



# Protective Effect of Swimming and Genistein on the Expression of microRNA 132, Insulin Growth Factor 1, and Brain-derived Neurotrophic Factor Genes, as well as Spatial Memory, in the Hippocampus of Diabetic Ovariectomized Rats

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Received: 07 Nov. 2020  
Accepted: 27 Jan. 2021  
ePublished: 01 Nov. 2021



## Background

The incidence and progression of type 2 diabetes increase the risk of cognitive impairment in postmenopausal women [1]. Some significant features, such as neuronal degeneration, apoptosis, and abnormal neurotransmitter secretion, perform a peculiar role in diabetic neuropathy [2] in menopausal women [3]. It is acknowledged that microRNAs, 18~24 nucleotides in length, play a critical role in the regulation of post-transcriptional gene appearance of target mRNA [4]. MicroRNAs and their target genes contribute greatly to dynamic biological processes, such as neuronal apoptosis and degeneration [5].

The related literature has demonstrated that unbalanced microRNA expression as an unbalanced

## Abstract

**Background and Objective:** The present study aimed to assess the effects of the combined use of exercise and genistein on the hippocampal expression of microRNA-132, IGF-1, and BDNF in type 2 diabetic ovariectomized rats.

**Materials and Methods:** Wistar female rats in the weight range of 180-220 gr (n=10) were assigned to six groups: sham, ovariectomy, ovariectomized diabetic, ovariectomized diabetic treated with genistein for eight weeks, diabetic ovariectomized treated with swimming for eight weeks, and a group that was treated with both genistein and swimming for eight weeks. The effect of those treatments was assessed by the determination of microRNA-132, insulin growth factor 1 (IGF-1), and brain-derived neurotrophic factor (BDNF) expression levels within the hippocampus. These genes were evaluated by real-time-polymerase chain reaction (RT-PCR) and spatial memory was assessed by the Morris water maze.

**Results:** Ovariectomy demonstrated a decrease in the expression of microRNA-132, IGF-1, and BDNF in the hippocampus, as well as spatial memory, in diabetic ovariectomized rats, which showed a greater reduction in the expression of those genes in rats ( $P<0.05$ ). Nevertheless, genistein administration, swimming training, and a combination of them significantly up-regulated microRNA-132, BDNF, and IGF-1 expression, as well as spatial memory ( $P<0.05$ ).

**Conclusions:** As evidenced by the obtained results, the combined use of genistein and swimming could prevent estrogen deficiency effects in the hippocampus of ovariectomized diabetic rats.

**Keywords:** Diabetes, Genistein, Ovariectomy, Spatial memory, Swimming

expression of microRNA-132 is involved in diabetes pathogenesis and menopausal neuropathy [2] [6]. Earlier studies have pointed out that microRNA-132 represses the expression of neurotrophic and growth factors, such as insulin-like growth factor 1 (IGF-1) and Brain-Derived Neurotrophic Factor (BDNF) within the brain [7]. High concentrations of BDNF exist in the cortex and hippocampus, [8] and its altered expression is recognized to play a major role in psychiatric disorders, synaptic plasticity, and the functions correlated with the hippocampus [9]. Furthermore, growth hormone (GH) and IGF-1 have been recently discovered to affect the central nervous system [10]. It has been established that IGF-1

deficiency is causally related to the cognitive impairment of adults and the rodent models of aging [11].

In addition, clinical and experimental observations have emphasized that estrogen therapy exerts a positive effect on learning and memory functions [12]. Nonetheless, these benefits of estrogen for the brain are offset by the negative severe risks due to its oncogenic and proliferative effects on the breast and the endometrium [13]. Numerous studies have been performed to find an alternative for estrogen with less or no side effects. Regular exercise is a non-pharmacological intervention which is claimed to boost cognitive tasks in postmenopausal women [9].

Studies have demonstrated that exercise has modulatory effects on microRNAs, thereby improving BDNF and IGF-1 expression [1]. Furthermore, genistein, as a phytoestrogen, has similar estrogenic functions in neuronal protection with minor side effects. It has been demonstrated that genistein improves hippocampus neuronal proliferation and cell viability *in vitro*. These neuroprotective properties are mediated by the BDNF-Trk pathway [14]. Nevertheless, to the best of our knowledge, the effects of swimming or genistein and both of them on the expression of microRNA-132, IGF-1, and BDNF within the hippocampus of diabetic ovariectomized rats have not been investigated yet.

