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The effect of silymarin supplementation on cognitive impairment induced by diabetes in rats

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Abstract:

Background: The objective of this investigation was to examine the impact of silymarin supplementation on locomotion, anxiety-related behavior, learning, and memory via several behavioral tests, such as open field, elevated plus maze, and Morris water maze tests in streptozotocin-induced diabetic rats.

Methods: The rats were divided into the control, diabetes, silymarin, and diabetes plus silymarin groups. On the 30th–35th days of the study, several behavioral tests were performed and blood and brain tissue samples were taken and brain-derived neurotrophic factor (BDNF) and histone deacetylase 3 (HDAC3) levels were analyzed.

Results: There was no significant difference in locomotor activity between the groups ($p = 0.534$). Spatial memory was lower ($p = 0.000$) but anxiety scores were higher ($p = 0.005$) in the diabetes group than in the control, silymarin, and diabetes plus silymarin groups. Plasma ($p = 0.000$) and brain tissue ($p = 0.007$) BDNF levels were lower in the diabetes group than in the control, silymarin, and diabetes plus silymarin groups; however, plasma ($p = 0.432$) and brain tissue ($p = 0.321$) HDAC3 levels did not significantly differ between the groups.

Conclusions: The findings obtained from this study suggest that silymarin supplementation could improve anxiety-related behavior, and learning and memory in diabetic rats by increasing the BDNF levels.

Keywords: BDNF, cognitive function, diabetes, HDAC3, learning, silymarin

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Introduction

Diabetes may lead to acute complications that result in death as well as chronic complications emerging as the failure and function loss of several organs because of long-term metabolic disorder. Together with more than one organ and tract, the brain also is exposed to the negative effects of diabetes [1]. Due to the damage to the central nervous system (CNS), diabetes leads to deteriorations in cognitive functions. In addition, diabetes can also induce dementia which affects synaptic plasticity and leads to inefficiency in cognition [2], [3], [4]. It has been known that there are significant deteriorations in the neurotransmitter levels, number of neurons, and apoptotic activity in the diabetic rats because of the occurrences of electrophysiological and structural disorders in the CNS [4]. Previous studies [5], [6] reported an impairment in spontaneous locomotor activity, aggression, anxiety and depression, and learning and social interaction behaviors of the diabetic rats.

Silymarin is a substance with antioxidant and anti-inflammatory properties used in the treatments of several dysfunctions, such as liver and gallbladder diseases, and its positive effects on the CNS have been also stated [7]. Silymarin has antioxidant activity in the CNS as it can pass the blood-brain barrier [8]. Silymarin supplementation increases the antioxidant enzyme activity and decreases the levels of lipid peroxidation in the erythrocytes exposed to hydrogen peroxide [9]. In addition, silymarin supplementation decreases protein oxidation in the cortex and hippocampus of old rats [10]. These data suggest that silymarin can have neuroprotective effects in diabetes via its antioxidant properties.

Silymarin also regulates glucose uptake in adipocytes with the blockage of GLUT-4. It is stated that silybin in the concentration range of 25–100 $\mu\text{mol L}^{-1}$ in the rat hepatocytes reduces glucose formation out of various gluconeogenic substances with its suppressive effects on pyruvate kinase activity [11]. In the cultured hepatocytes, silymarin supplementation at low doses decreased the formation of free radicals in mitochondria [12]. In randomized studies on type 1 and type 2 diabetic animal models [13] and type 2 diabetic patients [14], we

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found that silymarin decreased hemoglobin A1c levels by affecting the blood glucose and triglyceride levels [13], [14].

In studies [8], [9], [15], [16] carried out recently, the effect of silymarin supplementation on many diseases has been investigated; however, the data about its relation with cognitive dysfunction due to diabetes are inadequate. The purpose of this investigation was to evaluate the impact of silymarin supplementation on cognitive functions, based on the Open Field (OF), Elevated Plus Maze (EPM), and Morris Water Maze (MWM) tests, as well as on brain-derived neurotrophic factor (BDNF) and histone deacetylase 3 (HDAC3) levels in blood and brain tissue.

