TÜBİTAK

Turkish Journal of Zoology

http://journals.tubitak.gov.tr/zoology/

Research Article

Turk J Zool
(2020) 44: 114-125
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doi:10.3906/zoo-1911-16

The effects of feeding obese rats with bee bread on leptin and ghrelin expression

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Received: 12.11.2019 • **Accepted/Published Online:** 28.01.2020 • **Final Version:** 04.03.2020

Abstract: Obesity is an important public health problem and a risk factor for many chronic diseases, and its prevalence is increasing rapidly. In recent years there has been interest in the impact of nutrients on obesity and this has been addressed as a research topic. Bee bread is a bee product that has recently attracted attention with its macro- and micronutrients and the enzymes and biocomponents it contains. Although there are many studies on the biological properties and effects of bee bread, its effect on obesity with leptin and ghrelin has not been investigated. In this study, 40 Sprague-Dawley adult female rats were separated into 4 groups after they had become obese by having been fed with commercial high-fat food. While the 1st group was the control, the 2nd group was the obese control and bee bread was given to the 3rd and 4th groups (100 and 200 mg/kg bw/day), and in the 5th group metformin (300 mg/kg/day) was provided. Histopathological and immunohistochemical analysis was performed in hypothalamus tissues, and biochemical analyses were conducted of blood samples and hypothalamus tissues by ELISA methods. The apoptotic cell level was determined by the TUNEL method. The results of the immunohistochemical assessment of the hypothalamus tissues were consistent, and the levels of leptin and ghrelin were inversely correlated in the samples. Biochemical analysis showed that MDA levels increased with obesity whereas differences in SOD, CAT, and XOD levels were not statistically significant. Bee bread improved lipid peroxidation caused by obesity. It was shown that obesity caused apoptotic cell count increase and bee bread decreased it. Bee bread administration (200 mg/kg/day) had positive effects on weight control and other parameters.

Key words: Bee bread, leptin, ghrelin, obesity, apoptosis, oxidative stress

1. Introduction

Leptin and ghrelin are fasting and fullness hormones regulating the body weight by affecting the total calorie intake of the fasting-fullness mechanism (Cooper et al., 2010). Ghrelin/leptin concentrations are controlled with a "feedback" mechanism through Y neurons in the hypothalamus, and body weight is controlled in this way (Ozcan, 2009). While leptin is an appetite-suppressant peptide, ghrelin is an appetite-stimulant. They show opposite effects to each other in the regulation of energy balance (Yang et al., 2010), and they influence eating behavior and appetite by directly signaling to hypothalamic neuropeptide Y neurons (Zhang et al., 2016). Neuropeptide Y (NPY)-positive cells, located in the hypothalamic arcuate nucleus (ARC), are effect sites for both ghrelin and leptin. While ghrelin stimulates synthesis and secretion of NPY, leptin inhibits it (Parker and Bloom, 2012).

Diet comes to the fore in the treatment of obesity. Foods that are low in calories but have high nutritive value are gaining importance in the diet. For this reason, the desire for a natural and healthy diet is increasing every day. Bee products draw attention as an option for a natural and healthy diet. Bee bread mainly includes pollen, honey, and secretions of bees' salivary glands (Vasquez and Olofsson, 2009), and bees pack the components in the cells of the honeycomb, then secure the mixture with wax and honey (Barene et al., 2015). After this, pollen is subject to lactic fermentation in the environment of a bee nest. Fermented bee pollen is called bee bread (De Grandi-Hoffman et al., 2013; Fuenmayor et al., 2014; Kieliszek et al., 2018).

It is known that bee bread contains approximately 20% proteins, 3% lipids, 24%–35% carbohydrates, 3% minerals, and vitamins. Bee bread is composed of well-balanced proteins containing all essential amino acids, the full spectrum of vitamins (C, B1, B2, E, H, P, nicotinic acid, folic acid), pantothenic acid, pigments, and other biologically active compounds, like enzymes such as saccharase, amylase, phosphatases, flavonoids, carotenoids, and hormones. Bee bread also contains over

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25 different micro- and macroelements such as iron, calcium, phosphorus, potassium, copper, zinc, selenium, and magnesium (Nagai et al., 2005; Khalifa et al., 2019). It was reported that bee bread involves more reduced sugar than the pollen of the same plant, as well as vitamin K and microorganisms' digestive enzymes (Haydak, 1958). Bee bread is considered to be a beneficial food supplement. Therefore, in recent years, there has been significant attention paid to the use of bee bread to treat many illnesses. By means of the antimicrobial activity of bee bread, mold and fungus development are inhibited and thus the bee bread is protected better (Nagai et al., 2004). Although there are studies about the biological activities and chemical content of bee pollen having different botanic origins, studies on bee bread are in the early stages. In recent years, there has been significant interest in the use of bee bread to treat many illnesses (Khalifa et al., 2019) and it has been shown to exhibit antimicrobial (Veiga et al., 2017), antioxidant (Nakajima et al., 2009), anticancer (Liu et al., 2016), and antiinflammatory (Rimbach et al., 2017) activities. In addition, fatty acid contents of bee bread (Kaplan et al., 2016) and its antibacterial (Abouda et al., 2011) and antioxidant (Haydak, 1958; Bakour et al., 2017) activities are topics of studies in recent years. The antibacterial activity of samples of bee bread and bee pollen collected from Morocco was shown against bacteria including *E. coli*, *Staphylococcus aureus*, and *Bacillus cereus*, and it was reported that fresh bee pollen and bee bread showed higher antibacterial activity than dried ones (Abouda et al., 2011). It was also indicated that antioxidant activity was high in the samples in which water was used as a solvent from among hot water, water, and ethanol extracts of bee bread (Haydak, 1958). Bakour et al. (2017) indicated that bee bread has an important protective effect on aluminum-induced toxicity in rats with its antioxidant activity.

