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Bioflavonoids Effects of Ginger on Glomerular Podocyte Apoptosis in Streptozotocin-Induced Diabetic Rat

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Abstract

Objective: Ginger is a strong antioxidant and long-term treatment of streptozotocin (STZ)-diabetic animals, and it has been shown to reduce oxidative stress. Prevalence oxidative stress among urban life and changes in antioxidant capacity are considered asplay an important role in the pathogenesis of chronic diabetes mellitus.

Materials and Methods: Wistar male rat (n = 40) were divided into three groups, control group (n = 10) and Ginger Quercetin group that received 100 mg/kg (gavage), (n = 10), and diabetic group, which received 55 mg/kg intra peritoneal (IP) STZ (n = 20), which was subdivided to two groups of 10; STZ group and treatment group. Treatment group received 55 mg/kg (IP) STZ plus100 mg/kg ginger, daily for, 8 weeks, respectively; however, the control group just received an equal volume of distilled water daily (IP). Diabetes was induced by a single (IP) injection of STZ (55 mg/kg). Animals were kept in standard condition. In 28 day after inducing diabetic 5 cc blood were collected for total antioxidant capacity, malondialdehyde and oxidized low density lipoprotein levels and kidney tissues of rat in whole groups were removed then prepared for apoptosis analysis by terminal deoxynucleotidyl transferase mediated dUTP nick end labeling assay (TUNEL) method.

Results: Apoptotic cells significantly decreased in group that has received 100 mg/kg ginger ($P < 0.05$) in comparison to experimental groups ($P < 0.05$).

Conclusion: Since in our study 100 mg/kg ginger have significantly preventive effect on kidney cells damages by reducing number of apoptotic cells in kidney and hence it seems that using it can be effective for treatment in diabetic rat.

Keywords: Apoptosis, Diabetic, Ginger, Kidney, Rat, Streptozotocin

Introduction

Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular diseases (1-3). Enhanced oxidative stress and changes in antioxidant capacity are considered as play an important role in the pathogenesis of chronic diabetes mellitus (4,5). Although the mechanisms underlying the alterations associated with diabetes mellitus are presently not well-understood, hyperglycemia lead patients to increased oxidative stress because the production of several reducing sugars (through glycolysis and the polyol pathway) is enhanced (5). These reducing sugars can easily react with lipids and proteins (nonenzymatic glycation reaction), increasing the production of reactive oxygen species (ROS). Diabetes is the most common endocrine disease that leads to metabolic abnormalities involving regulation of carbohydrate metabolism. In addition to imbalanced carbohydrate metabolism, yet another major concern in diabetes is increased oxidative stress increaesd production of free radicals or ROS formation may induce oxidized low density lipoprotein, which is key step in the sequence of events leading to atherosclerosis sustained hyperglycemia and increased oxidative stress, are the major players in the development of

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secondary complications in diabetes. These abnormalities produce pathologies including vasculopathies, neuropathies, ophthalmopathies and nephropathies, among many other medical derangements (6). The balance of ROS and antioxidant is a major mechanism in preventing damage by oxidative stress. Therefore, the dietary supplement of antioxidants such as vitamins, flavonoids has been used to prevent the occurrence of many chronic diseases (7). Many herbal such as: Barberry, Estragon ginger, Allum cepa, Rhus coriaria, Cinnamomum zelanicum, Hypericum perforatum and onion known anti diabetic effects and use to patient treatment. Ginger is a well-known flavonoid and first flavonoid identified in ginger is called gingerol, but the root also contains other phenolic compounds such as flavones and flavonols. It is strong antioxidant and long-term treatment of streptozotocin (STZ)-diabetic animals and it has been shown to reduce oxidative stress (8). The aim of this study was seen the effect of ginger as a protective on kidney cells apoptosis ROS.

Materials and Methods

Forty adult Wistar albino male rats were 8 weeks old and weighed 250 ± 10 g, they were obtained from the animal facility of pasture institute of Iran. Male rats were housed in temperature controlled rooms (25 °C) with constant humidity (40-70%) and 12 h/12 h light/dark cycle prior to use in experimental protocols. All animals were treated in accordance to the Principles of Laboratory Animal Care. The experimental protocol was approved by the Animal Ethical Committee in accordance with the guide for the care and use of laboratory animals prepared by NIH. All rats were fed a standard diet and water. The daily intake of animal water was monitored at least 1 week prior to start of treatments in order to determine the amount of water needed per experimental animal. Thereafter, the rats were randomly selected and divided into control (n = 10) and ginger group that received 100 mg/kg (gavage), (n = 10), and diabetic group that received 55 mg/kg intra peritoneal (IP) STZ (n = 20), which was subdivided to two groups of 10; STZ group and treatment group. Treatment group received 55 mg/kg (IP) STZ plus100 mg/kg (gavage). The control group just received an equal volume of 1cc distilled water daily (IP). Diabetes was induced by a single IP injection of (STZ, Sigma, USA.) in 0.1 M citrate buffer (pH 4.0) at a dose of 55 mg/kg body weight. Quercetin (QR) injections were continued to the end of the study (for 8 weeks), (9).

