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Original Article

Effect of Hesperidin on serum Heart Marker, Myocardial Tissues Parameter and Histopathological of Heart in isoproterenol induced Myocardial infarction in Diabetic Rats

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Abstract

Present study was designed to evaluate Hesperidin on serum heart marker, myocardial tissues parameter and histopathology of heart in isoproterenol induced myocardial infarction in normal and Streptozotocin-Nicotinamide induced 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 rats on 29th and 30th day. At the end of experimental period (i.e. on the day 31) blood samples were collected and animals were euthanized. A heart tissue sample of each rat was collected and glycogen and nitrite 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), creatine kinase (CK), Glutamate oxaloacetate transferase (GOT), glycogen and nitrite whereas the levels of myocardial infarct size was found low to be significant ($p<0.05$). Treatment with Hesperidin (HES) significantly decreased change HbA1c, glucose level and no change in glycogen but significantly reduced CK ($p<0.05$), GOT ($P<0.01$) and nitrite ($p<0.01$) in compared to diabetic control group. The myocardial fiber, heavy neutrophil infiltration and cellular edema than non diabetic rats. The HES treated infarction in diabetic rats also led to severe splaying of muscle diabetic rats exhibited reduction in necrosis with less fragmentation of fibres as compared to diabetic control groups, which reflects the cardio protective effect of HES. This study concluded that HES at 100 mg/kg may show reduce experimentally induced myocardial infarction in type 2 diabetic rats.

***Keywords*:** Cardioprotective, Isoproterenol, Streptozotocin, Nicotinamide

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 (Kahn and Porte,1990; Leibowitz,1990). 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 (Uemura *et a*l.,2001) due to which there is alteration in vascular responsiveness to several vasoconstrictors and vasodilators (Senses *et al*.,2001). Recently, a protective effect of Hesperidin against oxidative stress in liver and kidney of diabetic rabbits (Gumieniczek,2003) has been reported.

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 the effect of Hesperidin on experimentally induced myocardial infarction in type 2 diabetic rats has not been studied. Hence, the purpose of the present study was to instigate the effect of Hesperidin treatment on serum heart marker, heart tissue parameter and histopathological alteration in Isoproterenol Induced myocardial infarction in type 2 diabetic rats.

Materials 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*.

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 or mice 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 (Masiello *et al*.,1998). 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 after isoproterenol induced myocardial infarction in rats on 29th and 30th day.

At the end of experimental period (i.e. on the day 31) blood samples were collected and animals were euthanized. A 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 and the treatment period for whole study was 4 weeks.

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

Group 3: 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 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 (200mg/kg, s.c.) on 29th and 30th day in normal saline.

Biochemical Estimation

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 Serum Markers

On 4th week blood samples were collected from retro-orbital plexus under light ether anesthesia and centrifuged at 2500 rpm for 20 minutes to separate serum. Glucose, HbA1c, CK and GOT were estimated using diagnostic kits (SPAN Diagnostics Pvt. India). *In vitro* quantitative determination of the activity of myocardial glycogen and myocardial nitrite (Guevara *et al*.,1998) levels.

Histological Examination

After decapitation, the heart was rapidly dissected out and washed immediately with saline and fixed in 8% buffered formalin. Hearts which were stored in 8% formalin were embedded in paraffin, sections cut at 5 µm and were stained with haematoxyline and eosin. The sections of the heart were observed under microscope (Olympus BX8) for histological changes.

Statistical Analysis

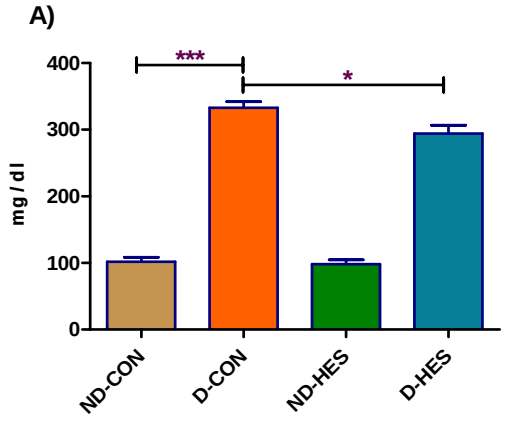
All of the data are expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using a computer-based

fitting program (Prism, Graphpad 5). Differences were considered to be statistically significant when $p < 0.05$.