### Objectives

In light of the aforementioned issues, the present study aimed to assess the effects of swimming and genistein on microRNA-132, IGF-1, and BDNF appearance, as well as spatial memory, within the hippocampus of ovariectomized diabetic rats.

### Materials and Methods

#### Animals

Wistar female rats (10-week-old, weighting 180-220 g, n=60) were obtained from the Experimental Animal Research Center, Medical Faculty, Tabriz University, Tabriz, Iran. All rats were retained under well-ordered conditions (temperature 22-24°C with 12:12 dark-light cycle) and received a normal diet and ad lib water. The research was approved by the University Ethics Committee.

#### Design of Experiment

Animals were assigned to six groups (n=10) as follows:

1. The sham control group underwent surgery without ovariectomy.
2. The OVX group were subjected to bilateral ovariectomy.

3. The OVX.D group was subjected to the induction of type 2 diabetes 10 days after bilateral ovariectomy.

4. The OVX.D.S group included animals that were treated with swimming, along with type 2 diabetes induction, 10 days after ovariectomy.

5. The OVX.D.G group received genistein (1 mg/Kg/day; Sub Cutaneous) through the induction of type 2 diabetes 10 days after ovariectomy.

6. The OVX.D.G.S group was treated with both genistein and swimming.

#### Instruction of Ovariectomy

For this procedure, the animals were initially anesthetized (5 mg/kg xylazine and 50 mg/kg ketamine), and a small abdominal cut was made. The ovaries were then situated, and a silk cord was firmly tied to the oviduct and ovarian blood vessels. The oviduct was split, and the ovary was detached; thereafter, the muscle wall and skin were sutured with silk cord [1,15].

#### Instruction of type 2 diabetes

To induce diabetes, 10 days after ovariectomy, diabetic groups were nourished by a high-fat diet (25% protein, 17% carbohydrate, and 58% fat), and ad lib for the initial retro of eight weeks. Thereafter, a low dose of STZ (35 mg/kg) was injected intraperitoneally in a citrate 0.1 M buffer with pH=4.5 [16]. The fasting glucose level in all rats after the generation of type 2 diabetes was measured by the Accu-Chek Active blood glucose meter. Animals with levels of glucose higher than 250 mg.dl<sup>-1</sup> were selected as diabetic rats [1].

#### Genistein administration protocol

Genistein was injected into the animals (one mg/kg/day; Sub Cutaneous). [Sigma Chemical Incorporation (St. Louis, MO, USA)] [17].

#### Swimming protocol

After the recovery period of ovariectomy, animals were habituated with a swimming bath (5-20 min/day) for five consecutive days. Thereafter, trained animals swam for six successive days (60 min/day) for eight weeks, while Sham control group rats failed to swim. The exercised rats were studied 24 h after their latest exercise session [1].

#### Morris Water Maze

Spatial learning and memory were determined by the Morris Water Maze (MWM) task. The water maze was a dark round pool with a height of 100 cm, a diameter of 136 cm, and full of water (20±1°C) to a depth of 60 cm. The maze was conceptually allocated into four quadrants and release points that were marked as N, E, S, and W.

A hidden square platform (10-10 cm) was placed within the center of the southwest quadrant which was submerged 1.5 cm below the surface of the water. Fixed additional visual signs were present at various locations all over the maze. A video camera was attached above the middle of the maze to record and observe the motions of rats. This technique was used to measure the escape latency, traveled path, and swimming speed [1].

### Total RNA extraction and real-time polymerase chain reaction

The whole RNA, including messenger RNA and microRNA, was taken out from the hippocampus by RNX-Plus resolution kit (Fermentase, Cinagen Co. Iran) and miR-amp kit (Parsgenome Co. Iran) correspondingly in agreement to the

manufacturer's orders. The expression of BDNF, IGF-1, and microRNA-132 genes was quantitatively measured by a real-time polymerase chain reaction (real-time PCR). The sequences of primers for every gene are presented in Table 1. The number of PCR products was standardized for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), mRNA samples, and microRNA-191 for microRNA samples (internal control).

### Statistical analysis

Assessment of groups was accomplished by Tukey test two-way Analysis of variance (ANOVA). All analyses were performed in SPSS software (version 16). A p-value of less than 0.05 was considered statistically significant. The results were presented as means $\pm$ SEM.

