Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(12)60090-2

Anti-oxidative role of quercetin derived from *Allium cepa* on aldehyde oxidase (OX-LDL) and hepatocytes apoptosis in streptozotocin-induced diabetic rat

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ARTICLE INFO

Article history:
Received 5 January 2012
Received in revised form 11 January 2012
Accepted 2 March 2012
Available online 28 July 2012

Keywords:
Apoptosis
Diabetic
Quercetin
Streptozotocin
Liver
Rat

ABSTRACT

Objective: To study the role of Quercetin in streptozotocin-induced diabetes in rats. **Methods:** Wistar male rat (n=40) were allocated into three groups, control group (n=10) and Quercetin (QR) group received 15 mg/kg (IP) QR, (n=10), and diabetic group that received 55 mg/kg (IP) streptozotocin (STZ) (n=20) which was subdivided to two groups of 10; STZ group and treatment group. Treatment group received 55 mg/kg (IP) STZ plus 15 mg/kg QR, daily for 4 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 streptozotocin (55 mg/kg). Animals were kept in standard condition. Twenty-eight days after inducing diabetic, 5 mL blood were collected for TAC, MDA and Ox-LDL levels and liver tissues of rat in whole groups were removed then prepared for apoptosis analysis by Tunel method. **Results:** Apoptotic cells significantly decreased in group that has received 15 mg/kg (IP) Quercetin (P<0.05) in comparison to experimental groups (P<0.05). **Conclusions:** Since in our study 15 mg/kg (IP) Quercetin have significantly Preventive effect on liver cells damages by reducing number of apoptotic cells in Liver, so it seems that using it can be effective for treatment in diabetic rat.

1. Introduction

Diabetes is a chronic disease mainly characterized as hyperglycemic. Hyperglycemic in long time have side effect in other tissues especially in liver. Liver dysfunction has seen in diabetic patients especially in patients with uncontrolled blood sugar level. Due attention has been paid to the search of effective drugs in the field of traditional Chinese medicine (TCM). 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]. Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus[2,3]. 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[3,4]. These reducing sugars can easily react with lipids and proteins (nonenzymatic glycation reaction), increasing the production of reactive oxygen species (ROS) [3-5]. 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 LDL (Ox-LDL), which is key step in the sequence of events leading to atherosclosis Sustained hyperglycemia and increased oxidative stress, are the major players in the development of secondary complications in diabetes. These abnormalities produce pathologies including vasculopathies, neuropathies, ophthalmopathies and nephropathies, among many other medical derangements[4-6]. 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

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Foundation Project: Supported by scientific society grant from the Research Vice Chancellor of Islamic Azad University, Tabriz Branch.

has been used to prevent the occurrence of many chronic diseases[7,8]. Many herbal such as: Barberry, Estragon, Rhus coriaria, Cinnamomum zelanicum, Hypericum perforatum and onion known anti diabetic effects and use to patient treatment. Quercetin is a well-known flavonoid and a strong antioxidant and long-term treatment of STZ-diabetic animals and it has been shown to reduce oxidative stress[8,9]. We plant to study the role of Quercetin in streptozotocin-induced diabetes in rats.

2. Material and Methods

2.1. Animals

Forty adult Wistar albino male rats were 8 weeks old and weighing (250±10) g, they were obtained from animal facility of pasture institute of Iran. Male rats were housed in temperature controlled rooms (25 ℃) with constant humidity (40%-70%) and 12h/12h 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 guidance for the care and use of laboratory animals prepared by Tabriz medical University. All rats were fed with a standard diet and water. The daily intake of animal water was monitored at least one 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 Quercetin (QR) group received 15 mg/kg QR (IP), (n=10), and diabetic group that received 55 mg/kg (IP) streptozotocin (STZ) (n=20) was subdivided into two groups of 10; STZ group and treatment group. Treatment group received 55 mg/kg (IP) STZ plus 15 mg/kg QR (IP). the control group just received an equal volume of 1 mL distilled water daily (IP). Diabetes was induced by a single intra peritoneal (I.P) injection of streptozotocin (STZ, Sigma- U.S.A.) 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 4 weeks) [9].

2.2. Induction of experimental type I diabetes

Experimental type I diabetes was induced in rats by intra peritoneal (I.P) injection of 55 mg/kg streptozotocin (STZ) in distilled water.

2.3. Blood glucose determination

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. One week after the induction of experimental diabetes, protocol was started.

