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BCL2 Expression in the Brains of Diabetic Rats Treated with Ginger

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Abstract

Objective: Diabetes is one of the most common high-risk diseases which causes many side effects in most body systems such as the nervous system. The medicinal plants, has been suggested to reduce the side effects of this diseases. In this experimental study, we examined the effect of ginger on BCL2 gene expression in the hippocampus of diabetic rats.

Materials and Methods: In this experimental study, we used 60 Wistar rats (200gr). We separated the rats into one healthy group and four groups of diabetics which after seven days of diabetes induction via Streptozotocin (60 mg/kg), received different mixture of insulin and ginger for 6 weeks. 24 hours after the last injection, brains of rats were removed and the hippocampus region was studied histologically and the BCL2 gene expression examined by Real-time PCR.

Results: According to the results, the blood glucose level in the ginger treated group (183.17) decreased significantly compared to the diabetic control group (232.5) ($P< 0.01$). In the expression of BCL2 gene, there was not any significant difference among the groups. In the histological examination of the hippocampus CA1 region, the ginger-insulin treated group had less pyknotic neurons than the diabetic control group.

Conclusion: According to results, the ginger, in addition to glycaemia reduction and despite any significant change in BCL2 gene expression, can cause the safe neurons with the bright nucleus and clear nucleolus remain in the treated group, which can indicate that maybe ginger could inhibit the creation of apoptosis.

Keywords: Apoptosis, Glycaemia, Ginger, Hippocampus

QR Code:

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Introduction

Hyperglycemia or diabetes is a common disorder in body metabolism which is followed by disorders of fats, proteins, and carbohydrates metabolism. The free radicals which are generated through the hyperglycemia and oxidative stress, are the important factors of diabetes pathogenesis (1). Nowadays it is known that diabetic people, in addition to a lot of problems related to their blood vessels, have some problems with their nervous tissues in different parts of central and peripheral nervous systems. One example of these issues is memory loss brought on by disorders resulting from damaged hippocampal neurons (2).

This disease can change the number and function of the neurons via apoptosis and causes central nervous system disorder. Hypothalamus, cerebral cortex malfunctions, cerebrovascular problems, stroke (brain attack), and also the hippocampus are among these disorders (3,4).

Nowadays in order to cure diabetes and reduce its side effects, besides chemical medicine, use of medicinal plants and non-medical factors such as antioxidants are applied for treatment and to reduce side effects in different parts of the body like hippocampus. These factors may cause an increased differentiation of neural precursor cells to adult stem cells and improvement of learning, memory, and cognitive functions (5,6). At the present age, most countries pay an especial attention to medicinal plants, because they have the least side effects due to their wide therapeutic application (7). Ginger (Zingiber officinale) is one of the plants which is used for diabetes treatment around the world. Its extract contains gingerol and shogaols antioxidants which remove superoxide and radicals of hydroxyl (8). It also has antitumor (9) and anti-inflammatory effects (10). The studies also have shown that ginger can cure inflammatory brain diseases (11) and can reduce the oxidative enzymes in some regions of the brain (12).

Apoptosis or programmed cell death is a regulated process associated with a gene which multiple gene groups such as BCL2 are involved in that process (13). In many diseases, such as diabetes, the increase of apoptosis occurs in different body tissues (14,15), and some of the negative effects of diabetes are related to the increase of apoptosis. Considering the subjects mentioned in this study, the effects of ginger on the apoptosis process of the cells in the hippocampus region of diabetic male rats using Streptozotocin were evaluated through examining the rate of BCL2 gene expression. Eventually, the amount of glycemia and histological specifications were evaluated, using crystal violet dying in the CA1 region of the hippocampus.

Materials and methods

In this experimental study which is conducted on 60 wistar rats, with a weight of 200 (+/-50) gr, they were divided into 5 groups (a randomized and blinded method was used for animal allocation in each group). All animals maintained in12:12-hour light/dark cycle under controlled temperature (21°C-23°C) (16). Seven days after inducing diabetes in all groups except the control group (second group), and assuring of that, their injections were begun; they received injections as follow: the first group or diabetic control with normal saline (include diabetic rats that injected only with normal saline), the third group with ginger, the fourth group with insulin plus ginger, and fifth group with insulin (17).

