MELLIFERA

JOURNAL OF BEEKEEPING | ARICILIK DERGİSİ

HACETTEPE UNIVERSITY

Volume/Cilt: 20 Issue/Sayı: 1 Year/Yıl: 2020
ISSN: 1302-5821

MELLIFERA

JOURNAL OF BEEKEEPING | ARICILIK DERGİSİ

Volume/Cilt: 20 Issue/Sayı: 1 Year/Yıl: 2020

ISSN: 1302-5821

MELLIFERA is a publication of the Hacettepe University Bee and Bee Products Research and Application Center.
MELLIFERA Hacettepe Üniversitesi Arı ve Arı Ürünleri Araştırma ve Uygulama Merkezi yayınıdır.

Published twice a year June and December. / Haziran ve Aralık aylarında olmak üzere yılda 2 kez yayımlanır.

Type of Publication / *Yayın Türü* International Periodical / Uluslararası Süreli Yayın

Publication Language / *Yayın Dili* English / İngilizce

Correspondence /*Yazışma Adresi* Hacettepe Üniversitesi Arı ve Arı Ürünleri Uygulama ve Araştırma Merkezi,Fen Fakültesi Biyoloji Bölümü, Beytepe, Ankara, Turkey
Tel: +90 (312) 297 80 43 - 297 71 61

Website / *Web Sayfası* www.mellifera.hacettepe.edu.tr

e-mail / *e-posta* mellifera@hacettepe.edu.tr, ozkirim@hacettepe.edu.tr

Online Publish Date / *Elektronik Basım* *29*th June, 2020 / 29 Haziran 2020

Online Publish Adress / *Elektronik Basım Adresi* http://dergipark.gov.tr/mellifera

Cover Photo / *Kapak Fotoğrafı* Aygün Schiesser

MELLIFERA EDITORIAL

National and Global Impact of COVID-19 on Beekeeping

Aslı ÖZKIRIM[1,2] / *Editor-in-Chief*

[1] Bee Health Laboratory, Department of Biology, Faculty of Science, Hacettepe University, Ankara, TURKEY

[2] Bee and Bee Products Application and Research Center, Hacettepe University, Ankara, TURKEY

** Corresponding author e-mail: ozkirim@hacettepe.edu.tr*

Received: 12th June, 2020; accepted:15th June, 2020; published: 29th June, 2020

The COVID-19 pandemic influenced our daily life and many people working in different fields[1]. Among these people were scientists. Especially for people who continue their research with field studies, not being able to go out has created an important challenge. Beekeeping and bee science are also based on field study. Even laboratory work in bee research is done by working on samples collected from field. Therefore, it is not possible for bee research to be affected by the COVID-19 pandemic. However, the degree of influence is not yet clear. Because all mechanism of action will be chained. Currently, the first link of the chain is experienced. Failure to go out, laboratory works to be done will result in not writing article. Economic interactions will reduce new project supports. Such effects are predictable. In addition, there are unforeseen effects. Therefore, it is considered that the real effects of COVID-19 will appear objectively in 2021.

Impact of COVID-19 outbreak on the beekeeping in Turkey, people over age 65 since the beginning of the pandemic has emerged with the curfew. The average age is 55 and over of people who are beekeeper in Turkey, has led to the majority of beekeepers affected by the curfew.

The first case to be seen in Turkey in March has a disadvantage due to the beginning of the beekeeping season. Activities such as maintaining apiary, cleaning hives, fighting Varroa, feeding were not done regularly. Neighbors or young friends took care of the bees of our beekeepers aged 65 and over. In addition, bee health and bee product analyzes could not be conducted due to the universities stopping their activities.

Migratory beekeeping as common beekeeping activities in Turkey, especially during the initial phase of a pandemic,

Cite as: ÖZKIRM, A (2020) National and Global Impact of COVID-19 on Beekeeping, Mellifera, 20(1):1-2.

outside the province could not be held due to travel restrictions. Later, farmers were able to travel with special permissions and hive transfers were made. Considering that beekeeping is the most important agricultural branch, negative feedback of the restriction of beekeeping activities will surely be in the COVID-19 pandemic.

In universities, COVID-19 pandemics negatively the most affected laboratory studies and face-to-face network environments such as congress, conference, symposium. Since lessons and graduate education are carried out online, they have been the least affected by the pandemic.

Looking across the world, the best work which summarizes the current situation was done by the COLOSS Association. In fact, although countries have different approaches, COVID-19 has shown similar results in almost all countries [2].

In the survey by COLOSS, 230 participants from 56 countries answered questions about COVID-19. This is the first study in global size and using measurable data for statistical analysis. The results will be published soon. This study will be an important resource for risk assessments in beekeeping for the next years.

All interactions show that national impact of COVID-19 is parallel with global one. Another conclusion we will consider is that it is still too early to fully talk about the effects of COVID-19. The reflections of the process we are currently experiencing will only emerge concretely next year.

Keywords: Covid-19, Corona virus, pandemi, apiculture, apicultural research, beekeeping

R E F E RE N C E S

[1] WHO (2020) Coronavirus disease (COVID-19) Situation Report – 141 (Situation Reports). Available at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200609-covid-19-sitrep-141.pdf?sfvrsn=72fa1b16_2 (Accessed at: 10.06.2020)

[2] Coloss Honey Bee Research Association (2020) Coloss Survey – Impact of Covid-19 On Research (Press Release). Available at: https://coloss.org/news/coloss-survey-impact-of-covid-19-on-research-results/ (Accessed at: 10.06.2020)

Cite as: ÖZKIRM, A (2020) National and Global Impact of COVID-19 on Beekeeping, Mellifera, 20(1):1-2.

MELLIFERA RESEARCH ARTICLE

Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats

Züleyha DOĞANYIĞIT[1*], Emin KAYMAK[1], Aslı OKAN[1], Dilek PANDIR[2], Sibel SİLİCİ[3]

[1]Yozgat Bozok University, Faculty of Medicine, Department of Histology and Embryology, Erdogan Akdag Campus, Yozgat, TURKEY

[2]Yozgat Bozok University, Faculty of Arts and Sciences, Department of Biology, Erdogan Akdag Campus, Yozgat, TURKEY

[3]Erciyes University, Faculty of Agriculture, Department of Agricultural Biotechnology, Kayseri, TURKEY

** Corresponding author e-mail: zuleyha.doganyigit@gmail.com*

Received: 30th November, 2019; accepted: 19th December, 2019; published: 29th June, 2020

A B S T R A C T

Apilarnil is a bee product that has attracted attention due to its beneficial biological properties recently. This study aimed to investigate the effect of apilarnil (API) on endotoxin-induced lung injury. For the study, 64 adult male Sprague dawley rats were divided into eight groups; control, 0.2, 0.4 and 0.8 g / kg API treated groups by gavage for 10 days, 30 mg / kg lipopolysaccharide (LPS) administered intraperitoneally (single dose), LPS + 0.2, LPS + 0.4 and LPS + 0.8 g / kg API applied groups. In histopathological evaluation, hyperemia, intra-alveolar hemorrhage, cellular infiltration, and increased cellular abnormal proliferation were observed in the lung samples of the LPS group. It was found that the lung samples of LPS + 0,4 and LPS + 0,8 API groups decreased statistically significant compared to the LPS group. The number of TUNEL positive cells observed in both LPS and API treated groups showed a statistically significant decrease compared to the LPS group. In comet test, 0,8 API group was found to be reduced more in tail % DNA and tail length when LPS + API treated groups were compared with LPS group. In conclusion, the API applied to rats can prevent LPS-induced lung injury.

Keywords: Apilarnil, LPS, TUNEL method, Comet test, rat

Introduction

Sepsis is a medical condition that describes the systemic immunological response of the body to an infectious process that can lead to end-stage organ dysfunction and death [1]. The annual incidence of severe sepsis and septic shock in the United States is 300 cases per 100,000 people [2]. It is estimated that more than 30 million people worldwide are affected by sepsis each year, resulting in 6 million deaths per year [3]. The highest

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

organ damage in sepsis seems in the lungs, liver, kidneys, heart, intestines, and brain [4, 5]. Awareness in the pathogenesis of sepsis is very important for new developments in diagnosis, follow-up, and treatment. The sepsis triad is inflammation, coagulation, and irregular fibrinolysis. Sepsis may be caused by bacteria, viruses, fungi or parasites or may develop in other events such as severe trauma, pneumonia, pancreatitis, and urinary tract infection [6]. Many mediators such as proinflammatory cytokines, chemokines, and free radicals are known to be involved in the process of sepsis [7, 8]. Therefore, compounds with anti-inflammatory properties are thought to be useful in the treatment of sepsis.

LPS obtained as a lyophilized powder is obtained from a large number of Gram (-) bacteria such as *Escherichia coli (E. coli), Salmonella typhimurium, Klebsiella pneumoniae, Pseudomonas aeruginosa*. However, experimental septic shock studies have focused on LPSs obtained from *E. coli*. Reducing inflammation induced by *E. coli* may be a potential therapeutic method for the treatment of sepsis [9].

API is a bee product that has attracted attention in recent years, and it is obtained by lyophilized male bee larvae. However, API is known to have antiviral, immune system enhancer, anabolic stimulant, body energy, vitality and regenerative potentiator properties [10]. Scientific studies related to API are mostly made in farm animals [10-12].

In this study, we aimed to determine the effect of apilarnil on endotoxin-induced lung injury, LPS was used to perform a septic shock model. For this purpose, histopathological analysis of lung samples, TUNEL analysis for the evaluation of apoptotic cells, and Comet assay to determine DNA damage after application were performed.

Materials and Methods

Chemicals

Lipopolysaccharide (Escherichia coli LPS, serotype 0127: B8) was obtained from Sigma Aldrich and Lyophilized Apilarnil is Nutral Therapy Ltd. Doses used for this study were determined from previous studies [10, 13].

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

Experiment Groups

Ethics committee approval was received from Erciyes University Animal Experiments Local Ethics Committee (HAYDEK) for this study (Protokol No: 18/063). Animals were housed at 22–24 °C in a continuously ventilated environment with a lighting period of 12 h dark and 12 h light. Throughout the study, the animals were provided with ad libitum rat feed and drinking water. Sixty-four adult male Sprague dawley rats weighing approximately 200-250 g were randomly divided into 8 groups (Table 1).

Six hours after the administration, the rats were anesthetized (ketamine hydrochloride; 50 mg/kg i.m/i.p ve % 2 xylazine hydrochloride; 10 mg/kg i.m/i.p) and lung tissues were removed.

Table 1. Experimental groups and treatment methods

Experimental Groups	Applied Chemical Agent	Dose Amount	Procedure of administration
Control	Saline	1 ml (0.9% NaCl)	i.p.
LPS	LPS	30 mg / kg / bw	i.p.
0.2 g/kg API	API	0.2 g / kg / bw	oral gavage for 10 days
0.4 g/kg API	API	0.4 g / kg / bw	oral gavage for 10 days
0.8 g/kg API	API	0.8 g / kg / bw	oral gavage for 10 days
0.2 g/kg API + LPS	API + LPS	0.2 g / kg API, 30 mg / kg LPS	API oral gavage for 10 days, after 60 min LPS single dose i.p.
0.4 g/kg API + LPS	API + LPS	0.4 g / kg API, 30 mg / kg LPS	API oral gavage for 10 days, after 60 min LPS single dose i.p.
0.8 g/kg API + LPS	API + LPS	0.8 g / kg API, 30 mg / kg LPS	API oral gavage for 10 days, after 60 min LPS single dose i.p.

Histologic Analysis

At the end of the study, rats were sacrificed, and the lung tissues were removed. After routine paraffin embedding, 5 μm thick paraffin sections were stained with Harris hematoxylin and eosin and examined under a light microscope for histopathological evaluation. Hyperemia/congestion, intra-alveolar hemorrhage, cellular infiltration,

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

and cellular abnormal proliferation were evaluated in the lung tissues [14]. Histopathological results in each category were scored as follows: 0 = none, 1 = mild, 2 = moderate, 3 = severe.

Tunel Analysis

Apoptotic cells in incisions, obtained from the subjects, were determined by using the Roche brand In Situ Cell Detection Apoptosis Fluorescein Kit. The staining operation was conducted in line with kit procedure. After tissue incisions, taken in 5 µm thickness, were first deparaffinized and then rehydrated, they were washed twice with PBS for 5 minutes, they were later kept at 0.01 M in 5% sodium citrate buffer in microwave oven at 350 W for 5 minutes for antigen recovery, and it was left for cooling in room temperature for 10 minutes. Tissues, which were washed twice with PBS for 5 minutes, were incubated in drying-oven for 60 minutes after they were placed into a moisture chamber at 37 ^{0}C with TUNEL reaction mixture which came out of the kit. Reverse staining was administrated with 4',6-diamidino-2-phenylindole (DAPI) to tissues, which were washed twice for 5 minutes, and the DAPI solution was used as closer for nuclear staining. Tissues, which were closed with glycerol closure solution, were displayed in Olympus BX51 model fluorescent microscope. Apoptotic cells were counted in the image J program from the images taken at 40X lens from each incision from fifteen different sites, to calculate the apoptotic index.

Evaluation of DNA Structure by COMET Assay

The lungs extracted from rats are centrifuged at 5000 rpm for 30 minutes and the resulting supernatant is mixed with low melting agarose (0.65%) and 75 µl of the prepared suspension is transferred onto slides that is coated with low melting agar (0.05%). Electrophoresis is carried out in buffer at 200 V for 4 min. The preparations are transferred to cold lysis solution for 1 hour after the electrophoresis buffer. The preparations from the lysis solution are washed in distilled water for 5 minutes. After washing, the preparations are stained with 80 µl ethidium bromide. Post-staining image analysis is performed using BS 200 ProP (BS 200 ProP, BAB Imaging System). A 40x objective is used on a fluorescent microscope to observe DNA damage. The result of imaging is to determine the percentage of tail DNA, tail length and tail

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

moment for 50 comet cells, and the differences between the groups are calculated statistically.

Statistical analysis

Experimental data were statistically analyzed in GraphPad Prism (version 6.0, GraphPad Software Inc., San Diego, California) and presented as mean ± SD. Data were analyzed using one-way ANOVA with Tukey's post hoc tests for multiple comparisons. P<0.05 was considered significant.

Results and Discussion

Histopathological evaluation

According to histopathologic evaluation, the normal histological structure was observed in the lung tissues of the control group and the groups receiving API in increasing doses. Hyperemia, intra-alveolar hemorrhage, cellular infiltration, and increased cellular abnormal proliferation were observed in the lung samples of the LPS group (Figure 1A). It was found that the lung samples of LPS + 0,4 and LPS + 0,8 API groups' injury score were decreased statistically significant compared to the LPS group (Figure 1B).

TUNEL Results

In TUNEL analysis, the number of TUNEL positive cells observed in the LPS group in the lung samples showed a statistically significant increase compared to the control and apilarnil treated groups (Figure 2). The number of TUNEL positive cells observed in both LPS and API treated groups showed a statistically significant decrease compared to the LPS group (Table 2).

Table 2. Numerical data of TUNEL analysis in the lung tissues of experimental groups

Gruplar	Kontrol	0.2	0.4	0.8	LPS	LPS+ 0.2	LPS+ 0.4	LPS+ 0.8	*p*
TUNEL	0.12±0.13[a]	0.13±0.33[a]	0.04±0.20[a]	0.24±0.52[a]	0.64±0.81[b]	0.14±0.35[a]	0.08±0.27[a]	0.0.5±0.21[a]	0.001

Data are expressed as mean ± standard deviation. There is no significant difference between the groups containing the same letter (a, b, c). *P* <0,05 was considered significant.

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

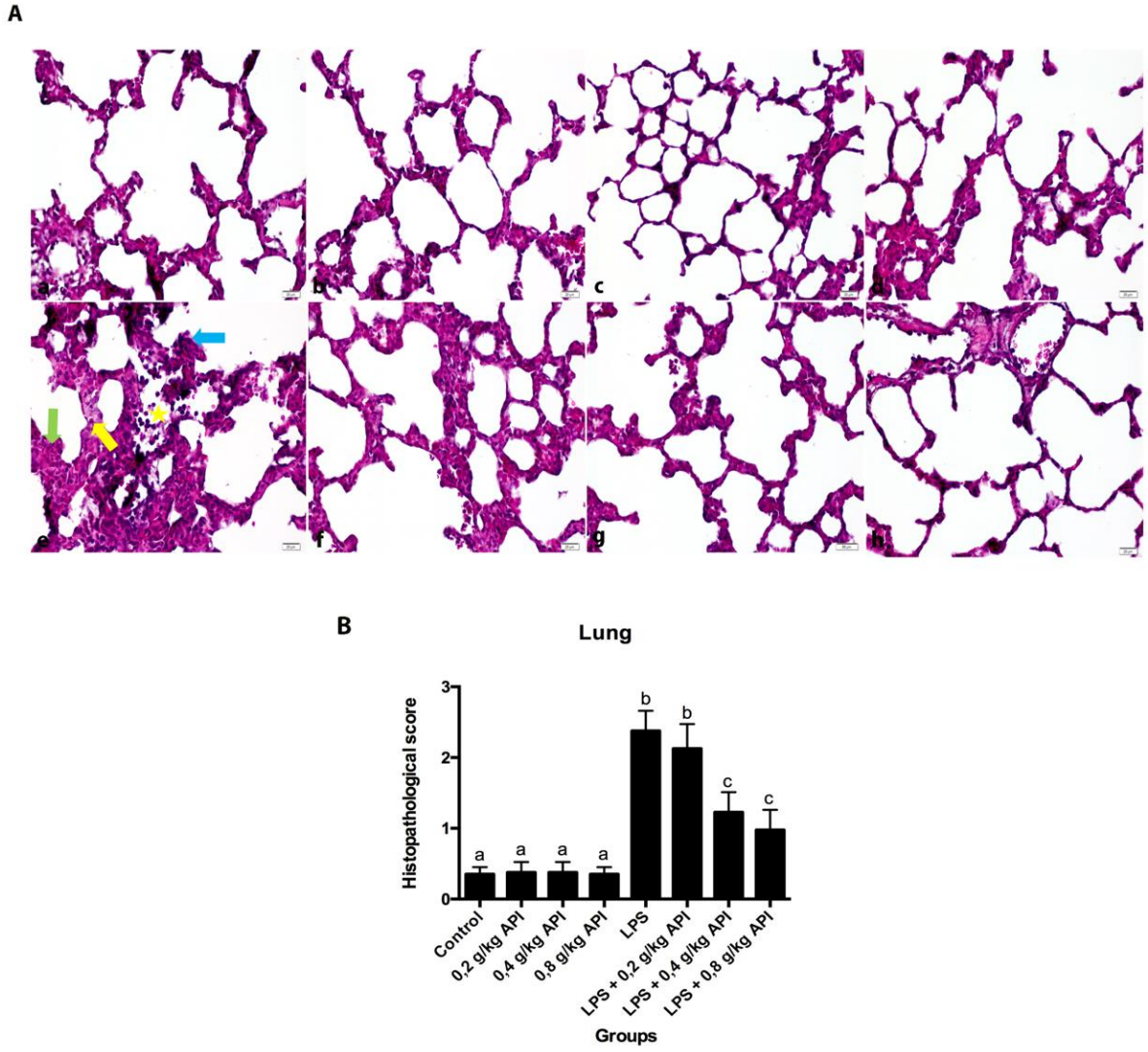

Figure 1. Histopathological evaluation of rat lung tissues of experimental groups by H&E staining method. **A.** Control (a), 0,2 g/kg body weight (bw) apilarnil (API) (b), 0,4 g/kg/ API (c), 0,8 g/kg/ bw API (d), LPS (e), LPS+ 0,2 g/kg/bw API (f), LPS+ 0,4 g/kg/bw API (g), LPS+ 0,8 g/kg bw (h) Magnification 40X, bar = 20µm (yellow arrow: congestion; star: intra alveolar hemorrhage; blue arrow: cellular infiltration; green arrow: cellular abnormal proliferation). **B.** The bar graph data are expressed as mean ± SD and compared by one-way ANOVA and TUKEY's multiple comparisons test. There is no significant difference between the groups containing the same letter (a, b, c). $P<0.05$ was considered significant.