In addition to the expense of traditional pharmaceutical drugs used in obesity treatment, the toxicity caused as a result of their long-term use and their side effects have increased the need for exploring new alternatives. In this study, it was aimed to determine the effects of bee bread, known for being natural, rich in nutrients, and healthy, on the expression of leptin and ghrelin hormones in the hypothalamus tissue of obese experimental animals at histological, immunohistochemical, biochemical, and molecular levels.

2. Materials and methods

2.1. Chemicals

Bee bread was purchased from Nutral Therapy Company (Kayseri, Turkey). The nutrient contents of the bee bread are provided in Table 1. Ash, protein, carbohydrate, dietary fiber, and energy content of bee bread were determined in line with AOAC official methods (AOAC, 2005). Commercial high-fat food rations was used (60 kcal, fat diet%, T-58Y1-58126, TestDiet, UK). Metformin (1,1-dimethylbiguanide, hydrochloride) was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz sc - 202000A; Heidelberg, Germany).

Table 1. Nutritional contents of bee bread.

Nutritional elements	Content
Ash	8.14 g/100 g
Protein	13.56 g/100 g (N×6.25)
Carbohydrate	30.60 g/100 g
Dietary Fiber	18.18 g/100 g
Fat	21.69 g/100 g
Energy	408 kcal/100 g

2.2. Experimental design

The protocol of this study was approved by the Ethic Committees of Erciyes University (Protocol No: 15/67). Sprague-Dawley adult female rats, raised in the Erciyes University Experimental and Clinical Research Center (DEKAM), were used in the study. Rats, which were kept in cages, were fed and provided water in conditions of 21 °C and 12 h light/dark in normal day order. Forty female rats weighing 200–250 g were arranged into a control (n = 8) group and obesity groups (n = 32) before they were divided randomly into five equal groups. The rats that were chosen to become obese (n = 32) were fed with the high-fat diet, which was provided as formerly prepared, for 4 weeks, and their weights were measured weekly and recorded on a regular basis. The rats in the control group were fed with standard rat food and their weights were also recorded (n = 8). Rats that had weights 10%–25% above those in the control group were regarded as obese (Hariri and Thilbault, 2010), and they were grouped as below. The animals continued receiving the high-fat diet while bee bread and metformin practice were applied. Bee bread was purchased from Nutral Therapy Company (Kayseri, Turkey). The nutrient contents of the bee bread are provided in Table 1. Ash, protein, carbohydrate, dietary fiber, and energy content of the bee bread were determined in line with AOAC official methods (AOAC, 2005). Bee bread was given to rats by oral gavage after it was dissolved in 1 mL distilled water. There were 8 animals in each group, and bee bread (Eraslan et al., 2009) and metformin (Kim et al., 2006; Yan et al., 2015) were provided by oral gavage to groups fed the high-fat diet. Metformin is an agent that improves insulin sensitivity and is known to reduce body weight (Kim, 2006; Malin and Kashyap, 2014). For this reason, it was used as a positive control.

The experimental groups were as follows:

1st Group: Control: Group fed with standard rat food,

2nd Group: Group (obese) fed with high-fat diet,

3rd Group: 100 mg/kg/day bee bread (Eraslan et al., 2009),

4th Group: 200 mg/kg/day bee bread (Eraslan et al., 2009),

5th Group: 300 mg/kg/day metformin (Kim et al., 2006; Yan et al., 2015).

Following the completion of the experimental protocol, blood and hypothalamus tissue samples were collected from the rats, the weights of which were recorded, and they were then investigated according to the following methods, after decapitation under ketamine and xylazine anesthesia.

2.3. Histological analysis

Tissue samples were determined with 10% formaldehyde solution for being used in the histological examination. They were embedded in paraffin by performing routine tissue follow-up stages after the operation. Slices of 5–6 μm were prepared with polylysine and flat slides, taken from paraffin blocks, and then the prepared slides were left in a drying oven for a while with standard histological methods, and paraffin was later removed with xylene. Next, slices were diluted by being passed through a graded alcohol series. Slices were examined under an Olympus BX51 microscope with hematoxylin and eosin (H&E) staining to see the general histological structure.

2.4. Immunohistochemical analysis

The avidin-biotin-peroxidase method was conducted immunohistochemically to specify ghrelin immunoreactivity in hypothalamus tissue. Sections taken from paraffin blocks in 5 μm thickness were passed though xylene and a decreasingly graded alcohol series and left in a drying oven for 1 night at 60 °C. Hydrogen peroxide practice was conducted for the slices, which were treated with citrate buffer for antigen recovery after they were washed with distilled water and PBS. The following operations were carried out using the Large Volume Detection System Kit. Ghrelin as a primary antibody (sc - 50297) was administrated for one night at 4 °C after serum block practice was performed for 5 min for the thin sections. After the primary antibody and biotinylated secondary streptavidin-HRP and DAB chromogen practices, reverse staining was conducted with Gill's hematoxylin. Finally, they were passed through a series of gradually ascending alcohol and xylene and closed with Entellan. The ImageJ program was used for immunoreactivity measurements from the images obtained from the prepared samples. Sixty different sites, from each group, were assessed for measurement.

The avidin-biotin-peroxidase method was also administrated immunohistochemically to determine leptin immunoreactivity in hypothalamus tissue. Slices taken from paraffin blocks in 5 μm thickness were kept in a drying-oven for 1 night at 60 °C, and then they were passed through xylene and a decreasing graded alcohol series. Furthermore, they were treated with citrate buffer for antigen recovery after they were washed with distilled water. Hydrogen peroxide practice was performed after washing with PBS. The following operations were conducted using the Large Volume Detection System Kit. Later, a serum block practice was performed for 5 min. After serum blocking, leptin (A20) (sc - 842) was applied as a primary antibody at 4 °C for one night. Afterward primary antibody and biotinylated secondary streptavidin-HRP and DAB chromogen practices, reverse staining was conducted with Gill's hematoxylin. Lastly, they were passed through a series of gradually ascending alcohol and xylene and was closed by Entellan. The ImageJ program was used for immunoreactivity measurements from the images obtained from prepared samples. Sixty different sites from each group were assessed for measurement.