**Table1.** Sequences of primers for genes

Genes	Accession number	Primers Sequence <sup>a</sup>
IGF-1	NM- 001082477	F: AAG CCT ACA AAG TCA GCT CG R: GGT CTT GTT TCC TGC ACT TC
BDNF	NM_012513	F: GCGGCAGATAAA AAGACT GC R: GCAGCCTTCCTTCGTGTA AC
GAPDH	NM_017008.4	F: TGCCGCTGGAGAAACCTGC R: TGAGAGCAATGCCAGCCCA
miR-132	MIMAT0017123	Target sequence b ACCGUGGCUUUCGAUUGUUACU
miR-191a	MIMAT0000866	CAACGGAAUCCAAAAGCAGCUG

<sup>a</sup> Sequences were derived from NCBI (www.ncbi.nlm.nih.gov)

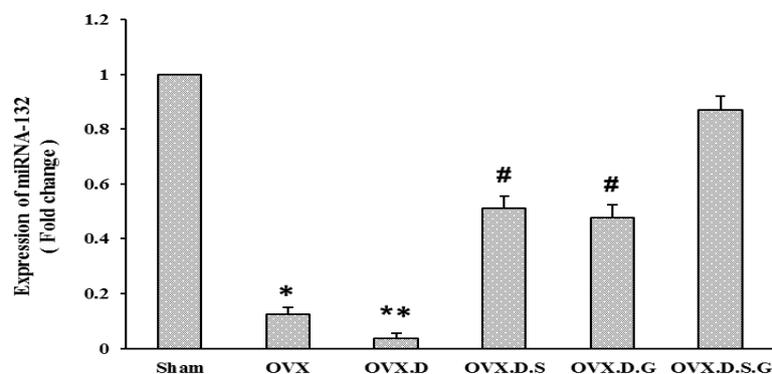
<sup>b</sup> Sequences were derived from miRBase (www.mirbase.org)

## Results

### MicroRNA-132 expression

As illustrated in Figure 1, the microRNA-132 appearance level was meaningfully diminished in the hippocampus of the OVX group, in comparison with that in the sham group ( $P<0.05$ ), and this decrease was more pronounced in the OVX.D group, as compared to the sham group ( $P<0.01$ ). Moreover,

Genistein and swimming treatment in OVX.D group significantly increased the microRNA-132 expression associated with OVX and OVX.D groups ( $P<0.05$ ). The combined use of swimming and genistein augmented hippocampal expression of microRNA-132, as compared to other groups (OVX, OVX.D, OVX.D.S, OVX.D.G), and this increase was statistically significant ( $P<0.05$ ).



**Figure 1.** MicroRNA-132 expression in the hippocampus of the studied groups

OVX: ovariectomized group, OVX.D: ovariectomized with type 2 diabetic group, OVX.D.E: ovariectomized diabetic group with eight-week exercise. OVX.D.G: ovariectomized diabetic group with eight-week genistein injection. OVX.D.G.E: ovariectomized diabetic group with eight-week swimming training and genistein injection. Data are stated as mean $\pm$ SEM.

\* Significant differences, compared to the Sham, OVX.D, and treatment groups ( $P<0.05$ )

\*\* Significant differences, in comparison with Sham, OVX, and treatment groups ( $P<0.05$ )

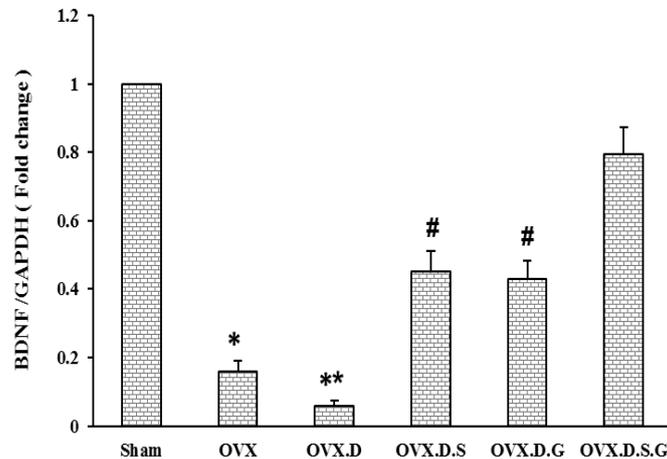
# Significant differences, compared to Sham, OVX, OVX.D, and OVX.D.G.S groups ( $P<0.05$ )



### Expression of brain-derived neurotrophic factor

The expression of BDNF is displayed in Figure 2. The expression level of BDNF was meaningfully reduced in the hippocampus of the OVX group ( $P<0.05$ ) and further declined in the OVX.D group, in comparison with the sham group ( $P<0.01$ ). The expression of BDNF was

meaningfully up-regulated in the hippocampus of OVX.D.S and OVX.D.G groups, as compared to that in OVX and OVX.D groups ( $P<0.05$ ). The combined use of swimming and genistein improved hippocampal expression of BDNF, in comparison with OVX, OVX.D, OVX.D.S, OVX.D.G groups ( $P<0.05$ ).



