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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

ARI EKMEĞİNİN ALZHEİMER SIÇAN MODELİNDE KARACİĞER 5HT2B ARACILI GLUKOZ DÜZENLEMESİ ÜZERİNE ETKİSİ

EFFECT OF BEE BREAD ON LIVER 5HT2B-MEDIATED GLUCOSE REGULATION IN ALZHEIMER'S RAT MODEL

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ÖZET

AMAÇ: Bu çalışmada Alzheimer hastalığının (AH) sıçan modelinde arı ekmeğinin insülin, serotonin (5-hidroksitriptamin, 5-HT) ve leptin hormonlarında meydana getireceği değişimin glukoz regülasyonu ve kilo değişimi üzerindeki etkisinin incelenmesi amaçlanmıştır.

GEREÇ VE YÖNTEM: Alzheimer hastalığı sıçan modeli, lateral ventriküllere intraserebroventriküler (i.c.v.) Streptozotosin (STZ) enjeksiyonu yoluyla oluşturuldu. Arı ekmeği uygulaması, STZ enjeksiyonundan sonra 3 hafta boyunca oral gavaj ile gerçekleştirildi. Plazmada leptin, insülin, 5-HT düzeyleri ile karaciğer dokusunda leptin, insülin, 5-HT, 5HT reseptör 2B (5HT2B), glukoz taşıyıcı 2 (GLUT2), glukoz 6-fosfataz (G6paz) düzeyleri Elisa kit ile ölçüldü. Açlık kan glukoz düzeyleri glukometre kullanılarak ölçüldü ve İnsülin Direnci İçin Homeostatik Model Değerlendirmesi (HOMA-IR) düzeyleri formül kullanılarak hesaplandı. Her bir sıçanın ağırlık değişimi, başlangıç ağırlıklarının son ağırlıklarından çıkarılmasıyla hesaplandı.

BULGULAR: AH grubunda bulunan sıçanların açlık kan glukoz, plazma insülin ve HOMA-IR düzeyleri ile karaciğer 5-HT, plazma 5-HT ve leptin düzeylerinin azaldığı, karaciğer 5-HT2B ve GLUT-2 düzeyleri ile kilo kaybının arttığı görüldü. Arı ekmeği tedavisinin bu hayvanlarda karaciğer 5-HT2B, G6paz düzeyleri ve plazma leptin düzeylerini önemli ölçüde artırdığı, ayrıca plazma 5-HT, karaciğer 5-HT ve GLUT-2 düzeyleri ile kilo kaybını belirgin şekilde artırdığı görüldü. Ayrıca arı ekmeğinin plazma insülin düzeyini etkilemeden açlık kan glukoz düzeylerini azalttığı saptandı.

SONUÇ: Bu sonuçlar, AH grubundaki sıçanların karaciğer dokusunda glukoz metabolizmasının anti-diyabetik savunma sistemi oluşturacak şekilde modüle edildiğini gösterdi. Arı ekmeği uygulamasının Alzheimer oluşturulmuş sıçanlarda leptin aracılı insülin duyarlılığını artırarak açlık kan glukoz düzeylerini azalttığı saptandı.

ANAHTAR KELİMELER: Alzheimer hastalığı, Karaciğer, Arı Ekmeği, 5-HT2B, Leptin.

ABSTRACT

OBJECTIVE: This study aimed to examine the effect of bee bread on glucose regulation and weight change through the change of insulin, serotonin (5-hydroxytryptamine, 5-HT), and leptin hormones in the rat model of Alzheimer's disease (AD).

MATERIAL AND METHODS: Alzheimer's disease rat model created via intracerebroventricular (i.c.v.) Streptozotocin (STZ) injection into the lateral ventricles. Beebread administration was performed with daily gavage for three weeks after the STZ injection. Leptin, İnsulin, 5-HT levels in plasma and leptin, insulin, 5-HT, 5HT receptor 2B (5HT2B), glucose transporter 2 (GLUT2), glucose 6-phosphatase (G6pase) levels in liver tissue were measured with Elisa kit. Fasting blood glucose levels were measured using a glucometer, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) levels were calculated using the formula. Each rat's weight change was calculated by subtracting their initial weight from their final weight.

RESULTS: In the AD-created rats, ıt was observed that blood glucose, plasma insulin, and HOMA-IR levels, liver 5-HT, plasma 5-HT, and leptin levels decreased, liver 5-HT2B and GLUT-2, and weight loss increased. In the AD-created rats, bee bread treatment significantly increased liver 5-HT2B, liver G6pase levels, and plasma leptin levels, also markedly increased plasma 5-HT, liver 5-HT, GLUT-2, and weight loss levels, and decreased fasting blood glucose levels without affecting plasma insülin levels in the AD group.

