage at the same time every day from the beginning of the study. The beehive air group of rats ($n = 8$) were treated with beehive air for 30 min once a day (9:00 h to 10:00 h). In the pollen group ($n = 8$), freshly prepared bee pollen at a dose of 200 mg/kg/day was dissolved in 0.5 ml of distilled water and injected to rats with a gavage syringe (No. 16) once a day (at the same time). Pollen doses were adjusted for the pollen group every 5 days according to the rats' live weights. The beehive air + pollen group of rats ($n = 7$) were treated with beehive air and pollen at a dose of 200 mg/kg/day that was dissolved in 0.5 ml of distilled water for 30 min (9:00 h to 10:00 h) and injected with a gavage syringe (No. 16) once a day (at the same time).

In the experiment, Yığılca bee pollen was used, which was produced commercially by the Beekeeping Research, Development, and Application Centre at Düzce University (DAGEM). The content analysis of the pollen sample used in the study was done in the Department of Biology, Faculty of Science, Düzce University (Table 1).

Beehive air treatment

The study was conducted in July–August. The beehives used in the study were procured from DAGEM, located in Yığılca District (Düzce, Türkiye). Düzce University is located at the coordinates of 40.847836 latitude and 30.939087 longitude. Two double-decker beehives were utilised for each group to be treated with beehive air. Since the number of bees in the hive was standardised by the number of slats, the number of slats in each hive was equalized using 10 slats (Plate IX, Figs 1 and 2).

Table 1. Chemical composition of Yığılca bee pollen used in the study.

Content	Minimum-maximum values
Proteins	18.04%
Humidity	19.92%
Phenols	33.21 mg GAE (Gallic acid equivalent)/g
Flavonoids	3.42 mg QE (Quercetin equivalent)/g
DPPH (2,2-diphenyl-1-picrylhydrazyl radical scavenging) values	3.05 mg TEAC (Trolox equivalent antioxidant capacity)/g
Palmitic acid	20.97%
Stearic acid	3.02%
Oleic acid	13.20%
Linoleic acid	19.21%
Linolenic acid	13.30%
Eicosanoid acid	10.15%
Total fatty acids	30.07%

Sample collection

At the end of the experiment, the rats were anaesthetized intramuscularly with ketamine and xylazine (90/10 mg/kg). Blood samples were drawn intracardially from the anaesthetized rats. At the end of the experiment, blood serum and BAL fluid samples were collected. Blood samples were centrifuged at $1,559 \times g$ at 4°C for 15 min; serum was extracted and stored at -80°C . Anaesthetized rats were fixed by their limbs on a surgical tray. The skin on the neck near the trachea was incised with a scalpel. The muscles around the trachea were incised to expose the trachea. Sterile saline (2 cc) was carefully injected between two cartilage rings with syringe no. 22 without harming the trachea. The saline was retracted by gently massaging the rat's thorax without harming it. The procedure was repeated twice, and BAL fluid was extracted and placed in tubes. The supernatant was stored at -80°C after centrifugation at $2,000 \times g$ at 4°C for 15 min. After blood and BAL samples were collected, the rats were euthanized by decapitation.

Biochemical analysis

Biochemical parameters from serum samples collected at the end of the experiment included concentrations of glucose, triglycerides, total cholesterol, gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid, creatinine, total protein, albumin, total bilirubin, sodium (Na), potassium (K), calcium (Ca), chlorine (Cl), inorganic phosphate (Pi), zinc (Zn), iron (Fe), and immunoglobulin G (IgG). Commercial kits were used to analyse GGT, ALT, ALP, total protein, albumin, Ca, Pi (Architect Abbott Laboratories, Princeton, USA), magnesium (Mg) (DiaLab, Wiener Neudorf, Austria), blood urea nitrogen (BUN), and Zn (Improgen Diagnostic Solutions, İstanbul, Türkiye) in BAL fluid using an autoanalyser device (Architect c8000, Princeton, USA).

Commercial ELISA kits were used to measure the concentrations of superoxide dismutase (SOD) (Cayman, Michigan, USA), malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), and immunoglobulin M (IgM) (Bioassay Technology Laboratory, Rat, ELISA Kit, Shanghai, China) using an ELISA reader (CLARIOstar 25, Ortenberg, Germany).

