Biomedicine & Preventive Nutrition 4 (2014) 511–517

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Antioxidative potential of chrysin, a flavone in streptozotocin–nicotinamide-induced diabetic rats

T.A. Anitha [a,*], M. Rajadurai [b]

[a] Department of Biochemistry, Muthayammal College of Arts and Science, Rasipuram, 637 408 Namakkal, Tamil Nadu, India
[b] Sri Venkateshwaraa College of Arts and Science, Dharmapuri, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 22 July 2014
Accepted 10 August 2014

Keywords:
Antioxidants
Diabetes
Chrysin
Streptozotocin–nicotinamide
Lipid peroxidation

ABSTRACT

Increasing evidence in both experimental and clinical studies suggests that oxidative stress has been suggested as a contributory factor in development and complications of both types of diabetes mellitus. The objective of the present study was to evaluate the protective effect of chrysin (5,7-dihydroxyflavone) against streptozotocin–nicotinamide (STZ–NA)-induced oxidative stress in male Wistar rats. Diabetes was induced by a single intraperitoneal (i.p.) injection of STZ (45 mg/kg body weight (b.w.)) dissolved in 0.1 mol/L citrate buffer (pH 4.5), 15 min after the i.p. administration of NA (110 mg/kg b.w.). The rats were divided into following groups: group 1: non-diabetic control, group 2: non-diabetic with chrysin (100 mg/kg b.w.), group 3: diabetic control, groups 4, 5 and 6 received chrysin as 25, 50, 100 mg/kg b.w., respectively. The oxidative stress was measured by examining the enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-*S*-transferase (GST) and the non-enzymatic antioxidants, such as vitamin C, vitamin E and reduced glutathione (GSH) in liver and kidney. They were decreased while increasing the levels of LPO markers were observed in liver and kidney tissues of diabetic control rats as compared to normal control rats. Oral administration of chrysin (100 mg/kg/day) for 45 days caused a significant increase in the activities of both enzymatic and non-enzymatic antioxidants when compared to those of diabetic rats. These biochemical findings were also supported by histological studies on liver and kidney tissues. In conclusion, chrysin, especially at the dosage of 100 mg/kg b.w. can act as a potent antioxidant and anti-inflammatory agent in type II diabetic rats.

1. Introduction

Diabetes mellitus (DM) is a complex and progressive disease in which the hallmark feature is elevated blood glucose levels resulting from defective insulin secretion, resistance to insulin action or both that associated with long-term complications and affects aorta, blood vessels, eyes and nerves system [1,2]. Diabetes has become a common global health problem that affects > 170 million people worldwide. It is one of the leading causes of death and disability. It is estimated that by 2030, the number will rise to 366 million [3].

Streptozotocin (STZ), an antibiotic produced by *Streptomyces achromogenes*, has been widely used for inducing diabetes in the experimental animals through its toxic effects on pancreatic β-cells and it is well studied that exposure of the insulin secreting cells to STZ results in DNA damage in pancreatic β-cells through the formation of alkylating free radicals leading to hasty necrosis of the β-cells, thereby the rate of insulin synthesis and secretion is decreased [4]. It has been reported that administration of NA, a poly-ADP-ribose synthetase inhibitor, protected the islets function, thereby partially reversing the inhibition of insulin secretion to prevent the aggravation of experimental diabetes following the administration of β-cell toxin, such as STZ. This model appears to be particularly suitable to test potential antidiabetic drugs as it shares some important features of type 2 diabetes mellitus [5,6].

Phytopharmaceuticals are gaining importance in allopathic as well as traditional medicine owing to their non-addictive and non-toxic nature [7]. Many pharmacological agents capable of ameliorating ROS in the vessel wall may result in better vascular health in diabetes. One class of such compounds garnering much attention in recent times is the flavonoids, which are polyphenolic compounds widely found in plant-based human diets, such as fruits and vegetables. They possess antioxidant potential both *in vivo* and *in vitro* by scavenging ROS [8,9].