CONCLUSIONS: These results showed that glucose metabolism was modulated to generate an anti-diabetic defense system in the liver tissue of AD-created rats. Beebread administration reduced fasting blood glucose levels by increasing leptin-mediated insulin sensitivity in the AD-created rats.

KEYWORDS: Alzheimer's disease, Liver, Bee Bread, 5-HT2B, Leptin.

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INTRODUCTION

Alzheimer's disease (AD) is characterized by accumulations of extracellular amyloid β-peptides (Aβ) and intracellular hyperphosphorylated tau (phospho-tau) proteins (1, 2). Aβ production is a physiological process, and the imbalance between Aβ production and clearance is essential in AD development. Recent evidence showed that most of the brain Aβ in the brain can be transported to the periphery (3), and most Aβ is cleared in the liver. Therefore, the first organ affected by AD pathology is the liver. The liver is the primary organ for maintaining peripheral glucose metabolism (4). Disruption of peripheral glucose metabolism leads to glucose metabolism disruption in the central nervous system and increased Aβ toxicity, Tau hyperphosphorylation, oxidative stress, neuroinflammation, and neurodegeneration (5). Therefore, well-coordinated hepatic glucose metabolism is crucial for health. Control of hepatic glucose metabolism is achieved by a complex integration of hormones produced in various tissues (6).

Serotonin (5-hydroxytryptamine or 5-HT) is derived from tryptophan. There are two separate 5-HT pools in the body, both central and peripheral (7). Peripheral 5-HT has complex effects on peripheral glucose regulation (8). Peripheral 5-HT encourages gluconeogenesis by fructose 1,6-bisphosphatase (FBPase) and glucose 6-phosphatase (G6pase), which are rate-limiting enzymes in gluconeogenesis through HTR2B during fasting in hepatocytes. In addition, 5HTR2B signaling promotes the degradation of glucose transporter 2 (GLUT2) in hepatocytes and prevents glucose uptake (9). Additionally, increased food intake increases peripheral 5-HT production, resulting in insulin secretion and leptin release. Leptin both increases insulin sensitivity in skeletal muscle and increases glucose uptake, glucose oxidation, and glycogen synthesis with insulin-like effects (10). It also acts as a key regulator of body weight and fat stores by modulating food intake and metabolism (11). However, Muck-Seler D et al. show that low platelet 5-HT concentration in the late stage of AD may indicate the severity and/or clinical progression of AD (12). Thus, modulation of the 5HT network may be an approach to ensuring peripheral and central glucose regulation in Alzheimer's disease.

Bee bread (Perga) is a fermented form of pollen collected by the honey bee and stored in the honeycomb (13). A recent study found that bee bread affected glycemic conditions, lipid profile, and hepatic functions in diabetic rats (14). It is thought that the rich nutrient content and positive health effects of bee bread, which is a functional food (13), will have an important impact on AD. In this study, we propose to explore the effects of 5-HT-5HT2B on body weight and hepatic glucose metabolism in the AD rat model. We also investigate whether bee bread can impact these changes.

MATERIALS AND METHODS

Animals and Treatment

Male albino Wistar rats aged three months were used throughout all experiments. During all experimental processes, animals were housed in steel cages under standard conditions and given standard rat chow and tap water ad libitum (humidity 50 ± 5% and 23 ± 1 °C) with 12:12 h light-dark cycles at all times. Streptozotosin (STZ, (2-deoxy-2(([methyl(nitroso)amino]carbonyl)amino)-(α and β)-D-glucopyranose) was purchased from Santa Cruz Biotechnology (catalog number: sc-200719). STZ solutions used in our study were prepared depending on previous studies. First, the body weight of animals was measured and noted. Then, the amount of STZ required for each rat was weighed individually in microcentrifuge tubes according to body mass. For example, 1.05 mg of STZ should be weighed and then diluted in 6 µl citrate buffer (0,05M) for a 350 g rat. This prepared solution corresponds to a dose of 2 mg/kg (15). Bee bread was purchased from Nutral Therapy (Nutral Therapy, Kayseri, Türkiye). The bee bread solution used in our study was prepared using capsules containing 800 mg of bee bread. Bee bread capsules are water-soluble. A bee bread capsule was dissolved in 20 ml of tap water to obtain a bee bread solution at a concentration of 40 mg/ml. The weight of each rat was determined, and the bee bread solution was administered by oral gava-

ge. Bee bread solutions used in our study were prepared based on previous studies (13, 16, 17).