Materials and methods

Animals and study protocol

After the approval of the experiment was taken from the Local Ethical Committee of Selçuk University, the investigation was carried out in the same center. In the study, 36 Wistar male rats (4-month-old) weighing between 400 and 450 g were used. The rats were housed in polycarbonate cages at a 12-h light:12-h dark cycle, temperature of $21 \pm 2^\circ\text{C}$, and 50% humidity conditions, with access to food and water *ad libitum*.

The animals were randomly separated into four groups as follows: control (C; $n = 6$), diabetes (D; $n = 12$), silymarin (S; $n = 8$), and diabetes plus silymarin (DS; $n = 10$).

The induction of experimental diabetes

In the present study, not all rats were included in the study. The rats whose glucose concentrations exceeded 250 mg dL^{-1} were included in the diabetic groups. In order to induce experimental diabetes in rats, streptozotocin (STZ) was used (S0130; Sigma Chemical Co., St. Louis, MO, USA). Before the STZ injection, the rats were fasted for 5 h. At the end of this period, the STZ, prepared freshly in 0.1 M sodium citrate buffer with pH 4.5, was administrated intraperitoneally in the single dose of 50 mg kg^{-1} . After the STZ injection, rats were given dextrose 5% instead of tap water for 3 days in order to prevent hypoglycemia. After 1 week of STZ injection, glucose concentrations were measured with a glucometer (eBsensor; Visgeneer, Hsinchu, Taiwan) and the rats whose glucose concentrations exceeded 250 mg dL^{-1} were included in the diabetic groups.

Silymarin supplementation

Silymarin supplementation started 7 days after STZ injection and this was considered the first day of supplementation. The silymarin-supplemented groups received silymarin (S0292; Sigma Chemical) via oral gavage at doses of $200\text{ mg kg body weight}^{-1}$ per day dissolved in maize oil for 35 days.

Behavioral tests

From the 31st day of the study onwards, the OF, EPM, and MWM tests for 5 days. All behavioral tests were performed in a sound- and light-insulated room by the same researcher and by taking care of the factors that will change the emotional condition of the animal (by eliminating different stimuli such as smell and noise and by wearing the same clothes in each application). The test room was enabled to be aired. After each rat completed the tests, the OF and EPM apparatus were cleared with alcohol to exclude olfactory cues such as smell, pile, or urine. All tests were applied same hours of the day.

Tests were taped online using a video camera. The obtained videos were assessed offline using software (Ethovision XT 9.0; Noldus Information Technology, Wageningen, The Netherlands).

OF test

The spontaneous locomotion of the animals was evaluated with the OF which is a black square wooden box ($80 \times 80\text{ cm}$) surrounded by walls (40 cm high). Each rat was placed in the center of the box and allowed to explore the environment freely for 5 min.

EPM test

The EPM apparatus contains two enclosed and two open arms elevated 50 cm above the floor. The enclosed and open arms of the assembly made of black plexiglas are of 50 cm length and 10 cm width. The animals were located one by one in the platform center in the way their face see one of the open arms, and during the 5-min test, they were allowed to move freely in the maze.

MWM test

Spatial memory was analyzed with MWM. The water temperature was settled at 25 ± 2 °C. The water was colored with non-toxic green paint. The tank was separated into four imaginary quadrants: northeast (NE), southeast (SE), northwest (NW), and southwest (SW). The hidden square platform (escape platform) was located below the water surface into the NW quadrant away from the side wall and kept constant during the test. Colored geometric cues were placed around the tank in the way the rats can see them in order to find directions and environmental insulation was enabled.

The animals were exercised on 4 days via four trials each day, and 24 h after the last exercise session a probe trial was applied. During the training, the rat was placed into the water each time from a different quadrant and expected to discover the platform in 60 s. If the rats were unsuccessful to find the platform within the allowed time period, it was physically placed on the platform by the experimenter for 30 s. In this way, it was aimed for the rats to recognize the area and learn the place of the platform. At the 24 h after the spatial navigation trials a single 90 s probe trial task was performed in which the platform was removed from the pool.

Sample collection

Twenty-four hours after the behavioral tests, all the groups were anesthetized with the intramuscular injection of ketamine and xylazine (50/10 mg kg⁻¹, respectively). Blood samples were obtained from the anesthetized rats by cardiac puncture. After drawing the blood, the rats killed with cervical dislocation and brain tissues were taken. After the tissue samples were cleaned from the remaining blood and tissues with the ice-cold physiological saline, they were dried with blotting paper and put into plastic tubes. Then, they were placed into a liquid nitrogen tank and quick-frozen. The blood and brain tissues were maintained at -80 °C until biochemical analysis was made.

