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MELLIFERA RESEARCH ARTICLE

Investigation of Some Metals in Honey Samples Produced in Different Regions of Bingöl Province by ICP-MS

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A B S T R A C T

Qualitative and quantitative analyzes of metals in honey are important for the quality and authenticity of honey. Therefore, in this study, the concentrations of some metals in honey samples obtained from beekeepers in 8 different regions of Bingöl province of Turkey (Merkez, Genç, Solhan, Yayladere, Karlıova, Yedisu, Adaklı ve Kığı) were determined by ICP-MS (Inductively Coupled Plasma-Mass Spectrometry). The concentrations of Al, As, Ba, Ca, Cd, Co, Cu, Fe, Hg, Mg, Mn, Se, Sr, Zn metals were investigated in honey samples. Al, Ba, Cu were not determined in Merkez and Genç honey samples, but other elements were detected. While Hg could not be determined in all honeys, other elements were detected. In honey samples, K element was detected at the highest concentration (442.56±1.8 mg/kg), while the lowest concentration was observed in As (6.0±1 µg/kg). It was observed that the As levels determined as a result of this study did not exceed the maximum limits accepted by the European Union, and the other metal concentrations were at acceptable levels. It was determined that honey samples produced in Bingöl region are rich in minerals, not completely devoid of heavy metals, but heavy metal concentrations are below the limits determined in the literature.

Keywords: ICP-MS, honey, heavy metal, mineral, Bingöl

Introduction

Honey is a natural sweet substance that honey bees collect, dry, store and leave in the honeycomb for maturation by combining them with specific substances they collect from the nectar of plants or from the secretions of living parts of plants or from the feces of plant sucking insects in the living parts of plants [1]. Honey contains many essential nutrients, especially carbohydrates, amino acids, vitamins, phenols, enzymes and minerals; therefore, it has an important place in human nutrition and medicine due to its nutritiousness and antibacterial, antifungal and antiviral effects [2]. The composition and properties of honey vary primarily depending on the botanical origin of the nectar or honey extract, climate, geographic

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regions, and honey bee species [3]. Honey is seen as a good source of essential minerals and trace elements that humans need. However, it is reported that some elements taken from food can be toxic, especially when heavy metals exceed their safety levels [4]. As a result of the accumulation of toxic heavy metals in the human body, many health disorders such as vomiting, bleeding, jaundice, anemia, kidney failure, mental disorders, skin lesions and fragile bone structure can be seen in humans depending on the type and amount of the metal. In addition, residues and contaminants, especially in our export products, negatively affect exports; this causes economic losses. Therefore, it is necessary to investigate the ways of contamination and prevention remedies for all kinds of substances (heavy metals, toxins, etc.) that may be harmful to human health [5].

Metals are divided into 4 groups by Domingo: 1. Argon (Ar), Cadmium (Cd), Lead (Pb), Mercury (Hg) 2. Essential trace metals Chromium (Cr), Cobalt. (Co), Manganese (Mn), Selenium (Se), Zinc (Zn) 3. Other metals of biological importance Nickel (Ni), Vanadium (Va) 4. Pharmacological metals Aluminum (Al), Calcium (Ca), Lithium (Li) [6].

Heavy metals; these are elements that show metallic properties, have relatively high density, and may show toxic effects even at low concentrations. In this group; there are more than 60 metals including lead, cadmium, chromium, iron, cobalt, copper, nickel, mercury and zinc. The most important feature that distinguishes heavy metals from other toxic substances is their natural presence in the earth's crust. That is, it can neither be created nor destroyed by humans. These metals, which are not necessary for the human body, are taken into the body through food, water or respiratory tract, and cause a "metal load" to form. Heavy metals are taken into the organism through the mouth, respiration and skin, and most of them cannot be excreted through the body's excretory pathways (kidney, liver, intestine, lung, skin) without special support. Therefore, most of the heavy metals accumulate in biological organisms. These metals, which concentrate in living things with their metal load, cause many chronic and degenerative diseases. In addition, these heavy metals can affect many biochemical reactions by binding to the functional groups of proteins, can take part in enzymatic activities in different pathways, and affect nuclear metabolism and ATP synthesis.

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The toxic effects of heavy metals vary according to the properties of each metal. However, all of them generally affect more than one organ and system. Toxic heavy metals; It damages nerves and bones, blocks the functions of important enzyme groups and causes cancer. Same time; As a result of studies conducted on subjects, behavioral disorders due to psychological and neurological effects, neurotransmitter production and irregularities in their functions were observed in people exposed to heavy metals. For example; when metallic mercury is taken into the body, it mixes with the blood and easily reaches all tissues including the brain and accumulates in the brain. Metallic mercury vapor is rapidly absorbed from the lung and distributed to the central nervous system; it may cause the development of central nervous system symptoms such as extreme irritability, forgetfulness, weakness, visual disturbances, tremors in the hands, arms, legs and head. The only way to help the body fight against heavy metals is to remove heavy metals from the body and eliminate the possibility of re-exposure [7].

The importance and some properties of ultra trace elements for human health in the study

Although it is generally accepted that Na (Sodium) is essential for human life, sodium salts are found in almost all foods and drinking water [8]. The formation of hypertension in different types of animals given high levels of sodium chloride in their diet is clearly demonstrated. [9]. The WHO recommends reducing sodium intake to reduce blood pressure in adults and the risk of cardiovascular disease, stroke and coronary heart disease. WHO recommends reducing sodium (5 g / day salt) by 2 g / day in adults [10].

K (Potassium) is a mineral and an electrolyte. It helps your muscles work, including those that control your heartbeat and breathing [11]. Total body potassium is approximately 3500 mmol. Approximately 98% of the total is intracellular, mainly in skeletal muscle and to a lesser extent in the liver. The remaining 2% (approximately 70 mmol) is in extracellular fluid (ECF) [12]. WHO recommends increasing potassium intake from food to reduce blood pressure and the risk of cardiovascular disease, stroke, and coronary heart disease in adults. WHO recommends a potassium intake of at least 90 mmol / day (3510 mg / day) for adults [13].

Aluminum (Al) atomic number is 13 and it has a silver appearance in color. For most

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people, aluminum is not as toxic as heavy metals. However, aluminum may rarely cause vitamin D-resistant osteomalacia, erythropoietin-resistant sufficiently high doses (>50 g / day microcytic anemia and central nervous system changes. Aluminum has been established as a neurotoxin, although the basis of its toxicity is unknown. Recently, it has been shown to alter the function of the blood-brain barrier (BBB), which regulates the exchange between the central nervous system (CNS) and peripheral circulation [14].

Arsenic (As) is a metalloid located between metal and nonmetal and has different chemical forms. Arsenic inhibits reductase enzyme activity that prevents the formation of reactive oxygen species (ROS) in the cell, causing ROS increase and damage by binding to DNA and other cell components, and various types of cancer occur [15,16]. After the International Agency for Research on Cancer (ICRA) classified arsenic as first degree carcinogens in 1980, the World Health Organization (WHO) reduced the upper limit value of arsenic allowed in drinking water from 50 ppb to 10 ppb [17].

Ba (Barium) is a soft silvery-white metal that is slightly golden when ultra pure [18]. Organs damaged by water-soluble barium compounds (i.e. barium ions) are the eyes, immune system, heart, respiratory system, and skin. In Canada, a maximum acceptable concentration (MAC) of 2.0 mg / L (2,000 µg / L) is recommended for total barium in drinking water [19].

Ca (Calcium) is the fifth element predominantly found in the body that is needed in large quantities. 99% of calcium in the body is found in the structure of bones and teeth, and 1% in intra and extracellular fluid. Calcium activates cell signaling in muscle contraction, nerve conduction, cell division, communication between cells, release of hormones [20]. The only source of calcium in the body is calcium from food. 2500 mg / day value is reported as the upper limit [21].

Cadmium (Cd) is a heavy metal that can cause serious problems in terms of human and living health due to its high mobility in soil, root and seed systems [22]. Cadmium is highly harmful in living things and is a metal that has toxic effects on almost every tissue [23]. It is known to cause reproductive and kidney disorders, hepatic toxicity, osteomalacia, cancer and cardiovascular diseases, and also affect protein, enzyme, carbohydrate and nucleic acid metabolism [66,67]. The Joint FAO / WHO Expert Committee on Food Additives has declared that 7 µg / kg body

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weight is tentatively acceptable weekly intake level [24].

Co (Cobalt) is also important in the nutrition of living things. Cobalt is the central building block of vitamin B_{12}. Cobalt is assimilated only with the intake of vitamin B_{12}, not in its ionic or metallic form. It is the most effective biocatalyst known to date. Cobalt deficiency increases the risk of anemia. The daily need for cobalt is 5 mg [68]. According to the assessment of the IARC, cobalt has a carcinogenic effect for humans. According to the report of the United States National Toxicology Program (NTP), cobalt sulfide significantly increases the incidence of lung tumors in animals compared to controls [25-27].

Cu (Copper) is one of the essential micronutrient trace elements that are abundant in various rocks and minerals. It plays an important role in iron metabolism. It is required for a wide range of metabolic processes in both prokaryotes and eukaryotes. There are at least 30 known copper-containing enzymes that have functions such as oxygen carriers (hemocyanin) or redox catalysts (cytochrome oxidase, nitrate reductase) [28]. Copper is essential as a trace dietary mineral for all living organisms as it is an essential component of the respiratory enzyme complex cytochrome c oxidase [29]. In copper deficiency, clinical disorders such as hypochromia anemia, neutropenia and ostewporosis can be seen. In the case of copper excess, clinical conditions such as Wilson (Hepatolenticular Degeneration), acute and chronic copper toxicity may occur [30]. The maximum value that copper salts can be found as particles in air is limited to 1 mg / m^3. [31].

It is necessary for the absorption of certain minerals such as Fe (Iron), copper and calcium, and for the production of red blood cells and various enzymes that carry oxygen in the blood. It also boosts the immune system. It is found in the organism hemoglobin, myoglobin, and respiratory enzymes. It is found in foods in the form of Fe 3. Excess iron is toxic to humans, because excessive intake of divalent iron (ferrous iron) reacts with peroxides in the body to form free radicals. Daily requirement is 8 - 10 mg [32]. The daily amount of iron that needs to be absorbed into the blood is around 1.5 mg in adults. Moderate to severe poisoning occurs when elemental iron intake exceeds 40 mg / kg. Intakes exceeding 60 mg / kg can be fatal [33].

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Hg (Mercury) is a metal that is liquid at room temperature. At sufficiently high doses, all types of mercury can produce toxicity [34]. Mercury vapor is monoatomic and can dissolve in lipids; therefore, 80% accumulation occurs in the organism. When metallic mercury is taken into the body, it mixes with the blood and easily reaches all tissues including the brain and accumulates in the brain [35]. The lethal mercury concentration in the air is up to 10 ppm in acute poisoning caused by inhalation in factories working on mercury. The lethal dose of mercury compounds is 1 gram [69]. It is dangerous if the maximum amount of mercury in food is more than 0.05 mg / kg [70].

Magnesium (Mg) is the fourth essential element in terms of the amount found in the human body (2000 mEq in a 70 kg human), second after potassium in terms of the amount found in the intracellular space, and is an essential element required for the function of more than three thousand enzymes [36]. 60% of the magnesium in the human body is in bones and teeth. The remaining 40% is contained in blood, tissue and other body fluids. It is more concentrated in the brain and heart than other tissues [37]. If the magnesium level in the serum is below normal, it means Hypomagnesemia. In this case, the serum magnesium concentration is below 1.6 mEq / L (< 1.9 mg / dl). [38]. If the magnesium level in the serum is above normal, it means Hypermagnesemia. Serum magnesium concentration above 2.1 mEq / L (> 2.5 mg / dl) [39].

Mn (Manganese) contains more than 300 minerals in nature that contain manganese [40]. It is among the minerals found in small amounts in the human body. In the body; there is a total of 10-20 milligrams of manganese, mainly in the kidneys, pancreas, liver and bones. Taking 2-3 milligrams of manganese a day is sufficient for health [41]. It plays a role in many processes in the body such as bone formation, hormone functions, blood sugar regulation, and immune system functions. It helps the healing of wounds, bone formation, absorption of nutrients [41].

An essential mineral, Se (Selenium) has an essential value for human life [42]. Selenium participates in the structure of many enzymes as a cofactor and plays a role in many events such as thyroid hormone mechanism, antioxidant enzyme defense, regulation of the immune system [43]. The minimum amount of selenium that should be taken to prevent deficiency symptoms in humans is 10 µg / day; The maximum

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tolerable intake is estimated at 400 µg / day [44]. Plasma selenium level should be on average 125 ng / mL [45].

Strontium (Sr) is a trace element common in nature and recommended for the treatment of osteoporosis. Its effect on bone is dose dependent. It disrupts bone mineralization in high doses. When using low doses for a long time, bone resorption decreases and formation increases. With Sr, there is an increase in both trabecular and cortical bone [71]. When strontium is administered at low doses (2-5 microg), cell differentiation and bone formation; intact nodule formation by changing mineralization at high concentration (20-100 microg); It causes the formation of hydroxyapatite when administered at a drug dose [46]. It is used in a daily dose of 2 g, it is safe and effective. Its effect on bone is dose dependent. When taken in high doses, it reduces calcitriol and bone mineralization [47].

Zn (Zinc) is an essential element for the organism. It plays a critical role for the structural and functional integrity of cells. It has functions in gene expression and growth. It protects from ultraviolet radiation, facilitates wound healing, contributes to immune and neuropsychiatric functions, and reduces the risk of cancer and cardiovascular disease. There is 1-2.5 gr of zinc in the human body [48]. Daily zinc requirement varies according to gender and age. The daily zinc requirement in adults is 15 mg [49,50].

Materials and Methods

Honey samples used in this study were obtained from beekeepers in Bingöl city (from Turkey) and its seven towns: Genç, Solhan, Karlıova, Adaklı, Yedisu, Kığı, and

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Yayladere. The location of the regions on the map is shown in Figure 1.

