

L-carnosine improves short-term spatial recognition memory and passive avoidance learning and memory in streptozotocin-diabetic rats

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ABSTRACT

Background and Objective: Diabetes mellitus (DM) affects synaptic plasticity in the hippocampus, leading to impairments in learning and memory. L-carnosine, an endogenous dipeptide, is reported to exhibit anti-diabetic and antioxidant effects. Therefore, this study was designed to evaluate its effect on learning and memory in diabetic rats.

Materials and Methods: In the present study, male Wistar rats (n=32) were randomly divided into four groups: control, control treated with carnosine (100 mg/kg), diabetic, and diabetic treated with carnosine (100 mg/kg). Diabetes was induced by streptozotocin (STZ) at a dose of 60 mg/kg. L-carnosine was injected *i.p.* at a dose of 100 mg/kg one week after STZ injection for 8 weeks. Blood sample was taken from retro-orbital plexus before STZ injection and 4 and 8 weeks after STZ injection to measure blood glucose level. Passive avoidance and Y maze tests were done to assess learning and memory deficits.

Results: After 8 weeks, diabetic rats showed a significant cognitive dysfunction in passive avoidance and Y maze tests that was significantly attenuated following carnosine treatment. In addition, carnosine showed a hypoglycemic effect.

Conclusion: This study demonstrated that treatment with carnosine for 8 weeks could improve cognitive functions in diabetic condition and part of its beneficial effect is due to its reduction of blood glucose.

1. Introduction

D iabetes mellitus (DM) accompanies debilitating neurological complications in both the peripheral (PNS) and the central (CNS) nervous system (1, 2). DM in long term is associated with some peripheral neural abnormalities like lowered motor nerve conduction velocity, axonal damage, disturbed nerve regeneration, and weakened axonal transport (3). In streptozotocin (STZ)-diabetic animals, the observed nerve damage is somewhat similar to nerve degeneration process in individuals with diabetic neuropathy (4). Different forms of neuropathies are one of the

major complications resulting in morbidity in DM patients. Pathological findings also show that DM is itself a main risk factor for dementia in Alzheimer's disease (5). Research evidence is increasing at a tremendous rate showing the effect of DM on the brain (6, 7). Passive avoidance learning and memory disturbance is observed in STZ-diabetic rats (8, 9). Deficit of spatial memory is also observed in DM (10, 11). Hippocampus-dependent synaptic plasticity is also affected in DM (12, 13). These evidences indicate that uncontrolled diabetes is associated with multiple deficits in the nervous system.

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Use of natural products for lowering devastating complications of DM has gained much attention (14). Carnosine (β -alanyl-L-histidine) is a dipeptide with great level in glial cells and neurons (15). Carnosine could exert protective effect against $A\beta$ -induced neurotoxicity due to its anti-oxidant and free-radical scavenging activity (15). Carnosine could also exert anti-aging effect, has anti-diabetic effect, and is able to improve memory (16-18). This study was performed to assess the efficacy of chronic carnosine on alleviation of learning and memory decline in STZ-diabetic rats using passive avoidance and Y-maze tasks.

2. Materials and Methods

Male albino Wistar rats (Pasteur's institute, Tehran, Iran) weighing 190-230 g were housed in an air-conditioned colony room on a light/dark cycle (a temperature of 21-23°C and a humidity of 30-40%) and freely supplied with standard pelleted diet and tap water. Procedures involving animals and their care were conducted in conformity with the NIH guidelines for the care and use of experimental animals.

The rats ($n = 32$) were randomly allocated into four groups: control, carnosine-treated control, diabetic and carnosine-treated diabetic. The rats were rendered diabetic by a single intraperitoneal injection of 60 mg/kg STZ (SigmaAldrich, USA) freshly dissolved in cold normal saline. Diabetes was confirmed by the presence of hyperglycemia, polyphagia, polydipsia, polyuria, and weight loss. One week after STZ injection, non-fasting blood samples were collected under light ether anesthesia from retro-orbital capillary plexus and serum glucose concentrations were measured using glucose oxidation method. Only those animals with non-fasting serum glucose level higher than 250 mg/dl were selected as diabetic for the following experiments. The day on which hyperglycemia had been confirmed was designated as day 0. L-carnosine (SigmaAldrich, USA) was administered i.p. at a dose of 100 mg/kg body weight one week after STZ injection for a period of 8 weeks. Carnosine was dissolved in normal saline. Changes in body weight were regularly observed during the experimental period. Behavioral tests including passive avoidance and Y-maze were performed at the end of study in experimental groups as described below.

