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Antihyperglycemic effect of hesperetin, a citrus flavonoid, extenuates hyperglycemia and exploring the potential role in antioxidant and antihyperlipidemic in streptozotocin-induced diabetic rats

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ABSTRACTS

Diabetes is the major health problem in modern civilization which occurs due to inadequate metabolism of carbohydrate and lipid could cause tremendous changes in the metabolic activities of liver. In this study, we investigated the antihyperglycemic, antioxidant and antihyperlipidemic effects of hesperetin, a citrus flavonoid against streptozotocin (STZ)-induced experimental rats. To stimulate diabetes mellitus, rats were injected with STZ intraperitoneally at a single dose of 45 mg/kg. STZ induced rats showed marked increase in the level of plasma glucose and significant reduction in the level of plasma insulin. The activities of carbohydrate metabolic enzymes, hepatic glycogen, lipid profiles, enzymic antioxidants in circulatory system and pancreas, hepatic and renal functional markers were explored. Supplementation with hesperetin (40 mg/kg b.w) to STZ-induced experimental rats for 45 days established a significant decline in plasma glucose and a marked improvement in plasma insulin and glycogen levels in STZ-induced rats. The altered activities of hepatic glucose metabolic enzymes, lipid profiles, enzymic antioxidants and serum biomarkers of liver and kidney toxicity were restored to almost normal. The acquired outcome were compared with glibenclamide (1 mg/kg b.w), a standard oral hypoglycemic drug. Hesperetin treatment was found to be efficient in protecting the normal histological manifestation of hepatic, renal and insulin positive β-cells in STZ induced rats. On the basis of current experimental findings, we concluded that administration of hesperetin attenuates the hyperglycemia and dyslipidemia through ameliorating antioxidant competence in STZ-induced experimental rats.

1. Introduction

Diabetes, a group of metabolic diseases in which blood sugar levels are abnormally high over a prolonged period, has become a serious public health problem with tremendous social and economic burden on society [1]. Hyperglycemia and hyperlipidemia are the common features for diabetes mellitus with alteration of glucose and lipid metabolism and modification of liver enzyme levels. Chronic hyperglycemia characterized by persistent and strangely high postprandial blood glucose levels, has been established as a key contributing factor to the onset of diabetes [2]. Insulin is the only hormone that controls plasma glucose homeostasis, and promotes glucose consumption, glycogenesis of hepatic tissue. The inconsistent regulation of glucose and deteriorated carbohydrate utilization that result from this flawed insulin production are the vital pathogenic actions in diabetes.

According to the latest statistics released by the International Diabetes Federation (IDF) that the prevalence of diabetes was estimated to be 8.8% in 2015 and predicted to rise to 10.4% in 2040 among which 87 million will be from India and 5 million people worldwide died from diabetes in 2015 [3]. The cytotoxic effect of STZ is connected with the development of reactive oxygen species (ROS) causing oxidative damage. The augmented oxidative stress, as determined by indices of enhanced Lipid peroxidation (LPO), reduction of endogenous antioxidant, and antioxidant enzymes in plasma and tissues, are commonly found in rats with STZ-induced diabetes [4]. Hyperlipidemia is metabolic complication of both clinical and experimental diabetes. Impairment of the biological action of insulin at the cellular level is supposed to be a fundamental and key underlying metabolic defect in the

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progress of the characteristic dyslipidemia observed in diabetes.

The pathogenesis of diabetes is still not very clear and the treatment with chemical drugs for it still conquered in clinic, but the chemical drugs have shown varying degrees of side effects and can exacerbate the symptoms of diabetes. Due to these reasons, the researchers have turned to natural therapies has inspired novel wave of research to look for more efficient agents with slighter side effects. The role played by dietary factors on health status has long been documented but recently that epidemiological and clinical studies have provided a clearer insight on the chemical and physiological mechanisms of the effects of bioactive foods on human health [5].

In this connection, a number of bioactive molecules found in fruits, vegetables, food constituents and other natural sources are being constantly discovered for their direct or indirect benefits in treatment diabetes [6]. There is a growing interest in the elucidation of the biological roles of flavonoid, the main components of some conventional medicinal plants. Hesperetin a 3′, 5, 7-trihydroxy-4′-methoxy flavanone belonging to the class of flavonoids named flavanones is one of the most plentiful flavonoids found in citrus fruits. Hesperetin possesses a huge array of pharmacological effects and is the aglycone of hesperidin, the predominant flavone glycoside found in citrus fruits [7]. Recently, we have found that hesperetin has an antidiabetic effect and also revokes the alterations in the levels of the glycoprotein components [8]. Although the competence of hesperetin to improve diabetic status has been reported, since, no systematic studies exist in the literature on the effect of hesperetin on glucose metabolic enzymes and lipid profile in diabetes in this view, we investigated to determine the antihyperglycemic and antihyperlipidemic efficacy of hesperetin on plasma and tissue in streptozotocin (STZ)-induced experimental rats.

