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EVALUATION OF ANTIDIABETIC ACTIVITY OF CITRUS FLAVANOID ON STREPTOZOTOCIN INDUCED DIABETIC MALE ALBINO RATS

D. Jeevan Mani Babu*, B. Ramesh Babu, Y. Rajasekhar Reddy, Ch.V. Satyanarayana, R. Mahalakshmi, N. Pavan Madhuri, Limiaa Adam Abdalkareem Mohamed, Fuada Abdelkarim Mohamed Farah, Mohamed Abdelraziq Adam Abdalla

Department of Pharmacology, Nova College of Pharmaceutical Education and Research, Jupudi, Andhra Pradesh, India

ABSTRACT

Aim of the study is to evaluate the antidiabetic activity of the Hesperdin of *Citrus aurantium* (family: Rutaceae) in experimatel animal models. The administration of streptozotocin produced a rise in the levels of glucose compared to control group($p<0.01$). Hesperidin treatment produced dose dependent decrease in the levels of glucose compared with the streptozotocin treated group($p<0.01$). These above data may indicate that the protective effect of hesperdin against renal damage in diabetic rats.

Key Words: Antidiabetic activity, Hesperidin, *Citrus aurantium*

Author for correspondence
D. Jeevan Mani Babu,
Department of Pharmacology, Nova College of Pharmaceutical Education and Research,
Jupudi, Andhra Pradesh, India.
Email: drjeevanbabu@gmail.com

INTRODUCTION

Diabetes mellitus is a disease which is characterized by increased blood glucose levels known as hyperglycemia due to abnormality either in insulin secretion, insulin action, or both. The severe hyperglycemia of diabetes leads to long-term damage, abnormality in function, and failure of major organs, especially the nerves, kidneys, eyes, heart, and blood vessels. Several pathogenic mechanisms are involved in the development of diabetes. The prevalence of affecting earlier aged people and number of deaths increased yearly. The diabetic population is increasing due to population growth, stress, food habits, obesity and decreased physical activity. India leads the world with largest number of diabetic individuals and termed the “diabetes capital of the world”. According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently is around 40.9 million and it may rise to 69.9 million by 2025 if preventive measures are not taken. The difference in the prevalence rate of diabetes between urban and rural was found in which environmental factors had a significant role to play. Untreated diabetes mellitus leads to increased prevalence of micro vascular and macro vascular diseases. With the increased diabetic population, the number of people suffering from the

vascular complications of diabetes will also increase. Genetic factors also may play a role in individual susceptibility to diabetic neuropathy and also nephropathy. The symptoms of marked hyperglycaemia include: Polyuria, Polydipsia, Polyphagia, Weight loss, Tiredness, Blurred vision, and Infections.

Type I diabetes is a multifactorial autoimmune disease due to severe insulin deficiency that is influenced by environmental and genetic factors. Type II diabetes is a non immune disorder with varying degrees of insulin resistance and impaired insulin secretion usually associated with obesity. Although there is difference in causation of both types of diabetes, it has been assumed that the neuropathy of types I and II diabetes result in hyperglycemia. But there are structural and electro physiologic differences exist between these two types of neuropathies, at least in experimental animals .The 10 countries estimated to have the highest numbers of people with diabetes in 2010 are represented in diagram. In the light of the observed increase in prevalence of obesity in many countries of the world and the importance of obesity as a risk factor for diabetes, the number of cases of diabetes in 2030 may be considerably more which leads to neuropathy and nephropathy.

