
The Role of Hesperetin in the Management of Diabetes Mellitus and Its Complications

J. Revathy^{1,*}, S. Sheik Abdullah²

¹Research and Development Centre, Bharathiyar University, Coimbatore, Tamilnadu, India

²Biochemistry Faculty, Department of Chemistry and Biosciences, Sastra University, Srinivasa Ramanujan Centre, Kumbakonam, Tamilnadu India

Email address:

revathy.reva@gmail.com (J. Revathy), sheikhaneef@yahoo.co.in (S. S. Abdullah)

*Corresponding author

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Abstract: Diabetes mellitus is a chronic metabolic disease which is a serious global problem. Many antidiabetic therapies focus on improving insulin sensitivity, increasing insulin production, and/or decreasing the level of blood glucose. Hesperetin is a flavanoid commonly found in many herbal medicines and food. Hesperetin seem to demonstrate adequate properties. Animal studies were carried out for 45 days and at the end of 45th day blood samples were collected and various biochemical parameters were measured using autoanalyser. They can inhibit enzymes involved in glucose metabolism, prevent the development of insulin resistance and normalize plasma glucose and insulin levels. This synthetic drug apart from producing a hypoglycemic effect has also been found to manifest hypolipidemic and anti-obesity activity. Hesperetin is also promising agents in the prevention of diabetic complications. They have strong antioxidant activity and inhibit the formation of advanced glycation end products, implicated in the pathogenesis of diabetic nephropathy, embryopathy, and neuropathy or impaired wound healing. Until now very few clinical studies have been concerned with the application of Hesperetin in treating diabetes. Our experimental findings with respect to the mechanism of action of synthetic compound in Streptozotocin induced diabetic rats suggest that it enhances insulin secretion by the islets of langerhans and enhances glucose utilization. However, due to their great therapeutic potential, these compounds deserve special attention.

Keywords: Hesperetin, Insulin, Hypoglycemic Effect, Hypolipidemic Activity

1. Introduction

Diabetes mellitus is a complex metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism that results from imperfections in insulin secretion, insulin action or both [1]. It is one of the most common metabolic disorders, and is considered to be one of the five leading causes of death worldwide [2, 3]. The disease is multifactorial that has a significant impact in the health, quality of life and life expectancy of patients, as well as on the health care system [4]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels [5]. Impairment of growth and susceptibility to certain

infections may also accompany with chronic hyperglycemia. Mortality and morbidity associated with diabetes is mainly due to complications arising from it which include neuropathy, nephropathy, vasculopathy and retinopathy [6, 7]. Flavanoids are non-nutritive dietary components that are widely distributed in plants, several types of vegetables and fruits, and it has been suggested that flavanoids are associated with potential health benefits [8]. The flavanoid hesperetin is the aglycone of hesperidin found in sweet oranges, other citrus fruits and some herbs. Biological activities of hesperetin include antioxidant, bone-sparing and lipid lowering effects [9]. Hesperetin also plays a significant role in inflammation and cancer inhibition. Chemical

compounds that selectively damage pancreatic β -cells constitute a class of diabetogenic agents. Among such compounds, alloxan and streptozotocin exhibit the most potent diabetogenicity, and have been widely used for induction of experimental diabetes [10]. The present study was undertaken to study the antidiabetic effect of hesperetin using *In vivo* analysis and to determine the biochemical parameters in normal and Streptozotocin induced diabetic rats.

2. Materials and Methods

2.1. Animal Management

Nine-week-old adult male albino rats of Wistar albino strain, weighing 120-150 g were acclimatized for one week at air conditioned room ($25 \pm 1^\circ\text{C}$) and relative humidity (55%) in a 12-hour light/dark cycle in a room under hygienic condition.

This study was carried out in the animal house of Srimad Andavan College, Tiruchirapalli and was approved by the Institutional Ethical Committee (SAC/IAEC/BC/2015/Ph.D-009). Animals were fed with pelleted rat chow and water *ad libitum*.

2.2. Source of Chemicals

The synthetic compound and all the chemicals and solvents were of analytical grade and purchased from Sigma-Aldrich Co and Himedia Laboratories Pvt.Ltd., Mumbai.

2.3. Induction of Diabetes

The rats were rendered diabetes by a single intraperitoneal injection of STZ (45mg/kg body weight) in a freshly prepared citrate buffer (0.1M, PH 4.5) after an overnight fast. STZ injected rats were given 20% glucose solution for 24hr to prevent initial drug-induced hypoglycemic mortality. After 72hrs of STZ injection rats exhibited massive glycosuria and hyperglycemia was confirmed by measuring the fasting blood glucose concentration. The rats with blood glucose levels more than 235mg/dL were considered diabetic and used for the experiment.

