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INSULIN AND NATURAL HONEY CHANGES HIPPOCAMPAL BCL-2 FAMILY GENE EXPRESSION, SUPEROXIDE DISMUTASE ACTIVITY, AND PASSIVE AVOIDANCE MEMORY IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

The present study was conducted to investigate the effects of insulin and honey on passive avoidance memory, expression of the Bcl-2 family of genes involved in apoptosis and the antioxidant activity of superoxide dismutase (SOD) in the hippocampus of streptozotocin (STZ)-induced diabetic male rats. The present study was conducted on 48 male Wistar rats in eight groups (six rats per group) as follows: healthy and diabetic rats treated with honey alone, insulin alone, both insulin and honey, or untreated. Diabetes was induced by injection of STZ (IP, 60 mg/kg). Three days after diabetes induction, the honey groups were treated by intraperitoneal injection with a 5 mg/kg dose of honey daily. The insulin groups received a daily subcutaneous injection of 2–3 U/kg doses of insulin, and the insulin + honey groups received a combination of the above two treatments at the same dosages. Control groups received normal saline. After four weeks of treatment, passive avoidance learning was assessed using a shuttle box apparatus and 24 hours later, the animals' memory was tested. The rats were then euthanized and their hippocampi were removed and Bcl-2 and Bax mRNA levels were assessed via semi-quantitative RT-PCR. SOD activity was assessed with a Randox SOD kit. The results showed that treatment of diabetic rats with insulin or honey separately and in combination led to increased learning and improved passive avoidance memory, reduced expression of Bax, increased expression of Bcl-2, increased Bcl-2/Bax ratio and increased SOD activity in the hippocampus region. It appears that treatment of these rats with insulin and honey separately or in combination can prevent apoptosis in the hippocampal region by reducing the expression of pro-apoptotic Bax and increasing the expression of anti-apoptotic Bcl-2, as well as by increasing SOD activity. Through inhibition of apoptosis, this treatment can potentially reduce learning and memory deficiencies caused by hippocampal damage in these rats.

KEYWORDS: Diabetes, insulin, honey, hippocampus, apoptosis, Passive-avoidance learning, Bax, Bcl-2, superoxide dismutase

INTRODUCTION

Diabetes is one of the most common chronic diseases in almost every country, and is of concern in the health community due to the number of people affected and the changes in lifestyle that lead to reduced physical activity and increased obesity. Diabetes is also associated with diminishing memory function and structural changes in the brain.[1] Neuropsychological experiments have shown that people with types I and II diabetes suffer from mild to moderate cognitive impairments compared to healthy people. The effect of diabetes on the brain has been known for about a hundred years. In the early twentieth century, researchers and clinicians noticed frequent complaints from diabetic patients about loss of memory and lack of concentration. In 1922, Mile et al. showed that people with diabetes had poor memory and concentration in behavioral-cognitive tests[2] and the term diabetic encephalopathy was introduced in 1950 to explain nervous system-related diabetes complications. Other terms such as brain dysfunction or central neuropathy have also been used to describe these phenomena.[3]

Diabetes affects both the peripheral and central nervous systems by increasing apoptosis in nerve cells.[4] Cognitive impairment due to damaged hippocampal neurons is also among the complications of diabetes. Furthermore, greater memory, learning and cognitive deficiencies have been reported in diabetics compared to healthy people.[5-7] As an important memory and learning center, the hippocampus is sensitive to increased blood sugar and its neurons are extremely vulnerable in type I diabetes.[8, 9] Even though many factors are involved in the process of apoptosis, its key elements are limited to only two groups, namely caspases and the Bcl-2 family of proteins.[10] The latter is divided into two main groups:

anti-apoptotic factor (such as Bcl-2) and pro-apoptotic factors (such as Bax).[11] Free radicals that include reactive species of oxygen (ROS) and nitrogen (RNS) have a major role in the pathogenesis of many chronic diseases, such as myocardial failure, degenerative diseases of the nervous system and diabetes. In healthy individuals, the body's antioxidant defense mechanism inhibits the production of free radicals and prevents their destructive effects. Naturally occurring compounds in the body with antioxidant activity fall into two groups: 1) non-enzymatic antioxidants such as vitamins A, E and C, carotenoids, etc.; 2) enzymatic antioxidants such as catalase, superoxide dismutase (SOD), glutathione peroxidase, etc. Increased blood sugar leads to the increased production of free radicals and the reduced levels and activity of antioxidants disturbs this balance, causing oxidative stress. Thus, diabetes is associated with the increased production of free radicals and an impaired antioxidant defense system. Previous studies have shown that in diabetic patients, SOD and antioxidants such as vitamin E and alpha-lipoic acid will reduce diabetes complications and continued insulin resistance and that oxidative stress can cause and exacerbate these symptoms through various signaling pathways. Free radicals and the resulting oxidative stress appear to cause lipid, protein and DNA damage.[12-17] Previous studies have shown that hyperglycemia-induced oxidative stress in *in vitro* and *in vivo* conditions can induce apoptosis in neurons and that use of antioxidants can prevent diabetes-induced oxidative stress and subsequent apoptosis.[17, 18]

SOD is an antioxidant enzyme with a major role in the elimination of free radicals from the body by dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen. SOD is responsible for the eradication of 90% of free radicals produced in the body; its activity therefore has a major role in regulating the complications of diabetes.[19] Meanwhile, studies have shown that honey, as an antioxidant compound, protects tissues against oxidative damage. Honey is a naturally occurring antioxidant that has long been of interest to physicians as a cure for many diseases, such that Avicenna, Canon of Medicine, compiled in 1025, refers to honey as a remedy for many ailments and a preserver of health due to its antioxidant and antimicrobial properties.[20] Honey is rich in antioxidants such as flavonoids, ascorbic acid, tocopherols and phenolic compounds, all of which have a synergistic antioxidant role in sweeping and eradicating free radicals.[21] Additionally, previous studies have demonstrated an anti-apoptotic property of honey in certain tissues such as the testis.[22] However, there is no clear information available about the anti-apoptotic property of honey as a major antioxidant in the central nervous system, especially the hippocampus. Jafari-Anarkooli et al., in their study using the TUNEL Assay method, showed the anti-apoptotic role of honey in the hippocampus.[23] However, no comprehensive study has been conducted on the effect of honey on the Bcl-2 family members involved in apoptosis in the central nervous system, including the hippocampus. Thus, the present study was conducted with the aim to investigate the effects of insulin and honey on passive avoidance memory using a shuttle-box apparatus, and also their role in preventing apoptosis in the hippocampal region in rats with type I diabetes through assessment of changes in expression of Bcl-2 family genes.

METHOD AND MATERIALS

The experimental study was conducted on 48 male Wistar rats (8–10weeks old) and weighing approximately 200–250g. Animals were purchased from Razi Institute in Tehran, and were transferred to animal house of Zanjan University of Medical Sciences (ZUMS), where they were kept for a week to adjust with the environment. During the study period, the animals had free access to food and water. The animal house was maintained on proper humidity, temperature ($23^{\circ C} \pm 3$) and an inverted 12 h light-dark cycle and. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals of ZUMS, Iran.

