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Research paper

Protective effect of esculetin on hyperglycemia-mediated oxidative damage in the hepatic and renal tissues of experimental diabetic rats

Dhamodaran Prabakaran, Natarajan Ashokkumar*

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India

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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 esculetin on the plasma glucose, insulin levels, tissue antioxidant defense system and lipid peroxidative status in streptozotocin-induced diabetic rats. Diabetic rats exhibited increased blood glucose with significant decrease in plasma insulin levels. Extent of oxidative stress was assessed by the elevation in the levels of lipid peroxidation markers such as thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (HP) and conjugated dienes (CD); reduction in the enzymic antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST); nonenzymic antioxidants 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 esculetin to diabetic rats for 45 days significantly brought back lipid peroxidation markers, enzymic and nonenzymic antioxidants to near normalcy. Moreover, the histological observations evidenced that esculetin effectively rescues the hepatocytes and kidney from hyperglycemia mediated oxidative damage without affecting its cellular function and structural integrity. These findings suggest that esculetin (40 mg/kg BW) treatment exerts a protective effect in diabetes by attenuating hyperglycemia-mediated oxidative stress and antioxidant competence in hepatic and renal tissues. Further, detailed studies are in progress to elucidate the molecular mechanism by which esculetin elicits its modulatory effects in insulin signaling pathway.

1. Introduction

Chronic hyperglycemia, a clinical hallmark to diabetes results in severe metabolic imbalances and pathological changes in many tissues. In 2011 there are 366 million people with diabetes, and this is expected to rise to 552 million by 2030 [1]. Oxidative stress plays an important role in the etiology of diabetes and its complications. Indeed, hyperglycemia is contemplated to generate reactive oxygen species (ROS) through various pathways, such as increased advanced glycation products, activation of protein kinase C, damage of the redox equilibrium or overproduction of mitochondrial superoxides that ultimately leads to oxidative stress in variety of tissues [2]. Decreased concentration of antioxidative enzymes and increased level of peroxidation products, and reactive oxygen species were detected in type 2 diabetes [3]. ROS, including superoxide radical ($O_2^{-\bullet}$), alkoxyl radical (RO^0), peroxyl radical (ROO•), hydroxyl radical ($\bullet OH^0$) and hydrogen peroxide (H_2O_2) are unstable molecules. When the generation of ROS exceeds cellular defense mechanisms, the ROS interact with biological macromolecules such as lipids, proteins and DNA, which could cause structural changes as well as functional abnormalities [4]. For this reason, oxidative stress has been considered to be a common pathogenic factor in the development of type 2 diabetes and its complications. Streptozotocin (STZ), an antibiotic produced by *Streptomyces achromogenes*, is frequently used to induce DM in experimental animals through its toxic effects on pancreatic β-cells. The cytotoxic action of STZ is associated with the generation of ROS causing oxidative damage [5].

Oxidative stress occurs when free radical production exceeds the antioxidant capacity of a cell. Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates and additives of variety of food to prevent or deter free radical-induced lipid oxidation. Most of these radicals are reactive oxygen species, such as hydroxyl radical, hydrogen peroxide, and superoxide anion, all of which can damage crucial cellular compounds, such as lipids, carbohydrates, proteins, and DNA [6]. These antioxidant defenses

* Corresponding author. Tel.: +91 4144 238343; fax: +91 4144 239141.
E-mail address: npashokkumar1@gmail.com (N. Ashokkumar).

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are extremely important since they represent the direct removal of free radicals, providing maximal protection for biological sites [7]. Clinical research has confirmed the efficacy of several plants and its active compounds in the modulation of oxidative stress associated with DM. Currently, the antidiabetic drugs in use for long term therapy are found to be associated with various toxicities owing to which the developmental process in antidiabetic drug discovery has shifted its focus to natural plant sources, which may alleviate oxidative stress, thus assist in managing diabetes mellitus [8].

Among the natural products, coumarin compounds are attracting the interest because of their beneficial effects in human health [9]. Esculetin (6,7-dihydroxycoumarin) (Fig. 1) is a coumarin derivative found in various plants that are used as folk medicines, such as *Artemisia scoparia*, *Artemisia capillaries*, *Ceratostigma willmottianum* and *Citrus limonia* [10]. They have been associated with the health benefits derived from consuming fruits and vegetables [11]. Esculetin has been reported to have several pharmacological effects, including anti-inflammatory, antioxidative and anti-tumor activities [12–14]. Some of the preliminary work has been revealed out the antihyperglycemic effect of *Matricaria chamomilla L.*, one of the active pytochemical esculetin present in the plant [15]. The biotransformation of esculetin occurs from 8 to 12 h and six esculetin metabolites were identified in rat urine; [16] drug bioavailability results indicate higher drug concentrations in bile than in blood [17]. Recently, we have reported that the antihyperglycemic effect of esculetin modulated carbohydrate metabolic enzymes activities in streptozotocin induced diabetic rats [18]. In vivo condition, esculetin has been showed LD 50 value greater than 2000 mg by mouth [19]. In the present study, we evaluated plasma glucose, insulin, lipid peroxidation (LPO) products, enzymic and nonenzymic antioxidants in the liver and kidneys of hyperglycemia mediated oxidative stress in STZ induced type 2 diabetic rats. In addition, the effects of esculetin on the histopathological alterations of liver and kidneys in control and diabetic rats were also studied.