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

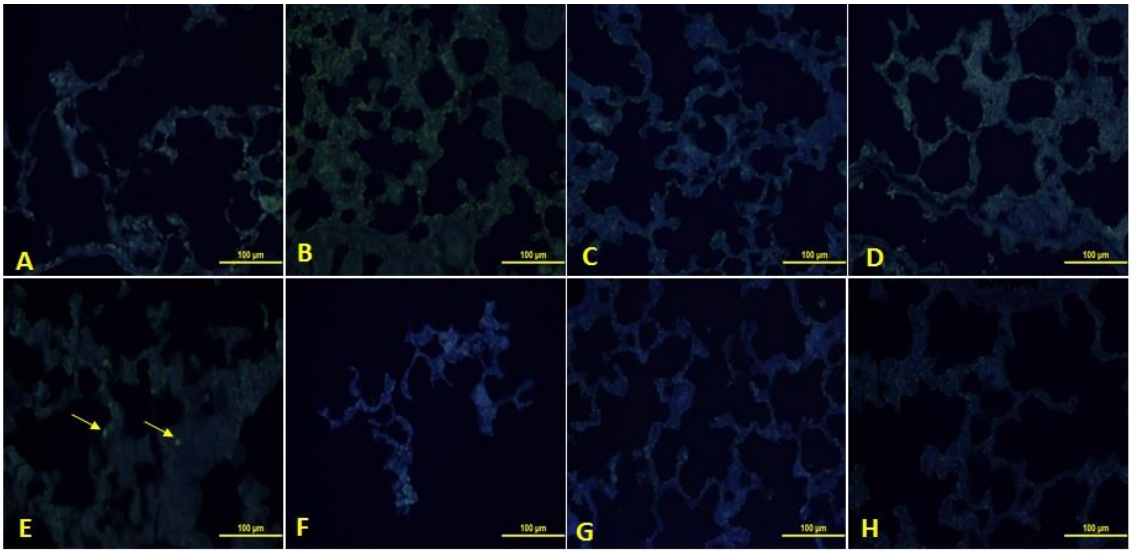

Figure 2. Evaluation of rat lung tissues of experimental groups by TUNEL method. Control (A), 0,2 g/kg/bw apilarnil (API) (B), 0,4 g/kg/bw API (C), 0,8 g/kg/bw API (D), LPS (E), LPS+ 0,2 g/kg/bw API (F), LPS+ 0,4 g/kg/bw API (G), LPS+ 0,8 g/kg/bw (H) Magnification: 40X, bar = 100μm.

Evaluation of Comet Assay Results

At the end of 6 hours LPS and/or API groups of rat lung tissues were taken for the comet method and tail %DNA, tail length and tail moment changes were determined (Figure 3 and Table 3).

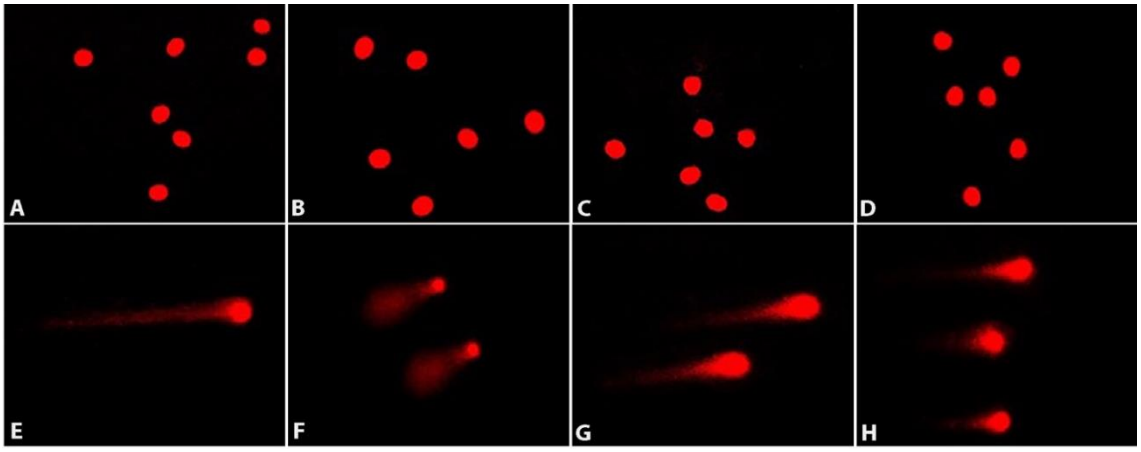

Figure 3. Determination of DNA damage caused by LPS administered to rat lungs cells and increasing doses of apilarnil. Control and API-treated group, (A-D); LPS-treated group (E), LPS + 0.2 g/kg API-administered group, (F) LPS + 0.4 g/kg API-administered group (G), LPS + 0.8 g/kg API-administered group (H).

No statistically significant difference was found in the DNA parameters of lung cells of rats in LPS-treatment groups and only increasing doses of API. Tail % DNA, tail

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

length and tail moment changes were determined in the LPS treated groups and it was found that there was a statistically significant increase in the studied parameters when the LPS treated groups and the control groups were compared. When LPS+API treated groups were compared, a statistically significant ($p < 0.05$) decrease was found in the studied parameters due to increasing concentrations of API. When 0.8 g/kg API+LPS applied group compared with 0.2 and 0.4 g/kg API+LPS, 0.8 g/kg API was found to be reduced more in tail % DNA and tail length according to 0.2 and 0.4 g/kg API applied group (Figure 3 and Table 3).

Table 3. Tail DNA%, Tail length and Tail moment values of lung cells exposed to LPS and increasing doses of API

	Tail DNA% Mean ± SD	**Tail Length Mean ± SD**	**Tail moment Mean ± SD**
Control	25.56±1.96[a]	16.40±1.22[a]	4.19±0.02[a]
0.2 g/kg API	24.48±3.25[a]	17.30±5.40[a]	4.23±0.17[a]
0.4 g/kg API	24.05±2.04[a]	16.79±2.02[a]	4.03±0.04[a]
0.8 g/kg API	23.55±2.31[a]	15.15±3.22[a]	3.56 ±0.07[a]
LPS	90.39±10.20[b]	82.94±8.15[b]	74.96±0.8[b]
LPS+0.2 g/kg API	70.82±6.53[c]	61.34±5.22[c]	43.44±0.34[c]
LPS+0.4 g/kg API	58.09±2.25[c]	25.12±4.85[c]	14.59±0.10[c]
LPS+0.8 g/kg API	39.06±3.12[d]	37.98±1.62[d]	14.83±0.05[d]

Data are expressed as mean ± standard deviation. There is no significant difference between the groups containing the same letter (a, b, c). $P < 0,05$ was considered significant.

Although many strategies have been developed to understand and treat the pathophysiological mechanisms of endotoxemia induced by endotoxins [15]. It is still a major problem in intensive care units. In recent years, alternative treatment options have gained increasing importance in addition to medical treatment, especially in the field of human medicine. API is one of the natural products that can be used for this purpose and contains many biologically active compounds. API related studies are very limited, but there are studies showing that API has positive effects on reproductive functions [10-12]. Besides, Meda et al. [16] reported that API has been

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

used successfully in South Africa (Burkina Faso) for gastrointestinal diseases, respiratory diseases, vertigo, ophthalmic diseases, toothache, muscle fatigue, wounds, burns and back pain, in particular, male infertility [16]. In this study, the effect of API on endotoxin-induced lung injury was investigated.

LPS application has been shown to cause tissue damage in many studies. Demiralay et al. [17] showed that LPS application in lung tissue increased inflammation, alveolar damage, vascular occlusion and the number of TUNEL positive bronchiolar and alveolar epithelial cells increased and LPS application resulted in induction of apoptotic cells characteristic of apoptotic cell death [17]. Wang et al. [18], investigated the role of bone marrow-derived mesenchymal stem cell (BMSC) transplantation on LPS-induced acute lung injury (ALI) in rats and they showed that LPS causes edema, severe damage to the alveolar wall, cellular abnormal proliferation, hyperemia and cellular infiltration [18]. Liu et al. [19] also showed that LPS administration causes serious damage to the lungs (a large amount of neutrophil and macrophage infiltration in the alveolar cavity, infiltration of the alveoli, edema, thickening of the alveolar wall and pulmonary interstitium), pulmonary alveoli, terminal bronchioles and whole lung tissue structure and in addition, LPS administration has been shown to significantly increase the number of apoptotic cells [19]. In the present study, it was observed that the lung samples of LPS + 0,4 and LPS + 0,8 API groups histopathological score were decreased statistically significant compared to the LPS group (Figure 1B). The data obtained at the end of the study are consistent with the existing literature.

LPS application increases the number of apoptotic cells in tissues [17-19]. As far as we investigate that there are no studies on the effects of API on increased apoptosis. The studies are related to other bee products. Cağlı et al. [20] investigated the effect of caffeic acid phenethyl ester (CAPE) on experimentally induced myocardial ischemia-reperfusion (I / R) injury and apoptotic changes and showed that pretreatment with CAPE reduced apoptosis in rat myocardium induced by I / R [20]. Yuluğ et al. [21] examined the effects of propolis on cisplatin-induced renal injury in mice and showed that tissue damage and increased apoptotic cell counts

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

due to CP were reduced due to the antioxidant and antiapoptotic effects of propolis [21, 22]. Kamijo et al. [22] showed that Cernitin pollen-extract (CN-009) significantly reduced tissue damage and apoptotic cell counts in the chronic nonbacterial prostatitis model [22]. As a result of TUNEL test in our study, we found that apilarnil has positive effects on increased apoptosis after LPS.

Comet test is one of the quick and easy test methods to determine the effects of various chemical agents that produce clastogenic effects in DNA [23, 24]. This method is used as a rapid and sensitive genotoxicity test to show DNA damage directly as well as single chain fractures and incomplete DNA repair sites [25]. The appearance of DNA migration generated by an electric current applied in the art is similar to a comet because it resembles a head and tail [26].

Agents with genotoxic effects cause damage to the DNA of prokaryotic and eukaryotic organisms. This damage is usually seen in the single and/or double helix in the DNA. Agents with genotoxic effects may cause carcinogenesis or chromosomal aberrations leading to sister chromatid changes, micronucleus, cell death and mutations [27]. In some studies, API has been reported to have antioxidant, antimicrobial and antiproliferative potential [28-30]. In this study, single and/or double-stranded DNA breaks were determined by comet test after LPS and/or API exposure time. Single and/or double-stranded DNA breaks were measured as tail% DNA, tail length and tail moment. Toxic effects of LPS on lungs cells of rats were evaluated by co-administration of API (0.2, 0.4 and 0.8 g/kg bw). There is no study showing the protective effect of API on DNA damage in lungs cells of rats in vivo. The toxic effects of LPS were determined by looking at tail DNA and tail length of lungs cells. At the end of 6 hours, it was determined that tail% DNA and tail length were increased in groups treated with LPS-treatment group compared to control group. In addition, in groups exposed to different concentrations of API, 0.8 g/kg API was found to be more protective than 0.2 and 0.4 g/kg API in lungs cells of rats.

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

Conclusion

In conclusion, we think that API prevented LPS-induced lung injury. We believe that our study will contribute to the literature in terms of demonstrating apilarnil's efficacy at tissue level. However, more comprehensive studies are needed.

Sıçanlarda lipopolisakkarit ile oluşturulan akciğer hasarına karşı apilarnilin koruyucu etkilerinin araştırılması

Öz: Apilarnil son yıllarda faydalı biyolojik özellikleri nedeniyle dikkat çeken bir arı ürünüdür. Bu çalışmanın amacı, apilarnil (API)' in, endotoksin ile indüklenen akciğer hasarı üzerine etkisini araştırmaktır. Çalışma için 64 adet Sprague dawley cinsi yetişkin erkek sıçan sekiz gruba ayrılmıştır; Kontrol, 10 gün boyunca gavaj ile 0.2, 0.4 ve 0.8 g/kg apilarnil (API) uygulan gruplar, intraperitoneal olarak 30 mg/kg lipopolisakkarit (LPS) uygulanan grup (tek doz), LPS + 0.2, LPS + 0.4 ve LPS + 0.8 g/kg API uygulanan gruplar. Histopatolojik değerlendirmede LPS uygulanan gruptaki ratların akciğer dokularında kanama, intra-alveolar hemoraji, hücresel infiltrasyon ve hücresel anormal proliferasyonda artış gözlendi. Oluşan doku hasarının özellikle LPS + 0.4 g/kg ve LPS + 0.8 g/kg API uygulanan gruplardaki ratların akciğer dokularında sadece LPS uygulanan grupla kıyaslandığında, istatistiksel olarak anlamlı derecede azaldığı tespit edildi. LPS ile birlikte API uygulanan gruplarda gözlenen TUNEL pozitif hücre sayısı ise LPS grubuna göre önemli oranda azaldı. Komet testinde, LPS+API ile tedavi edilen gruplar LPS grubu ile karşılaştırıldığında 0,8 g/kg API grubunun kuyruk % DNA'sının ve kuyruk uzunluğunun daha çok azaldığı bulundu. Sonuç olarak sıçanlara uygulanan API, LPS ile oluşturulan akciğer hasarını önleyebilmektedir.

Anahtar kelimeler: Apilarnil, LPS, TUNEL metodu, Komet testi, Sıçan

R E F E RE N C E S

[1] KAUKONEN, K M; BAILEY, M; SUZUKI, S, PILCHER, D; BELLOMO, R (2014), Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012, JAMA, 311(13), 1308-1316.

[2] GYAWALI, B; RAMAKRISHNA, K; DHAMOON, A S (2019), Sepsis: The evolution in definition, pathophysiology, and management, SAGE Open Med, 7, 2050312119835043.

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

[3] LEVY, M M; ARTIGAS, A; PHILLIPS, G S; RHODES, A; BEALE, R; OSBORN, T; VINCENT, J L; TOWNSEND, S; LEMESHOW, S; DELLINGER, R P (2012), Outcomes of the Surviving Sepsis Campaign in intensive care units in the USA and Europe: a prospective cohort study, Lancet Infect Dis, 12(12):919-24.

[4] POLAT, G; UGAN, R A; CADIRCI, E; HALICI, Z (2017), Sepsis and Septic Shock: Current Treatment Strategies and New Approaches, Eurasian J Med, 49(1):53-58.

[5] ADAM, N; KANDELMAN, S; MANTZ, J; CHRETIEN, F; SHARSHAR, T (2013), Sepsis-induced brain dysfunction, Expert Rev Anti Infect Ther, 11(2): 211-21.

[6] COHEN, J (2002), The immunopathogenesis of sepsis, Nature, 420: 885-91.

[7] NDUKA, O O; PARRILLO, J E (2009), The pathophysiology of septic shock, Crit Care Clin, 25, 677-702, vii.

[8] CINEL, I; OPAL, S M (2009), Molecular biology of inflammation and sepsis: a primer, Crit Care Med, 37: 291-304.

[9] POP-BEGAN, V, PAUNESCU, V, GRIGOREAN, V, POP-BEGAN, D; POPESCU, C (2014), Molecular mechanisms in the pathogenesis of sepsis, J Med Life, 7 Spec No, 2:38-41.

[10] ALTAN, O; YUCEL, B; ACIKGOZ, Z; SEREMET, C; KOSOGLU, M; TURGAN, N; OZGONUL, A M (2013), Apilarnil reduces fear and advances sexual development in male broilers but has no effect on growth, Br Poult Sci, 54:355-61.

[11] BOLATOVNA, K S; RUSTENOV, A; ELEQALIEVA, N; OMIRZAK, T; AKHANOV, U K (2015), Improving reproductive qualities of pigs using the drone brood homogenate, Biol Med (Aligarh), 7, BM-091-15.

[12] YÜCEL, B; AÇIKGÖZ, Z; BAYRAKTAR, H; SEREMET, Ç (2011), The effects of apilarnil (drone bee larvae) administration on growth performance and secondary sex characteristics of male broilers, J Anim Vet Adv, 10: 2263-2266.

[13] DOGANYIGIT, Z; KUP, F O; SILICI, S; DENIZ, K; YAKAN, B; ATAYOGLU, T (2013), Protective effects of propolis on female rats' histopathological, biochemical and genotoxic changes during LPS induced endotoxemia, Phytomedicine, 20: 632-9.

[14] HIRANO, Y; AZIZ, M; YANG, W L; WANG, Z; ZHOU, M; OCHANI, M; KHADER, A; WANG, P (2015), Neutralization of osteopontin attenuates neutrophil migration in sepsis-induced acute lung injury, Crit Care, 19:53.

[15] WILHELM, E A; JESSE, C R; ROMAN, S S; NOGUEIRA, C W; SAVEGNAGO, L (2009), Hepatoprotective effect of 3-alkynyl selenophene on acute liver injury induced by D-galactosamine and lipopolysaccharide, Exp Mol Pathol, 87: 20-6.

[16] MEDA, A; LAMIEN, C E; MILLOGO, J; ROMITO, M; NACOULMA, O G (2004), Therapeutic uses of honey and honeybee larvae in central Burkina Faso, J Ethnopharmacol, 95: 103-7.

[17] DEMIRALAY, R; GURSAN, N; ERDEM, H (2006), Regulation of sepsis-induced apoptosis of pulmonary cells by posttreatment of erdosteine and N-aceylcysteine, Toxicology, 228: 151-61.

[18] WANG, J; QIN, Y; MI, X (2016), The protective effects of bone marrow-derived mesenchymal stem cell (BMSC) on LPS-induced acute lung injury via TLR3-mediated IFNs, MAPK and NF-kappaB signaling pathways, Biomed Pharmacother, 79: 176-87.

[19] LIU, W A; PANG, G; WANG, S; SUN, A (2017), Protective effect of ulinastatin on severe pulmonary infection under immunosuppression and its molecular mechanism, EXPERIMENTAL AND THERAPEUTIC MEDICINE, 14:3583-3588.

[20] CAGLI, K; BAGCI, C; GULEC, M; CENGIZ, B; AKYOL, O; SARI, I; CAVDAR, S; PENCE, S; DINCKAN, H (2005), In vivo effects of caffeic acid phenethyl ester on myocardial ischemia-reperfusion injury and apoptotic changes in rats, Ann Clin Lab Sci, 35:440-8.

[21] YULUG, E; TUREDI, S; YILDIRIM, O; YENILMEZ, E; ALIYAZICIOGLU, Y; DEMIR, S; OZER-YAMAN, S; MENTESE, A (2019), Biochemical and morphological evaluation of the effects of propolis on cisplatin induced kidney damage in rats, Biotech Histochem, 94:204-213.

[22] KAMIJO, T; SATO, S; KITAMURA, T (2001), Effect of cernitin pollen-extract on experimental nonbacterial prostatitis in rats, Prostate, 49:122-31.

[23] SINGH, N P; MCCOY, M T; TICE, R R; SCHNEIDER, E L (1988), A simple technique for quantitation of low levels of DNA damage in individual cells, Exp Cell Res, 175:184-91.

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

[24] FAIRBAIRN, D W; OLIVE, P L; O'NEILL, K L 81995), The comet assay: a comprehensive review, Mutat Res, 339: 37-59.

[25] TICE, R R; VASQUEZ, G (1998), Protocol for the application of the pH>13 alkaline sigle cell gel (SCG) assay to the detection of DNA damage in mammalian cells, ILS: The pH>13 Alkaline SCG Assay,Available from: http://cometassay.com/Tice%20and%20Vasques.pdf (Data of access)

[26] KLAUDE, M; ERIKSSON, S; NYGREN, J; AHNSTROM, G (1996), The comet assay: mechanisms and technical considerations, Mutat Res, 363: 89-96.