Results and Discussion

Characterization of Type 2 Diabetes

Single intraperitoneal (i.p) injection of Streptozotocin (65mg/kg) followed by i.p administration of Nicotinamide (110 mg/kg) to rats produced severe hyperglycemia and increased HbA1c in 70 to 80 % the animals (Figure 1). The levels of glucose and HbA1c was significant (P<0.05) decreased after treatment with HES (100 mg/kg, p.o) alone and combination with HES (100 mg/kg, p.o) as compared to DB-CON rats.



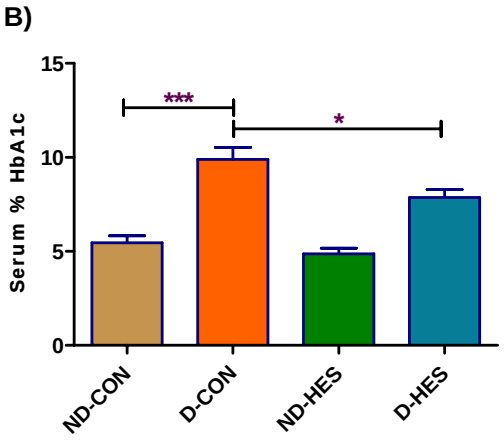


Fig.1: Effect of Hesperidin (100 mg/kg/day, p.o) on changes in serum glucose and HbA1c level in normal and STZ-NIC induced diabetic rats.

Body Weight and Heart Weight

Final body Weight of control animals was significant (P < 0.05) increased as compared to initial body weight. There was a significant reduction in final body weight as compared to initial body weight of D-CON diabetic group (Table 1). Hesperidin treatment had no significant effect on the body weight of D-CON group animals. There was a significant (P < 0.05) increased in heart weight of diabetic rats (D-CON). HES treatment could prevent increase in heart weight in diabetic rats (D-CON). Heart to body weight ratio of the entire group is show in (Table -1).

Table -1: Effect of Hesperidin (100 mg/kg/day, p.o) on changes in body weight, heart weight and heart to body weight ratio after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

Groups	Body Weight						Heart Weight (gm)			Heart to Body Weight ratio		
	Initial			Final								
ND-CON	240.6	±	12.5	261.6	±	15.4#	0.872	±	0.021	0.00333	±	0.00064
D-CON	249.2	±	17.4	224.4	±	16.1#	0.973	±	0.019*	0.00433	±	0.00027*
ND-HES	236.9	±	19.9	248.9	±	15.6	0.862	±	0.038	0.00346	±	0.00039
D-HES	242.6	±	18.4	254.3	±	14.8	0.942	±	0.046*	0.00370	±	0.00049

Values are expressed as mean ± SEM for six animals in the group. * P<0.05 compared to respective control group and # P<0.05 compared to initial weight.

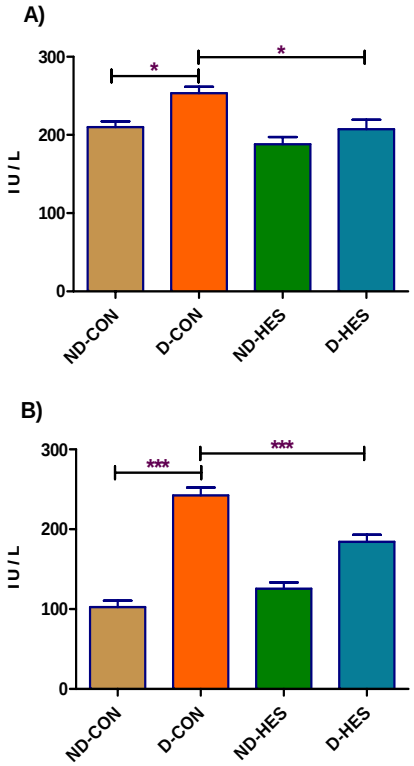


Fig. 2. Effect of Hesperidin (100 mg/kg/day, p.o) on changes in serum Creatine kinase (CK) and Glutamate oxalatoacetate transferase (GOT) level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

