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Sesamin: A promising protective agent against diabetes-associated cognitive decline in rats

Yaghoob Farbood[a], Shahab Ghaderi[a,*,1], Masome Rashno[a,*,1], Seyed Esmaeil Khoshnam[a], Layasadat Khorsandi[b], Alireza Sarkaki[a], Mohammad Rashno[b,c]

[a] Department of Physiology, Faculty of Medicine, Physiology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[b] Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[c] Department of Immunology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

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ABSTRACT

Aims: Hippocampal oxidative stress and apoptosis of CA1 neurons play significant roles in the pathophysiology of diabetes-associated cognitive decline (DACD). The present study was aimed to elucidate the putative effects of sesamin, a major lignan of sesame seed, against DACD, and possible involvement of anti-oxidative and anti-apoptotic mechanisms.

Main methods: Fifty adult male Wistar rats were randomly divided into control, control-sesamin (30 mg/kg/day), diabetic, diabetic-sesamin (30 mg/kg/day), and diabetic-insulin (6 IU/rat/day) groups. Diabetic rats were treated with sesamin (P.O.) or insulin (S.C.) for eight consecutive weeks. Cognitive performance was evaluated in a Morris Water Maze (MWM) test; in addition, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) concentrations were assayed in the hippocampus using assay kits. Moreover, hematoxylin-eosin (HE), TUNEL, and immunohistochemistry (IHC) stainings were conducted to evaluate histological changes, the apoptosis status and expression of pro- and anti-apoptotic proteins in the hippocampal CA1 neurons, respectively.

Key findings: The results showed that diabetes reduced the spatial cognitive ability in MWM, which was accompanied by decrease in SOD, CAT, and GPx activities and increase in MDA level in the hippocampus. Additionally, diabetes resulted in neuronal loss, enhanced apoptotic index, elevated the expression of pro-apoptotic Bax protein, and decreased the expression of anti-apoptotic Bcl-2 protein in the hippocampal CA1 neurons. Interestingly, sesamin treatment improved all the above-mentioned deficits of diabetes at a comparable level with insulin therapy.

Significance: The results suggest that sesamin could be a promising potential therapeutic agent against DACD, possibly through its intertwined anti-hyperglycemic, anti-oxidative, and anti-apoptotic properties.

1. Introduction

Diabetic encephalopathy (DE) is one of the most significant complications of diabetes mellitus (DM) [1], and diabetes-associated cognitive decline (DACD), the main element of DE [2], has attracted considerable attention in recent years. Although the mechanisms underlying DACD are not well understood, several factors such as hyperglycemia [3], oxidative stress [4], and neuronal apoptosis [5] are believed to play possible roles in the matter.

In diabetic patients, chronic hyperglycemia causes oxidative stress in tissues susceptible to complications [5]. The brain is inherently susceptible to oxidative damage due to its high energy requirement, high oxygen consumption, high lipid content, and low levels of free radicals scavengers [6,7].

Recent reports strongly suggest that hyperglycemia-induced apoptosis plays a crucial role in development of DACD [5,9]. Bcl-2 family, divided into two main groups of inducers (the head of Bax) and inhibitors (the head of Bcl-2) of apoptosis [9], is a key factor in the apoptotic processes. Reduction in Bcl-2 expression and enhanced Bax expression have been shown to be crucial factors in hyperglycemia-mediated toxicity which induces apoptosis of neuronal cells [10,11]. In accordance with the above-mentioned mechanism, an increased

* Corresponding authors at: Physiology Research Center, Department of Physiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

E-mail addresses: Ghaderi.shahab@yahoo.com (S. Ghaderi), Masome.rashno@yahoo.com (M. Rashno).

[1] Both corresponding authors equally contributed to this work.

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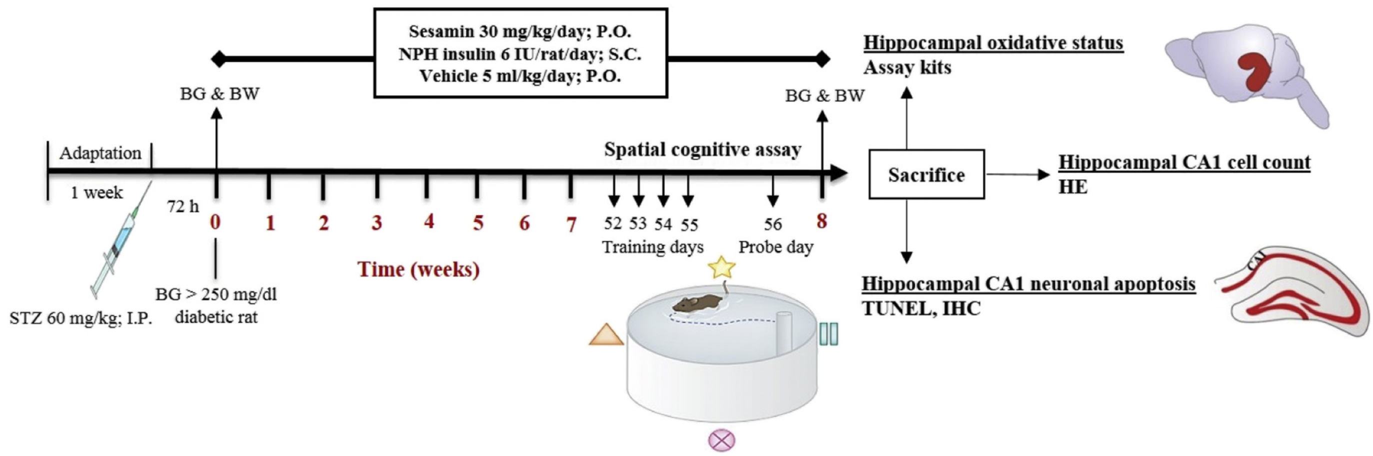

Fig. 1. Schematic timeline of experimental protocol. STZ: Streptozotocin; I.P.: Intraperitoneal; BW: Body weight; BG: Blood glucose; P.O.: Per oral; S.C.: Subcutaneous; CA1: Cornu ammonis 1; HE: Hematoxylin-eosin; TUNEL: Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick-end labeling; IHC: Immunohistochemistry.

number of TUNEL-positive cells have been found in different brain parts of diabetic animals [12].