After weighing, blood glucose level of all animals was measured through tail vein using a glucometer device (Accu-Chek, Germany). After 24 hours fasting, diabetes was induced in diabetic groups of rats through intraperitoneal injection (IP) of 60 mg/kg dose of STZ (Sigma, USA).[24] The injection solution was prepared by dissolving STZ in a 10 molar citrate buffer (pH=4.5). The stress effect due to injection was also created in control groups of rats by injection of saline. The STZ injection day was assigned as day zero and three days (72 hours) after STZ injection, blood sugar in diabetic groups was measured and animals with higher than 250 mg/dl blood sugar were considered diabetic[25] and those with blood sugar less than 250 mg/dl were excluded. Then STZ-induced diabetic rats were randomly divided into the following four groups (six each), (1) the diabetic group that received no treatment (D). (2) The diabetic group treated with insulin (D+I); rats belonging to this group received subcutaneous (SC) injections of protamine insulin (NPH) (Exir Borogerd Pharmacy, Iran). The dose administered was between 2 to 3 units per day. (3) The diabetic group treated with natural honey (D+H) (Natural Food Industries of Keshtzar-e Sabz Khansar, Iran); rats in this group received intraperitoneally (IP) daily doses of natural honey (5 mg per kg of body weight) diluted in distilled water.[26, 27] (4) The diabetic group treated simultaneously with insulin and natural honey (D+I+H); the daily doses of insulin and natural honey were the same as the doses mentioned in "2" and "3," respectively. Apart from these four groups, four control group was formed with rats who had not received STZ as follows (each group consisted of 6 rats), (1) the control group that received no treatment (C). (2) The control group treated with insulin (C+I) between 2 to 3 units per day (SC). (3) The control group treated with natural honey (C+H) 5 mg per kg of body weight (IP). (4) The control group treated with insulin and natural honey (C+I+H) the same as the doses

mentioned in "2" and "3," respectively. Group C and D received equal amounts of normal saline. The treatment regimen was continued for 4 weeks and at the end of the treatment period (end of four week counting from day zero), the blood sugar levels and the body weights of rats from each group were recorded.

Four weeks after treatment, passive avoidance learning in studied animals was assessed using shuttle box apparatus, and 24 hour later, their memory was tested. Four criteria were considered in assessment of passive avoidance learning and memory, including; 1- step-through latency in the acquisition trial; 2- number of trials; 3- step-through latency in the retention trial; and 4-time spent in dark compartment (in 10 minutes). After behavioral tests and recording of blood glucose and final weight, rats were anesthetized with chloroform and slaughtered. Their skulls were incised at midline and their brains were removed and placed on frozen cutting board, and meninges membranes were carefully removed. Then, hippocampi were carefully dissected, removed and stored in cryotubes at -70$^{°C}$ for later extraction of RNA and SOD activity measurement.

The semi-quantitative Reverse Transcription-Polymerase Chain Reaction: **RT-PCR**
Using the Tripure Isolation Reagent method, RNA was isolated from hippocampal tissue according to the manufacturer's protocol (Invitrogen). The optical absorbance of extracted RNA was determined using a Nanodrop 2000 (Thermo Scientific). mRNA levels of Bcl-2 and Bax genes were determined using semi-quantitative RT-PCR. PCR was performed using Accupower-RocketScript-RT-PCR premix kit (BIONEER Company). The reaction (final volume of 20 µl) contained 2 µl of mRNA, 10 pmol each of the Bcl-2 or Bax specific primers, and 10 pmol of GAPDH specific primer as an internal control (Table 1). Data relating to expression of Bcl-2 and Bax at the mRNA level were assessed according to the semi-quantitative RT-PCR method. ImageJ was used to assess the density of the PCR bands. Data from Bcl-2 and Bax were normalized with quantitative data from GAPDH.

Behavioral studies
Behavioral data relating to passive avoidance learning and memory of animals were collected by observing the shuttle box device and time criteria were recorded by a chronometer and then analyzed.

SOD activity measurement
To measure SOD activity, a Randox SOD kit in a Prestige 24i system was used according to the manufacturer's instructions. Briefly, xanthine and xanthine oxidase (XOD) are used to produce superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to produce a red color (formazone), which is measured at 505 nm. In the presence of active SOD, the superoxide radicals are converted into hydrogen peroxide and molecular oxygen, preventing color formation, and SOD activity is measured by the decrease in colorimetric signal.

Statistical analysis
Data obtained from behavioral and molecular studies were analyzed in SPSS using ANOVA and Tukey statistical tests at a significance level of $P \leq 0.05$.