Tissue homogenization

Before homogenization, the brain tissues kept at -80 °C were thawed respectively at -20 °C and +4 °C. Tissue samples were weighed and homogenized in 10 volumes of ice-cold Tris-HCl buffer (50 mmol L⁻¹, pH 7.5) using a homogenizer (Daihan HS, Seoul, South Korea). BDNF and HDAC3 levels were analyzed in this homogenate.

Biochemical analysis

The measurement of BDNF levels was carried out with the enzyme-linked immunosorbent assay (ELISA) using the BDNF ELISA Kit (Cat. No. CSB-E04504r; Cusabio, WuHan, China). HDAC3 levels were measured with the ELISA method using the Rat Histone Deacetylase 3 ELISA Kit (Cat. No. E1215Ra; Bioassay Technology Laboratory, Shanghai, Yangpu, China).

Data analysis

The data analysis was carried out with SPSS 20.0 (SPSS Inc., Chicago, IL, USA). The descriptive values of the scalar variables were stated with mean and standard error (SE). OF, EPM, and MWM probe trial variables, and biochemical parameters were analyzed via two-way analysis of variance (ANOVA) (diabetes × supplementation). Repeated-measures ANOVA was performed in order to compare the mean values of scalar parameters measured at different times. When the difference between groups is significant, the Tukey test with Bonferroni correction was used to measure particularly statistical significance. Student's *t*-test was used to evaluate the statistical difference between before and after supplementation. The level of significance was set as $p < 0.05$.

Results

Blood glucose levels of the groups are given in Table 1. There was an impact of diabetes on blood glucose levels in both before ($F = 577.410$ and $p = 0.000$) and after ($F = 285.371$ and $p = 0.000$) supplementation. Blood glucose levels were higher in the D and DS groups than in the C and S groups before and after the silymarin supplementation ($p < 0.05$). In addition, blood glucose levels were higher after supplementation than before supplementation in the D and DS groups ($p < 0.05$).

Table 1: Blood glucose levels of the groups before and after silymarin supplementation.

Groups	n	Before supplementation, mg dL ⁻¹	After supplementation, mg dL ⁻¹
C	6	127.7 ± 7.0	130.2 ± 4.7
D	12	531.1 ± 18.3 ^a	590.8 ± 6.9 ^{a,d}
S	8	130.5 ± 8.6 ^b	127.3 ± 5.3 ^b
DS	10	545.8 ± 17.9 ^{a,c}	569.7 ± 21.6 ^{a,c,d}

Data are expressed as mean ± SE. ^a $p < 0.05$ compared to the C group, ^b $p < 0.05$ compared to the D group, ^c $p < 0.05$ compared to the S group, ^d $p < 0.05$ compared to the before supplementation. C, control; D, diabetes; S, silymarin; DS, diabetes plus silymarin.

In the OF and EPM tests, the total distance traveled and average velocity are indicators of the locomotor activity. In addition, in the OF apparatus, time spent as a mobile is also a measure of locomotor activity. In the OF apparatus, the number of rearings and number of entries in the center are a measure of exploratory behavior. OF and EPM test results of the groups are demonstrated in Table 2 and Table 3, respectively. In the OF test, there was no significant difference in the total distance traveled ($p = 0.508$), average speed ($p = 0.654$), and number of entries ($p = 0.541$) in the center between the groups. There was an impact of supplementation × diabetes on the rearing number ($F = 5.535$ and $p = 0.025$), and time spent as a mobile ($F = 6.834$ and $p = 0.014$). Besides, further analysis demonstrated that the rearing number and time spent as a mobile did not differ between the groups. In the EPM test, there was an impact of supplementation ($F = 17.105$ and $p = 0.000$), diabetes ($F = 7.313$ and $p = 0.011$), and supplementation × diabetes ($F = 10.991$ and $p = 0.002$) on the total distance traveled. The total distance traveled was higher in the S group than in the C and D groups ($p = 0.000$), and was lower in the DS group than in the S group ($p = 0.001$). These data indicate that diabetes and/or silymarin supplementation have limited effects on locomotor activity and exploratory behavior.