[27] HIROSE, H; SAKUMA, N; KAJI, N; SUHARA, T; SEKIJIMA, M; NOJIMA, T; MIYAKOSHI, J (2006), Phosphorylation and gene expression of p53 are not affected in human cells exposed to 2.1425 GHz band CW or W-CDMA modulated radiation allocated to mobile radio base stations, Bioelectromagnetics, 27: 494-504.

[28] CAPUCHO, C; SETTE, R; DE SOUZA PREDES, F; DE CASTRO MONTEIRO, J; PIGOSO, A A; BARBIERI, R; DOLDER, M A; SEVERI-AGUIAR, G D (2012). Green Brazilian propolis effects on sperm count and epididymis morphology and oxidative stress, Food Chem Toxicol, 50: 3956-62.

[29] RIZK, S M; ZAKI, H F; MINA, M A (2014), Propolis attenuates doxorubicin-induced testicular toxicity in rats, Food Chem Toxicol, 67: 176-86.

[30] DOGANYIGIT, Z; SILICI, S; DEMIRTAS, A; KAYA, E; KAYMAK, E (2019), Determination of histological, immunohistochemical and biochemical effects of acute and chronic grayanotoxin III administration in different doses in rats, Environ Sci Pollut Res Int, 26: 1323-1335.

Cite as: DOĞANYİĞİT, Z; KAYMAK, E; OKAN, A; PANDIR, D; SİLİCİ, S (2020), Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Lung Injury in Rats, Mellifera, 20(1):3-15.

MELLIFERA REVIEW ARTICLE

Flavones (Apigenin, Luteolin, Chrysin) and Their Importance for Health

Fatma Özsel ÖZCAN[1*], Ozan ALDEMİR[2], Bülent KARABULUT[3]

[1]Beykoz Üniversity, Faculty of Art and Design, Department of Gastronomy and Culinary Arts, İstanbul, Turkey

[2]Ege University Faculty of Medicine, Department of Biochemistry, İzmir, TURKEY

[3]Ege University Faculty of Medicine, Department of Oncology, İzmir, TURKEY

** Corresponding author e-mail: fatmaozselozcan@beykoz.edu.tr*

Received: 14th March, 2020; accepted: 4th May, 2020; published: 29th June, 2020

A B S T R A C T

It has been shown in recent years that foods called functional foods may protect against certain types of cancer, cardiovascular diseases and cognitive dysfunctions. In the studies performed, the flavonoids (apigenin, chrysin, luteolin) which are subclass of flavonoids have been shown to have antioxidant, antiinflammatory, antiallergic, neuroprotective and cardioprotective effects and it is presented as the current treatment method in the treatment of some diseases. The structure function, nutritional resources and potential therapeutic properties of the flavones, which are also used as supplement in the compost, have been studied. The purpose of this review is to evaluate the therapeutic effects of flavones in certain diseases. The positive effect of flavones on health can be proven in many experimental studies and can be proven in the long run.

Keywords: Functional nutrients, flavones, apigenin, luteolin, chrysin

Introduction

Polyphenolic flavonoids are among the wide variety of phytochemicals found in the human diet. Current studies reveal that dietary flavonoids are inversely related to many cancers and age-related diseases [1]. Average flavonoid intake in the diet of humans varies between 20 and 1,000 mg / day [2]. Theories and randomized clinical studies on the cancer prevention mechanisms of flavonoids are still ongoing. The levels of total flavonoids in foods are affected by factors such as plant species, environment, genetics, light, maturity, harvest [3]. Flavones, a subset of flavonoids, form glycosylation and contain a hydroxylated β-ring. In preclinical models, especially Apigenin, Luteolin and Crisis have neuroprotective, anti-inflammatory, antioxidant effects [4]. It is estimated that the majority of metabolic diseases are caused by oxidative stress, so it is important that studies have shown the

Cite as: ÖZCAN, F Ö; ALDEMİR, O; KARADEMİR, B (2020), Flavones (Apigenin, Luteolin, Crhysin) and Their Importance for Health, Mellifera, 20(1):16-27.

positive effect of flavones on oxidative stress related diseases. It is necessary to examine the current approaches about flavones in order to create evidence for their use in the treatment of flavones.

Sources of Flavones, Bioavailability and Functions

Regular intake of flavones with nutrients is associated with a reduced risk of a number of chronic diseases, including cancer formation, cardiovascular diseases (CVD) and neurodegenerative disorders. Extensively consumed fruits, vegetables and beverages contain various amounts of flavone.

Fruit peels, celery, parsley, paprika, chamomile, mint, ginkgo biloba, red wine, buckwheat, tomato peel, paprika are rich sources of flavone. The accumulation of flavonoids in plants is positively associated with the amount of sunlight received. Flavones are synthesized from the anthocyanidine / proanthocyanidin pathway from flavanones as direct biosynthetic precursors [5,6]. Flavones are present in their natural form, both as O and C-glycosides. Flavones cannot be absorbed from the intestines at plasma concentrations of <1 μmol / L [7]. Flavones have antioxidant, anti-inflammatory, neuroprotective, cardioprotective and antiallergic effects. Flavones reduce the reactive species (hydroxyl, superoxide and nitric oxide) of intracellular free radicals, also the effect of preventing damage to biomolecules such as lipids, proteins and DNA.

Flavones can also inhibit the activity of free radical producing enzymes such as xanthine oxidase, nicotinamide adenine dinucleotide phosphate oxidase, or inducible nitric oxide synthase, and can modulate intracellular levels of the pro-oxidant [8].

Apigenin and Effects of Apigenin on Health

Apigenin is a flavonoid found in some plants, fruits, and vegetables (parsley, chamomile, celery, vine spinach, artichoke, and thyme). The genus *Apium* (celery, carrot or parsley family) is also known as Umbelliferae [9]. Apigenin was first described in 1900 and was synthesized in 1939. The naturally occurring glycoside conjugates in Apigenin are more water-soluble. In in vivo conditions, glycoside conjugates are digested and hydrolyzed by bacteria in the gut to form molecules of free apigenin [10]. There is only one review on the antimicrobial effect of apigenin in the

Cite as: ÖZCAN, F Ö; ALDEMİR, O; KARADEMİR, B (2020), Flavones (Apigenin, Luteolin, Crhysin) and Their Importance for Health, Mellifera, 20(1):16-27.

literature. Although there are insufficient studies, it has been shown that apigenin or its glycosides are divided into metabolites by certain gut bacteria and affect the health of gut [11]. There are many studies in the literature investigating the cancer prevention mechanisms of apigenin. Studies argue that apigenin can be used in the cell to prevent cancer types such as breast cancer, uterine cancer, colon cancer, lung cancer, ovarian-prostate cancer, skin cancer, liver cancer and stomach cancer [12]. Breast cancer can be prevented by inhibition of telomerase activity. Cisplatin, a chemotherapeutic drug, causes DNA damage. In a study, it was observed that the use of apigenin and cisplatin together in the treatment of breast cancer had a synergistic effect in reducing telomerase activity [13]. The mechanism underlying apoptosis-inducing effect of apigen in colon cancer treatment is thought to inhibit the transcription activator (STAT3) phosphorylation of the apigen and anti-apoptotic proteins (Bcl-xL and Mcl-1). Apigenin stimulates the proliferation of cancer cell lines, reproducing by division and apoptosis formation in a dose-dependent manner [14]. Treatment of apigenin significantly improves weakened heart functions. In one study, male rats were fed a high-fat diet for three months. Then, in order to create a Type 2 Diabetes model in these mice, 100 mg / kg STZ treatment was applied, and cardiac measurements were performed by administering 100 mg / kg apigenin daily for 4 months in diabetic mice. In this study, apigenin, Kaspaz3 and NF-κB / P65 ', influenced the signal path, regulated GSH-Px, MDA and SOD levels and was determined to be effective in regulating oxidative stress [15]. Apigenin prevents dopamine-related oxidative stress in melanocytes. In the treatment of vitiligo, an immune system disease, antioxidant apigenin can be used with its effects on Nrf2 expression and genes [16]. Another study investigating the roles of apigenin in diabetic cardiomyopathy has been reported to decrease in diabetes and cardiomyopathy markers with apigenin treatment in mice treated with streptozosine (50 mg / kg) for 5 days [17]. It has been suggested that apigenin can provide a clinically beneficial effect for these neurodegenerative disorders by targeting neuroinflammatory processes, such as cytokine suppressive anti-inflammatory drugs (CSAIDs). In a study investigating the therapeutic effect of apigen on glial fibril acidic protein-interleukin 6 (GFAP-IL6), it was found that the number of active microglia in the

Cite as: ÖZCAN, F Ö; ALDEMİR, O; KARADEMİR, B (2020), Flavones (Apigenin, Luteolin, Crhysin) and Their Importance for Health, Mellifera, 20(1):16-27.

cerebellum and hippocampus decreased by 25-30% [18]. In a study investigating the effect and possible mechanism of the combination of apigenin and ischemic conditioning on renal ischemia-reperfusion injury in rats, the combination has been shown to inhibit the TLR4 / NF-sinyalB signaling pathway in renal ischemia / reperfusion injury and provide great protection against renal ischemia / reperfusion injury in rats [19]. Apigenin shows a strong power in the treatment of paclitaxel resistant hypoxic tumors [20]. Another study investigating the potential anticancer properties of Apigenin on human breast cancer; while apigene does not act in normal cells, it has been found to be genotoxic in selected cancer cells, cells that have the potential to oxidize lipids. Combined with the low cytogenotoxic and pro-cell death activities of Apigenin and its low toxicity to normal cells, it is argued that this natural flavone may be used as an anticancer agent in the future [21]. In a study conducted to examine the growth inhibitory effects of apigenin, different doses of apigenin were given to HCT116 cells. Apigenin low concentrations (6.25 μM) didn't affect cell viability, whereas high concentrations of Apigenin (25 and 50 μM) were found to significantly reduce cell viability of HCT116 cells. Morphological and qualitative changes were also observed in the cells given apigenin [22]. In a study examining the effect of apigen on muscle atrophy due to sciatic nerve denervation, mice with impaired sciatic nerves were fed a diet containing 0.1% apigenin for 2 weeks. Muscle atrophy resulting from denervation was found less in apigenin given mice and it was observed that apigenin inhibits muscle atrophy caused by denervation due to its inhibitory effect on inflammatory processes in the muscles [23].

Luteolin and Effects of Luteolin on Health

Luteolin (LUT) is a common flavonoid that is abundant in many herbal products, including broccoli, peppers, thyme, peanuts, and celery. In vitro and in vivo studies have shown that LUT has neuroprotective effects [24]. LUT sensitizes cancer cells to cytotoxicity by suppressing cell survival pathways such as phosphatidylinositol 3-kinase (PI3K), nuclear factor kappa B (NF-κB), X-linked apoptosis inhibitor (XIAP) and stimulation of apoptosis pathways. The anticancer property of LUT has been associated with induction of apoptosis and inhibition of cell proliferation, metastasis and angiogenesis

[25]. Due to its bacteriostatic properties and potent antioxidant potential, LUT is valuable in the treatment of a variety of diseases, including peptic ulcers. With this antioxidant effect, LUT reduces kidney anemia by reducing oxidative stress in the kidney. In a study on wound healing in diabetic rats, different LUT concentrations were applied in MTT analysis on 3T3 fibroblast cells. Annexin V and cell cycle analyzes were performed. Significant improvement was observed in the groups treated with LUT compared to control. LUT increased the live population of 3T3 cells and the cell population in the G2M phase compared to the control group [26,27]. Another study shows that LUT treatment is able to reduce fat accumulation by acting on serotonin-related receptors ser-6 and mod-1 [28]. In a study examining the effects of LUT on colon cancer in obese mice; different groups were formed as normal diet (ND), high-fat diet (YYD), high-fat diet with 0.0025% LUT, high-fat diet with 0.005% LUT. As a result, it was observed that body weight, colon weight / height and tumor rate increased significantly in the YYD group compared to the ND group [29]. In YYD, LUT supplementation significantly reduced colon weight / length and the rate of colon tumors but did not change body weight. It has been reported that plasma tumor necrosis factor (TNF-a) levels and inducible nitric oxide synthase and cyclooxygenase-2 protein increase colonic expression in response to YYD, and these effects are suppressed by LUT supplementation [30].

In a study testing the effects of LUT on sunburn, LUT in human keratinocytes exposed to physiological doses of UVB has been shown to weaken cell death caused by UVB by inhibition of apoptotic signaling. LUT has inhibitory effects on UVB-induced release of inflammatory mediators, IL-1 and prostaglandin-E2. LUT increases the survival rate of normal keratinocytes [31].

Inhibition of osteoclast differentiation and bone resorption is considered an effective therapeutic approach in the treatment of postmenopausal bone loss. In one study, oral administration of LUT (5 and 20 mg / kg per day) to the ovarian-removed mice prevented the reduction of bone resistance while increasing the bone mineral density and bone mineral content in the femur. These data strongly demonstrate that LUT has the potential to prevent bone loss in postmenopausal osteoporosis by reducing

Cite as: ÖZCAN, F Ö; ALDEMİR, O; KARADEMİR, B (2020), Flavones (Apigenin, Luteolin, Crhysin) and Their Importance for Health, Mellifera, 20(1):16-27.

osteoclast differentiation and function [32]. In patients with heart transplantation, the hypothermic protection of the heart takes 4-6 hours and over time, calcium accumulation causes cell death. LUT protects the heart and vessels by reducing oxidative stress-induced damage in cells. Because of this feature, it is used in Chinese medicine to protect the donor hearts for a longer time. In a study investigating the protective role of LUT in modulating the cardiomyocyte calcium cycle, 7.5, 15 or 30 μmol / l LUT-supported solutions were used to protect cardiomyocytes. The results showed that three doses of LUT supplementation reduced calcium overload by providing a cardioprotective effect over a 6-hour protection period [33]. LUT increases the effect of cisplatin used in breast cancer treatment by decreasing Bcl-2 expression. Studies show that a combination of cisplatin and LUT supplements may be a potential treatment in ovarian cancer [34].

There are cytokine-neuropeptide interactions in the pathogenesis of diseases in the brain. Especially myalgic encephalomyelitis syndrome and autism spectrum disorder are negatively affected by the release of corticotropin hormone and neurotensin. Natural flavonoid LUT and tetrametoxyluteolin inhibit these processes and provide neuroprotective effect. Tetrametoxyluteolin is metabolically more stable and absorbed more [35]. In one study, mice fed a high-fat diet were given LUT supplement or celery fiber containing high amounts of luteolin. These supplements have been reported to reduce weight by reducing the activity of gastric inhibitory polypeptide and hepatic glucogenic enzymes, reducing insulin sensitivity, inflammation (IL1-6) and dyslipidemia [36].

LUT has been found to have an effective antiviral activity against the Japanese encephalitis virüs [37]. Another study reported that administration of LUT in hepatitis B virus (HBV) mice reduced hepatocyte nuclear factor 4α (HNF4α) and DNA replication. This study suggests that LUT can be used for anti-HBV treatment [38]. Regular LUT treatment can create an antidepressant effect by reducing stress in the endoplasmic reticulum [39]. Mangiferin, another polyphenol in combination with LUT, has been found to increase long-term supplementation at high and low doses during sprint, exercise

Cite as: ÖZCAN, F Ö; ALDEMİR, O; KARADEMİR, B (2020), Flavones (Apigenin, Luteolin, Crhysin) and Their Importance for Health, Mellifera, 20(1):16-27.

performance, muscle O_2 extraction and brain oxygenation [40].

Chrysin and Effects of Chrysin on Health

Chrysin, a natural flavone, is found in many plant extracts, including propolis and honey. It is one of the herbal medicines widely used in Asian countries. The chrysin has estrogenic, antiinflammatory, antibacterial, anti-diabetic, antitumor effects [41]. In humans, the acute dose of 400mg chrysin is not toxic. Daily doses of 0.5–3 g are recommended for the efficacy of the chrysin. Chrysin reduces toxicity in liver cells, inhibits novo DNA synthesis. The low toxicity and broad spectrum of antitumor activity underlines crystalline cancer treatment [42]. Doxorubicin (DOX) is one of the most effective chemotherapeutic drugs; however, the incidence of cardiotoxicity impairs its therapeutic index.

İn a study to investigate the protection of chrysin against DOX-induced acute cardiotoxicity, rats were given 25 and 50 mg / kg of chrysin for 12 days, and on the 12 th day, DOX (15 mg / kg) was given. It has been reported that doxorubicin triggers inflammatory responses by increasing levels of nuclear factor kappa-B (NF-κB), inducible nitric oxide synthase and cyclooxygenase-2, tumor necrosis factor-alpha, and the pre-doxorubicin chrysin significantly inhibits inflammatory responses [43].

Chrysin has beneficial effects on the brain due to antioxidant effect on neuronal activity disorder, memory impairment and neuronal cell death in rats [44]. Chronic cerebral hypoperfusion induced by occlusion of the bilateral carotid arteries is associated with neurological disorders and causes cognitive decline. In a study investigating the effects of chrysin on brain damage, it was seen that rats exposed to neuronal damage increased, Cell apoptosis was significantly reduced by treatment of chrysin (30 mg / kg) for a long time. Chrysin reduced lipid peroxidation, superoxide dismutase activation, and increased glutathione peroxidase activity. These effects have shown that chrysin may have therapeutic potential for the treatment of neurodegeneration and dementia caused by decreased cerebral blood flow [45].

Chrysin, an inhibitor of the aromatase enzyme that provides the balance of sex hormones, is found in high concentrations in honey and propolis. This flavonoid used

Cite as: ÖZCAN, F Ö; ALDEMİR, O; KARADEMİR, B (2020), Flavones (Apigenin, Luteolin, Crhysin) and Their Importance for Health, Mellifera, 20(1):16-27.

by athletes as supplements is thought to have testosterone-enhancing effects. In a study investigating the relationship between urine testosterone and chrysin consumption for 21 days in volunteer male subjects, no change in testosterone levels was observed in volunteer male subjects [46].

Plasma PAI-1 increases under inflammatory conditions such as infection, obesity and atherosclerosis. In a study that examined the increase in PAI-1 caused by inflammation, it was observed that chrysin inhibits the production of PAI-1 [47].

Benign prostatic hyperplasia (BPH) is a health problem in men over sixty years old. In the study investigating the protective effects of chrysin in testosterone-induced BPH, rats were given 50 mg / kg of chrysin. Chrysin reduced testosterone-induced oxidative stress, caspase-3 level, Bax / Bcl-2 ratio and mRNA expression of p53 and p21 to normal levels. Chrysin reduced the nuclear factor kappa and inhibited mRNA expression of IGF-1R. These data show that chrysin plays a protective role against BPH [48].

In another study, sperm motility, sperm concentration, and serum testosterone levels were significantly increased in mice given 50 mg / day of chrysin, while abnormal sperm rate was significantly reduced with chrysin therapy. It is thought that chrysin therapy can positively affect the reproductive system and can be used in the treatment of male infertility [49]. İn a study investigating the effect of chrysin on fertility, it was observed that blood testosterone level and sperm quality increased in roosters with increasing amount of chrysin supplements [50].

In a study with atopic dermatitis mice for use in chrysin skin allergies, serum IgE and IgG2a levels, mast cell infiltration, and serum histamine levels have been reported to decrease with treatment [51].

Chrysin has a similar effect to metformin, reducing blood glucose and triglyceride levels, reducing the secretion of pro-inflammatory cytokines and thus creating an antidiabetic and dyslipidemic effect [52]. Recently, animal models show that the dietary polyphenol chrysin is an effective inhibitor of fructose uptake by human intestinal epithelial cells. Reducing the effect of excessive amounts of fructose reduces metabolic syndrome parameter values [53].