Experimental Procedure

The animals were randomly divided into four groups (n = 6 for each group). Sham group (SH) received 2 µl citrate buffer (2µl/ventricle) via intracerebroventricularly (i.c.v) injection. On the 7th day, tap water was given by oral gavage for 21 days; (2) Bee bread (SHP) group received 2 µl citrate buffer (2µl/ventricle) via i.c.v. Injection. On the 7th day, bee bread solution was given by oral gavage for 21 days; (3) Alzheimer's disease (AD) group received two µl of STZ (STZ; 2mg/kg, 2µl/ventricle) via i.c.v. Injection and on the 7th day, tap water was given by oral gavage for 21 days; (4) Bee bread (perga) + AD group (ADP) received two µl of STZ (2mg/kg, 2µl/ventricle) via i.c.v. Injection. On the 7th day, bee bread solution by oral gavage for 21 days. The weight of the animals was recorded at the beginning and end of the experimental processes. To calculate weight gain, we subtracted the initial weight measurement from the final weight measurement of each rat. On the last day, feeding to the animals was stopped 12 hours before to measure fasting blood sugar.

Surgery Protocol

The animal model for Alzheimer's Disease (AD) was created according to a previously published protocol (15). Rats were anesthetized using intraperitoneal injections of ketamine (80 mg/kg) and xylazine (5 mg/kg). Afterward, the skulls were placed in a stereotaxic apparatus for skull surgery. Fasting blood glucose levels are measured with blood taken from the tail vein by glucometer (plusMED Blood GlucoseMeter, Accuro, pM1-300, Bionime Corporation, Taiwan) (5). Standard procedures were followed to sterilize a middle sagittal incision in the scalp. A dental drill was used to create bilateral holes in the skull over the lateral ventricles (AP: −0.8 mm, ML: ±1.4 mm, DV: −3.6 mm). Rats in the AD and ADP groups were given an i.c.v. Injection of 2 µl STZ (1 µl/min) and the SH and SHP groups were given an injection of 2 µl citrate buffer into the ventricle. The surgery had come to an end; the wound on the scalp was stitched up, and sulfamethoxazole was applied to prevent any infections. Additionally, penicillin (40,000 U) was applied to the gluteus muscle once a day for three days. After a week, the administration of substances through oral gavage began. The SH and AD groups were given tap water by oral gavage for 21 days, while the SHP and ADP groups were given bee bread solution (200 mg/kg/day) by oral gavage for 21 days (13, 17).

Tissue Collection and Biochemical Analysis

At the end of the experimental period, rats were anesthetized with ketamine (80 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). The blood samples were collected from their veins into tubes containing ethylenediaminetetraacetic acid (EDTA). The liver tissues were excised, flash-frozen in liquid nitrogen, and stored at -80°C for biochemical analysis. Tissue samples were homogenized in cold phosphate-buffered saline (PBS), centrifuged at 10,000×g for 5 minutes, and the resulting supernatants were stored at -80 °C for protein assays. Plasma was obtained by centrifuging the blood samples at 3000 rpm for 15 minutes at 2-8°C within 30 minutes after collection. 5-HT, 5HT2BR, Leptin, G6Pase, GLUT2 and insülin levels in the samples were measured with ELISA Kits (Sunred Biological Technologies Rat 5-HT ELISA Kit, Catalog No: 201-11-1683; Rat 5HT2BR ELISA Kit, Catalog: SRB-T-84698; Rat G6Pase ELISA Kit, Catalog No: SRB-T-84846; Rat GLUT2 ELISA Kit, Catalog No: 201-11-5377; Rat Insulin ELISA Kit, Catalog No: 201-11-0708; Rat Leptin ELISA Kit, Catalog No: 201-11-0261, China). Standard curves were created with the absorbance values of known amounts of standards. The results were expressed as pg/mg tissue protein for leptin levels, ng/mg tissue protein for 5-HT, GLUT2 and G6Pase, ng/g tissue protein for 5-HT2BR, and mIU/L for insulin levels. The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) evaluation was calculated using the following formula: insulin (mIU/L) multiplied by fasting blood glucose (mM) divided by 22.5 (18).

Protein measurements: Protein concentrations were quantified by modified Bradford assay (19).

Ethical Committee

Experimental procedures were performed following the guidelines established by the Institutional Animal Care and Use Committee at Akdeniz University (approval ID:1619/2023.08.005).

Statistical Analysis

The data obtained was analyzed using SPSS 23.0 (SPSS, Chicago, IL, USA) software for Windows. For normally distributed variables, a one-way ANOVA followed by Tukey's Post Hoc Test was used to analyze the biochemical parameters. For non-normally distributed variables, Kruskal-Wallis followed the Mann-Whitney U test was used.

RESULTS

Fasting Glucose Levels

Statistical analysis revealed a significant difference in the fasting glucose levels among the various groups, $X^2(3) = 8.405$, $p = 0.038$ **(Figure 1)**.