Short-term spatial recognition memory performance was assessed by recording spontaneous alternation behavior in a single-session Y-maze as described before (16). The maze was made of black-painted Plexiglas. Each arm was 40 cm long, 30 cm high and 15 cm wide. The arm converged in an equilateral triangular central area that was 15 cm at its longest axis. The procedure was basically the same as that described previously as follows: each rat, naive to the maze, was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded visually. Arm entry was considered to be completed when the base of the animal's tail had been completely placed in the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The number of maximum spontaneous alternation was then the total number of arms entered minus 2 and the percentage is calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus two).

Single-trial passive avoidance test was conducted 2-3 days after Y-maze task and was according to a previous study (16). The apparatus (BPT Co., Tehran) consisted of an illuminated chamber connected to dark chamber by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator. On the first and second days of testing, each rat was placed on the apparatus and left for 5 min to habituate to the apparatus. On the third day, an acquisition trial was performed. Rats were individually placed in the illuminated chamber. After a habituation period (5 min), the guillotine door was opened and after the rat entering the dark chamber, the door was closed and an inescapable scrambled electric shock (1 mA, 1 s once) was delivered. In this trial, the initial latency (IL) of entrance into the dark chamber was recorded and rats with ILs greater than 60 s were excluded from the study. Twenty-four hours later, each rat was placed in the illuminated chamber for retention trial. The interval between the placement in the illuminated chamber and the entry into the dark chamber was measured as step-through latency (STL up to a maximum of 90 s as cut-off).

All data were expressed as mean \pm S.E.M. For behavioral tests, parametric one-way ANOVA test was applied. Body weight and serum glucose levels at different weeks were analyzed using repeated measure one-way ANOVA. In all calculations, a difference at $p < 0.05$ was regarded as significant.

3. Results

After 8 weeks, the weight of the diabetic rats was found to be significantly lower versus control group ($p < 0.01$) and carnosine treatment

produced no decrease in diabetic rats as compared to diabetics. In addition, diabetic rats had also an elevated serum glucose level over those of control rats ($p < 0.001$) and treatment of diabetic rats with carnosine for 4 and 8 weeks caused a significant decrease in the serum glucose ($p < 0.05-0.01$) as compared to diabetics. Meanwhile, carnosine treatment of control group did produce a significant reduction regarding serum glucose level at week 8 ($p < 0.05$) (Fig. 1).

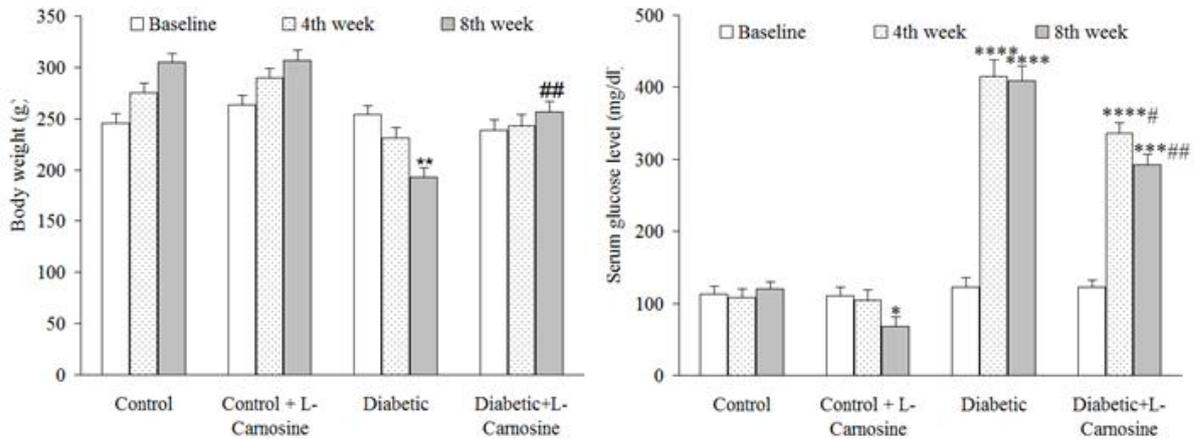


Fig. 1. Body weight and serum glucose concentration in different weeks (means \pm S.E.M)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$ (as compared to baseline in the same group)
$p < 0.05$, ## $p < 0.01$ (as compared to diabetics in the same week)

Figure 2 shows the results for the performance of rats in Y-maze task, in which short-term spatial recognition memory performance as alternation behavior can be tested. In this respect, the alternation score of the diabetic group was lower than that of the control ones at the end of the study ($p < 0.01$). In addition, carnosine-treated diabetic group showed a higher alternation score as compared to diabetic group ($p < 0.05$) at the end of study. Meanwhile, carnosine treatment of control rats did not produce any significant change regarding this parameter.