2.4. Treatment Group Protocol

The animals were divided into six groups, each comprised of nine rats.

Group I – Normal Rats

Group II – Rats were induced with intraperitoneal injection of STZ (45mg/kg body weight).

Group III – Rats were induced with intraperitoneal injection of STZ (45mg/kg body weight) and treated with Hesperetin (20mg/kg body weight in saline).

Group IV - Rats were induced with intraperitoneal injection of STZ (45mg/kg) and treated with Hesperetin (40mg/kg body weight in saline).

Group V – Rats were treated with Hesperetin (40mg/kg body weight in saline).

Group VI - Rats were induced with intraperitoneal injection of STZ (45mg/kg) and treated with Glibenclamide (1mg/kg body weight in saline).

2.5. Collection of Blood Sample

At the end of the experimental period, the rats were sacrificed. Plasma and serum were separated. Treatment continued for 45 consecutive days. Before the treatment (0^{th} , 3^{rd} , 15^{th} , 30^{th} day and the end 45 days plasma levels were estimated using the glucose oxidase method. At the end of the experimental period, the rats were sacrificed. Plasma and serum were separated from blood by centrifuging the samples at 5000 rpm for 10 min and stored in a refrigerator until analysed.

2.6. Biochemical Estimation

All biochemical estimations were done by using fully automated analyzer (ERBA-EM 200). For serum glucose estimation glucose oxidase and peroxidase (GOD-POD) method, for triglycerides Glycerol Phosphate Oxidase-peroxidase (GPO-POD), for total cholesterol, HDL and LDL, Cholesterol Oxidase-Peroxidase (CHOD-PAP) methods were employed. VLDL was estimated using the formula, $\text{VLDL} = \text{triglycerides} / 5$.

2.7. Histopathology

For histopathological study, rats from each group were perfused with cold physiological saline followed by formalin (10% formaldehyde). Then dehydrated on treatment with a series of different concentration of ethanol and embedded in paraffin wax and thick sections are cut using microtome and stained with hematoxylin and eosin. The specimens are evaluated with light microscope.

2.8. Statistical Analysis

Results are presented as mean \pm S.D for six rats in each group. Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using SPSS version 10 (SPSS, Chicago, IL, USA). The limit of statistical significance was set at $p < 0.05$.

3. Results

Table 1 showed the levels of plasma glucose, haemoglobin, insulin and glycosylated haemoglobin in normal control and experimental rats. The level of plasma glucose and glycosylated haemoglobin is significantly increased whereas plasma insulin and blood haemoglobin level was significantly decreased in diabetic control rats. Administration of hesperetin as well as glibenclamide brings a significant decrease in plasma glucose and increase in insulin level was observed at the end of the experimental period. Hesperetin at a dose of 40 mg/kg bw showed a highly significant effect than other dose (20 mg/kg bw).

Table 1. Effect of hesperetin on plasma glucose, haemoglobin, insulin and glycosylated haemoglobin levels in the control and experimental rats.

Groups	Plasma glucose (mg/dl)	Haemoglobin (g/dL)	Insulin (μ U/mL)	HbA _{1c} (%)
Group I (control)	89.00 \pm 1.20 ^a	15.20 \pm 0.12 ^a	16.12 \pm 0.08 ^a	4.64 \pm 0.02 ^a
Group II (diabetic)	215.67 \pm 1.41 ^a	9.86 \pm 0.19 ^b	7.23 \pm 1.01 ^b	8.38 \pm 0.02 ^c
Group III (diabetic+20mg/kg bw hesperetin)	150.17 \pm 1.69 ^b	11.47 \pm 0.06 ^c	11.13 \pm 1.01 ^c	6.91 \pm 0.02 ^b
Group IV (diabetic+40mg/kg bw hesperetin)	123.83 \pm 2.51 ^c	13.72 \pm 0.16 ^d	13.54 \pm 1.00 ^d	5.28 \pm 0.02 ^a
Group V (control+40mg/kg bw hesperetin)	94.00 \pm 5.98 ^a	14.61 \pm 0.10 ^a	17.21 \pm 0.08 ^a	5.00 \pm 0.02 ^b
Group VI (diabetic +glibenclamide 1mg/kg bw)	110.92 \pm 1.45 ^d	14.53 \pm 0.07 ^d	15.10 \pm 0.10 ^d	5.12 \pm 0.01 ^a

Values are given as means \pm S.D from six rats in each group. Values in each column, different superscript letters mean significant differences at $p < 0.05$ (DMRT).

