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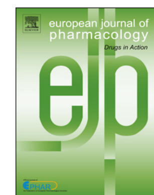

Endocrine pharmacology

Fisetin improves glucose homeostasis through the inhibition of gluconeogenic enzymes in hepatic tissues of streptozotocin induced diabetic rats

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ABSTRACT

Liver plays a vital role in blood glucose homeostasis. Recent studies have provided considerable evidence that hepatic glucose production (HGP) plays an important role in the development of fasting hyperglycemia in diabetes. From this perspective, diminution of HGP has certainly been considered for the treatment of diabetes. In the present study, we have analyzed the modulatory effects of fisetin, a flavonoid of strawberries, on the expression of key enzymes of carbohydrate metabolism in STZ induced experimental diabetic rats. The physiological criterions such as food and fluid intake were regularly monitored. The levels of blood glucose, plasma insulin, hemoglobin and glycosylated hemoglobin were analyzed. The mRNA and protein expression levels of gluconeogenic genes such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) were determined by immunoblot as well as PCR analysis. Diabetic group of rats showed significant increase in food and water intake when compared with control group of rats. Upon oral administration of fisetin as well as gliclazide to diabetic group of rats, the levels were found to be decreased. Oral administration of fisetin (10 mg/kg body weight) to diabetic rats for 30 days established a significant decline in blood glucose and glycosylated hemoglobin levels and a significant increase in plasma insulin level. The mRNA and protein expression levels of gluconeogenic genes, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), were decreased in liver tissues upon treatment with fisetin. The results of the present study suggest that fisetin improves glucose homeostasis by direct inhibition of gluconeogenesis in liver.

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1. Introduction

Liver is one of the chief storage organ for glucose reserve in the body and plays a crucial role in blood glucose homeostasis. Numerous studies have provided substantial evidence that hepatic glucose production (HGP) plays an important role in the development of fasting hyperglycemia in diabetes. From this perspective, diminution of HGP has certainly been considered for the treatment of diabetes (Morral, 2003). It has been reported that the diabetic condition decreases the activities of enzymes in the glycolytic and pentose phosphate pathways, while increasing the activities of gluconeogenic and glycogenolytic pathways (McAnuff et al., 2005). Therefore, the enzymes that regulate hepatic glucose metabolism are potential targets for controlling hepatic glucose balance and thereby blood glucose levels in diabetes.

Due to adverse side effects of the currently available oral antidiabetic medications, there have been persistent efforts to identify potential compounds that can “cure” DM, for example by stimulating β-cell regeneration and preventing apoptosis, leading to a return of endogenous control of glucose homeostasis. Naturally occurring plant compounds are attractive candidates because they are abundant in nature, inexpensive to produce and may have fewer side-effects than currently used pharmaceutical compounds (Chang et al., 2013). Some natural products have the ability to lower blood glucose and it should be safer than allopathic drugs if utilized over a prolonged period.

Epidemiological studies have shown that the consumption of diets rich in fruit and vegetables is associated with lower risks of many chronic diseases such as diabetes, cardiovascular disease (CVD) and cancer, as well as reducing the risk of stroke (O'Byrne et al., 2002; Lee et al., 2011). It is mainly attributed to the antioxidant capacities derived

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from flavonoid contents in fruits and vegetables (Halliwell, 1994). Strawberries have been reported to have the highest total antioxidant activity among fruits (Wang et al., 1996), which is conferred by the wide variety of flavonoids contained therein (Hannum, 2004). In fact, strawberry consumption either as an individual component (Tsuda et al., 2004), or as whole strawberries (Prior et al., 2008), has been specifically implicated in the effects on risk factors for cardiovascular disease.

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), antioxidant (Hanneken et al., 2006), anti-inflammatory effects (Higa et al., 2003). Fisetin lowers methyl glyoxal dependant protein glycation and limits the complications of diabetes (Maher et al., 2011).