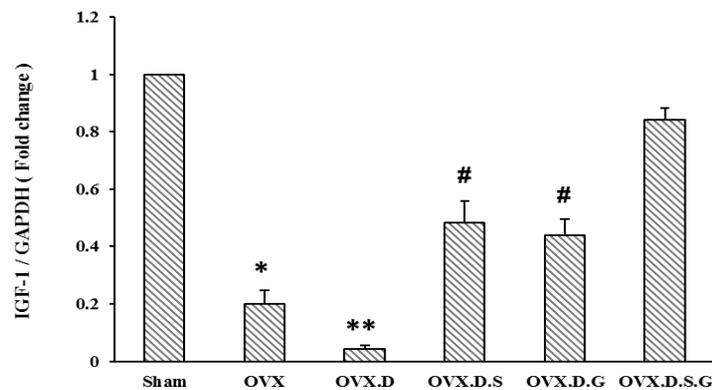
**Figure 2.** BDNF expression in the hippocampus of the studied groups

OVX: ovariectomized group, OVX.D: ovariectomized with type 2 diabetic group, OVX.D.E: ovariectomized diabetic group with eight-week exercise. OVX.D.G: ovariectomized diabetic group with eight-week genistein injection. OVX.D.G.E: ovariectomized diabetic group with eight-week swimming training and genistein injection. Data are stated as mean $\pm$ SEM.

\* Significant differences, compared to the Sham, OVX.D, and treatment groups ( $P<0.05$ )

\*\* Significant differences, in comparison with Sham, OVX, and treatment groups ( $P<0.05$ )

# Significant differences, compared to Sham, OVX, OVX.D, and OVX.D.G.S groups ( $P<0.05$ )



**Figure 3.** Expression of insulin growth factor 1 in the hippocampus of the studied groups

OVX: ovariectomized group, OVX.D: ovariectomized with type 2 diabetic group, OVX.D.E: ovariectomized diabetic group with eight-week exercise. OVX.D.G: ovariectomized diabetic group with eight-week genistein injection. OVX.D.G.E: ovariectomized diabetic group with eight-week swimming training and genistein injection. Data are stated as mean $\pm$ SEM.

\* Significant differences, compared to the Sham, OVX.D, and treatment groups ( $P<0.05$ )

\*\* Significant differences, in comparison with Sham, OVX, and treatment groups ( $P<0.05$ )

# Significant differences, compared to Sham, OVX, OVX.D, and OVX.D.G.S groups ( $P<0.05$ )

### Expression of insulin growth factor 1

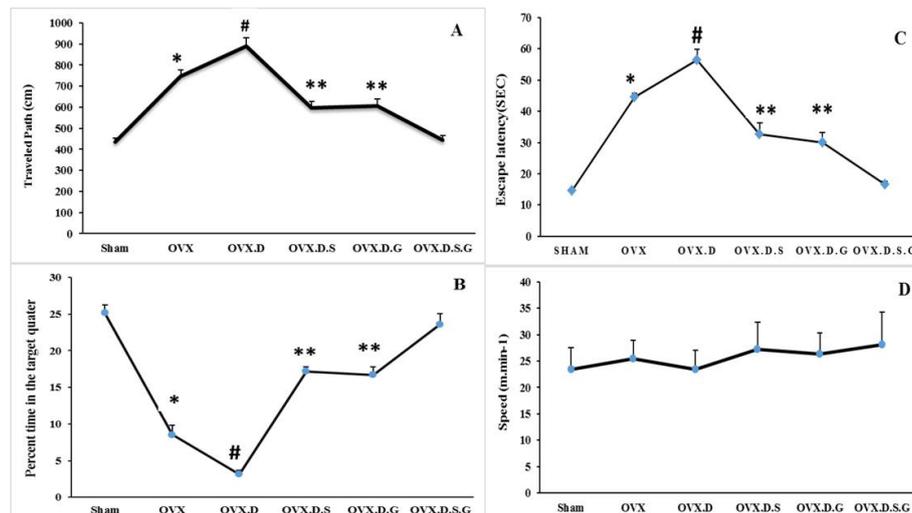
The expression of IGF-1 is depicted in Figure 3. The IGF-1 expression level was meaningfully reduced in the hippocampus of the OVX group, as compared to that in the sham group ( $P<0.05$ ), and this reduction was more pronounced in the OVX.D group, in comparison with the sham group ( $P<0.01$ ). Moreover, the result