It has been proved that streptozotocin (STZ)-induced diabetic rats could be a very useful model to determine the underlying causes of central nervous system (CNS) complications [13–15]. The hippocampus, highly susceptible to diabetes-induced hyperglycemia, plays a fundamental role in cognitive processes. Hence, it is considered a specific target tissue for the changes related to cognitive functions in animal models of diabetes [14,16]. Occurrence of oxidative stress and apoptosis in the hippocampus of STZ-induced diabetic rats has been suggested to be involved in induction of spatial learning and memory deficits [14].

Sesamin, a major lignin derived from sesame seed and its oil, has received a great deal of attention over the last few years. Sesamin has exhibited strong hypoglycemic effects in both animal models of diabetes and diabetic patients [17,18]. Furthermore, sesamin has shown reactive oxygen species (ROS) scavenging activity in brain disease models such as cerebral ischemia, seizure, and Parkinson's disease [19–22]. Sesamin suppresses STZ-induced apoptosis in INS-1 pancreatic β-cells through inhibition of nuclear factor kappa B (NF-κB) activation and regulation of Bcl-2 family protein expression [23]. Cognitive-improving effects of sesamin have been proposed in chronic stress-induced mice models [24,25]. It has also been shown that sesamin alleviates blood-brain barrier (BBB) disruption through its anti-oxidative and anti-apoptotic effects in a mouse model of traumatic brain injury (TBI) [26]. However, the effects of sesamin on cognitive function in diabetes have not yet been investigated by any researcher.

In view of this background, the present study attempts to answer the question that whether sesamin has a protective effect against DACD in STZ-induced diabetic rat model? If so, are the possible anti-oxidative and anti-apoptotic mechanisms of sesamin involved in this protective effect?

2. Material and methods

2.1. Chemicals and drugs

The following chemicals and drugs were used in the present study: Sesamin (purity > 98%) (Organic Herb Co., Changsha, China); Streptozotocin (STZ), carboxymethylcellulose (CMC), 3, 3-diaminobenzidine (DAB), proteinase K, citric acid, sodium citrate (Sigma-Aldrich Co., St, Louis, MO, USA); Phosphate-buffered saline (PBS), protease inhibitor cocktail (Roche Co., Basel, Switzerland); Protamine-Zinc insulin (NPH) (EXIR Pharmaceutical Co., Borujerd, Iran); Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), malondialdehyde (MDA) (ZellBio Co., Ulm, Germany); Bio-Rad protein assay (Bio-Rad Co., Hercules, CA, USA); Bcl-2-associated X protein (Bax), B-cell leukemia-2 (Bcl-2), m-IgGκ BP-HRP secondary antibody (Santa Cruz Biotechnology, Inc., Dallas, Texas, USA); Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) (In Situ Cell Death Detection kit, POD, Roche, Mannheim, Germany); Hematoxylin-eosin (HE), hydrogen peroxide (H_2O_2) (Merck Co., Darmstadt, Germany); Ketamine, and xylazine (Alfasan Co., Ja Woerden, Netherlands). STZ was dissolved in citrate buffer solution (0.1 M, pH 4.5), and all the procedures were performed on ice and away from light in order to prevent STZ degradation. Sesamin was freshly dissolved in 1% CMC to obtain an oral suspension. Insulin was freshly diluted with the physiological saline. All the other chemicals used in the study were of the analytical grade.

2.2. Animals and experimental design

Fifty adult male Wistar rats (200 ± 20 g) were purchased from the animal house of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. They were maintained under standard conditions of humidity (55–60%), temperature (22 ± 2 °C), and 12-h light/dark cycle, with free access to standard rodent pellet diet and tap water. All experimental protocols were approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (ethic code: IR.AJUMS.REC.1396.284), and were in accordance with the NIH guidelines. Every possible effort was exerted to minimize animal suffering and the minimum number of rats that were able to produce reliable data were used.

A schematic timeline of the experiment is shown in Fig. 1. The rats were randomly divided into five groups (n = 10 per group) as listed below:

Group 1 (Cont) consisted of normal control rats that received 1% CMC vehicle (5 ml/kg/day; P.O.).

Group 2 (Ses) consisted of normal rats that received sesamin (30 mg/kg/day; P.O.).

Group 3 (STZ) consisted of diabetic control rats that received 1% CMC vehicle (5 ml/kg/day; P.O.).

Group 4 (STZ + Ins) consisted of diabetic rats that received NPH insulin (6 IU/rat/day; S.C., 2 IU in the morning and 4 IU in the afternoon) as previously described by other researchers [27,28].

Group 5 (STZ + Ses) consisted of diabetic rats that received sesamin (30 mg/kg/day; P.O.).

All the treatments were continued for eight consecutive weeks. The selected dosage of sesamin was based on earlier studies on its anti-hyperglycemic, anti-apoptotic, anti-oxidative, and neuroprotective effects in different models of brain injury [17,19–21,26], as well as the pilot study in our laboratory. During the eighth-week period of the study, spatial learning and memory abilities of the rats were assessed for five consecutive days in the MWM test [13,28,29]. All the animals were sacrificed at the end of the study (57th day) for biochemical and histological evaluations of hippocampus [14,16].

2.3. Diabetes induction

Experimental diabetes was induced in rats by a single intraperitoneal injection of STZ (60 mg/kg in citrate buffer). 72 h after STZ injection, a blood sample was obtained by a tail-prick lancet, and then blood glucose levels were determined by a digital glucometer (Accu-Chek Active®, Roche, Germany). Only rats with blood glucose levels of over 250 mg/dl were considered to be diabetic animals and thus were selected for the study. The onset of study was considered the day on which hyperglycemia was confirmed. The normal control rats were injected with an equal volume of citrate buffer. Blood glucose levels and body weights were estimated at both the onset and the end of the study.

2.4. Assessment of cognitive function

2.4.1. Morris water maze (MWM) test

Spatial learning and memory abilities were estimated using MWM paradigm. The MWM apparatus with 150 cm diameter and 80 cm height was divided into four equal quadrants filled with water (23 ± 1 °C) to a depth of 40 cm.