2.4. Quercetin preparation

Quercetin powder was obtained from Sigma Chemical Company (St. Louis, MO, USA). It was dissolved and diluted with 20% glycerol in 0.9% normal saline, mixed vigorously and stored in a dark bottle at 4 ℃. The quercetin solution was freshly prepared each week.

2.5. Surgical procedure

On the 28th day, (at the end of the treatment period), the rats were killed with diethyl ether, and kidney tissues in control & experimental groups were immediately removed.

2.6. TUNEL analysis of apoptosis

The in-situ DNA fragmentation was visualized by TUNEL method. 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 ℃. The slides were then rinsed three times with PBS and incubated with secondary antifluorescein-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 liver tissues per specimen were assessed and the mean number of TUNEL positive apoptotic cells per cross- section was calculated[10].

2.7. Measurement of serum total antioxidant capacity (TAC)

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 $ABTS^{\cdot}$, 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 TAC measurement assays, and the assay results are expressed as trolox equivalent (mmol/L)[9].

2.8. Measurement of serum MDA

Tissue MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as mmol 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[9].

2.9. Measurement of Ox-LDL

Oxidized LDL level was measured by using a Mercodia Oxidized LDL ELISA kit (Lot No. 15904; Mercodia, Uppsala, Sweden). Mercodia Oxidized LDL Competitive ELISA is based on the monoclonal antibody 4E6.

2.10. Statistical analysis

Statistical analysis was done using the ANOVA and test for comparison of data in the control group with the experimental groups. The results were expressed as Mean ± SEM (standard error of means). P-value less than 0.05 were

considered significant and are written in the parentheses.

3. Results

3.1. Amount of apoptotic cells among kidney cells

Number of apoptotic cells colored brown, in diabetic group was (16.00±1.11) and in Quercetin received diabetic group and control group was (5.05±0.17) and (1.05±0.05) respectively. These changes was significant as p value less than 0.05 (P<0.05) (Table 1).

3.2. Results of total blood antioxidant capacity(TAC)

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. Statistic analysis Dunnett(one side) shows significant differences between experimental groups in comparison to control group(P<0.05).(table 1).

3.3. Results of MDA (malondialdehyde) level in blood

MDA level in control group was (0.25±0.04) mmol/L and in experimental groups was (0.300±0.212), (4.10±0.06), (1.10± 0.08) mmol/L respectively.

Statistic analysis Dunnett(one side) shows significant differences between experimental groups in comparison to control group(P<0.05)(Table 1).

3.4. Results of OX-LDL (aldehyde oxidase) level in blood

OX-LDL level in control group was (3.10±0.05) μ/L and in experimental groups was (3.0±0.45), (5.6±0.85), (4.9±0.80) μ/L respectively.

Statistic analysis Dunnett(one side) shows significant differences between experimental groups in comparison to control group(P<0.05)(Table 1).

3.5. Pathology of liver

Section of liver in light microscopic study in diabetic group showed congestion in central vein in portal lobe and irreversible cell death such as necrosis of hepatocyte. Liver cells in diabetic group that received fresh juice of onion congestion in sinusoidal area has decreased in portal lobe. The hepatocyte in control group showed normal architectural and vein without congestion (Figure 1).

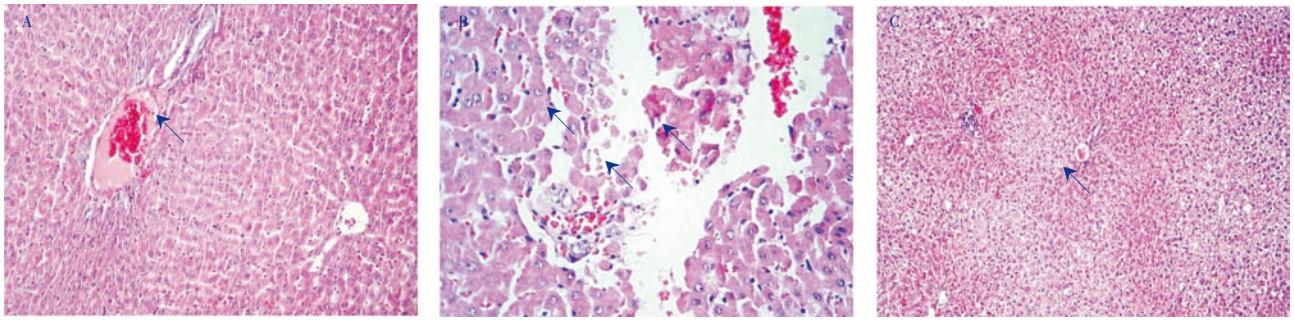

Figure 1. (A & B): section of liver in diabetic group that congestion in central vein in portal lobe has seen (arrow) and necrosis of hepatocyte (head of arrow), (H&E), X320. Photomicrograph, (c): section of liver in diabetic group that recived fresh juice of onion congestion in sinusoidal area has decreased in portal lobe has seen (arrow), (H&E), X320.