Table 2: OF test results of the groups.

n	C 6	D 12	S 8	DS 10
Total distance traveled, cm	570.2 ± 250.8	719.8 ± 339.5	572.4 ± 241.7	570.8 ± 209.2
Number of defecations	2.83 ± 1.16	4.50 ± 1.16 ^a	2.50 ± 2.07 ^b	2.67 ± 0.87 ^b
Number of groomings	3.17 ± 1.94	2.50 ± 2.46	3.25 ± 1.83	1.90 ± 1.07
Number of rearings	7.00 ± 1.89	11.00 ± 4.70	9.88 ± 6.10	7.40 ± 3.13
Time spent as a mobile, s	14.96 ± 8.45	26.80 ± 12.41	20.77 ± 5.23	15.72 ± 9.66
Average speed, cm s ⁻¹	1.90 ± 0.83	2.39 ± 1.13	2.44 ± 1.88	1.90 ± 0.69
Time spent in the center, s	192.4 ± 47.7	141.8 ± 53.9	152.6 ± 23.1	137.4 ± 85.2
Number of entries in the center	18.00 ± 11.45	15.67 ± 6.44	22.88 ± 12.10	17.40 ± 13.45

Data are expressed as mean ± SE. ^a $p < 0.05$ compared to the C group, ^b $p < 0.05$ compared to the D group. C, control; D, diabetes; S, silymarin; DS, diabetes plus silymarin.

Table 3: EPM test results of the groups.

	C	D	S	DS
Total distance traveled, cm	343.5 ± 170.9	435.6 ± 272.3	1466.6 ± 698.9 ^{ab}	559.2 ± 412.4 ^c
Number of entries in closed arms	0.67 ± 0.41	2.36 ± 1.12 ^a	1.63 ± 0.91	2.10 ± 0.99 ^a
Number of entries in open arms	1.67 ± 0.51	0.36 ± 0.27	2.00 ± 1.77	0.60 ± 0.49
Time spent in closed arms, s	221.4 ± 119.4	291.2 ± 8.7	263.9 ± 40.9	256.9 ± 92.8
Time spent in open arms, s	62.73 ± 35.11	4.09 ± 2.26 ^a	36.40 ± 23.6 ^b	27.98 ± 13.21 ^b

Data are expressed as mean \pm SE. ^a $p < 0.05$ compared to the C group, ^b $p < 0.05$ compared to the D group, ^c $p < 0.05$ compared to the S group. C, control; D, diabetes; S, silymarin; DS, diabetes plus silymarin.

In the OF apparatus, number of defecations, and groomings and time spent in the center are indicators of anxiety-like behavior. In the EPM apparatus, number of entries in closed and open arms and time spent in closed and open arms are also a measure of anxiety-like behavior. In the OF test, there was no significant difference in the number of groomings ($p = 0.534$) and time spent in the center ($p = 0.308$) between the groups. There was an impact of supplementation on the defecation number ($F = 6.210$ and $p = 0.018$). It was higher in the D group than in the C group ($p = 0.020$), and lower in the S and DS groups than in the D group ($p = 0.008$). In the EPM test, there was an impact of diabetes on the number of entries in the open ($F = 7.302$ and $p = 0.011$) and the closed ($F = 10.576$ and $p = 0.003$) arms. The number of entries in closed arms was higher in the D and DS groups than the C group ($p = 0.034$ and $p = 0.008$, respectively). However, further analysis demonstrated that the number of entries in open arms was not differing between the groups. There was an impact of diabetes ($F = 11.942$ and $p = 0.002$) and supplementation \times diabetes ($F = 6.698$ and $p = 0.015$) on the time spent in open arms. It was lower in the D group than in the other groups ($p = 0.001$). Time spent in closed arms did not differ between the groups ($p = 0.307$). These data indicate that diabetes induces an increase in the anxiety levels and silymarin supplementation attenuates severity of anxiety-like behavior.