Figure 1. The regions where the study was conducted: Bingöl city and its seven towns

Reagents

Ultrapure distilled water (18.3 MΩ cm − 1) was used throughout the entire experiment. Multi-Element Calibration Standard (Agilent Technologies, USA) and Internal Standard Mix (Agilent Technologies, USA) were used as the calibration standard. Nitric acid, 65% Suprapure Merck (Darmstadt, Germany) brand was used.

- The natural mortality of Varroa *:* Each hive is equipped with a lange, coated with greasy material, placed on the floor (the plateau) of hives. Each lange is protected by a metal grid preventing bees from accessing it. The count of dead Varroa is done every three weeks throughout the study period.

Sample preparation and analysis method for ICP-MS

In the elemental analysis performed with ICP-MS, firstly the samples were solubilized by burning supra pure with 65% nitric acid in a microwave cracker oven (CEM MARS6 One Touch USA). Completely dissolved samples were diluted with 1% supra pure nitric acid solution using 18.3 MΩ ultrapure water (Human Power I). ICP-MS calibration solutions mix standards (Agilent Technologies, USA) were prepared in seven different concentrations (Table 1) using 1% supra pure nitric acid-ultrapure water.

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Table 1. Calibration standards

	1.Standard 1 (ppb)	2.Standard 10 (ppb)	3.Standard 25 (ppb)	4.Standard 50 (ppb)	5.Standard 125 (ppb)	6.Standard 250 (ppb)	7.Standard 500(ppb)	Internal standard
Analytes	^{23}Na, ^{24}Mg, ^{27}Al, ^{39}K, ^{43}Ca, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{63}Cu							^{45}Sc
	^{66}Zn, ^{75}As, ^{82}Se, ^{88}Sr, ^{111}Cd, ^{138}Ba, ^{202}Hg							^{89}Y

ICP-MS (Agilent 7700X (Tokyo, Japan)) device was used for element analysis in the study, and ICP-MS calibration was performed before each measurement. ^{45}Sc, ^{89}Y internal standard elements were used for the control of calibration graph and element analysis. After the samples prepared and the calibration charts created, the analysis of the elements with the ICP-MS device was made as follows: The samples diluted in the standard calibration range using a peristaltic pump were sent to the cyclonic spraychember with high purity argon gas flow. High levels of helium gas have been used to prevent interference. In addition, for the reliability of the measurements, standard reading was made after each sample, and device control was provided. Operating conditions of the ICP-MS device are given in Table 2, and analytical parameters are given in Table 3.

Table 2. ICP-MS device working conditions

Parameter	Description / Value
Radio frequency power	1550 W
Radio frequency matching	1.80 V
Radio frequency	27.12 MHz
Plasma gas flow (Ar)	15 L/min
Plasma gas	Ar X50S 5.0
Makeup gas	0.9 L/min
Carrier gas (inner)	1.1 L/min
Sample intake	0.5 mL/min

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Spray chamber temperature	2°C
Nebulizer pump	0.1 rps
Resolution m/z	244 amu
Short-term stability	<3% RSD
Long-term stability	<4% RSD/2 h
Background	<5 cps (9 amu)
Cones	Ni
Rinse time	45 sec
Injector	2.0 mm

Table 3. Analytical parameters of the ICP-MS method

Element	Linear range (µg/kg)	Regression	Correlation coefficient (r)	Limit of detection (µg/kg)	Limit of quantification (µg/kg)
Na	0–500	y=0.049x+00	0.9997	0.2126	0.6982
Mg	0–500	y=0.023x+00	0.9999	0.1504	0.5248
Al	0–500	y=0.006x+00	0.9999	0.1122	0.4306
K	0–500	y=0.011x+00	0.9998	0.2012	0.6402
Ca	0–500	y=0.001x+00	0.9996	0.2015	0.6005
Mn	0–500	y=0.062x+00	0.9999	0.2200	0.6600
Fe	0–500	y=0.003x+00	0.9998	0.3006	0.9018
Co	0–500	y=0.247x+00	0.9999	0.0926	0.2896
Cu	0–500	y=0.203x+00	0.9998	0.4321	1.2894
Zn	0–500	y=0.027x+00	0.9997	0.9151	2.7526
As	0–500	y=0.004x+00	0.9999	0.2236	0.7446
Se	0–500	y=0.001x+00	0.9999	0.3685	1.2275
Sr	0–500	y=0.020x+00	0.9999	0.1242	0.4628
Cd	0–500	y=0.013x+00	0.9999	0.3255	0.8985
Ba	0–500	y=0.082x+00	0.9999	0.2018	0.6122
Hg	0–500	y=0.021x+00	0.9997	0.4186	1.2948

Values expressed are means ±standard deviation of three parallel measurements ($p < 0.05$).

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Results and Discussion

In this study, elemental analysis of honey samples obtained from 8 different regions of Bingöl province was performed. The results of the elemental analysis are given in Table 4 with their three parallel mean and standard deviation values.

Table 4. Elemental analysis results of honey

Sample Id	Na 23 (mg/kg)	K 39 (mg/kg)	Al 27 (µg/kg)	As 75 (µg/kg)	Ba 138 (µg/kg)	Ca 43 (mg/kg)	Cd 111 (µg/kg)	Co 59 (µg/kg)
Bingöl City	15,78±0,5	432,15±2	<LOD	6,5±0,3	<LOD	20,2±1,1	285,0±5	506,2±13
Genç	23,43±1,1	412,52±1,9	<LOD	6,0±0,1	<LOD	11,7±0,2	251,1±54	544,1±45
Solhan	19,62±1	442,56±1,8	2686,0±112	6,9±0,3	27,4±1,1	16,6±0,3	223,8±23	656,4±39
Yayladere	16,21±0,9	401,12±2	8183,3±442	6,9±0,3	766,8±29	42,4±1,9	232,0±49	800,2±69
Karlıova	10,25±0,7	298,55±1,4	1903,0±62	6,4±0,2	57,7±2,1	21,1±1,6	198,7±41	936,1±28
Yedisu	25,11±1,2	320,15±1,8	1831,6±507	7,5±3	67,4±12	30,6±4,6	171,1±18	994,1±8
Adaklı	26,21±1	391,52±1,5	48053,8±1175	5,8±2	1716,9±87	56,7±5,1	177,1±57	1024,6±39
Kığı	24,02±0,9	333,58±1,9	1520,5±256	6,7±2	117,3±11	18,3±1,1	265,9±18	955,9±34

Sample Id	Cu 63 (µg/kg)	Fe 57 (µg/kg)	Hg 202 (µg/kg)	Mg 24 (mg/kg)	Mn 55 (µg/kg)	Se 82 (µg/kg)	Sr 88 (µg/kg)	Zn 66 (µg/kg)
Bingöl City	<LOD	849,1±85	<LOD	29,2±0,9	1190,3±75	468,1±45	595,68±35	208,9±49
Genç	<LOD	438,8±69	<LOD	17,5±0,8	612,8±71	593,1±30	266,75±34	364,2±94
Solhan	636,7±70	5065,4±530	<LOD	56,4±1,9	921,2±87	323,9±36	425,44±33	4205,1±81
Yayladere	946,8±140	7566,5±317	<LOD	113,8±3	5306,7±126	319,4±65	1524,65±147	4927,3±176
Karlıova	439,7±66	2615,0±46	<LOD	33±1,4	631,7±57	348,2±110	367,09±22	1943,7±133
Yedisu	<LOD	2827,5±351	<LOD	67,6±2,3	2430,3±43	377,6±4	598,68±43	2206,2±110

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Adaklı	3464,2±157	3282,5±136	<LOD	117,7±1,9	12328,3±137	349,5±27	1487,15±83	1792,7±21
Kığı	<LOD	3007,2±115	<LOD	36,4±1,2	1509,9±41	281,6±72	615,33±39	1046,9±39

Since the concentrations of K, Na, Mg, and Ca are high, the results are given in mg/kg (ppm), and the concentrations of the other elements are given as µg/kg (ppb). In this study, Na, K, As, Ca, Cd, Co, Fe, Mn, Se, Sr, and Zn elements were detected in all honey samples, whereas Hg was not detected in any honey samples (<LOD). In the study, the minimum value of Na element in honey found in different regions is 10.25 ppm and the maximum value is 26.21 ppm. They found the amount of Na element between 0.0657-0.2791 ppm in the study by Bengü and Kutlu (2020) "Analysis of Some Basic and Toxic Elements in Honey Supplied from Bingöl with ICP-MS" [51]. In another study, the amount of Na in honey was found between 11.7 and 52 ppm [52]. Kek et al. (2017) found the minimum and maximum values of Na amount as 375.2-944.5 ppm, respectively, in their study on 8 different honey samples [53]. In another study, Na was found in the range of 52.38–289.20 ppm. The amount of K in our honey samples is in the range of 298.55- 442.56 ppm. Kek et al. (2017) found the minimum and maximum values of K amount as 95.4-1643.9 ppm, respectively, in their study on 8 different honey samples. In another study, the amount of K was found in the range 104.40-878.70 ppm [54]. They found the amount of K element between 2,612-6,871 ppm in the study "Analysis of Some Basic and Toxic Elements with ICP-MS in Honey Supplied from Bingöl" by Bengü and Mutlu (2020). In another study, the amount of K in honey is between 277-7382 ppm [52]. The minimum value of the Al amount found in honey samples is 1,520 ppm and the maximum value is 48,053 ppm. Tutun et al. (2019) found the amount of Al in the range of 1.00-25.80 ppm [55]. In another study in which element analysis was performed in honey samples, the amount of Al was found in the range of 2.11-8.04 ppm [56]. The amount of As in honey samples was determined as 7.5-5.8 ppb. Aghamirlou et al. (2015) found the amount of As in the honey sample as 4.68 ppm [57]. In the study conducted on Iranian honey, the amount of As was found in the range of 0.1-0.001 ppm [58]. Roman et al. (2011), the minimum amount of As in honey samples is 0.087 ppm and the maximum value is 1.238 ppm [59]. The amount of Ba we found in the

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study is in the range of 1716.9-27.4 ppb. Tutun et al. (2019) found the amount of Ba in the range of 0.03 ppm on average. In another study, they found the amount of Ba in honey 0.75 ppm [60]. Staniškienė et al. (2006) found the amount of Ba in the range of 5.8-71.3 ppb in their study [61]. The amount of Ca we find in honey samples is 56.7-11.7 ppm. In a study, the amount of Ca in honey was found to be 219.38 ppm [54]. They found the amount of Ca element between 52.90-199.97 ppb in the study "Analysis of Some Essential and Toxic Elements with ICP-MS in Honey Supplied from Bingöl" by Bengü and Kutlu (2020). In another study, the amount of Ca was determined as 113-858 ppm [52]. In the study conducted by Tutun et al. (2019), they found the amount of Ca in the range of 0.03-0.12 ppm. Kek et al. (2017) the amount of Ca in honey 157.1 ppm, Silva et al. (2013) found it in the range of 10.28-93.37 ppm [62]. In this study, the amount of Cd was found in the range of 171.1-285.0 ppb. Kek et al. (2017) Cd amount in honey 0.001-0.004 ppm, Bayır (2019) 4.301-6.898 ppb, Tutun et al. (2019) 0.20 ppb, Aghamirlou et al. (2015) found it to be 27.62-125.88 ppb, while Yücel and Sultanoğlu (2013) found it to be 0.008–0.12 ppm [72]. The amount of Co determined in our study is 506.2-1024.6 ppb. Yücel and Sultanoğlu (2013) found that Co amount 10–120 ppb, Tutun et al. (2019) found 0.17 ppb. In this study, Cu amount was found between 439.7- 3464.2 ppb. Kek et al. (2017), the amount of Cu in honey 0.621-2.931 ppm, Bayır (2019) 0.395 -0.950 ppm, Altundağ et al. (2016) 0.45-2.15 ppm, Tutun et al. (2019) 0.38-1.52 ppm, and Kasapoğlu (2006) determined it as 0.15-0.71 ppm. The amount of Fe detected in our study is 438.8-7566.5 ppb. Kasapoğlu (2006), Fe amount in honey 1.15-13.18 ppm, Bengü and Kutlu (2020) 10.90-61.15 ppb, Yücel and Sultanoğlu (2013) 7.40-92.38 ppm and Bayır (2019) found it as 6,266 -14,500 ppm. The amount of Mg we detected in honey samples is 17.5-117.7 ppm. Kek et al. (2017) determined the amount of Mg in honey 13.72-71.04 ppm, Tutun et al. (2019) found it as 9.28- 117.16 ppm, and Bengü and Kutlu (2020) found it as 71.84- 179.91 ppb. The amount of Se detected in the study is in the range of 281.6-593.1 ppb. In the study conducted by Altunatmaz et al. (2018), the amount of Se in honey was found to be 0.096-29.496 ppm, while Costa-Silva (2011) found 1.0-2.91 µg / 100 g [63, 64]. In our study, the amount of Sr was determined as 266.75-1524.65 ppb. Studies have found that the amount of Sr in honey samples is 0.12–2.46 ppm, 0.03 ppm and 1.45 ppm [54, 55] [65]. The amount of

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Zn detected in our study is in the range of 208.9-4927.3 ppb. In their studies, Kasapoğlu (2006) 0.72-9.8 ppm, Kek et al. (2017) 1.258- 4.566 ppm, Bayır (2019) 1.039-1.635 ppm, Altundağ et al. (2016) found it to be 0.80-64.49 ppm.

Conclusion

It is inevitable that our analysis results differ from the results in the literature, as the element content of honey may be affected by the plants visited by the bees, the difference in the raw materials they collect to produce honey, the environment, exhaust gases, industrial activities, and even bee races. The results obtained showed that Bingöl honey consumption will not cause any problem in terms of metal concentrations. Metal concentrations of the studied honeys were found to be in accordance with the limits of the foods consumed according to the WHO data and other literature data. In this study, it was determined that honeys are rich in minerals. It is evaluated that Bingöl honey, which is especially rich in Fe and Zn content, can be used as an important food supplement considering the importance of these minerals in terms of health. It is also pleasing that some of the metals such as As, Cd and Hg, which are considered to be the most dangerous heavy metals, were not detected or found in low amounts in Bingöl honeys. This situation can be considered as a result of the lack of industrial facilities that will cause environmental pollution in Bingöl province and the protection of its natural flora. This study is expected to contribute to the literature on the element analysis of honey.