2.5. TUNEL method

Apoptotic cells in thin sections, obtained from the subjects, were determined by using a Roche In Situ Cell Detection Apoptosis Fluorescein Kit. The staining operation was conducted in line with the kit's specified procedure. After tissue sections, taken in 5 μm thickness, were first deparaffinized and then rehydrated, they were washed twice with PBS for 5 min and later kept at 0.01 M in 5% sodium citrate buffer in a microwave oven at 350 W for 5 min for antigen recovery. They were then left for cooling at room temperature for 10 min. Tissues, which were washed twice with PBS for 5 min, were incubated in a drying oven for 60 min and placed into a moisture chamber at 37 °C with the TUNEL reaction mixture that came with the kit. Reverse staining was administrated with 4',6-diamidino-2-phenylindole (DAPI) to tissues, which were washed twice for 5 min, and the DAPI solution was used as a closer for nuclear staining. Tissues, which were closed with glycerol closure solution, were viewed with an Olympus BX51 fluorescence microscope. Apoptotic cells were counted in the ImageJ program from the images with a 40× lens from each slice from fifteen different sites to calculate an apoptotic index.

2.6. Biochemical analysis

Serum and tissue samples, obtained from rats, were used for biochemical analysis. Superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), xanthine oxidase (XOD), ghrelin, and leptin levels were measured in serum and hypothalamus tissue. The protocol of the kits of the manufacturer was followed to specify the levels of Rat SOD (Sunred Bio, Cat. No: 201-11-0169), rat CAT (Sunred Bio, Cat. No: 201-11-5106), rat MDA (Sunred Bio, Cat. No: 201-11-0157), rat XOD (Sunred Bio, Cat. No:

201-11-7522), rat LEP (Sunred Bio, Cat. No: 201-11-0562), and rat ghrelin (Cat. No: EZRGRT-91K). The results were measured with an ELISA reader device at 450 nm, and they were provided as ng/mL for SOD, CAT, XOD, and ghrelin and as nmol/mL for MDA and pg/mL for LEP.

2.7. Statistical analysis

SPSS 22 was used for statistical analysis. Results are shown as mean ± standard deviation. The Kolmogorov–Smirnov test was used to identify the normal distribution of data. Intergroup comparison was performed with one-way ANOVA tests, while the post hoc Tukey test was conducted for binary comparisons. Values of $P < 0.05$ were considered as statistically significant.

3. Results

3.1. Weight

Animal weights at the beginning of the experiment are shown in Table 2. There was no statistically significant difference between the initial weights of rats ($P > 0.05$). The rats (Groups 2–5) that were selected to become obese were fed with a high-fat diet, which was provided commercially, for 4 weeks, and their weights were also recorded weekly on a regular basis. Meanwhile, the rats in the control group were fed with standard rat food during this time and their weights were recorded. The rats whose weights increased by 10%–25% compared to the control group were regarded as obese (Hariri and Thilbault, 2010) (Table 2). In this manner, similar proportions of obesity were observed in all rats, apart from the ones outside the control group, at the end of the 4-week feeding process.

The latest recorded weights of obese rats at the end of 4 weeks are shown in Table 2. Although there was not a statistically significant difference, the weights of rats fed with the high-fat diet were numerically highest compared to the other groups.

The groups with the highest weight loss were Group 4, to which 200 mg/kg/day bee bread was provided, and Group 5, to which 300 mg/kg/day metformin was provided.

3.2. Histological and immunohistochemical analysis

The general structure of sites in the hypothalamus tissue is shown in Figure 1 with hematoxylin and eosin staining. While a normal structure is observed in the control group and Groups 3–5, structural irregularity is seen in the hypothalamus in Group 2. Histopathological differences were not witnessed in cells among the groups.

Immunoreactivity values with ghrelin and leptin in hypothalamus tissue were assessed among groups and the mean and standard deviation values are given in Table 3.

Ghrelin immunoreactivity decreased by a statistically significant amount in Groups 2–5 compared to Group 1 (Figure 2A). A statistically significant increase was

Table 2. Body weights of experimental groups.

Group	Group 1	Group 2	Group 3	Group 4	Group 5	P
Weight at beginning of experiment	190.75 ± 4.26	190.14 ± 4.46	188.28 ± 7.93	189.11 ± 9.36	189.25 ± 10.74	0.876
Weight change %	14.01 ± 3.274[a]	31 ± 9.63[b]	33.25 ± 7.34[b]	27.65 ± 2.26[b]	32.10 ± 6.79[b]	0.05
End of the experiment	226.00 ± 12.55	264.37 ± 36.81	239.75 ± 28.14	231.75 ± 31.12	228.50 ± 27.39	0.067

Group 1: Control - standard rat food; Group 2: Obese - high-fat diet; Group 3: High-fat diet and 100 mg bee bread; Group 4: High-fat diet and 200 mg bee bread; Group 5: High-fat diet and 300 mg metformin. Data are shown as ±standard deviation. $P < 0.05$ was accepted as significant. There is no statistically significant difference among groups marked by the same superscripted letters.

