Evaluation the Protective Effect of Zinc Supplementation on Depression and Mir 129-GPR39 Gene Expression in the Hippocampus of Diabetic Rat

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Abstract

Background: MicroRNAs (miRs) has growing evidence in the pathogenesis of diabetic depression. The present study evaluated the oral administration of zinc (Zn) on depression behaviors induced by alterations in the mir-129 and G protein-coupled receptor 35 (GPR35) in the hippocampus of diabetic rats.

Method: Rats were divided into six groups of 10 each. Healthy and diabetic group, healthy and diabetic group received 200 mg of Zn, and healthy and diabetic group received 350 mg of Zn. Animals were made diabetic by an intraperitoneal (i.p.) injection of newly ready Streptozotocin (STZ; 50 mg/kg body weight). After four weeks of oral gavage, the forced swim test (FST) was investigated to assess depression. The expression of the mir-129 and GPR35 gene level in the hippocampus was measured using the real-time PCR method. Data were shown as mean ± SEM and analyzed for comparison using one-way ANOVA in SPSS20 software.

Result: The analysis of behavioral tests demonstrated the improvement of immobility over the course of the test in the diabetic rats, whereas swimming behavior increased in rats received 200 and 350 mg of Zn (P-value<0.001). GPR35 and mir-129 gene expression dropped significantly in diabetic rats but Zinc supplementation did not restored their expression in them (P-value > 0.05). Expression of GPR35 gene in rats received 350 mg of Zn elevated significantly in compare to diabetic mice (P-value<0.001).

Conclusion: Our findings revealed that Zn has ability to induce an antidepressant-like effect and ameliorate the rat mobility, suggesting a possible antidepressant action of Zn in diabetic rats.

Keywords: Diabetes - mir-129 - GPR39 - Hippocampus - Forced swim test.

Tob Regul Sci.™ 2022; 8(1): 573-583
DOI: doi.org/10.18001/TRS.8.1.50

1- Introduction

Hyperglycemia is recognized as a chronic metabolic disease like diabetes affecting a remarkable percentage of the world population. The main subtypes of diabetes are type 1 (insulin deficiency) and type 2 (insulin resistance) (1).

In diabetes, a major difficulty is the atrophy of hippocampal formation, which is associated with learning and memory behaviors in mammals (2). Depression is also one of the most neglected problems in patients with diabetes and has a link with cognitive deficits and hippocampal degeneration (3). Depression considerably declines the quality of a great life and medical adherence, reduces mental and physical health, causes unsatisfactory diet, and even increases death (4).

Studies on magnetic resonance imaging (MRI) have suggested that in depression, brain is changed structurally, and the hippocampal formation is damaged (5). It seems that the level of shrinkage of hippocampus determines different phases of depression (6). In depression, insulin administration boosts memory and causes hippocampal neurogenesis (7). It has also been proven that trace metals have a connection with glucose metabolism and diabetes (8). Zinc (Zn) activates over 300 enzymes in the body and has been reported to play a key role in glucose maintenance and metabolism, insulin biosynthesis, and diabetes therapy (9). This element also has an essential role in the proper function of glucose metabolism in the hippocampus area, particularly in the brain (10). The central target area for both Zn oxide nanoparticles and Zn sulfate treatments has been shown to be hippocampus (11). It has also been affirmed that Zn deficiency increases depression symptoms; however, they can be ameliorated by antidepressants (10).

Micro-ribonucleic acids (miRNAs) are a group of small, single-stranded genes (having 20-32 nucleotides) discovered at the beginning of 1990s (12). These molecules have a vital function in biologic and physiologic processes viz proliferation, evolution, and differentiation and are considered among the genes involved in diabetes (13). MiR-129 is expressed in neural progenitor cells and cortical neurons during cortical neurogenesis (14). Previously, it has been reported that Zn deficiency lessens tissue Zn level and alters miRNA expression in rat tissues (15). MiR-129 is abundantly expressed in hippocampal neurons where it regulates the expression of the potassium channel $K_v1.1$, in order to control neuronal excitability (16, 17).

