

Administration of Salvianolic Acid B Attenuates Learning and Memory Deficits in Diabetic Rats: Involvement of Oxidative Stress

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Background and Objective: Based on the effect of chronic metabolic diseases including diabetes mellitus on the learning and memory, this study was designed to assess the usefulness of administration of salvianolic acid B on lessening of learning and memory deficits in streptozotocin (STZ)-diabetic rats.

Materials and Methods: Male Wistar rats were allocated into control, salvianolic acid B-treated control, diabetic and salvianolic acid B-treated diabetic groups. Salvianolic acid B was administered at a dose of 10 mg/kg/day for 8 weeks. Assessment of learning and memory was performed by Y maze and passive avoidance tests. Moreover, oxidative stress marker malondialdehyde (MDA) was also measured.

Results: The results showed that in diabetic rats, alternation score in Y maze and retention and recall in passive avoidance test decreased in comparison with control rats ($p < 0.0001$ - $p < 0.01$). After treatment with salvianolic acid B (10 mg/kg), alternation score and retention and recall of diabetic rats significantly improved ($p < 0.05$ - $p < 0.001$). On the other hand, the treatment of diabetic rats with salvianolic acid B significantly decreased level of MDA ($p < 0.01$).

Conclusion: Taken together, these results show that salvianolic acid B could improve learning and memory deficits in STZ-diabetic rats by reduction of lipid peroxidation.

1. Introduction

One of the most common endocrine disease is diabetes mellitus (DM), which based on the predictions made, its prevalence in the human population increases in the future (1). In diabetes mellitus, deficiency or relative decrease in the amount of insulin is associated with acute metabolic disorders such as diabetic ketoacidosis and hyperosmolar coma and chronic metabolic disorder and inappropriate long-term effects such

as different types of neuropathy, retinopathy, renal vascular involvement, skin lesions and disorders of the heart and circulatory system (2). Based on the research findings, diabetes is one of the major factors in incidence of dementia in old age (3).

There is low data about diabetes effects on the central nervous system in terms of structure and function (behavioral changes including learning and memory) found to be lower (4).

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Especially, type one diabetes causes occurrence of disturbances in learning, memory and cognition.

According to the microvascular and oxidative hypothesis, there is a close relationship between the incidence of laboratory animals DM and impaired learning and memory (5). Some studies have shown that diabetes causes the marked decrease in neuronal density in the dentate gyrus region that has important role in spatial memory and learning processing (3). Also, diabetes mellitus reduces the expression of nitric oxide synthase in neuronal hippocampus, which plays an important role in synaptic plasticity (6, 7). Although in the past two decades, natural products derived from medicinal plants have been introduced in treatment of diabetes mellitus and its complications, but we can not find a valid definitive evidence of their effectiveness in many research (8). Salvianolic acid is a soluble active ingredient extracted from the root of *Salvia miltiorrhiza* or Danshen. In Chinese medicine, this herb has been used to increase blood flow in angina pectoris, acute ischemia and hyperlipidemia (9-11). Among the salvianolic acids (Sal), Sal A and Sal B are the most abundant compounds. Due to their polyphenolic structure, salvianolic acids are able to collect free radicals. The Sal B collecting activity of free radicals such as OH[•], O²⁻ and DPPH is greater than vitamin C (12). Considering the antioxidant properties and free radicals scavenging activity of SalB, this study aims to evaluate the protective effect of salvianolic acid B on spatial learning and memory deficiency with emphasis on oxidative stress in STZ-induced diabetic rats.

2. Materials and Methods

2.1. Animals

In this experimental study, 40 male albino Wistar rats (obtained from local animal house) weighing 225–285 g were used. Animals were housed in an air-conditioned colony room on a light/dark cycle (21–23 °C) and supplied with free access to 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 laboratory animals.