Streptozotocin is a chemical substance specifically toxic to pancreatic β cells. It is taken into the pancreatic β-cells by glucose transporter 2 (GLUT-2). When injected into adult rats, streptozotocin can cause type 1 diabetes with severely elevated blood glucose level. However, when streptozotocin is administered to rats, the hyperglycemia within the first few days and the blood glucose gradually decreases thereafter. The surviving rats exhibit decreased β-cell mass and develop type 2 diabetes with the features described in type 2 diabetes patients (hyperglycemia, polyphagia, polydipsia, polyuria, insulin resistance and abnormal glucose tolerance) after adulthood. Therefore, this model provides an ideal platform for β-cell regeneration study and new anti-diabetic drug screening. Recently there have been growing interests in the application of natural components as antidiabetic agents. The diabetogenic agent streptozotocin inhibits insulin secretion and causes a state of insulin-dependent diabetes melliyus through its ability to induce a selective necrosis of the pancreatic beta cells.Both effects can be assorted to the alkylating potency of streptozotocin.The common chemical denominator of the two effects is the selective cellular uptake and the accumulation of streptozotocin by the beta cell. The toxic action of streptozotocin and chemically related alkylating compounds requires their uptake into ihe cells.Streptozotocin is less lipophilic and this significantly affects the cellular distribution.Thegreater lipophilicity of streptozotocin reduces itschance to enter cells without support and also prevent access to the brain via the blood-brain barrier. Streptozotocin has also been employed in the treatment o human islets-cell carcinomas and malignant carcinoid tumours.However clinical streptozotocin cancer treatment trypically. The selective pancreticbeta cell toxicity of streptozotocin and the resulting diabetic metabolic state are clearly related to the glucose moity in its chemical structure. Toxicity of streptozotocin and related compounds resides in theirability to alkylate biological macromolecules.It is generally assumed that the toxic activity of streptozotocin relates to the DNA alkylatingactivity of its methlnitrosourea moity.especially at the O-6 position of guanine (1-4).

Natural plant products have shown tremendous potential to serve as the alternative therapeutic agents so as to counter the side effects of various over the counter drugs. The main objective of the study was to evaluate the antidiabetic activity of the Hesperdin of *Citrus aurantium*(family: Rutaceae) in experimatel animal models

MATERIALS AND METHOD

Animals

Male Wistar rats weighing 160-240 g were obtained from National Institute of Nutrition Hyderabad. Animals were housed under standard laboratory conditions of 22 ± 3^0 c temperature and relative humidity 30% and 12 h light and dark cycle, free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol.

Route of administration

Streptozotocin was administerd through intraperitoneal route (dissolving in citrate buffer 0.1mol/L,pH 4.2)in a dose of 90mg/Kg bodyweight for 3 successive days. Hesperidin administered

through oral route (dissolved in tween 80) in a dose of 40mg/Kg&80mg/Kg bodyweight.

Induction of Diabetes (5-8)

A single dose of Streptozotocin 90 mg/kg body weight was dissolved in citrate buffer 0.1 mol/L,Ph 4.2 and given intraperitoneal as for induction of diabetes in rats after 12 hr fasting. following Streptozotocin administration, the animals were fed with standard pellets and water *ad libitum.* 5% dextrose in 0.9% NaCl solution was provided to the animals for 10 hrs to overcome hypoglycemia. After two days of injection, fasting blood glucose level was measured

Experimental design

Rats were divided into four groups with each group containing 6 animals. Group 1: Normal rats treated with saline (control), Group 2: Diabetic untreated rats, Group 3: Diabetic rats treated with Hesperdin 40mg/kg orally, Group 4: Diabetic rats treated with Hesperdin 80mg/kg orally.

Withdrawl of blood

Blood was withdrawn from tail vein of rats for measurement of blood glucose levels to confirm diabetes after induction of Streptozotocin. After the treatment period blood was withdrawn by retro orbital puncture into tubes after ethar anaesthesia

Estimation of Blood Glucose levels

Blood glucose levels were measured by GOD- POD method. All the reagents were brought to room temperature. Three dried test tubes were labeled as standard (S), test (T), blank (B) were taken. 10 µl of standard reagent, 10 µl of test sample and 10 µl of distilled water were added to the respective test tubes. 10 µl of working reagent was added to each of three test tubes. All tubes were mixed well and incubated at room temperature for 5 min. The concentration was determined directly in mg/dl using Erba Chem 5 Plus v2 advanced semi automatic analyzer.