Cite as: ÖZCAN, F Ö; ALDEMİR, O; KARADEMİR, B (2020), Flavones (Apigenin, Luteolin, Crhysin) and Their Importance for Health, Mellifera, 20(1):16-27.

A study investigating the oxidative stress induced by tert-butyl hydroperoxide and the mechanism of action of this stress in rat hepatocytes, Depending on the dose of luteolin, apigenin and chrysin, it has been reported to increase the intracellular glutathione content and inhibit oxidative stress by increasing gene transcription through ERK2 / Nrf2 / ARE signaling pathways in rat primary hepatocytes. Among the studied flavones, chrysin had the best effect [54].

The ubiquitin-proteasome pathway plays an important role in regulating apoptosis and cell cycle. In recent years, some flavonoids have been reported to inhibit proteasome activity in tumor cells. It has been observed that the effect of flavones on the proteasome inhibitor is higher compared to other flavonoids. In studies, it has been observed that the inhibitory effect of tumor cells decreases in the order of luteolin, apigenin, and chrysin [55].

Conclusion

Flavones are polyphenolic components included in the daily diet. Nowadays, researchers are turning to the mechanism of action of functional components in foods and their relationship to health. In this review, the positive effects of flavones (Apigenin, Luteolin, Chrysin) on health are revealed. In vivo and in vitro studies show that flavones shorten the treatment time in inflammatory processes. Apigenin, luteolin and crisis have apoptosis effect in many cancer types. These flavones additionally play an important role in improving cardiovascular conditions, stimulating the immune system and improving renal dysfunction, protecting against muscle atrophies, exceeding fertility, diabetes and cholesterol control. Chrysin, which is found in propolis, has a preventive and reproductive protective effects, as well as a therapeutic potential in neurodegenerative diseases. A diet rich in flavones, integration of its modification into the diet can be a chemopreventive strategy for individuals with high cancer risk. To determine whether the therapeutic effects are beneficial for patients, more research and scientific evidence needs to be produced.

Flavonlar (Apigenin, Luteolin, Krisin) ve Sağlık İçin Önemi

Öz: Son yıllarda fonksiyonel gıdalar adı verilen gıdaların belirli kanser türlerine, kardiyovasküler hastalıklara ve bilişsel işlev bozukluklarına karşı koruyabileceği gösterilmiştir. Yapılan çalışmalarda, flavonoidlerin alt sınıfı olan flavonoidlerin (apigenin, krisin, luteolin) antioksidan, antienflamatuar, antialerjik, nöroprotektif

Cite as: ÖZCAN, F Ö; ALDEMİR, O; KARADEMİR, B (2020), Flavones (Apigenin, Luteolin, Crhysin) and Their Importance for Health, Mellifera, 20(1):16-27.

ve kardiyoprotektif etkileri olduğu gösterilmiştir ve bazı hastalıkların tedavisinde mevcut tedavi yöntemi olarak sunulmuştur. Kompost takviyesi olarak da kullanılan flavonların, yapısal fonksiyonu, besin kaynakları, potansiyel tedavi edici özellikleri incenmektedir. Bu derlemenin amacı bazı hastalıklarda flavonların terapötik etkilerini değerlendirmektir. Flavonların sağlık üzerindeki olumlu etkisi birçok deneysel çalışma ile uzun vadede kanıtlanabilir.

Anahtar Kelimeler: Fonksiyonel besinler, flavonlar, apigenin, luteolin, krisin

R E F E RE N C E S

[1] GRAF, B A; MİLBURY, P E; BLUMBERG, J B (2005), Flavonols, Flavones, Flavanones, and Human Health: Epidemiological Evidence, Journal of Medicinal Food, 8(3):281–290.

[2] MULLİE, P; CLARYS, P; DERİEMAKER, P; HEBBELİNCK, M (2007), Estimation of daily human ıntake of food flavonoids, Plant Foods for Human Nutrition, 62(3):93–98.

[3] YAO, L H; JİANG, Y M; SHİ, J; T S-BARBER N, F A; DATTA, N; SINGANUSONG, R; CHEN, S S (2004), Flavonoids in food and their health benefits, Plant Foods for Human Nutrition, 59(3): 113–122.

[4] ARREDONDO, F; ECHEVERRY, C; BLASİNA, F; VAAMONDE, L; DİAZ, M; RİVERA, F; DAJAS, F (2015), Flavones and Flavonols in Brain and Disease, Bioactive Nutraceuticals and Dietary Supplements in Neurological and Brain Disease, Prevention and Therapy, pp. 229–236.

[5] JİANG, N; DOSEFF, A; GROTEWOLD, E (2016), Flavones: From Biosynthesis to Health Benefits, Plants, 5(2): 27.

[6] PANCHE, A N; Diwan, A D; Chandra, S R (2016) "Flavonoids: an overview," Journal of Nutritional Science, Cambridge University Press, 5: p. 47.

[7] HOSTETLER, G L; RALSTON, R A; SCHWATRZ, S J (2017), Flavones: food Sources, bioavailability, metabolism, and bioactivity advances in nutrition: An International Review Journal, 8(3):423–435.

[8] CATARINO, M D; ALVES-SİLVA, J M; PEREİRA, O R; CARDOSO S M (2015), Antioxidant Capacities of Flavones and Benefits in Oxidative-Stress Related Diseases, Curr Top Med Chem 15(2):05-19.

[9] SUNG, B; CHUNG, H Y; KİM, N D (2016), Role of apigenin in cancer prevention via the ınduction of apoptosis and autophagy journal of cancer prevention, J Cancer Prev. 21(4):216–226.

[10] BRUNO, A; SİENA L; GERBİNO, S; FERRARO, M; CHANEZ, P; GİAMMANCO, M; PACE, E (2011), Apigenin affects leptin/leptin receptor pathway and induces cell apoptosis in lung adenocarcinoma cell line, European Journal of Cancer, 47(13): 2042–2051.

[11] WANG, M; FİRRMAN, J; LİU, L; YAM, K (2019), A Review on Flavonoid Apigenin: Dietary Intake, ADME, Antimicrobial Effects, and Interactions with Human Gut Microbiota, Biomed Res Int.

[12] SHUKLA, S; GUPTA, S (2010), Apigenin: A Promising molecule for cancer prevention, Pharm Res; 27(6): 962–978.

[13] NOORFAİZA, A; AZİZA B; GABRİELE, R A (2018), Apigenin increases cisplatin inhibitory effects on the telomerase activity of triple negative breast cancer cells 80(1),123–132

[14] MAEDA, Y; TAKAHASHİ, H; NAKAİ, N; YANAGİTA, T; ANDO, N; OKUBO, T; TAKİGUCHİ, S (2018), Apigenin induces apoptosis by suppressing Bcl-xl and Mcl-1 simultaneously via signal transducer and activator of transcription 3 signaling in colon cancer, International Journal of Oncology. (52):1661-1673.

[15] LİU, H; YANG, Z; DENG, W; TANG, Q (2016), Apigenin attenuates the cardiac remodeling in

Cite as: ÖZCAN, F Ö; ALDEMİR, O; KARADEMİR, B (2020), Flavones (Apigenin, Luteolin, Crhysin) and Their Importance for Health, Mellifera, 20(1):16-27.

experimental diabetic cardiomyopathy, Journal of the American College of Cardiology,68(16):10.

[16] ZHANG, B; WANG, J; ZHAO, G (2020), Apigenin protects human melanocytes against oxidative damage by activation of the Nrf2 pathway, Cell Stress and Chaperones 25: 277–285.

[17] LİU, H-J; FAN, Y L; LİAO, H-H.; LİU, Y; CHEN, S; MA, Z-G; TANG, Q-Z (2017), Apigenin alleviates STZ-induced diabetic cardiomyopathy, Molecular and Cellular Biochemistry, 428(1-2): 9–21.

[18] LİANG, H (2017), Antiinflamatuar and neuroprotectıve effect of Apigenin: Studies in the GFAB-IL6 Mouse model of Chronic Neuroınflamatıon, Free Radical Biology and Medicine 108:1-510.

[19] LİU, Y; LİU, X; DU, Y; CHEN, Z; CHEN, H; GUO, J; WENG, X; WANG, X; WANG, M; WANG, Z; WANG L (2017), Combination of apigenin and ischemic postconditioning protects against renal ischemia/reperfusion injury in rat by inhibiting TLR4/NF-κB signaling pathway, Int J Clin Exp Med 10(5):7931-7939.

[20] Lİ, K; Lİ, M; LUO, Z; MAO, Y; YU, Y; HE, Y; CAİ, K (2020), Overcoming the hypoxia-induced drug resistance in liver tumor by the concurrent use of apigenin and paclitaxel, Biochemical and Biophysical Research Communications. 526(2):321-327.

[21] VROHOCAV MADUNIC, I; MADUNIC, J; ANTUNOVIC, M; PARADZICK, M; GARAJ-VRHOVAC, V; BRELİJAK, D; GAJSKİ, G (2018), Apigenin, a dietary flavonoid, induces apoptosis, DNA damage, and oxidative stress in human breast cancer MCF-7 and MDA MB-231 cells, Naunyn-Schmiedeberg's Archives of Pharmacology, 391(5): 537–550.

[22] LEE, Y; SUNG, B; KANG, Y J; KIM, D H; JANG, J Y; HWANG, S Y; KIM, N D (2014), Apigenin-induced apoptosis is enhanced by inhibition of autophagy formation in HCT116 human colon cancer cells, International Journal of Oncology, 44(5): 1599–1606.

[23] CHOİ, W H; JANG, Y J; SON, H J; AHN J; JUNG, C H; HA, T Y (2018), Apigenin inhibits sciatic nerve denervation-induced muscle atrophy, Muscle & Nerve, 58(2): 314–318.

[24] NABAVİ, S F; BRAİDY, N; GORTZİ, O; SOBARZO-SANCHEZ, E; DAGLİA, M; SKALİCHA-WOZNİAK, K; NABAVİ, S M (2015), Luteolin as an anti-inflammatory and neuroprotective agent: A brief review, Brain Research Bulletin, 119: 1–11.

[25] LİN, Y; SHİ, R; WANG X; Shen, H M (2008), Luteolin, a Flavonoid with Potential for Cancer Prevention and Therapy, Current Cancer Drug Targets, 8(7):634-46.

[26] PARK, J-E; KIM, E (2018), Effects of luteolin on chemical induced colon carcinogenesis in high fat diet-fed obese mouse, Journal of Nutrition and Health, 51(1): 14.

[27] Lİ, S; JİANG, H; HAN, B; KONG, T; LV, Y; YANG, Q; WU, P; LV, Z; ZHANG, Z (2020), Dietary luteolin protects against renal anemia in mice, Journal of Functional Foods,65.

[28] LİN, Y; YANG, N; BAO, B; WANG, L; CHEN, J; LİU, J (2020), Luteolin Reduces Fat Storage in Caenorhabditis elegans through Promoting Central Serotonin Pathway, Food & Function, 11: 730-740

[29] LIU, Y; FU, X; LAN, N; Lİ, S; ZHANG, J; WANG, S; ZHANG, L (2014), Luteolin protects against high fat diet-induced cognitive deficits in obesity mice, Behavioural Brain Research, 267:178–188.

[30] WEDLER, J; DAUBITZ, T; SCHLOTTERBECK, G; BUTTERWECK, V (2014), In Vitro Anti-Inflammatory and Wound-Healing Potential of a Phyllostachys edulis Leaf Extract – Identification of Isoorientin as an Active Compound, Planta Medica, 80(18): 1678–1684.

[31] VERSCHOOTEN, L; SMAERS, K; VAN KELST, S; PROBY, C; MAES, D; DECLERQ, L; GARMYN, M (2010), The Flavonoid Luteolin Increases the Resistance of Normal, but Not Malignant Keratinocytes, Against UVB-Induced Apoptosis, Journal of Investigative Dermatology, 130(9): 2277–2285.

[32] KIM, T.-H; JUNG, J W; HA, B. G; HONG, J M.; PARK, E K.; KIM, H-J; KIM, S-Y (2011), The effects of luteolin on osteoclast differentiation, function in vitro and ovariectomy-induced bone loss, The Journal of Nutritional Biochemistry, 22(1): 8–15.

[33] YAN, Q; Lİ, Y; YAN, J; ZHAO, Y; LİU, Y; LİU, S (2018), Effects of luteolin on regulatory proteins and enzymes for myocyte calcium circulation in hypothermic preserved rat heart, Experimental and Therapautıc Medicine, 15(2): 1433–1441.

[34] WANG, H; LUO, Y; QİAO, T; WU, Z; HUANG, Z (2018), Luteolin sensitizes the antitumor effect of cisplatin in drug-resistant ovarian cancer via induction of apoptosis and inhibition of cell migration and invasion, Journal of Ovarian Research, 11(1):93.

[35] THEORASİS, C; IRENE, T (2018), Tetramethoxyluteolin for the Treatment of Neurodegenerative Diseases, Current Topics in Medicinal Chemistry,18(21):1872 – 1882.

Cite as: ÖZCAN, F Ö; ALDEMİR, O; KARADEMİR, B (2020), Flavones (Apigenin, Luteolin, Crhysin) and Their Importance for Health, Mellifera, 20(1):16-27.

[36] KWON, E Y; KIM S Y (2018), MS Choi, Luteolin-Enriched Artichoke Leaf Extract Alleviates the Metabolic Syndrome in Mice with High-Fat Diet-Induced Obesity, Nutrients, 10(8):979.

[37] FAN, W; QIAN, S; QİAN, P; Lİ, X (2016), Antiviral activity of luteolin against Japanese encephalitis virus**,** Virus Res, 15(220):112-6.

[38] BAİ, L; NONG, Y; SHİ, Y; LİU, M; YAN L; SHANG, J; HUANG, F; LIN, Y; TANG, H (2015), Luteolin Inhibits Hepatitis B Virus Replication through Extracellular Signal-Regulated Kinase-Mediated Down-Regulation of Hepatocyte Nuclear Factor 4α Expression, Mol Pharm, 1;13(2):568-77.

[39] ISHİSAKA, M; KAKEFUDA, K; YAMAUCHİ, M; TSURUMA, K; SHİMAZAWA, M; TSURUTA, A; HARA, H (2011), Luteolin shows an antidepressant-like effect via suppressing endoplasmic reticulum stress, Biol Pharm Bull, 34(9):1481-6.

[40] GELABERT-REBATO, M; WİEBE, J C; MARTİN-RİNCON, M; GALVAN-ALVAREZ, V; CURTELİN, D; PEREZ-VALERA; M-Calbet, J A L (2019), Enhancement of Exercise Performance by 48 Hours, and 15-Day Supplementation with Mangiferin and Luteolin in Men, Nutrients, 11(2):344.

[41] KASALA, E R; BODDULURU, L N; MADANA R M; VAK GOGOİ , R; BARUA C C (2015), Chemopreventive and therapeutic potential of chrysin in cancer: mechanistic perspectives, Toxicol Lett. 4;233(2):214-25.

[42] MANİ, R; NATESAN, V (2018), Chrysin: Sources, beneficial pharmacological activities, and molecular mechanism of action, Phytochemistry ,145: 187-196.

[43] BADR, A; EL-DEMERDASH, A M (2014), Chrysin alleviates acute doxorubicin cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis, European Journal of Pharmacology, 728: 107–118.

[44] SHOOSSHTARİ, M K; SARKAKİ, A; MANSOURİ, S M T (2020), Protective effects of Chrysin against memory impairment, cerebral hyperemia and oxidative stress after cerebral hypoperfusion and reperfusion in rats, Metab Brain Dis 35: 401–412.

[45] Lİ HEA X; HUA-WANG, Y; BİA, M G; HUA DU, G (2012), Chrysin improves cognitive deficits and brain damage induced by chronic cerebral hypoperfusion in rats, European Journal of Pharmacology 680(1–3): 41-48.

[46] GAMBELUNGHE, C; ROSSİ, R; SOMMAVİLLA, M; FERRANTİ, C; ROSSİ, R., Ciculi, C., Rufini, S. (2003), Effects of Chrysin on Urinary Testosterone Levels in Human Males, Journal of Medicinal Food, 6(4), 387–390.

[47] OHKURA, N; TAKATA, Y; ANDO, K; KANAİ, S; WATANABE, E; NOHİRA, T; ATSUMİ, G (2012), Propolis and its constituent chrysin inhibit plasminogen activator inhibitor 1 production induced by tumour necrosis factor-α and lipopolysaccharide, Journal of Apicultural Research, 51(2): 179–184.

[48] SHOLİED, S M; ESMAT A; KHALİFA A E; ABDELRAHİM A B (2018), Chrysin attenuates testosterone-induced benign prostate hyperplasia in rats, Food and Chemical Toxicology 111: 650-659.

[49] CIFTCI, O; OZDEMIR, I; AYDIN, M; BEYTUR, A (2012), Beneficial effects of chrysin on the reproductive system of adult male rats, Andrologia ,44(3):181-6.

[50] AMIN, ALTAWASH, A S; SHAHHEK, A Z; MORAVEJ, H; ANSARİ, M (2017), Chrysin-induced sperm parameters and fatty acid profile changes improve reproductive performance of roosters, Theriogenology, 104:72–79.

[51] CHOİ, J K; JANG, Y H; LEE, S; LEE, S-R; CHOİ, Y-A; JİN, M; KIM, S-H (2017), Chrysin attenuates atopic dermatitis by suppressing inflammation of keratinocytes, Food and Chemical Toxicology, 110:142–150.

[52] RAMİREZ-ESPİNOSA, J; SALDANA-RIOS, J; GARCİA-JİMENEZ, S; VİLLALOBOS-MOLİNA, R, ÁVİLA-VİLLAREAH, G; RODRIGUEZ-OCAMPO, A; ESTRADA-SOTO, S (2017), Chrysin Induces Antidiabetic, Antidyslipidemic and Anti-Inflammatory Effects in Athymic Nude Diabetic Mice, Molecules, 23(1):67.

[53] ANDRADE, N; ANDRADE, S; SİLVA, C (2020), Chronic consumption of the dietary polyphenol chrysin attenuates metabolic disease in fructose-fed rats, Eur J Nutr 59:151–165.

[54] HUANG, C-S; Lİİ, C K; LİN, A-H; YEH, Y W; YAO, H-T; Lİ, C-C; CHEN, H-W (2012), Protection by chrysin, apigenin, and luteolin against oxidative stress is mediated by the Nrf2-dependent up-regulation of heme oxygenase 1 and glutamate cysteine ligase in rat primary hepatocytes, Archives of Toxicology, 87(1):167–178.

[55] WU, Y X; FANG, X (2009), Apigenin, Chrysin, and Luteolin Selectively Inhibit Chymotrypsin-Like and Trypsin-Like Proteasome Catalytic Activities in Tumor Cells, Planta Medica, 76(02):128–132.

Cite as: ÖZCAN, F Ö; ALDEMİR, O; KARADEMİR, B (2020), Flavones (Apigenin, Luteolin, Crhysin) and Their Importance for Health, Mellifera, 20(1):16-27.