RESULTS

Behavioral test results: passive avoidance learning and memory
To determine the presence of sensory and motor disorders in animals, step-through latency in the acquisition trial (STLa) was compared to step through latency in the retention trial (STLr). No significant difference in STLa or STLr was found between the groups. Memory tests conducted 24 hours after training showed no significant difference in the number of trials to acquisition (TA) in an intergroup comparison (non-diabetic groups or diabetic groups receiving treatment). A significant difference was found between the diabetic group with no treatment and the healthy control group ($P<0.05$), with increased acquisition trials between two compartments as a result of the induced diabetes. A significant difference was also observed between the diabetic group receiving no treatment and the diabetic groups receiving honey and insulin separately ($P<0.05$) and in combination ($P<0.01$) (Figure 1).

Memory tests conducted 24 hours after training showed no significant difference between the healthy groups receiving honey and insulin separately and in combination and the healthy control group in STLr, which indicates that receiving insulin and/or honey has no effect on latency in healthy rats. A significant reduction in latency was observed in the diabetic control group compared to the healthy control group ($P<0.05$), which shows that induced diabetes reduces latency in test time. There was a significant reduction in latency in the diabetic control group compared to the diabetic groups receiving honey and insulin alone or in combination ($P<0.01$). However, no significant difference was found between the diabetic groups receiving honey and insulin alone or in combination (Figure 2).

The results showed a significant difference between the groups in terms of total time spent in the dark compartment (TDC) in the shuttle box device ($P<0.05$). An inter-group comparison showed no significant difference between the healthy control group and the healthy groups receiving insulin and honey separately or in combination, but did show a significant difference between the healthy and diabetic control groups ($P<0.01$). A significant difference was also found between the diabetic control and the diabetic groups receiving insulin and honey separately and in combination ($P<0.01$). The difference between diabetic group treated with honey and diabetic control group was also significant ($P<0.05$) No significant difference was observed between the healthy groups receiving insulin

and honey separately or in combination and the diabetic groups receiving the same treatment (Figure 3).

RT-PCR results

According to RT-PCR assessment of hippocampus tissue, the diabetic control group had increased levels of Bax mRNA compared to the healthy control group. However, expression of Bax at the mRNA level decreased in diabetic groups treated with honey and insulin alone and in combination compared to the control diabetic group. No difference was observed in the expression of Bax at the mRNA level between the diabetic and healthy groups receiving honey and insulin separately and in combination (P<0.05) (Figures 4 and 6).

RT-PCR assessment also showed reduced expression of Bcl-2 at the mRNA level in the hippocampus in the diabetic control group compared to the healthy control group. The diabetic control group also had reduced Bcl-2 levels compared to the diabetic groups treated with insulin and honey (alone and in combination). No differences were observed in the expression of Bcl-2 at the mRNA level in the diabetic and healthy groups receiving honey and insulin (separately and in combination) (P<0.05) (Figure 5 and 6).

The results showed a significant increase in the Bax/Bcl-2 ratio in the hippocampus in the diabetic control group compared to the healthy control group (P<0.001). This ratio was significantly reduced in the diabetic groups treated with insulin and honey (separately and in combination) compared to the diabetic control group (P<0.001) (Figure 7).

The results of assessment of SOD activity

A comparison of SOD activity showed a significant difference between groups (P<0.05). An inter-group comparison showed no significant difference between the healthy control group and the healthy groups treated with honey and insulin (separately and in combination). However, comparison of the healthy control and diabetic control groups showed a significant difference (P<0.05). Similarly, there was a significant difference between the diabetic control group and the diabetic groups treated with insulin and honey (separately and in combination) (P<0.05). No significant difference was found between the healthy and diabetic groups receiving treatment (Figure 8).