Table 2. Effect of hesperetin on plasma glycoproteins levels in the control and experimental rats.

Groups	Hexose (mg/dl)	Hexosamine (mg/dl)	Sialic acid (mg/dl)	Fucose (mg/dl)
Group I (control)	14.69 \pm 0.06 ^a	4.91 \pm 0.01 ^a	2.87 \pm 0.03 ^a	3.08 \pm 0.04 ^a
Group II (diabetic)	28.25 \pm 0.12 ^b	12.78 \pm 0.06 ^b	10.60 \pm 0.10 ^b	8.04 \pm 0.06 ^b
Group III (diabetic+20mg/kg bw hesperetin)	19.22 \pm 0.03 ^a	10.44 \pm 0.19 ^a	6.40 \pm 0.08 ^a	6.86 \pm 0.03 ^a
Group IV (diabetic+40mg/kg bw hesperetin)	16.12 \pm 0.14 ^a	6.98 \pm 0.05 ^a	3.97 \pm 0.03 ^a	4.06 \pm 0.06 ^a
Group V (control+40mg/kg bw hesperetin)	15.21 \pm 0.11 ^a	6.05 \pm 0.07 ^a	3.83 \pm 0.04 ^a	3.06 \pm 0.02 ^a
Group VI (diabetic +glibenclamide 1mg/kg bw)	15.65 \pm 0.07 ^a	5.79 \pm 0.06 ^a	3.60 \pm 0.05 ^a	3.60 \pm 0.08 ^a

Values are given as means \pm S.D from six rats in each group. Values in each column, different superscript letters mean significant differences at $p < 0.05$ (DMRT).

Table 2 shows the changes in the levels protein bound hexose, hexosamine, fucose and sialic acid in plasma of control and experimental rats. Significantly higher levels of glycoprotein components were observed in the plasma of diabetic rats when

compared to normal rats. Oral administration of hesperetin as well as glibenclamide to diabetic rats resulted in a significant reduction of protein bound hexose, hexosamine, fucose and sialic acid in plasma when compared to diabetic control rats.

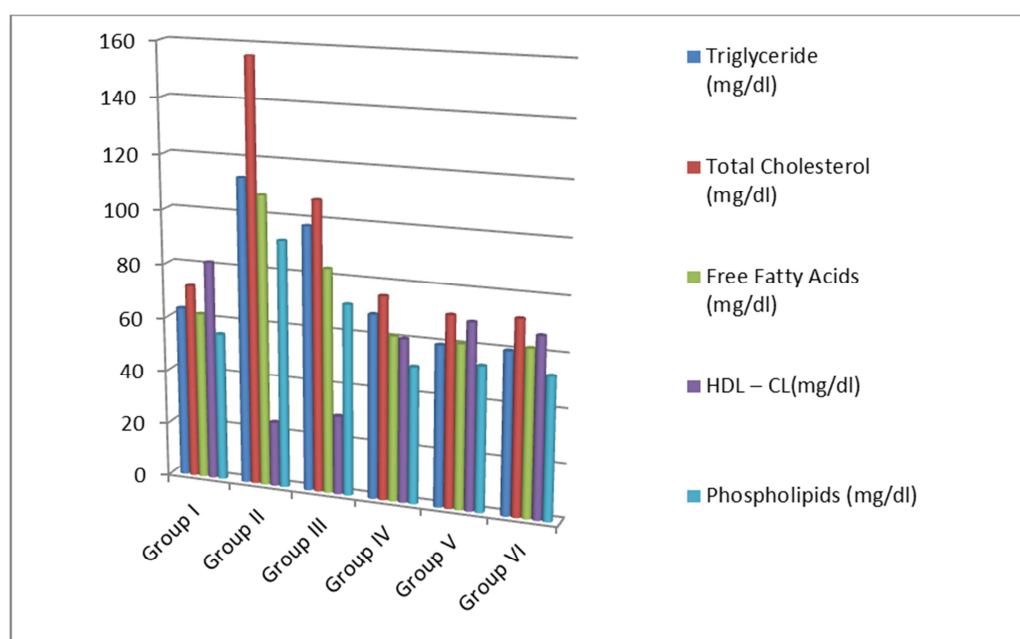
**Figure 1.** Effect of hesperetin on lipid profiles in the control and experimental rats.