Estimation of serum cholesterol levels

Cholesterol levels were measured by CHOD – PAP method. All the reagents were brought to room temperature. Three dried test tubes were labeled as standard (S), test (T), blank (B) were taken. 10 µl of standard reagent, 10 µl of test sample and 10 µl of distilled water were added to the respective test tubes. 10 µl of working reagent was added to each of three test tubes. All tubes were mixed well and incubated at room temperature for 5 min. The concentration of cholesterol was determined using Erba Chem 5 Plus v2 advanced semi automatic analyzer.

Estimation of serum triglycerides levels

Triglyceride levels were measured by GPO –PAP method. All the reagents were brought to room temperature. Three dried test tubes which are labeled as standard (S), test (T), blank (B) were taken. 10 µl of standard reagent, 10 µl of test sample and 10 µl of distilled water were added to the respective test tubes. 10 µl of working reagent was added to each of three test tubes. All tubes were mixed well and incubated at room temperature for 5 min. The concentration of triglycerides was determined by using Erba Chem 5 Plus v2 advanced semi automatic Analyzer.

Statistical analysis

Data are expressed as mean ± SEM. Analysis of data was done by One-way ANOVA followed by Tukey-Kramer comparison test. Graph Pad In Stat version 3.10 for Windows 2009 (Graph Pad Software) was used. The statistical significance was set as 0.05 ($p<0.05$).

RESULTS AND DISCUSSION

Hesperdin has shown significant effects on blood glucose levels, cholesterol and triglyceride levels in diabetic rats. Low dose (40mg/Kg) of hesperdin was not shown significant less effects on diabetes but higher dose (80mg/Kg) of hesperdin was shown significant antidiabetic effects. Longer duration of diabetes which is increased levels of blood glucose leads to several complications which are myocardial infarction, atherosclerosis, neuropathy, and nephropathy etc.[72]Streptozotocin causes the destruction of β-cells of the Langerhans, and inducing hyperglycemia by reducing insulin levels.[73] In present study Streptozotocin increased blood glucose levels indicating diabetes symptoms which are reduced by hesperdin effectively may be due to antioxidant effect of it. Here hesperdin decreased the formation of free radicals by Streptozotocin which inhibit insulin secretion.

Effect of Hesperidin on glucose levels in Streptozotocin induced diabetic rats

The administration of streptozotocin produced a rise in the levels of glucose compared to control group($p<0.01$). Hesperidin treatment produced dose

dependent decrease in the levels of glucose compared with the streptozotocin treated group($p<0.01$) (Fig-1).

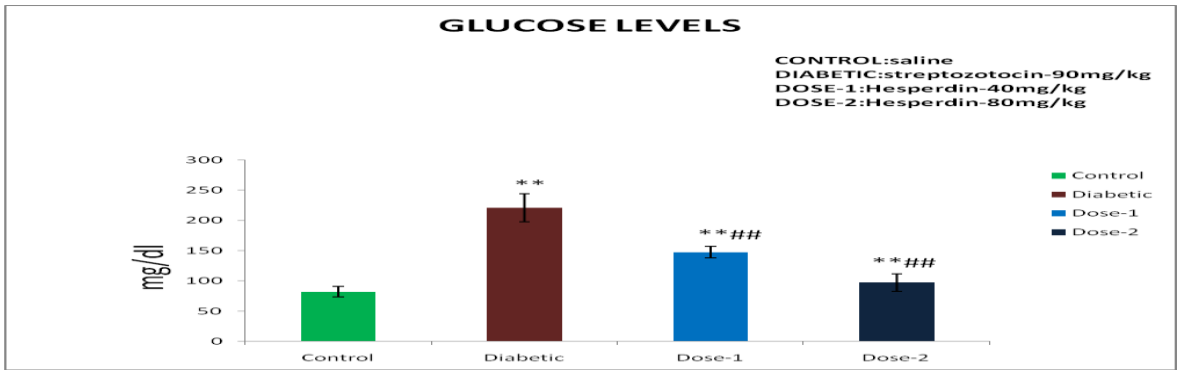

Fig-1 Effect of Hesperidin on glucose levels in streptozotocin induced diabetic rats

