Influence of hesperetin on glycoprotein components in diabetic rats

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ABSTRACT

Diabetes is a life-long disease and is considered as the second foremost reason to cause blindness and renal failure worldwide. It is a chronic disease caused by inherited or acquired deficiency in the production of insulin by the pancreas, or by ineffectiveness of the secreted insulin. This study is carried out to evaluate the effect of hesperetin on dearrangement in glycoprotein levels in the streptozotocin (STZ) induced diabetic rats. Diabetes was induced in male Wistar rats by a single intraperitoneal injection of STZ (45mg/kg bw). The levels of glycoproteins were altered in experimental diabetes mellitus. Hesperetin were administered to diabetic rats intragastrically at 20, 40mg/kg bw for 45 days. The effects of hesperetin on plasma glucose, insulin and glycosylated haemoglobin were determined. The plasma and tissue (liver and kidney) glycoproteins such as hexose, hexosamine, fucose and sialic acid were also studied. Oral administration of hesperetin (40mg/kg bw) for 45 days dose dependently improved the glycemic status in STZ-induced diabetic rats. The levels of plasma glucose were decreased with significant increase of plasma insulin level. The altered levels of plasma and tissue glycoprotein components were restored to near normal. The present findings suggest that hesperetin can potentially ameliorate glycoprotein components abnormalities in addition to its antidiabetic effect in experimental diabetes.

Keywords: Hesperetin, glycoprotein components, diabetes mellitus, streptozotocin

1. INTRODUCTION

Diabetes mellitus is one of the major health problems in both developed and underdeveloped countries. Diabetes mellitus is the most common serious metabolic disorder and it is considered to be one of the five leading causes of death in the world [1]. Diabetes mellitus a pervasive and multifactorial metabolic syndrome is characterized by imperfection in insulin secretion and insulin receptor or post receptor events with derangement in carbohydrate, protein and lipid metabolism and results in chronic hyperglycemia, a clinical hallmark of diabetes [2]. The chronic hyperglycemia caused by diabetes is associated with long term damage, dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart

and blood vessels [3]. The pathophysiology of diabetes involves a very complex cascade of several interrelated mechanism.

Glycoproteins can be simply defined as proteins that have carbohydrate moiety covalently attached to their peptide portion. They have multiple and complex function and are found as enzymes, hormones, blood group substances and as constituents of extracellular membranes [4]. The commonest glycoproteins are those in which the carbohydrate is linked to the protein by glycosyl linkages, usually hexose, hexosamine, fucose and sialic acid, joined together covalently linked to polypeptide chain. Hyperglycemia in experimental diabetic rats leads to a decreased utilization of glucose by insulin dependent pathways, thereby enhancing the formation of glycoproteins [5]. Chemical compound Streptozotocin exhibits the most potent diabetogenicity and has been widely used for induction of experimental diabetes [6].

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 [7, 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. Hesperetin also plays a significant role in inflammation and cancer inhibition. The aim of the present study was experimentally to validate the neutraceutical potential of hesperetin in the management of diabetes and also its effect on plasma and tissue glycoproteins in STZ-induced diabetic rats and its efficacy of hesperetin was compared with glibenclamide a standard oral antihyperglycemic drug.

2. MATERIALS AND METHODS

2.1 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.

2.2 Animals

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± 1°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*.

2.3 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, $_P$H 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 Experimental design

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

At the end of the treatment period, the rats were fasted overnight, anaesthetized with ketamine (24mg/kg bw) and killed by cervical decapitation. Blood sample was collected in a tube containing potassium oxalate and sodium fluoride (3:1) for the estimation of plasma glucose, insulin and glycoproteins. Liver and kidney were dissected out, washed in ice-cold saline, patted dry and weighed.

2.5 Statistical analysis

The values are expressed as mean values of six rats in each group±standard error mean (S.E.M). Data analysis was done with SPSS 17.0 (SPSS, Cary, NC, USA) student software. Hypothesis testing method included one way analysis of variance (Anova) followed by Duncan's Multiple Range Test (DMRT) [9]. Values are considered statistically significant when $P<0.05$.

3. RESULTS

3.1 Effect of hesperetin on the levels of plasma glucose, insulin and glycosylated haemoglobin levels.

Table 1 showed the levels of plasma glucose and insulin in normal control and experimental rats. The level of plasma glucose is significantly increased whereas plasma insulin level was significantly decreased in diabetic control rats. Administration of hesperetin as well as glibenclamide brings a significant decrease in plasma glucose, glycosylated haemoglobin and increase in insulin levels.

