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Isorhamnetin mitigates learning and memory disturbances in streptozotocin-induced diabetic rats

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ABSTRACT

Background and Objective: Diabetes as a metabolic disorder can cause memory and learning impairment. In recent years, the effect of plant extracts on the treatment of diabetes mellitus has been raised. The purpose of this study was to determine the effect of isorhmnetin administration on learning and memory disability in an experimental model of streptozotocin-induced diabetes mellitus in rats.

Materials and Methods: In the present study, for inducing diabetes, streptozotocin was administered at a dose of 60 mg/kg (intraperitoneal) inmale rats. Intraperitoneal injection of isorhmnetin (10 mg/kg) was performed after induction of diabetes (10 mg/kg) for 12 weeks. Control groups also received relevant doses. Y-maze and passive avoidance tests were used for assessing of learning and memory ability. The serum glucose and body weight were determined before and 12 weeks after diabetic development.

Results: Behavior data showed that compared to control rats, alternation percentage in Y-maze task ($p<0.01$) and step through latency in the passive avoidance test ($p<0.001$) reduced in the diabetic rats. Administration of isorhmnetin to diabetic rats improved alternation percent ($p<0.01$) and step through latency in the passive avoidance test ($p<0.001$).

Conclusion: This study reveals that isorhmnetin administration to diabetic rats attenuates learning and memory impairment.

1. Introduction

Diabetes mellitus is considered as the most common endocrine system diseases that its outbreak in the human community will increase in the future (1). Diabetes is one of the most important risk factors in developing of the Alzheimer's disease and dementia in old people (2). Although a lot of researches has been performed on the relationship between diabetes mellitus and neuropathy, but a few studies can be found about the effects of diabetes on central nervous system especially on the behavioral changes involving learning and memory (3). According to some studies, diabetes mellitus, especially type 1, impairs the process of learning, memory and cognition. The involved mechanisms of the incidence of these disorders is not well defined, though there is many evidence for microvascular hypothesis and oxidative stress caused by the formation of free oxygen radicals (4). In addition, diabetes mellitus causes neuronal loss in hippocampus that plays an important role in the process of spatial memory (2). Also, diabetes mellitus reduces expression of hippocampal nitric oxide synthase enzyme. This enzyme plays an important role in synaptic plasticity and learning and memory process (5,6). Regarding heterogeneity of diabetes, the need to find effective compounds with less side effects in preventing and treating diabetes is felt.

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Medicinal herbs and their derivatives have long been considered in the treatment of diabetes mellitus and its complications, but no reliable evidence has been available about their efficacy (7). Isorhamnetin is a flavonol aglycone and a metabolite of quercetin (8,9) that is found in *Hippophae rhamnoides L.*, *Oenanthe javanica* and *Ginkgo biloba L*. These plants are often used for treating different diseases (10). Some studies show that isorhamnetin can block the apoptotic pathway in heart myocytes exposed to H2O2 (11). It has also been reported that isorhamnetin protects microvascular endothelial cells of brain from oxygen glucose deprivation–induced cytotoxicity (12). Recently, some preclinical studies has been revealed that isorhmnetin can exerts anti-inflammatory and anti-oxidative activities (13-16)**.**

With regard to anti-inflammatory and antioxidant effects of isorhmnetin, this study was designed to evaluate the protective effect of isorhmnetin on learning and memory abilities in streptozotocin-induced diabetic rats.

2. Materials and Methods

2.1. Animals

This experimental study was performed on male albino Wistar rats (local animal house of IUMS, Tehran, Iran) with a weight range of 250-270 g (10–14 weeks old). Rats were kept in an air-conditioned colony room with a temperature of 21±2◦C. Three to four rats per cage were freely supplied with standard pellet diet and tap water. Procedures involving animals and their care were conducted in conformity with NIH guidelines for the Care and Use of Laboratory Animals.

2.2. Experimental protocol

Rats (n= 40) were randomly and similarly grouped into four groups: normal vehicle-treated control, isorhmnetin-treated control, vehicle-treated diabetic, isorhmnetin -treated diabetic. The rats were become diabetic by a single intraperitoneal injection of 60 mg/kg of STZ freshly dissolved in cold normal saline. One week after STZ injection, serum glucose concentrations were measured by spectrophotometer using glucose oxidation method (Zistshimi, Tehran, Iran). The animals with a non-fasting serum glucose level higher than 250 mg/dl were chosen as diabetic. Isorhmnetin dissolved in 10% Cremophor was injected intraperitoneally for 12 weeks at a dosage of 10 mg/kg body weight. Changes in body weight and blood glucose were recorded on the regular basis during the experimental period.