GPR39, identified as Zn^{2+}-sensing receptor (ZnR), is a member of a large family A of 7-transmembrane comprising G protein-coupled receptors and exists in all vertebrates. GPR39, a member of the ghrelin receptor subfamily, was cloned with GPR38 as structural homologues to the ghrelin receptor from human fetal brain (18). For GPR39, no endogenous peptide ligand has been explored yet (19). However, the presence of an ionic Zn receptor was unpredictably displayed after it was identified that extracellular Zn^{2+} is able to activate intracellular signaling Ca^{2+}. The receptor was then called ZnR, and it is now known as GPR39 (20). Later, it was indicated that the physiological concentrations of Zn^{2+} have ability to activate GPR39. The hippocampus and amygdala are the two brain regions with the highest levels of GPR39 (21). Initiation of GPCR signaling targets the expression of multiple miRNAs and demonstrates the ability of miRNAs to target 3 = -UTRs of different genes, thereby making miRNA the main amplifier of the GPCR signaling cascade (22). Besides, GPR39 causes the synthesis of proteins that are involved in neuronal plasticity and hippocampal-related antidepressant behaviors, (23). Earlier findings have reflected that Zn improves ZnR/GPR39 receptor characters in the

pathogenesis of Alzheimer's disease caused by streptozotocin (STZ) (24). Evidence has also demonstrated that Zn deficiency results in depressive-like behaviors, and impaired GPR39 neurotransmission is a probable mechanism of the pathophysiology of depression (25).
Considering the protective effects of Zn on the complications of diabetes on neurons in the hippocampus, it can be speculated that Zn may have neurogenesis and ameliorate depressive-like effects by a direct impact on the expression of mir-129-GPR39 genes. Therefore, the aim of this study was to evaluate the effect of mir-129-GPR39 expression and also to assess the effect of depression.

2- Materials and Methods

2-1- Experimental animals

A total of 60 adult male Wistar rats, weighing 200–250 g, were obtained from Kharazmi Laboratories (Tehran, Iran) and kept in groups of 10 with *ad libitum* access to food and water. Animals were preserved in a temperature-controlled area with a light/dark cycle of 12:12 h. Trials were carried out through a 12-light cycle and in accordance with the Ethics and Procedures of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animals were randomly divided into control (C), diabetic (D), diabetic treated with 200 mg of Zn (DZ200), control treated with 200 mg of Zn (Z200), diabetic treated with 350 mg of Zn (DZ350), and control treated with 350 mg of Zn (Z350). The Zn sulfate ($ZnSo4.H_2O$) lyophilized powder was purchased from Sigma Aldrich (USA; Catalog Number: 7-19-7446).

2-2- Induction of diabetes

Diabetes was induced in rats by a single i.p. dose of Streptozotocin (STZ; 50 mg/kg body weight) melted in a fresh 0.01 M citrate buffer, pH 4.522. After seven days of STZ usage, the blood glucose levels of all rats were measured, and a range of ≥250 mg/dL was considered as diabetic. Treatments were started on day eight after STZ injection. At the same time, Zn was supplemented by gavage at the doses of 200 and 350 mg/kg of body weight, which was considered day one of treatment and continued for four weeks (26). For blood glucose level measurement of fasted Rats was cleaned and injured, blood was taken using a glucometer strip, and blood glucose level result was registered using a glucometer. Twenty four hours after the last treatment, the fasted animals were deep anesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg) (27).
Blood from fasted rats was collected to define serum zinc concentration by atomic absorption spectrophotometry (Varian Spectrophotometer Spectr AA-20, air acetylene flame, 0.5-nm slit, wavelength of 213.9 nm, Perkin-Elmer, Norwalk, CT) (28).
The brain was excised immediately and washed in ice-cold physiological saline several times. Then the expression levels of the miR-129 and G protein-coupled receptor 35 (GPR35) protein in the hippocampus were measured by qRT-PCR.