A hidden circular platform with 10 cm diameter was submerged 1 cm below the water surface in the center of the designated target quadrant. All the animals (n = 10/group) went through four consecutive training trials per day for four consecutive days, with 10 min inter-trial intervals. Each trial lasted until the rat reached the hidden platform, or a maximum of 60 s was elapsed. Any rat that failed to reach the platform within the allotted time was guided to it and was allowed to remain there for 30 s. The escape latency to find the hidden platform was measured by a video-tracking software (Maze Router V3.1, Techniq Azma Co, Tabriz-Iran) and considered as learning process [30]. On the fifth day, in order to evaluate the consolidated memory, the animals were subjected to a probe trial, in which the platform was removed and the rats were freely allowed to swim for 60 s. The time spent in the target quadrant (as memory consolidation) and swimming speed (as motor performance) were evaluated [31].

2.5. Biochemical assay

At the end of the experiment, the animals (n = 6) were deeply anaesthetized by a ketamine-xylazine mixture (100 mg/kg–10 mg/kg, respectively). Their intact brains were rapidly removed and hippocampi were dissected on ice, homogenized in a cold PBS with pH 7.4, which contained protease inhibitor cocktail.

The samples were centrifuged at 10,000 ×g at 4 °C and aliquots of the supernatant were stored at −80 °C. Total protein concentration of the supernatant was determined using the Bio-Rad total protein assay reagent according to the manufacturer's instructions.

The concentrations of the studied oxidative stress markers including SOD, CAT, GPx, and MDA in the hippocampus were analyzed by commercial assay kits according to the manufacturer's protocols.

2.6. Histological assay

2.6.1. Hematoxylin-eosin (HE) staining

To determine the histological changes, four animals were randomly chosen from each group and transcardially perfused with 5% paraformaldehyde solution under deep anesthesia. Rats were decapitated, their brains were fixed with 10% paraformaldehyde solution for 72 h and embedded in paraffin. In the next step, tissue sections (5 μm thick) were stained by HE. Then intact neurons (normal cells) and dark neurons (dead cells) from hippocampal Cornu Ammonis 1 (CA1) region were evaluated under a light microscope (Olympus PX 50 F3 model, Japan) [16,28].

2.7. Assessment of apoptosis

2.7.1. Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) assay

In order to detect apoptosis in the hippocampal CA1 neurons, TUNEL staining was performed according to the manufacturer's protocol. Following deparaffinization samples were treated with proteinase K and immersed in H_2O_2 to block endogenous peroxidase activity. Thereafter, the samples were incubated in the TUNEL reaction mixture at 37 °C. Next, the slides were incubated with secondary anti- fluorescein-POD-conjugate and were stained with DAB chromogen substrate.

A cell was TUNEL-positive when the nuclear staining was brown, intense, and homogenous. Apoptotic index was calculated as TUNEL-positive cells/total number of cells × 100 [32].

2.7.2. Immunohistochemistry (IHC) assay

To evaluate the expression of pro-apoptotic Bax and anti-apoptotic Bcl-2 proteins in the hippocampal CA1 area, IHC assay was performed 57 days after diabetes induction. After deparaffinization and incubation in citrate buffer solution (98 °C), the tissue sections were blocked with goat serum. Thereafter, the sections were incubated overnight with monoclonal antibody against Bax or Bcl-2 in a humidity chamber at 4 °C. The tissue sections were treated with secondary antibody. Next, they were incubated with a DAB as chromogen substrate. Finally, the tissue sections were counterstained with hematoxylin and observed by light microscopy. Immunohistochemical slides were blindly assayed using H-score. The calculation of H-score for each section was found as follows: H-score = ΣPi (i + 1), where "i" is the intensity of staining (0 = negative, 1 = low, 2 = medium and 3 = high) and "Pi" is the percentage of stained cells for each intensity (0 to 100%) [32].

2.8. Statistical analysis

The escape latency to reach the hidden platform in training days of MWM was analyzed by repeated measures two-way analysis of variance (ANOVA), and differences between individual groups were determined by one-way ANOVA, using Statistical Package of Social Sciences (SPSS) software program, version 16 (SPSS; Chicago, IL, USA) and GraphPad Prism version 5.0 (GraphPad software, San Diego, CA, USA). Other data was analyzed by one-way ANOVA followed by Tukey's HSD test for multiple comparisons. The data is expressed as mean ± standard error of mean (SEM), and P value of < 0.05 was considered statistically significant.

3. Results

3.1. Treatment with sesamin decreases blood glucose level, but does not prevent weight loss in the diabetic rats

Three days after STZ or citrate buffer injection, the blood glucose levels of the animals, which had been treated with STZ, were markedly higher than those treated with sodium citrate buffer ($F_{4, 45}$ = 62.921; $P < 0.001$). At the end of the study, diabetes induction resulted in increased blood glucose levels ($F_{4, 45}$ = 95.67; $P < 0.001$), while they were markedly decreased in the groups treated with sesamin or insulin ($P < 0.001$), suggesting that sesamin or insulin consumption helps protecting against severe hyperglycemia in diabetic animals (Fig. 2A).

At the onset of the study, the non-diabetic rats and those which were going to receive STZ had similar body weights ($F_{4, 45}$ = 0.451; $P = 0.771$); while the STZ group grew poorly and had markedly lower