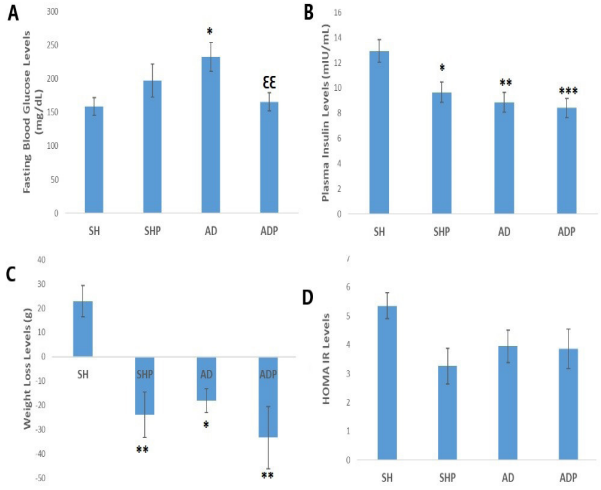

Figure 1: Plasma fasting blood glucose levels in SH and experimental groups. Statistical analysis was by Kruskal Wallis One Way Analysis of Variance on Ranks and all pairwise multiple comparison procedures were done by Mann-Whitney U test. B: Serum insulin levels in SH and experimental groups. C: Weight loss levels in SH and experimental groups. D: HOMA-IR levels in SH and experimental groups. Statistical analysis was by one-way analysis of variance (ANOVA) followed by Tukey's Post Hoc Test. All values are mean ± SEM and n=6 for each group. *, p<0,05 vs sham; **, p<0,01 vs sham; ***, p<0,001 vs sham; εε p<0,01 vs AD.

Fasting glucose levels are shown in **Table 1**. The AD group had significantly higher plasma glucose levels compared to the SH group (U:3, p=0.016). The administration of bee bread resulted in a marked increase in plasma fasting glucose levels of the SHP group as compared to the SH group, but the observed trend was not statistically significant (U:11.5, p=0.296). Additionally, the administration of bee bread significantly decreased plasma glucose levels of the ADP group in comparison with the AD group (U:1.50, p=0.008).

Table 1: Biochemical analysis results of the groups.

Parameters/Groups	AD Group	ADP Group	SH Group	SHP Group
Fasting Glucose Levels	232,50±21,662*	165,67±13,363$^{\varepsilon\varepsilon}$	158,67±13,152	197,33±24,41
Plasma Insulin Levels	8,866±0,796**	8,408±0,749***	12,934±0,8752	9,654±0,81*
Analysis of Weight Loss Levels	-18,00 ±4,926*	-33,33±12,80**	22,83 ± 6,405	-23,83 ± 9,38**
HOMA-IR Levels	3,947±0,566	3,853±0,690	5,352±0,4531	3,259±0,61
Liver 5-HT Levels	1,199±0.075**	1,700±0.164	1,950 ± 0.213	1,149±0.08**
Plasma 5-HT Levels	35,015±1,903**	42,476±4,894	44,142±1,034	39,539±1,23*
Liver 5-HT2BR Levels	16,489±1,67*	34,250±6,32**#$^{\varepsilon}$	9,569±2,150	17,561±2,63*
Liver GLUT-2 Levels	0.440±0.038*	0.503±0.034**	0.250±0.029	0.445±0.065*
Liver Glucose 6 Phosphatase Levels	0,296±0,033#	0,895±0,091***#$^{\varepsilon\varepsilon\varepsilon}$	0,289 ± 0,040	0,583±0,091*
Plasma Leptin Levels	198,61± 14,73***	267,59±11,52#$^{\varepsilon}$	294,07±19,92	199,16 ± 12,80***

All values are mean ± SEM and n=6 for each group. *, p<0,05 vs sham; **, p<0,01 vs sham; ***, p<0,001 vs sham; #, p<0,05vs. SHZ; ε, p<0,05 vs AD; εε, p<0,01 vs AD; εεε, p<0,001 vs AD. 5-HT, 5 hydroxytryptamine; 5-HT2BR, 5-hydroxytryptamine receptor 2 B; GLUT2, glucose transporter 2.

Plasma Insulin Levels

Statistical analysis revealed a significant difference in the levels of plasma insulin between various groups, F (3.20) = 6.378, p =0.003 Figure 1. Plasma insülin levels are shown in Table 1. The AD group had significantly lower plasma insulin levels when compared to the SH group (p=0.010). The administration of bee bread showed a significant reduction in plasma insulin levels in the SHP group when compared to the SH group (p=0.044). Additionally, the administration of bee bread slightly decreased the plasma insulin levels of the ADP group in comparison with the AD group(p=0.978).

