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Fisetin, a tetra hydroxy flavone recuperates antioxidant status and protects hepatocellular ultrastructure from hyperglycemia mediated oxidative stress in streptozotocin induced experimental diabetes in rats

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ABSTRACT

Oxidative stress is a biological entity quoted as accountable for several pathological conditions including diabetes mellitus. Chronic hyperglycemia in diabetes is associated with oxidative stress mediated tissue damage. The present study is aimed to explore the role of fisetin, in ameliorating hyperglycemia-mediated oxidative damage to liver in streptozotocin induced diabetic rats. In addition to the levels of blood glucose, plasma insulin, glycosylated hemoglobin, the extent of oxidative stress was assessed by hepatic lipid peroxides and hydroperoxides. The levels of reduced glutathione and the activities of enzymatic antioxidants were determined in the liver tissues. The activities of serum aminotransferases and alkaline phosphatase were assayed. A portion of liver was processed for histological and ultrastructural studies. Oral administration of fisetin (10 mg/kg b.w.) to diabetic rats decreased the levels of blood glucose and glycosylated hemoglobin and increased the plasma insulin level. A reduction in lipid peroxides and hydroperoxides were observed. The diminished activities of antioxidant enzymes and reduced glutathione in diabetic rats were improved upon fisetin administration. Thus, the results of the present study indicate that fisetin treatment protects the hepatocytes by improving the antioxidant competence in hepatic tissues of diabetic rats which is further evidenced from histological and ultra structural observations.

1. Introduction

The role of oxidative stress in the onset and progression of diabetes mellitus is well established. Therefore, diabetes represents an ideal candidate for studying the consequences of oxidative stress and its treatment. Chronic hyperglycemia has been hypothesized to contribute to oxidative stress either by excess generation of ROS or by altering the redox balance. This occurs via several well-studied mechanisms, including increased polyol pathway flux, increased intracellular formation of advanced glycation end products, activation of protein kinase C, or overproduction of superoxide anions by the mitochondrial electron transport chain (Brownlee, 2001; Ahmad et al., 2005).

Excess generation of highly reactive oxygen and nitrogen species is a key component in the development of complications invoked by hyperglycemia. Free radicals are formed in normal physiology but become deleterious, if not quenched by a cascade of antioxidants systems. Overproduction of ROS and inactivation of antioxidants, shifts the Oxidative stress/antioxidants balance in favor of stress. ROS are neutralized by a battery of antioxidants, which can be classified into two categories: enzymes (ex: superoxide dismutase SOD, glutathione peroxidase GPx and catalase) and non-enzymatic systems (ex: glutathione GSH, vitamins A, C and E) (Wiernsperger, 2003). Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance which can promote the development of primary and complications of diabetes mellitus (Maritim et al., 2003).

Liver is the crucial organ responsible for both oxidative and detoxifying processes (Stadler et al., 2003). The diabetogen, streptozotocin exerts its toxic effects on liver and other organs in addition to pancreatic β cells. Induction of STZ results in β cell necrosis which leads to hypoinsulinemia and hyperglycemia. This further enhances liver damage through free radical mediated lipid peroxidation of hepatocellular membrane (Kume et al., 2004). Also, liver mitochondria from STZ-induced diabetic rats were shown to

Abbreviations: ROS, reactive oxygen species; DMSO, dimethyl sulphoxide; STZ, streptozotocin; HbAlc, glycosylated hemoglobin; TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GST, glutathione S transferase; GR, glutathione reductase; GSH, reduced glutathione; LSD, least significant difference; SD, standard deviation.

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generate increased levels of ROS at the electron transport chain which serves as the major site for mitochondrial ROS generation (Kristal et al. 1997). It has also been proposed that an increase in oxidative stress could contribute to tissue damage in diabetes.

The ability of antioxidants to protect against the deleterious effects of hyperglycemia and also to improve glucose metabolism and uptake must be considered as strategy in diabetes treatment (Nicolle et al., 2011). Apart from the conventional antidiabetic treatment, antioxidant therapy may benefit in ameliorating the secondary complications of diabetes (Garg and Bansal, 2000). Plant derived products exhibits various pharmacological properties without any adverse side effects (Ahn et al., 2010, 2013; Chang et al., 2013).

Flavonoids are a diverse group of polyphenolic phytochemicals that are produced as secondary metabolites by various plants in appreciable quantities (Havsteen, 2002). Flavonoids contain the basic structure of benzo-γ-pyrone. Flavonoids, being structurally similar to vitamin E, are known to mimic antioxidant activity in membranes (Ozgová et al., 2003). The powerful antioxidant properties of flavonoids are attributed to the presence of phenolic hydroxyl groups in the flavonoid structure. Flavonoids exhibit a multitude of biological activities such as antioxidant, antibacterial, antiinflammatory, antiallergic, vasodilatory, anticarcinogenic (Rice-Evans et al., 1996). Due to their abundance in dietary products and their potential pharmacological and nutritional effects, the flavonoids are of considerable interest for drug as well as health food supplements (Khan et al., 2012). Flavonoids have also been recognized as the antidiabetic components in a number of traditional ethnic remedies (Jung et al., 2006). Recent reviews suggest that the benefit of antioxidants is not only attributed to their radical scavenging but to their ability to interact with many basic cellular activities (Finley et al., 2011). Therefore, recent attention has focused on the potential uses of flavonoid-based drugs for the prevention and therapy of free radical mediated diseases.