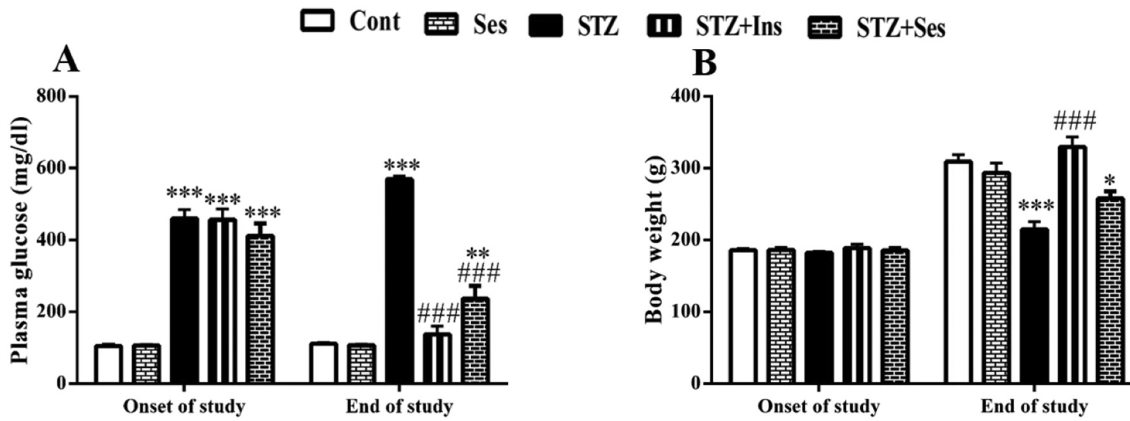

Fig. 2. Effects of sesamin (30 mg/kg/day; P.O., for 8 weeks) on blood glucose levels (A) and body weight (B) of diabetic rats. Each bar represents mean ± SEM of 10 animals per group; *P < 0.05, **P < 0.01, ***P < 0.001 vs. the Cont group; ###P < 0.001 vs. the STZ group.

body weights than the Cont group at the end of the study ($F_{4, 45} = 14.947$; $P < 0.001$, Fig. 2B). Body weight was significantly increased in the STZ group after insulin treatment ($P < 0.001$). However, sesamin did not affect the body weight of the STZ + Ses group, showing that sesamin consumption did not prevent weight loss in diabetic rats.

3.2. Treatment with sesamin improves cognitive impairment in the diabetic rats

To investigate the effects of chronic hyperglycemia associated with DM on the spatial learning and memory performance, animals were trained in the MWM task. In this paradigm, learning was associated with reduction in the escape latency to find the hidden platform during four consecutive training days.

Repeated measures two-way ANOVA analysis of the escape latency to reach the hidden platform demonstrated the overall significant effects of the treatment ($F_{4, 45} = 18.10$; $P < 0.001$), and day ($F_{3, 135} = 26.62$; $P < 0.001$). However, there was no significant effect of day × treatment interaction ($F_{12, 135} = 1.587$; $P = 0.4311$) for it in all the training days.

The following Tukey's post hoc test demonstrated that the STZ group had more escape latency than the Cont group in the training days 1 ($P < 0.01$), 2 ($P < 0.01$), 3 ($P < 0.05$), and 4 ($P < 0.05$), which indicating noticeable learning deficits in the diabetic rats. However, chronic treatment of diabetic animals with either of sesamin or insulin significantly decreased the escape latency in the training days 1 ($P < 0.01$ and $P < 0.05$, respectively), and 3 ($P < 0.001$ for each comparison, Fig. 3A and C).

In the probe session, one-way ANOVA showed that the STZ group spent the least amount of time in the target quadrant when compared with the Cont group ($F_{4, 45} = 4.605$; $P < 0.01$). However, this was increased following chronic administration of both sesamin and insulin in diabetic animals ($P < 0.05$ for each comparison, Fig. 3B and D). Furthermore, there was no significant difference in swimming speed among the five groups ($F_{4, 45} = 1.253$; $P > 0.05$, Fig. 3E). These results confirm that all changes observed in the acquisition and probe trials did not result from the changes in motor performance.

3.3. Treatment with sesamin ameliorates hippocampal oxidative status in the diabetic rats

As illustrated in Fig. 4A, B, and C, decreased activities of free radical scavenging enzymes, namely SOD ($F_{4, 25} = 11.78$; $P < 0.001$), CAT ($F_{4, 25} = 6.067$; $P < 0.01$), and GPx ($F_{4, 25} = 8.326$; $P < 0.01$), were statistically found in the hippocampus of the STZ group compared to the Cont group. Such reductions in the activity of these enzymes were restituted to the Cont group's level by sesamin or insulin treatment.

The free radical damage of the eight weeks following diabetes induction was evaluated by lipid peroxidation that was considered as MDA level. As illustrated in Fig. 4D, there was an elevated MDA level in the hippocampal tissue of the STZ group compared to the Cont group ($F_{4, 25} = 43.20$; $P < 0.001$). Sesamin treatment in the diabetic rats caused a significant decrease in the MDA level in comparison to the STZ group ($P < 0.001$); however, the level did not return to the Cont level ($P < 0.001$). Likewise, insulin therapy markedly decreased MDA level in diabetic animals ($P < 0.001$). No significant difference was detectable between the Cont and the Ses groups ($P > 0.05$), indicating sesamin per se did not alter MDA level in the hippocampus of the normal animals.

3.4. Treatment with sesamin prevents neuronal loss in the hippocampal CA1 area of diabetic rats

Based on the results shown in Fig. 5A and B, histological changes in the hippocampal CA1 area were assessed by HE staining. A significant decline was found in the number of intact neurons of the hippocampal CA1 ($F_{4, 15} = 23.42$; $P < 0.001$) area of the STZ group compared to the Cont group, indicating neuronal loss in diabetic animals. Sesamin treatment of the diabetic rats markedly protected the hippocampal CA1 neurons as compared to the STZ group ($P < 0.001$). Furthermore, insulin treatment significantly increased the number of intact neurons in this brain area when compared to the STZ group ($P < 0.01$).

3.5. Treatment with sesamin decreases apoptotic index in the hippocampal CA1 area of diabetic rats

The apoptotic cells in the brain slides were identified by TUNEL staining. In the normal animals, TUNEL-positive cells were absent or rare in the hippocampal CA1 region (Fig. 6A_1). Apoptotic index was significantly increased in the hippocampal CA1 ($F_{4, 15} = 23.30$; $P < 0.001$, Fig. 6A_2) area of the STZ group compared to the Cont group.

Apoptotic index was also increased in the STZ + Ses, and STZ + Ins groups compared to the Cont group ($P < 0.001$ for each comparison). Although, when compared to the STZ group, both sesamin and insulin treatments significantly reduced the apoptotic index in the hippocampal CA1 neurons of diabetic rats ($P < 0.001$).