Groups	Plasma Glucose (mg/dl)	Insulin (µU/mL)	HbA_1C(%)
Group I(control)	89.00±1.20^{a}	16.12±0.08^{a}	4.64±0.02^{a}
Group II(diabetic)	215.67±1.41^{a}	7.23±1.01^{b}	8.38±0.02^{c}
Group III(diabetic+20mg/kg bw hesperetin)	150.17±1.69^{b}	11.13±1.01^{c}	6.91±0.02^{b}
Group IV(diabetic+40mg/kg bw hesperetin)	123.83±2.51^{c}	13.54±1.00^{d}	5.28±0.02^{a}
Group V(control+40mg/kg bw hesperetin)	94.00±5.98^{a}	17.21±0.08^{a}	5.00±0.02^{b}
Group VI(diabetic +glibenclamide 1mg/kg bw)	110.92±1.45^{d}	15.10±0.10^{d}	5.12±0.01^{a}

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

3.2 Effect of hesperetin on the levels of plasma glycoproteins

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.

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±0.06[a]	4.91±0.01[a]	2.87±0.03[a]	3.08±0.04[a]
Group II (diabetic)	28.25±0.12[b]	12.78±0.06[b]	10.60±0.10[b]	8.04±0.06[b]
Group III (diabetic+20mg/kg bw hesperetin)	19.22±0.03[a]	10.44±0.19[a]	6.40±0.08[a]	6.86±0.03[a]
Group IV (diabetic+40mg/kg bw hesperetin)	16.12±0.14[a]	6.98±0.05[a]	3.97±0.03[a]	4.06±0.06[a]
Group V (control+40mg/kg bw hesperetin)	15.21±0.11[a]	6.05±0.07[a]	3.83±0.04[a]	3.06±0.02[a]
Group VI (diabetic +glibenclamide 1mg/kg bw)	15.65±0.07[a]	5.79±0.06[a]	3.60±0.05[a]	3.60±0.08[a]

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

3.3 Effect of hesperetin on the levels of tissue glycoproteins

Table 3 and 4 shows the levels of liver and kidney glycoproteins in control and experimental rats. The level of hexose, hexosamine and fucose were significantly increased whereas the level of sialic acid was significantly decreased and those levels were brought back to normal by treatment with hesperetin and glibenclamide.

Table 3. Effect of hesperetin on liver glycoproteins levels in the control and experimental rats

Groups	Hexose (mg/g)	Hexosamine (mg/g)	Sialic acid (mg/g)	Fucose (mg/g)
Group I (control)	15.43±0.07[a]	5.16±0.01[a]	3.01±0.03[a]	3.23±0.04[a]
Group II (diabetic)	29.66±0.12[b]	13.42±0.06[b]	11.13±0.11[b]	8.44±0.07[b]
Group III (diabetic+20mg/kg bw hesperetin)	20.18±0.03[a]	10.97±0.20[a]	6.72±0.08[a]	7.20±0.03[a]
Group IV (diabetic+40mg/kg bw hesperetin)	16.93±0.15[a]	7.33±0.05[a]	4.17±0.03[a]	4.27±0.06[a]
Group V (control+40mg/kg bw hesperetin)	15.97±0.11[a]	6.35±0.07[a]	4.02±0.05[a]	3.22±0.02[a]
Group VI	16.43±0.07[a]	6.08±0.06[a]	3.78±0.06[a]	3.79±0.09[a]

(diabetic +glibenclamide 1mg/kg bw)				

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

Table 4. Effect of hesperetin on kidney glycoproteins levels in the control and experimental rats

Groups	Hexose (mg/g)	Hexosamine (mg/g)	Sialic acid (mg/g)	Fucose (mg/g)
Group I (control)	9.95±0.04[a]	3.33±0.01[a]	1.42±0.01[a]	1.53±0.02[a]
Group II (diabetic)	19.14±0.08[b]	8.66±0.04[b]	5.25±0.05[b]	3.98±0.03[b]
Group III (diabetic+20mg/kg bw hesperetin)	13.02±0.02[a]	7.08±0.13[c]	3.17±0.04[a]	3.40±0.01[c]
Group IV (diabetic+40mg/kg bw hesperetin)	10.92±0.10[a]	4.73±0.03[c]	1.97±0.02[a]	2.01±0.03[c]
Group V (control+40mg/kg bw hesperetin)	10.31±0.07[a]	4.10±0.05[a]	1.90±0.02[a]	1.52±0.01[a]
Group VI (diabetic +glibenclamide 1mg/kg bw)	10.60±0.05[a]	3.92±0.04[c]	1.78±0.03[a]	1.79±0.04[c]

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

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 [10] who reported that administration of asiatic acid, a terpene to diabetic rats significantly decreased the plasma glucose level to near normal.

Glucose is the most important component for most living organisms. 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 [11].

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 [12]. Our results are finding in line with the study of reduced fucose by improved secretion of insulin in coumarin treated diabetic rats [13]. 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 [14]. 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 sialytransferase, which transfers the sialic acid residues to the glycolipids and glycoproteins [15]. Previous studies show that diosmin, produce the same effect in experimental diabetic rats [16].

From the above findings, we conclude that that oral administration of hesperetin exhibits great potential as an antidiabetic agent by improving hyperglycemia in STZ induced diabetic rats. It also improved plasma insulin levels and decreased glycoprotein components in plasma, liver and kidney. This can be used as an effective indicator to show the beneficial effects of hesperetin in controlling the progression and complications of diabetes.

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