Bingöl İlinin Farklı Bölgelerinde Üretilen Bal Örneklerinde Bazı Metallerin ICP-MS ile Araştırılması

Öz: Balda bulunan metallerin kalitatif ve kantitatif analizleri, balın kalitesi ve güvenilirliği açısından önemlidir. Bu nedenle, bu çalışmada Türkiye'nin Bingöl ilinin 8 farklı bölgesindeki (Merkez, Genç, Solhan, Yayladere, Karlıova, Yedisu, Adaklı ve Kığı) arıcılardan temin edilen bal örneklerinde bazı metallerin konsantrasyonları ICP-MS (İndüktif Eşleşmiş Plazma-Kütle Spektrometresi) ile belirlenmiştir. Toplanan bal örneklerinde Al, As, Ba, Ca, Cd, Co, Cu, Fe, Hg, Mg, Mn, Se, Sr, Zn metallerinin konsantrasyonları araştırıldı. Merkez ve Genç'ten toplanan bal örneklerinde, Al, Ba, Cu metalleri belirlenmezken diğer elementler ise tespit edildi. Balların

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tamamında ise Hg belirlenemezken diğer elementler tespit edilmiştir. Bal örneklerinde K elementi en yüksek konsantrasyonda (442,56±1,8 mg/kg) tespit edilirken, en düşük konsantrasyon ise As'de (6,0±1 µg/kg) görüldü. Bu çalışma sonucunda belirlenen As düzeylerinin Avrupa Birliği tarafından kabul edilen maksimum limitleri aşmadığı, diğer metal konsantrasyonlarının ise kabul edilebilir düzeylerde olduğu görüldü. Bingöl bölgesinde üretilen bal örneklerinin mineral yönünden zengin olduğu, ağır metallerden tamamen yoksun olmadığı ancak ağır metal konsantrasyonlarının literatürde belirlenen limitlerin altında olduğu tespit edilmiştir

Anahtar Kelimeler: ICP-MS, bal, ağır metal, mineral, Bingöl

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MELLIFERA RESEARCH ARTICLE

Protective Effect of Propolis and Bee Bread in Experimental Gastric Ulcer Model

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A B S T R A C T

Gastric ulcer is the most common disease of the upper digestive system. The pathophysiology of the disease is associated with environmental factors and non-steroidal anti-inflammatory drugs. These factors weaken the gastric mucosal barrier and increase acid secretion, causing damage to the gastric epithelium. Although medical treatment approaches are widely used, side effects are seen. Propolis and bee bread are natural compounds obtained from the products of honeybees. Studies have shown that these compounds have antioxidant, antimicrobial and anti-inflammatory effects. In this study, it was aimed to investigate the therapeutic effects of propolis and bee bread in an animal model of gastric ulcer induced by indomethacin. 48 male Wistar rats were used in the study. Six random groups were formed as the control group, the indomethacin-treated ulcer group, the low and high-dose bee bread (perga) groups, the low and high-dose propolis groups. After injections and treatment applications, stomach tissues of animals were examined histologically and blood samples biochemically. According to the obtained findings, propolis and bee bread prevented epithelial damage in gastric ulcer induced by indomethacin and formed a histopathological structure similar to the control group ($p < 0.05$). These data indicate that propolis and bee bread have protective and therapeutic effects in the treatment of gastric ulcers.

Keywords: Bee bread, Propolis, Stomach, Ulcer, Histology

Introduction

Gastric ulcer is the most common disease of the upper digestive system. The prevalence of gastric ulcer in the western population is % 2.4 [1]. In addition, the annual incidence rates range from % 0.10 to 0.19 [2]. The most common ulcer area in the stomach is the small curvature. Moreover, Ulcerative lesions might be seen anywhere from pylori to cardia of the gastric tissue [3]. Causes of gastric ulcer contain environmental factors

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(smoking, alcohol use and microorganisms) and non-steroidal anti-inflammatory drugs (NSAID) [4]. The reduction of protective factors in the gastric physiology (prostaglandin release, gastro-mucosal barrier, and blood flow) and the increase of degenerative secretions play a role in the main pathophysiology [5]. Under normal circumstances, the gastric tissue can tolerate high concentrations of hydrochloric acid, refluxing bile salts, alcohols and nutrients at varying temperatures. This tolerance is maintained by the repair ability of the mucosal barrier. The production of prostaglandins (PG) regulates mucosal defense and increases the resistance of the stomach layer [6]. The production of bioactive PGs is provided by the enzyme cyclooxygenase (COX). In addition, NSAIDs [7], such as Aspirin and Indomethacin, prevent the production of PGs by inhibiting the production of COX. With these effects, NSAIDs have been shown to play a role in gastric ulcer development. Although treatment approaches aim to reduce acid secretion from gastric cells, side effects are commonly occur [8]. Due to these side effects, new treatment procedures based on bee products have been investigated in recent years.

Bee bread is a type of fermented pollen collected by the honeybee and stored in the honeycomb [9]. Bee bread contains approximately %24-35 carbohydrate, % 3 lipid, % 20 protein, % 3 vitamins and minerals. The bee bread contains all of the essential amino acids that the human body cannot biosynthesize, as well as proteins, vitamins such as C, B, B2, E, H, P, nicotinic acid, folic acid, pantothenic acid, sucrose, pigments, enzymes such as amylase, phosphatase, flavonoids, carotenoids and hormones [10]. The antibacterial and antioxidant properties of the fatty acid content of bee bread have been proven by scientific studies [11-13]. In addition, the positive effects of bee bread on stomach tissue have been shown in studies [14].

Propolis, a resinous substance, is prepared by honeybees to cover cracks, smooth walls in the hive and to keep humidity and temperature constant. Propolis is a natural sticky substance that honeybees collect from the resins of flowers, trees and plants and mix them with their saliva [15].

Propolis is frequently used in studies according to chemical composition and therapeutic properties [16]. The chemical content of propolis has identified more than 300 compounds [17]. Studies have shown that the observed effects of propolis may be

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a result of the synergistic effect of the complex components [18]. These data show that Propolis and its extracts have many applications in the treatment of various diseases due to anti-inflammatory, antiseptic, antioxidant, antibacterial, antimycotic, antifungal, anti-ulcer, anticancer and immunomodulatory effects. In the light of this information, effects of propolis and bee bread on gastric epithelium were investigated in the experimental gastric ulcer model in the present study.

Materials and Methods

Animals

48 Wistar albino male rats aged 8-10 weeks were used in the study. Rats were obtained from Erciyes University Experimental and Clinical Research Center (DEKAM). Ethics committee approval required for the study was obtained from Erciyes University Animal Experiments Local Ethics Committee (decision dated 15.11.2017 and numbered 17/114). Throughout the study, rats were kept in cages in groups of four, where they had unlimited access to food, on a 12-hour light and 12-hour dark cycle at 21 + 1 ^{o}C room temperature.

Chemicals

The present study used popular type propolis collected from the vicinity of the Kayseri province in central Anatolia (Turkey). Bee bread was purchased from the Nutral Therapy Company in Kayseri (Turkey) and stored at -20°C. Using the AOAC method, the moisture content of bee bread was determined by drying it gravimetrically at 105 °C to constant weight in a convection oven. Crude protein value was determined. A conversion factor of 6.25 was used to convert the percentage of nitrogen to the percentage of crude protein. The crude oil was extracted using a soxhlet apparatus and diethyl ether. The crude fiber content of bee bread was analyzed. Ash content of the samples was determined gravimetrically. The total carbohydrate content was calculated according to the expression: total carbohydrates = 100- (% moisture + % protein + % fat + % ash).

Experimental Groups

Control group: Saline (0.9% NaCl) 1 ml intraperitoneally (i.p.) administered group (n = 8).

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END (Indomethacin) group: 1 ml volume of 25 mg kg 1 i.p administered Indomethazcin group (n = 8) [19].

High-dose Perga (bee bread) (PY) group: 1 ml volume of perga was administered 100 mg / kg by oral gavage (n = 8) [14, 20].

Low-dose Perga (PD) group: 1 ml volume of perga was administered 50 mg / kg by oral gavage (n = 8) [20].

High-dose Propolis (EPY) group: 100 mg / kg olive oil-based propolis administered by oral gavage in a volume of 1 ml (n = 8) [20, 21].

Low-dose Propolis (ELP) group: 50 mg / kg olive oil-based propolis administered by oral gavage in a volume of 1 ml (n = 8) [20].

Histological procedures

Stomach tissues were fixed with 10% formaldehyde solution to be used in histological examinations. After fixation, tissues were embedded in paraffin by applying routine further steps.

5-6 μm sections from the paraffin blocks were taken on flat slides. The prepared slides were kept in the oven for a certain period using standard histological methods. The paraffin was removed with xylene and diluted after passing through graded alcohol series. The sections were stained with Hematoxylin-Eosin (H&E) to see the general histological structure. Gastric tissue damage scoring was made considering criteria such as surface epithelial degeneration, presence of necrotic cells, shortening of the mucosa from 50 areas. According to these criteria, 0: no damage, 1: mild damage, 2 moderate damage, 3 severe damage. The examinations were examined under the Olimpus BX51 microscope.

Biochemical analysis

At the end of the experiment, blood samples taken from all groups were taken into EDTA tubes and the hematological parameters of WBC, RBC, HGB, HTC, MCV, MCH, MCHC, EOS, LYM were analyzed.

Statistical analysis

For the biochemical data obtained as a result of the research, SPSS 20.0 (Statistical Package for the Social Sciences) Software package program was used. Arithmetic Mean ($\bar{x}$) for descriptive statistics and standard error ($S\bar{x}$) is used to observe the difference between the groups by comparing the data with each other. The data of the variables were determined by the Shapiro-Wilk normality test, and the data of the parametric variables were analyzed by one-way ANOVA test. While Post-hoc

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Tukey test is used for data showing homogeneity of variance in one-way ANOVA test; Games-Howell Post-Hoc test was applied to the data without variance homogeneity. The level of significance was set at $\alpha = 0.05$ for all statistical tests.

Results and Discussion

Histological Results

Normal histological structure was observed in the gastric tissue of the control group. In the END group, degeneration of surface epithelial cells and shortening of the mucosa and glands were observed. A significantly similar structure to the control was observed in the PY, PD, ELP and EPY groups (Figure 1, Table 1). Perga and propolis have been shown to provide a significant improvement over the END group at both low and high doses.

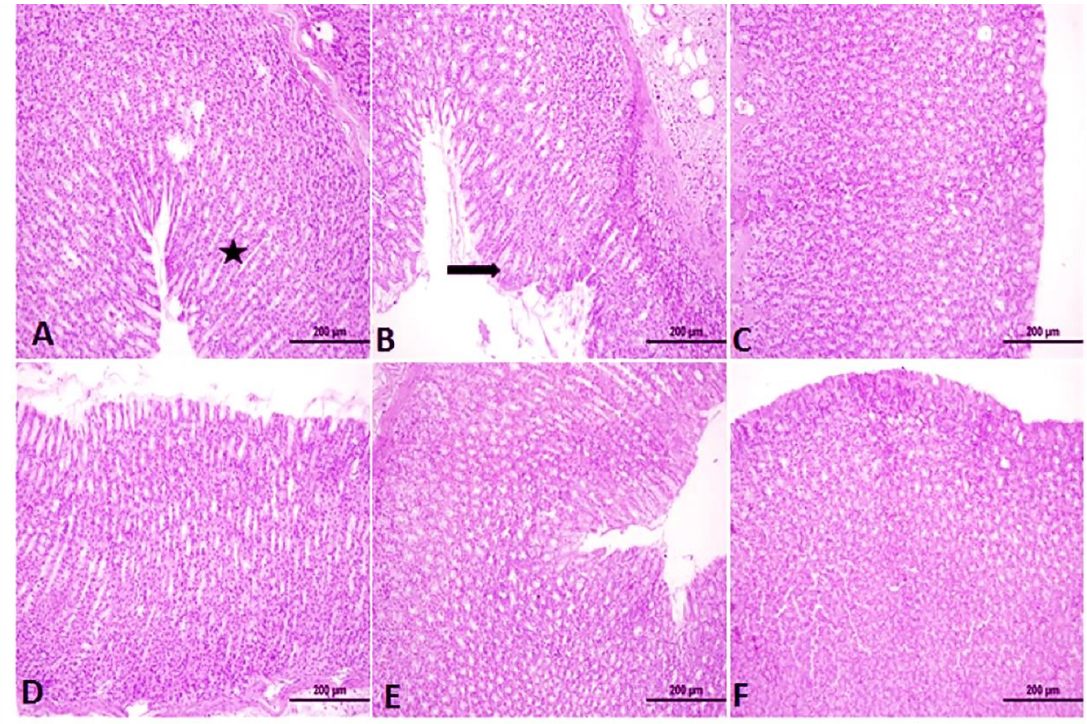

Figure 1. The normal stomach structure of the control group (A) is shown, the END group (B) showed disruption in the surface epithelium and shortening of the mucosa. PY group (C), PD group (D), ELP group (E), EPY group (F). H&E. Image magnification X200

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Table 1. Histopathological evaluation results of rat stomach tissues.

Gruplar	Kontrol	END	PY	PD	ELP	EPY	*p*
Histopathological score	**0.20±0.61^{a}**	**1.06±0.92^{b}**	**0.20±0.55^{a}**	**0.13±0.34^{a}**	**0.46±0.62^{a}**	**0.50±0.57^{a}**	**0.001**

Datas are expressed as mean ± standard deviation. P <0.05 was considered significant. There is no significant difference between the groups containing the same letter (a-b).

Biochemical Results

In the biochemical analysis of blood samples, a significant decrease was detected in the number of red blood cells, hemoglobin, hematocrit, mean erythrocyte hemoglobin and lymphocyte in the indomethacin group compared to the control group. A significant decrease in lymphocyte count was observed in the perga and propolis groups compared to the indomethacin group. The mean erythrocyte cell volume was significantly changed in the propolis groups compared to the indomethacin group. In addition, a significant decrease in eosinophil levels was found in the propolis and perga groups compared to the indomethacin group (p <0.05) (Table 2).

Table 2. Biochemical analysis results of rat blood samples.