3.6. Treatment with sesamin regulates Bcl-2 family protein expression in the hippocampal CA1 neurons of diabetic rats

To evaluate the anti- and pro-apoptotic effects of sesamin in STZ-induced diabetic model, expression of pro-apoptotic Bax and anti-

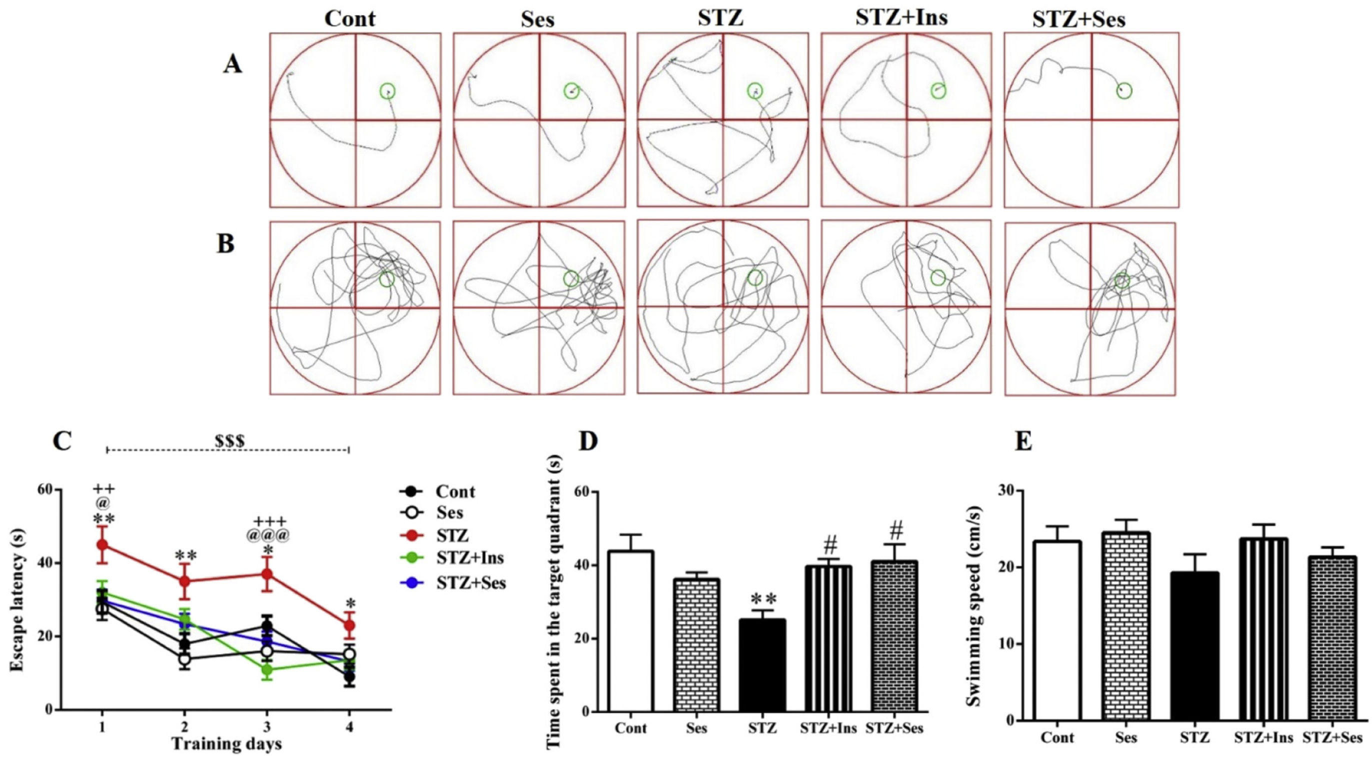

Fig. 3. Effects of sesamin (30 mg/kg/day; P.O., for 8 weeks) on spatial learning and memory of diabetic rats by MWM test. (A) Represents swimming traces of the rats on the 4th training day. (B) Represents swimming traces of the animals in the probe trial. (C) Escape latency during four consecutive training days. (D) Time spent in target quadrant in the probe session. (E) Swimming speed in the probe session. Each bar represents mean ± SEM of 10 animals per group. $$$P < 0.001 (repeated measures two-way ANOVA); *P < 0.05, **P < 0.01 Vs. the Cont group; + +P < 0.01, + + +P < 0.001 Vs. the STZ + Ses group; @P < 0.05, @@@P < 0.001 vs. the Ins + Ses group; #P < 0.05 vs. the STZ group (one-way ANOVA).