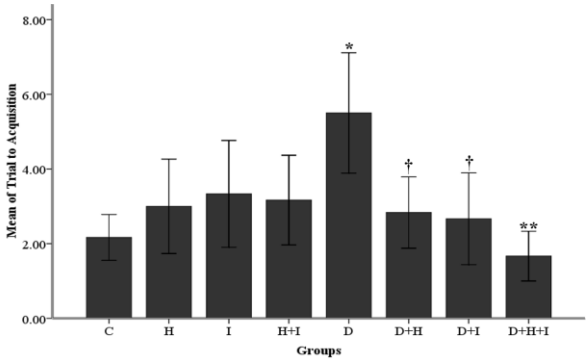

Figure 1: The effects of insulin or honey or both on Trial Acquisition (TA) of animals in passive avoidance learning test in healthy and diabetic groups (P<0.05)*, comparing untreated diabetic control group with healthy control group (P<0.01), comparing untreated diabetic control group with diabetic group treated with insulin and honey groups both together. (P<0.05)†, comparing untreated diabetic control with diabetic groups separately treated with insulin and honey**.

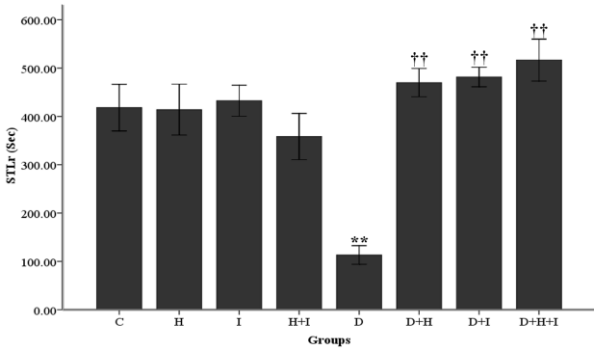

Figure 2: The effects of insulin or honey or both on STLr in passive avoidance learning test in healthy and diabetic groups (P<0.05) **, comparing healthy control group with untreated diabetic control group. (P<0.001)††, comparing untreated diabetic control group with diabetic groups treated with insulin or honey or both together.

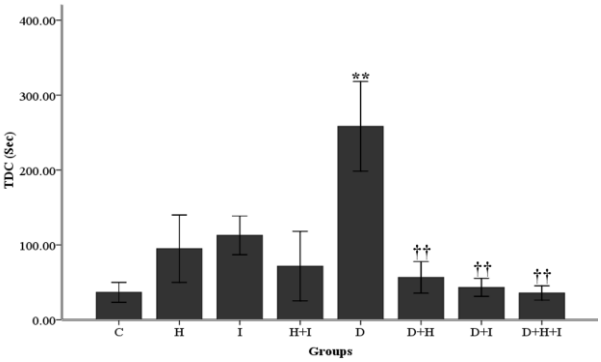

Figure 3: The effects of insulin or honey or both on TDC in passive avoidance learning test in control and diabetic groups. (P<0.05) **, comparing untreated diabetic control group with healthy control group. (P<0.01)††, comparing untreated diabetic control group with diabetic groups treated with insulin, honey, or both together.

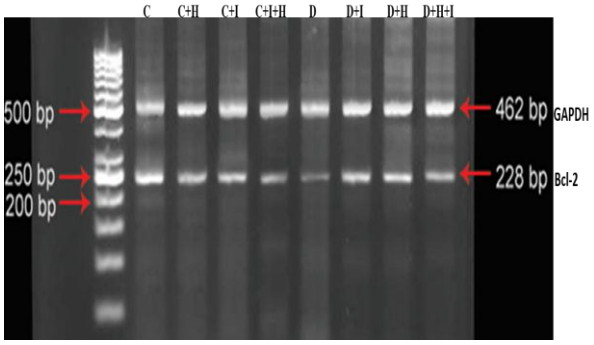

Figure 4: The effects of insulin or honey or both on expression of Bcl-2 gene at mRNA level in hippocampal region of rats. Expression of mRNA in RT-PCR method minus bands. Data normalized with GAPDH gene as internal control.

