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RESEARCH ARTICLE

EVALUATION OF ANTIDIABETIC EFFECT OF HESPERETIN ON NORMAL AND STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Hesperetin is a flavanoid commonly found in many herbal medicines and food. The antidiabetic effect of hesperetin was determined. The animals were divided into six groups such as normal, diabetic untreated, diabetic treated with (20mg hesperetin/kgbw in saline), diabetic treated with (40mg hesperetin/kgbw in saline), normal rat treated with 40mg of hesperetin/kgbw in saline) and diabetic treated with reference drug Glibenclamide (1mg/kgbw in saline). 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. The experiment is carried out for 45 days. After 45days, the rats were fasted for 12hr, anaesthetized and sacrificed by cervical decapitation. Blood samples were collected by and various biochemical parameters were measured using auto analyser. The levels of plasma glucose, insulin, blood haemoglobin and glycosylated haemoglobin were determined. The level of lipid profiles was also determined. Histopathology of pancreas was also determined. 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.

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INTRODUCTION

Diabetes mellitus is a complex metabolic disorder that involves chronic alterations in the carbohydrate, fat and protein metabolism, basically resulting from an imbalance between the biological action and normal secretion of insulin. It is known in the chronic form by elevated blood glucose concentration if untreated leads to severe thirst, profuse urination, polyphagia, weight loss and stupor (Akubue, 2000). Diabetes is associated with an increased risk of retinopathy, diabetic neuropathy, and colon cancer, thrombotic, atherosclerotic and cardiovascular diseases. There are two types of diabetes Type 1diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of beta cells of the endocrine pancreas. Type 2 diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency (American Diabetes Association, 2010).

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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 (Cao *et al*., 2007; Sharma *et al*., 2008). 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 (Horcajada *et al*., 2008) Hesperetin also plays a significant role in inflammation and cancer inhibition. Chemical compound Streptozotocin exhibits the most potent diabetogenicity and has been widely used for induction of experimental diabetes (Szkudelski, 2001). 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.

MATERIALS AND METHODS

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± 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*.

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.

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 (Shalini *et al.,* 2010). 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.

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) (Kalavathy *et al*., 2014).

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 45days plasma levels were estimated using the glucose oxidase method (Trinder, 1969). 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.

Histopathology

For histopathological study, rats from each group were perfused with cold physiological saline followed by formalin (10% formaldehyde).

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

Groups	0^{th} day	3^{rd} day	15^{th} day	30^{th} day	45^{th} day
Group I (control)	86.33±0.71	94.00±0.58	92.80±0.58^{a}	89.50±0.22^{a}	89.00±1.20^{a}
Group II (diabetic)	88.67±0.33	235.67±0.21	227.67±0.21^{b}	221.67±0.21^{b}	215.67±1.41^{b}
Group III (diabetic+20mg/kg bw hesperetin)	82.33±0.84	222.83±0.54	198.50±0.67^{c}	181.50±0.67^{c}	160.00±2.33^{c}
Group IV (diabetic+40mg/kg bw hesperetin)	86.67±0.49	215.67±0.80	180.67±1.02^{d}	155.67±1.02^{e}	127.50±1.31^{e}
Group V (control+40mg/kg bw hesperetin)	78.00±0.45	84.32±0.21	94.32±0.21^{a}	88.32±0.21^{a}	92.32±0.21^{a}
Group VI (diabetic +glibenclamide 1mg/kg bw)	84.67±0.33	217.00±0.37	175.50±1.26^{d}	139.50±1.26^{e}	115.92±1.45^{e}

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 2. Effect of hesperetin on haemoglobin, insulin and glycosylated haemoglobin in control and experimental rats