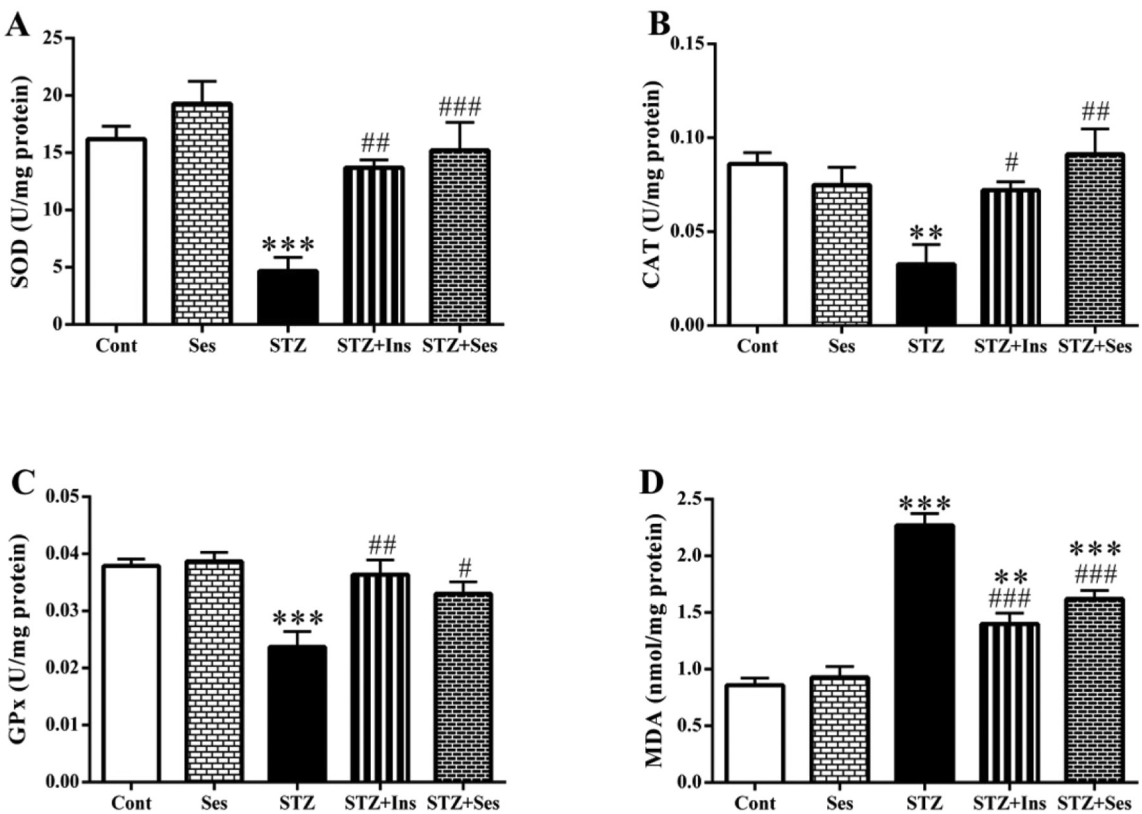

Fig. 4. Effects of sesamin (30 mg/kg/day; P.O., for 8 weeks) on SOD (A), CAT (B), and GPx (C) activities and MDA level (D) in the hippocampus of diabetic rats. Each bar represents mean ± SEM of six rats per group. *P < 0.05, **P < 0.01, ***P < 0.001 vs. the Cont group; #P < 0.05, ##P < 0.01, ###P < 0.001 vs. the STZ group.

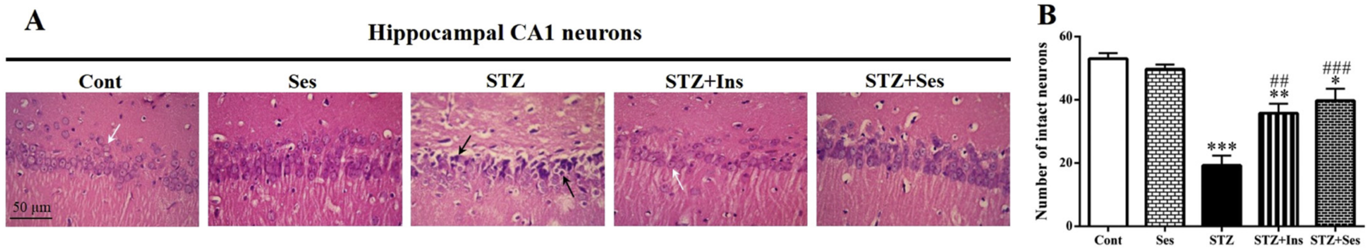

Fig. 5. Effects of sesamin (30 mg/kg/day; P.O., for 8 weeks) on histological changes in the hippocampal CA1 region (H & E stain, 400 x magnification, scale bar 50 μm) of STZ-induced diabetic rats. "A" includes the revealing photographs of intact neurons (clear cells with distinct round nuclei, identified by white arrows) and dark neurons (shrinkage of cells with pyknotic nuclei, identified by black arrows), and "B" represents the quantitative data of the number of intact neurons. Each bar represents mean ± SEM of four animals per group. *P < 0.05, **P < 0.01, ***P < 0.001 vs. the Cont group; ##P < 0.01, ###P < 0.001 vs. the STZ group.

apoptotic Bcl-2 proteins were assayed in the hippocampal CA1 area, eight weeks after STZ-diabetes induction. There was a significant elevation in the expression of Bax protein ($F_{4, 15} = 269.5$; $P < 0.001$, Fig. 6$B_{1, 2}$) and reduction in the expression of Bcl-2 ($F_{4, 15} = 50.45$; $P < 0.001$, Fig. 6$C_{1, 2}$) in the hippocampal CA1 area of the diabetic group compared with the Cont group.

This imbalance of Bcl-2 family (increased Bax and decreased Bcl-2) was also detected in the STZ + Ses and the STZ + Ins groups in comparison to the Cont group ($P < 0.001$ for each comparison). Although, when compared to the STZ group, both sesamin and insulin treatments of diabetic animals markedly decreased Bax expression and increased Bcl-2 expression in the hippocampal CA1 neurons ($P < 0.001$, and $P < 0.01$, respectively).

4. Discussion

The present study showed that STZ-induced diabetes impaired spatial learning and memory of rats, which was associated with hippocampal oxidative stress, and the apoptosis and neuronal loss of hippocampal CA1 region. Interestingly, treatment with sesamin for eight consecutive weeks improved spatial cognitive impairment, ameliorated hippocampal oxidative status, and prevented the apoptosis and neuronal loss of hippocampal CA1 area in the diabetic rats at a comparable level with insulin therapy, which is the standard anti-diabetic drug.

The current study revealed that STZ-induced diabetes resulted in spatial learning impairment in the MWM test, since an elevation in the escape latency to reach the hidden platform was observed in the diabetic rats during four consecutive days of training trials. In addition, the