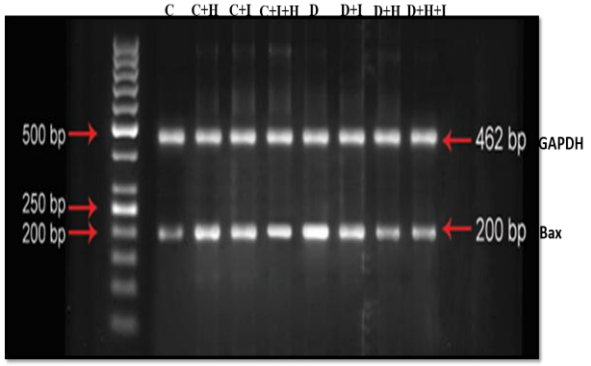

Figure 5: The effects of insulin or honey or both on expression of Bax gene at mRNA level in hippocampal region of rats. Expression of mRNA in RT-PCR method minus bands. Data normalized with GAPDH gene as internal control.

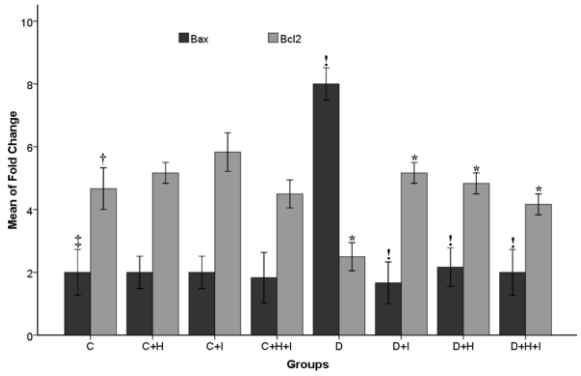

Figure 6: The effects of insulin or honey or both on expression of Bax and Bcl-2 genes at mRNA level in hippocampal region of rats. (P<0.05)* Comparing Healthy control group and diabetic treated with insulin or honey or both with untreated diabetic control group. (P<0.05)[!] Comparing untreated diabetic control group with healthy control and diabetic control treated with insulin, honey or both together. (P<0.05)[‡] comparing Healthy control group with untreated diabetic control group. (P<0.05)[†] Comparing untreated diabetic control group with healthy control.

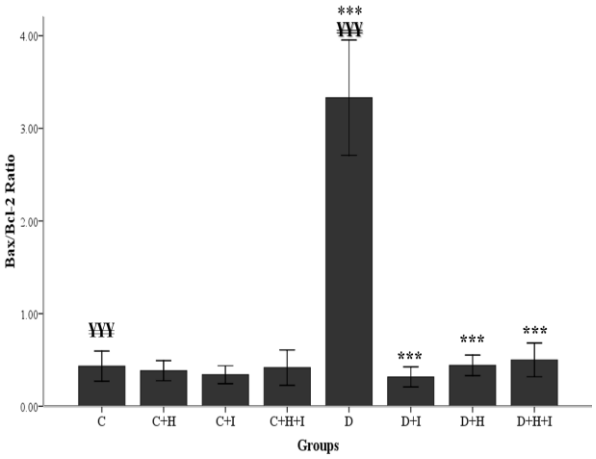

Figure 7: The effects of insulin or honey or both on Bax/Bcl-2 ratio at mRNA level in hippocampus region in rats. (P<0.001)*, Comparing diabetic group treated with insulin, honey or both together with untreated diabetic control group. (P<0.001)[¥¥¥], Comparing untreated diabetic control group and healthy control group.**

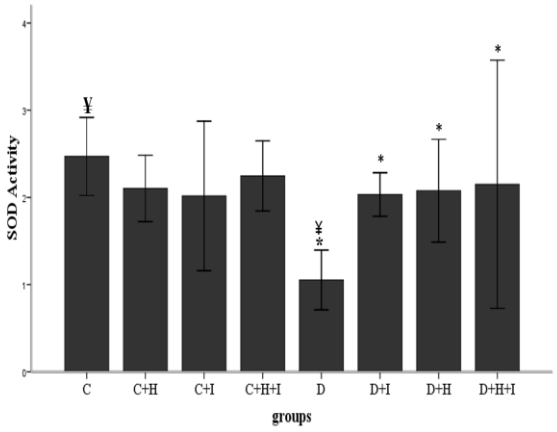

Figure 8: The effects of insulin or honey or both on SOD activity in hippocampus region in rats. (P<0.05)[¥], comparing health control and untreated diabetic control groups. (P<0.05)*, comparing untreated diabetic control group with diabetic group treated with insulin, honey, or both together.