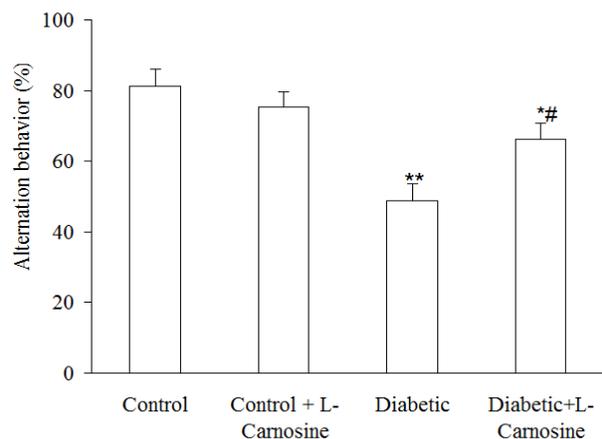


Fig. 2. Alternation behavior of treated-control and -diabetic rats in Y-maze task in different groups

* $P < 0.05$, ** $p < 0.01$ (as compared to control)
$p < 0.05$ (as compared to diabetic)

Figure 3 shows the performance of treated-control and diabetic rats in passive avoidance task as indicated by initial (IL) and step-through (STL) latencies. Regarding IL, there was no significant difference among the groups. In addition, diabetic group showed a significant impairment in retention and recall in passive avoidance test ($p < 0.01$), as it is evident by a lower STL and carnosine treatment significantly improved it ($p < 0.05$). Furthermore, retention and recall of carnosine-treated control group was not significantly different from control group.

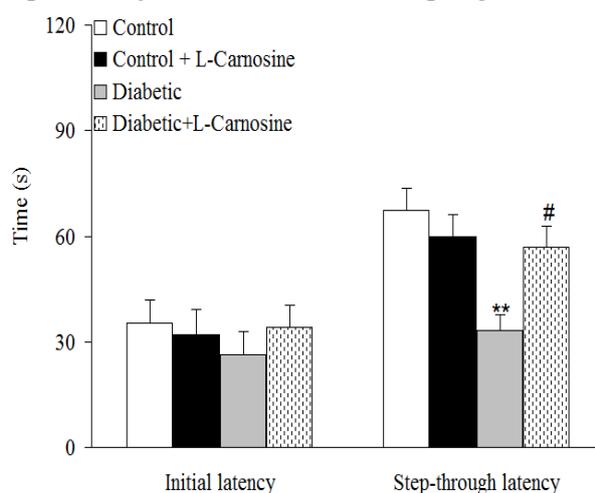


Fig. 3. Initial (IL) and step-through (STL) latencies of treated-control and -diabetic rats in single-trial passive avoidance test ** $p < 0.01$ (vs. control) # $p < 0.05$ (as compared to diabetic)

4. Discussion

The main findings of this research were two-fold. First, chronic DM leads to deficits in animal performance in passive avoidance and Y-maze tests as was verified by a lower STL and alternation score, respectively. Second, 8-week administration of carnosine improved short-term spatial recognition memory performance in Y-maze and learning and memory in passive avoidance test in diabetic group.

Although the main mechanisms responsible for cognitive and memory impairments in diabetic state is still under research investigation, several factors like metabolic derangement, vascular abnormalities, oxidative stress, and neuroinflammation have been suggested (19). Chronic hyperglycemia in DM is the main cause of most of its complications. It has been known

that chronic hyperglycemia is associated with cognitive decline in DM (16, 20, 21). Thus, normalization of some cognitive abilities in diabetic group in our study may be partly attributed to the ability of carnosine to reduce hyperglycemia. In addition, since brain vascular abnormalities also contributes to the pathophysiology of some cognitive impairments in DM (22-24), thus, carnosine could have increased vascular endothelial nitric oxide synthase activity and improved vascular function (25, 26) and this may have been responsible for improvement of spatial recognition memory and conditioned learning and memory in our research study. Furthermore, oxidative damage is also responsible for cognitive dysfunction in DM (27, 28). Thus, treatment with antioxidants could be a therapeutic strategy in various kinds of neurodegenerative disorders (27, 28). Since oxidative stress plays a critical role in development of memory impairments in DM (27, 28), the antioxidant properties of carnosine (16) is responsible for its nootropic effects in our study. In addition, DM causes apoptosis-induced neurodegeneration in the hippocampus and to a lesser extent in the frontal cortex of rodents which is associated with cognitive decline (29). Since carnosine has shown anti-apoptotic potential (30, 31), this may also have attenuated memory decline in diabetic group in our study.

In conclusion, findings of this study demonstrated that treatment with carnosine for 8 weeks could improve cognitive functions in diabetic condition and part of its beneficial effect is due to its reduction of blood glucose. Further research studies are strongly warranted to explore the detailed involved mechanisms.

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