Life Sciences 158 (2016) 57–62

Contents lists available at ScienceDirect

Life Sciences

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

The effects of genistein supplementation on fructose induced insulin resistance, oxidative stress and inflammation

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A R T I C L E   I N F O

Article history:
Received 11 March 2016
Received in revised form 12 June 2016
Accepted 15 June 2016
Available online 24 June 2016

Keywords:
Genistein
Fructose
Insulin resistance
Inflammation
Oxidative stress
TNF-α
IL-6
Visfatin
8-isoprostane

A B S T R A C T

Aims: This experimental study was designed to investigate the effects of 10 weeks genistein administration on oxidative stress and inflammation in serum and liver of rats fed with fructose.
Main methods: 6–8 weeks old, 40 male Sprague-Dawley rats were included. Group 1 (control) was fed with standard chow food and 100 μl/kg/day/rat dimethyl sulfoxide (DMSO) administered subcutaneously; group 2 (genistein) with standard chow food and 0.25 mg/kg/day/rat genistein; group 3 (fructose) with standard chow food and drinking water 20% fructose, group 4 (fructose + genistein) with standard chow food, drinking water with 20% fructose and 0.25 mg/kg/day/rat genistein. TNF-α, IL-6, visfatin as inflammatory markers and 8-isoprostane as a oxidative stress marker were measured by ELISA, glucose, triglyceride, total cholesterol, LDL-cholesterol and HDL-cholesterol by enzymatic colorimetric method, AST and ALT by kinetic UV method.
Key findings: Significantly high 8-isoprostane levels in serum ($p < 0.001$) and liver ($p < 0.05$) in group 3 compared to control group indicate that presence of oxidative stress. Significantly high TNF-α and IL-6 levels in serum ($p < 0.05$) and liver ($p < 0.01$) and visfatin levels in serum ($p < 0.001$) of group 3 indicate inflammation accompanying insulin resistance and oxidative stress. Genistein administration to fructose group causes a significant decrease in HOMA-IR ($p < 0.001$) and LDLC ($p < 0.05$) level. Significantly lower serum 8-isoprostane ($p < 0.01$) level indicates the antioxidant effect of genistein and significantly lower liver TNF-α ($p < 0.01$), serum, liver IL-6($p < 0.01$) and serum visfatin ($p < 0.01$) levels reflect the antiinflammatory effects of genistein.
Significance: Genistein administration to rats fed with fructose causes an ameliorating effect on HOMA-IR values and lipid status markers in addition to its antioxidant and antiinflammatory effects.
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1. Introduction

Genistein, an isoflavone phytoestrogen, is a multifunctional agent found in soybeans. There is accumulating evidence that it regulates many biological activities. Genistein has been investigated for its beneficial effects on cancer treatment, cognitive function and cardiovascular and skeletal health, with a primary focus on its potential hypolipidemic, antioxidative, antiinflammatory and estrogenic effects [1].

The beneficial effects of genistein administration on the regulation of glucose homeostasis have been shown in several experimental and human studies [2–4].

Genistein administration is reported to significantly reduce fasting glucose and insulin concentrations in osteopenic postmenopausal women [5], to ameliorate glycaemia and improve glucose tolerance in diabetic animals and stimulate insulin signaling pathways in hepatocytes [3,6,7].

In contrast to its antidiabetic effects, genistein administration has also been shown to inhibit insulin-stimulated glucose uptake by down-regulation of GLUT-4 translocation and to counteract the antilipolytic action of insulin in rat adipocytes. The negative regulation of insulin action by genistein was mostly derived from in vitro studies. Further studies need to be performed to elucidate its effects in vivo [2].

In an attempt to gain further insights into the effects of genistein administration on oxidative stress, insulin resistance, lipid status and inflammatory state; this experimental study was carried out in fructose-induced insulin resistance model in rats. To serve this purpose; 8-

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http://dx.doi.org/10.1016/j.lfs.2016.06.014
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isoprostane was measured as a sensitive and specific marker of oxidative stress, TNF-alfa, IL-6 and visfatin as inflammatory markers, triglyceride, LDL-cholesterol, total cholesterol and HDL-cholesterol as lipid status markers both in serum and liver tissues of fructose- induced insulin resistance (IR) rats, genistein administered IR rats and in control groups.

2. Materials and methods

2.1. Animals and treatments/study design

Forty male Sprague-Dawley rats, six weeks of age and weighing 150–200 g were provided from The Experimental Animal Center of Bezmialem Vakıf (Foundation) University. The research was conducted according to protocols approved by The Institutional Ethical Committee (approval no: 2012/418). All experimental procedures were implanted in accordance with The Institutional Guidelines for Animal Experiments.

The rats were kept under standard conditions of illumination (12-h light/dark cycle), temperature (18–21 °C) and relative humidity (48–55%). After one week of adaptive feeding, the rats were randomly divided into 4 groups, consisting of 10 rats per group. All rats got free access to standard diet (pellet chow) and water ad libitum.

Rats in group 1 served as the normal control and were injected dimethyl sulfoxide sc (100 μl/kg/rat/day). Group 2 served as the genistein control. Genistein was injected sc (0.25 mg/kg/rat/day). Group 3 served as fructose induced insulin resistance (IR) group. Drinking water containing 20% fructose was given. Group 4 served as genistein supplemented insulin resistance (G + IR) group. In addition to drinking water with 20% fructose, genistein (0.25 mg/kg/rat/day) was injected sc.

