The Effect of Exercise Training and *Portulaca Oleracea* on Neurobehavioral Dysfunction in Type 2 Diabetic Rats

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Abstract

Background: Diabetes mellitus is one of the most important causes of Alzheimer's disease and dementia. *Portulaca oleracea* (*P.oleracea*) is a rich source of antioxidants, which reduces inflammation and oxidative stress in diabetic rats. Exercise training has also been shown to improve mental function and enhance learning and memory efficacy. Therefore, this study was designed to explore the potential combined effect of *P. oleracea* and exercise training on neurobehavioral dysfunction in streptozotocin (STZ)-induced diabetic male rats.

Methods: For this purpose, 50 male Wistar rats were divided into five groups: 1) healthy control group (Con), 2) sedentary diabetic group (D), 3) diabetic rats treated with *P. oleracea*(D+Po), 4) diabetic rats treated with exercise training (D+Ex), and 5) diabetic rats treated with *P.oleracea* and exercise training (D+Po+Ex) simultaneously. Animals in the exercise groups were subjected to progressive swimming training for 12weeks. *P.oleracea* was mixed with standard pellet food for 12weeks. Neurobehavioral dysfunction was investigated by elevated plus-maze, shuttle box, open field, and novel object recognition tests.

Results: Compared with the normal control group, rats in the sedentary diabetic group showed a more passive avoidance memory deficit and more anxiety, and less exploration. Due to exercise training and treatment with *P. oleracea*, the neurobehavioral deficit in the trained diabetic rats receiving *P. oleracea* reached the normal levels of those in the healthy group.

Conclusion: These data demonstrated that diabetes causes significant neurobehavioral deficit. Nevertheless, swimming training and *P. oleracea* synergistically ameliorate and reverse the neurobehavioral deficit in STZ-induced diabetic male rats.

Background

Diabetes mellitus (DM) is one of the most common diseases of the endocrine system, which according to the prospective studies, its global prevalence due to the lifestyle of people will be increased in the coming decades [1]. The disease is caused by an impairment in the metabolism of carbohydrates, fats, and proteins, leading to the lack of insulin secretion or reduced tissue sensitivity to insulin [2]. Diabetes causes side effects, such as kidney and liver failure, cardiovascular complications, skin complications, and also an increase in the risk of peripheral nerve disorders and neurological complications [3]. In this regard, previous studies have demonstrated that hyperglycemia lasted for a long time leads to decreased nerve conduction velocity, axonal shrinkage, impaired nerve regeneration, and deficient axonal transport [4].

Diabetes leads to motor and cognitive impairments and learning disabilities. The molecular mechanism, through which diabetes could mediate these effects is not yet clear. In general, diabetes via promotion of reactive oxygen species (ROS) production leads to systemic inflammation and oxidative stress resulting in mitochondrial DNA damage [5]. On the other hand, brain tissue is rich in phospholipids that can be

attacked by ROS to initiate lipid peroxidation [6]. Lipid peroxidation can lead to the production of toxic dialdehydes. Malondialdehyde is one of the most toxic dialdehydes. Malondialdehyde is strongly associated with functional disorders in the central nervous system (CNS) [7]. Thus, after the onset of diabetes, cognitive dysfunction and synaptic plasticity gradually appear in the dentate gyrus of the hippocampus [5, 8]. Therefore, Alzheimer's disease and dementia are common in diabetics.

Now, the fundamental and unresolved question is how to prevent cognitive deficit and dementia in diabetic patients. One of the most effective factors is physical activity. Recently, several studies have been conducted on the effects of exercise on brain function, and several biological mechanisms have been proposed on the protective effects of exercise and physical activity on brain abilities. Physical activity is widely accepted as a behavioral strategy to enhance general health, including mental function. One of the important effects of exercise on brain function is memory improvement [9]. Therefore, brain health is one of the most important goals in human life, which can be achieved by physical activity [10]. There is growing evidence that exercise training increases insulin sensitivity (increased insulin secretion and GLUT-4), plasticity, and neurogenesis in the brain [11, 12]. On the other hand, exercise increases the amount of blood flow to the brain. Exercise-induced increased blood flow to the brain appears to slow the process of brain cell loss, which approximately begins at the age of 40 and improves brain function [10]. Exercise training represents one of the main extrinsic factors that can profoundly increase hippocampal neurogenesis in adults, by altering neurochemistry and function of newly generated neurons [13].