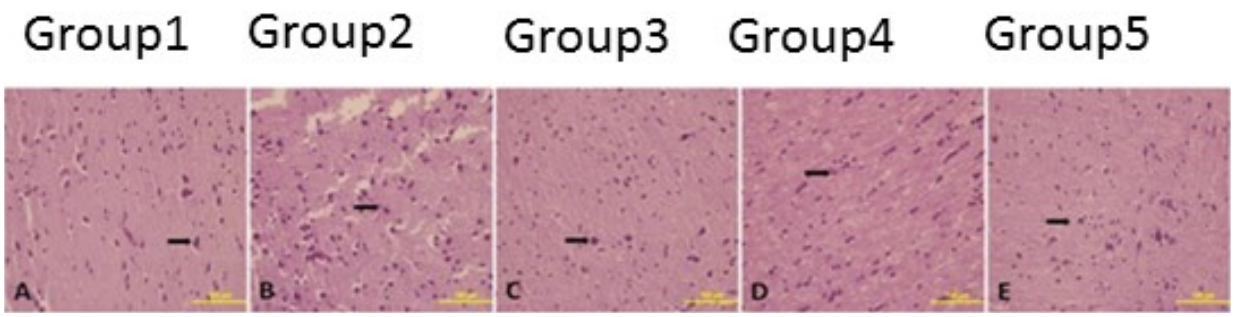


Figure 1. Cells, stained with hematoxylin and eosin in hypothalamus tissue are shown with black arrows. Image zoom 400×. A: Group 1 (fed with standard rat food), B: Group 2 (obese - fed with high-fat diet), C: Group 3 (high-fat diet and 100 mg/kg/day bee bread), D: Group 4 (high-fat diet and 200 mg/kg/day bee bread), E: Group 5 (high-fat diet and 300 mg/kg/day metformin).

observed in other groups compared to the control group when considering leptin immunoreactivity results ($P < 0.01$) (Figure 2B).

3.3. TUNEL analysis

Apoptotic cell numbers in hypothalamus tissue were assessed with the TUNEL method (Table 4; Figure 3). It was detected that apoptotic cell numbers increased statistically significantly in Group 2 compared to the other 4 groups (Table 4; Figure 3).

3.4. Biochemical analysis

The difference between the groups was statistically significant regarding SOD activity ($P < 0.001$). While there was no difference among the control, the obese control, and the group to which 100 mg/kg bee bread was provided, the SOD activity of the groups to which 200 mg/kg bee bread and metformin were given was higher than the other groups. The SOD activities of the groups to which 200 mg/kg bee bread and metformin were given were found as similar. There was no statistically significant difference between the groups in terms of CAT and XOD activities. However, even if there was no statistical difference between the groups regarding MDA level, the fall in MDA levels for the groups to which 200 mg/kg bee bread and metformin were given was remarkable when compared numerically to the obese control group (Table 5).

Ghrelin amounts in Group 2, 3, and 4 decreased statistically significantly compared to Group 1. Although an increase was observed in the leptin amount for Groups 2, 3, 4, and 5 compared to Group 1, a statistically significant difference was only found between the obese

Table 3. Immunoreactivity values of ghrelin and leptin in hypothalamus tissue.

Group	Group 1	Group 2	Group 3	Group 4	Group 5	P
Ghrelin	82.37 ± 6.03^{a}	78.44 ± 2.42^{b}	78.84 ± 3.03^{b}	78.92 ± 3.62^{b}	80.02 ± 3.38^{b}	0.001
Leptin	90.264 ± 3.48^{a}	93.66 ± 1.74^{b}	92.91 ± 3.45^{b}	92.69 ± 3.20^{b}	92.210 ± 2.98^{b}	0.001

Group 1: Control - standard rat food; Group 2: Obese - high-fat diet; Group 3: High-fat diet and 100 mg bee bread; Group 4: High-fat diet and 200 mg bee bread; Group 5: High-fat diet and 300 mg metformin. Data are shown as ±standard deviation. $P < 0.05$ was accepted as significant. There is no statistically significant difference among groups marked by the same superscripted letters.