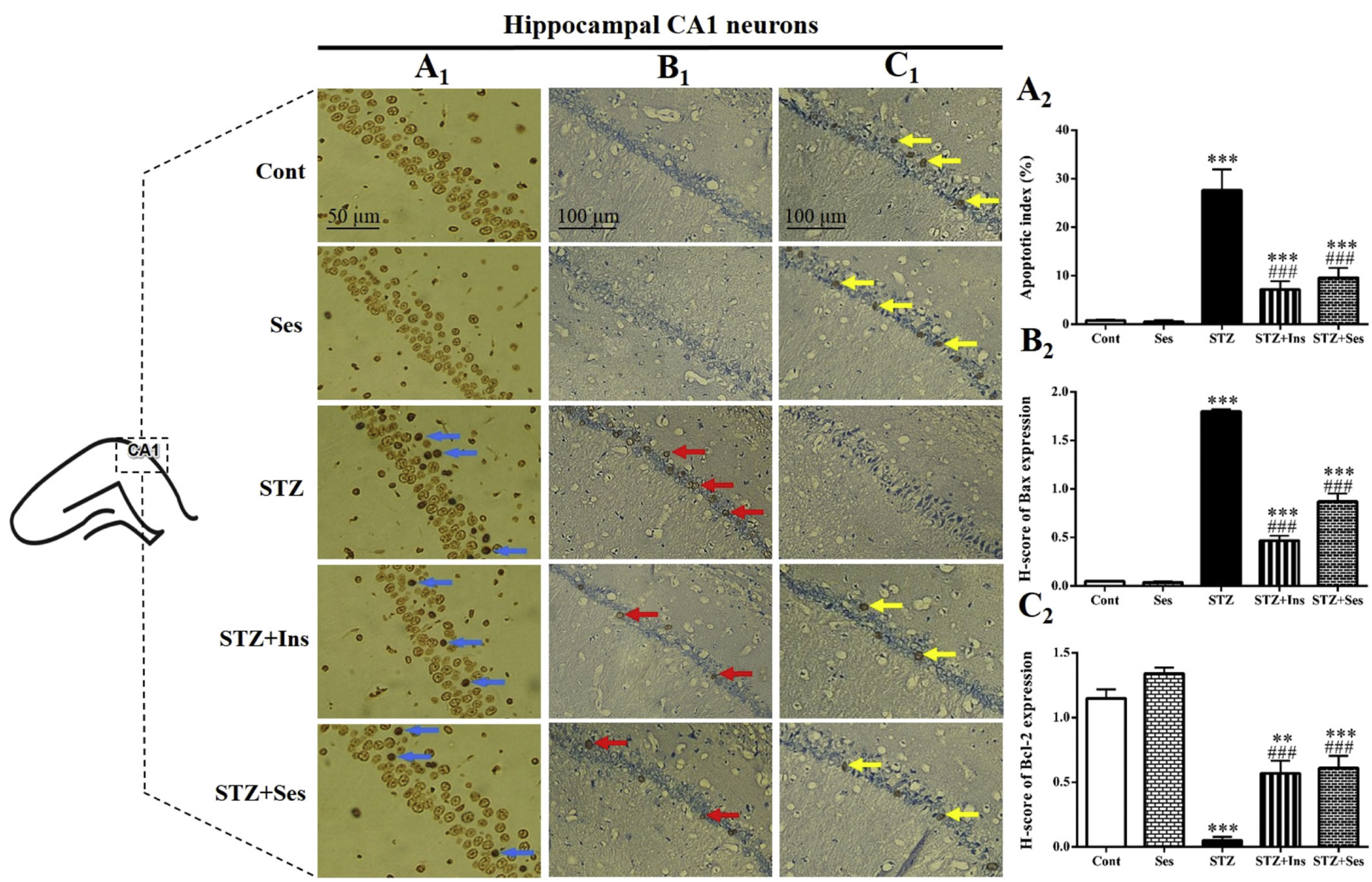

Fig. 6. Results of TUNEL staining (TUNEL-positive cells identified by blue arrows; A_1) and IHC staining on the Bax (positive expression of Bax identified by red arrows; B_1), and Bcl-2 expressions (positive expression of Bcl-2 identified by yellow arrows; C_1) in the hippocampal CA1 neurons of diabetic rats. Apoptotic index (%) (A_2), H-score of Bax (B_2), and Bcl-2 (C_2) expressions in all the groups. Data was obtained from six sections per animal and four animals per group, and is presented as mean ± SEM. ***P < 0.001 vs. the Cont group; ###P < 0.001 vs. the STZ group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

time spent in the target quadrant by the diabetic rats was significantly reduced in the probe trial, representing poorer memory consolidation. These results are in line with previous studies [28,33]. However, treatment with sesamin shortened the escape latency to reach the hidden platform and increased the time spent in the target quadrant in the diabetic rats. Collectively, these findings suggest that sesamin could effectively improve diabetes-induced spatial cognitive deficits in the MWM paradigm. Numerous experimental and clinical studies have reported that sesamin is able to improve many neurobehavioral deficits [19,22,24,25,34]. However, to the best knowledge of the authors, this is the first study that examines the influences of sesamin on DACD.

Similar to previous studies [16,29], the results of the present study showed that STZ-induced diabetes resulted in significant elevation of blood glucose levels along with reduction of body weight in rats. This diabetogenic effect of STZ is probably due to its damaging effect on pancreatic β-cells. Prolonged sesamin treatment significantly decreased the blood glucose levels in the diabetic animals, which is precisely in accordance with previous experimental [17,35] and clinical [18] studies. Chronic hyperglycemia is one of the main causes of DM complications, including cognitive deficits [36]. In contrast, anti-hyperglycemics and insulin-sensitizing agents could be effective in preventing or slowing the progression of cognitive dysfunction in diabetic condition [37]. Hence, the amelioration of DACD may be partly due to the ability of sesamin to alleviate hyperglycemia; although its efficacy was lower than insulin therapy. The possible hypoglycemic mechanisms of sesamin are related to its ability to increase the number of low-affinity insulin receptors, enhance insulin sensitivity [35], elevate insulin secretion capacity [23], reduce the oxidative stress generated by STZ in pancreatic β-cells [38], and to protect against pancreatic β-cells damage by means of its anti-apoptotic property [39].

Hyperglycemia-induced oxidative stress plays a critical role in cognitive and behavioral deficits [16]. Oxidative stress, resulting from excessive levels of ROS, contributes to increased neuronal damage and death by oxidizing proteins, and damaged DNA and lipids in cellular membranes [40]. Oxidative damage to synapses in the hippocampus of rats has been linked to cognitive disabilities [41,42]. Learning and memory deficits were associated with changes in the hippocampal synaptic plasticity of diabetic rats [43,44]. Diabetes-induced oxidative stress in the hippocampus of rats has been previously reported as a factor that contributes to cognitive dysfunction [16,45]. In the present study, the activities of SOD, CAT, and GPx (as endogenous antioxidants) were decreased, whereas MDA level (as an indicator of lipid peroxidation) was increased in the hippocampus of the diabetic rats, which is in agreement with previous findings [15,16]. However, chronic sesamin treatment improved the hippocampal oxidative status by increasing the SOD, CAT, and GPx activities, and inhibiting the elevation of MDA levels, indicating its free radical scavenging property. Similarly, the antioxidant and free radical scavenging capabilities of sesamin have been reported in different models of brain injury [19–22]. Therefore, the improvement of DACD by sesamin may be partly due to its protective effects against diabetes-induced hippocampal oxidative stress.