Statistical analysis

Kolmogorov Smirnov and Shapiro-Wilk's tests were used to analyse normality of data and one-way analysis of variance was used to compare more than two groups for which the assumption of normality was met. When there was a difference between the groups, Duncan multiple comparison test was run to analyse the difference, and $P < 0.05$ was considered as significant. Statistical analyses were done using the IBM SPSS Statics 20.0, Corporation, Armonk, New York, USA packaged software.

Results

No significant difference ($P > 0.05$) was found between the groups in live weight gain at the end of the study. No significant difference ($P > 0.05$) was found between the control and sham groups in terms of all biochemical parameters (Table 2).

Table 2. Serum values in control and experimental groups (mean $\pm$ SEM).

Indicator	Groups				
	Control (n = 7)	Sham (n = 8)	Apiair (n = 8)	Pollen (n = 8)	Apiair + pollen (n = 7)
Glucose (mg/dl)	246.29 $\pm$ 27.61	194.25 $\pm$ 8.04	245.88 $\pm$ 27.11	196.13 $\pm$ 19.03	212.00 $\pm$ 31.78
Triglycerides (mg/dl)	60.58 $\pm$ 2.91	61.00 $\pm$ 2.67	61.13 $\pm$ 3.57	60.13 $\pm$ 4.39	68.57 $\pm$ 1.21
Cholesterol (mg/dl)	65.43 $\pm$ 2.97	68.88 $\pm$ 2.01	67.25 $\pm$ 2.81	71.38 $\pm$ 2.80	68.00 $\pm$ 2.44
GGT (U/l)	1.71 $\pm$ 0.29	1.88 $\pm$ 0.30	1.75 $\pm$ 0.16	2.00 $\pm$ 0.19	2.14 $\pm$ 0.34
AST (U/l)	120.57 $\pm$ 8.43	129.63 $\pm$ 9.03	129.38 $\pm$ 8.39	133.38 $\pm$ 8.47	136.14 $\pm$ 10.69
ALT (U/l)	61.00 $\pm$ 3.15	53.75 $\pm$ 2.66	64.88 $\pm$ 2.23	58.38 $\pm$ 5.66	60.29 $\pm$ 2.37
ALP (U/l)	206.29 $\pm$ 17.05	218.38 $\pm$ 18.16	243.75 $\pm$ 24.83	216.13 $\pm$ 18.63	208.43 $\pm$ 17.97
BUN (mg/dl)	46.43 $\pm$ 3.46 ^b	48.75 $\pm$ 4.17 ^b	41.63 $\pm$ 0.60 ^b	61.75 $\pm$ 3.01 ^a	47.00 $\pm$ 1.56 ^b
Uric acid (mg/dl)	0.83 $\pm$ 0.05 ^{ab}	0.86 $\pm$ 0.04 ^{ab}	0.79 $\pm$ 0.05 ^b	0.99 $\pm$ 0.09 ^a	0.79 $\pm$ 0.06 ^b
Creatinin (mg/dl)	0.49 $\pm$ 0.01	0.48 $\pm$ 0.01	0.48 $\pm$ 0.01	0.49 $\pm$ 0.01	0.42 $\pm$ 0.06
Total protein (g/dl)	6.21 $\pm$ 0.06	6.44 $\pm$ 0.13	6.38 $\pm$ 0.07	6.48 $\pm$ 0.10	6.39 $\pm$ 0.11
Albumin (g/dl)	2.87 $\pm$ 0.03 ^b	2.99 $\pm$ 0.04 ^{ab}	3.01 $\pm$ 0.01 ^a	2.95 $\pm$ 0.03 ^{ab}	2.99 $\pm$ 0.07 ^{ab}
Total bilirubin (mg/dl)	0.14 $\pm$ 0.01	0.15 $\pm$ 0.00	0.13 $\pm$ 0.01	0.14 $\pm$ 0.00	0.15 $\pm$ 0.01
Na (mmol/l)	139.29 $\pm$ 0.57 ^{ab}	138.38 $\pm$ 1.21 ^b	140.50 $\pm$ 0.65 ^{ab}	138.63 $\pm$ 0.71 ^b	141.71 $\pm$ 0.68 ^a
K (mmol/l)	4.99 $\pm$ 0.20	4.96 $\pm$ 0.08	4.93 $\pm$ 0.28	5.31 $\pm$ 0.17	5.06 $\pm$ 0.23
Ca (mg/dl)	9.94 $\pm$ 0.10 ^b	9.93 $\pm$ 0.11 ^b	10.01 $\pm$ 0.09 ^{ab}	9.86 $\pm$ 0.06 ^b	10.26 $\pm$ 0.09 ^a
Cl (mmol/l)	98.43 $\pm$ 0.87 ^{ab}	96.88 $\pm$ 1.03 ^b	99.75 $\pm$ 0.77 ^a	96.38 $\pm$ 0.71 ^b	100.57 $\pm$ 0.57 ^a
Pi (mg/dl)	7.39 $\pm$ 0.15 ^b	7.21 $\pm$ 0.17 ^b	8.20 $\pm$ 0.22 ^a	7.14 $\pm$ 0.22 ^b	7.94 $\pm$ 0.16 ^a
Mg (mg/dl)	1.94 $\pm$ 0.06	1.93 $\pm$ 0.06	2.00 $\pm$ 0.04	2.09 $\pm$ 0.05	2.06 $\pm$ 0.0
Zn (ug/dl)	121.54 $\pm$ 8.13 ^b	120.93 $\pm$ 5.73 ^b	144.03 $\pm$ 2.43 ^a	125.89 $\pm$ 4.26 ^{ab}	141.11 $\pm$ 3.34 ^a
Fe (ug/dl)	157.00 $\pm$ 2.80 ^b	157.00 $\pm$ 2.33 ^b	170.88 $\pm$ 5.99 ^a	147.25 $\pm$ 3.85 ^b	175.00 $\pm$ 2.36 ^a