Forced swim test

Each rat was forced to swim in a chamber (40 cm height and 15 cm diameter) for 15 min; the chamber was filled up with fresh water up to a height of 30 cm. This process was regarded as the 'pretest' swim. After 24 h, rats were re-exposed to the swimming status in the chamber for 6 min ('test session'). In the last five minutes of test session, the entire duration of swimming, climbing,

and immobility was recorded for all animals. Swimming and climbing behaviors were defined as frequently horizontal movement and upward directed movement of the forepaws throughout the swimming chamber, respectively. Immobility included no extra movement except that needed for the rat's head above the water (29).

Hippocampal RNA extraction and qRT-PCR analysis of miRNA-129 and GPR35

Hippocampal tissues of rats were selected, and hippocampal total RNA was extracted using Trizol kit according to the manufacturer's instructions. An ultraviolet spectrophotometer was used to determine the purity and concentration of total RNA, and formaldehyde denaturing agarose gel electrophoresis was performed to detect the integrity. Total RNA was stored at -80°C until use (30). Primers sequences employed in RT-PCR were as follows: forward: 5'-ACCACTGGTGTTGAGACGCC-3' and reverse: 5'-TCTGGGTCTTTGAACTCGCTG-3' (31) for GPR35, and forward: 5'-ACACTCCTTTTTGCGTCTGGGCTTGC-3' and reverse: 5'-TGGTGTCGTGGAGTCG-3' for miR-129 (32). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcription levels were utilized as an internal reference. RevertAid First Strand cDNA Synthesis Kit (Life Technologies, Thermo Fisher Scientific Inc., Waltham, MA, USA), and 5 μg of RNA were employed for reverse transcription and for each sample, respectively. For RT-qPCR, we used RT-qPCR Master Mix (Toyobo Co., Ltd., Osaka, Japan), which was run on an ABI 7500 fast real-time fluorogenic quantitative RT-PCR system (Life Technologies, Thermo Fisher Scientific). The analysis of RT-qPCR experimental data was conducted by the aid of an ABI 7900 system SDS software (Life Technologies, Thermo Fisher Scientific), and relative quantitative was analyzed by the $2^{-\Delta\Delta CT}$ method. The following formula was used to calculate the index quantifying expression difference (multiple) from the control: Relative quantity (RQ) $=2^{-\Delta\Delta CT}$ and $\Delta\Delta CT= (CT1-CT2) - (CT3-CT4)$. The threshold cycle numbers of target genes and reference genes of experimental samples are shown by CT1 and CT2 and those of control samples are indicated by CT3 and CT4, respectively (33).

Statistical analysis

All data were stated as mean standard error. Results were analyzed by ANOVA, followed by Tukey's post hoc test. All statistical analyses were performed using SPSS 20 (IBM, Chicago, IL, USA). P values <0.05 were considered statistically significant.

3- Results

3-1- Effect of STZ injection on glucose measurements

Three groups of non-diabetic rats and three groups of STZ diabetic rats were prepared. The blood glucose values were determined for four weeks. Measurements of blood glucose were made from the tails and at weekly intervals (Fig. 1). The loss of normal glucose homeostasis was obvious in STZ diabetic rats, especially in the first day of trial (P-value <0.001). At the highest gavage Zn dose (350 mg/kg), glucose level decreased significantly after four weeks of experimentation in the diabetic group (P-value <0.001), while 200 mg/kg of Zn gavage reduced relatively lower level of glucose level (P-value <0.05).