2.2. Experimental procedure

In this study, those rats were used in which in the normal and fasting state (overnight), the amount of glucose level was less than 250 mg/dl. In this regard, the blood sampling was from tail. The rats (n = 56) were randomly divided into seven similar groups: vehicle-treated control, salvianolic acid B-treated control at a dose of 10 mg/kg, vehicle-treated diabetic, and salvianolic acid B-treated diabetic groups at a dose of 10 mg/kg. The rats were rendered diabetic by a single intraperitoneal injection of 60 mg/kg of streptozotocin (STZ) (Pharmacia and Upjohn, USA) freshly dissolved in cold normal saline. Control animals received an injection of an equivalent volume of normal saline. One week after STZ injection, overnight fasting blood samples were collected and serum glucose concentrations were measured using glucose oxidation method (Zistshimi, Tehran) using a spectrophotometer (Spectronic 20, USA). Only those animals with a fasting serum glucose level higher than 250 mg/dl were selected as diabetic for the following experiments. Gradually, in the next days, obvious signs of diabetes such as polydipsia, diuresis and weight loss were seen in some rats. The day on which hyperglycemia had been confirmed was designated as day 0. Salvianolic acid B (Sigma, USA) was administered i.p. at a dose of 10 mg/kg/day 1 week after STZ injection for a period of 6 weeks. Dose of salvianolic acid B was chosen according to our pilot study and our previous studies (not published). Salvianolic acid B was dissolved in normal saline and freshly administered. Behavioral tests including Y-maze and passive avoidance and measuring of some oxidative stress indices were performed at the end of study as described below.

2.3. Single-trial passive avoidance test

This test was conducted 2 days after Y-maze task and was according to a previous study (13). For evaluation of passive avoidance behavior, it was used of a device with dimensions 20 × 80 × 20 cm (shuttle box) that has a clear and a dark compartment. Metal rods on the floor of dark chamber was used for shocking to the animal's foot. A specific device (Behboud Pardaz, Tehran) was used for dark chamber stimulation. Therefore, a single stimulation (1 mA, 1 ms) was applied. In this research, passive avoidance behavior was assessed in three stages of

adaptation, acquisition and retention and recall of information and two parameters of initial latency (IL) and step-through latency ((STL), STL up to a maximum of 600 s as cut-off) was measured.

2.4. Y-maze task

Short-term spatial recognition memory performance was assessed by recording spontaneous alternation behavior in a single-session Y-maze (as described before) 2-3 days before performance of passive avoidance test (13). The Y-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, 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 -2 and the percentage is calculated as the ratio of actual to possible alternations (defined as the total number of arm entries -2).

2.5. Determination of hippocampal MDA concentration

The rats were anesthetized with ketamine (100 mg/kg) and decapitated. Hippocampal blocks were isolated and blotted dry and then weighed and prepared as a 5% tissue homogenate in ice-cold 0.9% saline solution. After centrifugation ($1000 \times g$, 4 °C, 10 min), the supernatant was aliquoted and stored at -80 °C until assayed. The concentration of malondialdehyde (MDA) used as a marker of lipid peroxidation was calculated by measuring thiobarbituric acid reactive substances (TBARS) in the supernatant as described previously (13). Briefly, trichloroacetic acid and TBARS reagent were added to aliquots of the supernatant, which were subsequently mixed and incubated at 100 °C for 80 min. After cooling on ice, the samples were centrifuged at $1000 \times g$ for 10 min, and the absorbance of the supernatant was read at 532 nm. The results of TBARS measurements were expressed as MDA

equivalents, using tetraethoxypropane as standard.

2.6. Protein assay

The protein content of the supernatant was measured by the Bradford method, using bovine serum albumin (Sigma Chemical, St. Louis, MO) as the standard (13).

2.7. Statistical analysis

All results were expressed as mean \pm S.E.M. The nonparametric Kruskal–Wallis test was used to analyze the behavioral tests, and if a difference was found to be significant, pair-wise comparison was done using the Mann–Whitney U-test. Parametric one-way ANOVA was used to assess the results of biochemical tests. 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

3.1. Body weight and serum glucose

According to acquired data, there was no significant difference in weight between sham, unpretreated and pretreated diabetic rats (Figure 1-A). Serum glucose level in untreated diabetic and salvianolic acid B treated diabetic rats was more than that of control rats ($p < 0.001$). Pretreatment of diabetic rats with salvianolic acid B lowered serum glucose level in comparison with diabetic ones ($p < 0.05$; Figure 1-B).