To investigate the effects of silymarin supplementation on diabetes-induced cognitive impairment, we examined spatial learning and memory using the MWM test. Figure 1 shows MWM training session results of the groups. Total distance traveled, latency, and thigmotactic behavior reduced over time ($F = 48.978$ and $p = 0.000$, $F = 50.863$ and $p = 0.000$, and $F = 30.193$ and $p = 0.000$, respectively). In all groups these parameters reduced with time and did not differ between the groups, significantly (Figure 1A, B, and D). Average swimming velocity did not change over time, remaining almost the same during the exercise sessions ($p = 0.659$), and did not differ between the groups (Figure 1C).

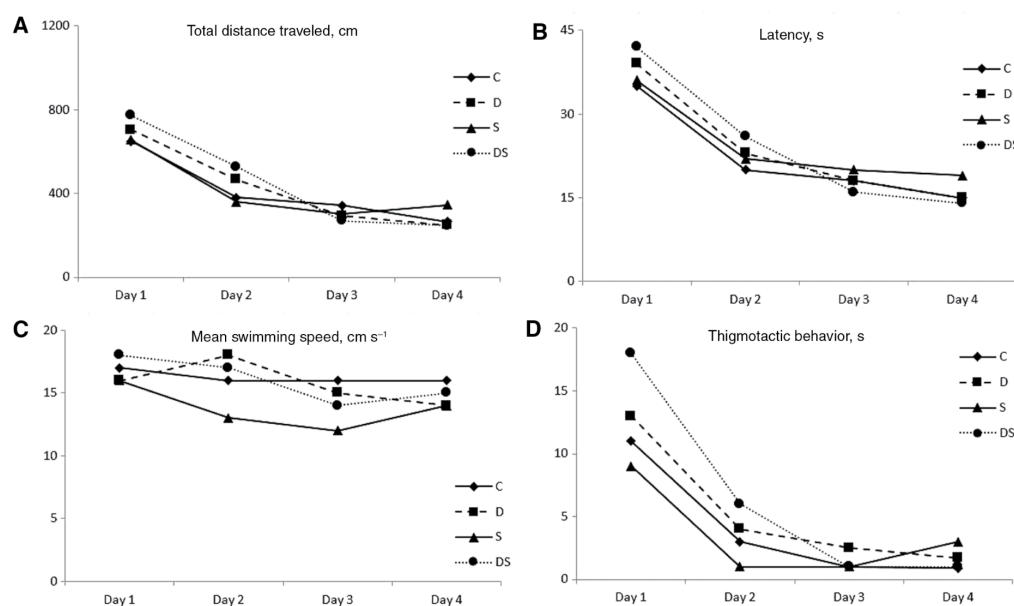


Figure 1: The effect of silymarin supplementation and/or diabetes on (A) total distance traveled (B) latency, (C) mean swimming speed, and (D) thigmotactic behavior during MWM training sessions. Data are expressed as mean \pm SE. C, control; D, diabetes; S, silymarin; DS, diabetes plus silymarin.

MWM training session results of the groups.

Table 4 shows MWM probe trial results. There was an impact of supplementation \times diabetes on mean velocity ($F = 4.973$ and $p = 0.033$) and total distance traveled ($F = 4.789$ and $p = 0.036$). Total distance traveled and mean velocity were lower in the DS group than in the D group ($p = 0.013$, and $p = 0.011$, respectively). There was an impact of supplementation ($F = 5.162$ and $p = 0.030$), diabetes ($F = 22.572$ and $p = 0.000$), and supplementation \times diabetes ($F = 7.861$ and $p = 0.036$) on the platform crossing number. It was lower in the D group than in the C group ($p = 0.000$), and higher in the S and DS groups than the D group ($p = 0.000$). Time spent in the NW quadrant was higher in all the groups, but it did not differ between the groups. These data suggest that diabetes impairs the learning ability of the rats and silymarin supplementation prevents this impairment.

Table 4: MWM probe trial results of the groups.

