Effect Of Hesperidin On Serum Glucose, HbA1c And Oxidative Stress In Myocardial Tissue In Experimentally Induced Myocardial Infarction In Diabetic Rats

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ABSTRACT

Present study was designed to evaluate in vitro antioxidant activity in heart and serum Glucose, HbA1c of Hesperidin on isoproterenol induced myocardial infarction in normal and diabetic in rats. Hesperidin (100 mg/kg, p.o) was administered for 28 days in rats injected with single dose of Streptozotocin (65 mg/kg, i.p, STZ) and nicotinamide (110 mg/kg, i.p, NIC) and after isoproterenol (200mg/kg, s.c., ISO) induced myocardial infarction in diabetic rats on 29th and 30th day. At the end of experimental period (i.e. on the day 31) heart tissue sample of each rat was collected and antioxidative parameter carried out for further estimations. Administration of STZ–NIC in rats showed a significant ($P<0.001$) increased in the levels of serum glucose, glycosylated heamoglobin (HbA1c). At the end of experimental period the level of malondialdehyde formation/ lipid peroxidation (LPO) and nitrite level in heart tissue was significantly increased. Whereas, the activity of biomarkers of oxidative stress such as reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were found to be decreased significantly compared to control rats. Treatment with Hesperidin significantly restored GSH level, SOD as well as catalase activity and reduced lipid peroxidation and nitrite in compared to diabetic control group. This study concluded that HES at 10 mg/kg may show reduced oxidative stress in heart on isoproterenol induced myocardial infarction in type 2 diabetic rats.

Keywords: Hesperidin, antioxidant, isoproterenol, Type 2 diabetic.

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INTRODUCTION

Three major metabolic abnormalities contribute to the development of hyperglycemia in Type 2 diabetes mellitus such as impaired insulin secretion in response to glucose, increased hepatic glucose production and decreased insulin-stimulated glucose uptake in peripheral tissues. The latter 2 abnormalities are primarily due to insulin resistance (1, 2). Type 2 Diabetes Mellitus is mainly characterized by the development of increased morbidity and mortality for cardiovascular disease. Cardiovascular disease is one of the leading causes of death in the western world and diabetes mellitus has been identified as a primary risk factor (3), due to which there is alteration in vascular responsiveness to several vasoconstrictors and vasodilators (4).

Oxidative stress has been associated with the pathogenesis of chronic diabetic complications including cardiomyopathy. The ability of antioxidants to inhibit these injuries has raised the possibility of newer therapeutic treatment for diabetic heart diseases.

Hesperidin (HES) is an abundant and inexpensive byproduct of Citrus cultivation and isolated from the ordinary orange Citrus aurantium and other species of the genus Citrus (family: Rutaceae). It is reported to have anti-allergic, radio protective, immunomodulator, anti-hypertensive and anti-oxidant properties. When hesperidin is administered orally, it is hydrolyzed by intestinal micro flora to yield a major active metabolite hesperidin.

So far in vitro antioxidant activity in heart of effect of Hesperidin on isoproterenol induced myocardial infarction in normal and diabetic in rats has not been

studied. Hence, the purpose of the present study was to instigate the effect of Hesperidin treatment on in vitro antioxidant heart tissue parameter alteration in Isoproterenol induced myocardial infarction in normal and type 2 diabetic rats.

MATERIAL AND METHODS

Drugs and Chemicals

Hesperidin was obtained from ACROS Lab, US. STZ and NIC were obtained from SIGMA, St. Louis, MO, USA. All other chemicals and reagents used in the study were of analytical grade.

Experimental Animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Dharmaj Degree Pharmacy College, Anand. Sprague Dawley rats (210 ± 15 g) were housed in-group of 3 animals per cage and maintained under standardized laboratory conditions (12- h light/dark cycle, 24°C) and provided free access to palleted CHAKKAN diet (Nav Maharashtra Oil Mills Pvt., Pune) and purified drinking water *ad libitium*. The animal experiment was approved by Animal Ethical Committee of the Institute (1163/a/08/CPCSEA).

Experimental Induction of Type 2 Diabetes in Rats

Type 2 Diabetes was induced in rats by a single intraperitoneal (i.p) injection of Streptozotocin (65 mg/kg, STZ) in overnight fasting rats followed by the i.p administration of Nicotinamide (110 mg/kg, NIC) after 15 minutes. STZ was dissolved in citrate buffer (pH 4.5) and NIC was dissolved in normal saline. After 7 days following STZ and NIC administration, blood was collected from retro-orbital puncture and serum samples were analyzed for blood glucose (5). Animals showing fasting blood glucose higher than 300 mg/dL were considered as diabetic and used for the further study. Hesperidin (100 mg/kg, p.o) was administered for 28 days in diabetic rats and afterisoproterenol induced myocardial infarction in rats on 29th and 30th day.

At the end of experimental period (i.e. on the day 31) heart tissue sample of each rat was collected and carried out for further estimations.

Experimental Protocol

Animals were divided into following groups, each group containing 6 animals.

Group 1: Non-diabetic control [0.5 % Sodium CMC (1 ml/kg/day, p.o) as vehicle for 4 weeks and (ND-CON)] and normal saline subcutaneously on 29th and 30th day.

Group 2: Non-diabetic control treated with HES (100 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (ND-HES)] and normal saline subcutaneously on 29th and 30th day.

Group 3: STZ-NIC diabetic control [0.5 % Sodium CMC (1 ml/kg/day, p.o) as vehicle for 4 weeks (D-CON)] and received ISO (200 mg/kg, s.c.) on 29th and 30th day in normal saline.

Group 4: STZ-NIC diabetic rats treated with HES (100 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (D-HES)] and received ISO (200 mg/kg, s.c.) on 29th and 30th day in normal saline.

BIOCHEMICAL ESTIMATIONS

Characterization of Type 2 Diabetes Model

Type 2 diabetes was confirmed by measuring fasting serum glucose using standard diagnostic kit (SPAN diagnostics Pvt., India) and the degree of uncontrolled diabetic state was confirmed by measuring HbA1c (Ion Exchange Resin method). After 4 weeks, diabetes was confirmed by measuring glucose and HbA1c as mentioned above.

Estimation of biomarkers of Oxidative stress

The excised liver was then weighed and homogenized in chilled Tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000 × g at 0 °C for 20 min using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assay of following antioxidant parameters. The levels of Lipid peroxidation (LPO) formation and the activities of endogenous antioxidant enzymes such as catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD) were estimated by the method of Slater and Sawyer (6) Hugo Aebi as given by Hugo (7) Moron et al (8) and Mishra and Fridovich (9). The clear supernatant was used for estimation of nitrite level (10).

Statistical Analysis

All of the data are expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using a computer-based