Another factor that can be used to treat neurobehavioral dysfunction in diabetics is using herbal remedies with hypoglycemic and anti-inflammation effects. *Portulaca oleracea* belongs to the Portulacaceae family and has hypoglycemic effects. It is widely found in North Africa, the Middle East, India, Malaysia, Australia, and United States. *P. oleracea* contains significant amounts of vitamins A and C, flavonoids, alkaloids, polysaccharides, terpenoids, sterols, calcium, iron, magnesium and potassium, and omega-3 [14]. The neuroprotective, antimicrobial, antidiabetic, antioxidant (vitamins A, C, and E, β -carotene, and glutathione), anti-inflammatory, antiulcerogenic, and anticancer effects of the *P. oleracea* have been reported [14, 15]. Previous studies have shown that *P. oleracea* increases insulin sensitivity and ameliorates impaired glucose tolerance in type 2 diabetic rats [16]. This herbal medicine can be used as an adjunctive and alternative therapy to ameliorate memory disorders in diabetics.

To our knowledge, no study has yet addressed the simultaneous effect of exercise training and treatment with *P. oleracea* on neurobehavioral dysfunction in diabetic rats. Therefore, the aim of the present study was to evaluate the effect of exercise training and *P. oleracea* on the behavioral deficit in type 2 diabetic rats.

Materials And Methods

Animals and Study Design

Fifty male Wistar rats (250 to 300 g) were purchased from the animal laboratory of Hamadan University of Medical Sciences. The animals were kept under 12 hours of light and 12 hours of darkness cycles at a temperature of $22 \pm 2^{\circ}\text{C}$ and relative humidity of 55–60%. The rats were then randomly divided into the following five groups: 1) healthy control group (Con, $n = 10$), 2) diabetic group (D, $n = 10$), 3) diabetic rats treated with *P. oleracea* (D + Po, $n = 10$), 4) diabetic rats treated with exercise training (D + Ex, $n = 10$), and 5) diabetic rats treated with *P. oleracea* and exercise training (D + Po + Ex, $n = 10$). All the animals were adapted to the atmosphere of the animal physiology laboratory for a week. The experiment timeline is shown in Fig. 1.

Diabetic Induction

At the end of the adaptation period, the rats fasted for more than overnight to prepare for type 2 diabetes inductions. For this purpose, a single dose of 6 mg/kg streptozotocin (STZ) (Sigma-Aldrich, Saint Louis, MO) dissolved in 0.1 mol citrate buffer (pH 4.5) was intraperitoneally injected into the rats. After 12 days, blood samples were taken from all rats (tail area), and fasting blood glucose level was determined by a glucometer. Subjects with fasting glucose levels above 126 mg/dL (FBS > 126 mg /dL) were diagnosed as diabetic rats.

The diet containing *P. oleracea*

P. oleracea was purchased from perfumeries located in the Hamedan market and identified and authenticated by Dr. Ramazan Kalvandi at the Botanic Institute of the Hamadan University of Medical Sciences. This plant has been used in accordance with the rules of IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. A voucher specimen was deposited at the Department of Pharmacognosy and Biotechnology, School of Pharmacy, Hamadan University of Medical Sciences (NO: 1398/284). *P. oleracea* was mixed with standard pelleted food at a weight ratio of 5%. The rat food was weighed daily by scales at a specified time. The difference between the amount of food left from the previous day was determined as the amount of food consumed per day by the rats. The weight of the rats was also measured weekly and on a specific day.

Exercise Training

Diabetic rats were subjected to swimming training for five 1-hour sessions per week for 12 weeks in water at a temperature of $32\text{--}35^{\circ}\text{C}$. To ensure continuous swimming, a weight equivalent to 2% of body weight was attached to the subjects' tails. First, to prepare the rats, swimming for 15 minutes was performed in the first two weeks, and then, from the third to the tenth week, the duration of the training sessions gradually increased to 50 minutes. Finally, at weeks eleventh and twelfth, the rats swam for 60 minutes [17]. The sequence of training days was as follows: three days of training, one day of rest, then, two days of training, and one day of rest.

Elevated Plus-Maze (EPM)

In order to measure anxiety in rats, an EPM test was used [18–20]. In this test, an elevated, plus-shaped (+) apparatus with two open ($50 \times 10 \times 50$ cm) and two enclosed (10×50 cm) arms is connected by a central square (10×10 cm) and the maze is elevated 80 cm from the floor. As previously described, each rat was placed in the center of the apparatus facing one of the closed arms and allowed to search inside the apparatus for 600 seconds. Video recording of each rat was later analyzed for time spent in closed arms [21].

