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Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

EFFECT OF HESPERETIN ON HYPERGLYCEMIA-MEDIATED OXIDATIVE DAMAGE IN THE HEPATIC AND RENAL TISSUES OF EXPERIMENTAL DIABETIC RATS

J. Revathy[1] and S. Sheik Abdullah[2]

[1]Research Scholar, Research and Development Centre,
Bharathiyar University, Coimbatore-641046, Tamilnadu, India
[2]Biochemistry Faculty, Department of Chemistry and Biosciences,
Sastra University, Srinivasa Ramanujan Centre,
Kumbakonam – 612 001, Tamilnadu India.

Abstract

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. Hyperglycemia-mediated oxidative stress plays a crucial role in diabetic complications. Hence, this study was undertaken to evaluate the protective effect of hesperetin on the plasma glucose levels and on the antioxidant defense system in streptozotocin-induced diabetic rats. Oxidative stress was assessed by the reduction in the enzymic antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and nonenzymic antioxidant Vitamin C, E and reduced glutathione (GSH) were observed in the liver and kidney tissues of diabetic control rats as compared to control rats. Oral supplementation of hesperetin to diabetic rats for 45days significantly brought back the enzymic and nonenzymic antioxidants to normalcy. These findings suggest that hesperetin (40mg/kg bw) exerts a protective effect in diabetes by attenuating hyperglycemia-mediated oxidative stress and antioxidant competence in hepatic and renal tissues.

Key words: Hesperetin, enzymic and nonenzymic antioxidants, plasma glucose.

INTRODUCTION

Chronic hyperglycemia a clinical hallmark to diabetes results in metabolic imbalances and pathological changes in many cases [1]. Oxidative stress plays an important role in the etiology of diabetes and its complications. Decreased concentration of antioxidant enzymes and increased level of peroxidation products and reactive oxygen species were detected in type 2 diabetes [2].

Free radicals have significant role in the causation of several diseases like diabetes

Disease [3, 4]. Diabetes as an oxidant milieu evokes free radical generation via [5, 6, 7, 8] a) Non-enzymatic glycosylation of protein (apolipoproteins, enzyme and receptors) leads to chronically elevated lipids and lipoprotein b) Increased glucose metabolism through aldose reductase pathways altering the intracellular redox balance. Increased *de novo* synthesis of diacylglycerol and subsequent attenuation of antioxidant defense system creating oxidative stress.

These effects resulted in increased levels of lipids and lipoproteins to free radical generation. Increased production of reactive O2 species and N2 species (ROS/RNS) in human with diabetes contribute to endothelial, vascular and neurovascular dysfunction [9] and the changes in structural and functional integrity of subcellular organelles and membrane [10]. Oxidative stress causes damage to DNA, Lipids, Proteins, disruption in cellular
homeostasis and accumulation of damaged metabolites results [10]. The defence system established to diminish the damaging effect of free radical species includes enzymes such as Catalase, Glutathione peroxidase, Superoxide dismutase and non enzymatic constituents and vitamins. Recent reports state that dietary supplementation with natural antioxidants such as vitamin C and E, melatonin, flavanoids and amino acid L-arginine diminish the oxidative stress in diabetic state [11, 12, and 13] and improves endothelial cell function [14].

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 [15, 16] .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.

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, pH 4.5) after an overnight fast[7]. 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).

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. 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.

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

Table 1 shows a significant ($p<0.05$) increase in the level of blood glucose. Oral administration of hesperetin to diabetic rats significantly ($p<0.05$) decreased the levels of blood glucose.

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

Groups	0th day	15th day	30th day	45th day
Group I (control)	86.33±0.71	92.80±0.58^{a}	89.50±0.22^{a}	89.00±1.20^{a}
Group II (diabetic)	88.67±0.33	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	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	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	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	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 represents significant ($p< 0.05$) decrease in catalase, SOD and GP_X activities in the liver and kidney of diabetic rats compared with normal rats. Diabetic rats with hesperetin treatment showed significant ($p< 0.05$) increase in CAT, SOD and GP_X activities which reflect restoration of the antioxidant enzyme systems to near normal values.

Table 3 represents the levels of Vitamin C, Vitamin E and reduced glutathione in the liver and kidney tissues of diabetic control rats. Diabetic rats exhibited a significant ($p< 0.05$) fall in the levels of low molecular weight antioxidants when compared to normal control. Oral administration of hesperetin to diabetic rats shows improvement in the antioxidant level.