Analysis of Weight Loss Levels

According to statistical analysis, there was a significant difference in the levels of weight loss across the various groups, F (3.20) = 7.734, p =0.001 Figure 1. The body weight levels are shown in Table 1. The body weight levels of the AD group were significantly reduced compared to the SH group (p=0.020). The administration of bee bread significantly decreased the body weight levels of the SHP group when compared to the SH group (p=0.044). Additionally, the administration of bee bread markedly decreased the body weight levels of the ADP group in comparison to the AD group (p=0.644). However, this decreasing trend did not reach the level of significance.

Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) Levels

There was no significant difference in the levels of HOMA-IR observed in the different groups, F (3.20) = 2.273, p =0.111 (Figure 1D). The HOMA-IR levels are shown in Table 1. The AD group

showed lower levels of HOMA-IR as compared to the SH group. Administration of bee bread showed a marked decrease in HOMA-IR levels of the SHP group as compared to the SH group. However, this decreasing trend did not reach a significant level. The administration of bee bread did not affect HOMA-IR levels in the ADP group as compared to the AD group.

Liver 5-HT Levels

Statistical analysis revealed a significant difference in the levels of liver 5-HT between various groups, F (3.20) = 7.050, p =0.002 **(Figure 2)**. Liver 5-HT levels are shown in Table 1. The liver 5-HT levels were found to be significantly lower in the AD group as compared to the SH group (p=0.009). The use of bee bread in the SHP group significantly reduced liver 5-HT levels in comparison with the SH group (p=0.005). On the other hand, the administration of bee bread markedly increased liver 5-HT levels in the ADP group compared to the AD group, although the trend did not reach the level of significance (p=0.107).

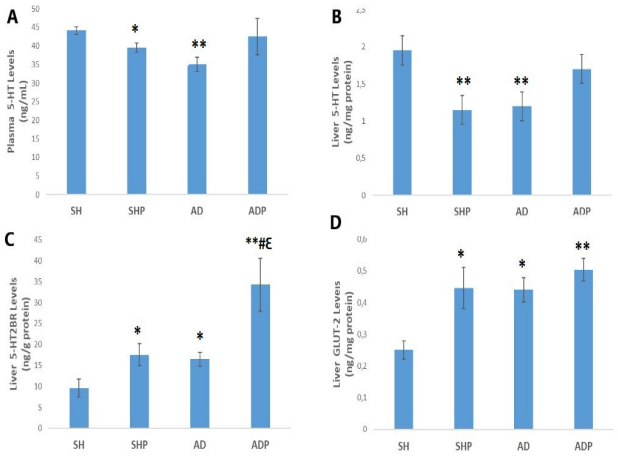

Figure 2: Livers 5-HT levels in SH and experimental groups. Statistical analysis was by one-way analysis of variance (ANOVA) followed by Tukey's Post Hoc Test. B: Plasma 5-HT levels in SH and experimental groups, and C: Livers 5-HT2B levels in SH and experimental groups. Statistical analysis was by Kruskal Wallis One Way Analysis of Variance on Ranks and all pairwise multiple comparison procedures were done by Mann-Whitney U test. D: Livers GLUT-2 levels in SH and experimental groups. Statistical analysis was by one-way analysis of variance (ANOVA) followed by Tukey's Post Hoc. All values are mean ± SεM and n=6 for each group. *, p<0,05 vs sham; **, p<0,01 vs sham; ***, p<0,001 vs sham; #, p<0,05 vs. SHZ; ,εp<0,05 vs AD; εεp<0,01 vs AD; εεε, p<0,001 vs AD.

Plasma 5-HT Levels

Statistical analysis revealed a significant difference in the levels of plasma 5-HT between various groups $X^2(3) = 9.817$, p = 0.020 Figure 2. Plasma 5-HT levels are shown in Table 1. The study found that plasma 5-HT levels were significantly lower in the AD group than in the SH group (U:2, p=0.010). When bee bread was administered, it significantly reduced plasma 5-HT levels in the SHP group compared to the SH group (U:5, p=0.036). However, there was no significant difference in plasma 5-HT levels between the ADP and AD groups after bee bread administration (U:8.50, p=0.128).

Liver 5-HT2BR Levels

There was a notable difference in the levels of 5-HT2BR among the various groups, $X^2(3) = 12.927$, p = 0.005 Figure 2. Liver 5-HT2BR levels are shown in Table 1. The liver 5-HT2BR levels in the AD group were significantly higher than those in the SH group (U:4, p=0.025). The administration of bee bread resulted in a significant increase in the liver 5-HT2BR levels in the SHP group compared to the SH group (U:4, p=0.025). Additionally, the administration of bee bread resulted in a significant increase in the plasma 5-HT2BR levels of the ADP group compared to the AD group (U:5.00, p=0.037). Furthermore, the liver 5-HT2BR levels in the ADP group were significantly higher than those in the SH group (U:1, p=0.006) and the SHP group (U:5, p=0.037).