Values are Mean ± SEM; n= 6,$^{**}p<0.01$compared to control group, $^{\#\#}$ $p<0.01$compared to diabetic group.

Effect of Hesperidin on insulin levels in streptozotocin induced diabetic rats

With streptozotocin administration the insulin levels decreased compared to the normal control group($p<0.01$).However treatment with hesperidin produced increasein the insulin levels compared with the diabetic group($p<0.01$) (Fig-2).

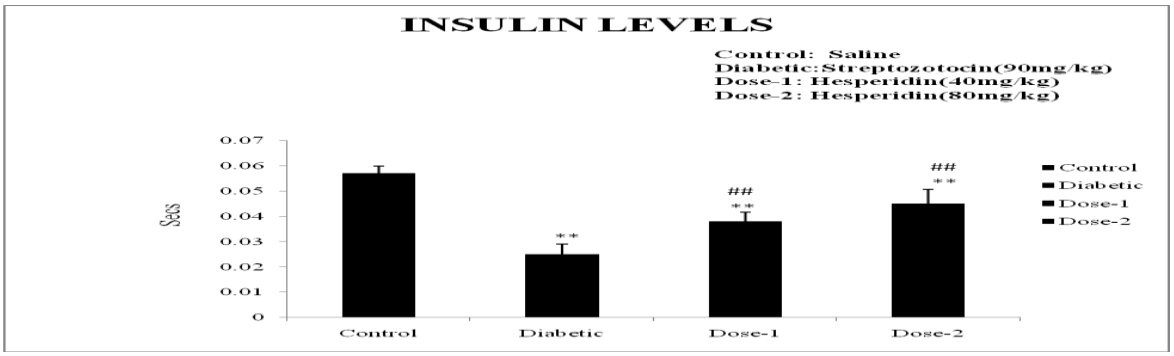

Fig-2 Effect of Hesperidin on insulin levels in streptozotocin induced diabetic rats

Values are Mean ± SEM; n= 6;$^{**}p<0.01$compared to control group,$^{\#\#}$ $p<0.01$compared to diabetic group

Effect of hesperidin on triglyceride levels in streptozotocin induced diabetic rats

Streptozotocin administrationcoused a rise in the levels of triglyceride compared with normal control group ($p<0.01$). Ttreatment with hesperidin these levels decresed in a dose-dependent manner compared to diabetic group.($p<0.01$) (Fig-3).

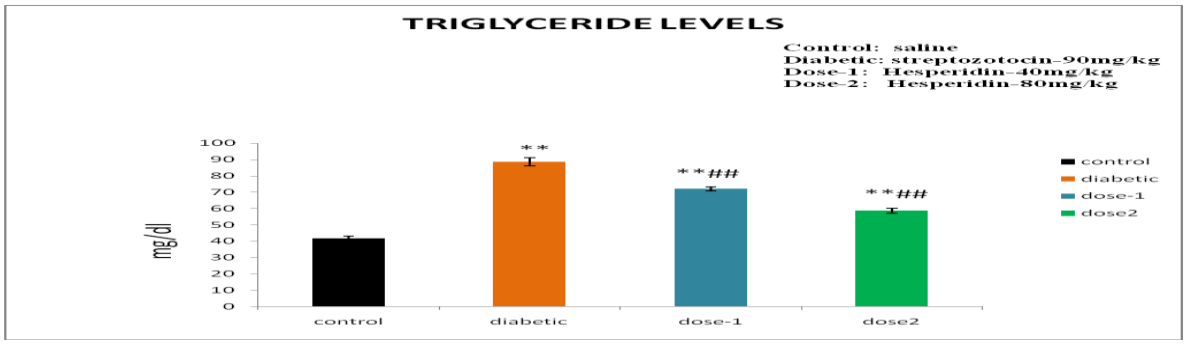

Fig-3 Effect of hesperidin on triglyceride levels in streptozotocin induced diabetic rats