	Groups					
	Control	**END**	**PY**	**PD**	**ELP**	**EPY**
WBC	9.66±3.79^{ab}	6.47 ±0.85^{b}	9.37 ±0.93^{ab}	9.65 ±1.89^{ab}	10.48±0.24^{a}	11.42±0.50^{a}
RBC	9.18±0.28^{c}	8.27 ±0.13^{b}	9.36 ±0.29^{a}	9.17 ±0.14^{a}	9.40 ±0.21^{a}	9.16 ±0.14^{a}
PLT	602.24±31.20	595 ±84.19	764 ±53,04	628 ±17.20	898 ±30,17	925 ±40.0
HGB	11.40±2.22^{b}	15.68 ±0.27	15.66 ±0.86	16.22 ±0.19	15.30 ±0.35	16.00 ±0.25
HCT	50.88±3.94^{c}	51.36 ±0.74	53.36 ±1.04	53.15 ±0.55	53.96 ±1.02	52.20 ±0.75
MCV	57.99±1.23^{ab}	60.35 ±0.55^{a}	57.06±0.99^{ab}	57.95±0.61^{ab}	57.73±0.37^{ab}	55.60 ±1.40^{b}
MCH	17.68±0.52^{b}	17.85 ±0.16	17.50 ±0.40	17.67 ±0.25	17.33 ±0.16	17.66 ±0.03
MCHC	30.50±0.68^{a}	29.65 ±0.27^{b}	30.66 ±0.16^{a}	30.52 ±0.33^{a}	30.0 ±0.12^{ab}	30.66 ±0.23^{a}

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LYM	69.49±3.01[b]	74.65 ±2.20[ab]	69.63 ±1.32[b]	81.60 ±1.99[a]	74.79±2.94[ab]	56.76 ±3.67[c]
EOS	0.50±0.02[a]	0.45 ±0.08[ab]	0.06 ±0.03[b]	0.57 ±0.13[a]	0.33 ±0.03[ab]	0.06 ±0.03[b]

Datas are expressed as mean ± standard deviation. P <0.05 was considered significant. There is no significant difference between the groups containing the same letter (a-b).

Findings from the study show that propolis and bee bread have positive effects on the epithelium in the gastric ulcer model induced with indomethacin (Table 1). In histopathological examinations, the treatment groups were statistically similar with the control group (Figure 1). In addition, no significant difference was observed between the varying doses of propolis and bee bread. Accordingly, it might be speculated that bee bread and propolis application has therapeutic potential in terms of epithelial damage in the treatment of gastric ulcers.

In another study conducted in 2021, a gastric ulcer model was created in mice with intragastric Indomethacin injection. The therapeutic effects of Mexican propolis were investigated in the study. With the findings obtained, it has been reported that propolis reduces the mucosal damage in the stomach, protects the mucosal content and decreases the pro-inflammatory cytokine levels. In addition, in this study we investigated the therapeutic effect in terms of pro-inflammatory markers such as tumor necrosis factor alpha (TNF-α), interleukin 1-beta (IL-1ß) and IL-6, and the effectiveness was tested [22]. However, blood hemogram analyzes such as white blood cells, platelet, lymphocyte, and eosinophil levels highlight the strength of our study.

In another study conducted in 2020, the effects of propolis on the damage caused by *Helicobacter pylori* were investigated *in vitro*. In the results obtained, it has been shown that propolis reduces the levels of pro-inflammatory cytokines such as IL-8, IL-12, IL-1ß and TNF-α. In addition, it has been reported that propolis upregulates antioxidant enzymes [23]. These findings demonstrate that propolis has therapeutic potential for ulcers developed with *H. pylori* infection as well as medical ulcerative lesions *in vitro*. However, our study provides preliminary information for the next step as *in vivo*.

In a study conducted in 2020, an acute and chronic gastric ulcer model was created with ethanol and acetic acid in rats. The protective and therapeutic effects of green

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propolis were investigated in the model. In the findings obtained, it has been shown that the application of propolis prevents gastric epithelial damage. In addition, it has been reported that it decreases the recovery time and shows an antioxidant effect by reducing oxidative stress markers [24]. The therapeutic effect against gastric ulcer may have been occurred by anti-inflammatory activity of propolis. In line with these findings, analyzing oxidative stress markers, pro-inflammatory cytokines, and blood hemogram values may be important in evaluating the therapeutic effect in future studies in terms of monitoring the efficacy of the treatment.

In a 2020 study, the gastroprotective effects of “red propolis” and an anti-inflammatory Formononetin were investigated in ethanol and indomethacin-induced gastric ulcer models. In the results obtained, reduced ulcer lesion areas have been shown that propolis applied at doses of 50, 250, 500 mg/kg. In addition, increased mucus production, antioxidant and anti-helicobacter pyloric activity were determined using propolis and formononetin combination [25]. These data suggest that the combination of propolis with anti-inflammatory agents can potentiate its therapeutic effect against gastric ulcers. In another study conducted in 2021, the protective effects of "red propolis" were investigated in gastric ulcer models induced with ethanol and hydrochloric acid. Histologically therapeutic effects at varying doses (30, 100, and 300 mg/kg) against ulcers have been shown that propolis provides. In addition, increasing levels of mucin secretion have been determined by propolis treatment. In addition, propolis has been observed to reduce oxidative stress [26]. These findings show that propolis may have different efficacy according to its local species. Furthermore, different dose administrations are important in determining the therapeutic effect. In this direction, the evaluation of different local propolis species by large dose groups in future studies may provide more robust results.

In a study, a gastric ulcer model was inducing with the application of acetic acid in rats. It has been reported that honey and pollen application to animals showed positive effects by preventing painful gastric motility [27]. Pollen known to be inhibited the lipid peroxidation, scavenges free oxygen radicals, [28, 29] and kills bacteria in bacteria studies *in vitro* [30]. However, our findings show that bee bread obtained from pollen has positive effects against gastric ulcer induced by

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indomethacin by reducing epithelial damage, for the first time. Limitations of the study include the lack of evaluation of propolis and bee bread in terms of pro-inflammatory cytokines and oxidative stress-related markers. In future studies on this subject, it is important to examine these parameters in terms of evaluating the therapeutic effect.

Conclusion

In conclusion, gastric ulcer, which has a wide spectrum of etiology such as alcohol, smoking, microorganisms and NSAIDs, is a common disease that reduces the quality of life of patients. New approaches are needed due to the side effects and insufficiency of commonly used treatments. In this context, honey products propolis and bee bread, which are known to have antioxidant, anti-inflammatory, and antimicrobial effects, should be targeted in terms of gastric ulcer treatment. In addition, in further studies, evaluation of different dose groups of propolis and bee bread is necessary to determine the therapeutic window. Our findings show that the gastric epithelium damaged in gastric ulcer was significantly improved as a result of the application of propolis and bee bread. In future studies, approaches in which a wider spectrum of doses and markers to elucidate the mechanism of the therapeutic effect are evaluated can be implemented.

Deneysel Gastrik Ülser Modelinde Propolis ve Arı Ekmeğinin Koruyucu Etkisi

Öz: Mide ülseri (gastrik ülser) üst sindirim sisteminin en yaygın hastalığıdır. Hastalığın patofizyolojisi çevresel etmenler ve non-stereoid anti-inflamatuar ilaçlarla ilişkilendirilmektedir. Bu faktörlerin mide mukoza bariyerini zayıflatması ve asit salınımını arttırması ile mide epitelinde hasar meydana gelmektedir. Medikal tedavi yaklaşımları yaygın olarak kullanılmakla beraber yan etkiler görülmektedir. Propolis ve arı ekmeği bal arılarının ürünlerinden elde edilen doğal bileşiklerdir. Yapılan çalışmalarda bu bileşiklerin antioksidan, antimikrobiyal ve anti-inflamatuar etkilere sahip olduğu gösterilmiştir. Bu çalışmada indometazin ile indüklenen gastrik ülser hayvan modelinde propolis ve arı ekmeğinin terapötik etkilerinin araştırılması amaçlanmıştır. Çalışmada 48 adet Wistar cinsi erkek ratlar kullanılmıştır. Kontrol grubu, İndometazin uygulanan ülser grubu, düşük ve yüksek doz arı ekmeği (perga) grupları, düşük ve yüksek doz propolis içeren gruplar olmak üzere 6

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rastgele grup oluşturulmuştur. Enjeksiyonlar ve tedavi uygulamaları sonrasında hayvanların mide dokuları histolojik olarak ve kan örnekleri biyokimyasal olarak incelenmiştir. Elde edilen bulgulara göre propolis ve arı ekmeğinin, indometazin ile oluşturulan mide ülserinde epitel hasarını önleyerek kontrol grubu ile benzer histopatolojik yapı oluşturduğu gözlemlendi ($p<0.05$). Bu veriler propolis ve arı ekmeğinin gastrik ülser tedavisinde umut vadeden etkilere sahip olduğunu doğrulamaktadır.

Anahtar Kelimeler: Arı ekmeği, Propolis, Mide, Ülser, Histoloji

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MELLIFERA RESEARCH ARTICLE

Nutrition and Health Conditions of Beekeepers in Turkey: A Pilot Study

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A B S T R A C T

The apiculture sector turned to be a fast-growing commercial field but beekeepers requiring strenuous efforts and arduous work were not addressed adequately in terms of job safety, health, and nutrition. This study is to assess the conditions of nutrition, health and job safety in the context of a pilot study. This cross-sectional and descriptive study was carried out using a survey and observation on 276 stationary and migratory beekeepers registered with the Kayseri Beekeepers Association in 14 districts of Kayseri, in the context of a pilot study. The study ascertained that beekeepers had in general a low level of income, 44,9% of beekeepers were suffering from chronic diseases and only 24,6% of beekeepers had regular health checks. Risky health behaviors were common among beekeepers. 19,8% of migratory beekeepers declared that their health conditions were affected negatively and 39,6% stated that their psychology was adversely influenced on performing this profession. Also, beekeepers were troubled by challenges in terms of water and food security. The study proposes the establishment of a system in which, as in the case of other fields of work, beekeepers would be offered economic, health, and nourishment support, be provided with safe working conditions and have access to periodic health examinations.

Keywords: Beekeeping, health status, nutritional status, stationary beekeepers, migratory beekeepers, work safety

Introduction

Apiculture (beekeeping) turned to be a sector that experienced tremendous progress and contributed to the economies of countries with a significant commercial value. 486.568 tons of honey that worth 464.166.019.000 Euros were exported in the world in 2017 [1]. In 2017, with the production of 551 thousand tons of honey,

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China ranked the first in the world whereas Turkey ranked the second [2]. According to the Turkish Statistical Institute, 114.471 tons of honey was produced in Turkey and 1.236 tons of honey worth 24.720.000 Dollars were exported from Turkey in 2017 [3].

Apiculture is not primarily based on agricultural land and so can act as a source of income for rural dwellers with insufficient agricultural land. Moreover, it can be said that it is the only agricultural activity that can inexpensively and effortlessly create employment. However, apiculture is practiced in a vast geographical landscape, extending from low lands at sea level to plateaus that are thousands of meters high, and in distant regions from residential areas. This situation makes apiculture an arduous endeavor and causes honey collection to be performed in harsh and socially isolated conditions. In this process, beekeepers are challenged with quite a few problems. On top of that, migratory beekeepers need to relocate the apiary to different regions, and this relocation requires sacrifice and efforts to be made at specific time intervals throughout the entire year.

The regulation on apiculture released by the Ministry of Agriculture and Forestry of Turkey addressed the 'Terms and Conditions of Migratory Apiculture' and endeavored to solve such problems as safety and accommodation of beekeepers in Turkey [4]. However, it is argued that meeting basic needs such as nutrition and healthcare is still a matter of critical importance.

However, it is possible to find several scientific research on apiculture and honey bee products in the literature. Nevertheless, researches that assess the working conditions of beekeepers practicing apiculture as a profession and so are directly in charge of the production of honey and bee products are almost inexistent.

The aim of this research is to assess nutrition, health, and job safety conditions of stationary and migratory beekeepers around the environs of Kayseri in where no sufficient knowledge before. The reason why this province was chosen as a pilot region is that Kayseri is a province rich in flowers and suitable for beekeeping with its rich flora.

Environs of Kayseri mainly happen to be on the migratory apiculture route extending from around Mersin province on the coast of the Mediterranean Sea to the provinces of East Anatolia and Black Sea regions of Turkey.

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Additionally, as there is no previous research on this region, the results of this research will likely contribute to the development of apiculture as a profession and set an example to other regions where apiculture is practiced.

Materials and Methods

In the form of descriptive research, this study was conducted in Kayseri province and its environs from July 1, 2016, to July 1, 2017.

The study included 180 stationary beekeepers and 96 migratory beekeepers, all of whom were registered to Kayseri Beekeepers Association, and who were from Yahyalı, Develi, Tomarza, Pınarbaşı, Sarız, Bünyan, Hacılar, Özvatan, Yeşilhisar, Sarıoğlan, İncesu, Melikgazi, Kocasinan and Talas districts of Kayseri. Approximately 380 beekeepers from these districts were registered to the Kayseri Beekeepers Association and 73% of them were reached in the study.

Research data were collected by a team of 3 students who studied at the Agricultural Biotechnology Department of Agriculture Faculty of Erciyes University, Kayseri, and instructed about research techniques and application of surveys before the research.

Just as noted in the regulation of the Ministry of Agriculture and Forestry of Turkey on apiculture, migratory beekeepers refers to those who relocate their bee

At the beginning of the research, the necessary permission was obtained from the department where the study was conducted and Kayseri Beekeepers Association and it was carried out according to the working principles of TUBITAK2204. 'Informed Consent Form for Human Volunteers' was prepared in full compliance with the Declaration of Helsinki for each beekeepers taking part in the research voluntarily and written approvals of beekeepers were received.

Through the face-to-face interview technique, the research team applied the survey form composed of questions on socio-demographic characteristics, general health conditions, medical backgrounds, health checks, health-aware eating habits, and specifics of health conditions of beekeepers to collect research data. The height was measured by the beekeepers' statement, and the weight was measured with a portable Tanita scale.

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colonies to benefit from different blooming periods of plants to the full extent and to protect their bees from winter conditions whereas stationary beekeepers relates to those who keep their bee colonies at the same location all through the year [4].