Passive avoidance learning (PAL) test: Shuttle box

Passive avoidance memory was evaluated by shuttle box test. The method of working with the device and the process were fully mentioned in our previous papers [22–24]. The device had two lighted and dark compartments with similar dimensions ($20 \times 20 \times 30$ cm) with a grid stainless-steel rod floor connected to a shock generator and a guillotine door separating two compartments [25, 26]. At First, for acclimatization, the rat was placed in a lighted section and then, the guillotine door was opened and 30 seconds after the entrance of the rat into the dark section, it was returned to its home cage. After 30 minutes, this test was repeated. When the animal had its whole body in the dark section, the entrance latency to the dark section (step-through latency, STLa) was measured. The guillotine door between two sections was closed and then, an electrical shock (0.8 mA) was applied to the rat for 2 seconds. Then, 30 seconds after receiving the electrical shock, the rat was returned to its home cage. The test was performed again after 2 min. Each time the rat re-entered the dark section, it received an electric shock. When the rat remained in the dark section for 120 seconds, the test was completed and the number of trials was recorded [22, 23]. The retention test was executed 24 hours following the acquisition trial, in which the rat was placed in the light section and the guillotine door was opened to the rat for 5 seconds and then the step-through latency (STLr) and the time spent in the dark section (TDC) were measured for 600 seconds.

Open field (OF) test

To measure locomotion, exploration, stress, and anxiety in diabetic rats OF test was used [27]. This task measures the innate responses of the subjects to open spaces apart from their explorative drive. The OF apparatus is a square arena with the bottom divided into four identical squares on the floor of the arena. When the animal is anxious, its natural tendency is to be next to the high walls of the apparatus and avoids going to the center of the apparatus and searching there, and when its anxiety and depression disappear, it walks everywhere to get to know more about the apparatus. The animal was placed in the center of the instrument and its behavior was recorded for 600 seconds. For this purpose, the EthoVision video tracking system (Noldus, Leesburg, VA, USA) recorded the total distance traveled (locomotor activity) by the animal.

Novel object recognition (NOR) test

This test measures the visuospatial memory of rodents [28, 29]. Rodents have a natural tendency to spend more time exploring novel objects than familiar objects. The NOR test can be evaluated by the differences in time spent exploring novel and familiar objects. The test is done in three days with three

phases: habituation, training, and testing. During training, conditions are provided for the rat to explore two identical objects. On the test day, one of the training objects is replaced by another object that is different in appearance (shape and color). Exploration time of each object (time spent sniffing or touching the object but not standing, sitting on, or leaning against the object) was recorded. The discrimination index ($DI = (TNO - TFO) / (TNO + TFO)$), was calculated - where TNO is the exploration time of the novel object and TFO is the exploration time of the familiar object [30].

Statistical analyses

SPSS software (IBM SPSS Statistics) version 21 was used for statistical analysis. The normal distribution of data was confirmed by the Shapiro-Wilk test. The statistical difference between different groups was evaluated by one-way ANOVA. Tukey's post-hoc test was used for comparison between groups. Data were reported as mean $\pm$ SEM. Data with p -values of smaller than 0.05 were considered statistically significant.

Results

Elevated plus-maze test

As shown in Fig. 2, statistical analyses showed that the time spent in close arm in the EPM test was not significantly different between the experimental groups ($p > 0.05$).

Shuttle box test

Step-through latency was significantly different between groups ($p \leq 0.05$). Tukey's test showed that STLa in diabetic rats was lower than healthy rats. This result indicated that DM decreased STLa in the shuttle box test. Exercise training and *P. oleracea* alone did affect STLa in diabetic rats but exercise training and *P. oleracea* synergistically increased STLa compared with the diabetic rats. Also, there was no significant difference between the D + Po + Ex and Con groups (Fig. 3).

As shown in Fig. 4, the number of trials to acquisition was not significantly different between the experimental groups. Diabetic rats that not received both treatments (group D) were found with an increase in the number of trials to acquisition compared with other groups, but this difference was not significant.

STLr was different between groups ($p \leq 0.05$). Diabetic rats showed a decrease in STLr than healthy rats. Swimming training and treatment with *P. oleracea* synergistically increased STLr; however, it was still lower than healthy rats (Fig. 5).