The first and third groups received 200 mg/kg ginger extract of their body weight intraperitoneal injections every other day for six weeks. The fourth and fifth groups received 4-6 units/kg of insulin subcutaneous injections daily for six weeks (18,19). The rats in the second group (healthy/control group) did not receive anything.

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In order to induce diabetes in these animals, they received an intra-peritoneal injection of 60 mg/kg Streptozotocin (STZ) of their bodies. Three days after STZ injection, diabetes induction was confirmed through examining the signs and symptoms, such as polyphagia, polydipsia, frequent urination, and also blood glucose measurement in these animals. In addition, we considered those rats which their blood glucose level was above 220mg/dl as diabetic rats (17). The rhizome of ginger was obtained from the Isfahan plant herbarium and then the alcoholic extract was provided.

24 hours after the last injection, all rats were anesthetized using ether and their brains were removed immediately. The samples of their left hemispheres were removed by homogenizers in the sterilized and homogenized condition and mRNA was extracted to produce cDNA (20). The right hemispheres were taken out from fixative after 24 hours and different stages of dehydration, clarification, infiltration with paraffin were done. After preparing paraffin-embedded block, some 5 microns cuts were provided and were examined histologically, using crystal violet dying.

Statistical analysis

The results are presented as mean ± SD, and the differences between groups assessed using One Way ANOVA followed by Post-Hoc Tukey test was used to analyze the data obtained from histological parameters and also mRNA expression of BCL2. The normality of the frequency distribution of the data was assessed using the Kolmogorov-Simonov test. The distribution of the quantitative variable was considered normal. Levene's test was also used to check the assumption of homogeneity of variance. All analyses were performed using SPSS version 22. $P< 0.05$ were considered statistically significant.

Ethical considerations

All animal procedures were conducted in accordance with the animal care and use protocol approved by the Ethics Committee of the Rafsanjan University of Medical Sciences (IR.KMU.REC.1393.384).

Results

BCL2 Gene expression based on real - time PCR

The sequence of primers for Real-time PCR is shown in Table 1. In order to examine the BCL2 gene expression rate, the results of real-time PCR are shown in Figure 1. As it can be seen in this figure, the BCL2 gene expression did not show significant changes between different groups, but the obtained values were somehow to confirm our theories. In ginger-insulin group and also in diabetic group treated with insulin, for example, the gene expression is at its highest level. The group treated with ginger shows the lowest gene expression. The gene expression level in the normal control group is close to the level of the diabetic control group (Figure1).

Comparing the groups using One-way ANOVA followed by Tukey's analysis, it was determined that there was a significant difference between the diabetic control group and the ginger-insulin and insulin groups (P= 0.0001), and a similar significant difference was found between the normal control group and the ginger-insulin and insulin groups (Table 2).

Histologic evaluation of CA1 region

In a histologic examination of CA1 region in the hippocampus using crystal violet dying, the neurons can be seen in large number with the pyknotic nucleus, surrounded with a white halo in the form of empty space in a diabetic group without treatment.

Table1. The sequence of primers for Real-time PCR

Gene	Forward primer	Reverse primer
Bcl2	ATCGCTCTGTGGATGACTGAGTAC	AGAGACAGCCAGGAGAAATCAAAC
β-actin	GATCAGCAAGCAGGAGTATG	GTGTAACGCAACTAAGTCATAG

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Such neurons also were observed in other diabetic groups in few numbers, but most of the neurons have normal vesicle nucleus (Figure 2).