Data were analyzed using IBM SPSS Statistics 15.0 (IBM Corp., Armonk, New York, USA). While analyzing the data, as descriptive statistical methods; a number of units (n) and percentage (%) in qualitative parametric variables; In the comparison of categorical/qualitative variables, "Chi-square" or "Fisher's Exact" tests were used according to the suitability of the data; Bonferroni correction was applied in the Post-Hoc complementary analyzes performed to determine the differences. In all statistical analyses, the “Confidence Interval” (Confidence Interval or CI) was 95% and a $p<0.05$ value was considered statistically significant.

Results and Discussion

The general characteristics of the beekeeping enterprises were given in Table 1. Beekeepers taking part in the research had an overall mean age of 51±12 years old (<30 years 5,1%; 31-50 years 39,1%; >51years 55,3%) The mean age of beekeepers is usually above 50 years as noted in other studies conducted in Turkey [5-7]. This may suggest that apiculture is more practiced as a profession by elder members of society. The fact that apiculture is more preferred by the old generation rather than the young generation, does not only jeopardize the transfer of apiculture as a profession to the next generations and its sustainability in the future but also raises health risks as chronic diseases to increase along with age.

Table 1. General characteristics of beekeepers

	Beekeepers				**Total**		χ^2	**p**
	Stationary		**Migratory**					
Education	**n**	**%**	**n**	**%**	**n**	**%**	5,334	0,149
Primary School	110	61,1	50	52,1	160	58		

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Secondary School	18	10,0	17	17,7	35	12,7		
High School	25	13,9	18	18,8	43	15,6		
University	27	15,0	11	11,5	38	13,8		
Profession								
Only Apiculture	21	11,7	58	60,4	79	28,6	72,830	**0,000**
Others	159	88,3	38	39,6	197	71,4		
Retiree	70	44,0	19	50,0	89	45,2	3,308	0,191
Farmer	28	17,6	10	26,3	38	19,3		
Others*	61	38,4	9	23,7	70	35,5		
Income (Turkish Liras)**								
With no regular income	18[#]	10,0	65	67,7	83	30,1	106,117	**0,000**
Below minimum wage	103[#]	57,2	21	21,9	124	44,9		
Above minimum wage	59	32,8	10	10,4	69	25		
Total	**180**	**100**	**96**	**100**	**276**	**100**		

*: These consist of civil servants, shopkeepers, worker, and who do not have a regular job or work with their families.

**: In 2016 when the research was carried out, Net Minimum Wage was 1.300.99 Turkish Liras, and 1 Dollar was equal to 2.9322 Turkish Liras (Central Bank of Turkey).

[#]: Post-Hoc Analysis, $p<0,05$

The fact that all beekeepers in this study were males implies that apiculture is carried out as a male-dominated business. On the other hand, it is a generally acknowledged fact that the number of females engaged in apiculture gradually increases, and the women are actively involved in apiculture even though they are still less involved than males [8]. For instance, women make 17.5% of beekeepers in Ardahan, an eastern province of Turkey [9].

This study demonstrates that apiculture is preferred more often as an additional income. Retirees and farmers prefer

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apiculture, and members of each profession successively make 45,2% and 19,3% of beekeepers participating in the study. Also, 44,6% of beekeepers reported that their incomes were below the minimum wage, and 30,1% of beekeepers claimed that they did not have a regular source of income and this percentage was 67,7% for migratory beekeepers ($p<0,05$) (Table 1).

Apiculture is a vital agricultural activity that has high economic value and whose products are traded locally and globally. On the other hand, it is a field of work engaged primarily by individuals with low socioeconomic status in society. This study exhibits that beekeepers have low socioeconomic status too. 58% of beekeepers said that they were primary school graduates, 30,1% of them declared that they had no regular income and almost half of them (44,9%) remarked that their monthly income was below the minimum wage ($p<0,05$) (Table 1).

As a repercussion of the capitalist economic system, individuals with low socioeconomic status tend to make efforts in the beekeeping business but earn a low level of income from honey production despite their endeavors. However, apiculture is still perceived as a source of hope for alleviating poverty. A study conducted in Tanzania ascertained that the average income of individual beekeepers was 856 Dollars (639 Euros) in 2009 and argued that apiculture buttressed the alleviation of poverty and the struggle to earn a living [10]. In a study performed to promote the development of apiculture as a profession, it was decided that there would be a 10% increase in income per capita for the poorest households with a net annual profit of 389 Dollars in Nueva Esperanza, Lima, Peru [11]. Besides, it was discerned that tens of thousands of households earned 25% of their annual income through the trade of honey and beeswax in Zambia and beekeepers increased their annual household income by 100-400 Dollars by selling honey and beeswax [12]. An economic analysis conducted in Africa found that a farmer could make an annual net income of 85.70 Dollars per beehive [13].

Today, the relationship between income, social inequality, and health risks is indisputably revealed. Whichever socio-economic indicators (such as income, social class, poverty level, and education level) are taken into consideration, those who are challenged socioeconomically are faced with risks and poor health conditions [14,15].

Non-communicable diseases are the leading global causes of death. They strike

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hardest at the low- and middle-income populations of the world and are preventable through the reduction of behavioral risk factors such as tobacco use, physical inactivity, harmful use of alcohol, and unhealthy diet [16].

Risky health behaviors were common among beekeepers. More than half of beekeepers (53,6%) were overweight, 98,2% of beekeepers did no physical exercise regularly, and 41,7% of beekeepers smoked. 65,2% of the smoker beekeepers were heavy smokers and smoked 20-25 cigarettes per day. 40,8% of beekeepers alleged that they increased smoking more during beekeeping activity. The percentage of smokers raising the number of cigarettes smoked during beekeeping activity was even higher for stationary beekeepers and reduced smoking during beekeeping activity ($p<0,05$) (Table 2).

Table 2. Behaviors of beekeepers to healthcare

	Beekeepers				**Total**		χ^2	**p**
	Stationary		**Migratory**					
BMI*	**n**	**%**	**n**	**%**	**n**	**%**		
Normal	57	31,7	39	21,6	96	34,8	4,617	**0,032**
Overweight	105[#]	58,3	43	44,8	148	53,6		
Obese	18	10,0	14	14,6	32	11,6		
Total	**180**	**100**	**96**	**100**	**276**	**100**		
Doing exercise regularly**								
Yes	5	2,8	-	-	5	1,8	5,162[a]	0,66
No	175	97,2	96	100,0	271	98,2		
Total	**180**	**100**	**96**	**100**	**276**	**100**		
Alcohol consumption								
No	179	99,4	92	95,8	271	98,2	[a]	0,051
Yes	1	0,6	4	4,2	5	1,8		
Total	**180**	**100**	**96**	**100**	**276**	**100**		
Smoking (in number of cigarettes)								

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Never	71[#]	39,4	56	58,3	127	46,0	8,945 [a]	**0,020**
Constantly	85[#]	47,2	30	31,2	115	41,7		
Occasionally	2	1,1	-	-	2	0,7		
Quitted despite smoking previously	22	12,2	10	10,5	32	11,6		
Total	**180**	**100**	**96**	**100**	**276**	**100**		
Number of cigarettes smoked per day								
3-5	3	3,5	1	3,3	4	3,5	20,778	0,236
10-20	23	27	3	10,0	26	22,6		
20-30	54	63,6	21	70,0	75	65,2		
30 and above	5	5,9	5	16,7	10	8,7		
Total	**85**	**100**	**30**	**100**	**115**	**100**		
Smoking during beekeeping activity								
Yes	76	89,4	27	90	103	89,6	[a]	0,177
No	9	10,6	3	10	12	10,4		
Total	**85**	**100**	**30**	**100**	**115**	**100**		
Smoking during beekeeping activity								
Smoking more	28	36,8	14	51,9	42	40,8	7,617 [a]	**0,017**
Smoking less	48[#]	63,2	13	48,1	61	59,2		
Total	**76**	**100,0**	**27**	**100**	**103**	**100**		

***:** BMI: Bady Mass Index https://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi
****:** Moderate physical activities for a minimum of 150 minutes per week.
Source: https://hsgm.saglik.gov.tr/depo/birimler/saglikli-beslenme-hareketli-hayat-db/Turkey_Dietary_Guidelines_2015.pdf
[a]:Fisher's exact test
[#]: Post-Hoc Analysis, p<0,05

The Labor class that also includes beekeepers are threatened by risks in society and it is necessary to pay particular attention to their nutrition. A sufficient and well-balanced diet has significant implications on both the health of workers and the enhancement of their work

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performance. In connection with the job which they are occupied with, workers need to have access to nourishment that is satisfactory in terms of amount and quality. If the nutritional needs of workers are not met properly, the efficiency and production pace of workers are likely to decrease and the risk of getting a chronic disease increases for them. A study by International Labor Organization indicated that a 1% increase in daily calorie intake by a worker increased his/her productivity by 2.27% [17]. This study displayed that the eating habits of beekeepers were adversely affected. The number of meals decreased and instead, they tended to consume more snacks, especially during beekeeping activities. Stationary beekeepers said they skipped meals because they could not find enough time, and migrant beekeepers said they had lack of sufficient meals (respectively 86,1% and 87%) (Table3).

They consumed foodstuff brought from home and tried to content themselves with homemade food during beekeeping activity. Although homemade food was the most preferred choice in their routine life, particularly migratory beekeepers tended to consume canned food, bakery, and pastry products and frequently had to have breakfast as toasted sandwiches and pastry during beekeeping activities. Access to clean drinking water was also an important problem for beekeepers. Beekeepers met this need with natural spring water or transported water (Table 3). Nevertheless, this type of nourishment has a high potential to offer risks based on the issue of food safety besides the sufficiency of its amount and quality. Beekeepers should be able to obtain an adequate amount of calories and nutrients necessary for performing activities efficiently through their diets, they should have 3 meals and 1-3 snacks per day, there should be milk, ayran, lemonade, herbal tea, fruit juice, different types of sandwiches, cakes, biscuits, pastry, bakery, fruit, walnut, hazelnut, dried fruit in their meal boxes, and the sufficient consumption of antioxidant vitamins, minerals, and herbal chemicals should be ensured during the day through the daily intake of diverse types of fruit and vegetable [18].

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Table 3. As a result of apiculture changing nutritional status in the apiary

	Types of Beekeepers								**Total**			
	Stationary				**Migratory**							
	In Routine Life		**During beekeeping activity**		**In Routine Life**		**During beekeeping activity**		**In Routine Life**		**During beekeeping activity**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Number of main meals												
Two	11	6,2	59	32,8	10	10,4	37	38,5	21	7,6	96	32,4
Three	169	93,8	121	67,2	86	89,6	59	61,5	255	92,4	200	67,6
Number of snacks												
Never	38	21,1	32	17,8	21	21,9	13	13,5	59	21,4	45	16,8
One	79	43,9	80	44,4	35	36,4	26	27,1	114	41,4	106	38,4
Two	56	31,1	62	34,5	36	37,5	50	52,1	92	33,3	112	40,6
Three and above	7	3,9	6	3,3	4	4,2	7	7,3	11	3,9	13	4,7
Most frequently skipped meal(s)												
Breakfast and dinner	7	3,9	6	3,3	1	2	2	2,1	8	2,9	8	2,9
Lunch	150	83,3	153	85	89	92,7	88	91,7	239	86,6	241	87,3
Snacks	23	12,8	21	11,7	6	6,3	6	6,2	29	10,5	27	9,8
Reason for a skipped meal(s)												

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Lack of sufficient time	148	82,2	155	86,1	84	3,1	1	1,1	232	84,1	156	56,6
Aversion to food	22	12,2	16	8,9	6	6,3	4	4,2	28	10,1	20	7,3
Lack of sufficient meals	3	1,7	4	2,2	3	87,5	87	31,5	6	2,2	91	32,9
Others*	7	3,9	5	2,8	3	3,1	4	4,2	10	3,6	9	3,2
Daily water intake (in water glasses)												
5	14	7,8	4	2,2	2	2,1	-	-	16	5,8	4	1,5
8-12	87	48,3	20	11,1	34	35,4	5	5,2	121	43,8	25	9,1
13-20	74	41,1	122	67,8	48	50	51	53,2	122	44,2	173	62,7
20 and above	7	3,9	32	17,8	13	13,5	39	40,6	20	7,2	71	25,7
Source for meeting the water need												
Tap water	177	98,3	7	3,9	91	94,8	10	10,4	268	97,1	17	6,2
Natural spring water	2	1,1	116	64,4	5	5,2	65	67,7	7	2,5	181	65,6
Transported water	1	0,7	57	31,7	-	-	21	21,9	1	0,4	78	28,3
Food consumed at lunch and dinner												
Homemade food	158	87,8	-	-	65	67,7	-	-	223	80,8	-	-
Restaurant food	22	12,2	1	0,7	31	32,3	-	-	53	19,2	1	0,4

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Homemade sandwichies, canned food	-	-	173	96	-	-	44	45,8	-	-	217	78,6
Home-made pastry, bakery, etc.	-	-	6	3,3	-	-	52	54,2	-	-	58	21
Total	**180**	**100**	**180**	**100**	**96**	**100**	**96**	**100**	**276**	**100**	**276**	**100**

*: Others pertained to the lack of eating habits and the practice of getting by on fast food.

All stationary and migratory beekeepers have conventional breakfast by consuming tea, cheese, olives, and eggs. Migratory beekeepers stated that they had breakfast mostly by eating toasted sandwiches and pastries during beekeeping activities. Both groups of beekeepers generally preferred bread made of white wheat flour and consumed olive oil. Furthermore, 33,3% of beekeepers added extra salt to their meals without even checking the taste of their food. 64,4% of stationary beekeepers reported that their eating habits did not change during beekeeping efforts and the rest of them claimed that they began to have unhealthy nutrition. This ratio was even higher for migratory beekeepers (61,5%) ($p<0,05$) (Table 4). This situation increases the risk of having a chronic disease and has negative effects on the remission of existing diseases.

