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Original article

The effect of hesperidin and quercetin on oxidative stress, NF-κB and SIRT1 levels in a STZ-induced experimental diabetes model

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ABSTRACT

Objective: The aim of this study is to investigate the roles of SIRT1 and NF-κB in the pathogenesis of diabetes mellitus in rats with STZ-induced diabetes and determine the effects of hesperidin and quercetin on oxidative stress and on the levels of SIRT1 and NF-κB.
Materials and methods: The experimental animals were divided into four groups, each group comprising ten rats designated as follows: group 1 served as control rats (C); group 2 served as diabetic rats (DM); group 3 served as diabetic rats administered hesperidin (DM + HSP) (100 mg/kg b.w.) in aqueous suspension orally for 15 days; and group 4 served as diabetic rats administered quercetin (DM + Q) (100 mg/kg b.w.) in aqueous suspension orally for 15 days.
Results: In diabetic group, liver and kidney SIRT1, SOD and CAT activities were significantly lower than control group ($p < 0.05$). Hesperidin and quercetin caused significant increase in the SIRT1, SOD and CAT activities of both DM + HP and DM + Q groups kidney tissues compared to DM group ($p < 0.05$). Liver SOD activies were not found to differ significantly between DM, DM + Q and DM + HP groups ($p > 0.05$). In DM + HP group, liver CAT activities were significantly higher than DM ($p < 0.05$), but there was no significant difference in liver CAT activities between DM and DM + Q ($p > 0.05$). In diabetic group, liver and kidney NF-κB and MDA levels were increased compared to control group ($p < 0.05$), and groups of DM + HP and DM + Q had lower NF-κB and MDA levels than diabetic group ($p < 0.05$).
Conclusion: As a conclusion, based on the results we obtained from this study and the literature data discussed above, we determined in STZ-induced diabetic rats that, increased glucose levels and liver and kidney damage markers decreased significantly after administration of hesperedin and quercetin, and that oxidative stress and NF-κB levels increased while SIRT1 levels decreased in the diabetic group.

1. Introduction

Diabetes mellitus (DM) is a life-long metabolic disease of high morbidity and mortality as well as high economic burden for the society, which requires continuous monitoring and treatment, and reduces the patient's quality of life due to acute and chronic complications. As a result of widespread improper dietary habits and obesity, the incidence of diabetes mellitus is increasing throughout the world. It can cause disorders in carbohydrate, protein and fat metabolisms as a result of absolute or relative insulin insufficiency or ineffectiveness, leading to micro- and macro-vascular complications in all systems [1,2]. It has been reported in the literature that oxidative stress has an important role in the pathogenesis and progression of diabetes, and that free oxygen radicals and lipid peroxidation increase significantly [3]. Increased free radicals interact with lipids, proteins and nucleic acids, leading to loss of membrane integrity, structural or functional changes in proteins and to genetic mutations. In addition to all these, malondialdehyde (MDA), one of the most important end products of lipid peroxidation, affects ion exchange from the cell membranes, leading to cross-linking of the compounds located in the membrane as well as adverse consequences such as changing of enzyme activity in parallel with ion permeability. The human organism has some enzymatic

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and non-enzymatic antioxidant defense systems to cope with the effects of these harmful radicals [4,5]. Antioxidant enzyme systems such as superoxide dismutase (SOD) and catalase (CAT), having cytoplasmic, mitochondrial and extracellular forms destroy these oxidant molecules in living organisms [6,7].

Nuclear factor kappa B (NF-κB) is defined as a eukaryotic nuclear transcription factor that binds to the promoter region on the immunoglobulin kappa light chain gene in the nuclei of B lymphocytes. In all cells, NF-κB keeps quiet in the cytoplasm and only passes to the nucleus in case of activation, and there it functions as a transcription factor, which is an important regulator of a large number of genes responsible for inflammation, immunological response, proliferation and apoptosis [8,9]. Nuclear factor kappa B, having a key role in the pathophysiology of clinically important diseases, also plays an important role in the cellular response given to stimuli such as stress, free radicals, ultraviolet and radiation [10,11].

Sirtuin-1 (SIRT1) is a member of the sirtuin family and it is an enzyme responsible for the deacetylation of proteins involved in cellular regulation. It is thought to play an important role in various matters such as cell proliferation, cell differentiation, senescence, regulation of gene expression, mitochondrial biogenesis, fatty acid oxidation, apoptosis, autophagy and control of cellular metabolic balance [12–14]. Recent studies reported that SIRT1 regulates many metabolic adaptations associated with obesity as well as the expression of adipokines, suppresses the efficacy of factors essential for maturation of adipocytes, regulates plasma glucose levels by regulating insulin secretion [1,15,16].