Hyperglycemia-induced apoptosis of hippocampal neurons is of great importance in learning and memory deficits [8,14,46]. Among the various hippocampal subfields, CA1 is especially susceptible to diabetes-induced hyperglycemia [46], oxidative stress [47], and apoptosis [8], and it is also closely related to learning and memory. It has been demonstrated that degeneration and apoptosis of hippocampal CA1 neurons are closely associated with spatial learning and memory deficits in STZ-induced diabetic rats [8,14]. In the current study, the increased apoptotic cell death in the hippocampal CA1 neurons of the diabetic rats might be related to the elevated expression of pro-apoptotic Bax protein, and the diminished expression of anti-apoptotic Bcl-2 protein, which is probably mediated by the mitochondrial (intrinsic) pathway. In this regard, it has been suggested that the imbalance of Bcl-2 family protein expression is the main mechanism behind hippocampal neuronal apoptosis in STZ-induced diabetic model [10,11]. Since sesamin decreases the activation of caspase-3 in neuronal cells in the hyperglycemic condition, a portion of its anti-apoptotic activity could be related to this effect [48]. Interestingly, chronic sesamin treatment effectively suppressed hippocampal neuronal apoptosis in the diabetic animals, and this is perhaps attributed to its regulation of Bcl-2 family protein expression. It has been suggested that one of the main mechanisms behind the apoptotic inhibitory effect of sesamin is mediated by regulation of Bcl-2 family protein expression [23]. Thus, it is possible that the alleviation of DACD following sesamin treatment was mediated, at least partially, by its anti-apoptotic activity against diabetes-induced apoptosis of hippocampal CA1 neurons.

In the present study, histological examination revealed that STZ-induced diabetes results in neuronal loss of hippocampal CA1 region, which is congruent with previous studies [13,16,28]. Surprisingly, treatment with sesamin was able to prevent neuronal loss in the hippocampal CA1 area of the diabetic animals. It seems that prevention of hippocampal neuronal loss in the present study is due to improvements in hyperglycemia, amelioration of hippocampal oxidative status, and reduction of hippocampal neuronal apoptosis that occurred after treating the diabetic rats with sesamin.

The mechanism underlying DACD seems to be a multifactorial process. Accumulating evidence has demonstrated that neurotransmitters' changes and loss of neurotrophic support are involved in the pathophysiology of DACD [12,28,49]. It has been suggested that the cognitive-improving effects of sesamin are partly mediated by preventing the decrease of monoamine neurotransmitters (serotonin and noradrenaline), and potentiation of neurotrophic factors (brain-derived neurotrophic factor and neurotrophin 3) [25]. It has been confirmed that even a low level of neuroinflammation can lead to damage in the synaptic function that might result in cognitive decline [50]. Recent evidence in different models of brain injury is indicative of the potential anti-inflammatory properties of sesamin against neuroinflammation and inflammatory mediators [20,21]. Several reports have shown that elevated inflammation in the hippocampus of diabetic animals may induce neuronal cell death and trigger cognitive decline [51,52]. Studies on both the animal models of diabetes and diabetic patients have concluded that treatment with sesamin can reduce the levels of inflammatory markers including tumor necrosis factor-α (TNF-α), intercellular adhesion molecule 1 (ICAM-1), nitric oxide synthase (iNOS), ionized calcium-binding adapter molecule 1 (Iba-1), and interleukin-6 (IL-6) [17,18]. Hence, the amelioration of DACD may be also partly due to the ability of sesamin to modulate neurotransmitters' changes and inflammation status as well as neurotrophic properties; although the mechanisms of the above-mentioned action were not investigated in this study; therefore, it is strongly recommended to include such measurements in future research works.

The metabolism and tissue distribution of sesamin have been studied in both human and animal models [53–55]. After consumption of sesamin, it enters the liver and then moves to the brain and other tissues, suggesting that sesamin possesses BBB permeability [53]. Furthermore, it has been shown that administration of sesamin at the dose of 30 mg/kg could also attenuate TBI-induced BBB disruption through increasing tight junction proteins zonula occludens (ZO)-1 and occludin expression in a mouse model [26]. Hyperglycemia-induced neurotoxicity and cognitive deficit in DM are notably linked to the presence of BBB hypermeability [56,57]. In the current study, sesamin attenuated DACD, and this beneficial effect may be linked to its protective role in BBB in the brain, which should be further examined in future research works.

In the present study, both sesamin and insulin treatments had parallel beneficial effects on spatial cognitive deficit, hippocampal oxidative stress, and the apoptosis and neuronal loss of hippocampal CA1 region in the diabetic rats, except for the hypoglycemic effect of insulin that was stronger than the hypoglycemic effect of sesamin. It seems that the alleviation of DACD by insulin in the current study is related to its interrelated anti-hyperglycemic, anti-oxidative, and anti-apoptotic activities, and this is in conformity with earlier reports [16,58]. On the other hand, insulin has anti-inflammatory [28], neuroproliferative [59], and neuroplastic [60] properties and some of the beneficial effects of insulin may be contributing to these properties.

5. Conclusion

The results of the present study demonstrated that chronic sesamin treatment could improve DACD, possibly through reduction in blood glucose level, amelioration of hippocampal oxidative status (increased activities of SOD, CAT, GPx, and decreased MDA content), prevention of hippocampal CA1 neuronal loss, and inhibition of apoptosis by modulation of Bcl-2 family protein expression (increased Bcl-2 and decreased Bax) in the hippocampal CA1 neurons. The present study suggests that sesamin could be a novel, promising, and accessible therapeutic agent against DACD. Further research is required to investigate the involved mechanisms in details.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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