Liver GLUT-2 Levels

Statistical analysis revealed a significant difference in the levels of liver GLUT-2 between various groups, F (3.20) = 6.206, p =0.004 Figure 2. Liver GLUT2 levels are shown in Table 1. The liver GLUT-2 levels were significantly higher in the AD group compared to the SH group (P=0.031). The administration of bee bread significantly increased liver GLUT-2 levels in the SHP group when compared with the SH group (P=0.026). Additionally, the administration of bee bread increased liver GLUT-2 levels in the ADP group compared to the AD group, but the increase was not statistically significant (P=0.743).

Liver Glucose 6 Phosphatase Levels

Statistical analysis revealed a significant difference in the levels of liver G6P between various groups, F (3.20) = 16.817, p =0.000 **(Figure 3)**.

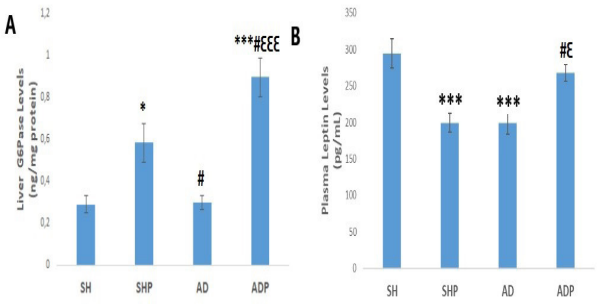

Figure 3: Livers G6Pase levels in SH and experimental groups. B: Plasma Leptin levels in SH and experimental groups. Statistical analysis was by one-way analysis of variance (ANOVA) followed by Tukey's Post Hoc Test. All values are mean ± SEM and n=6 for each group. *, p<0,05 vs sham; **, p<0,01 vs sham; ***, p<0,001 vs sham; #, p<0,05 vs. SHZ; ε, p<0,05 vs AD; εε, p<0,01 vs AD; εεε, p<0,001 vs AD.

Liver G6Pase levels are shown in Table 1. The liver G6P levels were not changed in the AD group compared to the SH group (p=1.00). Administration of bee bread significantly increased liver G6P levels of the SHP group in comparison with the SH group (p=0.036). Furthermore, the administration of bee bread increased liver G6P levels of the ADP group in comparison with the AD group (p=0.000).

Plasma Leptin Levels

There was a statistically significant difference in plasma leptin levels between groups, F (3.20) = 10.339, p =0.000 Figure 3. Plasma leptin levels are shown in Table 1. The plasma leptin levels were significantly decreased in the AD group compared to the SH group (p=0.001). Administration of bee bread significantly decreased plasma leptin levels of the SHP group in comparison with the SH group (p=0.001). Furthermore, the administration of bee bread increased plasma leptin levels of the ADP group in comparison with the AD group (p=0.020).

DISCUSSION

The effect of bee bread supplementation on liver glucose metabolism was evaluated in an experimental AD model created via i.c.v. STZ injection. The STZ and bee bread doses used in our study were determined by reference to previous studies. The injection of STZ into the brain causes insulin resistance and other changes that are similar to those seen in Alzheimer's Disease. Therefore, this injection technique is useful for studying the metabolic changes that occur in Alzheimer's Disease and for developing new treatment approaches (15).

Previous reports have shown that patients with Alzheimer's disease (AD) and mouse models of AD often display impaired glucose-insulin homeostasis and body weight loss prior to the onset of AD symptoms (5, 20). Our study investigated various markers related to peripheral glucose metabolism, including fasting blood glucose, serum insulin levels, and HOMA-IR levels. We found that plasma insülin levels significantly decreased while fasting blood glucose levels significantly increased in the AD group. Insulin is a hormone produced by pancreatic β-cells. It enhances glucose uptake in tissues such as muscle and fat. Thus, it has a primary function of maintaining peripheral glucose homeostasis (21). Epidemiological data indicate that both hyper- and hypoinsulinemia increase the risk of developing AD (22). Moreover, Rivera et al. showed that the levels of insulin and IGF-I polypeptide genes and their corresponding receptors are significantly reduced in advanced AD compared to aged control brains (23). Impairment of insulin secretion by β cells of the pancreatic islets (β cell dysfunction) leads to hypoinsulinemia and hyperglycemia that characterize Type 2 Diabetes Mellitus (T2DM) (24). Diabetes is a disease that often has no symptoms and typically occurs in later life. Insulin resistance can occur years before DM and its complications (25, 26). However, Bondar et al. showed that patients with reduced function of β cells had lower indices of insulin resistance and were characterized by a more prolonged duration of diabetes and high fasting glycemia (27). Consistent with this information, we observed a significant reduction in HOMA-IR levels in the AD group compared to the SH group. In conclusion, the information obtained indicates that the findings obtained from our study reflect the findings of T2DM in the AD group (28).