Values are Mean ± SEM; n= 6;$^{**}p<0.01$compared to control group, $^{\#\#}$ $p<0.01$compared to diabetic group

Effect of hesperidin on total protein levels in streptozotocin induced diabetic rats

On administration of streptozotocin total protein levels decreased as compared to the normal control group ($p<0.01$). Treatment with hesperidin increased the levels significantly (Table-1).

Table-1 Effect of Hesperidin on total protein levels in streptozotocin induced diabetic rats

S.No	Groups	Treatment	Duration	Total Protein(mg/g)
1	Control	Normal saline	30 DAYS	133.5±2.54
2	Diabetic	Streptozotocin(90mg/kg)		75.7±1.254**
3	Dose-1	Hesperidin(40mg/kg)	30 DAYS	109.1±1.55**##
4	Dose-2	Hesperidin(80mg/kg)	30 DAYS	127.3±2.26**##

Values are Mean ± SEM; n= 6; ****p<0.01compared to control group,** $^{\#\#}$ **p<0.01compared to diabetic group**

Effect of hesperidin on superoxide dismutase (SOD)levels in streptozotocin induced diabetic rats

On streptozotocintreatment,superoxide levels were decreased in the diabetic group as compared to the normal control group. Treatment of rats with hesperidin significantly increased the kidney SOD levels compared to the diabetic group (Table-2).

Table-2 Effect of Hesperidin on superoxide dismutase levels in streptozotocininduced diabetic rats

S.NO	Groups	Treatment	Duration	SOD(U/mg)
1	Control	Normal saline	30 DAYS	32.04±1.29
2	Diabetic	Streptozotocin(90mg/kg) + Saline	3DAYS+ 27 DAYS	17.12±0.52**
3	Dose-1	Hesperidin(40mg/kg)	30 DAYS	24.14±1.47**##
4	Dose-2	Hesperidin(80mg/kg)	30 DAYS	27.73±2.12**##

Values are Mean ± SEM; n= 6; ****p<0.01compared to control group,** $^{\#\#}$ **p<0.01compared to diabetic group**

Effect of hesperidin on catalase(CAT)levels in streptozotocin induced diabetic rats

On the streptozotocin treatment, catalase levels were decreased in diabetic group as compared to the normal control group. Treatment of hesperidin significantly increased the catalase levels compared to the diabetic group (Table-3).

Table-3 Effect of Hesperidin on catalase levels in streptozotocin induced diabetic rats

S.NO	GROUPS	TREATMENT	DURATION	CATALASE(U/mg)
1	Control	Normal saline	30 DAYS	17.51±1.75
2	Diabetic	Streptozotocin(90mg/kg) +Saline	3 DAYS+ 27 DAYS	5.06±0.526**
3	Dose-1	Hesperidin(40mg/kg)	30 DAYS	12.52±1.24**##
4	Dose-2	Hesperidin(80mg/kg)	30 DAYS	16.22±1.66**##

Values are Mean ± SEM; n= 6; ****p<0.01compared to control group,** $^{\#\#}$ **p<0.01compared to diabetic group**

CONCLUSION

Present study revealed the ability of the hesperdin to reduce levels of blood glucose, cholesterol and triglycerides. On the basis of our study hesperdin has significant potential to treat diabetic symptoms. We suggest that hesperdin could be considered an additional weapon in the management of diabetes and its complications. These above data may indicate that the protective effect of hesperdin against renal damage in diabetic rats.

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