demonstrated that exercise training and genistein up-regulated the expression of IGF-1 in the OVX.D group, in comparison with with OVX and OVX.D groups ( $P<0.05$ ), and this up-regulation was more pronounced in the hippocampus of the OVX.D.G.S group, as compared to other ovariectomized animals ( $P<0.05$ ).

### Effect of swimming and genistein on spatial memory in different groups

Swimming and genistein treatment decreased path length (Figure 4A), travel latencies of searching for the hidden platform on acquisition trial (Figure 4B), and an increased percentage of spent time in the target quadrant (Figure 4C), and this variance was statistically significant ( $P < 0.05$ ). There were no significant changes in swimming speed among all exercised groups (Figure 4D).

Ovariectomy significantly impaired spatial learning performance in the MWM ( $P < 0.05$ ), and this impairment was more noticeable in the OVX.D group ( $P < 0.05$ ). Genistein and swimming enhanced spatial learning in OVX rats, in comparison with OVX and OVX.D groups ( $P < 0.05$ ). The improvement of spatial learning in the OVX.D.G.S group was within the range of the sham group. There was no important variance among the groups in swimming speed.



**Figure 4.** Effects of genistein and/or exercise on learning acquisition as measured through escape latencies (A), memory retention during the probe trial (B), the percentage of time spent in the target quadrant (C), and swimming speed (D) detected in the MWM. Ovariectomy meaningfully decreased spatial learning presentation in the MWM ( $P < 0.05$ ), and this impairment was more pronounced in the OVX.D group ( $P < 0.05$ ). Spatial learning in OVX rats was also improved by the exercise and genistein injection in OVX.D, in comparison with OVX.D and OVX groups ( $P < 0.05$ ). In OVX.D.G.E group, spatial learning improved and no significant difference with Sham group. No significant difference was found among the groups in swimming speed.

OVX: ovariectomized group, OVX.D.E: diabetic ovariectomized group with eight-week exercise. OVX.D.G: diabetic ovariectomized group with eight-week genistein injection. OVX.D.G.E: diabetic ovariectomized group with eight-week swimming and genistein injection. Data are stated as mean  $\pm$  SEM.

\* Significant differences, compared to the Sham, OVX.D, and treatment groups ( $P < 0.05$ )

\*\* Significant differences, in comparison with Sham, OVX, and treatment groups ( $P < 0.05$ )

# Significant differences, compared to Sham, OVX, OVX.D, and OVX.D.G.S groups ( $P < 0.05$ )

### Discussion

The present study was the first to determine whether exercise and/or genistein play a role in appearance changes of microRNA-132, IGF-1, and BDNF in the hippocampus of diabetic ovariectomized rats. Therefore, in this research, rat models of ovariectomy, diabetic ovariectomized rats, and diabetic ovariectomized rats that were treated with eight weeks of swimming and/or genistein administration were compared with the sham group. The major findings were as follows: 1) ovariectomized rats with or without diabetes markedly down-regulated microRNA-132, and 2) swimming and genistein increased the expression of these biomarkers; however, the combined use of them had a more significant effect.

The studies in the last few years have focused on the analysis of variance expression of microRNAs in heart failure, and the key part of the mammalian genome is controlled by microRNAs with great

tissue specificity [4]. Synaptic smoothness, neuronal cell growth, swelling, and angiogenesis are associated with microRNA-132 [18]. A large amount of data, in vivo and in vitro, support the role of microRNA-132 in the mechanisms of memory formation and activity-stimulated gene expression [19]. Nevertheless, the expression of microRNA-132, as well as the effect of genistein and/or swimming in the hippocampus of the diabetic ovariectomized rat, has not been studied yet.

Ang (2006) pointed out that ovariectomy decreased the latency and distance to obtain platform in Morris Water Maze; nonetheless, this reduction was significantly more pronounced in the diabetic group. The studies have suggested that the irregular microRNAs expression is also involved in the pathogenesis processes in menopause and diabetic neuropathy, such as miRNA-1, -133, -141, -206, -223, -373, and -132 [20,21]. It seems that

microRNAs control neuronal survival, synaptic plasticity, and neurotransmitter secretion through numerous mechanisms. It has been shown that BDNF and IGF-1 can increase microRNA-132 expression [22]. The BDNF improves memory and plays a key role in the expansion of dendritic spines, differentiation, and survival for extensive types of neuronal cells [23].