Table 2: Effect of hesperetin on enzymic antioxidants in control and experimental rats

Groups	Catalase (mM of H_2O_2 hydrolyzed/g tissue)		SOD (µM of Adrenochrome formed/g tissue)		GPx (µM of GSH oxidized/g tissue)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Group I (control)	1.66±0.01 a	0.60±0.00 a	3.17±0.01a	1.67±0.01 a	3.68±0.02 a	0.82±0.00 a
Group II (diabetic)	0.34±0.02 b	0.12±0.01 b	11.35±0.01 b	9.85±0.01 b	0.84±0.00 b	0.19±0.00 b
Group III (diabetic+20mg/kg bw hesperetin)	0.63±0.01 c	0.23±0.00 c	7.46±0.01c	5.96±0.01 c	1.18±0.03 c	0.26±0.01 c
Group IV (diabetic+40mg/kg bw hesperetin)	1.24±0.07 c	0.45±0.03 c	4.85±0.01c	3.35±0.01 c	2.96±0.02 c	0.66±0.01 c
Group V (control+40mg/kg bw hesperetin)	1.63±0.01 a	0.59±0.00 a	4.09±0.01a	2.59±0.01 a	3.44±0.03 a	0.76±0.01 a
Group VI (diabetic +glibenclamide 1mg/kg bw)	1.49±0.01 c	0.54±0.00 c	4.17±0.01c	2.67±0.01 c	2.96±0.02 c	0.66±0.01 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)

Table 3: Effect of hesperetin on non-enzymic antioxidants in control and experimental rats

Groups	Vitamin C (µg/g tissue)		Vitamin E (µg/g tissue)		Reduced Glutathione (µg/g tissue)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Group I (control)	235.58±0.44a	188.47±0.35a	20.54±0.09a	16.43±0.07a	11.16±0.02a	7.86±0.02a
Group II (diabetic)	208.66±0.21b	166.92±0.17b	12.40±0.03b	9.92±0.02 b	3.84±0.02 b	0.54±0.02b
Group III (diabetic+20mg/kg bw hesperetin)	215.30±0.30c	172.24±0.24c	16.44±0.04c	13.15±0.03c	7.26±0.03 c	3.96±0.03c
Group IV (diabetic+40mg/kg bw hesperetin)	226.82±0.56c	181.46±0.45c	18.54±0.06c	14.83±0.05c	8.82±0.09 c	5.52±0.09c
Group V (control+40mg/kg bw hesperetin)	223.57±0.37a	178.85±0.30a	19.34±0.06a	15.47±0.05a	9.82±0.02 a	6.52±0.02a
Group VI (diabetic +glibenclamide 1mg/kg bw)	228.74±0.26c	182.99±0.21c	18.46±0.07c	14.77±0.05c	10.75±0.02c	7.45±0.02c

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)

DISCUSSION

Diabetes mellitus is due to abnormality of carbohydrate metabolism and it is mainly linked with less insulin level or insensitivity of target organs to insulin [17]. The oral administration of hesperetin to diabetic rats reduced the plasma glucose levels to near normal glycemic concentration and is an essential trigger for the liver to revert its normal homeostasis during diabetes.SOD is an enzymatic antioxidant which catalyzes the conversion of superoxide radical to hydrogen peroxide and molecular oxygen. Other enzymic antioxidant CAT catalyzes the reduction of hydrogen peroxides and protects the tissues against reactive hydroxyl radicals. In the present study, the low levels in the activity of SOD, CAT and GP_X in diabetic rats indicated diabetes-induced stress and a significant elevation of these antioxidant enzymes activities was observed in the hesperetin treated diabetic rats which might be due to its scavenging property.

Vitamins C and E are diet derived and detoxify free radicals directly. [18] Reported that Vitamin C depletion leads to formation of hydroperoxides and this may due to increased utilization in trapping the oxyradicals. Vitamin E a liphophilic antioxidant transfers its phenolic hydrogen to a peroxyl free radical of peroxidized polyunsaturated fatty acids, thereby breaking the radical chain reaction and preventing the peroxidation of membrane lipids [19]. The administration of hesperetin to diabetic group of rats significantly reverted back the altered back the altered levels to near normalcy, which in turn reveals the antioxidant potential of hesperetin.GSH functions as free radical scavenger and in the repair of free radical caused biological damage [20].

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