TDC in the shuttle box test was affected by exercise training and *P. oleracea*. In this regard, statistical analyses showed that TDC was different between groups. Diabetic rats showed a longer TDC than healthy rats. Swimming training and *P. oleracea* significantly decreased TDC compared with the D group. There was no significant difference between the D + Po and D + Ex groups (Fig. 6).

Open Field test

Distance traveled as a locomotor activity and curiosity index was different between groups in response to the treatments. As shown in Fig. 6, the traveled distance decreased in diabetic rats compared with the Con group. Swimming training and *P. oleracea* for 12 weeks increased traveled distance in OF test compared with the diabetic rats (Fig. 7).

Novel object recognition test

Comparisons of DI in the NOR test showed different results between the experimental groups. As revealed in Fig. 8, diabetic rats were found with a decrease in DI than healthy control rats ($p \leq 0.05$). Swimming training and *P. oleracea* alone did not affect DI, but exercise training and *P. oleracea* synergistically increased DI in diabetic rats. Also, DI in the D + Po + Ex group was more than the D + Ex and D + Po groups.

Discussion

This study was designed to investigate the potential neuroprotective effect of swimming training and *P. oleracea* in STZ-induced diabetic male rats. Recent clinical studies have indicated that DM causes neurobehavioral deficit [31, 32]. As expected, our findings are in line with previous studies, indicating that DM damages neurobehavioral index by 38% reduction in STLa, 58% reduction in STLr, 93% elevation in TDC, 29% reduction in distance traveled, and 40% reduction in DI.

According to recent findings, the onset of DM is associated with several structural and functional changes in the CNS and peripheral nervous system, including slowing of nerve conduction, impaired regeneration in the body's peripheral nerves, and morphological changes in nerve fibers, and eventually, these changes lead to neurobehavioral complications, such as dementia [33]. Although several studies have been done on the association between DM and peripheral neuropathy, very little is known about the effects of diabetes on the CNS [34]. In this regard, there is a close relationship between the incidence of DM and the occurrence of neurobehavioral deficits, although the mechanisms responsible for the occurrence of these disorders are not well defined.

Oxidative stress and inflammation promotion in diabetic patients are two important and vital responsible mechanisms in this regard [35, 36]. In addition, DM significantly reduces neuronal density in the dentate gyrus, which plays an important role in memory and spatial learning processes. Also, DM reduces the expression of the neuronal nitric oxide synthase enzyme, which plays an important role in synaptic plasticity in the hippocampus, leading to a neurobehavioral deficit in diabetics. On the other hand, neural cell adhesion molecule reduction in different areas of the brain of diabetic animals, including the hippocampus, cerebellum, and cortex, may well explain some of the neurobehavioral deficits associated with diabetes [37].

In contrast, regular exercise has been shown to counteract many of the effects of diabetes. Exercise training, especially aerobic training, such as swimming is the most important non-pharmacological strategy for the treatment of type 2 diabetes [38]. Previous studies have confirmed that swimming training promoted insulin sensitivity, ameliorated glucose homeostasis, and minimized inflammation and stress oxidative by ROS reduction, Sirtuin1 elevation, and Wnt3a/ β -catenin pathway modulation [39, 40].

On the other hand, beta-amyloid and Tau phosphorylation play an important role in neurobehavioral dysfunction in diabetics and patients with Alzheimer's diseases. In this regard, Diegues et al. showed that swimming training (5 days/week, 1 h/day) for 6 weeks promoted some proteins related to the insulin/IGF-1 pathway and reduced Tau phosphorylation and amyloid precursor protein expression in the hippocampus resulting in an improvement in spatial learning and memory of diabetic rats [41]. Our results showed that swimming training insignificantly (-0.3% time spend in close arms, 25% STLa, 23% STLr, -10% TDC, and 17% distance traveled) ameliorated neurobehavioral dysfunction in type 2 diabetic rats. Probably, reduced oxidative stress and inflammation, an elevation in neurotrophic factors, and an increase in synaptogenesis and neurogenesis can mediate these deteriorative effects in neurological impairment.