Fisetin supplementation decreases cardiovascular risks by ameliorating hepatic steatosis and lowering circulating glucose concentrations (Cho et al., 2013). Fisetin supplementation has been reported to exhibit hypocholesterolemic effects by modulating the expression of genes associated with cholesterol and bile acid metabolism (Shin et al., 2013). Fisetin has been reported to downregulate both glycogenolysis and gluconeogenesis in vitro (Constantin et al., 2010). The experiments performed and the results obtained so far were typically based on in vitro studies. Very few reports were available in the literature on experimental animal models. Recently, we have evaluated the antidiabetic and antioxidant activity of fisetin in streptozotocin induced experimental diabetes in rats (Sriram Prasath and Subramanian, 2011; Prasath and Subramanian, 2011, 2013; Prasath et al., 2013). However, the mechanism by which fisetin maintains glucose homeostasis still remains to be elucidated. Hence, the present study is framed to analyze the modulatory effects of fisetin on glucose homeostasis in STZ induced experimental diabetes in rats.

2. Materials and methods

2.1. Experimental animals

Male albino Wistar rats weighing 160–180 g were purchased from Tamil Nadu 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 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 & Environment, Government of India and Institutional Animal Ethical Committee guidelines [IAEC no. 01/079/09]. The rats were acclimatized for 1 week before initiating the experiments.

2.2. 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 this study were of analytical grade and were obtained from standard commercial suppliers. The antibodies for the study were procured from Santa Cruz Biotechnology, USA. The secondary antibodies (anti-rabbit–HRP conjugated) were purchased from Genei, Bangalore India.

2.3. Induction of experimental diabetes

Experimental diabetes was induced in overnight fasted rats by single intraperitoneal injection of streptozotocin (50 mg/kg b.w) freshly prepared in 0.1 M of cold citrate buffer (pH 4.5) (Rakieten et al., 1963). Since 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 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. Rats with uniform diabetic status were grouped.

2.4. Experimental design

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.

During the experimental period, body weight, blood glucose, food and water consumption and physical examinations were determined at regular intervals. The dosage was adjusted every week according to any change in body weight to maintain similar dose per kg body weight of rat over the entire period of study for each group. 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

2.5. Biochemical parameters

Glucose was measured by glucose oxidase method (Trinder, 1969). Plasma was separated and used for insulin assay using ELISA kit for rats (Linco Research, Inc., USA). Levels of hemoglobin and glycosylated hemoglobin were estimated according to methods of Drabkin and Austin (1932) and Nayak and Pattabiraman (1981). Urine sugar was detected using urine strips. (Diastix (2804B); manufactured in India by Siemens Ltd. Gujarat, India.)

2.6. Preparation of tissue homogenate

The liver tissue was excised, rinsed in ice cold saline. Known amount of the tissues was homogenized in Tris–HCl buffer (100 mM, pH 7.4) at 4 °C, in a Potter–Elvehjem homogenizer with a Teflon pestle at 600 rpm for 3 min. The homogenate was then centrifuged at 12,000*g* for 30 min at 4 °C. The supernatant was collected as tissue homogenate, which was used to assay of various parameters. The protein content in the tissue homogenate was estimated by the method of Lowry et al. (1951).

2.7. Western blot analysis

The PEPCK and glucose-6-phosphatase expressions were analyzed by SDS-PAGE and Western blotting. Western blotting was carried out according to the method of Towbin et al. (1979). Liver tissue homogenate was taken for the study. The protein content in the tissue homogenate was estimated by the method of Lowry et al. (1951). The proteins (30 μg) were resolved by 10% SDS-PAGE. After electrophoresis, the polyacrylamide gel was equilibrated with a solution containing 25 mM-Tris (192 mM Glycine, pH 8.3) for 30 min at room temperature. The proteins were then electrophoretically transferred to nitrocellulose paper which was performed for 3.5 h at 10 °C. Free binding sites on the paper were blocked with 2% BSA in 0.1% TBST.

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