Table 1
Modulating effects of quercetin on OX-LDL, hepatocytes apoptosis, TAC and MDA.

Groups	control(n=10)	Quercetin (15mg/kg(IP)) (n=10)	STZ(55mg/kg(IP))(n=10)	Quercetin +Stz55mg/kg (IP) streptozotocin lus15mg/kg Quercetin(n=10)
Apoptotic cells	1.05±0.05	1.05±0.05	16.00±1.11*	5.05±0.17*
(TAC) (mmol/mL)	0.70±0.03	0.75±0.03*	0.32±0.04*	0.61±0.05*
(MDA) (mmol/mL)	0.25±0.04	0.30±0.21*	4.10±0.06*	1.10±0.08*
OX-LDL (aldehyde oxidase) (μ /L)	3.10±0.05	3.00±0.45	5.60±0.85	4.90±0.80

Data are presented as mean ± SEM.* P< 0.05 comparing with the control group.

4. Discussion

Worldwide studies have been done to make use of herbal medicine in different fields of medicine. Base on ancient Persians traditional books Use of herbal medicine has positive effect on treatment of different diseases especially on diabetes mellitus. Onion contains A, B, C vitamins, flavonoids and selenium which their antioxidant role has been proved[8,9]. Make use of onion and Quercetin in diabetic patient treatment has been experimented[11]. Investigations show onion and Quercetin decrease serum glucose level[12] but this reduction with onion has been significant[13]. Also Quercetin decreases oxidative stress and blood vessels damage in diabetic rats[14]. Other investigations show Quercetin increases the level of blood insulin and serum Ca^{2+} and Mg^{2+}[15]. Investigations show liver has an important role in carbohydrate metabolism since it is responsible for the balance of blood glucose level by means of glycogen sis and glycogenolysis therefore impaired hepatic function impairs metabolic homeostasis of glucose[1,16]. In the presence of impaired glucose metabolism and occurrence of hyperglycemia, genes involved in fatty acid storage were activated[17]. 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[16]. Insulin resistance occurs in muscular and adipose tissues combined

with hyper insulinemia are pathophysiological bases of diabetes in liver disease[1,16]. The etiology of liver disease is important in the incidence of diabetes mellitus since non alcoholic fatty liver disease (NALFD), alcohol, hepatitis C virus (HCV) and hemachromatosis are frequently associated with diabetes mellitus[16]. Investigations show liver tissue damage and apoptosis induced by diabetes mellitus increase active O2 specious. Flavonoids as an antioxidant factor found in nutrient such as fruit, vegetables, tea and black burgundy grape. Flavonoids value in daily meal varies from 16 mg to 1000 mg. Quercetin as an important and main flavonoids found in human meal. Investigations show Quercetin absorbs in small bowel. Useful effect of Quercetin in human health involves prevention of diabetes induced cataract, reduced blood vessels fragility, anti microbial, anti viral, anti allergy, and anti inflammatory effects and prevention of platelet aggregation. One of the Quercetin anti oxidant mechanism is removal of free radical such as xanthine superoxide and xanthine oxidase[18,19]. Investigations show Quercetin in the chicken spermatogonial cell culture not only doesn't have harmful effects but also increases amount of spermatogonial cells and decreases oxidative effects. In this study like pervious investigations Quercetin decreases malondialdehyde and increases serum antioxidant capacity[20,21]. Pervious investigations show O_2 lacked specious cause body tissue damage in diabetic rats[22]. Studies had been done on serum surface of glutathione , catalase , superoxide dismutase, and fat peroxidation in liver, brain, kidney tissue show Quercetin as an antioxidant agent not only decreases free O_2 specious and LDL oxidase in diabetic rats but also has therapeutic potential[23]. Therefore suggested, increased use of herbal medicine, fruit, vegetables, onion, tea and black burgundy grape which are full of flavonoids and Quercetin can decrease side effects of diabetes mellitus on liver tissue in diabetic patient complicated with hepatic diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This research was supported by scientific society grant from the Research Vice Chancellor of Islamic Azad University, Tabriz Branch. We gratefully thank for their help and financial support.

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