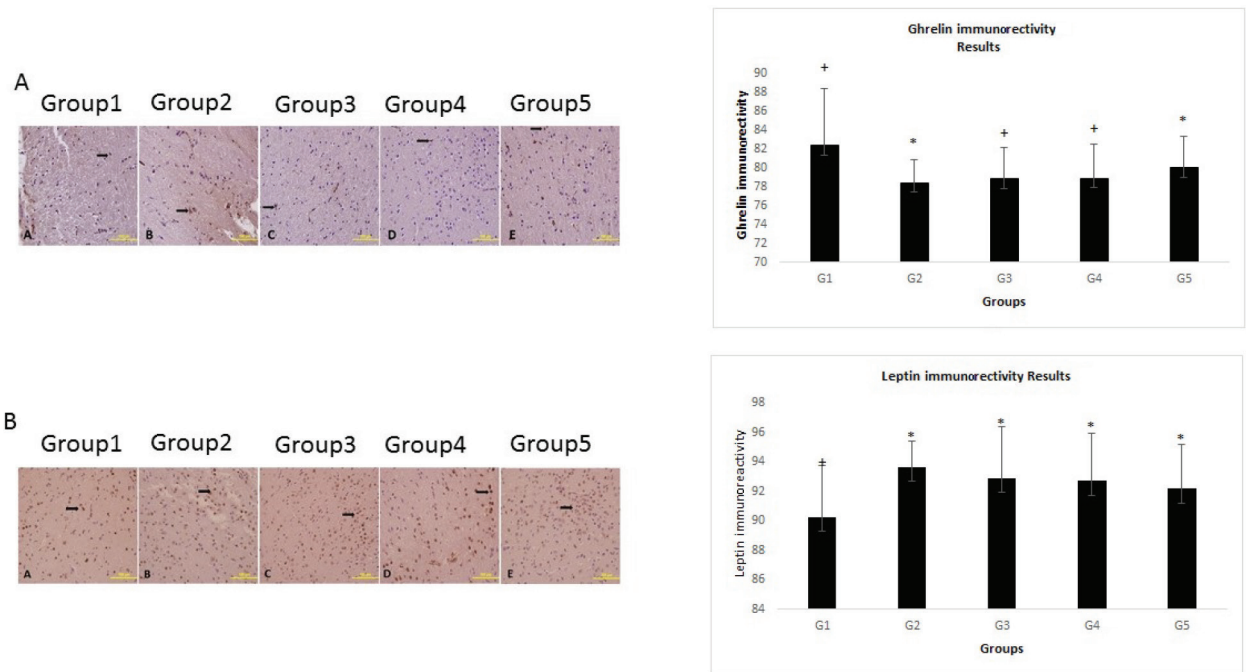


Figure 2. Cells stained with ghrelin (A) and leptin (B) in hypothalamus tissue are shown with black arrows. A: Group 1 (fed with standard rat food), B: Group 2 (obese - fed with high-fat diet), C: Group 3 (high-fat diet and 100 mg/kg/day bee bread), D: Group 4 (high-fat diet and 200 mg/kg/day bee bread), E: Group 5 (high-fat diet and 300 mg/kg/day metformin). Image zoom 400×.

Table 4. Comparison of TUNEL results in groups.

Group	Group 1	Group 2	Group 3	Group 4	Group 5	P
TUNEL	0.36 ± 0.75[a]	1.03 ± 1.88[b]	0.33 ± 0.62[a]	0.43 ± 0.88[a]	0.25 ± 0.65[a]	0.001

Group 1: Control - standard rat food; Group 2: Obese - high-fat diet; Group 3: High-fat diet and 100 mg bee bread; Group 4: High-fat diet and 200 mg bee bread; Group 5: High-fat diet and 300 mg metformin. Data are shown as ±standard deviation. $P < 0.05$ was accepted as significant. There is no statistically significant difference among groups marked by the same superscripted letters.

control group and the group to which 100 mg/kg bee bread was administrated (Table 5). The results were very close to each other in the groups to which 200 mg/kg bee bread and metformin were administered.

SOD increased in Group 4 and Group 5 according to the biochemical analyses performed for antioxidant capacity in the hypothalamus tissue of rats. No statistically significant difference was observed in CAT activity among the groups. An increase was seen in the obese groups compared to the control in terms of MDA levels. Groups 3, 4, and 5 showed a statistically significant decrease compared to Group 2 ($P < 0.001$). There was no statistically significant difference among the groups with respect to tissue XOD activity (Table 6).

Ghrelin levels were highest in the control group. When comparing the groups fed with the high-fat diet, there was no difference between the group in which no treatment was applied and the group to which 100 mg/kg bee bread was given, but the ghrelin level was high in groups to which 200 mg/kg bee bread and metformin were provided.

No difference was observed between ghrelin levels of the groups to which 200 mg/kg bee bread and metformin were given. No statistically significant difference was seen between the groups regarding leptin hormone levels ($P > 0.001$). However, while leptin levels were numerically lowest in the normal control group, leptin levels of the groups to which 200 mg/kg bee bread and metformin were given were higher than those of the control obese group and the group to which 100 mg/kg bee bread was administrated, similar to ghrelin levels (Tables 5 and 6).

4. Discussion

Obesity is one of the most important health problems of the modern age. Many of the methods developed to treat this health problem are related to dietary substances. Bee products are accordingly becoming increasingly important as a natural and healthy diet option. Since ancient times, bee bread has been used in different cultures for several nutritional and therapeutic purposes. Different biological effects and dietary properties have been attributed to bee bread, including antimicrobial, antioxidant, anticancer, and antiinflammatory activities (Khalifa et al., 2019). The chemical content of bee bread has been revealed in various studies (Nagai et al., 2005; Kaplan et al., 2016). Bee bread has a much better composition than many animal protein products due to the presence of all the essential amino acids and significant amounts of proteins, vitamins, and phenolic compounds such as natural antioxidants (Nagai et al., 2005; Abouda et al., 2011). Bee bread is reported to help regulate lipid metabolism (Nagai et al., 2004). Unsaturated fatty acids (FAs) have many beneficial health effects, such as the reduction of triglyceride (Von Schacky and Harris, 2007) and cholesterol levels in the blood (Simopoulos, 2004). Polyunsaturated FAs are required for the body to function. The ratio of unsaturated/saturated FAs in bee bread varies between 1.38% and 2.39%, suggesting that bee bread can be used as a good source of unsaturated FAs (Kaplan et al., 2016). Because of all these features, we investigated the effects of bee bread on obesity based on the hypothesis that bee bread can play a therapeutic role in the treatment of obesity.

In this study, after completing an experimental protocol in obese rats, it was concluded that although there was no statistically significant difference among the groups according to the performed weight measurement results ($P > 0.05$), the weights of animals were highest in the obesity group (Group 2), and the highest weight loss was seen in Group 4, to which 200 mg/kg/day bee bread was given, and Group 5, in which metformin was administrated. According to the obtained results, bee bread supplementation did not lead to weight gain in rats. A dose of 200 mg/kg/day showed a weight-reducing effect, though it was not as effective as metformin. Bee bread, which is the fermented state of bee pollen, contains important nutrients such as proteins, amino acids, lipids, minerals, carbohydrates, and vitamins (Campos et al., 2003). It was indicated that bee bread increased growth performance and feed intake in rabbits, and it could be beneficial for feed conversion rates (Attia et al., 2011). In another study, it was determined that a fresh bee pollen formula had useful biological activities such as the recovery of muscle protein and energy metabolism in rats that suffered from severe food restrictions. Results obtained from prior studies revealed that fresh bee pollen has good anabolic