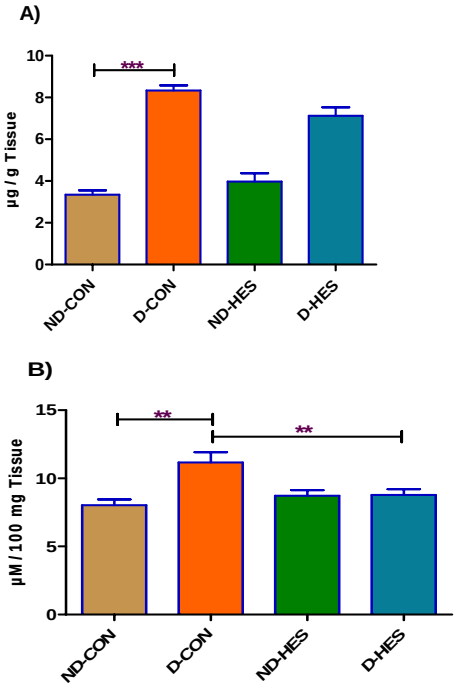


Fig.3. Effect of Hesperidin (100 mg/kg/day, p.o) on myocardial changes in Glycogen (A) and Nitrite (B) level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

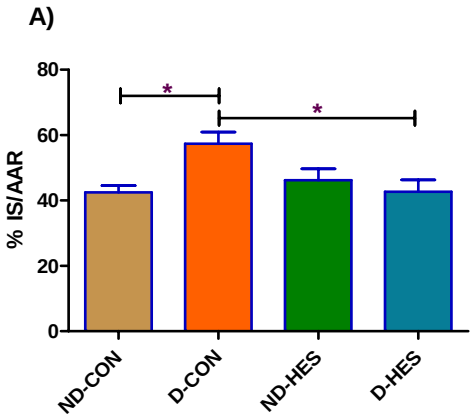


Fig.4. Effect of Hesperidin (100mg/kg/day, p.o) on myocardial infact size changes after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

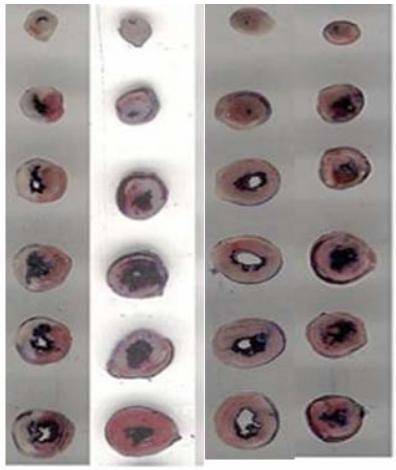


Fig.5. Effect of Hesperidin (100 mg/kg/day, p.o) on TTC stained myocardial sections changes after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

Histopathology of Heart

The photomicrographs revealed that induction of myocardial infarction caused more necrotic damage along with focal loss and fragmentation of muscle fibres of myocardial in diabetic rats (D-CON) than non diabetic rats (ND-CON) (fig. 6). The myocardial infarction in diabetic rats (D-CON) also led to severe splaying of muscle fiber, heavy neutrophil

infiltration and cellular edema than non diabetic rats (ND-CON). The HES treated diabetic rats (D-HES) exhibited reduction in necrosis with less fragmentation of fibres as compared to D-CON groups, which reflects the cardio protective effect of HES (Fig. 6). However, HES treatment could protect myocardial infarction against in non diabetic rats (ND-HES).

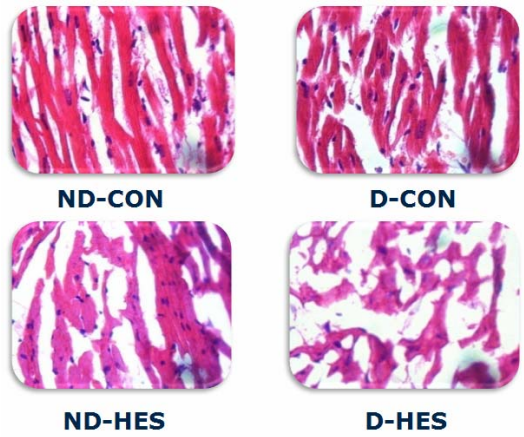


Fig. 6: Effect of Hesperidin (100 mg/kg/day, p.o) on light micrographs of histopathological section of heart changes after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

Discussion

The present study was under taken with the objective of exploring the Hesperidin Reduces on experimently induced myocardial infarction in diabetic rats. Recent studies have suggested that prevalence of type 2 diabetes is rapidly increasing. Heart failure of myocardial infarction or ischemic origin is more frequent and severe in patients with diabetes. Diabetes is an independent risk factor for cardiac failure (Kannel and Mcgee,1979), although its detrimental impact on the myocardium remains to be identified. The significant amount of myocytes loss in this model of non insulin dependent diabetes mellitus is consistent with a greater vulnerability of the diabetic heart to cardiac processes.