	C	D	S	DS
Total distance traveled, cm	2383.9 ± 323.3	2794.2 ± 300.9	2249.4 ± 344.8	2502.2 ± 435.2 ^b
Time spent in NW quadrant, s	44.33 ± 10.49	40.60 ± 4.90	42.65 ± 10.00	44.14 ± 8.39
Time spent in NE quadrant, s	14.36 ± 4.60	15.68 ± 5.50	17.37 ± 10.43	17.56 ± 2.66
Time spent in SW quadrant, s	20.36 ± 4.69	22.55 ± 7.35	18.97 ± 6.88	18.12 ± 5.12
Time spent in SE quadrant, s	11.13 ± 3.48	11.37 ± 3.63	11.20 ± 4.81	10.36 ± 4.66
Mean velocity, cm s ⁻¹	26.87 ± 3.65	32.01 ± 5.87	27.86 ± 3.59	25.5 ± 3.81 ^b
Number of platform crossings	5.17 ± 0.98	1.75 ± 0.86 ^a	5.50 ± 0.75 ^b	4.10 ± 1.79 ^b

Data are expressed as mean ± SE. ^ap < 0.05 compared to the C group, ^bp < 0.05 compared to the D group. C, control; D, diabetes; S, silymarin; DS, diabetes plus silymarin.

The effects of silymarin and/or diabetes on BDNF and HDAC3 concentrations in plasma and brain tissue of the rats are shown in Table 5. There was an effect of diabetes and supplementation × diabetes on plasma ($F = 8.455$ and $p = 0.007$, and $F = 9.636$ and $p = 0.004$, respectively) and brain tissue ($F = 8.190$ and $p = 0.007$, and $F = 17.966$ and $p = 0.000$, respectively) BDNF concentrations. Plasma BDNF concentrations were lower in the D group than the C group ($p = 0.001$), and were higher in the S and DS groups than the D groups ($p = 0.006$, and $p = 0.002$, respectively). Brain tissue BDNF concentrations were lower in the D group than the C group ($p = 0.005$). Neither supplementation nor diabetes affected plasma and brain tissue HDAC3 levels ($p = 0.432$, and $p = 0.321$, respectively).

Table 5: The effects of silymarin supplementation and/or diabetes on BDNF and HDAC3 levels in both plasma and brain tissue.

	C	D	S	DS
<i>BDNF</i>				
Plasma, ng mL ⁻¹	0.89 ± 0.16	0.46 ± 0.10 ^a	0.79 ± 0.22 ^b	0.80 ± 0.26 ^b
Brain, ng mg protein ⁻¹	0.18 ± 0.05	0.10 ± 0.05 ^a	0.13 ± 0.03	0.15 ± 0.03
<i>HDAC3</i>				
Plasma, ng mL ⁻¹	4.80 ± 0.62	4.34 ± 0.99	4.74 ± 1.32	5.05 ± 0.85
Brain, ng mg protein ⁻¹	0.40 ± 0.12	0.46 ± 0.12	0.47 ± 0.09	0.39 ± 0.08

Data are expressed as mean ± SE. ^ap < 0.05 compared to the C group, ^bp < 0.05 compared to the D group. C, control; D, diabetes; S, silymarin; DS, diabetes plus silymarin.

Discussion

Diabetes is a disease characterized by a number of symptoms that occur because of the structural defect of insulin and decreased insulin secretion such as polydipsia, polyuria, polyphagia, weight loss and fatigue [17]. Diabetes also adversely affects many organs. Alvarez et al. [18] stated that there is a remarkable correlation between hippocampus and diabetes in behavioral alterations. In our study, the fact that the rat's defecations to create itself a safer area is more in the diabetic group than the silymarin-supplemented groups shows that there is an alteration of behavior in the diabetic rats and diabetes increase the anxiety levels. In the current investigation, silymarin implementation reduced anxiety-related behaviors in diabetic rats.

In the EPM test, the behaviors of the rats' exhibit in the open and enclosed arms and the time spent in these arms provide data about anxiety levels [19]. It has been detected that there was a difference between the groups in terms of total distance traveled, the number of entries into the closed arms, and the time spent in the open arms. While the silymarin group becomes the one traveling the most distance, there was no significant difference between the other groups. The number of entries into the closed arms is higher in the diabetes groups than the control group; however, there was no difference between the other groups. The time spent in the open arms is quite a bit lower in the diabetes group than in the control group, and no significant difference was found between the other groups. Landgraft [20] stated in their study on the anxiety and depression in rats that anxiety has an effect that makes it harder to learn. The fact that the number of entries into the enclosed arms was higher and the time spent in the open arms was lower in the diabetes group than those in the control group shows the presence of anxiety in the diabetic rats.