Genistein was (98% in purity) was purchased from Sigma-Aldrich and was solved in dimethyl sulfoxide (DMSO) (0.1% *v*/*v*).

At the end of 10 week of the study, animals were fasted overnight and total perfusion was applied under ketamine/xylazine (35 mg/kg and 5 mg/kg respectively) anesthesia prior to sacrifice. Blood samples were extracted from right ventricle into dry tubes, centrifuged at 2500 $\times g$ at 4 °C for 10 min; serum was collected and stored at −80 °C until further analysis.

Under anesthesia, perfusion solution was given through cannula placed in left ventricle approximately 90 cm above rats. Femoral artery was dissected and perfusion continued until all blood was removed. Liver tissues were then dissected out immediately and frozen in liquid nitrogen at −80 °C. Frozen liver samples (500 mg) were homogenized in 100 mmol/L phosphate buffer (pH: 7.4) containing sodium azide (0.05%), for 1 min on ice and then centrifuged at 20,000 g, at +4 °C for 15 min and supernatants were obtained.

Liver index was calculated as; liver weight/total body weight [8].

2.2. Biochemical analysis

TNF-alfa and IL-6 levels in serum and liver tissues were determined by solid phase sandwich ELISA using commercial kits (Invitrogen Corporation, CA 93012). For these parameters, intra- and inter CVs were <6.2%, <8% and <2.8% and <5.7% respectively.

Visfatin and 8-isoprostane levels in serum and liver tissues were measured by solid phase sandwich ELISA (Hangzhou Eastbiofarm Corporation). Intra- and inter CVs were <10%, < 12% and <2.8% and <3.2% respectively.

Fasting blood glucose, total cholesterol, HDL-C, LDL-C and triglyceride levels were measured by enzymatic, colorimetric methods, aspartate amino transferase (AST) and alanine aminotransferase (ALT) by kinetic UV methods in Roche Modular System.

Insulin level was measured by solid phase sandwich ELISA (DRG instruments GmbH, Germany). Insulin resistance was assessed using the homeostasis model assessment for insulin resistance (HOMA-IR), which was calculated using the following formula: glucose (mg/dl) × insulin (μIU/ml)/22.5.

2.3. Histopathological examination

The fixed portion of liver tissues was processed by routine histopathological procedures and then embedded in paraffin. Tissue sections (5 μm) were stained with hematoxylin and eosin (H&E) and were examined under Nikon Eclipse Ni-U photomicroscope. All histopathological examinations were performed by an experienced histologist, blinded to the experimental group, in the Histopathology Department of Bezmialem Vakıf (Foundation) University.

2.4. Statistical analysis

All statistical analyses were performed using the statistical analysis software package Statistical Package for the Social Sciences 11.0 for Windows. Data were expressed as mean + standard deviation (SD). Kruskal–Wallis test was performed for the abnormally distributed data, and one way variance analysis (ANOVA) followed by post-hoc Tukey multiple comparison test was applied for the normally distributed parameters.

Relations among the parameters were assessed with Pearson's correlation analysis for normally distributed variables and Spearman's correlation analysis for the variables that were distributed otherwise (cholesterol and liver visfatin).

$p < 0.05$ was considered statistically significant.

3. Results

Findings related to anthropometric measurements, lipid status and HOMA-IR (insulin resistance), liver index and AST, ALT levels. Oxidative stress and inflammatory markers in blood and liver tissues of rats in study groups are shown in Tables 1–4 respectively.

At the end of 10 week experimental study period, increases in weight (g), skin thickness (cm) and waist circumference (cm) were observed in control group (group 1) (95.57%, 32.14% and 39.50% respectively); in group 2 (88.97%, 53.85% and 45.25% respectively), in group 3 (115.06%, 73.08% and 47.18% respectively) and in group 4 (102.40%, 68% and 45.37% respectively). In fructose group (group 3); skin thickness and waist circumference measurements were significantly higher ($p < 0.01$, $p < 0.05$ respectively) than the control group. (Table 1).

With respect to lipid status and insulin resistance (IR) parameters; in fructose group TG (<0.01) and VLDL-C ($p < 0.05$) values were found to be significantly higher and HDL-C ($p < 0.001$) lower than the control group. Glucose ($p < 0.001$) and insulin ($p < 0.001$) levels and HOMA-

Table 1
Levels (means ± SD) of weight, skin thickness and waist circumference in study groups at the beginning and at the end of 10 week study period; and statistical significances.

	Weight (gr)		Skin thickness (cm)		Waist circumference (cm)	
	0 week	10 weeks	0 week	10 weeks	0 week	10 weeks
Group 1 (C)	175.11 ± 11.08	344.22 ± 25.11	0.28 ± 0.05	0.37 ± 0.05	11.09 ± 0.65	15.47 ± 0.66
Group 2 (C + G)	172.22 ± 16.25 a	325.44 ± 18.99	0.26 ± 0.06	0.4 ± 0.03	11.01 ± 0.59	15.99 ± 0.63
Group 3 (F)	172.56 ± 16.10	371.11 ± 27.73	0.26 ± 0.07	0.45 ± 0.05 b**	11.19 ± 0.57	16.47 ± 0.73 b*
Group 4 (F + G)	170.56 ± 16.86	345.22 ± 43.20	0.25 ± 0.05	0.42 ± 0.04	11.02 ± 0.83	16.02 ± 0.97

b: group 1 vs 3 *$p < 0.05$ **$p < 0.01$.

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