3.2. Passive avoidance test

In the passive avoidance test, no significant difference was seen in IL between different groups (Fig. 2-A). Also, The STL as an index for evaluation of the animal's ability to store information in memory storage, show a significant reduction in diabetic rat as compared to sham ones. Pretreatment of diabetic rats with salvianolic acid significantly increased the STL ($p < 0.05$; Figure 2-B).

3.3. Spatial recognition memory in Y-maze

The results of Y-maze test that is an indicator of the type of recognition in short-term spatial

memory, showed that in diabetic rats, alternation percent was significantly reduced. Pretreatment of these animals with salvianolic acid had no significant effect on their alternation percent

(Figure 3-A). Meanwhile, no difference was observed in the total number of arm entries in Y-maze between the groups (Figure 3-B).

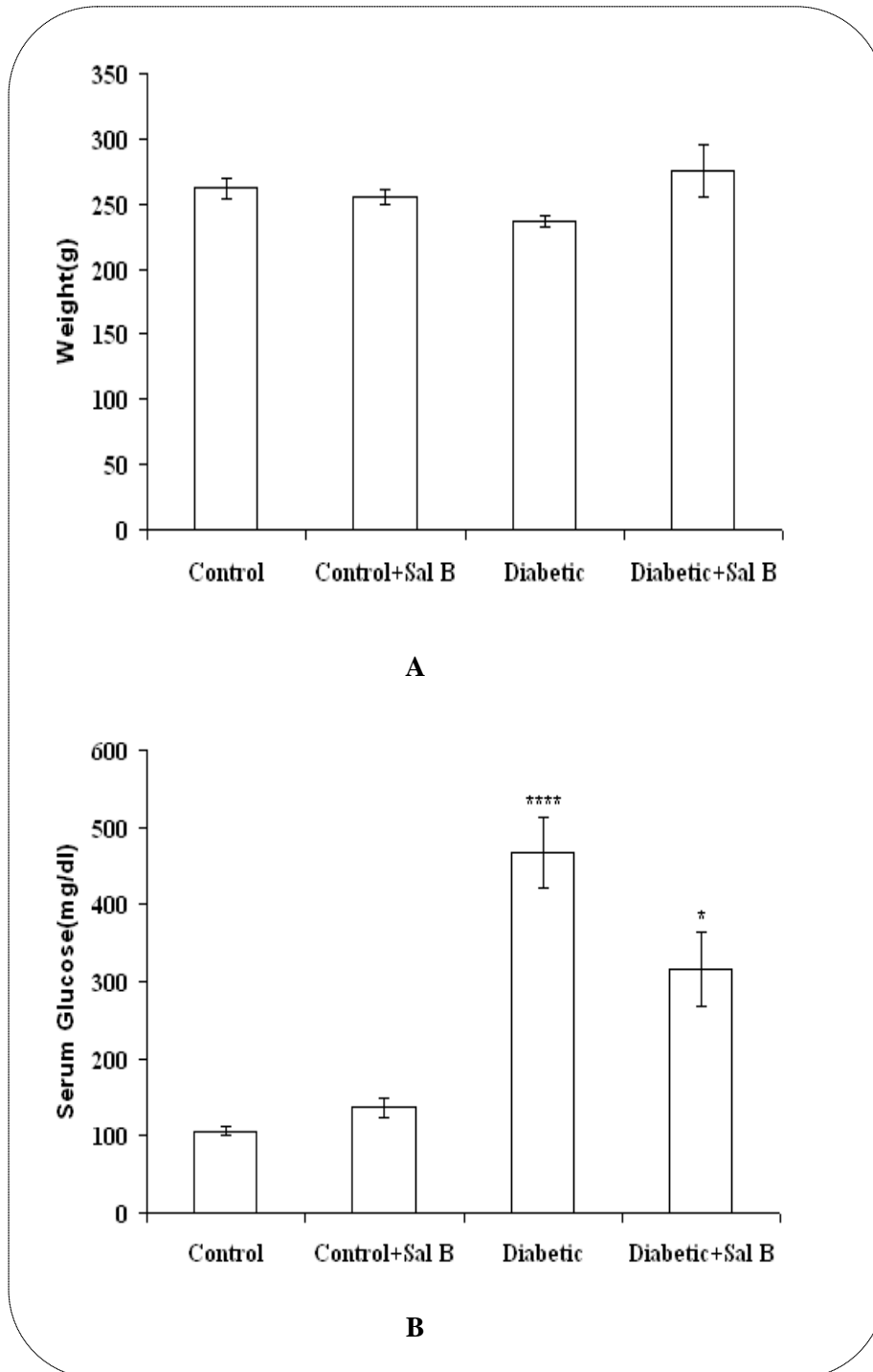


Figure 1. Body weight (A) and serum glucose (B). Values are means \pm SEM. **** $P < 0.0001$ (vs. Control); * $P < 0.05$ (vs. Diabetic)

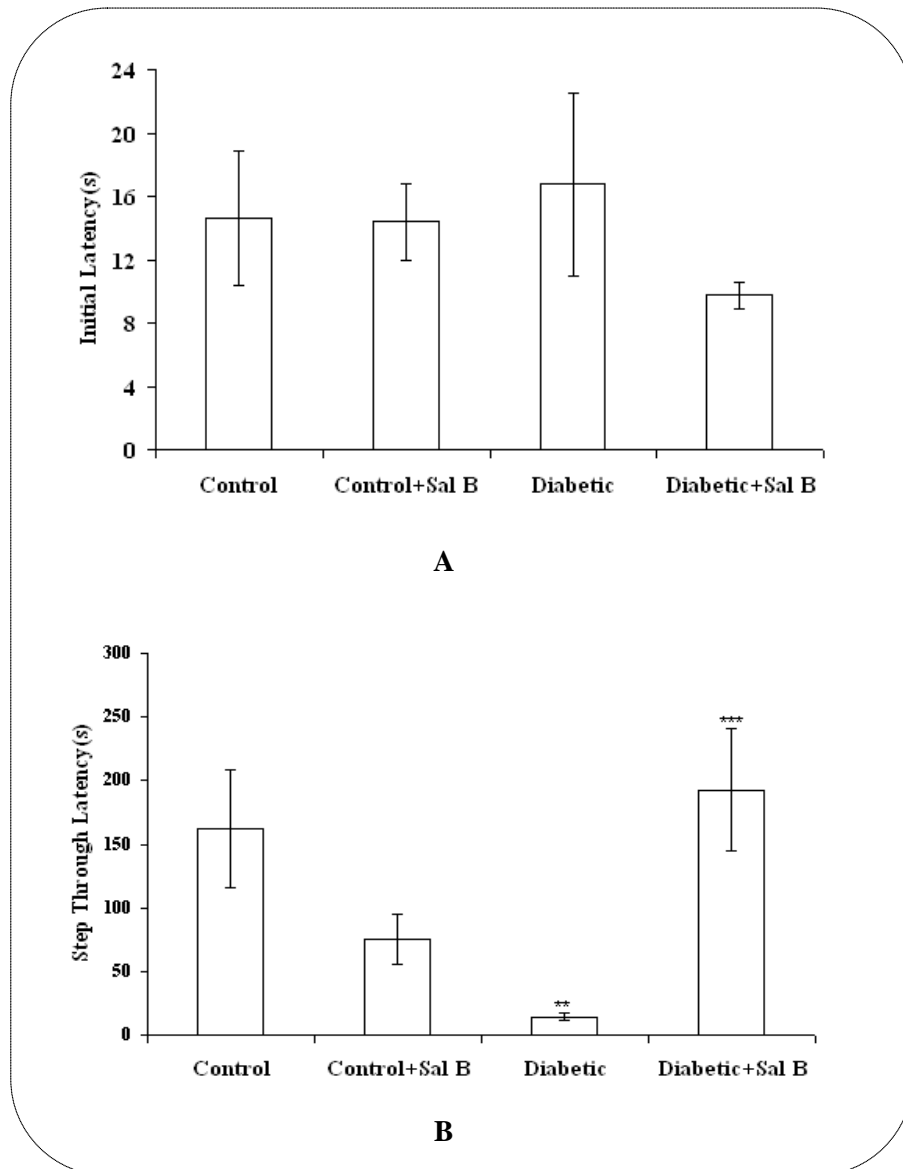


Figure 2. Initial latency (A) and step-through latency (B) recorded in a single-trial passive avoidance test for rats. Values are means \pm SEM; ** $P < 0.01$ (vs. Control); *** $P < 0.001$ (vs. Diabetic).