MELLIFERA RESEARCH ARTICLE

Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane

Nilgun DOĞAN[1*], Hakan ADANACIOĞLU[2], Gamze SANER[2], Ciğdem TAKMA[3]

[1] Gumushane University, Aydın Dogan Vocational School, Kelkit Gumushane, TURKEY

[2]Ege University, Faculty of Agriculture, Department of Agricultural Economics, Izmir, TURKEY

[3]Ege University, Faculty of Agriculture, Department of Animal Science, Izmir, TURKEY

[*]*Corresponding author e-mail: nilgundogan@gumushane.edu.tr*

Received: 28th March, 2020; accepted: 4th May, 2020; published: 29th June, 2020

A B S T R A C T

The aim of this study is to determine the effects of socio-economic factors on the profitability of the beekeeping enterprises in the province of Gümüşhane in Turkey. The relationship between gross profit and some socio-economic characteristics were investigated, and the effects of socio-economic factors on profitability were analyzed by the decision tree method. The results showed that the socio-economic factors affecting the gross profit of beekeepers were the non-beekeeping income, the production of the other bee products except honey, the beekeeping experience, the number of the hives and the years of education. Additionally, if the beekeeping is performed as a second source of income and with more experience, more education and working with fewer beehives will produce positive results on profitability. For producers who did not have any other income, other bee products provided more gross margin per hive. Therefore, other bee products besides the honey production would increase their profitability. The low amount of the other bee products such as propolis, royal jelly, bee pollen, bee bread (perga), apilarnil, bee venom, etc. were result from some socio-economic factors that had been identified in the research area and lack of adequate training. Interventions should aim at trainings that overcome production, management practices and marketing constraints in the value chain.

Keywords: Beekeeping, decision tree, profitability, socio-economic factors, Turkey.

Introduction

Beekeeping is a branch of production that can be carried out with other agricultural activities in rural areas, and it is also one of the most important agricultural activities because of the importance of bee products in the human diet, their use in the pharmaceutical, traditional medicine usage in treatment and the role of bees in

Cite as: DOĞAN, N; ADANACIOĞLU, H; SANER, G; TAKMA, Ç (2020), Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane, Mellifera, 20(1):28-40.

improving product quality in crop production.

According to FAO's data on the number of bee colonies, with 9 million 148 thousand colonies, China is in the first place, while the second place is occupied by Turkey with 8 million 331 thousand colonies. China takes part in the first place in the world in terms of the number of colonies with 502 thousand tons in honey production, and it is followed by Turkey (114 thousand tons), the United States (73 thousand tons) and Russia (69 thousand tons) [8]. Although Turkey, both in the number of hives and in the production of honey in the world, comes after China, the value of its exports remains relatively low in comparison to other countries.

Although beekeeping may be practiced almost anywhere around the country, the honey yield per hive is still low in Turkey. According to the 2018 data from Republic of Turkey Ministry of Agriculture and Forestry [21], the yield is only 14 kg per hive in Turkey. Despite the increase in the number of beehives over the years in Turkey, the yield per hive has decreased. The yield per hive decreased from 17 kg in 2005 to 13 kg in 2016 [30]. Productivity is closely related to the production technique applied in beekeeping. Due to the lack of technical methods, serious financial losses occur in beekeeping. Achieving technical beekeeping increases the economic value of the activity of beekeeping and ensures that it becomes profitable for the beekeeper.

In the world and Turkey, several studies examining the economic aspects of beekeeping have been carried out so far. Beekeeping techniques in various provinces of Turkey have aimed at solving economic problems, and there are many studies about the significance of beekeeping [2, 5, 6, 7, 10, 11, 13, 14, 17, 20, 25, 26, 27, 28-31].

Despite the significance of the beekeeping enterprises, there was no any empirical evidence on potentials and challenges of the beekeeping enterprises in the study area. Republic of Turkey Ministry of Agriculture and Forestry is currently focusing attention on how to increase agricultural production with providing employment opportunities for the local people in rural areas. So, production of the honey and the other bee products in Gümüşhane is important for the national honey market, and this affects a profitable enterprise in this context.

Cite as: DOĞAN, N; ADANACIOĞLU, H; SANER, G; TAKMA, Ç (2020), Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane, Mellifera, 20(1):28-40.

The aim of this study is to determine the effects of socio-economic factors on the profitability of beekeeping farms in the province of Gümüşhane and the relationship between gross profit and some socio-economic characteristics by Decision Tree-CRT algorithm. The socio-economic factors that have an effect on profitability will be identified in this study, and this information will fill the gap in the literature. Furthermore, introducing the optimum type of beekeeping enterprise in Gümüşhane will be a guide for decision-makers and beekeeping enterprises that need such information.

Research questions

a.What are the socio-economic characteristics of the beekeepers?

b.What are the production characteristics of the beekeeping enterprises?

c.What is the contribution of beekeeping to beekeepers' household income?

d.What is the contribution of beekeeping enterprises to poverty alleviation?

Research hypothesis

i.There is no significant relationship between selected socio-economic characteristics and poverty status.

ii.There is no significant relationship between beekeeping enterprises' production characteristics and present status

iii.There is no significant relationship between the contribution of beekeeping enterprises and poverty status.

Materials and Methods

The main material of this study was obtained from a survey conducted with beekeeping enterprises. The secondary sources of the study were previous national and international studies and research reports.

Gümüşhane was chosen as the research area where the survey was conducted. There were about 41 thousand hives and approximately 615 tons of honey production in 2018 [21]. When the number of hives and honey production in the province of Gümüşhane were analyzed based on the district, the Kelkit district had the largest share of production in the province with approximately 17 thousand hives and 441 tons of honey production (Table 1). Therefore, the Kelkit district was included in this study.

Cite as: DOĞAN, N; ADANACIOĞLU, H; SANER, G; TAKMA, Ç (2020), Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane, Mellifera, 20(1):28-40.

Although beekeeping was common in the Kelkit district of Gümüşhane, where the survey was conducted, healthy data on the number of hives could not be obtained. For this reason, it was found appropriate to use proportional sampling method in the study. In addition, the fact that this study was carried out with the own financial resources of the researchers and that there was a time constraint in choosing this method.

The sample size was calculated by using the proportional sampling method. In terms of this method, the sample according to the known or predicted ratio (p) of the population size N is given below [22].

$$n = \frac{Np(1-p)}{(N-1)\sigma^2_{\hat{p}_x} + p(1-p)}$$

n= Sample size

N= Number of beekeepers in the Kelkit district

p= Proportion of beekeepers on an adequate level (0.50 for maximum sample volume)

$\sigma^2{}_{px}$ = Variance of rate

There were 110 registered beekeepers in the Kelkit district in the Bee Registration System (BRS) of the Ministry of Agriculture and Forestry. Beekeepers in the Beekeepers' Association, producers who were not in BRS, beekeepers with fewer than 30 hives and beekeepers who came to Kelkit from outside (migratory) were also included in the study, and as a result, the population size was calculated as 190 producers. According to the proportional sampling method, the sample size was calculated as 60 with a 90% confidence interval and a 10.5% error rate The beekeeping enterprises surveyed were selected randomly.In this study, the effects of socio-economic factors on the profitability of the enterprises were analyzed by the decision tree method in this study. The explanatory variables were: the level of education of the producer, age, the beekeeping experience, non-beekeeping income, the size of the producer's household, the type of beekeeping production, bee breeds, number of hives, status, use of consultancy and production of other bee products except honey. Gross profit per hive was used as the dependent (continuous) variable (Table 2).

The gross margin for a beekeeping enterprise is one measure of profitability that is useful for enterprise planning.

Cite as: DOĞAN, N; ADANACIOĞLU, H; SANER, G; TAKMA, Ç (2020), Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane, Mellifera, 20(1):28-40.

Calculation of gross margins may be the starting point for construction of cash flow budgets and assessment of the whole farm's profitability. Gross margin profit is the difference between the annual gross income for that enterprise and the variable costs directly associated with the enterprise [9].

The Classification and Regression Trees (CRT) algorithm is used to construct decision trees. A decision tree is a classification method consisting of decision nodes and leaf nodes in the form of a tree structure. A decision tree algorithm develops a dataset consisting of categorical and/or numerical data by dividing it into small pieces. In a decision tree, the first node is called the root node, and the other branches are called the decision nodes. A decision node may include one or more branches. According to the contributions of the independent variables in classification of the dependent variable, child nodes are formed. Various algorithms are used to construct the tree. The CRT (Classification and Regression Tree) algorithm is widely used among these algorithms that have been developed. In the CRT algorithm, the contribution of the independent variables to classification of the dependent variable is determined by their importance [3].

Results and Discussion

Socio-Economic Characteristics of Beekeeping Enterprises

The socio-economic characteristics of the beekeeping enterprises were given in Table 3. The average age of the beekeepers was 52 years, their mean years of education were 8.5 years and the period of beekeeping experience was 19 years. This age result explained that beekeeping was maintained by an older generation and did not attract young people enough in Kelkit area. A similar result on the age factor was obtained in the beekeeping study of Affognon et al. [1]. The average age of beekeepers was found as 51. Makri et al. [18] found the mean year of education of the beekeepers was 10 years, and the beekeeper age changed from 40 to 50 years. In the study of Öztürk [26], the average period of education of beekeepers were found to be only 5.35 years that was the lower finding from this study.

Approximately 42% of the beekeeping enterprises (25 enterprises) took part in the

Cite as: DOĞAN, N; ADANACIOĞLU, H; SANER, G; TAKMA, Ç (2020), Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane, Mellifera, 20(1):28-40.

animal breeding or the crop production other than the beekeeping, the period of their average agricultural experience was 25 years, and the average period of the beekeeping experience was found as 19 years. This average beekeeping experience value was less than 21 years determined by Ceyhan and Canan [35]. On the other hand, in the study performed by Kalanzi et al. [12], 56.3% of the surveyed beekeepers had less than 10 years of beekeeping experience. In this study, the average household size was found as 4 people. However, in the study that was published by Mbah [19]on the topic of the profitability of honey production, the average size of the households was found as 12 persons.

The average number of hives per farm was 146. The mean sales value obtained from bee products in the production period was calculated as US $13 930.

The majority of the beekeeping enterprises (66.70%) did not produce other bee products. Only 33.30% of the investigated enterprises produced 1 to 2 other bee products including honey (Table 4). Similar result was obtained in Kebede and Tadesse's [15] study, and the beekeepers (86.4%) reported that they did not produce any bee products apart from honey.

The interviewed producers (66.70%) stated that they did beekeeping as additional activity. On the other hand in the study by Okpokiri et al. [23], 70% of the beekeepers who participated in the survey reported that they took part in honey production as their main source of livelihood. To the study of Ceyhan and Canan [35]; 64% of Turkish beekeepers do the beekeeping as the main source of income. But this result was obtained different in this study. The main reasons for the beekeeping as a second job by the majority of Kelkit beekeepers were that it was easier to produce in comparison to other production activities (crop and animal), they aimed to provide the employment opportunities for the family members, and it was seen as a profitable activity. When we considered the mean age of the beekeepers in the research area, this finding was an expected result. As a result, it was understood that the training activities could be carried out continuously in order to encourage the beekeeping to the target group of the young or middle age groups.

In order to ensure the economic feasibility of beekeeping, it was necessary to defuse

Cite as: DOĞAN, N; ADANACIOĞLU, H; SANER, G; TAKMA, Ç (2020), Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane, Mellifera, 20(1):28-40.

the missing technical knowledge of the producers about this production activity. In this context, it was important that beekeepers receive basic training in apiculture and seek consultancy from experts during their activities. The findings obtained from this study showed that the level of technical knowledge about beekeeping of the interviewed beekeepers was generally good. As a matter of fact, 83.30% of the beekeepers stated that they participated in a course or a training program on beekeeping in the past. The percentage of the beekeepers receiving consultancy or assistance to obtain technical information on beekeeping was 43.30% at present. However, Kebede and Tadesse [15] showed that the most important problem faced by beekeepers was lack of adequate training on beekeeping.

According to the results, 78.30% of the interviewed producers were members of the Beekeepers Association as it is shown in Table 4. This was a positive result that shows that the producers depended on producer organizations.

Gross Margin Analysis

The variable costs of the beekeeping enterprises were firstly determined in this section. The variable costs associated with honey production per colony were given in Table 5.

The total variable costs included subsequently feed costs (sugar and cake), medication (parasite and disease control), wax foundation, transportation of hives, labor, location rental fees, and packaging of honey, repairs and maintenance, interest on variable costs. The total variable cost per hive was determined US$69.14. Labor cost and feed cost were identified as the significant cost items among the variable costs in this study. In a similar study conducted by Vaziritabar and Esmaeilzade [32] on the profitability of apiculture in the Karaj region of Iran, the variable cost per hive was found as about US$60.10. Variable costs were obtained as US$18.53 per hive in the study by Aydın et al. [34] and as US$94.25 in the study by Adanacıoğlu et al. [33]. These results showed that the beekeeping enterprises' operating costs was higher in the study area. According to these results in order to increase the economic performance of the beekeeping enterprises, the feed and the labor cost had to be reduced.

Cite as: DOĞAN, N; ADANACIOĞLU, H; SANER, G; TAKMA, Ç (2020), Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane, Mellifera, 20(1):28-40.

The gross revenue and variable costs associated with honey production are given in Table 6. While the gross revenue per hive was US$124.22, the total variable costs per hive was calculated as US$69.14 in the beekeeping enterprises. Therefore, the gross margin was calculated to be US$55.08 per hive.

Analysis of The Effects of Socio-Economic Factors on The Profitability

In this section, the effects of socio-economic variables on the gross profit obtained by beekeeping enterprises were shown by the decision tree method. In this context, the effects of the producer's education years, age, beekeeping experience, non-beekeeping income, the size of the producer's household, the type of beekeeping, the bee breed used in production, the number of hives, the type of honey produced, the status of receiving training on beekeeping and the effects of the production of other bee products except honey on the gross profit were analyzed. As a result of the CRT algorithm that was used, the non-beekeeping income of the beekeeper, the beekeeping experience of the producer, the production of other bee products except honey and the number of hives were found to be more effective than the other factors. Whereas among the evaluated predictors, only two ones "age of enterprise" and "non-beekeeping income" were effective in the study of Aksoy et al. [2].

The non-beekeeping income of the beekeepers was found to be the most effective. The mean gross profit per hive for the producers who had non-beekeeping income was found to be higher (Node 1= US$58.28 (280.54 TL) than the producers who did not have non-beekeeping income (Node 2= US$23.92 (115.17 TL). On the other hand, it was seen that beekeeping experience was important for beekeepers with non-beekeeping income.

According to a single beekeeper with less than 1.5 years of experience in beekeeping, the experience variable was subdivided into sub-categories, and the gross profit was found to be lower among the beekeepers with little experience. The gross profit of the producer was found as US$61.05 (293.86 TL) (Node 4). According to the results of Kutlu [17] on determination of socio-demographic and economic factors that affect honey production, an increase in the beekeeping experience of beekeepers

Cite as: DOĞAN, N; ADANACIOĞLU, H; SANER, G; TAKMA, Ç (2020), Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane, Mellifera, 20(1):28-40.

had a positive effect on honey production. The same finding was reached in the study by Onuç et al. [24]. They found that the professional experience of the beekeeper was an important factor. In our study, in addition to honey, production of other bee products was found to be a significant factor for the producer. The mean gross profit per hive for the producers who produced other bee products was US$53.99 (259.89 TL) (Node 6), while the mean was US$ 4.60 (22.14 TL) (Node 5) for the producers who did not.

As another variable, the number of hives was found to be effective on the producers with more beekeeping experience. The gross profit for the producers with less than 98 hives was US$ 70.59 (339.77 TL) (Node 7), and for those with more than 98, this was US$ 36.25 (174.50 TL) (Node 8). The years of education was an effective factor for the producers with a low number of hives. The producers with more education years had more gross profit per hive. The gross profit for producers who had more than 5 years of education was US$ 129.16 (621.67 TL) (Node 10), whereas, for those who had less than 5 years of education, it was US$ 62.95 (303 TL) (Node 9) (Fig. 1 Tab. 7).

The factors that affected gross profit per hive and their importance values were shown in Table 8 and Fig. 2 (importance and normalized importance values of the independent variables). Among these factors, the non-beekeeping income of the producer was determined as the first and 100% effective factor on gross profit. In a similar study by Aksoy et al. [2], the age indicator was a 100% effective factor. Production of other bee products than honey (79.8%), the producer's beekeeping experience (76.7%), number of hives (75.2%), the producer's education years (69.5%), the producer's age (34.8%), the size of household (18.6%), beekeeping type (15.6%) and honey type (13.6%) followed these. However, the variables on the producer's age, size of household, beekeeping type and honey type were not included in the decision tree diagram

The results showed that the socio-economic factors affecting the gross profit of the beekeeping farms were the income of other bee products except honey, the beekeeping experience of the beekeeper, the number of hives and the education year.

In the regression tree analysis, it was found that the beekeepers who had non-

Cite as: DOĞAN, N; ADANACIOĞLU, H; SANER, G; TAKMA, Ç (2020), Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane, Mellifera, 20(1):28-40.

beekeeping income had more gross profit per hive than the beekeepers who did not. However, it was determined that the beekeepers who had non-beekeeping income, those with fewer hives, and those with high education levels made higher gross profits. The results of this study showed that, if beekeeping was performed as a second job, it was expected that more experience, high education and fewer beehives would have positive results on profitability. In this situation, we might state that, as the activity of beekeeping was carried out as a second job by the majority of beekeeping enterprises, a high number of beehives would limit the effective management of the hives.

According to the findings, non-beekeeping income beekeepers who produced other bee products than honey had more gross profit per hive. Unfortunately, other bee products than honey were not widely known. There are many products such as propolis, royal jelly, bee pollen, bee bread (perga), apilarnil, and bee venom. All these products may be used effectively in the world in apitherapy and alternative medicine. Without doubt, all the bee products could be used effectively to make more profit by the beekeeping enterprises in the research area. Therefore, production of the other bee products as a side activity to the primary honey production in Gümüşhane would increase the profitability of the beekeeping enterprises.

Conclusion

In conclusion, despite having adequate advantages such as the natural resources, the gross profit, and the yield in the research area, the number of beekeeping enterprises are still low. This is due to some insufficient management practices and lack of adequate training. In this context, aiming to improve the beekeeping management and the increasing profitability through identifying the socio-economic factors, providing the training courses, improving the marketing bee products except honey will be very vital to all the governmental and non-governmental organizations. Organizations are essential areas of intervention to utilize the management practices and the training.

Beekeeping in Kelkit area should be promoted to improve the employment and as a main income with the young/middle aged local people. Additionally, further

Cite as: DOĞAN, N; ADANACIOĞLU, H; SANER, G; TAKMA, Ç (2020), Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane, Mellifera, 20(1):28-40.

study need to be conducted for improving the technical efficiency of the beekeeping enterprises.

Türkiye'de Arıcılık İşletmelerinin Karlılığına İlişkin Sosyo-Ekonomik Belirleyiciler: Gümüşhane'nin Kelkit İlçesinde Uygulamalı Bir Çalışma

Öz: Bu çalışmanın amacı, Gümüşhane ilinde arıcılık işletmelerinin karlılığına etki eden sosyo-ekonomik faktörleri belirlemektir. Brüt kar ile bazı sosyo-ekonomik özellikler arasındaki ilişki araştırılmış ve sosyo-ekonomik faktörlerin karlılık üzerindeki etkileri karar ağacı yöntemi ile analiz edilmiştir. Elde edilen sonuçlar göstermiştir ki, arıcıların brüt kârını etkileyen sosyo-ekonomik faktörler sırasıyla arıcılık dışı gelir, bal hariç diğer arı ürünlerinin üretimi, arıcılık deneyimi, kovan sayısı ve eğitim yılıdır. Ayrıca, yüksek arıcılık deneyimi, yüksek eğitim seviyesi, az kovan sayısı ve arıcılığın ek gelir olarak yapılması faktörlerinin arıcılık işletmelerinin karlılığını olumlu etkileyeceği saptanmıştır.

Bal hariç propolis, arı sütü, arı poleni, arı ekmeği (perga), apilarnil, arı zehiri,...gibi diğer arı ürünlerinin düşük miktarda üretilmesinin nedeni, araştırma alanında tespit edilen bazı sosyo-ekonomik faktörlerden ve yetersiz eğitim etkinliklerinden kaynaklandığı anlaşılmaktadır. Bundan dolayı, bal üretim değer zincirinde yapılması gereken müdahaleler; üretim, yönetim uygulamaları ve pazarlama kısıtlamalarının üstesinden gelecek şekilde hedeflenmelidir.