The release of ROS in the early phase of myocardial, in combination with the infarction induced decrease in anti-oxidant activity, renders the myocardium vulnerable. Previous studies proved that, ROS produced during myocardial infarction could trigger myocyte apoptosis by activating MAPK and produces DNA damage by activation of the nuclear enzyme poly (ADP ribose) polymerase, which consumes cellular Nicotinamide dinucleotide and adenosine triphosphate.

In the present study, an increase in the levels of serum glucose and HbA1c in STZ-NIC treated rats confirmed the induction of diabetes mellitus. Significant decreased was observed in the glucose and HbA1c level in diabetic rats after treatment with HES (100 mg/kg) when compared with D-CON rats at the end of experimental period. There was a significant increase in heart weight in STZ-NIC diabetic rats which may be due to cardiomyopathy associated with diabetes. It was reflected by increase in serum CK and GOT levels along with heart weight to body weight ratio. Hesperidin could protect the heart from cardiomyopathy associated with STZ-NIC diabetes. This may be the reason for decreased serum CK and GOT level in D-HES group. Myocardial infarction causes further reduction in nitric oxide due to endothelial dysfunction. Hesperidin reduced myocardial infarct size in STZ-NIC diabetic rats. The glycogen deposition in heart is increased in STZ-NIC diabetic rats which may be due to reduction in glucose utilization. HES reduced cardiac glycogen content in STZ-NIC diabetic rats (D-HES) by increasing glucose utilization after myocardial infarction. Therefore, another possibility for cardioprotection by HES may be shifting of energy substrate metabolism from fatty acid to glucose. Reduction in oxidative stress and increase in NO level in Hesperidin treated control group showed significant improvement in CK and GOT.

There may be several mechanisms for cardioprotective by HES against myocardial infarction. It may be due to improvement in NO availability in STZ-NIC diabetic rats. Administration of STZ caused increase in serum CK, GOT and Hesperidin (100 mg/kg, p.o) could reduce them. This study concluded that HES at 100 mg/kg may show reduced on experimentally induced myocardial infarction in diabetic rats.

References

Kahn, S.E. and Porte, D.J.1990. The pathophysiology of type II (noninsulin-dependent) diabetes *mellitus*: Implications for treatment. In: Rifkin, H., Porte, D.J., eds. Ellenberg and Rijkin's Diabetes Mellitus:

Theory and Practice. New York: Elsevier Science. 436-456.

Leibowitz, H.E. 1990. Oral hypoglycemic agents. In: Rifkin H, Porte, D.J., eds. Ellenberg nd Rijkin's Diabetes Mellitus: Theory and Practice. New York: Elsevier Science. 554-574.

Uemura, S., Matsushita, H., Li. W., Glassford, A.J., Asagami, T., Lee, KH., *et al*.2001. *Diabetes mellitus* enhances vascular matrix metalloproteinase activity: role of oxidative stress. *Circ Res*., 88:1291– 1298.

Senses, V., Ozyazgan, S., Ince, E., Tuncdemir, M, Kaya, F., Ozturk, M., *et al*.,2001. Effect of 5-aminoimidazole-4-carboxamide riboside (AICA-r) on isolated thoracic aorta responses in streptozotocin-diabetic rats. *J. Basic. Clin Physiol Pharmacol*., 12:227– 248.

Gumieniczek, A.2003. Effect of the new thiazolidinedione - Hesperidin on the development of oxidative stress in liver and kidney of diabetic rabbits. *Life Sci*., 74:553-562.

Masiello, P., Broca, C., Gross, R., Roye, M., Manteghetti, M., Hillaire-Buys, D., Novelli, M. and Ribes, G. 1998. Experimental NIDDM: development of a new model in adult rats administered Streptozotocin and Nicotinamide. *Diabetes*, 47, 224–229.

Guevara, S. M., Iwanejko, J., Dembinaka-Kiec, A., Pankiewicz, J., Wanat A, Anna P, *et al*., 1998. Determination of nitrite/nitrate in human biological material by the simple Griess reaction. *Clin Chim Acta*., 274: 177-88.

Kannel, W. B., Mcgee, D. L., 1979. Diabetes and cardiovascular disease. The Framingham study. *JAMA*, 241: 2035-2038.