2.3. Y-maze task

When isorhmnetin injection period was over, the recording of spontaneous alternation behavior in a single-session Y-maze was performed for evaluation of spatial memory (18). After putting of each of rats at the end of one arm of three arms Y-maze, the total number of arms entered freely for an 8-min session was recorded. Successive entries into the three arms on overlapping triplet sets was defined as alternation. The total number of arms entered − 2 is the maximum number of spontaneous alternation. The percentage is calculated as the ratio of actual to possible alternations (defined as the total number of arm entries −2).

2.4. Single-trial passive avoidance test

Two to three days after Y-maze, according to a previous study (19), single-trial passive avoidance test was done. The apparatus of this test included a light chamber and a dark chamber that were separated from each other by a guillotine door. The initial latency (IL) was defined as the time it took the rat to go from light chamber to dark chamber. After entering the rat into the dark chamber, the guillotine door was closed and a single electric shock (1 mA, 1 s) was delivered. After 24 hours, the interval between placement of the rats in the light chamber and entry into the dark chamber was measured as step-through latency (STL).

3. Results

3.1. Body weight and serum glucose level

After 12 weeks, diabetic rats showed a non-significant decrease in body weight but a significant increase in serum glucose as compared to control rats ($p < 0.001$).

Treatment of diabetic rats with isorhmnetin cause a significant improvement in body weight and serum glucose level relative to diabetics ($p < 0.05$- $p < 0.01$; Table 1). Administration of isorhmnetin to control rats for 12 weeks significantly raised body weight ($p<0.05$).

Table 1. Body weight and serum glucose level of experimental groups

	Body weight(g)		**Serum glucose(mg/dt)**	
	1 week before	After 12 weeks	1 week before	After 12 weeks
Control	278.3±5.4	251.8±18.92	122.3±8.5	103.4±5.7
Control+Isorhamnetin	277.2±6.7	446.25±25.12*	134.1±9.3	125±13.3
Diabetic	268.1±6.9	123±5.06	129.6±8.5	495±46.99***
Diabetic+ Isorhamnetin	259.3±7.6	312.94±43.38#	141.3±8.9	189.55±52.79##

Data are represented as mean±SEM.
* P< 0.05 (vs. Control); # P < 0.05 (vs. Diabetic); *** P< 0.001 (vs. Control);
P < 0.01 (vs. Diabetic)

3.2. Y-maze test

In this study, with recording of the alternation percent in Y-maze test, the short-term spatial recognition memory was assessed. In the STZ-induced diabetic rats, the alternation percent decreased considerably comparing to the control group (P<0. 01). Administration of isorhmnetin to diabetic rats at a dose of 10 mg/kg significantly raised alternation percentage (p<0. 01). In addition, it was revealed that total arms entrance number, as a locomotor activity index, had no significant difference between different groups (Fig. 1).

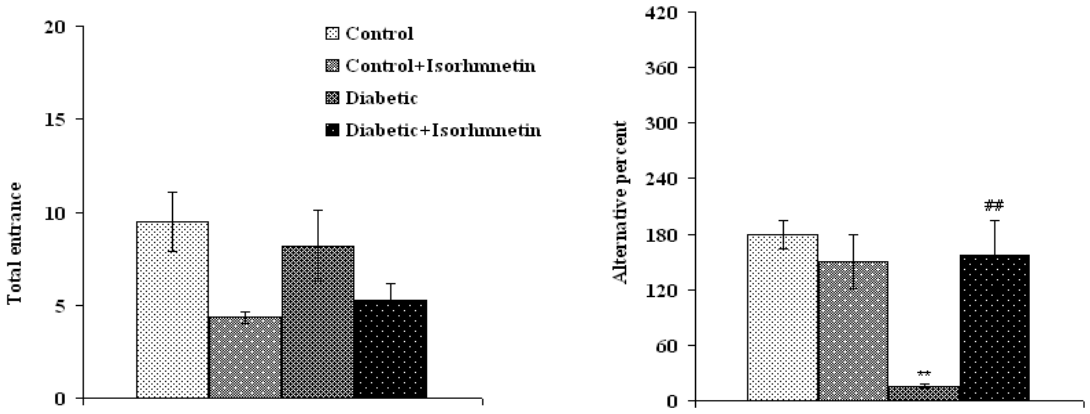

Fig. 1. Total entrance (A) and alternation behavior (B) displayed in the Y-maze by rats. Values are means ± SEM.
** P< 0.01 (vs. Control); ## P < 0.01 (vs. Diabetic)

3.3. Passive avoidance test

The results of passive avoidance test showed that there was no meaningful difference in initial latency in experimental groups, but in the STZ-induced diabetic rats, STL decreased apparently in comparison to control group ($p<0.001$). Applying of isorhmnetin to diabetic rat significantly increased STL ($p<0.001$).