Anahtar Kelimeler: Arıcılık, Karlılık, Karar ağacı, Sosyo-ekonomik faktörler, Türkiye

R E F E R E N C E S

[1] AFFOGNON, H; KINGORI, W S; OMONDI, A I; et al (2015), Adoption of Modern Beekeeping and Its Impact on Honey Production in the Former Mwingi District of Kenya. Inter J of Tropical Insect Sci., 35: 96-102.

[2] AKSOY, A; ERTÜRK, Y E; EYTURAN, E; ERDOĞAN, S; TARIQ, M M (2018), Estimation of Honey Production in Beekeeping Enterprises from Eastern Part of Turkey Through Some Data Mining Algorithms. Pakistan J of Zoo., 50: 2200-2207.

[3] BREIMAN, L; FRIEDMAN, J; OLSHEN, R; STONE, C (1984), Classification and regression trees. Chapman and Hall. Wadsworth Inc.; New York pp.216-228.

[4]BÜMKO **(**2018), Average Exchange Rates. Available at http://www.bumko.gov.tr/TR,147/ekonomik-gostergeler.html. (20.02.2019)

[5] ÇEVRİMLİ, M B; SAKARYA, E (2018), Structural Characteristics and Problems of Beekeeping Enterprises in Aegean Region Example. Eurasian Journal of Veterinary Sciences, 34: 83-91, 2018.

[6] DEMEN, H; KARACAOĞLU, M; KOÇ, A (2015**),** Beekeeping structure and problems in Diyarbakir province. Available from http://dergi.adu.edu.tr//tralleis, (10.10.2018) [in Turkish with English abstract]

[7] ENGİNDENİZ, S.; UÇAR, K.; BAŞARAN, C. 2014 Economic Aspects and Problems of Beekeeping in Izmir

Cite as: DOĞAN, N; ADANACIOĞLU, H; SANER, G; TAKMA, Ç (2020), Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane, Mellifera, 20(1):28-40.

Province. J of Agric. Econ., 20: 113-120 [in Turkish with English abstract]

[8] FAO (Food and agriculture organization of the United Nations). Retrieved from: http://www.fao.org/countryprofiles/en/ (14.12.2018)

[9] GRDC: Farm Gross Margin and Enterprise Planning Guide (2018), Available at https://grdc.com.au/data/assets/pdf_file/0038/294959/Farm-Gross-Margin-and- İnterprise-Planning-Guide-2018.pdf. (Accessed Nov 10, 2018)

[10] GÜRER, B; AKYOL, E (2018), An Empirical Analysis of Technical Efficiency Determinants in Beekeeping Farms: Evidence and Policy Implications from Nigde, Province, Turkey. J of Agric. Environ for Intern Develop., 112: 343-359. [in Turkish with English abstract]

[11] KADİRHANOĞULLARI, İH; KARADAŞ, K; KÜLEKÇİ, M. (2016), Socio-Economic Status of Beekeepers in Iğdır Province. Bee J., 16: 2-11. [in Turkish with English abstract]

[12] KALANZİ, F; NANSEREKO, S; BUYİNZA, J; KIWUSO, P; TURINAVO, Y. et al (2015), Socio-Economic Analysis of Beekeeping Enterprise in Communities Adjacent to Kalinzu Forest, Western Uganda. International Journal of Research on Land-Use Sustainability, 2: 81-90. ISSN:2200-5978.

[13] KARLIDAĞ, S; KÖSEMAN, S (2015), The Role and Importance of Beekeeping in Turkey and Malatya. Ministry of Food, Agric. Econ and Livestock J., 28-40. [in Turkish with English abstract]

[14] KARAKAYA, E; KIZILOĞLU, S (2015), Honey Production in Bingöl. Igdır Univ. Sci&Tech., 5:25-31. [in Turkish with English abstract]

[15] KEBEDE, H; TADESSE, G (2014), Survey on Honey Production System, Challenges and Opportunities in Selected Areas of Hadya Zone Ethiopia. J Agric. Biotech Sustainable Development, 6: 60-66.

[16] KEKEÇOĞLU, M; RASGELE, P; ACAR, F; Kaya, S (2014), Structure of Beekeeping in Düzce Province and a Research on Beekeeping Activities. Düzce Univ. Sci. and Tech J., 2: 1-15. [in Turkish with English abstract]

[17] KUTLU, MA (2014), Determination of Beekeeping in Gaziantep Province, Problems and Solutions. Turkish J of Agricultural and Natural Sci., 1: 481-484.[in Turkish with English abstract]

[18] MAKRI, P; PAPANAGİOTOU, P; PAPANAGİOTOU, E (2014), Efficiency and Economic Analysis of Greek Beekeeping Farms. Bulgarian Journal of Agricultural Science, 21: 479-484.

[19] MBAH, S O (2012), Profitability of Honey Production Enterprise in Umuahia Agric. Zone of Abia State Nigeria. J of Agric and Rural Development, 15: 1268-1274.

[20] MERDAN, K (2017), Economic and Social Aspects of Beekeeping in Gumushane. Gumushane Univ. Social Sci. Institute J., 8: 184-202. [in Turkish with English abstract]

[21] MINISTRY of AGRICULTURE and FORESTRY DATA Available from https://www.tarimorman.gov.tr/sgb/Belgeler/SagMenuVeriler (05.12.2018)

[22] NEWBOLD, P. (1995),: Statistics for business and economics. England: Prentice Hall International Editions.

[23] OKPOKIRI, C I; NWACHUKWU, I N; ONWUSIRIBE, C N (2015), Determinants and Profitability of Honey Production in Ikwuano Local Government Area, Abia State, Nigeria. Scientific Papers Series Management, Economic Engineering in Agric Rural Development, 15:211-216.

[24] ONUÇ, Z; YANAR, A; SANER, G; GÜLER, D (2018), An Analysis on Economical Aspect of the Beekeeping Enterprise: A Case of Kemalpasa District-Izmir/Turkey.[in Turkish with English abstract]

[25] ÖZBAKIR, G; DOĞAN, Z; ÖZTOKMAK, A (2016), Examination of Beekeeping Activities in Adıyaman Province. Harran Agric. and Food Sci. J., 20: 119-126. [in Turkish with English abstract]

[26] ÖZTÜRK, A İ (2017), Determination of Some Technical Properties of Beekeeping in Ula District of Mugla Province. Animal Production, 58: 52-57. [in Turkish with English abstract]

[27] SEZGİN, A; KARA, M (2011), A Research on Determining the Factors Effecting Yield Increase in Beekeeping: The Example of TRA2 Region. J Agric. Faculty, 15: 31-38. [in Turkish with English abstract]

[28] SIRALI, R (2017), Major Problems of Ordu Apiculture and Solutions. Beekeeping J., 17: 35-43. [in Turkish with English abstract]

[29] STEINBERG, D.; COLLA, P. CART–Classification and regression trees. Salford Systems. San Diego: California, 1997.

[30] TURKSTAT. (Turkey Statistic Institution) Database (2017),. Available at from

Cite as: DOĞAN, N; ADANACIOĞLU, H; SANER, G; TAKMA, Ç (2020), Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane, Mellifera, 20(1):28-40.

http://www.turkstat.gov.tr/PreTablo. (Accessed November 20, 2018)

[31] UZUNDUMLU, A S; AKSOY, A; IŞIK, H B (2011), The Existing Structure and Fundamental Problems in Beekeeping enterprises; A Case Bingöl. J Agric. Faculty of Ataturk Univ., 42: 49-55. [in Turkish with English abstract]

[32] VAZIRITABAR, S; ESMAEILZADE, S (2016), Profitability and Socio-Economic Analysis of Beekeeping and Honey Production in Karaj State Iran. J Entomology and Zoo Studies, 4: 1341-1350.

[33] ADANACIOĞLU, H; KOSOĞLU, M; SANER, G; TOPAL, E; YÜCEL, B (2019), Economic Feasibility of Package Beekeeping Application in Turkey: A Case Study of Edirne Province. Kafkas Univ Vet Fak Derg. 25(5): 651-658.

[34] AYDIN, B; AKTÜRK, D; ARSOY, D (2020), Economic and Efficiency Analysis of Beekeeping Activity in Turkey: Case of Çanakkale Province. Ankara Üniv Vet Fak Derg, 67, 23-32.

[35] CEYHAN, V; CANAN, S (2017), Economic Performance of Beekeepers under Different Colony Management System in Turkey. Turkish Journal of Agricultural and Natural Sciences. 4(4): 516-522. [in Turkish with English abstract]

Cite as: DOĞAN, N; ADANACIOĞLU, H; SANER, G; TAKMA, Ç (2020), Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane, Mellifera, 20(1):28-40.

MELLIFERA RESEARCH ARTICLE

Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey

Yusuf ÇAKIR[1], Duygu Nur ÇOBANOĞLU[2,3], Gökhan DERVIŞOĞLU[4], Serhat KOÇYIĞIT[2], Davut KARAHAN[2*], Sedat YELKOVAN[2]

[1]Department of Dairy Technology, Faculty of Agriculture, Bingöl University, Bingöl, TURKEY

[2]Pilot University Coordination Center Unit, Bingöl University, Bingöl, TURKEY

[3]Department of Crop and Animal Production, Vocational School of Technical Sciences, Bingöl University, Bingöl, TURKEY

[4]Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Bingöl University, Bingöl, TURKEY.

[*]*Corresponding author e-mail: dkarahan@bingol.edu.tr*

Received: 14th June, 2020; accepted: 19th June, 2020; published: 29th June, 2020

A B S T R A C T

Honey has been used in traditional medicine since ancient times. Many parameters such as botanical, geological, climatic features constitute the characteristic features of honey. For this reason, it is important to reveal the chemical, palynological and antibacterial properties of honey in regional studies with honey. In this study, antimicrobial, palynological and chemical properties of some honey samples produced in Turkey were determined. The effects of honey samples of Rize (Anzer), Gümüşhane and Sivas (Zara) provinces on some pathogens were investigated via antimicrobial test. According to this test, all of the honey samples was effective *Staphylococcus aureus and Saccharomyces cerevisiae*, only honey from Anzer region was effective on *Escherichia coli*, and none of the honey samples showed activity on *Listeria monocytogenes* and *Candida albicans*. In the palynological analysis, 36 pollen taxa were determined. Chemical compositions of honey samples were determined by GC-MS analysis. GC-MS results showed all of the honey samples had antibacterial and antioxidant properties. The structure types of honey samples were determined by FTIR analysis and chemical bond types in FTIR correlated with the chemical compositions of GC-MS results.

Keywords: Palynological characteristics; chemical composition; honey; antimicrobial activity

Introduction

Honey is a natural sweet substance that is collected by the honey bees (*Apis mellifera*) from nectars of plants, living parts of plants secretions or excretions of insects on the

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

living parts of plants and mixed with their own substances and dehydrated and matured [1]. Honey is a natural product that varied structure affected by like botanical and geographical source factors, intensity of nectar flow, climatological situations beekeepers management, packing process, storage time and the storage conditions [2]. Although it varies depending on these factors, honey is mainly composed of around 81% carbohydrates (31% glucose, 38% fructose and 12% from other carbohydrates like maltose and sucrose), 17% water and 1–2% of other enzymes/compounds. These 1-2% other substances are very significant contributors to the bioactive characteristics of the honey such as antimicrobial activity, antioxidant activity and determines variability of honey [3, 4]. Honey is widely known for its wound-healing properties and has been used since ancient times. One of the most widely known positive effects is that it has antibacterial properties [3, 5]. As a result of the spread of antibiotics, the clinical use of honey has almost been abandoned in modern medicine, and therefore the use of large amounts of antibiotics recently has resulted in the formation of common resistant bacteria. However, the increase in the spread of antibiotic-resistant bacteria, the antimicrobial activity of honey is increasingly valued and is still used as a clinical application in many cultures [5]. Factors of the comprehensive spectrum antimicrobial activity of honey are very high in nature. The most well-known causes of honey antimicrobial activity are high levels of osmolarity - ~ 80% (w / v) of solids, naturally low pH, hydrogen peroxide and the presence of phenolic acids, flavonoids and other substances from some flower sources. [4, 6, 7]. The strong antimicrobial effect of honey against bacteria that are resistant to antibiotics and its positive results in the treatment of wound infections that do not heal with long-term antibiotic treatment have attracted considerable attention [5, 8]. Honey acts in a broad spectrum against antibiotic-resistant and biofilm-forming bacteria in different environments [3, 9]. Abd-El Aal et al. stated that honey exhibited a more appeared inhibition effect (approximate 85%) on bacteria and especially on Gram negative bacteria compared to commonly used antimicrobial agents. Moreover, they stated that honey shows a synergistic effect in combination with other antimicrobial agents in both Gram negative and Gram

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

positive bacteria. For example, it has been reported that 100% inhibition against Gram positive bacteria such as methicillin-resistant *S. aureus*, compared to the use of honey with other antimicrobial agents and antibiotic alone [7]. Al Somal et al. stated that Manuka Honey has a preventive effect on *Helicobacter pylori* growth [10]. In the study by some researchers it was found that honey is very effective against various clinical bacterial isolates and increases the effect of existing antibiotics as a result of synergistic effect when applied with antibiotic discs [7, 11]. Honey has also been reported by Molan, to have antifungal activity and so can have possible as a local antifungal agent [12]. In Turkey, Kahramanmaraş honey samples proved the most effective inhibitors against *Bacillus megaterium* and *S. aureus*, also honey samples showed significant antifungal effects on *C. albicans* [13]. The honey samples from Western Turkey were more effective antimicrobial *against Baccilus megaterium*, *Baccilus subtilis*, *C. albicans* [14].

Pollen analysis in honey have a great importance in controlling the quality of honey. Honey is a natural product that contains the pollen grains of the plant species, nectar and secretions collected by honey bees, and honeydew elements such as algae and fungal spores. Therefore, pollen analysis is the most effective method used to determine the botanical source of honey [15].

The aim of the present study was to determine and compare the antimicrobial activity, chemical composition (by GC/MS), structure types/vibrations (by FTIR) and palynological properties of honey samples from different localities in Turkey.

Materials and Methods

Materials

Honey samples was procured through a beekeeper were from Anzer-Rize (H1), Gümüşhane (H2), Zara-Sivas (H3) Regions, Turkey. Ethyl alcohol (~96% v/v), which was used as a solvent in extraction and was produced from agricultural products was purchased from Alkomed Kimya Ltd. Sti., Turkey. Sarthorius BP210S branded analytical balance was used for the weighing of honey samples in

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

the preparation of the extraction. The extraction was mixed with the help of magnetic fish on the Heidolph MR Hei-Standard branded magnetic stirrer.

Preparation of Honey Extractions

Honey samples was planned to be taken in solution at the rate of 10% (m / m). In other words, 5 g honey sample was added to 50 mL ethanol-water solution (50% v/v) sample and was extracted by mixing during 4 hours with magnetic stirrer at room temperature. In this extraction method, most of the ingredients in honey samples were provided to pass into alcohol. The mixture was filtered on Whatman cellulose filter paper (no:2, diameter: 125 mm) and then the remaining mass on the filter was discarded.

Palynological analysis method

Sorkun method was used for qualitative pollen analysis of honey samples [17]. 20 mL pure water was added on 10 g honey, after the obtained solution was homogenized and centrifuged at 3500 rpm for 45 minutes. Supernatant solution is spilled and the remaining solution was transferred onto the slide by 1-2 mm^3 glycerin-gelatin with basic fucsin. Microscope slides were examined under Labomed light microscope. For determining the importance degree of the botanical origin of honey samples, we used the classification given by the count of 200 pollen grains per sample [17]. Determined pollen types were classified in four classes of frequency [18, 19], (i) predominant pollen types (>45% of the total pollen content); (ii) secondary pollen types (16-45%); (iii) important minor pollen types (3-15%); and (iv) minor pollen types. Samples with pollen grain frequencies of a given plant, above 45%, were called as monofloral.

Characterization methods

For GC-MS measurement, Agilent brand 7890A model GC, 5975C model MS and FID detector were used synchronically. The column brand was BPx90 and the column dimensions were 100 mm x 0.25 mm x 0.25 μm. The flow rate was adjusted to 1 mL/min using column carrier with helium gas. Under chromatographic conditions, the samples increased 5 °C per minute and reached from 120 °C to 254 °C and were kept at this temperature for about 16 minutes. MS results were determined by comparing with WHILEY and NIST

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

libraries in the memory of the device. A part of the filtered clear solution was used for GC-MS measurement and the following procedures were carried out at this stage, respectively:

1. 100 µL of sample was mixed with 10 ml of hexane and vortexed.
2. 100 µL of 2 N KOH was added to the resulting solution and vortexed.
3. The solution was centrifuged for 10 minutes at 4500 rpm.
4. It was placed on the GC-MS device using the clear part in the centrifuged tubes and made ready for measurement.

Perkin Elmer Spectrum 100 branded device was preferred for FT-IR measurement and provided to detect the organic bond structures and properties of different chemical molecules in the sample.

Antimicrobial activity

All chemicals and reagents were procured from Merck (Darmstadt, Germany) and prepared in HPLC grade. The water extract of H1, H2 and H3 was tested for their antimicrobial activity against *E. coli* ATCC 25922, *S. cerevisiae* ATCC 76521, *S. aureus* ATCC 29213, *L. monocytogenes* NCTC 5348, and *C. albicans* ATCC 90028.

Antimicrobial activity tests were performed using the agar disc diffusion method described in [16] with some modifications. The water extract of honey samples was adjusted to different concentrations as 125, 250, and 500 mg/mL via sterile pure water. The tested microorganism suspensions (10^6 CFU) were seeded onto Mueller-Hinton agar plates. 40 µL of different concentrations (125, 250 and 500 mg/mL) of honey extract were impregnated to the sterile paper discs (6 mm diameter, 3mm thickness). After that, the discs were put onto the surface of inoculated agar plates. The ampicillin sulbactam 10/10 µg (SAM) disc was also used as positive control. Petri plates were incubated at 37 °C for 24 hours after that, at 4 °C for 1 hour. The determination of antimicrobial activity was determined with measuring the inhibition zone.

Statistical analysis

All measurements were repeated three times, and values are the average of triplicate and expressed as mean ± SD.

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

Results and Discussion

Characterization results

GC-MS graphs of H1, H2 and H3 samples were given in Figure 1, the compounds and their properties of H1, H2, H3 samples were given in Table 1. According to the GC-MS results, the groups of alkanes (docosane, tetratriacontane), cycloalkenes (1,5,9-cyclododecatriene, 1,5,9-cyclododecatriene,(E,Z,Z), 5,6-divinyl-1-cyclooctene), ketones (2-t-butyl-6-[2-hydroxy-2-(4-methoxyphenyl)ethyl]-[1,3]dioxin-4-one, N-allylmaleimide, trans-3-ethylidene-1-vinyl-2-pyrrolidone), aromatic acid (ethyl-(2E)-3-[2-(diethoxyphosphoryl)-4-(dimethylamino) phenyl]-2-propenoate), amide (oleamide), aldehydes (9-octadecenal), phenolic compounds (3-hydroxycarbofuranphenol, cis-3-ethyl-2-(4-methoxyphenyl)-4-methyleneoxolane), esters of saturated acid (4-[[4-(4-bromo-phenyl)-thiazol-2-yl]-methyl-amino]-butyric acid), heterocyclic compound (8-azahypoxanthine) were seen over of area as 1%. Especially, oleamide (bioactive fatty acid ester) were found in all samples as the common compound, and this compound had antibacterial and antioxidant properties [20,21]. Different phenolic compounds were seen in the samples and it was known that these compounds and aromatic acids had antibacterial and antioxidant properties in the view of literature data [22, 23, 24]. Also ketone groups and fatty acids methyl esters were obtained antibacterial activity and useful for antibiotics [25, 26]. There is also a concordance with the literature values and the differences in compound names were found in honey species, but they were similar as compound groups. Generally, the compounds of H1, H2 and H3 samples had antibacterial activity and this result was compatible with antibacterial activity tests.