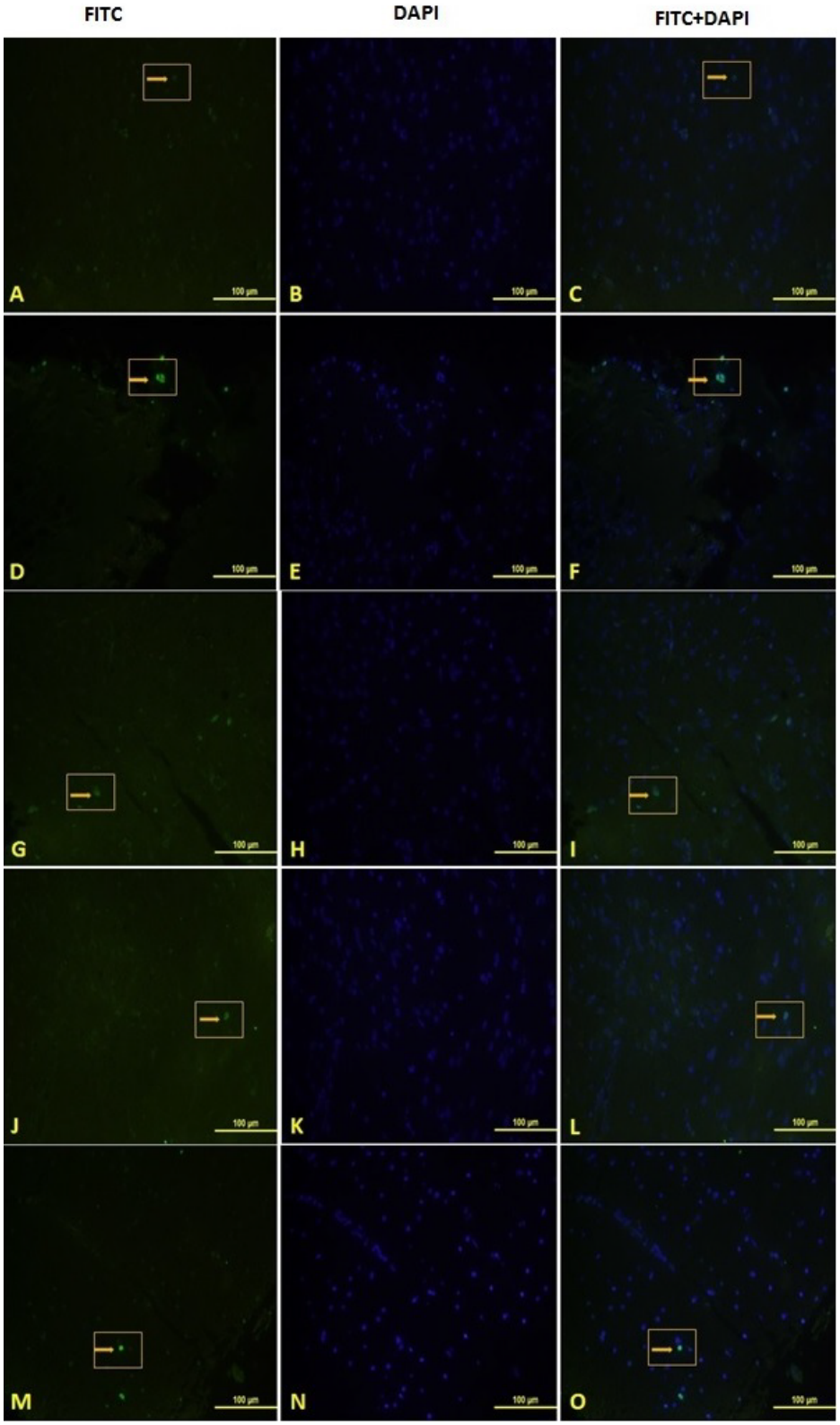


Figure 3. Cells stained as TUNEL-positive in hypothalamus tissue in Group 1 (fed with standard rat food) (A–C), Group 2 (obese group - fed with high-fat diet (D–F), Group 3 (high-fat diet and 100 mg/kg/day bee bread) (G–I), Group 4 (high-fat diet and 200 mg/kg/day bee bread) (J–L), and Group 5 (high-fat diet and 300 mg/kg/day metformin) (M–O) are shown with yellow arrows. Image zoom 400×.

Table 5. Results of biochemical analysis of blood serum.

Group	Group 1	Group 2	Group 3	Group 4	Group 5	P
SOD	5.57 ± 3.60[a]	5.24 ± 2.38[a]	7.06 ± 1.97[a]	21.65 ± 4.39[b]	21.47 ± 7.45[b]	0.001
CAT	19.93 ± 3.02[a]	22.58 ± 6.78[a]	23.30 ± 5.79[a]	26.87 ± 8.44[a]	30.36 ± 13.97[a]	0.194
MDA	4.17 ± 2.04	6.86 ± 0.72	4.40 ± 1.68	5.25 ± 2.44	5.77 ± 2.71	0.123
XOD	5.74 ± 1.97	6.11 ± 2.34	5.92 ± 1.80	5.36 ± 1.94	4.17 ± 1.85	0.668
Ghrelin	1.90 ± 0.60[a]	0.76 ± 0.09[b]	0.87 ± 0.33[b]	1.22 ± 0.30[bc]	1.4 ± 0.45[ac]	0.001
Leptin	187.17 ± 115.35[a]	360.71 ± 32.32[bc]	357.92 ± 31.57[bc]	296.54 ± 56.16[ac]	252.95 ± 60.52[ac]	0.002

Group 1: Control - standard rat food; Group 2: Obese - high-fat diet; Group 3: High-fat diet and 100 mg bee bread; Group 4: High-fat diet and 200 mg bee bread; Group 5: High-fat diet and 300 mg metformin. Data are shown as ±standard deviation. $P < 0.05$ was accepted as significant. There is no statistically significant difference among groups marked by the same superscripted letters.

Table 6. Results of biochemical analysis of hypothalamus tissue.