2. Materials and methods

2.1. Animals

Male albino (9 week-old) rats of Wistar strain with a body weight ranging from 180 to 200 g, were procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamilnadu, India and all the animals used in this experiment were acclimatized at the above place for one week prior to start the experiments and were maintained in an air conditioned room (25 ± 1 °C) with a 12 h light/12 h dark cycle. Standard pellet diet and water were provided *ad libitum* to all the animals. The study protocols were approved by the Institutional Animal Ethics Committee (Reg No.160/1999/CPCSEA, Proposal number: 712), Annamalai University, Annamalainagar, Tamilnadu, India.

2.2. Chemicals

Streptozotocin and esculetin were purchased from Sigma–Aldrich, St. Louis, MO, USA. All other chemicals were of analytical grade and obtained from E. Merck and Himedia, Mumbai, India.

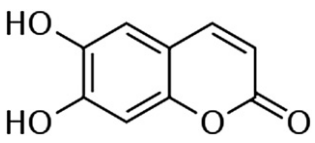

Fig. 1. The structure of esculetin (Molecular weight: 178.14).

2.3. Experimental induction of diabetes

The animals were rendered diabetic by a single intraperitoneal injection of streptozotocin (40 mg/kg BW) in freshly prepared citrate buffer (0.1 M, pH 4.5) after an overnight fast. Streptozotocin injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality [20]. Streptozotocin-injected animals exhibited hyperglycemia within a few days. Diabetes was confirmed by measuring the fasting blood glucose level more than 235 mg/dl, after 72 h of injection.

2.4. Experimental design

The animals were randomly divided into six groups of six rats each as given below. Esculetin (98% purity) was dissolved in aqueous solution and administered orally using intragastric tube once in a day in the morning after the food delivery for 45 days.

Group 1:	Control rats
Group 2:	Control + esculetin (40 mg/kg body weight (BW))
Group 3:	Diabetic control rats
Group 4:	Diabetic + esculetin (10 mg/kg BW)
Group 5:	Diabetic + esculetin (20 mg/kg BW)
Group 6:	Diabetic + esculetin (40 mg/kg BW)

After 45 days of treatment, the 12 h fasted animals were anaesthetized between 08.00 a.m. and 09.00 a.m., using ketamine (24 mg/kg BW. intramuscular injection) and sacrificed by decapitation. Blood was collected in tubes with ethylenediamine tetra acetic acid (EDTA) for the estimation of plasma glucose and insulin levels.

2.5. Tissue homogenate preparation

Liver and kidney tissues (250 mg) were sliced into pieces and homogenized in appropriate buffer in cold condition (pH 7.0) to give 20% homogenate (w/v). The homogenates were centrifuged at 1000 rpm for 10 min at 0 °C in cold centrifuge. The supernatant was separated and used for various biochemical estimations.

2.6. Biochemical analysis

2.6.1. Measurement of plasma glucose and plasma insulin

Plasma glucose was estimated using a commercial kit (Sigma Diagnostics Pvt. Ltd., Baroda, India) by the method of Trinder [21]. Insulin in the rat plasma was assayed by the solid phase system amplified sensitivity immunoassay using reagent kits obtained from Medgenix INS-ELISA, Biosource, Europe S.A., Belgium by the method of Burgi et al. [22].

2.6.2. Estimation of LPO in liver and kidney

The concentration of TBARS in the tissues was estimated by the method of Nichans and Samuelson [23]. In this method, 0.5 mL of sample was diluted with 0.5 mL of double distilled water and mixed well, and then 2.0 mL of TBA–TCA–HCl reagent was added. The mixture was kept in a boiling water bath for 15 min, after cooling, the tubes were centrifuged at 1000 *g* for 10 min and the supernatant was estimated. A series of standard solution in the concentrations of 2–10 nmol were treated in a similar manner. The absorbance of the chromophore was read at 535 nm against a reagent blank. The values were expressed as mmol/100 g of tissues.