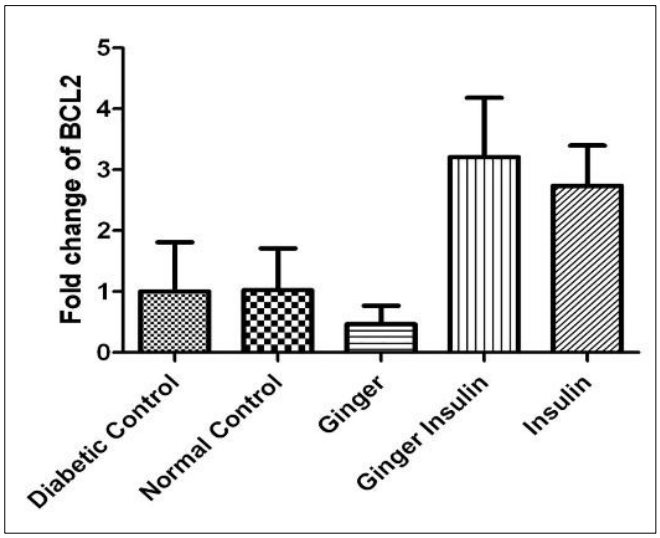

Figure 1. Comparison of BCL2 gene expression after 6 weeks receiving an injection of Ginger or saline in different groups, Note that there can't be seen any significant difference between groups in relation to the expression of this gene.

Table 2. Mean and standard deviation of different groups

Groups	Mean ±SD	*P*-value
Diabetic control	0.97 (±0.89)	
Normal control	1.01 (±0.84)	
Ginger	0.37 (±0.24)	0.0001
Ginger insulin	3.08 (±0.84)	
Insulin	2.73 (±0.53)	

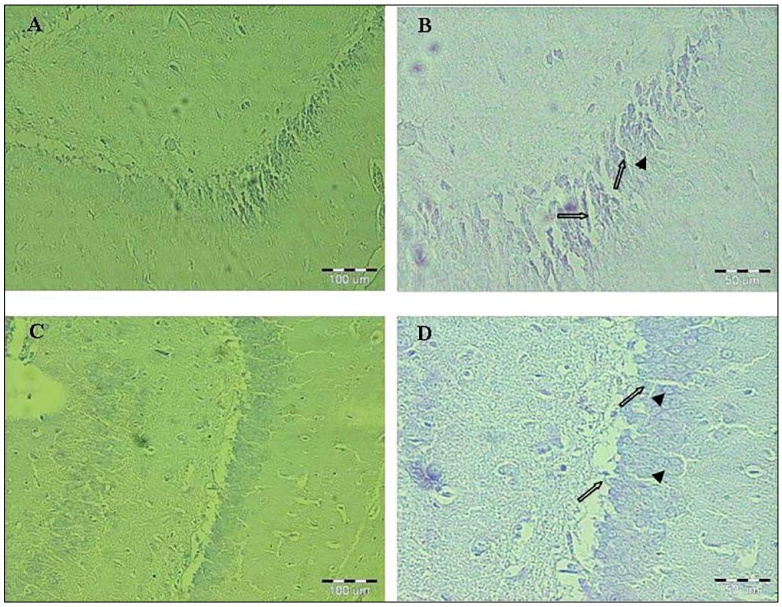

Figure 2. Histologic cuts of hippocampus CA1 region after six weeks of receiving ginger extract plus insulin injection, using crystal violet dying. A) Magnification (20x) of the hippocampus to show the general appearance of the region. B) Hippocampus of diabetic rats without treatment in which the neurons with a pyknotic nucleus and empty space around the nucleus can be seen (40x). C) Hippocampus of treated rats with ginger-insulin (20x). D) In some parts of the hippocampus, neurons can be seen with a normal vesicle appearance (tip of fleshes), magnification 40x.

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Normal cells count

Also, the necrotic and normal cells in two groups counted, and a significant difference was observed between the groups (Figure 3).

Discussion

The reduction in blood glucose, which is the most important effect of ginger in treating diabetes, seems to be related to its protective effect on pancreatic beta cell. This protective effect can modulate and increase insulin secretion. Scientists also found antioxidant compounds to reduce her blood sugar levels and reduce their negative effects on tissues, including apoptosis (19).