It is known that the control of glucose homeostasis is achieved by the complex integration of hormones produced in various tissues. Emerging evidence suggests that 5-HT in the periphery impacts liver glucose metabolism depending on physiological conditions. We investigated how 5-HT affects hepatic glucose homeostasis through HTR2B. In our study, it was

observed that the 5-HT levels were significantly reduced in both the serum and liver tissue of AD group rats. Also, our results showed that the 5HT2B levels increased in the liver of AD rats. Similarly, a study by Paulose et al. showed that there was a decreased 5-HT increase in 5-HT receptors in the brains of pyridoxine-deficient young rats (29). In this respect, our results were found to be compatible with studies showing a negative correlation between 5-HT receptor level and 5-HT concentration (30). Furthermore, in our study, it was observed that GLUT2 levels increased in parallel with the increase in 5HT2B levels in the liver of AD rats, but G6Pase levels did not change. GLUT2 is the main member of the GLUT family in hepatocytes. It plays a crucial role in controlling the uptake of glucose in the liver cells, which is dependent on the levels of glucose in the bloodstream. After entering the cell, glucose is quickly transformed into glucose-6-phosphate by the enzyme glucokinase. It is then either metabolized by glycolysis or stored as glycogen (31). Therefore, our findings revealed that 5 HT2BR promoted glucose entry into hepatocytes by increasing GLUT2 levels in the liver of AD rats. Our results are based on previous findings showing the effect of 5-HT2B receptor activation on glycogen synthesis in astrocytic cells (32). Moreover, when the results were evaluated together, it is possible to say that as an adaptation mechanism, hepatic GLUT2 level was increased by 5HT2BR in the AD. To the best of our knowledge, our study is the first to demonstrate that 5-HT2BR may regulate glucose metabolism as an adaptation mechanism in Alzheimer's disease by increasing glucose entry in the liver. Additionally, our findings are from previous studies showing the reduction of peripheral 5-HT in AD (33).

The effect of beef bread supplementation on hepatic glucose metabolism in an AD model created via STZ injection was evaluated in this study, with a focus on 5-HT2B-mediated effects. We found that bee bread administration caused a marked increase in 5-HT levels in plasma and liver tissue and also significantly increased 5HT2B levels in the liver of the ADP group. It is known that the presence of the tryptophan (Trp) substrate is critical in serotonin synthesis, and dietary Trp increases 5-HT synthesis (7, 34). Bee bread is known to contain more than 300 compounds, such as free amino acids, sugars, fatty acids, minerals, organic acids, polyphenols, and vitamins. Additionally, Bayram et al. showed that bee bread contains many amino acids, including tryptophan (17). This information suggests that the tryptophan content of bee bread increases peripheral 5HT synthesis in the ADP group. Additionally, our research indicates that when there is an increase in 5H levels, hepatic 5HT2B levels may also increase. We found that administering bee bread significantly increased G6Pase levels while slightly increasing GLUT2 levels in the livers of rats in the ADP group. This suggests that glucose output from the liver tissue of ADP group rats increased significantly. However, contradictory results have been produced by studies examining the impact of 5-HT on hepatic gluconeogenesis, glycogen storage, glucose uptake, and glycolysis (35). Studies performed in rodents reported that the injection of 5-HT or 5-HT receptor agonists results in hyperglycemia (36). Another study demonstrated that the neurotransmitter 5-HT plays a role in regulating brain glycogen levels in rainbow trout. This results in a breakdown of glycogen when the fish are in a normoglycemic or hypoglycemic state but not in a hyperglycemic state (37). Lee et al. have shown that 5-HT elicited hyperglycemic responses in Procambarus clarkii in a dose-dependent manner (38). In this respect, our results are consistent with studies showing that the effect of 5-HT on glucose metabolism may vary depending on the physiological state. Therefore, we concluded that bee bread administration promotes gluconeogenesis by increasing the level of G6Pase through the 5-HT-HTR2B pathway in the ADP group. However, interestingly, it was observed that bee bread administration reduced the fasting glucose levels of ADP group rats. As far as we know, our study is the first to demonstrate that bee bread administration reduced the fasting glucose levels of Alzheimer's rats created by i.c.v. STZ injection. Moreover, we found that bee bread administration slightly reduced plasma insulin levels without affecting HOMA-IR levels. Therefore, we examined the effect of leptin levels on peripheral glucose levels. Leptin can independently reduce blood gluco-