There is growing evidence that *P. oleracea* has beneficial effects on diabetes due to its contents, including polyunsaturated fatty acids, flavonoids, and polysaccharides. The omega-3 fatty acid is required for normal neurodevelopment and brain health [42]. Preclinical evidence suggests that omega-3 fatty acids in *P. oleracea* reduces the levels of beta-amyloid [43, 44] and prevents or delays neurobehavioral deficit [42]. In this regard, previous studies have shown that crude *P. oleracea* polysaccharide could significantly increase β -cell mass, and therefore, reduced the fasting blood glucose level, and elevated the fasting serum insulin level and insulin sensitivity index value in diabetic rats [45, 46]. In general, recent studies have linked the positive effects of *P. oleracea* on the treatment of diabetes to the anti-inflammatory and anti-stress oxidative effects of this plant [47]. Given that oxidative stress and inflammation are the most important factors to cause behavioral disorders in diabetic patients, this plant possibly improves neurobehavioral decline by reducing inflammation and oxidative stress.

A notable finding in this study is that swimming training and *P. oleracea* synergistically ameliorated neurobehavioral dysfunction ($\uparrow$ 52% STLa, $\downarrow$ 12% number of trials to acquisition, $\uparrow$ 79% STLr, $\downarrow$ 31% TDC, $\uparrow$ 29% distance traveled, and $\uparrow$ 81% DI) in type 2 diabetic rats. The simultaneous effect of exercise and *P. oleracea* on memory improvement is much greater than their effects when used alone. These results indicate that exercise training and *P. oleracea* simultaneously have greater effects on the process of neurogenesis development and suppression of neuronal degradation in diabetic patients.

Conclusions

the results of this study showed that diabetes significantly impaired brain abilities and swimming training and *P. oleracea* synergistically reversed and ameliorated neurobehavioral dysfunction in type 2 diabetic rats.

Declarations

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Author contributions

All authors have assumed responsibility for data integrity and accuracy of the data analysis. Study concept and design: H. P., A. K. Data acquisition: T. S., H. P. Data analysis and interpretation: K. R., A. K., T. S. Drafting of the manuscript: A. K., K. R. Critical revision of the manuscript for important intellectual content: A.K. and H. P. Statistical analysis: H. P., T. S., K. R. Study supervision: H. P., A. K.

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Availability of data and materials

The data generated or analyzed during this study are included in the article.

Ethical approval

All of the experiments and animal care methods were confirmed by the Veterinary Ethics Board of the Hamadan University of Medical Science (IR.BASU.REC.1398.031) and carried out according to Guidelines of the National Institutes of Health on the principles of laboratory animal care (NIH Publication 80-23, 1996). The study was also carried out in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

The experimental timeline. Type 2 diabetes was induced by a single IP injection of streptozotocin (65 mg/kg) and nicotinamide (120 mg/kg), and approved by a fasting glucose level of ≥ 250 mg/dL three days later. Swimming training was started one day after confirmation of diabetes. The rats underwent 12 weeks of progressive swimming training. During swimming training, the treated groups received *Portulaca oleracea* mixed with standard pelleted food at a weight ratio of 5% for 12 weeks. To assess cognitive memory, the novel object recognition (NOR) and elevated plus maze (EPM) tests were used shuttle box test was used to measure aversive (acquisition and retention) learning and memory after the training programs, and the open field test was employed to measure locomotor activity.

Figure 2

Time spent in close arm between the experimental groups in the elevated plus-maze test. Values are presented as mean $\pm$ SEM.

Figure 3

Comparison of the step-through latency in the shuttle box test between groups. Values are presented as mean $\pm$ SEM. * Significant differences vs. the control group ($p \leq 0.05$) and † significant differences vs. the diabetes group ($p \leq 0.05$).

Figure 4

The number of trials to acquisition was not significantly different between the experimental groups. Values are presented as mean $\pm$ SEM.

Figure 5

Step-through latency in the retention phase between the experimental groups. Values are presented as mean $\pm$ SEM. * Significant differences vs. the control group ($p \leq 0.05$) and † significant differences vs. the diabetes rats ($p \leq 0.05$).

Figure 6

Time spent in the dark section in the shuttle box test was different between groups. Values are presented as mean $\pm$ SEM. * Significant differences vs. the control group ($p \leq 0.05$) and † significant differences vs. the diabetic rats ($p \leq 0.05$).

Figure 7

Distance traveled, as an indicator for locomotor activity and curiosity index, was different between groups. Values are presented as means $\pm$ SEM. * Significant differences vs. the control group ($p \leq 0.05$) and † significant differences vs. the diabetic rats ($p \leq 0.05$).

Figure 8

Discrimination index was different between groups. Values are presented as means $\pm$ SEM. * Significant differences vs. the control group ($p \leq 0.05$), † significant differences vs. the diabetic rats ($p \leq 0.05$), and # significant differences vs. the D+Po+Ex group.