Fisetin (3,3′,4′,7-tetrahydroxy flavone) is a major flavonoid present in strawberries, onion, persimmon at a concentration of 2–160 μg/g (Arai et al., 2000). It exerts wide pharmacological properties such as anticancer (Suh et al., 2010), neurotrophic (Maher, 2006), anti-inflammatory (Higa et al., 2003), and antiangiogenic (Fotsis et al., 1998) effects. Fisetin lowers methyl glyoxal dependant protein glycation and limits the complications of diabetes (Maher et al., 2011). Recently, we have evaluated the antidiabetic and pancreatic tissue protective nature of fisetin in streptozotocin induced experimental diabetic rats (Sriram Prasath and Subramanian 2011; Prasath and Subramanian 2011; Prasath et al., 2013).

In the present study, an attempt has been made to explore the role of fisetin, a tetrahydroxyflavone in ameliorating hyperglycemia-mediated oxidative damage to liver in streptozotocin induced experimental diabetic rats.

2. Materials and methods

2.1. Chemicals

Fisetin was purchased from Sigma Aldrich, Ultra-sensitive ELISA kit for rat insulin (Linco Research, Inc., St. Charles, MO), Streptozotocin, and all other chemicals used in the study were of analytical grade and were obtained from standard commercial suppliers.

2.2. Experimental animals

Male albino Wistar rats weighing (160–180 g) were purchased from Tamilnadu Veterinary and Animal Sciences University (TANUVAS), Chennai. The rats were housed in polypropylene cages lined with husk. The rats were fed with commercial pelleted rats chow (Hindustan Lever Ltd., Bangalore, India), and had free access to water *ad libitum*. The diet pellet composed of 5% fat, 21% protein, 55% nitrogen-free extract, and 4% fiber (w/w) with adequate mineral and vitamin levels for the animals. The experimental rats were maintained in a controlled environment (12:12 h light/dark cycle and temperature (30 ± 2 °C). The experiments were designed and conducted in strict accordance with the ethical norms approved by Ministry of Social justices and Empowerment, Government of India and Institutional Animal Ethical Committee guidelines [IAEC NO: 01/079/09]. The rats were acclimatized for one week before commencing the experiments.

2.3. Induction of experimental diabetes

For successful induction of experimental diabetes, a freshly prepared solution of STZ (50 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 (Rakieten et al., 1963). As STZ is capable of inducing fatal hypoglycemia due to massive pancreatic insulin release, the rats were provided with 10% glucose solution after 6 h of STZ administration for the next 24 h to overcome drug induced hypoglycemia (Fischer and Rickert, 1975). Neither any death nor any other adverse effect was observed. After a week time, for the development and aggravation of diabetes, rats with moderate diabetes (i.e. fasting blood glucose concentration, >250 mg/dl) that exhibited hyperglycemia and glycosuria were selected for the experiment. STZ treated rats that failed to reach the required level of hyperglycemia were eliminated from the study.

2.4. Experimental protocol

The animals were divided into four groups, comprising a minimum of six animals in each group as follows:

Group 1 – control rats receiving 10% DMSO orally.
Group 2 – STZ induced diabetic rats receiving 10% DMSO orally.
Group 3 – diabetic rats orally treated with fisetin (10 mg/kg b.w/day) dissolved in 10% DMSO for 30 days.
Group 4 – diabetic rats treated with gliclazide (5 mg/kg b.w/day) in aqueous solution orally for 30 days.

At the end of 30 days, rats were fasted overnight, anesthetized with ketamine (80 mg/kg b.w. i.p.) and killed by cervical decapitation. Blood was collected with and without anticoagulant for plasma and serum separation respectively.

2.5. Biochemical analysis

Fasting plasma glucose level was determined by glucose oxidase peroxidase diagnostic enzyme kit (Span Diagnostic Chemicals, India) and plasma insulin was assayed using rat ELISA kit (Millipore, St. Charles, USA) according to the manufacturer's instructions. The levels of HbAlc were estimated in the control and experimental groups of rats by the method of Nayak and Pattabiraman (1981).

2.6. Preparation of hepatic tissue homogenate

Hepatic tissues from control and experimental groups of rats were excised, rinsed with ice-cold saline and homogenized in Tris–HCl buffer (100 mM, pH 7.4) using Teflon homogenizer and centrifuged at 12,000*g* for 30 min at 4 °C. The supernatant was pooled and used for the estimations. The protein content in the tissue homogenate was measured by the method of Lowry et al., 1951.

2.7. Assay of aminotransferases and alkaline phospatase

The activities of pathophysiological enzymes such as AST, ALT and ALP were assayed in the liver homogenate of control and experimental groups of rats (King, 1965a,b).

2.8. Determination of antioxidant status

The levels of lipid peroxides and hydroperoxides were determined in the liver tissue homogenate (Ohkawa et al., 1979; Jiang et al., 1992). The activities of enzymatic antioxidants such as SOD (Misra and Fridrovich, 1972), catalase (Takahara et al., 1960), GPx (Rotruck et al., 1973), GST (Habig et al., 1974) and GR (Carlberg and Mannervik, 1985) were assayed in the hepatic tissue homogenate of control and experimental groups of rats. The levels of non-enzymatic antioxidant reduced GSH (Sedlak and Lindsay, 1968) was measured in the liver tissue homogenate of control and experimental groups of rats.

2.9. Histological observations of liver

A portion of liver tissue was fixed in 10% formalin for one week at room temperature. The specimens were then dehydrated in a graded series of ethanol, cleared in xylene and embedded in paraffin wax. Tissue blocks were sectioned into 5 μm thickness using a rotary microtome. The sections were stained by hematoxylin-eosin (Kleiner et al., 2005). Histological changes in the stained sections were viewed under the light microscope by a qualified pathologist.