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Full Length Research Paper

Modulating effects of quercetin on aldehyde oxidase (OX-LDL) and hepatocytes injury in Streptozotocin-induced diabetic rat

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Quercetin (QR) is a strong antioxidant and long-term treatment for STZ-diabetic animals and it has been shown to reduce oxidative stress. Antioxidants have essential effect on liver diseases. Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus. Wistar male rat (n = 40) were allocated into three groups, control group (n = 10) and (QR) groups that received 15 mg/kg, (n = 10) and diabetic group that received 55 mg/kg streptozotocin (STZ) (n = 20) which was subdivided to two groups of 10; STZ group and treatment group. Treatment group received 55 mg/kg STZ plus 15 mg/kg QR, daily for 4 weeks, respectively; however, the control group just received an equal volume of distilled water daily. Diabetes was induced by a single injection of STZ (55 mg/kg). Animals were kept in standard condition. In 28 day after inducing diabetic, serums were collected for total antioxidant capacity (TAC), malondialdehyde concentration (MDA) and oxidized low density lipoprotein (Ox-LDL) levels and liver tissues of rat in whole groups were removed then prepared for apoptosis analysis by Tunel method. TAC was significantly decreased in diabetics group (P<0.05) in comparison to control and (QR) groups, MDA and Ox-LDL were significantly increased in diabetics groups (P<0.05). Conclusively, in our study 15 mg/kg QR, has significant preventive the effect on liver cells damages so it seems that using it can be effective for treatment in diabetic rat.

Key words: Antioxidant, cell injury, diabetes.

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. Hyperglycemia in long time have side effect in other tissues especially in liver. Liver dysfunctional has seen in diabetic patients especially that with uncontrolled blood sugars level. Due attention has been paid to the search of effective drugs in the field of traditional Chinese medicine (TCM) (Tappy and Minehira, 2001)

Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus (Baynes and Thorpe, 1999; Wolff et al., 1991). 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 (Palmeria et al., 2001). These reducing sugars can easily react with lipids and proteins (nonenzymatic glycation

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reaction), increasing the production of reactive oxygen species (ROS) (Palmeria et al., 2001). 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. Increased production of free radicals or ROS formation may induce oxidized LDL (Ox-LDL), 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 secondary complications in diabetes. These abnormalities produce pathologies including vasculo-pathies, neuropathies, ophthalmopathies and nephropathies, among many other medical derangements (Sexton and Jarow, 1997). 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 (Peluso, 2006). Many herbal such as: Barberry, Estragon, *Rhuscoriaria*, *Cinnamomum zelanicum*, *Hypericum perforatum* and onion known anti diabetic effects are use for patient treatment. Quercetin is a well-known flavonoid and a strong antioxidant and long-term treatment of Streptozotocin-diabetic animals and it has been shown to reduce oxidative stress (Mahesh and Menon, 2004). We plan to study the antioxidant effect of QR on hepatcytes cells injury after induces diabetes.

MATERIALS AND METHODS

Animals

Forty adult Wistar albino male rats with 8 weeks old and weigh 250±10 g were obtained from animal facility of Pasture Institute of Iran. Male rats were housed in temperature controlled rooms (25°C) with constant humidity (40 to 70%) and 12 /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 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 all treated animal.

Induction of experimental type 1 diabetes

Experimental type 1 diabetes was induced by a single intraperitoneal (IP) 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.

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.

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°C. The quercetin solution was freshly prepared each week.

Groups and treatments

Thereafter, the rats were randomly selected and divided into control (n = 10) and (QR) group that received 15 mg/kg QR intraperitoneal (IP), (n = 10), and diabetic group that received 55 mg/kg (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 plus 15 mg/kg QR (IP). The control group just received an equal volume of distilled water daily. (QR) injections were continued to the end of the study (for 4 weeks), (Coskun et al., 2005; Khaki et al., 2010).

Surgical procedure

On the 28th day, (at the end of the treatment period), the rats were decapitation, and liver tissues in control and experimental groups were immediately removed.

Measurement of serum total antioxidant capacity (TAC)

TAS 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 TAS measurement assays, and the assay results are expressed as Trolox equivalent (mmol/L), (Feng et al., 2001).

Measurement of serum malondialdehyde concentration (MDA)

Tissue MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as mmol MDA formed/ml. Plasma Measurement of serum malondialdehyde concentration (MDA) A calibration curve was prepared by using 1,1',3,3'-tetramethoxypropane as the standard (Quintanilha et al., 1982).

Measurement of oxidized LDL level (Ox-LDL)

Oxidized LDL level was measured by using a Mercodia Oxidized LDL ELISA kit .Mercodia Oxidized LDL Competitive ELISA is based on the monoclonal antibody 4E6 (Khaki et al., 2010).