Figure 1 depicts the levels of triglycerides, total cholesterol, free fatty acids and phospholipids were significantly ($p < 0.05$) increased, whereas the levels of HDL-Cholesterol (HDL-C) was significantly ($p < 0.05$) decreased in STZ induced diabetic rats (group II) compared to control rats. Oral supplementation with hesperetin (40mg/kg body weight) shows the pronounced effect.

Figure 2 A-E represents the photomicrographs of hematoxylin-eosin staining of pancreatic tissues of control and experimental rats. Fig. 2A shows the section of pancreatic

tissues of control and experimental rats. Fig. 2 A shows the section of pancreatic tissue of control rats shows normal pancreas with both exocrine and endocrine including islets. Fig. B shows the pancreas of diabetic rats exhibiting mild degenerative changes of islets. Fig. D shows normal group treated with hesperetin (40mg/kg bw). Fig. C and E shows more pronounced in the rats supplemented with hesperetin (40mg/kg bw) and the reference drug glibenclamide (1mg/kg bw).

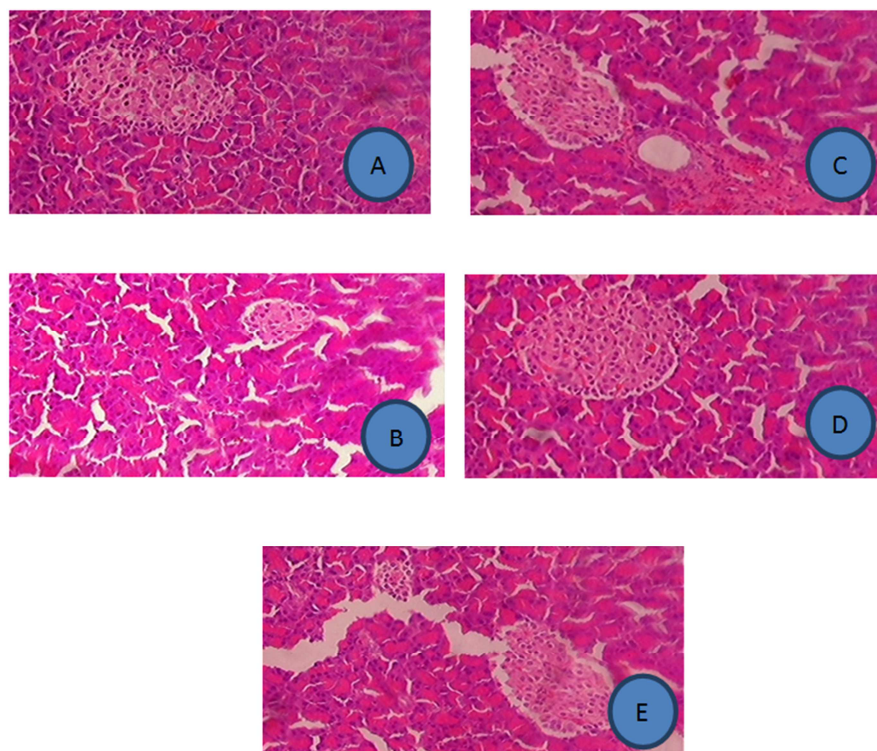


Figure 2. Photomicrographs of hematoxylin-eosin staining of pancreatic tissues of control and experimental rats.

Histological photograph of (A) control, (B) diabetic control, (C) diabetic+ hesperetin (40mg/kg bw), (D) control+ hesperetin (40mg/kg bw), (E) glibenclamide (1mg/kg bw).