In the current investigation, the total distance traveled, time spent to reach the platform, and the duration of thigmotaxis decrease gradually from the first day until the fourth day in each group. As a result, in the

probe trial of the MWM test, learning was achieved in all of the groups. Long-term potentiation (LTP) is the phenomenon of synaptic plasticity essential for the execution of memory functions. Kamal et al. [21] noticed a difference in the synaptic plasticity in the hippocampal cross section of the experimental diabetic rats. They stated that LTP alterations marked in the 6 months to 8 months of diabetic rats were observed in the hippocampal CA1 region and the errors in the occurrence of LTP reach at the highest level and become permanent in the 12-month diabetic rats. Yonucu et al. [22] stated that learning occurs much harder in the rats with diabetes.

On the MWM probe trial of our study, the difference between groups in terms of average speed, the number of passes over the platform, and total distance traveled is found as significant. The velocity of the diabetes group is higher than that of the diabetes plus silymarin group; however, there was no difference between other groups. The total distance traveled is higher in the diabetic group than the diabetes plus silymarin group. The number of passes over the platform was lower in the diabetic group than the control and silymarin groups. Because of the fact that the number of passes over the platform was lower in the diabetes group, we have suggested that learning disorder occurs in this group and the fact that these parameters were higher in the diabetes plus silymarin group indicated that 200 mg kg⁻¹ silymarin supplementation can cure the diabetes-related cognitive dysfunctions. Consistent with our findings, Neha et al. [23] showed that silymarin ameliorates memory deficits in the mouse model of high-fat-diet-induced experimental dementia. In addition, in a recent review, Oh [24] reported that silymarin may be a novel therapeutic agent for the treatment of neuronal cell death in diabetes.

BDNF as one of the most essential neurotrophic factors provides the protection and growth of neurons and the persistence of synaptic plasticity. BDNF promotes the development of noradrenergic and serotonergic neurons by protecting them from toxic damage [25]. Palizvan and Sohya [26] implemented BDNF into the neuron culture obtained from the cortex of rats, and as a result, they reported that the development in the dendrites and synapses increases. In some studies [27], [28], it has been reported that oxidative damage reduces the BDNF levels and causes cognitive functions to decrease. In our study, plasma BDNF levels were lower in the diabetes group than the other groups. Moreover, brain tissue BDNF levels were also lower in the diabetes group than the control group. It has been well known that chronic diabetes significantly downregulated BDNF expression in the hippocampus as compared to control mice [29]. Consistent with our findings, Zhen et al. [30] showed that low BDNF levels in patients with type 2 diabetes are associated with cognitive deficits. The fact that the BDNF levels of the diabetes plus silymarin group were higher than that of the diabetes group could be related to the antioxidant effect of silymarin [12], [31], [32], [33], [34]. It has been reported that the neuroprotective and BDNF levels increasing effects of silymarin are mediated in part by its effects on the hypothalamic-pituitary-adrenal axis, oxidative stress, increasing monoamines, and reducing cytokine level in the hippocampus and cerebral cortex [35], [36], [37].

Histone modifications are one of the major epigenetic mechanisms that provide changes in gene expression [38]. Wang et al. [39] stated that in the diabetic rat brains, an alteration was not observed in the histones of the class HDAC1, but together with the increases in the histone levels in the class HDAC2, errors of gene expression were observed. In our study, no significant difference was found between the groups in terms of the levels of HDAC3 of plasma and brain tissue. In the present study we evaluated HDAC3 levels, because HDAC3 is necessary for proper brain development [40]. In addition, it has been reported that HDAC3 negatively regulates spatial memory in rodents [41], [42]. One limitation of our study is that although NAD⁺ depletion [43] and increased oxidative stress [44] are the main features of neurodegenerative disorders, we could not examine these variables in this study.

Conclusions

As a result, the fact that there was a difference in learning between the diabetic group and the diabetes plus silymarin group in the behavioral tests suggested the curative effect of silymarin on diabetes-induced cognitive dysfunctions. Our study is the first to be conducted in this field. Along with the data we obtained, new studies are needed to be carried out with different dosages and biomarkers in order for the effect of silymarin on diabetes and cognitive functions to be revealed in detail. In addition, clinical investigations should be beneficial for assessing the neuroprotective effect of silymarin in diabetes.

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