Oxidative stress is the main cause of the effects of diabetes neuropathy. The oxidative stress caused by the increase in the amount of intracellular glucose causes excessive production of nitric oxide and active oxygen species, which in turn leads to oxidative damage to proteins, lipids and intracellular DNA. These injuries inhibit normal cellular activity and disrupt homeostasis and increase the activity of active proteins in cell apoptosis, including bcl2. In this study, the relative expression of BCL2 gene as a gene involved in cell apoptosis (Figure 1) as well as the status and level of necrotic cells (Figure 2,3) was investigated in diabetic specimens**.** However, no significant difference was observed between the groups. Perhaps, the effect of diabetes on the development of apoptosis is due to the increase of other proteins involved in this process in this study. Because oxidative stress is the result of an imbalance between the production of free radicals and reactive oxygen species, there must be an antioxidant defense mechanism to reduce the harmful effects of these invasive agents (21). Therefore, an appropriate and ideal drug for the treatment of diabetes should also have antioxidant properties, in addition to its reduced blood glucose profile. For this purpose, it must be able to withstand free radicals and active oxygen species. Medicinal plants are a good source of natural antioxidants. Meanwhile, herbal drugs impose a lower financial burden on the economy of patients and their families (22). In ancient China and India, ginger has been used to treat various diseases. Ginger also can block serotonin receptors and reduce blood sugar. Ginger contains a lot of antioxidants, including Jinjarrol, Shugual and it removes radicals (18). The positive effect of ginger on reducing apoptosis in other neural tissues, including the cerebellum of diabetic rats, is similar to our study (23).

Ginger, with these antioxidants, can be effective in treating other diseases that are particularly affected by the nerve tissue (such as multiple sclerosis). In similar studies, the expression of proteins such as inducible Nitric Oxid (iNOS), Caspase-3 (Glial Fibrillary Acidic Protein) GFAP, and acetyl cholinesterase has been investigated in diabetic rats treated with ginger in brain tissue.

The results of these studies showed decreased expression of proteins such as iNOS

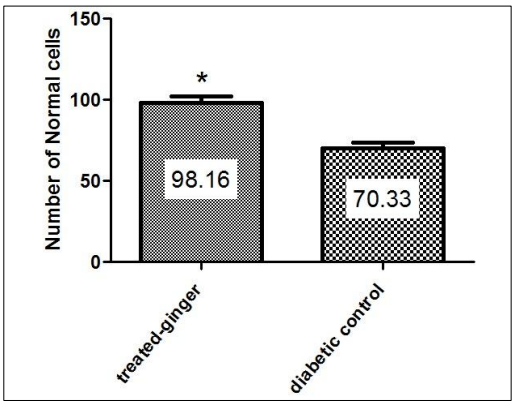

Figure 3. Normal cells count in treated-ginger group and diabetic control group

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and Caspase-3, which confirms the antioxidant capacity of ginger. Instead, increasing the rate of necrotizing factor tumor after treatment with ginger shows the anti-inflammatory effect of this herbal medicine (18).

In the histopathological changes of neurons in the CA1 region of the hippocampus using Crisel Villeh staining, the necrotic sites in the hippocampus of diabetic rats treated with ginger were lower than the control group without treatment and necrotic state in the nucleus of CA1 hippocampus neurons decreased in ginger-insulin group compared with non-treated diabetic group (Figure 2,3) (17). Such histological changes have been reported in similar studies that evaluated other parts of the brain. For example, Shanmugam's study reported similar changes in the cortex, cerebellum, hypothalamus and El-Akabawy in the forearm cortex. One of the limitations of our study is the investigation of only one gene involved in this pathway, which suggests that other genes and cellular pathways should be evaluated in more comprehensive studies.

Conclusions

This study showed that ginger, in addition to lowering blood glucose levels, may be effective in reducing the expression of BCL2 gene and can be used as an herbal remedy with less side effects in the treatment of diabetes problems.

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Conflict of Interest

The authors declare no conflict of interest.

Author contributions

Conceptualization and Supervision: MM.T, SH.EV and A.M.

Investigation and writing original draft: Z.T, A.Sh and A.B.

Data collection: I.N and A.M.

All the authors critically revised the manuscript, agree to be fully accountable for the integrity and accuracy of the study, and read and approved the final manuscript.

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