Different superscripts (^{a, b}) in the same row denote significantly different values ($P < 0.05$).

SEM - Standard error of the mean; GGT - gamma-glutamyl transferase; AST - aspartate aminotransferase; ALT - alanine aminotransferase; ALP - alkaline phosphatase; BUN - blood urea nitrogen; Na - sodium; K - potassium; Ca - calcium; Cl - chloride; Pi - inorganic phosphate; Mg - magnesium; Zn - zinc; Fe - iron

Compared to the control, Pi, Zn, and Fe concentrations in the beehive air and beehive air + pollen groups, and BUN concentrations in the pollen group were significantly higher ($P < 0.05$) (Table 2). Uric acid concentrations were higher ($P < 0.05$) in the pollen group compared to the beehive air and beehive air + pollen groups, Na concentrations were higher in the beehive air + pollen group compared to the pollen group ($P < 0.05$), and Cl concentrations were higher in the beehive air and beehive air + pollen groups compared to the pollen group ($P < 0.05$).

Concentrations of IgG and IgM were significantly higher ($P < 0.05$) in the pollen group compared to the control (Table 3).

Table 3. Serum IgG and IgM values in control and experimental groups (mean $\pm$ SEM).

Indicator	Groups				
	Control (n = 7)	Sham (n = 8)	Apiair (n = 8)	Pollen (n = 8)	Apiair + pollen (n = 7)
IgG (mg/dl)	242.43 $\pm$ 7.40 ^b	261 $\pm$ 8.29 ^{ab}	253.25 $\pm$ 9.47 ^b	283 $\pm$ 9.94 ^a	250 $\pm$ 9.86 ^b
IgM (ng/dl)	1221.7 $\pm$ 79.5 ^b	1286.5 $\pm$ 79.5 ^{ab}	1432.2 $\pm$ 75.9 ^{ab}	1480.9 $\pm$ 62.9 ^a	1270.4 $\pm$ 56.2 ^{ab}

Different superscripts (^{a, b}) in the same row denote significantly different values ($P < 0.05$).

SEM - Standard error of the mean; IgG - immunoglobulin G; IgM - immunoglobulin M

When all groups were analysed among themselves, SOD enzyme activity was found to be higher ($P < 0.05$) in pollen and beehive air + pollen groups compared to the beehive air group (Table 4).

Albumin and Ca concentrations were considered in BAL fluid compared to the control group, it was determined that the values of the beehive air groups were higher and the Pi concentration was lower in the beehive air group ($P < 0.05$).

Table 4. Serum antioxidant concentrations in control and experimental groups (mean $\pm$ SEM).