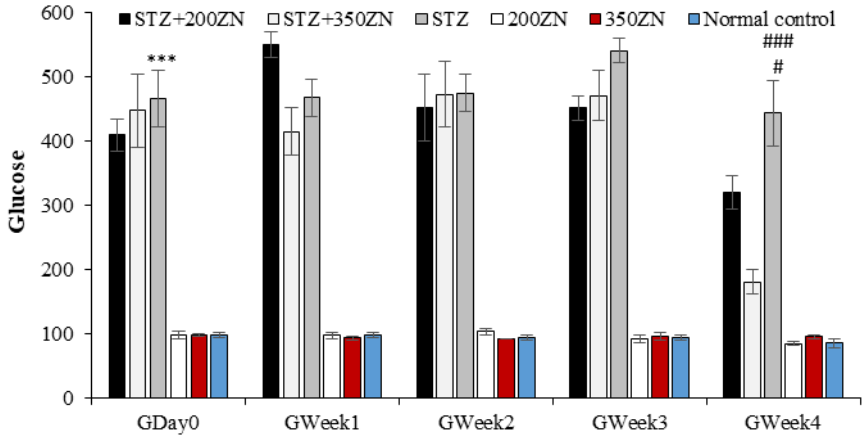


Fig. 1. Glucose (G) level changes in non-diabetic compared to STZ diabetic rats in four continuous weeks. Data points were expressed as mean ± SEM (n=10). ***P-value <0.001 vs. the control group; ###P-value <0.001 vs. DZ350 group; #P-value <0.05 vs. DZ200.

3-2- Increased level of Zn in the blood of non-diabetic and diabetic rats

Zn has a main part in supporting neuronal function by neurogenesis. As depicted in Figure 2, the level of Zn increased in the blood serum of rats. In addition, Zn serum level results indicated an obvious elevation with high dose treatment in both the non-diabetic and diabetic rats. Zn oral gavage also enhanced the Zn serum level in the blood of both rat groups. However, in untreated diabetic rats, the serum level of Zn decreased (P-value <0.001). At the time when the rats received 350 mg/kg of oral gavage of Zn, the serum level significantly elevated (P-value <0.01).

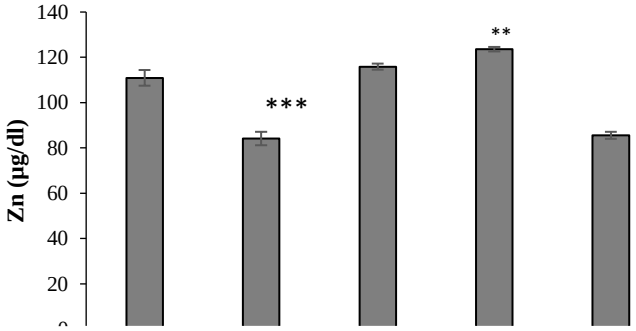


Fig. 2. Zn serum level changes in non-diabetic rats compared to STZ diabetic rats at week four. Data are shown as the mean ± SEM (n = 10). ***P-value < 0.001 vs. control group, **P-value < 0.01 vs. control group; ##P-value < 0.01 vs. diabetic group; #P-value < 0.05 vs. diabetic + Zn 200 mg/kg group; control (C), diabetic (D), diabetic treated with 200 mg of Zn (DZ200), control treated with 200 mg of Zn (Z200), diabetic treated with 350 mg of Zn (DZ350), and control treated with 350 mg of Zn (Z350).

3-3- Effect of Zn administration as an antidepressants

Based on forced swim test (FST), Zn meaningfully increased the swimming behavior and reduced immobility time, as represented in Figure 3. In the control rat received 200 mg/kg of Zn, the duration of immobility was significantly shortened (P-value < 0.001). In the FST, the administration of Zn supplement (200 and 350 mg/kg) for four week made decreased immobility in diabetic rats (P-value < 0.001).

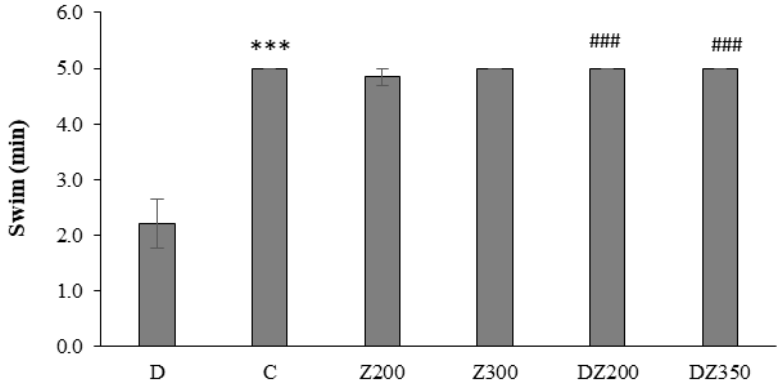
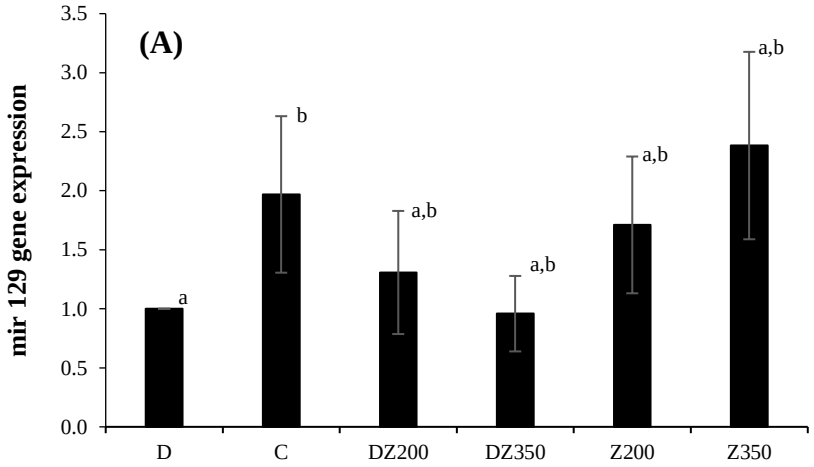