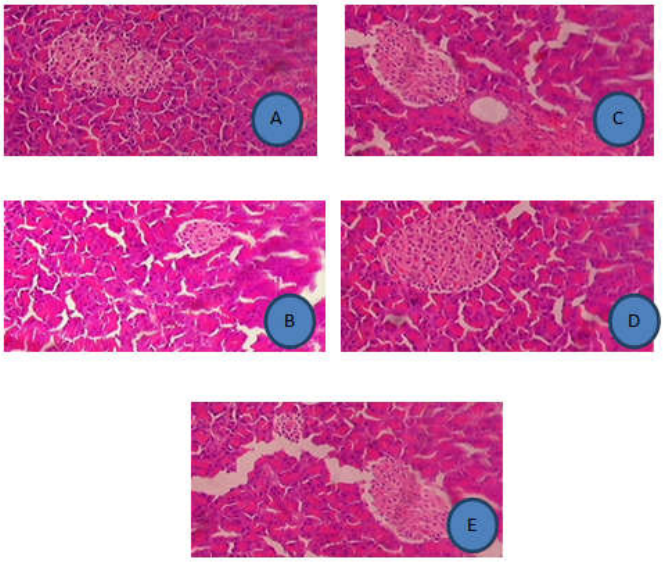
Groups	Haemoglobin (g/dL)	Insulin (µU/mL)	HbA_1C(%)
Group I (control)	15.20±0.12^{a}	16.12±0.08^{a}	4.64±0.02^{a}
Group II (diabetic)	9.86±0.19^{b}	7.23±1.01^{b}	8.38±0.02^{c}
Group III (diabetic+20mg/kg bw hesperetin)	11.47±0.06^{c}	11.13±1.01^{c}	6.91±0.02^{b}
Group IV (diabetic+40mg/kg bw hesperetin)	13.72±0.16^{d}	13.54±1.00^{d}	5.28±0.02^{a}
Group V (control+40mg/kg bw hesperetin)	14.61±0.10^{a}	17.21±0.08^{a}	5.00±0.02^{b}
Group VI (diabetic +glibenclamide 1mg/kg bw)	14.53±0.07^{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)

Table 3. Effect of hesperetin on triglycerides, total cholesterol, free fatty acids, high density lipoprotein-C and phospholipids

Groups	Triglyceride (mg/dl)	Total Cholesterol (mg/dl)	Free Fatty Acids (mg/dl)	HDL – CL (mg/dl)	Phospholipids (mg/dl)
Group I (control)	63.43±0.75^{a}	72.09±0.13^{a}	61.92±0.31^{a}	81.50±1.47^{a}	55.10±0.17^{a}
Group II (diabetic)	113.27±0.86^{b}	155.91±0.34^{b}	107.49±0.27^{b}	24.17±0.80^{b}	91.80±0.06^{b}
Group III (diabetic+20mg/kg bw hesperetin)	97.84±0.60^{c}	107.33±0.15^{c}	83.18±0.04^{c}	29.33±1.24^{b}	70.87±0.07^{c}
Group IV (diabetic+40mg/kg bw hesperetin)	68.25±0.25^{d}	75.17±0.16^{d}	61.19±0.03^{d}	60.50±1.02^{a}	50.76±0.09^{d}
Group V (control+40mg/kg bw hesperetin)	59.69±0.28^{a}	70.79±0.04^{a}	60.90±0.14^{a}	69.00±0.67^{a}	53.75±0.08^{a}
Group VI (diabetic +glibenclamide 1mg/kg bw)	60.14±0.18^{d}	71.88±0.06^{d}	61.85±0.44^{d}	66.67±0.69^{a}	52.75±0.19^{d}

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)



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

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.

Statistical analysis

Results are presented as mean ± 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 $p< 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the levels of plasma glucose measured at 15 days interval in control and experimental rats till the end of the experimental period. Significant ($p<0.05$) increase in the level of blood glucose was observed in Streptozotocin treated diabetic rats compared to control rats. Oral administration of hesperetin (20mg and 40mg) daily for a period of 45days to diabetic rats significantly ($p<0.05$) decreased the levels of blood glucose. The maximum glucose reduction was observed at the 45^{th} day rather than 15^{th} and 30^{th} days. The more pronounced effect was observed in the hesperetin treated diabetic rats at a dose of 40mg/kg body weight (Group IV).

Table 2 represents the levels of plasma insulin and blood haemoglobin were significantly decreased ($p<0.05$) and the levels of glycosylated haemoglobin were significantly ($p<0.05$) increased in diabetic rats compared to normal rats while the levels of these parameters in diabetic rats supplemented with hesperetin were reversed as compared to the diabetic rats (Group II). The effect of hesperetin was more pronounced in the rats supplemented with 40mg kg/bw of hesperetin (Group IV).

Table 3 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 (Marinangeli *et al*., 2006). Figure 1 A-E represents the photomicrographs of hematoxylin-eosin staining of pancreatic tissues of control and experimental rats. Fig1A 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 Cand E shows more pronounced in the rats supplemented with hesperetin (40mg/kg bw) and the reference drug glibenclamide (1mg/kg bw).

Conclusion

It may be concluded that the alterations of impaired blood glucose level status in diabetic condition have been restored to normal by administering synthetic compound and also indicates the protective role. The synthetic compound hesperetin increases insulin secretion, enhances insulin stimulated glucose uptake by modulating lipid and carbohydrate metabolism and hence it is concluded that hesperetin lowers the blood glucose level in diabetes.

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