Indicator	Groups				
	Control (n = 7)	Sham (n = 8)	Apiair (n = 8)	Pollen (n = 8)	Apiair + pollen (n = 7)
MDA (nmol/ml)	0.63 $\pm$ 0.06	0.58 $\pm$ 0.04	0.51 $\pm$ 0.04	0.51 $\pm$ 0.06	0.63 $\pm$ 0.07
CAT (ng/ml)	9.10 $\pm$ 1.35	8.48 $\pm$ 0.80	9.97 $\pm$ 0.45	9.94 $\pm$ 1.22	10.95 $\pm$ 0.88
SOD (U/ml)	16.85 $\pm$ 1.17 ^{ab}	16.91 $\pm$ 0.95 ^{ab}	14.41 $\pm$ 1.50 ^b	20.01 $\pm$ 2.04 ^a	21.24 $\pm$ 1.11 ^a
GSH (mg/dl)	402.14 $\pm$ 10.50	409.17 $\pm$ 15.13	387.50 $\pm$ 11.66	428.33 $\pm$ 21.35	413.33 $\pm$ 12.29
GPx (ng/ml)	25.41 $\pm$ 2.86	23.87 $\pm$ 1.52	22.23 $\pm$ 2.20	24.55 $\pm$ 1.71	19.73 $\pm$ 2.74

Different superscripts (^{a, b}) in the same row denote significantly different values ($P < 0.05$).

SEM - Standard error of the mean; MDA - malondialdehyde; CAT - catalase; SOD - superoxide dismutase; GSH - reduced glutathione; GPx - glutathion peroxidase

Table 5. BAL fluid values in control and experimental groups (mean $\pm$ SEM).

Indicator	Groups				
	Control (n = 7)	Sham (n = 8)	Apiair (n = 8)	Pollen (n = 8)	Apiair + pollen (n = 7)
GGT (U/l)	3.14 $\pm$ 0.46	3.63 $\pm$ 0.32	4.25 $\pm$ 0.53	4.63 $\pm$ 1.00	4.43 $\pm$ 0.9
ALT (U/l)	1.71 $\pm$ 0.29	2.25 $\pm$ 0.56	2.00 $\pm$ 0.19	2.25 $\pm$ 0.49	2.14 $\pm$ 0.51
ALP (U/l)	36.43 $\pm$ 4.00	37.50 $\pm$ 4.16	45.13 $\pm$ 3.99	40.75 $\pm$ 2.19	33.43 $\pm$ 6.30
Albumin (mg/dl)	0.859 $\pm$ 0.002 ^b	0.860 $\pm$ 0.001 ^b	0.867 $\pm$ 0.002 ^a	0.856 $\pm$ 0.001 ^b	0.860 $\pm$ 0.001 ^b
Total protein (mg/dl)	2.65 $\pm$ 0.0 ^{ab}	2.62 $\pm$ 0.02 ^b	2.66 $\pm$ 0.01 ^a	2.64 $\pm$ 0.01 ^{ab}	2.66 $\pm$ 0.01 ^a
Ca (mg/dl)	3.11 $\pm$ 0.02 ^b	3.14 $\pm$ 0.03 ^{ab}	3.19 $\pm$ 0.02 ^a	3.16 $\pm$ 0.03 ^{ab}	3.11 $\pm$ 0.02 ^b
Pi (mg/dl)	2.99 $\pm$ 0.02 ^a	3.06 $\pm$ 0.07 ^a	3.03 $\pm$ 0.03 ^a	3.05 $\pm$ 0.02 ^a	2.83 $\pm$ 0.09 ^b
Zn (ug/dl)	30.06 $\pm$ 0.61	28.31 $\pm$ 2.21	30.85 $\pm$ 2.14	33.84 $\pm$ 1.39	31.05 $\pm$ 2.00

Different superscripts (^{a, b}) in the same row denote significantly different values ($P < 0.05$).

SEM - Standard error of the mean; GGT - gamma-glutamyl transferase; ALT - alanine aminotransferase; ALP - alkaline phosphatase; Ca - calcium; Pi - inorganic phosphate; Zn - zinc

Discussion

This study investigated the effectiveness of beehive air, pollen, and the combination of beehive air + pollen on live weight gain and changes in selected biochemical, immunoglobulin, and oxidative stress parameters in serum and BAL fluid of rats. Moreover, the effectiveness of apiair treatment in asthma and COPD patients was assessed with the data gathered.