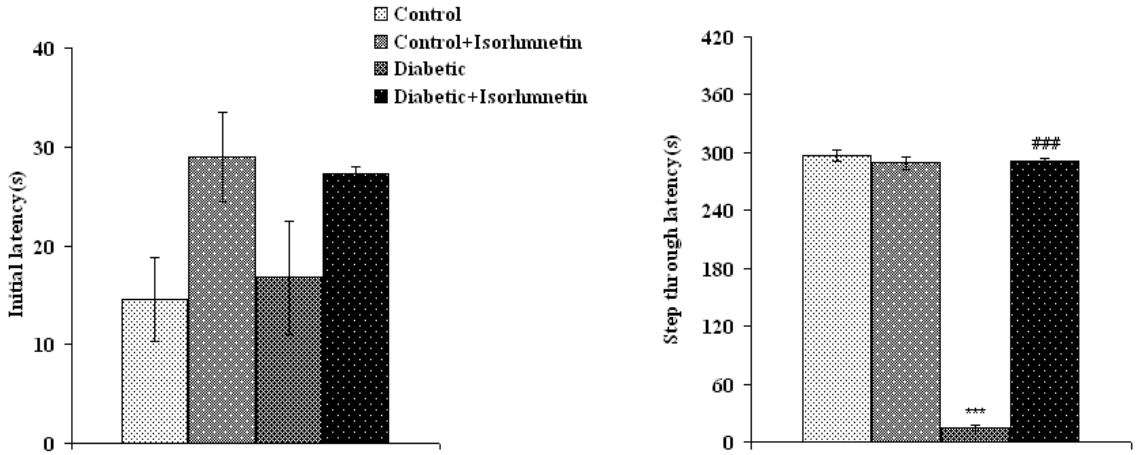

Fig. 2. Initial latency (A) and step-through latency (B) recorded in a single-trial passive avoidance test for rats. Values are means ± SEM
*** P< 0.001 (vs. Control); ### P < 0.001 (vs. Diabetic)

4. Discussion

Present study was designed for evaluating the effect of isorhamnetin on learning and memory efficiency in rat model of STZ-induced diabetes. The chief findings of this study were as follows: 12 weeks post-STZ injection (1) the diabetic rats exhibited a considerable decrease in body weight and increase in serum glucose level, (2) also alternation percent and STL in the STZ -injected rats decreased considerably.

Based on previous studies, diabetes in lab animals (such as rat) and humans is accompanied with cognition and memory disturbances, brain atrophy and increase the chance of getting dementia. Brain cortex and hippocampus are two regions in central nervous system associated with cognition process that are largely affected by diabetes (17, 18). A large body of studies show that in diabetic rats, the level of insulin-like growth factor, brain-derived neurotrophic factor and capability for consolidation and retention of stored information are reduced (19, 20). The same results was obtained in our study, so that 12 weeks after induction of diabetes in rats, the learning and memory ability were impaired. According to available evidence, deficiency in learning and memory ability could be attributed to change in hippocampal synaptic plasticity and disturbing the long term potentiation process. Recent investigations show that diabetes impairs learning of new and complex skills (21, 22).

In the present study, long-term administration of isorhmnetin at a dose of 10 mg/kg improved learning and memory in passive avoidance test and spatial memory in Y-maze. It also significantly decreased diabetic rats' body weight and serum glucose. Previously, it has been revealed that in brain cortex and hippocampus of small rodents such as rats, STZ-induced diabetes intensifies oxidative stress resulted from increased reactive oxygen species (ROS) formation (17,18). ROS has a major role in cellular lipids, proteins, and nucleic acids impairment that ultimately leads to cell death (23). Studies have demonstrated that isorhamnetin could attenuate oxidative stress through nuclear factor E2-related factor 2 (Nrf2) and heme-oxygenase activation (24). In the brain, one of the major intermediators for getting excitotoxic injury is

nitric oxide (25). NO promotes peroxidation of lipid and mitochondrial enzymes disturbance (26). In a recent study, it has been revealed that in an ischemic model of cortex, isorhamnetin restricts the expression of iNOS and reduced NO production. In addition, in an ischemic stroke model, isorhmnetin attenuated the

IL-1b, IL-6, and TNF-α levels as inflammatory cytokines (27-30). According to some studies, it has been determined that activation of N-methyl-D-aspartate receptor (NMDAR), as an ionotropic glutamate receptor, results to neuronal apoptosis

through increasing the amount of intracellular Ca^{2+}, caspases and

calpains activity. In mice isorhamnetin protects mice against ischemic stroke, by inhibition of the expression of NR1, a useable subunit of NMDAR.

In conclusion, based on the present results, it was suggested that administration of isorhmnetin could attenuates learning and spatial recognition memory deficiency in STZ-induced diabetic rats. However, its mechanism of function should be investigated in future studies.

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