FTIR plots which had range as 4000-650 cm^{-1} and resolution as 4 cm^{-1} of H1, H2 and H3 samples are given in Figure 2. Overall, looking at the FTIR results, three samples were found to show similar peak values. The peaks at 3700-2970 cm^{-1} have the feature of O-H stretching vibration [27]. The features of CH3 asymmetric stretching vibration, C-O stretching and C-N stretching (amide group), aliphatic CH2, CH3 bending, O-H bending in –COOH or CH3 bending were showed the peak values at 2948, 1640, 1425, 1380 cm-1,

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

respectively [1]. The peak at 1240 cm-1 was related to C=O, C-O stretching in RC(=O)-OH and P+O asymetric stretching in RO-P(-O2)-OR [27, 28].

Table 1. Chemical composition of honey samples via GC-MS analysis

Region	RT	Area %	Library/ID	Ref#	CAS#	Qual	Chemical Group
H1	25.213	0.88	Docosane	69204	000629-97-0	83	Alkane
	30.912	1.2	Tetratriacontane	75175	014167-59-0	83	Alkane
	51.752	0.17	Benzyl Methyl Ether	64536	000000-00-0	89	Ether
	57.096	0.19	1-Octadecene	26418	000112-88-9	90	Alkene
	62.898	0.37	3-Hydroxycarbofuranphenol	287816	017781-15-6	83	Phenolic compounds
	75.486	5.45	2-t-Butyl-6-[2-hydroxy-2-(4-methoxyphenyl)ethyl]-[1,3]dioxin-4-one	288224	999288-22-7	87	Ketone
	76.551	11.23	Oleamide	83151	000301-02-0	91	Amide group
H2	16.373	1.87	6-aza-5,7,12,14-tetrathiapentacene	507397	000000-00-0	86	Polycyclic aromatic hydrocarbon
	50.201	1.1	Ethyl-(2E)-3-[2-(diethoxyphosphoryl)-4-(dimethylamino) phenyl]-2-propenoate	507399	066564-08-7	94	Aromatic acid-Phenolic compound
	55.122	1.06		507399	066564-08-7	90	
	58.435	0.57	1,5,9-cyclododecatriene	52147	002765-29-9	90	cycloalkene
	63.196	0.23	Cyclooctacosane	70123	000297-24-5	90	cycloalkane
	75.675	3.23	1,5,9-cyclododecatriene,(E,Z,Z)-	52526	002765-29-9	86	cycloalkene
	76.562	7.4	Oleamide	83149	000301-02-0	92	Amide group

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

	77.678	0.79	5,6-divinyl-1-cyclooctene	52519	046045-35-6	83	cycloalkene
	78.067	0.42	1,2,4-Triazolo[4,3-B][1,2,4]-triazin-7(8H)-one	286124	000874-40-8	83	Carbocyclic compound
H3	32.411	4.23	Ethyl-(2E)-3-[2-(diethoxyphosphoryl)-4-(dimethylamino) phenyl]-2-propenoate	507399	066564-08-7	83	Aromatic acid-Phenolic compound
	44.628	2.99	4-[[4-(4-bromo-phenyl)-thiazol-2-yl]-methyl-amino]-butyric acid	472704	999472-71-6	91	Esters of saturated acids
	50.195	2.59	Ethyl-(2E)-3-[2-(diethoxyphosphoryl)-4-(dimethylamino) phenyl]-2-propenoate	507399	066564-08-7	95	Aromatic acid-Phenolic Compound
	59.562	2.65		507399	066564-08-7	90	
	67.258	1.43	8-Azahypoxanthine	287663	002683-90-1	80	Heterocyclic compound
	73.672	1.57	9-octadecenal	11106	005090-41-5	83	Aldehyde
	74.17	0.75	cis-3-ethyl-2-(4-methoxyphenyl)-4-methyleneoxolane	286688	000000-00-0	83	Phenolic compounds
	75.629	1.08	N-allylmaleimide	52476	002973-17-3	81	Ketone
	75.858	0.76	5,6-divinyl-1-cyclooctene	52519	046045-35-6	92	cycloalkene
	76.551	3.05	Oleamide	83152	000301-02-0	92	Amide group
	76.997	1.53	trans-3-ethylidene-1-vinyl-2-pyrrolidone	52475	999052-47-7	80	Ketone

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

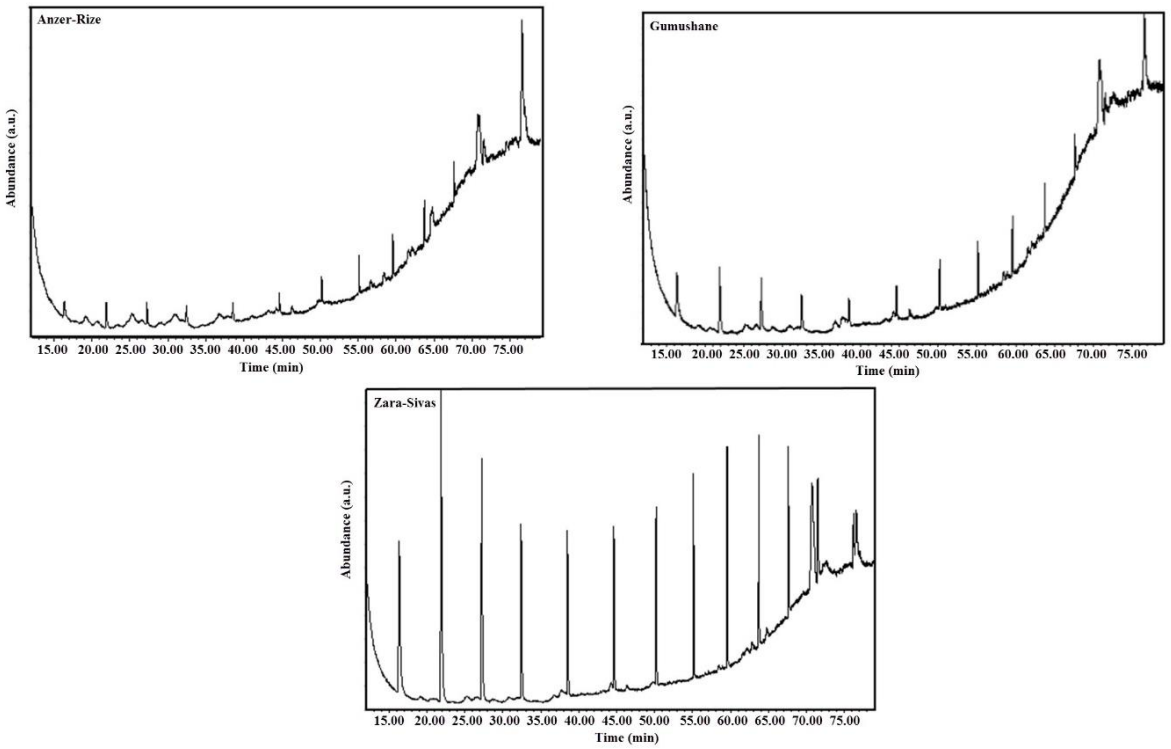

Figure 1. GC-MS graph of honey samples

The peak values of 1030 and 1060 cm-1 showed the property of C-O stretch in -C-O-C-, -C-OH compounds, C-S stretching aryl-S-aryl compounds and C=S stretching [1,2]. These values were compatible with GC-MS results which had the compounds such as amide groups, ketones, ethers, fatty acids, phosphoric compounds, sulfonic compounds etc. Any degradation or different bond types were not observed.

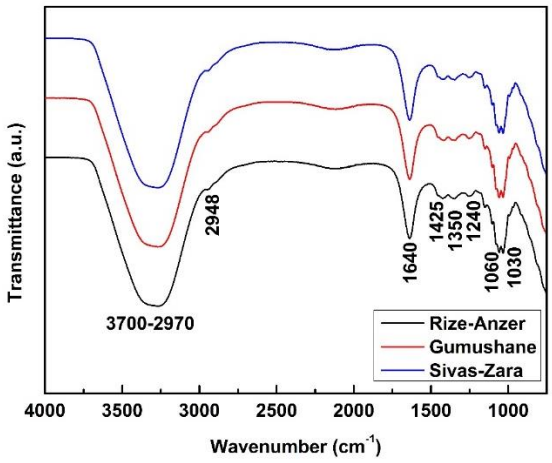

Figure 2. FTIR graph of honey samples

Antimicrobial analysis

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

Table 2 indicates the results of the antimicrobial activity of honey samples present at different concentrations (125, 250, 500 mg/mL) against *E. coli, S. cerevisiae, S. aureus, L. monocytogenes,* and *C. albicans* by disc diffusion assay. Also, SAM (ampicillin/sulbactam 20 µg) used as a control and it showed different antimicrobial activities on these microorganisms.

In this study, the antimicrobial activity values of H1, H2 and H3 for 500 mg/mL extract concentration on *S. aureus* were found as 10.0-8.5-8.0 mm the zones of inhibition, respectively. The antimicrobial activity values of H1 and H2 for 250 mg/mL extract concentration on *S. aureus* were found as 6.0-6.5 mm the zones of inhibition, respectively. The antimicrobial activity values of H3 for 250 mg/mL extract concentration on *S. aureus* were not in detectable levels the zones of inhibition. The antimicrobial activity values of H1, H2, and H3 for 125 mg/mL extract concentration on *S. aureus* were not in detectable levels the zones of inhibition. The antimicrobial activity of SAM for H1, H2, and H3 were found as 11.5-13.0-12.0 mm zones of inhibition on *S. aureus*, respectively (Table 2.). The antimicrobial activity values determined for *S. aureus* in this study were lower than the antimicrobial activity values reported for *S. aureus* by Aksoy and Dığrak, Alan et al, Silici et al, Mercan et al, Ekici et al, Sheikh et al, Merces et al [29-35]. The antimicrobial activity values determined for *S. aureus* in this study were higher than the antimicrobial activity values reported for *S. aureus* by Silici et al [31]. The antimicrobial activity values determined for *S. aureus* in this study were similar to the antimicrobial activity values reported for *S. aureus* by Sagdic et al [36]. The antimicrobial activity values of H1, H2, and H3 for 125-250 and 500 mg/mL extract concentrations on *L. monocytogenes* were not in detectable levels the zones of inhibition. The antimicrobial activity of SAM for H1, H2 and H3 honey samples were found as 34.5-34.0-37.0 mm zones of inhibition on *L. monocytogenes*, respectively (Table 2.). The antimicrobial activity values determined for *L. monocytogenes* in this study were lower than the antimicrobial activity values reported for *L. monocytogenes* by Silici et al, Ekici, Sagdic et al [31, 33, 36].

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

The antimicrobial activity values of H1 for 500 mg/mL extract concentration on *E. coli* were found as 6.0 mm the zones of inhibition. The antimicrobial activity values of H2 and H3 for 500 mg/mL extract concentration on *E. coli* were not in detectable levels the zones of inhibition. The antimicrobial activity values of H1, H2, and H3 for 125-250 mg/mL extract concentration on *E. coli* were not in detectable levels the zones of inhibition. The antimicrobial activity of SAM was found as 11.0-9.5-8.0 mm zones of inhibition on *E. coli* (Table 2.). The antimicrobial activity values determined for *E. coli* in this study were lower than the antimicrobial activity values reported for *E. coli* by Aksoy and Dığrak, Alan et al, Silici et al, Mercan et al, Ekici,et al, Sheikh et al, Merces et al [29- 35]. The antimicrobial activity values determined for *E. coli* in this study were similar to the antimicrobial activity values reported for *E. coli* by Sagdic et al [36].

The antimicrobial activity values of H1, H2 and H3 honey samples for 500 mg/mL extract concentration on *Saccharomyces cerevisae* were found as 9.0-7.5-6.0 mm the zones of inhibition, respectively. The antimicrobial activity values of H1, H2 and H3 honey samples for 125-250 mg/mL extract concentrations on *Saccharomyces cerevisae* were not in detectable levels the zones of inhibition. The antimicrobial activity of SAM for H1, H2 and H3 honey samples were found as 10.5-11.5-7.5 mm zones of inhibition on *Saccharomyces cerevisae*, respectively (Table 2.). The antimicrobial activity values determined for *Saccharomyces cerevisae* in this study were higher than the antimicrobial activity values reported for *Saccharomyces cerevisae* by Alan et al, Silici et al , Ekici et al, Sagdic et al [29, 31, 33, 36].

The antimicrobial activity values of H1, H2 and H3 honey samples for 125-250-500 mg/mL extract concentrations on *Candida albicans* were not in detectable levels the zones of inhibition. The antimicrobial activity of SAM for H1, H2 and H3 honey samples were found as 31.5-27.5-28.5 mm zones of inhibition on *Candida albicans*, respectively (Table 2.). The antimicrobial activity values determined for *Candida albicans* in this study were lower than the antimicrobial activity values reported for *Candida albicans* by Aksoy and Dığrak, Mercan et al [26, 29]. The antimicrobial

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

activity values determined for *Candida albicans* in this study were similar to the antimicrobial activity values reported for *Candida albicans* by Alan et al, Silici et al, Ekici et al, Sagdic et al, Sheikh et al, Merces et al [30- 35].

Table 2. Antimicrobial activity of H1, H2, and H3 samples

Tested microorganism	H1				H2				H3			
	Zone of inhibition (mm)[a]											
	500[b]	250[b]	125[b]	SAM	500[b]	250[b]	125[b]	SAM	500[b]	250[b]	125[b]	SAM
Gram (+)												
S. aureus	10.0 ± 1.4	6.0 ± 0.0	-	11.5 ± 0.7	8.5 ± 0.7	6.5 ± 0.7	-	13.0 ± 1.4	8.0 ± 0.0	-	-	12.0 ± 1.4
L. monocytogenes	-	-	-	34.5 ± 0.7	-	-	-	34.0 ± 1.4	-	-	-	37.0 ± 1.4
Gram (-)												
E. coli	6.0 ± 0.0	-	-	11.0 ± 1.4	-	-	-	9.5 ± 0.7	-	-	-	8.0 ± 1.4
Yeast												
Saccharomyces cerevisae	9.0 ± 0.0	-	-	10.5 ± 0.7	7.5 ± 0.7	-	-	11.5 ± 0.7	6.0 ± 0.0	-	-	7.5 ± 0.7
Fungi												
C. albicans	-	-	-	31.5 ± 2.1	-	-	-	27.5 ± 0.7	-	-	-	28.5 ± 2.1

–: No activity.
a Values are the average of triplicate and expressed as mean ± SD.
b Honey extract concentration (mg/mL); SAM, Ampicillin/Sulbactam (20 µg/disc).

Melissopalynological analysis

Melissopalynology was used to determine the botanical origin of honey, which is important for its traceability [37]. The pollen analysis results are presented in Table 3, Table 4 and Figure 3. In this study, a total of 36 pollen types belonging to 18 families were identified. The pollen grains of the honey samples belonging to the family Apiaceae, Asteraceae, Betulaceae, Boraginaceae, Brassicaceae, Campanulcaeeae, Caprifoliaceae, Caryoplyllaceae, Cistaceae, Ericaceae, Fabaceae, Lamiaceae, Moraceae, Onagraceae, Plantaginaceae, Ranunculaceae, Rosaceae, Salicaceae were identified at different rates. As a result of the pollen analysis, 18 plants were identified at the family level, 18 of them are genus and 1of them is species. The families with the highest numbers of pollen types situated in the honey samples were Asteraceae, Cistaceae, Fabaceae,

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

Rosaceae and Salicaceae families. All of the samples were classified as multifloral because they contains the pollen of many plant taxa.

Table 3. Pollen frequency percentages and taxa recovered from the honey samples

Pollen Taxa	Pollen Frequency (%) of Honey Samples From Turkey		
	H1 %	***H2 %***	***H3%***
Apiaceae	0.8		1.9
Type I			0.6
Type II			0.6
Asteraceae		0.7	19.6
Centaurea			17.7
Betulaceae		0.7	
Boraginaceae	3.1	0.7	
Myosotis	9.3		
Shymphytum			0.6
Brassicaeae	3.9	4.48	1.2
Campanulaceae			
Campanula	0.8		0.6
Caprifoliaceae	2.3		
Scabiosa			0.6
Caryophyllaceae		0.7	
Cistaceae			0.6
Helianthemum	18.6	17.9	
Ericaceae			0.6
Rhododendron	1.6		
Fabaceae	11.7	5.9	13.9
Acacia			0.6
Astragalus	1.6	5.2	0.6
Lathyrus	0.8		
Lotus	3.9		
Onobrychis	24.3	6.7	20.9
Trifolium	1.6	8.9	
Lamiaceae	1		0.6
Type I		2.2	
Type II		4.48	
Lamium	0.8	2.2	5.1
Teucrium			0.6
Moraceae			
Morus	0.8		
Onagraceaea	0.8		5.7
Plantaginaceae			
Plantago lanceolata	0.8		
Ranunculaceae			
Ranunculus	5.4		
Rosaceae	1.6	13.4	3.8
Salicaceae			
Salix	4.7	26.12	3.8

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

The secondary group of pollens were the following taxa in three honey samples; *Helianthemum* sp., *Salix* sp. for H2 sample, *Onobrychis* sp *Helianthemum* sp. for H1 sample and *Centaurea* sp., *Onobrychis* sp. for H3 sample. Şık et al. (2017) were examined pollen content of honey samples from Ardahan, (Northeast Anatolia). They found 23 different taxa from 13 families. *Astragalus* spp., Apiacaee, Brassicaceae, Fabaceae pollen grains were the most abundant in honey samples. All of the samples were multifloral [38].