Experimental type 1 diabetes was induced in rats by IP injection of 55 mg/kg STZ in distilled water. Control rats were received distilled water, only.

Blood samples were collected from the tail vein. Basal glucose levels were determined prior to STZ injection, using an automated blood glucose analyzer (glucometer elite XL). Sample collections were then made 48 h after STZ injection and blood glucose concentrations were determined and compared between groups. Rats with blood glucose concentrations above 300 mg/dl were declared diabetic and were used in the experimental group. 1 week after the induction of experimental diabetes, protocol was started.

Ginger root was changed to powder by mixer and 100 mg/kg of this powder was solved in 3 cc distilled water and used daily.

In the 56th day, (at the end of the treatment period), the rats were killed with diethyl ether, and kidney tissues in control and experimental groups were immediately removed.

The in-situ DNA fragmentation was visualized by terminal deoxynucleotidyl transferase mediated dUTP nick end labeling assay (TUNEL) method (10). Briefly, dewaxed tissue sections were predigested with 20 mg/ml proteinase K for 20 min and incubated in phosphate buffered saline solution (PBS) containing 3% H_2O_2 for 10 min to block the endogenous peroxidase activity. The sections were incubated with the TUNEL reaction mixture, fluorescein-dUTP (in situ cell death detection, POD kit, Roche, Germany), for 60 min at 37°C. The slides were then rinsed three times with PBS and incubated with secondary anti fluorescein-POD-conjugate for 30 min. After washing three times in PBS, diaminobenzidine-H_2O_2 (DAB, Roche, Germany) chromogenic reaction was added on sections and counterstained with hematoxylin. As a control for method specificity, the step using the TUNEL reaction mixture was omitted in negative control serial sections, and nucleotide mixture in reaction buffer was used instead. Apoptotic cells were quantified by counting the number of TUNEL stained nuclei per liver tissues cross sections. Cross sections of 100 kidney tissues per specimen were assessed and the mean number of TUNEL positive apoptotic cells per cross-section was calculated (PBS) TUNEL-transferase-mediated fluorescein-dUTP.

Total antioxidant capacity (TAC) was measured in serum by means of a commercial kit (Randox Co-England). The assay is based on the incubation of 2,2'-azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS) with a peroxidase (methmyoglobin) and hydrogen peroxide to produce the radical cation ABTS+, which has a relatively stable blue-green color, measured at 600 nm. The suppression of the color is compared with that of the trolox, which is widely. Used as a traditional standard for total antioxidant status measurement assays, and the assay results are expressed as trolox equivalent (mmol/l), (10).

Tissue MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as nmol MDA formed/ml. Plasma MDA concentrations were determined with spectrophotometer. A calibration curve was prepared by using 1,1',3,3'-tetramethoxypropane as the standard (11).

Statistical analysis was performed using the analysis of variance and test for comparison of data in the control group with the experimental groups. The results were expressed as mean ± standard error of mean ($P < 0.05$) were considered as significant and are written in the parentheses.

Results

Amount of apoptotic cells among kidney cells

Number of apoptotic cells colored brown, in the diabetic group, was (12.25 ± 1.14) and in QR received diabetic group and control group was (6.15 ± 8.17) and (1.05 ± 0.41) respectively. These changes were significant as ($P < 0.05$).

Results of total blood anti-oxidant capacity

Amount of total blood anti-oxidant capacity in control group was (0.70 ± 0.03 mmol/ml) and in experimental groups was 0.75 ± 0.03, 0.32 ± 0.04 and 0.61 ± 0.05 mmol/ml respectively. Statistical analysis dunnett (one side) shows significant differences between experimental groups in comparison to control group ($P < 0.05$) (Table 1).

TAC

1.ABTS

2. TBA

Results of MDA (malondi aldehyde) level in blood

MDA level in control group was 0.25 ± 0.04 mmol/l and in experimental groups was 0.30 ± 0.212, 4.1 ± 0.06, 1.1 ± 0.08 mmol/l respectively.Statistical analysis dunnett (one side) shows significant differences between experimental groups in comparison to control group ($P < 0.05$) (Table 1).