Fig. 3. Immobility changes of non-diabetic rats compared to STZ diabetic rats at week four. Data are shown as the mean ± SEM (n = 10). $^{***}p < 0.001$ vs. control group; $^{###}p < 0.01$ vs. diabetic group; control (C), diabetic (D), diabetic treated with 200 mg of Zn (DZ200), control treated with 200 mg of Zn (Z200), diabetic treated with 350 mg of Zn (DZ350), and control treated with 350 mg of Zn (Z350).

3-4- Effects of Zn upregulation on mir-129 and GPR35

The descending effects of diabetes on the miR-129 and GPR35 are displayed in Figure 4. The results showed a significant increase of GPR35 gene in the Zn 350 mg/kg received rats in compare to diabetic rats. However, 350 and 200 mg/dl of Zn treatment in diabetic group induced a minor elevation in the expression of miR-129 and GPR35 genes, no statistically significant changes were observed in miR-129 and GPR35 levels among diabetic groups during four weeks of regular Zn feeding.



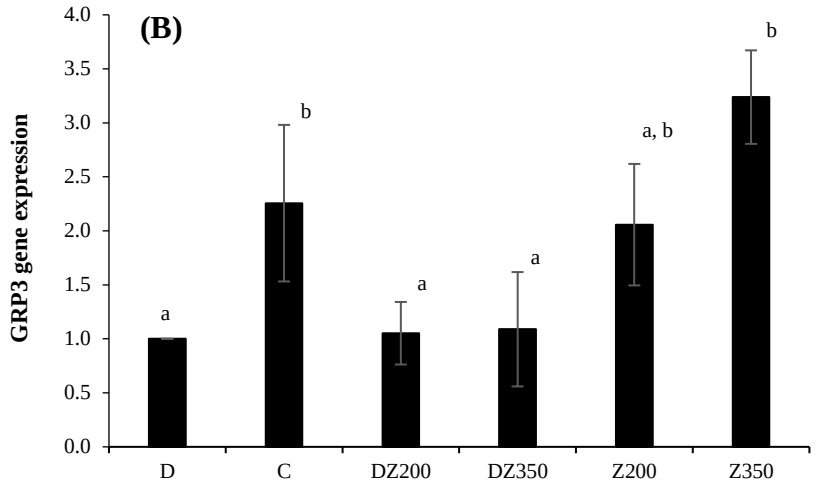


Fig. 4. Changes of (A) miR-129 and (B) GPR35 levels changes in non-diabetic rats compared to STZ diabetic rats at week four. Data are shown as the mean ± SEM (n = 10). Control (C), diabetic (D), diabetic treated with 200 mg of Zn (DZ200), control treated with 200 mg of Zn (Z200), diabetic treated with 350 mg of Zn (DZ350), and control treated with 350 mg of Zn (Z350). Same letters indicate non-significant difference; non-same letters indicate significant (P-value < 0.05) difference between groups.