Table 1: Primers used and length of segments resulting from PCR

Genes	Primers	Product size
Bcl-2	F: 5'-CTG GTG GAC AAC ATC GCT CTG-3'	228 bp
	5'-GGT CTG CTG ACC TCA CTT GTG-3' R:	
Bax	5'-TTC ATC CAG GAT CGA GCA GA-3' F:	200 bp
	R: 5'-GCA AAG TAG AAG GCA ACG-3'	
GAPDH	F: 5-'GGC CAA GAT CAT CCA TGA CAA CT-3'	462 bp
	R: 5'-ACC AGG ACA TGA GCT TGA CAA AGT-3'	

DISCUSSION

The present study results showed that STZ-induced diabetes in rats impairs their cognitive and memory functions. In a passive avoidance test, the mean number of trials to and from dark and light compartments in the shuttle box increased in the untreated diabetic group, which suggests learning deficiency in these animals. In a memory test, STLr reduced and TDC increased, which shows memory deficiency in STZ-induced diabetic rats. Learning and memory deficiencies have frequently been reported in both diabetic animals and humans.[2,28,29] In recent years, many human and animal studies have reported association of learning and memory complications due to diabetes with structural and neurochemical impairments in the nervous system.[30-32] The results obtained from this study showed those four weeks after the induction of diabetes, a significant difference was observed in all behavioral criteria for the assessment of learning and memory in a shuttle box device between diabetic animals that were treated with honey and/or insulin and an untreated diabetic group. Research has shown that injection of insulin affects processing and memory by affecting cholinergic nerves, increasing the concentration of serotonin and acetylcholine in pre-synaptic neurons, and reducing the concentration of dopamine. Rasoul et al. studied the effect of insulin on cognitive functions of the brain, and showed that insulin prolongs latency of entering the dark compartment, which indicates improved memory function in rats.[33] Similarly, in relation to the positive effect of honey on memory, a study by Akanmu showed that generally, honey strengthens the central nervous system and improves memory.[34] In agreement with the present study, Chepulis et al. showed that honey likely reduces anxiety and improves spatial memory in middle-aged people.[35]

The present study showed that STZ-induced diabetes caused apoptosis in hippocampal neurons in rats by increasing the expression of the pro-apoptotic Bax gene and reducing the expression of the anti-apoptotic Bcl-2 gene and that use of honey and/or insulin was able to inhibit apoptosis caused by STZ-induced diabetes by reducing the expression of Bax and increasing the expression of Bcl-2. Rizk et al. have shown that the use of insulin before inducing ischemia in the mid-cerebral artery in STZ-induced diabetic rats can dramatically prevent apoptosis in cortex neurons.[36] Guo et al. reported that insulin can prevent apoptosis in ganglia neurons in STZ-induced diabetic rats.[37] The anti-apoptotic effects of insulin have also been reported in non-neural tissues.[38] In a study on Alloxan-induced diabetic rats, Muriach et al. reported that two weeks of insulin administration can maintain the activity of oxidative markers at their normal levels.[39] The results from the present study as well as the studies referenced above emphasize the neuroprotective role of insulin. A study conducted by Srinivasan et al. on posterior spinal root ganglia neurons in STZ-induced diabetic rats showed that a two-week insulin treatment in rats to control blood sugar significantly increased the expression of the anti-apoptotic protein Bcl-2.[40] Insulin may be able to inhibit the release of cytochrome C and other apoptogenic factors by preventing the repression of Bcl-2 caused by STZ-induced type I diabetes. Therefore, the Bcl-2/Bax expression ratio is highly important as a determinant in the inhibition or induction of apoptosis. The present study shows that honey can inhibit apoptosis in a diabetes model through regulating the Bcl-2/Bax ratio. This is in agreement with several previous studies showing that honey inhibits apoptosis in testicular germinal cells.[22] The anti-apoptotic property of honey was confirmed in a study conducted by Jafari-Anarkooli using the TUNEL Assay, H&E staining, and trypan blue vital staining on the hippocampi of STZ-induced diabetic rats.[23] Similarly, this effect has been shown on pyramidal cell proliferation in CA1, CA2 and CA3 areas and the hippocampal dentate gyrus and honey improved long- and short-term memory.[41] Honey is rich in enzymatic and non-enzymatic antioxidants such as catalase, ascorbic acid, flavonoids and alkaloids[42-44], which function by preventing oxidative factors from bonding with saturated fatty acids[43] and preventing the oxidation of proteins.[45] As shown in the present study, activity of the antioxidant enzyme SOD was reduced as a result of STZ-induced diabetes in the hippocampus of rats and treatment with insulin, honey, or both increased its activity. Hence, these other studies confirm the results of this study. As discussed earlier, hyperglycemia and increased oxidative stress is the most important cause of neural complications of diabetes.[46] Previous studies have also shown that type I diabetes increases hippocampal oxidative stress in STZ-induced diabetic rats.[47,48]