Group	Group 1	Group 2	Group 3	Group 4	Group 5	P
SOD	31.90 ± 6.09[a]	27.78 ± 14.29[a]	25.85 ± 4.15[a]	32.83 ± 3.50[a]	30.34 ± 7.02[a]	0.720
CAT	58.31 ± 17.02[a]	40.05 ± 13.55[a]	48.02 ± 15.37[a]	45.67 ± 12.03[a]	49.03 ± 10.74[a]	0.485
MDA	7.83 ± 1.31[ab]	13.33 ± 4.03[a]	3.00 ± 2.95[b]	6.94 ± 3.62[b]	6.92 ± 1.56[b]	0.003
XOD	14.60 ± 1.71	12.77 ± 2.21	9.83 ± 3.27	11.87 ± 2.67	9.17 ± 2.98	0.064
Ghrelin	2.03 ± 0.59[a]	0.32 ± 0.20[bc]	0.98 ± 0.73[ac]	1.21 ± 1.06[ac]	1.48 ± 0.38[ac]	0.032
Leptin	353.40 ± 37.55[a]	428.17 ± 55.97[b]	314.82 ± 8.92[a]	353.90 ± 20.17[a]	381.62 ± 39.42[ab]	0.007

Group 1: Control - standard rat food; Group 2: Obese - high-fat diet; Group 3: High-fat diet and 100 mg bee bread; Group 4: High-fat diet and 200 mg bee bread; Group 5: High-fat diet and 300 mg metformin. Data are shown as ±standard deviation. $P < 0.05$ was accepted as significant. There is no statistically significant difference among groups marked by the same superscripted letters.

and metabolic activity, and it may be beneficial in the prevention or recovery of malnutrition as it has good anabolic and metabolic activity (Salles et al., 2014).

Obesity, which is a significant risk factor for the development of diabetes mellitus, cardiovascular diseases, and some cancer types, generally originates from a chronic positive energy balance due to an increasingly static lifestyle with genetic and epigenetic background and unlimited access to food (Cui et al., 2017). The ability of the hypothalamus to control the energy balance is compromised and degraded in many obese individuals (Williams, 2012). There is a mechanical connection between overeating, especially for long-chain FAs with inflammatory responses, which can reduce the running of the hypothalamus in obese individuals, and systemic formation (Williams, 2012; de Git and Adan, 2015). Ghrelin is produced by gastric mucosa, which mainly stimulates the appetite, leading to body weight increase and causing a positive energy balance (Kirsz and Zieba, 2011). Leptin is basically secreted by adipocytes of white adipose tissue and it may diminish the appetite, and both of them can also decrease fat formulation and deposition, which are closely related to the formation of type 2 diabetes mellitus (Zhang et al., 2013). Furthermore, NPY-positive cells in the hypothalamic ARC are effective sites both for ghrelin and leptin. While ghrelin stimulates synthesis and secretion of NPY, leptin inhibits it (Parker and Bloom, 2012). However, changes in hypothalamic NPY content and its relationship with insulin, leptin, and ghrelin levels has not yet been reported, along with its correlation to the potential mechanism underlying their interactions during the development of the type 2 diabetes mellitus rat model. The NPY system is accepted as the last mutual pathway for expression of appetite in the hypothalamus. Leptin is an appetite-suppressant peptide while ghrelin is an appetite-stimulant. They show opposite effects in the regulation of energy balance (Yang et al., 2010), and they regulate appetite and eating behavior by giving signals directly to hypothalamic NPY neurons (Zhang et al., 2013). In the study by Zhang et al. (2013), they investigated the change of NPY in the hypothalamus and its correlations with insulin, leptin, and ghrelin in the development of a type 2 diabetes

mellitus rat model; levels of both fasting insulin and leptin serum (ng/mL) significantly increased and fasting plasma ghrelin concentration considerably reduced its content of hypothalamic NPY (pg/mg) during the development of the type 2 diabetes mellitus rat model. Hypothalamic NPY concentration showed a positive correlation with changes in serum insulin and leptin, and a negative correlation with plasma ghrelin. The same researchers also indicated in another study that serum leptin concentrations and adipocyte leptin production increased significantly with the gain in body weight of animals while their serum and gastric ghrelin levels decreased significantly with obesity (Zhang et al., 2013). As has been stated before, many studies reported that the average serum ghrelin level is generally lower in obese patients compared to thin individuals (Abdemur et al., 2014). Metformin is an agent taken orally as an adjuvant of insulin sensitivity. It not only inhibits the production of hepatic glucose; it also increases the effects of insulin on glucose uptake in the skeletal muscle and adipocytes, and it diminishes the absorption of glucose from the intestines. Although its mechanism of action is still not fully explained, it is known that metformin decreases body weight (Campos et al., 2003; Kim, 2006; Malin and Kashyap, 2014). In addition, instead of using drugs for the treatment of obesity, healthy eating and physical activity are recommended. Therefore, metformin was chosen as a positive control since it is not a conventional drug for treatment. Our study, in line with the related literature, revealed that the amount of ghrelin was diminished in both serum and hypothalamus tissue in the obesity group, and the levels of tissue and serum leptin increased. It was observed that metformin and 200 mg/kg bee bread relieved these impacts.

As it is known in accordance with prior studies that obesity leads to oxidative stress, serum SOD and CAT activities increased in Groups 3, 4, and 5 compared to the control group as the result of analysis of enzymes that are oxidative stress parameters, but this rise was not found as statistically significant ($P > 0.05$). When evaluating ELISA results of tissue samples, no statistically significant difference was seen in SOD activity ($P > 0.05$). CAT activity decreased in Groups 2, 3, 4, and 5 compared to the control group. When evaluating MDA results, an increase was witnessed in Group 2 compared to the control group, and it diminished statistically significantly in Groups 3, 4, and 5 compared to Group 2 ($P < 0.05$). No statistically significant difference ($P > 0.05$) was noted between blood or tissue samples in terms of XOD activity ($P > 0.05$).