Table 4. General eating habits of beekeepers

	Beekeepers				**Total**		χ^2	**p**
	Stationary		**Migratory**					
	n	**%**	**n**	**%**	**n**	**%**		
Bread consumption								
White bread	167	92,8	89	92,7	256	92,8	0,135[a]	1
Brown bread	7	3,9	4	4,2	11	3,9		

Whole wheat bread	6	3,3	3	3,1	9	3,3		
Oil consumption								
Butter	27	15	7	7,3	34	12,3	3,844 [a]	0,273
Margarine	4	2,2	2	2,1	6	2,2		
Olive oil	103	57,2	63	65,6	166	60,1		
Vegetable oil	46	25,6	24	25	70	25,4		
Adding extra salt to the food								
Without even tasting the food	64	35,6	28	29,2	92	33,3	1,150	0,284
After tasting the food	116	64,4	68	70,8	184	66,7		
Daily honey consumption (gram)								
25-50	31	17,2	16	16,7	47	17	6,100	**0,010**
70-100	85	47,2	38	39,6	123	44,6		
150-200	25	13,9	15	15,6	40	14,5		
250 and above	9	5	6	6,3	15	5,4		
No consumption	30	16,7	22	22,8	51	18,5		
Change in eating habits as a result of apiculture								
No change	116[#]	64,4	37	38,5	153	55,4	9,300	**0,010**
Irregular consumption of food	27[#]	15	36	37,5	63	22,8		

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Excessive consumption of food	10	5,6	13	13,5	23	8,3		
Insufficient consumption of food	27[#]	15	10	10,5	37	13,4		
Total	**180**	**100**	**96**	**100**	**276**	**100**		

[a]:Fisher's exact test
[#]: Post-Hoc Analysis, p<0,05

Beekeepers taking part in this research are faced with problems akin to those experienced by other beekeepers in Turkey regarding the satisfaction of their basic needs [9, 19-22]. Migratory beekeepers having accommodation and nutrition inconvenient for a healthy life, lack of access to clean drinking water and insufficient hygienic conditions and increase in smoking have negative effects on the health conditions of beekeepers and so can lay the groundwork for the emergence and development of chronic diseases.

Most beekeepers taking part in this study thought that they were healthy enough although almost one-third of them suffered from a critical chronic disease such as hypertension, diabetes and were supposed to use medication regularly, 47,8% of migratory beekeepers were diagnosed with hypertension. Hypertension is the most serious risk factor for ischemic and hemorrhagic strokes. The risk of having a stroke is three or four times as high for hypertensive patients and one-and-a-half times high for borderline hypertensive patients [23]. Four-fifths of patients suffering from strokes were diagnosed with hypertension [24].

In a similar vein, uncontrolled levels of blood glucose can cause short-term (immediate) or long-term complications and even at times result in death [25]. Fluctuations in blood pressure associated with hypertension or blood sugar due to diabetes can cause serious health problems for migrant beekeepers with chronic illnesses who spend most of the year alone away from home.

Migratory beekeepers participating in this research stated that their health and psychology were also negatively affected because of spending 3-6 months of the year away from home (Table 5).

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Table 5. General information on migratory beekeepers

	Migratory Beekeepers	
	n	**%**
Time spent away from home in a year		
1-2 month(s)	6	6,3
3-6 months	80	83,3
More than 6 months	10	10,4
Total	**96**	**100**
Negative effect on health		
Yes	19	19,8
No	77	80,2
Total	**96**	**100**
Adverse influence on the psychology		
Yes	38	39,6
No	58	60,4
Total	**96**	**100**
Response when faced with a health risk*		
Calling a friend	25	28,4
Creating his solution	63	71,6
Total	**88**	**100**
Proposed measure		
Transportation support	27	28,1
Access to clean drinking water	27	28,1
Equipment support	24	25,0
Accommodation support	18	18,8
Total	**96**	**100**

Periodic health checks are an indispensable part of for healthcare. However, beekeepers tend to be insensitive to this fact. Only 24,6% of beekeepers had health checks

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regularly, 79,2% of migratory beekeepers did not get regular health checks at all. In addition, stationary beekeepers use their medicines more regularly and more visits to a health facility ($p<0,05$)(Table 6). Periodic health checks which are effective for reducing morbidity and mortality by identifying risk factors and early symptoms of curable diseases play a critical role in the prevention of workforce loss and deaths. As well as enhancing overall health, the periodic health check is an important instrument also for measuring the state of a person's satisfaction with his/her job and his/her overall well-being, addressing the hardships experienced by the person, discussing the physical and psychological effects of the job on the person and forwarding the person to a health facility for his/her health issues or referring the person to a specialist physician [26].

Table 6. General state of health of beekeepers and their healthcare behaviors

	Beekeepers				**Total**		χ^2	**p**
	Stationary		**Migratory**					
Visits to a health facility	**n**	**%**	**n**	**%**	**n**	**%**		
6 months ago	137[#]	76,1	49	51	186	67,3	17,723	**0,001**
7-11 months ago	11	6,1	8	8,3	19	6,8		
1-3 years ago	18[#]	10	18	18,7	36	13		
More than 3 years ago	14[#]	7,7	21	21,8	35	12,6		
Self-evaluation of health state								
Good	169	93,9	88	91,7	257	93,1	0,482	0,487
Average	11	6,1	8	8,3	19	6,9		
Existence of a chronic disease								
Hypertension	23	35,4	11	47,8	34	38,6	7,499[a]	0,357
Diabetes	16	24,6	4	17,4	20	22,7		

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Other endocrine diseases	8	12,3	2	8,7	10	11,4		
Cardiovascular diseases	7	10,8	2	8,7	9	10,2		
Musculoskeletal disorders	5	7,7	4	17,4	9	10,2		
Respiratory system diseases	4	6,2	-	-	4	4,5		
Digestive system diseases	2	3,1	-	-	2	2,3		
Total	**65**	**36,1**	**23**	**23,8**	**88**	**31,9**		
Regular use of medication								
Yes	63	35	19	19,8	82	29,7	[a]	**0,009**
No	117[#]	65	77	80,2	194	70,3		
Periodic Health Checks								
Yes	48	26,6	20	20,8	68	24,6		
No	132	73,4	76	79,2	208	75,4	1,142	0,285
Total	**180**	**100**	**96**	**100**	**276**	**100**		

[a]:Fisher's exact test
[#]: Post-Hoc Analysis, p<0,05

Therefore, the health conditions of beekeepers can be improved by employing occupational physicians in professional chambers or associations where beekeepers are registered, and by arranging periodic health control for beekeepers. On the other hand, all beekeepers taking part in this research were interested in the organization of awareness promotion activities about healthcare and they wanted to attend a training program on the issue.

In the international conference held by the World Health Organization in Alma-Ata in 1978, it was underlined that all individuals had the right to health, and fulfillment of this right was the most fundamental duty of the governments. In 2008, it was highlighted that health was under the threat of globalization and injustice in income distribution, and the conference stressed the importance of social justice, an extension of the right to health to every human being, participation, equality and solidarity [27].

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Promote the health of the workplace and workers in this respect, it is essential to take every measure such as maintaining the social, spiritual, and physical health of beekeepers to the full extent, providing them with healthy working conditions, eliminating risks and offering training and instructions to them just as in the case of other fields of work [28].

Each profession is likely to pose a potential risk to human health. Apiculture is a field of work not only with commercial value but also it is one of the agricultural activities with high risk. Beekeepers are more frequently challenged with high risks in terms of both healthcare and job safety. This research also ascertained that 13% of beekeepers were confronted with risks such as bee stings, assault by wild animals, robbery, diseases, and terror attacks and forced to deal with these challenges through their means. It is necessary to identify locations convenient for the accommodation of migratory beekeepers at the provincial level, to develop infrastructure such as road, electricity, water, and waste management in these locations, and to build permanent or portable housing units

Conclusion

Apiculture occupies a strategically significant place in Turkey. Nevertheless, people involved in apiculture are faced with more various difficulties than those experienced by people engaged in agriculture and livestock farming. Beekeepers especially the migratory ones are supposed to live in locations far from family and sociable environs and have trouble having access to a sufficient and well-balanced diet. It is indispensable to provide beekeepers with economic, health, and nourishment support, offer safe working conditions to them and create systems ensuring their periodic health checks.

Acknowledgement

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Türkiye'de Gezginci ve Sabit Arıcıların Beslenme ve Sağlık Durumları: Pilot Çalışma

Öz: Arıcılık sektörü hızla büyüyen bir ticari faaliyet alanı olmasına rağmen, zorlu çaba ve yoğun emekler ile çalışan arıcılar iş güvenliği, sağlığı ve beslenme açısından yeterince ele alınmamıştır. Bu çalışmanın amacı; Kayseri yöresinde sabit ve gezginci arıcılık yapanların beslenme, sağlık ve iş güvenliği durumlarının pilot bir çalışma kapsamında değerlendirilmesidir. Kesitsel ve tanımlayıcı nitelikte olan bu çalışma, Kayseri' nin 14 ilçesinde, Kayseri Arıcılar Birliğine kayıtlı 276 sabit ve gezginci arıcı üzerinde anket ve gözlem yolu ile gerçekleştirilmiştir. Çalışmada arıcıların düşük gelir düzeyinde oldukları, %44,9'unda teşhis edilmiş kronik hastalığın bulunduğu, ancak sadece %24,6' sinin düzenli olarak sağlık kontrollerini yaptırdığı saptanmıştır. Arıcılar arasında riskli sağlık davranışlarının yaygın olduğu, gezginci arıcıların %19,8'i bu işi yaparken sağlıklarının ve %39,6'i de psikolojilerinin olumsuz yönde etkilendiği belirtilmiştir. Ayrıca suya erişim ve besin güvenliği konusunda sıkıntılar yaşandığı ifade edilmiştir. Arıcıların diğer iş kollarında olduğu gibi, ekonomik, sağlık ve beslenme yönünden desteklenmeleri, güvenli çalışma koşullarının sağlanması ve periyodik sağlık muayenelerinin yapılmasını sağlayacak sistemlerin oluşturulması önerilmektedir.

Anahtar Kelimeler: Arıcılar, beslenme durumu, sağlık durumu, sabit arıcılar, gezginci arıcılar, iş güvenliği.

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MELLIFERA RESEARCH ARTICLE

Preliminary Trials on The Efficacy of Propolis in The Control of The*Varroa destructor* (Mesostigmata:Varroidae) Ectoparasite of The Honey Bee *Apis mellifera intermissa* (Hymenoptera: Apidae)

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A B S T R A C T

Honey bee colonies, *Apis mellifera intermissa* in particular, are affected by *Varroa destructor* mites which threaten their existence. Several chemical treatments are used, but the parasite has become resistant to them. In recent years, plant origin substances have been used as a natural alternative means for combating the parasite. In this study, the acaricidal effect of propolis is evaluated in the laboratory. Seven Varroa mites were placed on filter paper (3 ×3cm) in petri dishes. Subsequently, the filter paper was impregnated with a dose of 0.2 ml (200 µl) of the propolis and ethanol solution. Four repetitions were carried out with a control without treatment. The results showed that propolis extract with 70% ethanol (EEP) is highly toxic against Varroa. The mortality rates of the Varroa mite were 40% to 100% after 30 and 120 minutes of exposure, respectively. On the other hand, treating mites with 100 mg of propolis powder showed mortality rates of 20% to 100% after 12 to 24 hours of exposure. Finally, when bees were exposed to EEP or propolis powder, they were not affected by the treatment.

Keywords: *Apis mellifera, Varroa destructor,* Parasite, Propolis, control, toxicity

Introduction

Propolis is a resinous, gummy substance with a viscous consistency collected by bees on certain parts (buds and bark) of plants, mainly trees [1]. It contains almost 50% resin and vegetable balm, 30% wax, 10% essence and aromatic oils, 5% pollen

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and 5% organic debris [2]. Propolis is rich in biochemical constituents, comprising mainly a mixture of flavonoid polyphenols, aglycon flavonoids and phenolic acid [3].

Propolis is used as a biocide by bees; it is responsible for the low incidence of bacteria and mold inside the hive. Bees may use it to coat the entrance and frames and to prevent drafts from entering the hive. It is also used to enbalm killed enemies if they are too large to be removed by worker bees from the hive [4] .

Propolis also plays an essential role in apitherapy. Indeed, it is known for its antimicrobial ([5][6], anti-inflammatory [7], antiviral [8][9] and antifungal characteristics [10]. However, a few studies have determined the insecticidal and acaricidal action of propolis [11, 12].

This study aims at determining the role of propolis in the biological control of the *V.destructor* mite. Hence, *V. destructor* is considered a formidable parasite in the collapse of *A.mellifera* bee colonies world wide.

Materials and Methods

Harvesting Propolis

The harvest of propolis is carried out during spring of 2018 on bee colonies at the educational apiary of the vocational training center of Tizi Ouzou (Algeria). With a Mediterranean climate and a temperate sub-humid bioclimatic stage.

The harvest of propolis is carried out by two methods:

- scraping hive and the frames: The obtained propolisis cleaned fromthe debris stuck to it (bees, wood, wax cover, Varroa etc.), weighed and put in boxes.

- using a particular grid called a "propolis grid"which is a food-grade plastic grid made frommany small interstices. It is placed on the body of the hive under the frame cover. Once these interstices are closed with propolis, the grid is removed and placed in a refrigerator. When cold, the propolis becomes brittle, and the twisting of the grid will detach the small pieces.

Preparation of Propolis and Ethanol Solutions

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For the preparation of the propolis solution, the protocol by Strehlet al. (1994) [13] was followed:

- The harvested propoliswas first heated in a saucepan filled with water to remove the debris (wax, pollen, etc.) stuck to it.

- The mixture of propolis and water was filtered with a colander. The propolis was stored in the freezer at -4°C for about two days and then crunched into a fine powder.

- This powder was added to ethanol in a ratio of 1 g of propolis to 10 ml of solvent.

- The mixture was left for maceration for a week, with frequent shaking.

- After maceration, the mixture was heated in a water bath at 70°C for 30 minutes then filtered. The resulting liquid extract was called Ethanolic Propolis Extract (EEP), stored in the refrigerator for later use.

Varroa Sampling

Mites were collected from infested colonies. A sample of 100 cells of capped brood was opened using forceps. The nymphs were removed to collect the present Varroaeither at the bottom of alveolus or stuck on the nymph as shown in Figure 1.