Ghouizi et al. (2020) treated rats with three different (100, 250, and 500 mg/kg/day) doses of pollen and found that BUN concentrations were high in all three groups ($P < 0.05$). Laaroussi et al. (2020) studied the effect of pollen at different doses (100 mg/kg/day, 200 mg/kg/day) on diabetic rats. They reported that BUN and creatinine concentrations lowered ($P < 0.001$) in diabetic groups treated with pollen compared to untreated diabetic groups, while there was no difference in BUN and creatinine concentrations in groups treated only with pollen. On the other hand, this study revealed that BUN concentrations

were higher ($P < 0.05$) in the group treated with pollen compared to the control group (Table 2). Bee pollen is a nutrient rich in protein content and varies according to the geographical region, climate type, and bee genotype (Thakur and Nanda 2020). The differences in BUN values obtained in these studies may be due to different contents of protein and amino acids in the pollen used therein.

Selmanoğlu et al. (2007) treated rats with 3 different pollens (A, B, and C) at a dose of 60 mg/kg/day and reported that Pi concentrations lowered significantly ($P < 0.05$) in pollens B and C. This study showed a significant ($P < 0.05$) increase in Pi values in the other groups (beehive air, beehive air + pollen groups) except for the pollen group (Table 2) compared to the control group. While this result contradicts the data of Selmanoğlu et al. (2007), it could be asserted that it is a quite remarkable finding when beehive air treatments are evaluated for increased Pi concentrations.

The study conducted by Selmanoğlu et al. (2007) using three types of pollen (A [Fabaceae - *Trifolium* spp], B [Brassicaceae - *Raphanus* spp], and C [Cistaceae - *Cistus* spp]) (60 mg/kg/day) reported that there was no difference in pollen A, while there were significant ($P < 0.05$) decreases in Zn concentrations in pollen B and C groups, compared to the control group. This study revealed no difference in the pollen group, whereas Zn concentrations were elevated significantly in the beehive air and beehive air + pollen (200 mg/kg) groups ($P < 0.05$) compared to the control (Table 2). This result for the pollen group in our study was compatible with the pollen A group in the study by Selmanoğlu et al. (2007), while the difference in the results for pollen B and C was thought to depend on the differences in pollen content.

A study conducted in COPD patients (Sarıbal 2006) reported a significant decrease in serum Zn and Fe concentrations in the COPD group compared to the control group. It was reported that serum Zn concentrations were lower in severe COPD patients compared to mild-moderate COPD patients (Karadag et al. 2004), and another study reported that serum Zn concentrations were significantly lower in COPD patients compared to healthy individuals (Kırkıl et al. 2008). It was reported that Zn concentrations were significantly ($P < 0.01$) lower in patients with asthma compared to healthy subjects and that low Zn concentrations were among the elements that might contribute to allergic diseases due to their role in the synthesis of some antioxidants or their effects on the immune system (Al-Fartusie et al. 2021). Considering the studies in which Zn concentrations were found to be low in COPD and asthma patients (Karadag et al. 2004; Sarıbal 2006; Kırkıl et al. 2008; Ariaee et al. 2016; Al-Fartusie et al. 2021) and the report that it would be beneficial to include Zn in the treatment (Ariaee et al. 2016), along with the finding that Zn and Fe concentrations were higher ($P < 0.05$) in the apiary-treated group compared to the control group in this study, it may explain the effectiveness of beehive air used as supportive treatment in these diseases.

A study conducted by Haro et al. (2000) to investigate the effect of 10 g/kg/day multifloral bee pollen supplementation to a standard diet on Fe metabolism in healthy rats and those with nutritional ferropenic anaemia reported that bee pollen elevated the haemoglobin concentration and lowered the platelet count. This study showed a significant ($P < 0.05$) increase in Fe concentrations in the other groups (beehive air, beehive air + pollen groups), except for the pollen group, compared to the control (Table 2). These findings are different from those reported by Haro et al. (2000), who found that pollen significantly alleviated the negative consequences resulting from Fe deficiency, had a restorative effect, and improved the absorption of Fe. This may be associated with the amount of pollen administered. In fact, while 200 mg/kg/day pollen was administered in this study, Haro et al. (2000) administered as much pollen as 10 g/kg/day. However, it is quite remarkable that the beehive air treatment alone or in combination with pollen elevated the Fe concentrations, just like the elevation in Zn concentrations.