Discussion

Ginger root is the rhizome of the plant Zingiber officinale, consumed as a delicacy, medicine, or spice. It lends its name to its genus and family (Zingiberaceae). According to the American Cancer Society, ginger has been promoted as a cancer treatment "to keep tumors from developing," but "available scientific evidence does not support this." They add: "recent preliminary results in animals show some effect in slowing or preventing tumor growth." Although these results are not well-understood, they deserve further study. Still, it is too early in the research process to say whether ginger will have the same effect in humans (12,13). In the presence of impaired glucose metabolism and occurrence of hyperglycemia, genes involved in fatty acid storage were activated (14). On the other hand, liver diseases can induce diabetes mellitus. This type of diabetes mellitus is clinically different from that of Type II diabetes mellitus since it is less frequently associated with microangiopathy. Insulin resistance occurs in muscular and adipose tissues combined with hyper insulinemia are pathophysiological bases of diabetes in liver disease. The etiology of liver disease is important in the incidence of diabetes mellitus since non-alcoholic fatty liver disease, alcohol, hepatitis C virus and hemochromatosis are frequently associated with diabetes mellitus. Investigations show liver tissue damage and apoptosis induced by diabetes mellitus increase active O2 specious. Antioxidants have also been shown to be effective in the protection of Kidneys (15). Kidneys are susceptible to various kinds of injuries such as ischemia-reperfusion (16). Mitochondrial damage to kidneys has been approved as the main destructive effect in kidney injuries (17). MitoQ is an antioxidant developed to target mitochondrial protection against damage caused by free radicals (18). MitoQ also decreases kidney ischemic injury (19). Mitochondria-targeted therapy also prevents diabetic nephropathy (20). Mitochondria transfers apoptosis signaling in ishchemic hearts (21). MithoQ has been shown to be effective in decreasing ischemic, oxidative, and other damages to kidneys (22). Administration of natural antioxidants has also been shown to be effective against nephrolithiasis (23). Flavonoids as an antioxidant factor found in nutrient such as fruit, vegetables, tea, ginger and black burgundy grape (24). Flavonoids value in daily Mail varies from 16 mg to 1000 mg. Ginger as an important and main flavonoids found in human Mail (25). Investigations show ginger absorbs in digestive. Useful effect of ginger in human health involves prevention of diabetes induced cataract, reduced blood vessels fragility, anti-microbial, anti-viral, ant-allergy, and anti-inflammatory effects and prevention of platelet aggregation (26,27). One of the ginger ant-oxidant mechanism is removal of free radical such as xanthine superoxide and xanthine oxidase (28). The results of this study showed that ginger in spermatogonial cell not only does not have harmful effects, but also increases the amount of spermatogonial cells and decreases oxidative effects.

Conclusion

In this study like pervious investigations ginger decreases malondy aldeid and increases serum anti-oxidant capacity (28-30). According to our results, it seems this as an anti-oxidant can protection kidney cells from cell injury by modulating TAC and MDA in diabetic conditions, and it advise to using it in diabetes diseases.

Ethical issues

Since this study involved animal and not humans as subject of study, ethical issue is not necessary.

Conflict of interests

We declare that we have no conflict of interests.

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Table 1. Compare of malondialdehyde (MDA) level between control group and experimental group

Groups	Control (n = 10)	QR [15 mg/kg (IP)] (n = 10)	STZ [55 mg/kg (IP)] (n = 10)	QR + STZ 55 mg/kg (IP) STZ plus15 mg/kg QR (n = 10)
Apoptotic cells	1.05 ± 0.41	1.01 ± 8.17	12.25 ± 1.14* (0.006)	6.15 ± 8.17* (0.004)
TAC (nmol/ml)	0.70 ± 0.03	0.75 ± 0.03* (0.006)	0.32 ± 0.04* (0.003)	0.61 ± 0.05* (0.004)
MDA (nmol/ml)	0.25 ± 0.04	0.30 ± 0.212* (0.004)	4.1 ± 0.06* (0.008)	1.1 ± 0.08* (0.005)

Data are presented as mean ± SE; * $P < 0.05$ were considered significant and are writing in the parentheses, (compared with the control group)
SE: Standard error; QR: Quercetin; STZ: Streptozotocin; IP: Intraperitoneal; TAC: Total antioxidant capacity; MDA: malondialdehyde

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