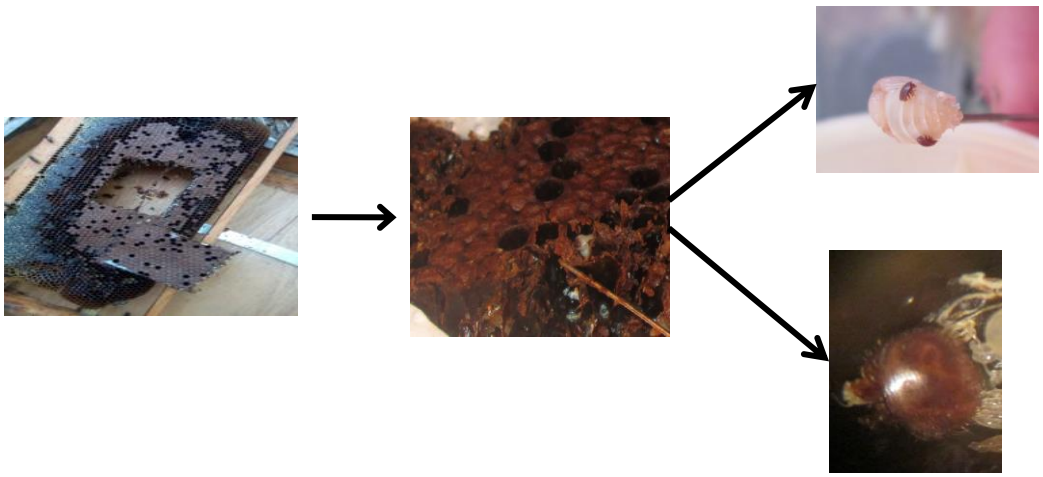

Figure 1. Removal of Varroa from the brood

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Application of the EEP Solution

Against Varroa: To test the effect of propolis on Varroa, the method of Garedew et al. (2002) [14] was adopted as follows:

Seven Varroamites were placed on filter paper (3 ×3cm) in petri dishes. Subsequently, the filter paper was impregnated with a dose of 0.2 ml (200 µl) of the EEP solution. Four repetitions were carried out with a control without treatment.

Varroa mites were left in contact with the EEP solution for 30 seconds. Nymphs of bees, which served as food for the mites, were introduced into the Petri dishes.

The mites' behavior was observed under a magnifying glass for 10, 30 and 60 minutes and every hour after treatment. The test was carried out at ambient temperature of approximately 22 to 25°C, and the treated mites were incubated at 28 ± 1 ° C, and 60% RH.

Each mite was classified as mobile or an inactive mite showing no movement of the legs or the rest of the body [15]. If a mite has remained inactive after 8 hours from the start of treatments, it is considered dead.

Against bees: To determine the effect of propolis on bees, each container's filter paper was impregnated with 0.2 ml (200 µl) of the ethanolic propolis extract (EEP) solution. Four repetitions were carried out with an untreated control. The results were read after 10, 30 and 60 minutes of exposure and every hour after treatment.For each exposure time and each repetition, the dead bees were counted under the EEP solution's effect.

Application of PropolisPowder

Against Varroa: Seven Varroa mites were placed in Petri dishes. Each Varroawas directly sprinkled with approximately 500mg of propolispowder. Parasite mortality was monitored in the same way mentioned before with the EEP solution.

Against bees: 50 bees were placed in crates. The bees were directly sprinkled with about 500mg of propolis powder. The bee mortality monitoring was carried out in the same way as mentioned before with the EEP solution.

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Results and Discussion

Effect of Propolis on Varroa

The average rates of mortality of Varroa mite observed as a function of the duration of exposure with the solution of ethanolic extract of propolis and propolis powder were presented in Figure2.

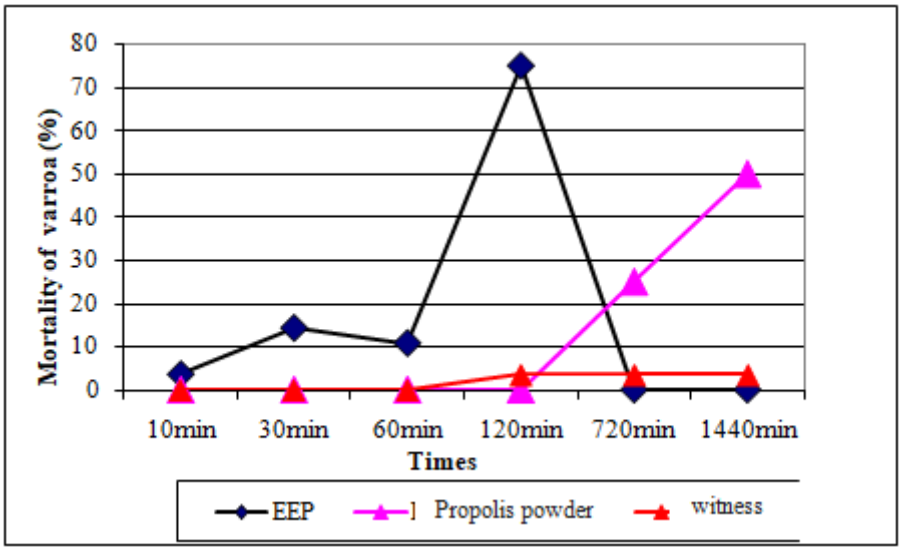

Figure 2. Evolution of Varroa mortality according to the type of treatment.

After 10 minutes of exposure to EEP, 3% of the Varroa was recordeddead. This mortality rose to 75% after 120 minutes of exposure. With the propolis powder, the parasites did not die until after 12 hours of exposure, with a rate of 25%. At 1440 minutes of contact with the treatment, the mortality of the Varroamite reached 50%. In the control batch, low mortality was observed (3.57%) after 120 minutes.

Effect of Propolis on Bees

The average rates of mortality in bees recorded according to the duration of exposure with the ethanolic extract solution of propolis and propolis powder were presented in Figure 3. After 720 minutes of exposure to EEP and propolis powder, the bee mortality rates were meager.

During this study, the acaricidal efficacy of propolis was tested against the parasite *V. destructor*. This bee product was used in two forms: as a liquid solution which is the ethanolic extract (EEP), and as a solid, which is propolis powder. The results showed that the EEP was very effective due to the total mortality of 100% after 120 minutes of exposure. Indeed, the

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harmful effect of this extract was quickly observed by the Varroa throughout the exposureduration, the mortality rate of the Varroawent from 3.57% to 75%.

On the other hand, propolis powder had a slow effect on Varroa because it was only at 720 minutes of exposure; the first mortality rate of 25% was recorded. The mortality rate only reached 50% after 1440 minutes of contact. According to Garedew et al. (2002) [14], the extraction of propolis in 70% ethanol made it possible to obtain and release most of the biologically hydrophobic active components. Furthermore, they suggested that contact with propolis solutions could lead tothe rigidity of the cuticle of mites, facilitating the entry of the active compounds present in propolis.

The death percentage of Varroa mites by EEP varied between 60.5% and 90% after 30 seconds of exposure in different regions of Argentina, and they assumed that the acaricidal activity of propolis extracts was due to the various bioactive components and the phenolic constituents present in the propolis [12] .

Popova and al. (2014) [16] suggested that propolis's acaricidal effect against Varroa mites resulted from its highly variable chemical composition, which largely depends on the plant harvested.

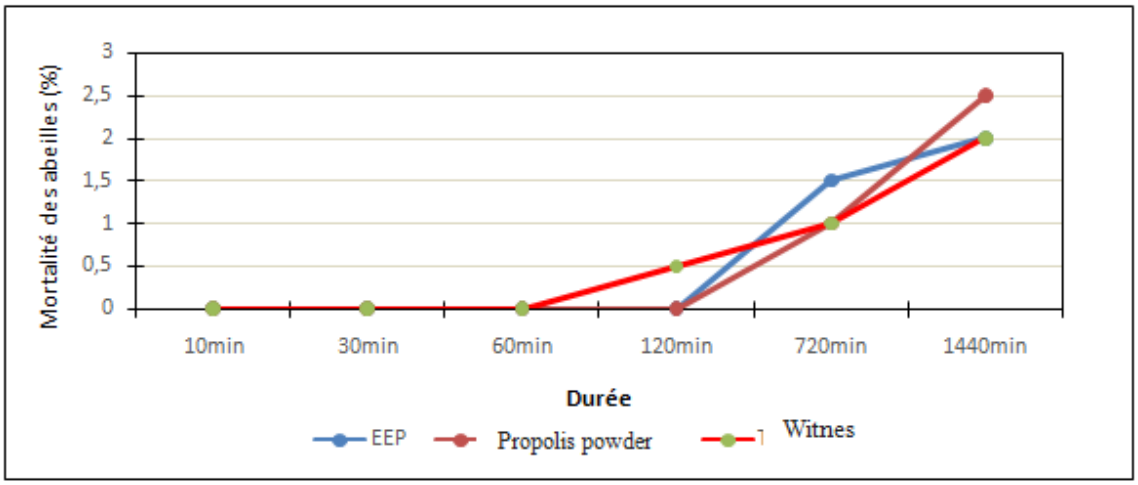

Figure 3. Evolution of bee mortality according to the type of treatment

The biological activity of propolis against several microorganisms was also demonstrated by Burdock (1998) [17]. Other studies have shown the inhibitory effect of propolis on Gram

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+, Gram- strains [18,19], and anaerobic bacteria [20, 21]. This effect depends on the investigated strain, the propolis's origin, and the used solvent [22]. Besides, propolis has antifungal [23,24], antiviral, antiprotozoal and antiparasitic properties due to its composition of polyphenols and flavonoids [25, 26]. Another work has reported that a thin-layer chromatography screening showed pinocembrin, caffeic acid, kaempferol, phenethylcaffeate, chrysin and galangin in Iranian propolis. The total flavonoids and phenolics are 7.3% and 36% respectively, which suggests that Iranian propolis's intense antimicrobial activity may be due to high levels of phenol and flavonoid compounds.

The antimicrobial activities of propolis have been the subject of several studies against different bacteria [27, 28], yeasts [29, 30],and parasites [31]. In vitro, propolis can act directly on microorganisms, and *in vivo*, it can stimulate the immune system by activating the mechanisms involved in the destruction of microorganisms.

Bastos et al. (2008) [32]. reported that extracts of propolis from different regions of Brazil inhibit the growth of *Paenibacillus* bacteria. This activity depends much more on the botanical origin of the propolis than on the applied concentration. These results were confirmed by Wilson et al. (2015) [33]who tested propolis samples collected from 12 different regions of the United States of America against bee pathogens: *Paenibacillus larvae* and *Ascosphaeraapis.*

The extraction methods of propolis can also influence its activity due to the various compounds' solubility properties [34]. The most widely used solvents in bioassays are ethanol and water. However, ethanolic extract shows more significant antimicrobial activity than water extracts or volatile compounds. It has all water and extractable ethanol, which are biologically active components. Also, the ethanolic extract contains several bioactive components that have not been found in other solvents.

In addition, Antunez et al. (2008) [35] studies on the virtue of propolis showed its antimicrobial effect on American foulbrood. These authors also revealed that bees tolerated high concentrations of ethanolic extract of propolis administered orally with the addition of syrup.

Drescher et al. (2017) [36] reported that propolis appears to be active against a range of honey bee pests and pathogens and can be considered an immune defense mechanism in the colony. Furthermore,

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they revealed that the propolis used in its natural form, deposited massively on the frames inside the hive, has no impact on *V. destructor* but the infestation of bees by the virus of deformed wings (DWV) and the sacciform brood virus (SBV) decreased considerably. This indicates that propolis is likely to play an essential role in maintaining the health of bee colonies.

Conclusion

The evaluation of propolis's acaricidal effect was used in two forms: an ethanolic extract and propolis powder, which provided impressive preliminary results for this study. In this study, the ethanolic extract was effective and caused the death of 100% of Varroa after 120 minutes of exposure. Propoliswas proved to be promising in the control of *V. destructor*and an accessible way of control. This beehive product that bees collect to embalming intruder in the hive or plug any existing void is a product that any beekeeper can easily acquire from his/her apiary.

Bal Arısı *Apis mellifera intermissa* (Hymenoptera: Apidae) Ektoparaziti Varroa destructor (Mesostigmata:Varroidae) Kontrolünde Propolisin Etkinliği Üzerine Ön Denemeler

Öz: *Apis mellifera intermissa* başta olmak üzere bal arısı kolonileri varlıklarını tehdit eden *Varroa destructor* akarlarından etkilenir. Birkaç kimyasal işlem kullanılmaktadır, ancak parazit bunlara karşı dirençli hale gelmiştir. Son yıllarda bitki kökenli maddeler parazitle mücadelede doğal bir alternatif araç olarak kullanılmaktadır. Bu çalışmada propolisin akarisit etkisi laboratuvar ortamında değerlendirilmiştir. Petri kaplarına filtre kağıdı (3x3cm) üzerine yedi Varroa akarı yerleştirilmiştir. Ardından, filtre kağıdına 0.2 ml (200 ul) propolis ve etanol solüsyonu emdirilmiştir. Tedavi uygulanmadan kontrol ile dört tekrar yapılmıştır. Sonuçlar, %70 etanol (EEP) içeren propolis ekstraktının Varroa'ya karşı

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oldukça toksik olduğunu göstermiştir. Varroa akarının ölüm oranları, sırasıyla 30 ve 120 dakikalık muameleden sonra %40 ila %100 olmuştur. Öte yandan, akarların 100 mg propolis tozu ile tedavi edilmesi, 12 ila 24 saatlik muameleden sonra %20 ila %100 arasında ölüm oranları göstermiştir. Son olarak, arılar EEP veya propolis tozuna maruz bırakıldıklarında etkilenmemişlerdir.