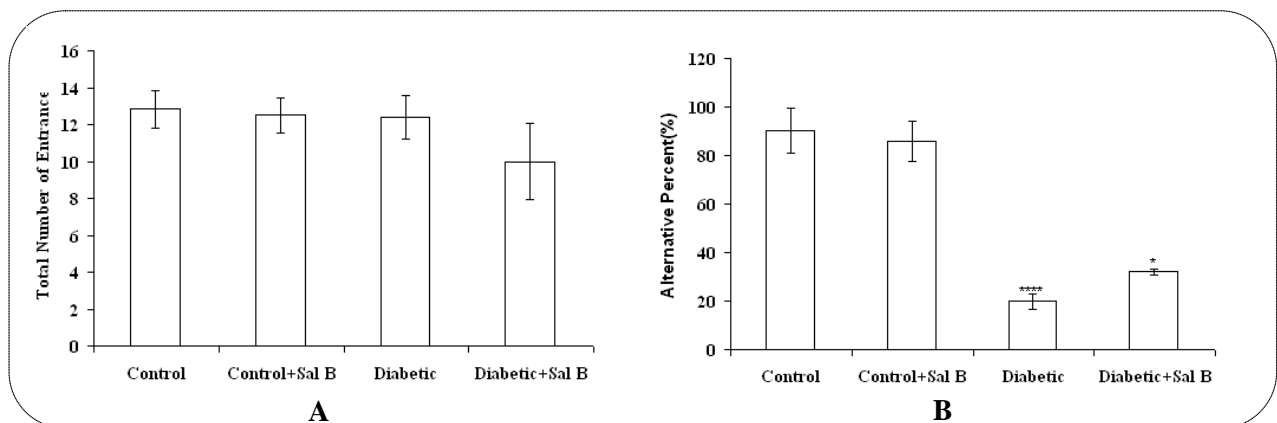


Figure 3. Total entrance (A) and alternation behavior (B) displayed in the Y-maze by rats. Values are means \pm SEM.

**** $P < 0.0001$ (vs. Control); * $P < 0.05$ (vs. Diabetic).

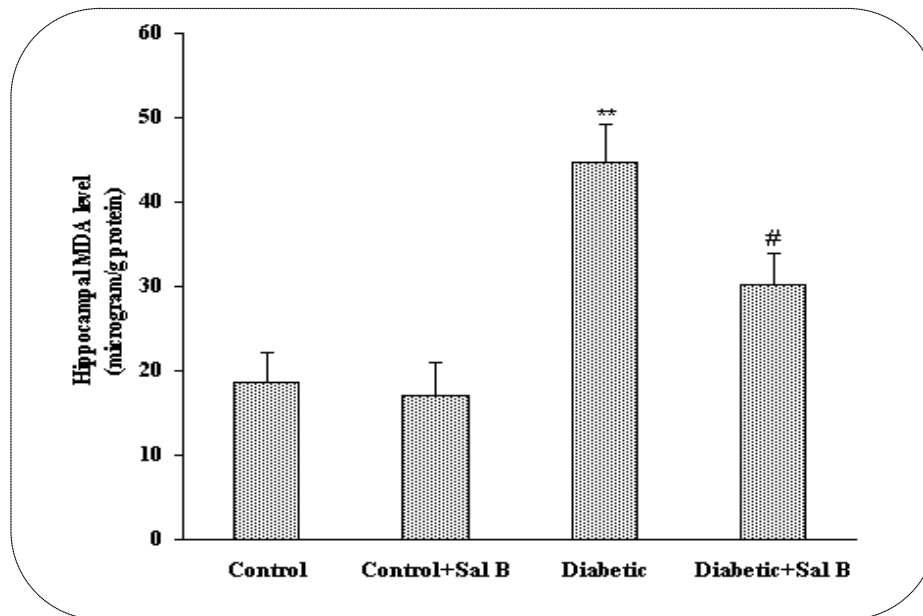


Figure 4. Malondialdehyde (MDA) concentration in hippocampal homogenate from different groups. Values are means \pm SEM.