Furthermore, IGF-1 is associated with pro-insulin, is an effective factor for oligodendrocytes and neurons, as well as neural elaboration, differentiation, and growth [19,24]. A large amount of data, in vivo and in vitro, support the role of microRNA-132 in the mechanisms of memory formation and activity-stimulated gene expression [19]. Nevertheless, the expression of microRNA-132, as well as the effect of swimming and/or genistein treatment in the hippocampus of the diabetic ovariectomized rat, has not been studied yet. Similar to other studies, the current research indicated that using Morris water maze protocol, ovariectomy increased the latency and distance to the obtain platform, compared to sham rats, and this increase was significantly more pronounced in the diabetic group. Moreover, to the best of our knowledge, for the first time, the obtained data demonstrated that the microRNA-132, IGF-1, and BDNF expression is down-regulated in the hippocampus of ovariectomized rats with or without diabetes.

Hormone therapy, such as estrogen, improves cognition and stops the progress of dementia in postmenopausal women, while other studies have not established the benefit of estrogen application [25,26]. In addition, the results of extensive investigational trials have indicated that hormone replacement treatments, such as estrogen-alone therapy, increase the risk of breast cancer in postmenopausal women [9,27]. A great deal of effort has been made to prevent cognitive dysfunction, as well as learning and memory failures, in postmenopausal women mostly with diabetes, among which swimming and genistein have been recommended as substitutes for estrogen replacement to stop cognitive failure [9,14].

In the present study, swimming and genistein treatment for eight weeks improved spatial memory, and this improvement was significantly more preannounced in the combined use of swimming and genistein treatment, as compared to OVX with or without diabetes group. In this study, eight weeks of regular swimming enhanced the expression of microRNA-132, IGF-1, and BDNF in the ovariectomized diabetic rats, in comparison with other groups. In addition, eight weeks of swimming enhanced spatial learning and

memory on the MWM task in OVX.S, as compared to OVX animals.

The findings of the present study confirm the prior investigation, indicating that exercise training can disturb the risk factors of cognitive dysfunction. Cell proliferation can be enhanced by exercise which can inhibit apoptosis in the hippocampus and improves cognitive function despite estrogen deficiency [28]. Exercise can raise the expressions of IGF-1 and BDNF by increasing the expression of microRNA-132. Moreover, the expression of microRNA-132, IGF-1, and BDNF was boosted by eight weeks of genistein injection in the ovariectomized rats. In this study, genistein administration enhanced spatial learning and memory on the MWM task in OVX.G, as compared to OVX animals.

It has been proven that genistein, which has estrogenic and anti-diabetic effects, could be used as a substitute for estradiol to protect from central nervous system degeneration in diabetic postmenopausal women. The addition of genistein to the diet of ovariectomized rats enhanced the proteins in the brain [29]. Moreover, studies have pointed to the special effects of genistein on microRNAs [1]. Phytoestrogens, such as soy, improve cognition, which might be associated with the increase in the expression of BDNF and synaptogenic proteins in the hippocampus of ovariectomized rats [30].

The effects of daidzein and genistein on the expression of BDNF and hippocampus neuronal cell proliferation have been studied [31]. Furthermore, the combined use of exercise and genistein prevents cognitive impairment risk factors by increasing microRNA-132 gene expression, which may be related to an increase in IGF-1 and BDNF expressions in diabetic ovariectomized rats, as compared to other groups. The obtained data indicated that in estrogen-deficient states with diabetes, regular moderate exercise combined with genistein administration is the most effective treatment for cognitive function disturbances.

### Conclusions

The results of the current study pointed to the beneficial effects of the combined use of swimming and genistein treatment on the improvement of spatial memory in OVX diabetic rats. These beneficial effects could also be caused by the increased microRNA-132 expression which is related to the up-regulation of IGF-1 and BDNF genes within the hippocampus of rats. Furthermore, a combination of exercise and genistein appears to be highly effective in inhibiting the expansion of cognition dysfunction.

### Acknowledgments

The funding for this study was supported by the Drug Applied Research Center of Tabriz University of Medical Sciences, Tabriz, Iran.

### Conflicts of Interest

The authors declare that they have no conflict of interest.

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