Anahtar Kelimeler: *Apis mellifera*, *Varroa destructor*, Parazit, Propolis, kontrol, toksisite

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MELLIFERA RESEARCH ARTICLE

The Effect of Royal Jelly Supplementation for Three Months on Bone Markers in Postmenopausal Osteoporotic Women

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A B S T R A C T

This study aimed to investigate the effect of three months of Royal Jelly (RJ) treatment on bone formation and resorption markers in postmenopausal women diagnosed with osteoporosis. The study included 80 postmenopausal women with osteoporosis (T-score <-2.5) randomly separated into two groups as the RJ group (n: 50) and the control group (n: 30). The RJ group took 1 gram of RJ in 100 ml of liquid every morning for three months. The control group was given a placebo in 100 ml of liquid. Basal bone-formation marker N-terminal propeptide of type 1 procollagen (P1NP) and bone resorption markers, C-terminal telopeptide of type 1 collagen (CTX), sclerostin levels were compared between the groups after three months. No statistically significant difference was determined between the groups regarding age, menopause duration, body mass index (BMI), and lumbar spine bone mineral densitometer (BMD) T-score. There was no statistically significant difference in calcium, 25-hydroxyvitamin D, bone production marker P1NP, bone destruction markers CTX and sclerostin parameters in both groups. This study showed that although RJ has an intense estrogenic effect when given orally to postmenopausal women with osteoporosis for 3 months, it did not affect bone formation and resorption parameters.

Keywords: Royal jelly, osteoporosis, postmenopausal, estrogen

Introduction

Osteoporosis is a progressive metabolic bone disease characterized by low bone mass and micro-architectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture [1].

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Osteoporosis affects many people of both sexes and races, and the prevalence will increase as the population ages [1]. Since most patients are women in the postmenopausal period, osteoporosis-related fractures impose a substantial burden of disability, costs, and mortality on postmenopausal women and older men. [2]. The key challenge in assessing the impact of osteoporotic fractures on mortality, disability, and costs is distinguishing the effects of the fractures themselves from the comorbidities and other risk factors that contribute to both the fracture and the outcome [3].

Estrogen plays a pivotal role in bone metabolism, and relative estrogen deficiency during the menopausal period contributes to the development of osteoporosis [4]. Although estrogen treatment effectively prevents and treats osteoporosis, hormone replacement therapy is not generally recommended due to the increased risk of breast cancer and cardiovascular events [*5, 6*]. In addition to estrogen replacement, other pharmacological and non-pharmacological treatment methods for preventing and treating postmenopausal osteoporosis are widely used. However, an effective and generally accepted treatment, completely safe, has not yet been described [7, 8]. This situation leads people to seek alternative treatments.

Phytoestrogens are defined as plant substances that cause estrogen effects in the human body, and they are increasingly consumed as a natural alternative to hormone replacement therapies. Numerous studies have examined bone effects in societies that consume the most known phytoestrogen, soybean. Although phytoestrogens are an area mentioned in osteoporosis treatment, more studies are required on this subject [1, 9].

Royal Jelly (RJ) is a glandular white-yellowish secretion produced from the hypopharyngeal and mandibular salivary glands of young nurse bees (aged between 5 and 14 days) [10]. RJ contains a considerable amount of proteins, free amino acids, lipids, sugars, and vitamins of very high biological value, and RJ has been demonstrated to possess numerous functional properties such as antibacterial activity, anti-inflammatory activity, vasodilative and hypotensive activities, disinfectant action, anti-oxidant activity, antihypercholesterolemic activity, anti-tumor activity, and estrogenic activities [11]. It has also been determined that RJ is effective in reducing postmenopausal symptoms in women [12].

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10-hydroxy-trans-2-paternoicacid (10H2DA), known as royal jelly acid or queen bee acid, is a unique medium-chain unsaturated fatty acid found only in RJ [13]. 10H2DA constitutes the vast majority of the RJ lipid content (0.75% to 3.39%) and represents one of RJ's main bioactive components [14]. RJ has been shown to have weak estrogenic activity since it competes with 17 beta-estradiol to bind to the estrogen receptor alpha and beta. It has been suggested that this estrogen receptor modulation is probably associated with fatty acids in RJ, such as 10H2DA [15].

In the postmenopausal period, estrogen treatment reduces fracture risk in women at high risk for fracture by increasing bone mineral density (BMD) and preventing bone loss [5]. This study aimed to investigate RJ's effects, which has been determined in previous studies to have estrogenic effects on bone formation/destruction in the postmenopausal period.

Materials and Methods

This study was conducted on postmenopausal women diagnosed with osteoporosis who presented at the Internal Medicine Endocrinology and Metabolism Outpatient Clinic of Suleyman Demirel University Faculty of Medicine Practice and Research Hospital. The study was approved by the Suleyman Demirel University Scientific Research Ethics Committee (Approval number: 11/02/2015 - 41). The purpose, benefit, and risk of this study were explained to each participant, and a signed informed consent form was obtained from all participants.

The study included a total of 80 female patients, aged 48-74 years, who were in the postmenopausal period and had a lumbar vertebra BMD total T-score of ≤ -2.5. Patients were excluded from the study if they had hypogonadism, hypothyroidism, hyperthyroidism, diabetes mellitus, chronic kidney failure, or any other disease that could lead to secondary osteoporosis.

The patients were randomly separated into the RJ group (n: 50) and the control group (n: 30). The RJ group took 1 gram of RJ in 100 ml of liquid every morning for three months. The control group was given a placebo in 100 ml of liquid every morning for three months.

Bone specific markers and Bone mineral density

At the beginning of the study and the end of 3 months, peripheral venous blood samples were taken from the patients between 08.00-10.00 hours, after overnight fasting

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of at least eight hours. Serum was obtained by centrifuging these blood samples at 3500 rpm for 4 minutes. Serums were stored at -80°C until assay of N-terminal propeptide of type 1 procollagen (PINP), C-terminal telopeptide of type 1 collagen (CTX), and sclerostin. The frozen serum samples were thawed back to room temperature, and the P1NP, CTX, and sclerostin levels were determined at 0 and 3 months values using separate commercial kits (Cloud Clone Corp. Enzyme-linked immunosorbent assay (ELISA) kit, Houston, TX, USA, Precoated plate). BMD was measured at the lumbar spine and total hip using dual X-ray absorptiometry (DXA; Hologic Discovery, Hologic, Inc., Bedford, MA, USA).

Statistics

The data obtained in the study was analyzed statistically using SPSS 19 for Windows (SPSS, Chicago, IL, USA). The Student's *t*-test and Wilcoxon signed-rank test were used to evaluating the changes in biochemical parameters before and after treatment. Results were stated as mean ± standard deviation (SD) values. A value of $p < 0.05$ was considered statistically significant.

Results and Discussion

The evaluation was made of 50 patients in the RJ group and 30 in the control group. No statistically significant difference was determined between the two groups regarding baseline anthropometric and laboratory data (Table 1).

Table 1. Comparison of baseline clinical. anthropometric. and laboratory characteristics between postmenopausal women receiving Royal Jelly supplementation and placebo.

	Groups	
Parameters	**Royal Jelly**	**Placebo**
n	50	30

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Age (years)	61.6±7.8	62.8±6.8
Duration of Menopause (years)	12.2±6.6	12.9±6.1
Body mass index (kg/m2)	27.5±3.3	28.2±2.6
Lumbar spine BMD (g/cm2)	-2.9±0.38	-2.8±0.24

*Mean (standard deviation) values.

When the calcium levels were compared in the RJ and control groups, there was no significant difference between the baseline and 3-month values (p= 0.314, p= 0.475, respectively).

When the 25-OHD3 levels were compared in the RJ and control groups, there was no significant difference between the baseline and 3-month values (p= 0.906, p= 0.900, respectively).

When the s-CTX levels were compared in the RJ and control groups, there was no significant difference between the baseline and 3-month values (p= 0.06, p= 0.489, respectively).

When the P1NP levels were compared in the RJ and control groups, there were no significant differences between the baseline and 3-month values (p= 0.475, p= 0.639, respectively).

When the sclerostin levels were compared in the RJ and control groups, there was no significant difference between the baseline and 3-month values (p= 0.445, p= 0.546, respectively).

The baseline and 3-month values of calcium, 25-OHD3, s-CTX, P1NP, and sclerostin of both groups are shown in Table 2.

Table 2. Comparison of the bone markers between postmenopausal women receiving Royal Jelly supplementation or placebo at baseline and after 3 months of intervention.

Bone markers	**Groups**	**Baseline**	**3 months**	**P values**
Calcium (mg/dl)	RJ	9.4±0.4	9.4±0.4	0.314
	Control	9.4±0.35	9.3±0.31	0.475
25-OHD3 (ng/mL)	RJ	16.7±7.8	16.8±7.3	0.906
	Control	17.8±8.6	17.6±7.7	0.900
s-CTX (ng/mL)	RJ	0.16±0.17	0.21±0.09	0.06

	Control	0.21±0.29	0.25±0.14	0.489
P1NP (ng/mL)	RJ	46.1±65.5	38.0±45.4	0.475
	Control	36.4±38.8	41.6±46.6	0.639
Sclerostin (pg/mL)	Basal	310.8±352	215.7± 216	0.445
	Control	251.2±390	207±202	0.546

Mean (standard deviation) values.
S-CTX: serum C-terminal cross-linked telopeptides of type I collagen.
P1NP: amino-terminal propeptide of type 1 procollagen

RJ's effects on bone formation/resorption markers in postmenopausal osteoporotic women were investigated in this study. The results demonstrated that three months of RJ treatment did not affect bone-formation marker, P1NP, bone resorption markers, CTX, and sclerostin.

Several studies have shown that alternative treatment methods are effective in postmenopausal osteoporosis. The effects of tea consumption on osteoporosis were investigated in postmenopausal osteoporotic patients. The positive effects on BMD were determined depending on the type of tea (green tea, oolong tea, black tea, or others] and amount [16]. In recent years, herbal drinks such as Persimmon (Diospyros kaki L.f.) leaves, Noni leaf, Herba Epimedii, Salvia miltiorrhiza, icariin, and tanshinones, and products made from these are very useful in postmenopausal osteoporosis due to anti-oxidant effects and the inhibition of osteoclastic activity [17]. A meta-analysis showed that the isoflavones found in some legumes are selectively modulating estrogen receptors. Isoflavone treatments effectively maintain BMD and reduce accelerated bone resorption in postmenopausal osteoporosis patients [18]. In a rat model study of oophorectomy-induced osteoporosis, the Cissus quadrangularis plant, a phytoestrogen, was determined to significantly increase the bone level formation marker, P1NP, due to the flavonoid content [19]. Previous studies have shown that RJ also has bone-sparing properties in postmenopausal osteoporosis. In a rat model of postmenopausal osteoporosis by oophorectomy, oophorectomized rats supplemented with 50 mg/kg RJ for twelve weeks exhibited higher BMD than those who did not receive RJ [20].

Similarly, in rats undergoing oophorectomy and in tissue culture, RJ is as effective as 17 beta-estradiol in correcting bone mineral

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density [21]. RJ has also been shown to stimulate the proliferation of mouse osteoblast-like cell lines and the production of type-1 collagen. It has been suggested that these effects are made by increasing the osteoblastic activity through interaction with the estrogen receptors of a component or components in the content of RJ [22]. Similarly, Moutsatsou et al. showed that the fatty acids found in RJ lead to mineralization in osteoblasts. These effects were demonstrated to be an estrogen receptor-mediated activity since it was inhibited when an estrogen receptor antagonist was added [23]. We have not evaluated the effects of RJ at the molecular level. If studies were done at the molecular level, probably the difference could be detected.

In the current study, no significant change was determined in circulating bone turnover parameters. In a similar previous study, 150 g / day of RJ was administered for three months to patients in the postmenopausal period but without osteoporosis. No significant change was found in bone-formation marker P1NP and bone-resorption marker CTX, and no bone-protective efficacy of RJ was demonstrated [24]. Although it has been shown that 10 mg/kg of RJ does not have bone-sparing activity in rats with oophorectomy-induced postmenopausal osteoporosis [25], RJ has been shown to improve lipid metabolism, erythropoiesis, glucose intolerance, and mental health in studies performed with 3-g and 6-g daily dose [26,27]. Since these parameters were not included in our study, non-osteoblast effects could not be evaluated.

Unfortunately, there is no definitive standard formula for RJ. The composition of RJ may vary depending on seasonal and regional nutritional conditions [10]. It is necessary to ensure RJ's standards, and further studies are required to determine the most suitable dose for humans and investigate its effectiveness in more extensive clinical studies.

This study's limitations can be considered primarily the low number of patients enrolled in the study and that the most effective dose of RJ for a person is unknown. Furthermore, the follow-up duration was short. Larger series with a long period of follow-up duration is required to show RJ's effectiveness on osteoporosis.

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Conclusion

Although RJ has been previously shown to have estrogenic activity, the results of this study demonstrated that a daily dose of 1 gr did not affect bone formation/resorption markers in postmenopausal osteoporotic women.

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Postmenopozal Osteoporozu olan Kadınlarda 3 aylık Arı Sütü (Royal Jelly) Kullanımının Kemik Belirteçleri Üzerine Etkisi

Öz: Bu çalışmada postmenopozal dönemdeki osteoporozu olan kadınlarda 3 aylık arı sütü (royal jelly) kullanımının kemik yapım ve yıkım belirteçlerini değerlendirerek kemik üzerindeki etkilerinin değerlendirilmesi amaçlanmıştır. Postmenopozal seksen kadın katılımcı, rastgele olarak arısütü verilenler (n=50) ve verilmeyenler (n=30) olarak ikiye ayrılmıştır. Arısütü alan gruba üç ay boyunca içerisinde 1 gram arısütü olan 100 mL sıvı verilmiştir. Kontrol grubuna ise yine 100 mL sıvı olacak şekilde plasebo verilmiştir. Kemik yapım belirteci olan N-terminal propeptit tip 1 kollajen (P1NP) ve kemik yıkım belirteci olan C-terminal telopeptit tip1 kollajen (CTX), sklerostin seviyeleri 0. ve 3. Ayda olmak üzere her iki grupta incelenmiştir. Sonuç olarak yaş, menopoz süresi, vücut kitle indeksi ve lomber vertebra kemik mineral dansitometri T skoru açısından benzer iki grupta kemik yapım ve yıkım beliteçleri açısından fark saptanmamıştır. Östrojenik etkisi yüksek olan arı sütünün 3 aylık süre boyunca kullanımı ile osteoporoz üzerine etkisi gösterilememiştir.

Anahtar Kelimeler: Arı sütü, osteoporoz, postmenopoz, östrojen

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