3- Discussion

Medical research has supported the role of Zn in the inhibition and treatment of depression. The continuous use of Zn has been demonstrated to enhance the effectiveness of antidepressant drug therapy in patients suffer from depression (34).

The results of this study indicated that the chronic orogastric gavage of rats with 350 mg/kg of Zn could increase the blood serum level Zn in both non-diabetic and diabetic rats. As a previous study has shown, diabetic depression is associated with the metabolic changes of prefrontal cortex, hypothalamus, and hippocampus in rats (4). Our results also suggested that Zn supplement compound could reduce the blood glucose concentration in STZ-induced diabetic rats in doses of 200 and 350 mg after four weeks of experimentation. The above-mentioned findings support the concept that Zn may positively work on blood glucose control in diabetes patients, in order to prevent depression behaviors directly linked to hippocampus. Cavalcanti et al. confirmed our work and showed that supplementation with Zn compounds has neurobehavioral benefits, such as antidepressant and neuroprotective effect, in diabetic animals (10). Dou et al.'s study also proved that zinc and folic acid combination can improve the depression symptoms of rats, and its mechanism is related to the increased levels of 5-hydroxytryptamine, dopamine, and norepinephrine in the brain (35).

The FST revealed longer immobility time in diabetic than Zn-treated diabetic rats and reflected depressive behavior (36). Type 2 diabetes has been linked to anxiety disorders and increased risk of depression (37). In our study, shorter immobility time was observed in groups received 200 and 350 mg/kg of Zn compared to the diabetic group, indicating Zn antidepressant activity. The reason for such observation is likely that immobility time is considered as an index of depression in rodent subjects (38).

Locomotor activity results revealed a significant increase of swimming time in both the DZ200 and DZ350 compared to the D group. Locomotor activity times in the Z200 and Z350 groups, unlike the C group, did not present a statistically significant difference. In this regard, we speculate that the positive effect of Zn supplementation occurs only in diabetic animals because

Zn supplement does not significantly alter the behavior of healthy animals throughout the behavioral tests (36). As per glutamatergic depression theory, Zn can possibly be a beneficial antagonist for NMDA receptor and develop fast-acting antidepressants via the GPR39 receptor (39). Mice and rats received Zn supplement could reduce depressant-like behaviors, as measured by the FST and tail suspension test (40).

GPR39 role in depression has been indicated by Zn deficiency, which downregulates cAMP response element-binding protein and brain-derived neurotropic factor in the hippocampus (20). It has been disclosed that an exterior stimuli or GPCR-ligand coupling motivates the transcription of miRNA in the nucleus (22). It has also been exhibited that miR-129-5p reduces depressive-like behaviors by negatively regulated MAPK in the hippocampus of chronic unpredictable mild stress mice (41). Considering the set of results, we hypothesize that Zn can interact with the GPR39 signaling, which activates miR-129, a key player in the reduction of depressive-like behaviors. The survey of Zeng et al. displayed that high miR-129-5p decreases the apoptosis and increases the proliferation of neuronal cells. They also emphasized that high miR-129-5p can alleviate the neuronal wound and inflammatory response in rats with Alzheimer's disease (42). A study by Wang et al. signified that miR-129-3p can protect against oxidative stress and apoptosis of hippocampal neurons exposed to increased glucose thorough decreasing calcium uniporter (43). In the present study The same as previous investigations, we found lower expression of miR-129-5P and GPR35 in hippocampal tissue of diabetic rats, which could be considered as a potent reason for causing movement problems and depressive behaviors in these animals. Although prescription of Zn had restoring effect on locomotor activities and depression –like behaviors, expressions of miR-129 and GPR35 did not have significantly such an effect on diabetic rats receiving Zn supplement. Given that our results showed that zinc supplementation at the concentration of 350 mg/dl led to a sharp increase in miR-129 and GPR35 genes expression in healthy rats, we speculate that more treatment of Zn may be needed to restore the expression of those genes in diabetic mice, however, this needs further investigation.

Our findings provide new insight into further study of the pathogenesis of diabetic depression. We believe that by using this method, additional connection of miR-129 with GPR39 can be discovered in patients with diabetic depression, which provides a more rational basis for scientific conclusions.

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