Table 4. Polen taxa in honey in percentages

Samples	Honey Type	Predominant pollen (> 45%)	Secondary pollen (16-45%)	İmportant minor pollen (3-15%)	Minor pollen (<3%)
H2	Multifloral	-	*Helianthemum*, *Salix*	Brassicaceae, Fabaceae, *Astragalus*, *Onobrychis*, *Trifolium*, Lamiaceae, Rosaceae	Asteraceae, Betulaceae, Boraginaceae, Caryophyllaceae, *Lamium*
H1	Multifloral	-	*Onobrychis*, *Helianthemum*	Boraginaceae, Brassicaceae, *Myosotis*, Fabaceae, *Ranunculus*, *Lotus*,*Salix*	Apiaceae, *Campanula*, Caprifoliceae, *Rhododendron*, *Astragalus*, *Lathyrus*, *Trifolium*, Lamiaceae, *Lamium*, *Morus*, Onagraceae, *Plantago*, Rosaceae
H3	Multifloral	-	Asteraceae, *Centaurea*, *Onobrychis*	Fabaceae, *Lamium*, Onagraceae, Rosaceae, *Salix*	Apiaceae, Brassicaceae, Campanulaceae, *Scabiosa*, Cistaceae, Ericaceae, *Acacia*, *Astragalus*, Lamiaceae, *Teucrium*

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

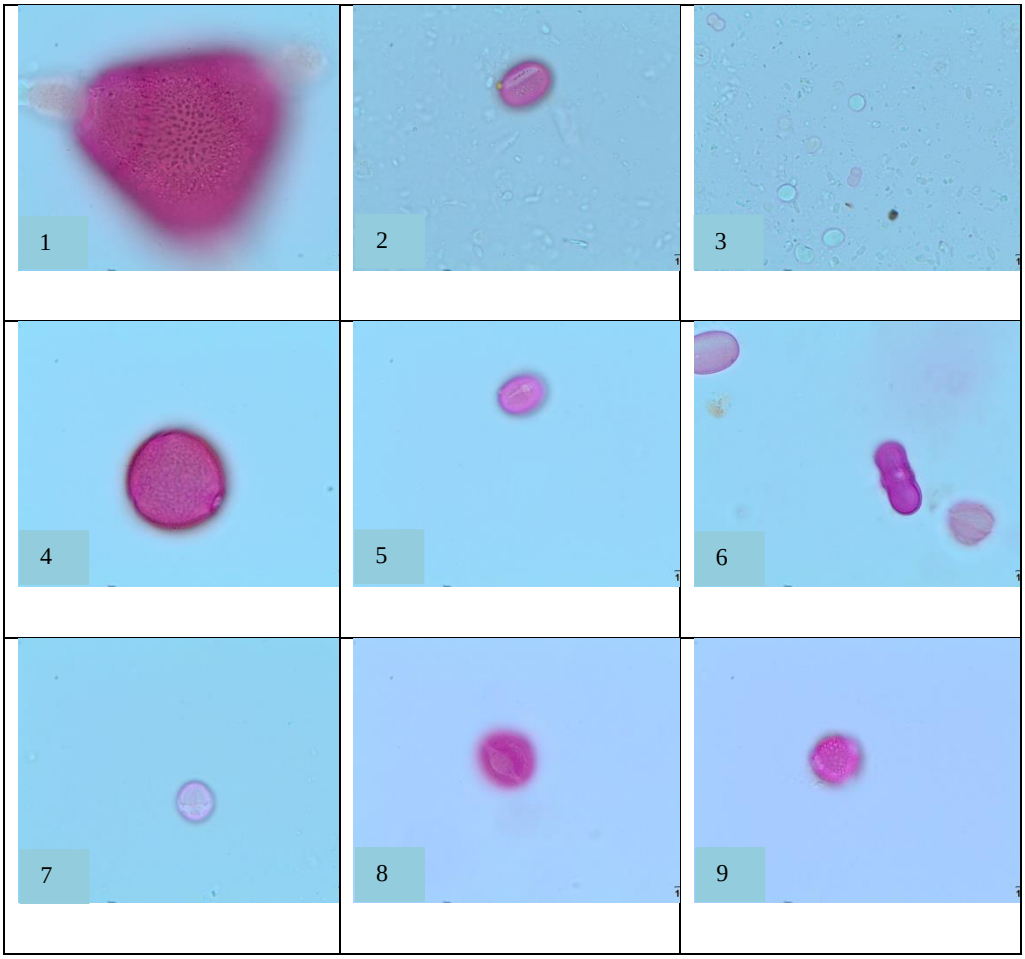

Figure 3. Light photomicrographs of characteristic pollen types from honey samples (*1.Scabiosa (x100)* 2. *Onobrychis (x100)* 3. *Myosotis (x100)* 4. Cistaceae *(x100)* 5. *Astragalus (x100)* 6. Apiaceae *(x100)*7. *Shymphytum (x100)* 8. *Trifolium (x100)* 9. *Salix (x100))*

Pollen spectrum profile determined in the study indicated predominance of the families Fabaceae, Asteraceae presenting a great diversity of botanical taxons. Tümerdem et al. (2016) also observed the predominance of the Fabaceae in honey samples from Ankara province in Turkey [39].

In another study, the melissopalynologic analyses indicated that 61 taxa (41 at the genus level and 20 at the species level) belonging to 34 families were identified. *Astragalus* sp., *Trifolium* sp., *Myrtus communis* and *Castanea sativa* were the predominant taxa in the unifloral honey samples [40]. Özler et al. (2018) observed in the analyzed honey samples from South

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

Anatolia that the findings of this study demonstrated that pollen grains of the family of Fabaceae, Rosaceae, the genera of Eucalyptus and Centaurea were in nectar and pollen sources for honey production [41].

According to the our pollen analysis results of H2, *Helianthemum* sp., *Salix* sp., *Astragalus* sp., *Onobrychis* sp., *Trifolium* sp. are the more preferred plant taxa by honeybees likewise in another study 12 honey samples collected from Gümüşhane region, *Astragalus* sp., *Trifolium* sp. taxa were determined dominant by melissopalynologic analysis [42]. Sorkun and Doğan (1995), were made pollen analysis of Anzer honey sample, they were determined that 35 different plant pollen types. Fabaceae, Asteraceae, Boraginaceae are some families that they were found. In our study, Fabaceae appears as the most common pollen family in 32,2% of the H1 honey sample and also Fabaceae family members were traced as being the dominant pollen group according to Sorkun and Doğan (1995). They were found 9% *Myosotis* pollens in 11 honey samples similar to our study [43]. In contrast to our study, *Papaver* sp. pollens were found to be dominant in H3 sample [44]. Fabaceae and Asteraceae were in a large quantity pollen grains in our honey sample from H3.

Conclusion

GC-MS results showed that alkanes, cycloalkenes, ketones, aromatic acid, amide, aldehydes, phenolic compounds, saturated acid esters, heterocyclic compound have over of area as 1%. By and large, the compounds of H1, H2 and H3 samples had antibacterial activity and this result was compatible with antibacterial activity tests. Oleamid (bioactive fatty acid ester) having antibacterial and antioxidant properties appeared as a common component in all samples. In addition, FTIR values were compatible with GC-MS results which had the compounds such as amide groups, ketones, ethers, fatty acids, phosphoric compounds, sulfonic compounds.

H1, H2 and H3, at 125 mg/mL concentration, showed no antimicrobial activity against *E. coli, S. cerevisiae, S. aureus, L. monocytogenes*, and *C. albicans*.

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

Among honey samples, H1 and H2 honey samples at 250 mg/mL concentration showed antimicrobial activity only against *S. aureus*. H3 sample at 250 mg/mL concentration did not exhibit any antimicrobial activity on *E. coli*, *S. cerevisiae*, *S. aureus*, *L. monocytogenes*, and *C. albicans*. H1, H2 and H3 honey samples, at 500 mg/mL concentration, exhibited inhibitions on *S. aureus* and *S. cerevisiae*, while they did not show any antimicrobial activity against *L. monocytogenes* and *C. albicans*. Also, H1 honey, at 500 mg/mL concentration, exhibited low antimicrobial activity on *E. coli*, while H2 and H3 honey samples did not show antimicrobial activity on *E. coli*.

Botanical, geographical origin and pollen composition of honey samples are an important on its biological activities.

According to pollen analyses a total of 36 pollen types belonging to 18 families were identified. Additionally, 18 genera and 1 species from 18 plant families were identified. Asteraceae, Cistaceae, Fabaceae, Rosaceae and Salicaceae families were determined to have the most pollen type. All honey samples are classified as multifloral, because they have more than one type of pollen.

Türkiye'deki Bazı Bal Örneklerinin Antimikrobiyal Aktivitesinin, Palinolojik Özelliklerinin ve Kimyasal Bileşiminin Belirlenmesi

Öz: Bal eski zamanlardan beri geleneksel tıpta kullanılmaktadır. Botanik, jeolojik, iklimsel özellikler gibi birçok parametre, balın karakteristik özelliklerini belirlemektedir. Bu nedenle balla yapılan bölgesel çalışmalarda balın kimyasal, palinolojik ve antimikrobiyal özelliklerini ortaya koymak önemlidir. Bu çalışmada, Türkiye'de üretilen bazı bal örneklerinin antimikrobiyal aktivitesi, palinolojik ve kimyasal özellikleri belirlenmiştir. Antimikrobiyal test yoluyla, Rize (Anzer), Gümüşhane ve Sivas (Zara) illerindeki balların bazı patojenler üzerindeki etkileri araştırılmıştır. Bu teste göre, bal örneklerinin tümü *S. aureus* ve *S. cerevisae* üzerinde etkili olmuş, sadece Anzer bölgesinden gelen bal *E. coli* üzerinde etkili olmuş ve bal örneklerinin hiçbiri *L. monocytogenes* ve *C. albicans* üzerinde aktivite göstermemiştir. Palinolojik analizlerde 36 polen taksonu belirlenmiştir.

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

Bal örneklerinin kimyasal bileşimleri GC-MS analizi ile belirlenmiştir. GC-MS sonuçları, tüm bal örneklerinin antibakteriyel ve antioksidan özelliklere sahip olduğunu göstermiştir. Bal örneklerinin kimyasal yapı türleri FTIR analizi ile belirlenmiş olup, FTIR'daki kimyasal bağ türleri GC-MS sonuçlarının kimyasal bileşimleri ile bağlantılı olmuştur.

Anahtar Kelimeler: Palinolojik özellikler; kimyasal bileşim; bal; antimikrobiyal aktivite

R E F E RE N C E S

[1] Joint FAO/WHO Food Standard Programme Codex Alimentarius Commission Twenty-Fourth Session Geneva, 2-7 July 2001. Available from: http://www.fao.org/input/download/report/277/Al01_25e.pdf (05.06.2020).

[2] THRASYVOULOU, A; TANANAKI, C.; GORAS, G; KARAZAFIRIS, E; DIMOU, M; LIOLIOS, V; GOUNARI, S (2014) Legislation of Honey Criteria and Standards. Journal of Apicultural Research, 57(1), 88-96.

[3] ANAND, S; DEIGHTON, M; LIVANOS, G; MORRISON, PD; PANG, ECK; MANTRI, N (2019), Antimicrobial Activity of Agastache Honey and Characterization of Its Bioactive Compounds In Comparison With Important Commercial Honey samples. Front. Microbiol. 10:263. doi: 10.3389/fmicb.2019.00263.

[4] ALMASAUDI, S B; AL-NAHARI, A A; EL SAYED, M; BARBOUR, E; AL MUHAYAWI, S M; AL-JAOUNI, S; HARAKEH, S (2017), Antimicrobial Effect Of Different Types Of Honey On *Staphylococcus aureus*, Saudi Journal Of Biological Sciences, 24(6): 1255-1261.

[5] KWAKMAN, P H; TE VELDE, A A; DE BOER, L; SPEIJER, D; VANDENBROUCKE-GRAULS, C M; ZAAT, S A (2010), How Honey Kills Bacteria. The FASEB Journal, 24(7): 2576-2582.

[6] WAHDAN, H A L (1998), Causes of the Antimicrobial Activity of Honey. Infection, 26(1), 26-31.

[7] ABD-EL AAL, A; EL-HADIDY, M; EL-MASHAD, N; EL-SEBAIE, A (2007) Antimicrobial Effect of Bee Honey in Comparison To Antibiotics on Organisms Isolated From Infected Burns, Ann. Burns Fire Disasters 20: 83.

[8] EFEM, S E E (1988), Clinical Observations on the Wound Healing Properties of Honey, British journal of Surgery, 75(7): 679-681.

[9] WANG, R; STARKEY, M; HAZAN, R; RAHME, L G (2012), Honey's Ability To Counter Bacterial Infections Arises from Both Bactericidal Compounds and QS Inhibition, Front. Microbiol. 3:144. doi: 10.3389/fmicb.2012.00144.

[10] AL SOMAL, N; COLEY, K; MOLAN, P; HANCOCK, B (1994), Susceptibility of Helicobacter Pylori to the Antibacterial Activity of Manuka Honey, J. R. Soc. Med. 87: 9–12.

[11] KWAKMAN, P H S; VAN DEN AKKER, J P C; GÛÇLÛ, A; ASLAMI, H; BINNEKADE, J M; DE BOER, L; BOSZHARD, L; PAULUS, F; MIDDELHOEK, P; TE VELDE, A A; VANDEBROUCKE-GRAULS, C M J E; SCHULTZ, M. J; ZAAT, S A J (2008), Medical-Grade Honey Kills Antibiotic-Resistant Bacteria In Vitro and Eradicates Skin Colonization, Clin. Infect. Dis. 46: 1677–1682.

[12] MOLAN, P C (1992), The Antibacterial Activity of Honey. 1. The nature of the antibacterial activity, Bee World, 73: 5-28.

[13] CENET, M (2019), Pollen Analyses and Antimicrobial Properties of the Natural Honey from the East Mediterranean Part of Anatolia, Pakistan J. Zool., vol. 51(2): 541-548.

[14] ÇENET, M; TOROĞLU, S; KESKIN, D; BOZOK, F (2015), Pollen Analysis and Antimicrobial Properties of Honey Samples Sold in Western Turkey. Pakistan J. Zool., 47(1): 269-273.

[15] VON DER OHE, W; PERSANO ODDO, L; MORLOT, P. M. (2004), Harmonized methods of melissopalynology, Apidologie, 35:18–25.

[16]RADJI, M; AGUSTAMA, R A; ELYA, B; TJAMPAKASARI, C R (2013), Antimicrobial Activity of Green Tea Extract Against Isolates of Methicillin–

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

Resistant Staphylococcus Aureus and Multi–Drug Resistant *Pseudomonas aeruginosa*, Asian Pacific Journal of Tropical Biomedicine, 3:663-667.

[17] SORKUN, K (2008), Türkiye'nin Nektarlı Bitkileri ve Polenleri, Ankara: Palme Yayıncılık.

[18] LOUVEAUX, J; MAURIZIO, A; VORWOHL, G (1978), Methods of Melissopalynology, Bee World, 59: 139-157. doi: 10.10 80/0005772X.1978.11097714.

[19] BARTH, O.M. (1989), O pólen do mel brasileiro. Rio de Janeiro: Luxor, 226 p.

[20] FARHA, AK; HATHA, AM (2019), Bioprospecting Potential and Secondary Metabolite Profile of A Novel Sediment-Derived Fungus *Penicillium* sp. Arcspf From Continental Slope of Eastern Arabian Sea, Mycology. Jun;10(2):109-117 DOI: 10.1080/21501203.2019.1572034.

[21] DEVI, JAI; MUTHU, AK (2014) Gas Chromatography-Mass Spectrometry Analysis of Bioactive Constituents in the Ethanolic Extract of *Saccharum spontaneum* Linn., Int J Pharm Pharm Sci. 6:755–759.

[22] MANDAL, SM; DIAS, RO; FRANCO, OL (2017), Phenolic Compounds in Antimicrobial Therapy, J. Med Food, 20 (10):1031-1038. doi:10.1089/jmf.2017.0017

[23] OUERGHEMMI, I; REBEY, I. B; RAHALI, F. Z; BOURGOU, S; PISTELLI, L; KSOURI, R; TOUNSI, M S (2017), Antioxidant and Antimicrobial Phenolic Compounds From Extracts of Cultivated and Wild-Grown Tunisian *Ruta chalepensis*, Journal of Food and Drug Analysis, 25(2):350-359.

[24] LUCCHINI, J J; CORRE, J; CREMIEUX, A (1990), Antibacterial Activity of Phenolic Compounds and Aromatic Alcohols, Research in Microbiology, 141(4): 499-510.

[25] MEA, PINTO; SG, ARAÚJO; MI, MORAIS; NP, SÁ; CM, LIMA; CA, ROSA; E P, SIQUEIRA; LARS, LIMA (2017), Antifungal and Antioxidant Activity of Fatty Acid Methyl Esters From Vegetable Oils, Anais da Academia Brasileira de Ciências 89(3): 1671-1681.

[26] ANDRUS, M B; MENDENHALL, K G; MEREDITH, E L; SEKHAR, B S (2002), Glycolate Aldol Reactions With Boron Enolates of Bis-4-Methoxyphenyl Dioxanone, Tetrahedron letters, 43(10):1789-1792.

[27] KUPTSOV, A H; ZHIZHIN, G N (1998), Handbook of Fourier Transform Raman and Infrared Spectra of Polymers. Volume 45 1st Edition Elsevier Science.

[28] EL-SAKHAWY, M; MILICHOVSKY, M (2000), Oxycellulose Modification, Polymer International, 49(8): 839-844.

[29] AKSOY, Z; DIĞRAK M (2006), Bingöl Yöresinde Toplanan Bal Ve Propolisin Antimikrobiyal Etkisi Üzerinde In Vitro Araştırmalar. Science and Eng. J of Fırat Univ. 18 (4): 471-478.

[30] ALAN, Y; ATALAN, E; ERBIL, N; BAKIR, O; ORMAN, Z; KANIK, PELDA (2014), Antimicrobial Activity of Honey and Propolis Collected In Muş and Bitlis Region, Muş Alparslan University Journal of Science, 2 (1): 221-229.

[31] SILICI, S; SAGDIC, O; EKICI, L. (2010) Total Phenolic Content, Antiradical, Antioxidant and Antimicrobial Activities of Rhododendron Honeys, Food Chemistry, 121:238-243.

[32] MERCAN, N; GUVENSEN, A; CELIK, A; KATIRCIOGLU, H (2006), Antimicrobial Activity and Pollen Composition of Honey Samples Collected From Different Provinces In Turkey, Natural Product Research, 21(3):187-195.

[33] EKICI, L; SAGDIC, O; SILICI, S; OZTURK, I (2014), Determination of Phenolic Content, Antiradical, Antioxidant and Antimicrobial Activities of Turkish Pine Honey, Quality Assurance and Safety of Crops & Foods, 6 (4): 439-444.

[34] SHEIKH, D; ZAMAN, S U; NAQVI, S B; SHEIKH, M R; ALI, G (1995), Studies on Microbial Activity of Honey. Pakistan Journal of Pharmaceutical Science, 8(1): 51-62.

[35] MERCÊS, M D; PERALTA, E D; UETANABARO, A P T; LUCCHESE, A M (2013), Antimicrobial Activity of Honey From Five Species of Brazilian Stingless Bees, Ciência Rural, Santa Maria, 43(4): 672-675.

[36] SAGDIC, O; SILICI, S; EKICI, L (2013), Evaluation of the Phenolic Content, Antiradical, Antioxidant, and Antimicrobial Activity of Different Floral Sources of Honey, International Journal of Food Properties, 16:658-666.

[37] MORAES, FJ; GARCİA, RC; GALHARDO, D; CAMARGO, SC; PİRES, BG; PEREİRA, DJ; SOUSA, PHAA (2019), Pollen Analysis Of Honey Samples Produced In the Counties of Santa Helena and Terra Roxa, Western Region of Paraná, Southern Brazil, Sociobiology 66(2):327-338

[38] ŞIK, L; GÜVENSEN, A; DURMUŞKAHYA, C; EROL, O (2017), Pollen Analysis of Honeys from

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.

Ardahan/Turkey, Biological Diversity and Conservation – 10: 2.

[39] TÜMERDEM, Ç; PEHLİVAN, S; DOĞAN, C (2016), Pollen Analysis of Honeys from Beypazarı District of Ankara Province (Turkey), Mellifera; 16(1):19–26.

[40] CENET, M (2019), Pollen Analyses and Antimicrobial Properties of the Natural Honey from the East Mediterranean Part of Anatolia Pakistan J. Zool., 51(2): 541-548.

[41] ÖZLER, H (2018), Pollen Analysıs of the Honey from South Anatolıa, Uludağ Arıcılık Dergisi – Uludag Bee Journal, 18 (2): 73-86.

[42] TÜRKER, M (1993) Gümüşhane Ballarında Polen Analizi. Yüksek Lisans Tezi Yüzüncü . Yıl Üni..Fen Bilimler Enstitüsü. Biyoloji A.B.D. 35 sayfa.

[43] SORKUN, K; DOĞAN, C (1995), Pollen Analysis Rize –Anzer Turkish Honey, Apiacta, 3:75-81.

[44] MERCAN, N; GUVENSEN, A; CELIK, A; KATIRCIOGLU, H (2007), Antimicrobial Activity And Pollen Composition Of Honey Samples Collected From Different Provinces In Turkey, Nat Prod Res, 21:187–195.

Cite as: ÇAKIR, Y; ÇOBANOĞLU, D N; DERVİŞOĞLU, G; KOÇYİĞİT, S; KARAHAN, D; YELKOVAN, S (2020), Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey, Mellifera, 20(1):41-60.