2. Materials and methods

2.1. Chemicals

Hesperetin (CAS Number: 69097-99-0, Product Number: W431300, Formula: $C_{16}H_{14}O_6$, Purity: (GC) ≥ 95.0%, Color: Faint yellow to Light yellow to Light beige, Appearance (Form): Powder with a Light Tan Cast, Specification: PRD.1.ZQ5.10000010204) and STZ were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used in the experiments were of analytical grade and high purity.

2.2. Animals

The use of animals was approved by the Institutional Animal Ethical Committee formed by CPCSEA, Ministry of Social Justices and Empowerment, Government of India, (Reg. No: SAC/IAEC/BC/2015/Ph.D-009). Male albino Wistar strain rats, weighing about 170–200 g were housed in steel cages at room temperature (28 ± 2 °C) with a 12 h dark-light cycle and had free access to food and water ad libitum.

2.3. Induction of diabetes

For successful induction of experimental diabetes, a freshly prepared solution of STZ (45 mg/kg b.w) dissolved in 0.1 M ice-cold citrate buffer (pH 4.5) was administered intraperitoneally immediately after preparation to overnight fasted rats. As STZ is capable of inducing fatal hypoglycemia due to massive pancreatic insulin release, the rats were given 10% glucose solution after 6 h of STZ administration for the next 24 h to overcome drug-induced hypoglycemia. After 72 h, the animals with a blood glucose level above 250 mg/dL confirmed as diabetic and preferred for the experiment.

2.4. Experimental protocol

Hesperetin dissolved in a vehicle solution of 1 mL of 0.9% saline was intragastrically given to rats daily for 45 consecutive days. All animals were randomly divided into 5 groups, comprising a minimum of 6 animals in each group as follows. Group I: served as normal untreated control rats given standard pellet diet; Group II: normal rats received hesperetin (40 mg/kg b.w) intragastrically; rats with STZ induction of 45 mg/kg b.w were considered diabetic, and randomly divided into 3 equal groups, Group III: diabetic control; Group IV: hesperetin treated (40 mg/kg b.w); Group V: glibenclamide treated (1 mg/kg b.w) and the treatment of groups IV and V were initiated after 3 days of STZ induction.

At the end of the experimental period, the animals were deprived of food overnight and sacrificed by decapitation. The blood was collected with and without anti-coagulants for plasma and serum separation, respectively. The liver, kidney and pancrease were excised, weighed and separated into portions for biochemical estimations and histological appraisal.

2.5. Biochemical analysis

The level of plasma glucose was estimated spectrophotometrically using commercial diagnostic kits (Sigma Aldrich, Bangalore, India) by the method of Trinder [9]. Plasma insulin level was determined using the sensitive rat insulin radioimmunoassay kit (Linco Research, Inc., St. Charles, MO). The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatinine and uric acid were assayed using commercially available diagnostic kits (Agappe Diagnostic Pvt. Ltd., India) according to the manufacturer's protocol. The estimation of protein was determined according to the method of Lowry et al. [10].

2.6. Determination of hepatic glucose metabolic enzymes activities and glycogen content

Liver was homogenized in ice-cold isotonic physiological saline (0.1 g mL^{-1}) and centrifuged (3000 × *g*) at 4 °C for 10 min to obtain the required supernatant for the assays of key enzymes of glucose metabolism. Glucokinase activities were determined by the method of Brandstrup [11]. Glucose 6 phosphate dehydrogenase was assayed by the method of Bergmeyer [12]. The activities of glucose 6 phosphatase and fructose 1, 6 bisphosphatase were estimated according to the methods of Koide and Oda [13] and Dziewulska Szwajkowskai et al. [14] respectively. Glycogen content of liver was measured according to previously originated method of Ong and Khoo [15].

2.7. Assay of circulatory and pancreatic antioxidant system

Pancreatic tissues were homogenized in 0.1 moi/L Tris HCI buffer, pH 7.4, and centrifuged at 12000 × *g* for 30 min at 4 °C. The activity of antioxidant enzymes were evaluated by standard protocols with minor modification. Plasma and pancreatic supernatant was collected and used for assays to determine the activities of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione peroxidase (GPx) according to methods described previously [16–19].

2.8. Measurement of hepatic lipid profiles

The total cholesterol was carried out following the protocol of Searcy and Bergquist [20]. Free fatty acids (FFAs) were evaluated by the method of Falholt et al. [21]. The levels of triglycerides (TGs) were measured by the method of Fossati and Lorenzo [22]. The phospholipids (PLs) concentration was estimated by the ferrous ammonium thiocyanate method [23].

2.9. Histopathological assessment

Liver and kidney tissues from each group were fixed in 4% formaldehyde solution for 24 h. After regular tissue processing, the tissues