se levels, especially in hyperglycemic models of leptin or insulin deficiency. Although leptin does not increase insulin levels, it has been shown to strongly increase insulin sensitivity in Type1 Diabetes (T1D) models (39, 40). Bee bread administration increased leptin levels in the plasma of the ADP group. Moreover, bee bread administration reduced glucose levels in the plasma of the ADP group without affecting insulin levels. Doğanyigit Z. et al. showed that the administration of bee bread increased the leptin immunoreactivity in the gastric tissue of obese rats (13). In this respect, our findings are consistent with previous studies. When these data are evaluated together, it can be said that the net effect of bee bread administration in experimental Alzheimer's model rats is to reduce plasma glucose levels by increasing leptin levels in the plasma. Moreover, previous findings show the circulating levels of leptin are significantly lower in patients with AD than in controls (41). Supporting this information, in our study, leptin levels decreased in the plasma of AD group rats.

In both human patients with Alzheimer's disease and Tg2576 mice, which are a special type of mouse that has been genetically modified to produce extra amyloid precursor protein (APP), there is an early reduction in body weight and plasma leptin levels. This occurs even before the formation of amyloid plaques or cognitive dysfunction. This weight loss was associated with the inhibition of hypothalamic neuropeptide Y (NPY) neurons by Aβ (42). In this respect, the findings obtained from our study were consistent with other studies showing that early weight loss occurs in AD disease. Additionally, in our study, the weight loss of ADP group rats increased due to bee bread administration, which is consistent with studies showing that Leptin can impact body weight by affecting hypothalamic neurons (11). Leptin treatment has consistently been shown to decrease Aβ levels by targeting all aspects of Aβ metabolism (43-45). Based on these results, we propose that the decreased levels of leptin in the blood may play a role in the deterioration of cognitive function and the worsening of Alzheimer's disease. Considering the peripheral and central effects of leptin on AD pathogenesis, it can be said that bee bread may reduce AD pathology. For example, propolis, a honey bee product, has been used in traditional medicine for many years (46). Recently, the effect of natural bee products on the pathogenesis of AD has been demonstrated. (47, 48). Nisa et al. suggested that phytoligand molecules obtained from honey bee products could be novel β-site APP-cleaving enzyme 1 (BACE-1) inhibitors for Alzheimer's disease. (47). "As far as we know," our study is the first to show the effect of bee bread on the hormones that regulate glucose in the pathogenesis of AD.

Nevertheless, administration of bee bread slightly increased the fasting glucose levels and significantly decreased the insülin levels in the plasma of healthy rats. Also, HOMA-IR levels markedly decreased in the SHP group. These results indicate that administering bee bread may cause hyperglycemia by disrupting insulin secretion in the pancreas. In addition, bee bread administration significantly increased the 5-HT2BR, GLUT2, and G6Pase levels in the liver of the SHP group. It is suggested that bee bread affects 5-HT2B-mediated glucose metabolism in the liver of SHP rats. However, interestingly, it was observed that bee bread administration significantly decreased the 5-HT levels in the plasma and liver tissue of SHP group rats. Moreover, our study observed that the administration of bee bread significantly decreased the leptin levels in the plasma of SHP group rats and increased weight loss. It is known that both Leptin and central 5-HT play a role in the regulation of nutritional signals and energy metabolism (7). According to the results obtained, it is seen that bee bread affects energy metabolism.

In particular, increased plasma Trp/large neutral amino acids (LNAA) ratio enhances brain Trp uptake and 5-HT synthesis (7). İmportantly, central 5-HT is known to play a role in regulating nutritional signals and energy metabolism. Disruption of central serotonin signaling via 5-HT2C receptors induces hyperphagia in mice, leading to obesity, insulin resistance, and impaired glucose tolerance independent of leptin (7). The amount of leptin released into the bloodstream is directly proportional to the amount of fat stored in the body. The circulating leptin levels also decrease as the body loses weight and fat stores decrease. Reduced leptin levels

signal the brain to increase food intake and decrease energy expenditure to restore body weight (11). In light of this information, our results suggest that bee bread may increase brain 5-HT levels, leading to weight loss and decreased leptin release from adipocytes. When these data are evaluated together, it is thought that the bee bread agent has an increasing effect on plasma glucose levels by reducing the leptin and insulin levels in the plasma of SHP rats, similar to the AD group. It also affects 5-HT2B-mediated glucose metabolism in the liver of SHP rats. In addition, the findings suggest that it is necessary to investigate the effects of healthy-oriented natural bee products on brain tissue.

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