Massion trichrome of Pancreas

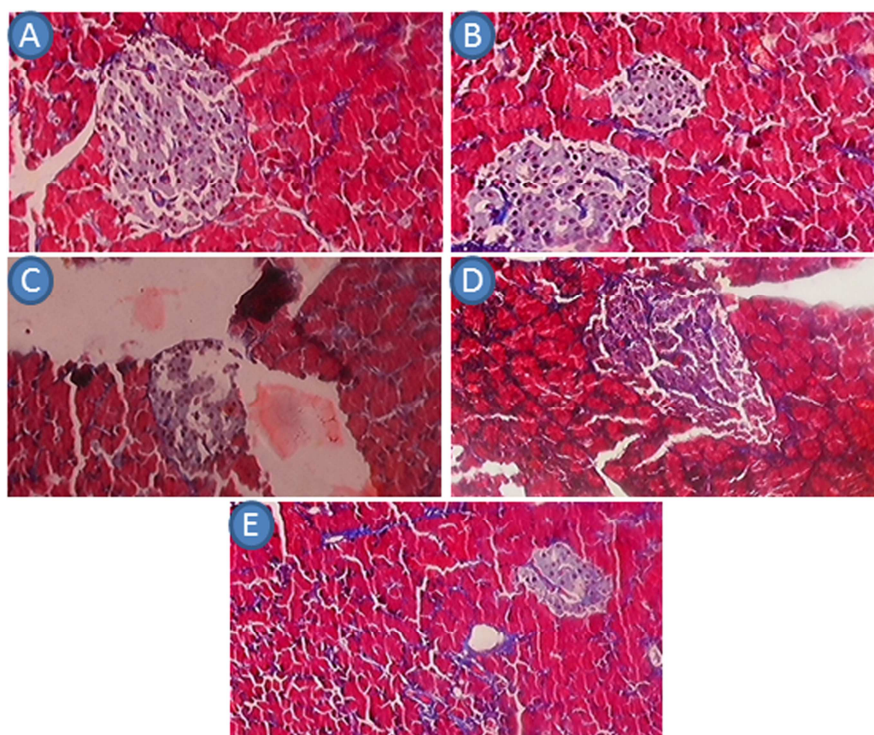


Figure 3. Photomicrographs of Massion trichrome staining of pancreatic tissues of control and experimental rats.

Histological photograph of (A) control, (B) diabetic control, (C) diabetic+ hesperetin (40mg/kg bw), (D) control+ hesperetin (40mg/kg bw), (E) glibenclamide (1mg/kg bw).

Figure 3 A-E represents the photomicrographs of hematoxylin-eosin staining of pancreatic tissues of control and experimental rats. Fig. 3A shows the section of pancreatic tissues of control and experimental rats. Fig. 3 A shows the section of pancreatic tissue of control rats shows normal pancreas with both exocrine and endocrine including islets. Fig. B shows the pancreas of diabetic rats exhibiting mild degenerative changes of islets. Fig. D shows normal group treated with hesperetin (40mg/kg bw). Fig. C and E shows more pronounced in the rats supplemented with hesperetin (40mg/kg bw) and the reference drug glibenclamide (1mg/kg bw).

4. Discussion

In the present research, we found that oral administration of hesperetin for 45 days resulted in a significant reduction in plasma glucose concentrations and an increase in insulin levels in diabetic rats. The antidiabetic effect of hesperetin may be due to the increased release of insulin from the existing β -cells and/or regenerated β -cells of pancreas, restored insulin sensitivity or inhibition of intestinal absorption of glucose or enhanced the utilization of glucose by peripheral tissues. These results are in agreement with Ramachandran and Saravanan [11]. Hexoses of which glucose is one example evolved as energy sources critical for life. The elevated level of hexoses in diabetic rats may be associated with disturbances with carbohydrate metabolism. Treatment with hesperetin and glibenclamide in diabetic rats showed significant reduction in hexoses due to improved glycemic control. The level of hexosamine, increased significantly in the plasma and tissues of diabetic rats which may be due to insulin deficiency, this leads to depressed utilization of glucose by insulin-dependent pathway, thereby enhancing the formation of hexose and hexosamine [12].

Fucose is a member of the group of eight essential sugars the body requires for optimal function of cell-to-cell communication and its metabolism appears to be altered in various diseases such as diabetes mellitus [13]. Our results are finding in line with the study of reduced fucose by improved secretion of insulin in coumarin treated diabetic rats [14]. Sialic acid is the terminal residue of the oligosaccharide side chain of glycoproteins and widely occurs in the exposed positions of molecules like hormones, enzymes and also on tissues [15]. Various factors cause elevation in the concentration of plasma sialic acid. Among various factors first increase in synthesis of sialic acid in insulin-independent tissues, such as the liver and the brain, and the second is an increase in the activity of sialyltransferase, which transfers the sialic acid residues to the glycolipids and glycoproteins [16]. Previous studies show that diosmin, produce the same effect in experimental diabetic rats [17].

Insulin has a potent inhibitory effect on lipolysis in adipocytes. Insulin deficiency leads to excess lipolysis and increased influx of free fatty acids to the liver [18, 19]. In the

present study we have observed higher levels of cholesterol, triglycerides due to increased mobilization of free fatty acid from peripheral deposits, as insulin inhibits the hormone-sensitive lipase. Pancreas of diabetic rats showed shrinkage of islets with mild degenerative changes and treatment with hesperetin showed normal islets with reduced fatty changes (H&E).

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