Light microscopic study

Liver samples after collected fixed in formalin 10%, and prepared to

light microscopic study and 5 µm sections were obtained and stained with H&E. Cross sections of 100 liver tissues per specimen were assessed and the mean number of necrotic cells per cross- section was calculated.

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 ± S.E.M (standard error of means). P-value less than 0.05 were considered significant.

RESULTS

Amount of apoptotic cells among liver cells

Number of apoptotic cells 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 ($P<0.05$).

Results of TAC, MDA and Ox-LDL level in blood

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.

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.

Ox-LDL level in control group was 3.1±0.05 u/L and in experimental groups was 3.0±0.45, 5.6±0.85, 4.9±0.80 u/L respectively.

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

Results of light microscopic study

The percentage of necrotic cells in control group was (1.05±0.05) and in experimental groups was 1.25±0.05, 22.00±0.01 and 10.05±0.12 ($P<0.05$) (Table 1).

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 (Jiang, 1996). Onion contains vitamins A, B, C, flavonoids and selenium which their antioxidant role has been proved. The use of onion and quercetin in diabetic patient treatment has been experimented (Machha et al., 2007). Investigations show onion and quercetin decrease serum glucose level (Cunha et al., 2008), but this reduction with onion has been significant (Mehmet et al., 2007). Also quercetin decreases oxidative stress and blood vessels damage in diabetic rats (Kato et al., 2008; Custro et al., 2001). Other investigations show quercetin increases the level of blood insulin and serum $Ca^{2}+$ and $Mg^{2}+$ (Lecube et al., 2004). Investigations show liver has an important role in carbohydrate metabolism since it is responsible for the balance of blood glucose level by means of glycogensis and glycogenolysis therefore impaired hepatic function impairs metabolic homeostasis of glucose (Holstein et al., 2002; Tappy et al., 2001). In the presence of impaired glucose metabolism and occurrence of hyperglycemia, genes involved in fatty acid storage were activated (Baynes and Thorpe, 1999). 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 (Lecube et al., 2004). Insulin resistance occurs in muscular and adipose tissues combined with hyper insulinemia are pathophysiological bases of diabetes in liver disease (Skibola and Smith, 2000). 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 (Lecube et al., 2004). Investigations show liver tissue damage and apoptosis induced by diabetes mellitus increase active O_2 specious. Flavonoids as an antioxidant factor found in nutrient such as fruit, vegetables, tea and black burgundy grape (Manach et al., 1998). Flavonoids value in daily mail varies from 16 to 1000 mg. Quercetin as an important and main flavonoids found in human mail (Formica and Regelson, 1995). Investigations show quercetin absorbs in small bowl. 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 (Hertog and Hollman, 1996; Bors et al., 1977; Dok-Go et al., 2003). One of the quercetin anti-oxidant mechanism is removal of free radical such as xanthine superoxide and xanthine oxidase (Mi and Zhang, 2007). Investigations show quercetin in the chicken spermatogonial cell culture not only does not have harmful effects but also increases amount of spermatogonial cells and decreases oxidative effects. In this study, like previous investigations quercetin decreases malondialdehyde and increases serum anti-oxidant capacity (Mi and Zhang, 2007; Chandel et al., 2008). Pervious investigations show O_2 lacked specious cause body tissue damage in diabetic rats (Naziroğlu, 2003; Sanders et al., 2001). Studies had been done on serum surface of glutathione, catalase, superoxide dismutase, and fat peroxidation in liver, brain, kidney tissue show

Table 1. Modulating effects of quercetin on Ox-LDL, hepatocytes necrosis, TAC and MDA.

Groups	Control (n = 10)	Quercetin (15 mg/kg (IP)) (n = 10)	STZ (55 mg/kg (IP)) (n = 10)	Quercetin + Stz 55 mg/kg (IP) streptozotocin plus 15 mg/kg Quercetin (n = 10)
Hepatocytes necrosis (%)	1.05±0.05	1.25±0.05	22.00±0.01* (0.005)	10.05±0.12* (0.003)
(TAC) (mmol/ml)	0.70±0.03	0.75±0.03* (0.006)	0.32±0.04* (0.003)	0.61±0.05* (0.004)
(MDA) (mmol/ml)	0.25±0.04	0.30±0.212* (0.004)	4.1±0.06* (0.008)	1.1±0.08* (0.005)
OX-LDL (aldehyde oxidase (u/L))	3.1±0.05	3.0±0.45 (0.108)	5.6±0.85 (0.061)	4.9±0.80 (0.055)

Data are presented as mean ± SE. * P-value less than 0.05 were considered significant and are writing in the parentheses, (compared with the control group).

quercetin as an antioxidant agent not only decreases free O2 specious and LDL oxidase in diabetic rats but also has therapeutic potential (Drobiova et al., 2009). Therefore, we suggest that 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.

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