It has been stated that pollen is an immunomodulator that is responsible for primary and secondary humoral immunity (IgM and IgG) (Dudov et al. 1994). A study conducted by Amr et al. (2013) on the immune conditions of hyperglycaemic rats by adding 1% and 2% bee pollen to the diet determined that bee pollen improved immune indicators (IgM and IgG) which were significantly higher in diabetic rats supplemented with 2% bee pollen ($P < 0.01$) compared to diabetic rats supplemented with 1% bee pollen ($P < 0.05$). The elevated serum IgG and IgM concentrations (Table 3) in the pollen group in this study confirm the immunomodulatory effect of pollen, similar to the result of Amr et al. (2013).

The study conducted by Bharti et al. (2018) on pollen treatment (100 mg/kg) showed that there was no significant difference between the control group and the pollen group in terms of SOD activity. In our study, no significant difference was observed in the pollen group compared to the control group, which is similar to the data of Bharti et al. (2018). Furthermore, although it was not statistically significant, it is quite striking that a reduction in SOD activity was noted in our study only in the beehive air group, whereas an increase in SOD activity was noted in the beehive air + pollen group (Table 4). It would be useful to test beehive air + pollen combinations at different rates in future studies.

This research is the first scientific study in which rats and bees were brought together as experimental animals, and where experimental animals were treated with beehive air (30 min/day). It was determined that the treatment with beehive air alone or in combination with pollen had no negative effect on rats in terms of the blood parameters investigated. However, although no difference was observed in serum creatinine values in the pollen group, the high BUN concentrations are a finding that should be taken into account in future studies. The elevated blood albumin, Fe, Zn, and Pi concentrations caused by beehive air treatment ($P < 0.05$) and elevated blood Fe, Zn, Pi, and Ca concentrations caused by beehive air + pollen treatment ($P < 0.05$) indicated that beehive air alone or in combination with pollen may be an effective supportive treatment option in cases of Zn deficiency disorders, iron deficiency anaemia, hypoalbuminaemia and hypocalcaemia. Furthermore, the finding of high ($P < 0.05$) Zn and Fe concentrations in beehive air may explain the effect of beehive air treatment on lung disorders such as asthma and COPD with low blood Zn and Fe concentrations.

Conflict of interest

The authors declare no conflict of interest.

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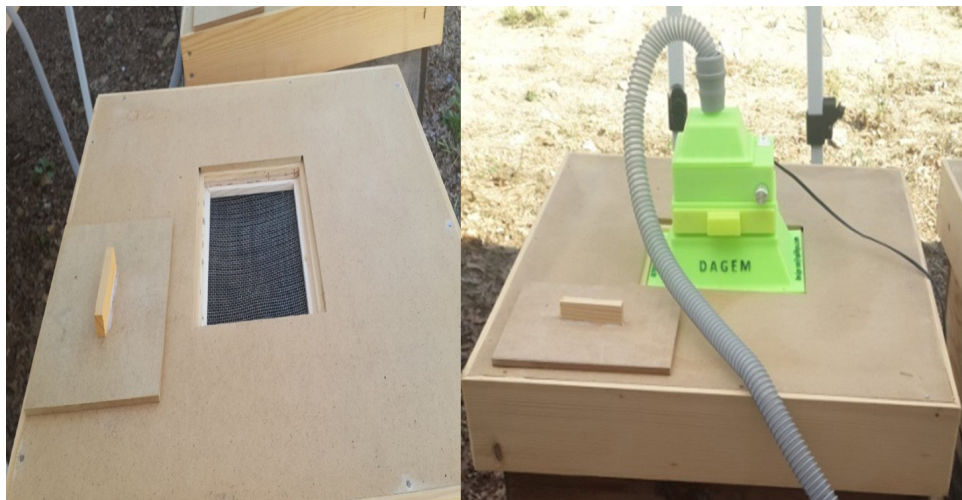


Fig. 1. Set-up for treatment by beehive air used in the study



Fig. 2. Treatment by beehive air during the study