Various alternative and complementary approaches to diabetes prevention or control, particularly those including dietary manipulations are essential factors in the treatment of diabetes. Flavonoids, with their highly antioxidant potential, have been long used by researchers in the treatment or prevention of various diseases [17]. Flavonoids have various biological effects in the cellular system such as antimicrobial, antiviral, antiulcerogenic, cytotoxic, antineoplastic, mutagenic, anti-inflammatory and antioxidant properties [18]. Quercetin is a flavonoid, a phenolic pigment product found in plants. Quercetin, which is a natural antioxidant, inhibits oxidative damage and cell death by various mechanisms such as xanthine oxidase inhibition and lipid peroxidation. Quercetin is also known to have cardioprotective, antiulcerative, antiinflammatory, antiallergic, antiviral and antibacterial properties [18]. Hesperidin, which has various biological and pharmacological properties, was reported to reduce the occurrence of cardiovascular diseases, play a protective role against colon, bladder and breast cancers, and show positive effects on lipid metabolism [19].

The aim of this study is to investigate the roles of SIRT1 and NF-κB in the pathogenesis of diabetes mellitus in rats with STZ-induced diabetes and determine the effects of hesperidin and quercetin on oxidative stress and on the levels of SIRT1 and NF-κB.

2. Materials and methods

2.1. Chemicals

STZ, quercetin and hesperidin were purchased from Sigma Chemicals (Sigma Chemicals Co., St. Louis, MO, USA), stored at 2–4 °C and protected from sunlight. All other chemicals were of analytical grade and were obtained from standard commercial supplies.

2.2. Experimental animals and procedures

This study was carried out in the Karadeniz Technical University 's Experimental Animal Laboratory of the Medical and Experimental Application and Research in accordance of the Karadeniz Technical University's Local Ethical committee decision of date 12.04.2016 and the project number of 2016/63. Male Wistar albino rats weighing about 200–250 g and 4–6 weeks of age were used in the experiments. The animals were monitored under standard laboratory conditions of 22–25 °C and controlled photoperiod of 12:12 h light:dark in the Animal Laboratory of the Experimental Research Unit of Karadeniz Technical University. During the experiment, the animals were fed with standard laboratory diet and tap water. Rats were fed a basal diet and the basal diet was formulated to meet nutrient requirements (Table 1). The experimental animals were divided into four groups, each group comprising ten rats designated as follows: group 1 served as control rats (C); group 2 served as diabetic rats (DM); group 3 served as diabetic rats administered hesperidin (DM + HSP) (100 mg/kg b.w.) [20] in aqueous suspension orally for 15 days; and group 4 served as diabetic rats administered quercetin (DM + Q) (100 mg/kg b.w.) [21] in aqueous suspension orally for 15 days.

The experimental diabetes model was administered as single-dose intraperitoneal injection of STZ at a dose of 60 mg/kg dissolved in a 0.4 mL (0.1 M) sodium citrate buffer (pH: 4.5) using a 26 gauge insulin injector. After 72 h, blood was taken from the tail vein of the rats and measured by a glucometer [22]. Rats with fasting blood glucose over 200 mg/dl were considered as diabetic [23]. Blood samples were taken around 09.00–10.00 in the morning after 8–10 h of fasting to determine fasting blood glucose levels in rats.

2.3. Preparation of serum and tissue homogenates samples

At the end of the experiment, blood samples were taken by cardiac route to obtain sera samples of the rats which were administered ketamine (60 mg/kg) and xylazine (12 mg/kg) intraperitoneally and afterwards cervical dislocations were performed liver and kidney tissues were removed. Blood samples were allowed for 20 min in the separation tubes then centrifuged for 10 min at 3500 rpm; and the supernatant was kept in the eppendorf tubes at −80 °C in the freezer until being analyzed for glucose, AST, ALT, urea, creatinine and BUN.

2.4. Analysis of malondialdehyte

The level of MDA, a measure of lipid peroxidation, was measured spectrophotometrically by the method described by Okhawa et al. [24]. Then, 0.5 mL of 8.1% sodium dodecyl sulfate (SDS), 0.5 mL of 0.8% thiobarbituric acid (TBA), 1.0 mL of 10% of trichloracetic acid (TCA), 1.0 mL of 2% glacial acetic acid/sodium hydroxide (NaOH) (pH = 3.5) and 50 μL of 2% butylhydroxytoluene (BHT) were added to the serum samples (1.0 mL) and this mixture was thoroughly mixed and kept in a water-bath at 95 °C for 60 min. After the tubes were chilled, a mixture of 4.0 mL of butanol/

Table 1
Composition and analysis of control diet.

Ingredient	Mk/kg	Chemical composition	
Mangan sulfate	144.78	Dry matter – DM(%)	87.00
Iron sulfate	375.58	Crude protein – CP (%)	23.00
Zinc oxide	151.53	Crude fiber (%)	3.19
Copper sulfate	33.51	Fat (%)	2.00
Cobalt carbonate	0.41	Crude ash (%)	6.00
Sodium selenite	1.07	Crude cellulose (%)	5.00
Calcium iodate	2.00	Phosphorus (%)	0.47
	0.30	Metabolic energy – M (kcal/kg)	2600