** $p < 0.01$ (vs. Control); # $p < 0.05$ (vs. Diabetic).

3.4. Hippocampal MDA concentration

Diabetic rats significantly showed an elevated level of MDA (44.8 ± 4.5 nmol/mg protein; $p < 0.01$) in hippocampal tissue as compared to the sham-operated group (MDA, 18.7 ± 3.5 nmol/mg protein) (Fig. 4). Pretreatment of diabetic rats with salvianolic acid B at a dose of 10 mg/kg significantly attenuated the increased MDA content (30.1 ± 3.8 ; $p < 0.05$).

4. Discussion

The results of this study showed that STL and alternative percent decreased in diabetic rats. Pretreatment of diabetic rats with salvianolic acid B (10 mg/kg) improved STL, but not alternative percent. Also, diabetic rats exhibited a significant increase in MDA of hippocampal tissue and pretreatment of rats significantly lowered this parameter. Based on previous findings, the incidence of diabetes mellitus in laboratory animals (such as rats) and the humane society are associated with the disturbances in cognitive processes and memory, brain atrophy and increased risk of dementia (14). Although the mechanisms of these disorders in the diabetic population is not well known but it has been found that diabetes affects the two main areas of the cerebral cortex and hippocampus that have

major roles in related disorders (14). Due to this, the incidence of diabetes mellitus exacerbates oxidative stress and lipid peroxidation in some brain areas including the hippocampus (14, 15). Also, the levels of hippocampal insulin-like growth factor and brain-derived neurotrophic factor decrease (16, 17). Moreover, diabetes reduces the ability of animals in the consolidation and recall stored information (19). As in the present study, similar results were obtained. Based on available evidence, the resulting changes of these abilities can be attributed to the synaptic plasticity of hippocampus and thus interfere with the process of the long-term potentiation. However, these changes are involved in information consolidation, but based on the new research evidence it may be implicated in learning of complicated and new skills (18, 19). In the present study, administration of salvianolic acid B at a dose of 10 mg/kg improves learning and memory in passive avoidance test. In explaining the beneficial effects of salvianolic acid B, it has been specified that the incidence of diabetes in laboratory animals, after a few weeks, increases the activity of nitric oxide producing neurons in some brain areas, especially the hippocampus (20). Since salvianolic acid B is able to reduce the nitric oxide synthase expression in some areas of the body (21), it is possible that it has

improved memory in diabetic rats by this effect. In addition, the incidence of diabetes in streptozotocin-diabetic rats increases oxidative stress-induced reactive oxygen radical formation in the brain, particularly in the hippocampal and cortical areas that is considered as main regions of learning and memory (14). In the present study, measurement of malondialdehyde (MDA), which is one of the obvious indicators of lipid peroxidation in tissues showed that in diabetic rats, hippocampal MDA level increased. Administration of salvianolic acid B reduced oxidative stress and lipid peroxidation and in this way it may protect neurons against the harmful elements.

Moreover, the intensification of activities of microglia in some area of the brain, including the hippocampus has been observed in diabetic rats (22). In a previous work, we showed that salvianolic acid B could improve astrogliosis in kainic acid-induced temporal lobe epilepsy model (not published). Hence, it is likely that salvianolic acid B exerts some protective effect through diminishing this process.

Altogether, this study showed that although administration of salvianolic acid B at a dose of 10 mg/kg could improve the capability of the storage and recall of diabetic animals in avoidance test, but has no effect on short term spatial memory. It seems that salvianolic acid B exerts a part of its protective effect through reducing lipid peroxidation.

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