Different photochemical compounds have been shown to have scavenging properties, which can activate key antioxidant enzymes in the brain, thus breaking up the vicious cycle of oxidative stress and preventing tissue damage. Several supplementary studies report that neuroprotective compounds like the polyphenols in honey have a role in many important brain activities such

as protection against oxidative stress, reducing neuroinflammation, increasing memory, learning and cognitive function and protection against damage due to neurotoxins.[49,50] For instance, apingenin, a common flavonoid found in honey, protects neurons against oxygen deficiency and blood sugar in damage caused by reperfusion of cultured primary hippocampal neurons and also improves activity of Na/K-ATPase in addition to scavenging free radicals.[51] In agreement with the present study, Rezai et al. have proposed that honey may have neural protective and disease recovery properties in different types of neurodegenerative disorders. In addition, honey stimulates adult neurogenesis and thus affects learning and memory.[52] In a study by Azman et al., gavage of 0.2 g/kg body weight of honey in animals as a supplementary food reduced the noise-induced stress that causes pseudo-depressive behaviors and reduced cognitive function.[53]

It has also been shown that other phenolic compounds found in honey such as chlorogenic acid have a dose-dependent protective effect against apoptosis in PC12 cells. The protective activity of this compound is said to be mediated via reduced ROS production and reduced apoptosis through activation of caspase 3.[54] The neuro-protective property of this compound was studied by Kwon et al. in scopolamine-induced memory and learning disorders in several behavioral tests such as the Y-maze, passive avoidance and the Morris water maze and it was found that this compound improved memory function in all behavioral tests. It was therefore concluded that this compound performs its antiamnesic activity through inhibition of acetylcholinesterase and malondialdehyde in the hippocampus and frontal lobe. It has also been shown that the compounds in honey are able to improve learning and memory impairment in addition to inhibiting cholinesterase, increasing SOD activity, and reducing the concentrations of glutamic acid and malondialdehyde in the hippocampus in rats. These results show that the antioxidant activity of honey may be attributed to improving the brain's cholinergic system and inhibiting neural damage by stimulating release of amino acids.[55,56]

This study found that following increased blood sugar due to STZ-induced diabetes, neural damage occurs with increased apoptosis in the hippocampus in rats, which is accompanied by increased expression of pro-apoptotic Bax, reduced expression of anti-apoptotic Bcl-2 and reduced activity of the antioxidant enzyme SOD. This also led to impaired learning and memory, including passive avoidance memory, and treatment with insulin, honey, or both inhibited the progress of apoptosis in the hippocampus of rats by reducing the expression of Bax and increasing the expression of Bcl-2, leading to increased antioxidant activity of SOD, thus reducing learning and memory impairments. Further *in vitro* and clinical trials are needed to better demonstrate these effects.

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