A high-fat diet triggers reactions including an increase in oxidative stress reagents, inflammation, endoplasmic reticulum (ER) stress, and autophagy defects and changes in apoptosis and neuronal regeneration rate in the hypothalamus (de Git and Adan, 2015). In many of the studies conducted on obese patients, it has been reported that oxidative stress reagents increased in the body, and antioxidant defense enzymes decreased, and as a result, obesity led to inflammation and chronic oxidative stress in the body (Vincent and Taylor, 2006). Increasing oxidative stress in obesity was put forward as the primary reason for tissue and function disorders (endothelial dysfunction, increased platelet aggregation, atherogenesis, etc.) that appeared in those patients (Uzun et al., 2004). It was indicated that induction of hypertriglyceridemia in rats increased with mitochondrial respiration in the hypothalamus in parallel with the rise in ROS production (Benani et al., 2007). It was also specified that ROS is significant in both glucose and lipid detection by the hypothalamus (Leloup et al., 2006), and ROS, increased in the hypothalamus of rats, had a correlation with abnormal glucose sensitivity (Colombani et al., 2009). Regulation of the melanocortin system in the hypothalamus requires ROS as an acute effect of its stimulation by POMC (proopiomelanocortin) neurons, and it also causes reduction of food intake, while suppression of ROS leads to activation of AgRP (agouti-related peptide)/NPY neurons and increased nutrition (Schrader and Fahimi, 2006). It was also reported that diabetic and high-fat diets evoke central leptin resistance and an inflammatory reaction in the hypothalamus that promotes the development of obesity (de Git and Adan, 2015).

Obesity is associated with increased lipid peroxidation; MDA is a biological reagent reflecting the level of lipid peroxidation. It was stated that MDA levels seemed to have a positive correlation in obese and nonobese healthy individuals with body mass index (Yilmaz et al., 2007). Increased lipid peroxidation in obesity may compromise permeability as it affects the structure and integrity of cell membranes. Proinflammatory cytokines, secreted from increased fat tissue in obesity, cause lipid peroxidation by producing high amounts of free oxygen radicals (Trayhurn and Wood, 2004). Growing epidemiological evidence indicates that metformin, which is the most appropriate first-step antidiabetic drug, reduces the incidence and severity of stroke. In addition, a clinical study found that metformin decreased oxidative stress by diminishing ROS production and developing the antioxidant reserve (Esteghamati et al., 2013). Al-Osaimi et al. (2018) aimed to assess the therapeutic and protective effects of pollen in improving the toxic effects of MeHg by measuring biochemical parameters selected due to oxidative stress in the brain homogenates of newborn male offspring, energy metabolism, and neurotransmission. They found that while MeHg administration increased lipid peroxidation and catalase activity, it decreased the levels of glutathione in an insignificant manner. Bee pollen administration was quite effective in both normalization of Mg^{2+}, K^{+}, lipid

peroxidation, and glutathione levels and the recovery of the activities of catalase, lactic dehydrogenase, and creatine kinase. Moreover, it was concluded that the oxidative stress of bee pollen could be used safely to improve metal ion defects and neuronal death along with weak detoxification, and it is a critical mechanism in the etiology of multiple neurological disorders. In another study Mohamed et al. (2018) indicated that both bee pollen and palm pollen had antioxidant and antihyperglycemic effects. Bakour et al. (2017) specified that bee bread has an important protective effect in rats against aluminum-induced toxicity by exerting antioxidant activity. In recent years, the potential for antioxidant usage, especially natural antioxidants, has drawn much interest. Antioxidants may have a protective effect in the prevention of aging and heart and liver diseases, or in reducing their severity. This protective effect is attributed to their ability of combatting the ROS produced during oxidative stress. Therefore, it is well known that antioxidants are beneficial in protecting cellular components against oxidative damage.

It is also known that obesity causes apoptosis (Li et al., 2018). In an investigation carried out by Sa-nguanmoo et al. (2018), it was detected that obesity insulin resistance developed with an increase in systemic inflammation, brain mitochondrial dysfunction, rise in brain apoptosis, and disruption and cognitive reduction in hippocampal plasticity in rats fed a high-fat diet. Chunchai et al. (2018) indicated that long-term high-fat diet consumption induced metabolic disruption, cognitive disruption, glial morphological changes, increased hippocampal oxidative stress, and cell apoptosis in both the hippocampus and cortex, and obesity. When assessing the apoptosis results of our study, it was witnessed that it was effective in the groups to which bee bread was given upon statistically increasing apoptosis in the obese groups compared to both the control and metformin groups. Kolesarova et al. (2013) reported the positive effects of bee pollen on ovarian cell apoptosis and proliferation. According to the study carried out by Huang et al. (Huang et al., 2017), it was put forward that *Schisandra chinensis* bee pollen extract decreased oxidative stress levels, and it may diminish liver and kidney injury caused by cisplatin by increasing the antioxidant, antiinflammatory, and antiapoptotic capacity of the body.

In addition to this, antiproliferative and antiapoptotic properties of metformin, which we used as a positive control in our study, were indicated in various studies (Jia et al., 2015; Lai et al., 2018). It is known today that ROS and oxidative stress emerging as a result of ROS play a significant role in apoptosis, while antioxidants can delay or prevent this process (Kannan and Jain, 2004).

For this reason, the activity of bee products, which have strong antioxidant activity, should be taken into consideration. The role of bee products, which provide versatile support with their natural and beneficial biological effects to promote treatment of important diseases such as obesity, must be revealed. In this study, the effect of bee bread on obesity was attempted to be assessed with applications of two different doses. We believe that this study will be useful for future studies concerning the determination of the role of bee products in the treatment of obesity. As a result of this study, it is thought that bee bread will have advantages compared to traditional pharmaceuticals or complementary components for nanoparticle production since it has therapeutic potential in obesity and other metabolic processes associated with it. In addition, rationally designed bee products can be utilized for the treatment of many diseases, including cancer, with future research and developing technology.

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