* Corresponding author. Department of Biochemistry, Muthayammal College of Arts and Science, Rasipuram 637 408 Namakkal, Tamil Nadu, India.
E-mail address: anithasarvan@gmail.com (T.A. Anitha).

http://dx.doi.org/10.1016/j.bionut.2014.08.008
2210-5239/

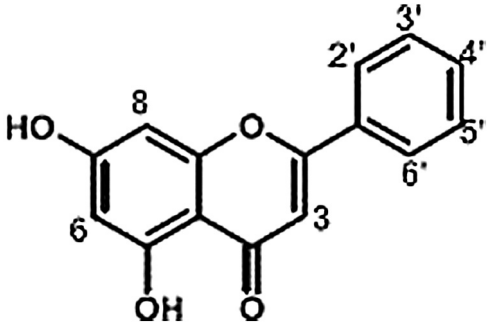

Fig. 1. Chrysin (5,7-dihydroxyflavone).

Chrysin, a 5,7-dihydroxyflavone (Fig. 1), is a naturally wide distributed flavonoid that occurs naturally in many plants, passiflora, pelargonium, pinacaeae, silver linden, some geranium species and in honey, bee pollen and propolis [10–12] and, in low concentrations, in fruits, vegetables, and beverages [13,14]. Chrysin is the main component of *Oroxylum indicum*, which is one of the most common herbal medicines used by China and other East Asian countries. It has been reported to have many different biological activities and pharmacological effects, such as anti-viral, anti-cancer, anti-bactericidal, anti-inflammatory, anti-allergic, anti-mutagenic, anti-anxiolytic and antioxidant effects [15]. Chrysin also has the clinical and therapeutic applications against the physiological and biochemical effects of aging [16]. The biological activities of chrysin are mainly attributed to hydroxyl and keto groups in its rings [17].

Based on these considerations, the present study was designed to investigate the effects of a flovone, chrysin on the oxidative stress induced by STZ–nicotinamide induced diabetes in an animal model using male Wistar rats. Histopathological studies were also carried out to assess the effect of chrysin on liver, kidney and pancreatic cells against STZ–NA damage in rats.

2. Materials and methods

2.1. Experimental animals

The whole experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by the Animal Ethical Committee of Muthayammal College of Arts and Science (Approval No. 03,2012-13) Rasipuram, India. The study was conducted on thirty-six male albino Wistar rats weighing 150–170 g, obtained from the Venkateswara Enterprises, Bangalore. They were housed in polypropylene cages (47 × 34 × 20 cm), (six rats per cage) lined with husk, renewed every 24 hours under a 12:12 hours light/dark cycle at around 22 °C and had free access to tap water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The pellet diet consists of 22.02% crude protein, 4.25% crude oil, 3.02% crude fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen free extract (carbohydrates). The diet provided a metabolisable energy of 3600 KCal.

2.2. Chemicals

Chrysin and STZ were purchased from Sigma Chemical Company, St. Louis, MO, US and NA was obtained from Ranbaxy chemicals Ltd., Mumbai, India. All other chemicals and reagents used in the experiments were of analytical grade and of the highest purity.

2.3. Induction of type 2 diabetes mellitus

Non-insulin dependent diabetes was induced in overnight fasted male Wistar rats by a single intraperitonial (i.p.) injection of 45 mg/kg streptozotocin, 15 min after the i.p. administration of 110 mg/kg of nicotinamide dissolved in normal saline. streptozotocin (STZ) freshly dissolved in 0.1 M citrate buffer (pH 4.5). After 72 hours, diabetic rats were identified by measuring fasting plasma glucose (FPG) levels. The animals with blood glucose concentration more than 250 mg/dL were used in this study [18].

2.4. Experimental design

In this study, a total of 36 rats were divided into six groups of six rats in each group and had free access to food and water and were treated for 45 days as follows:

- group I: normal control (vehicle treated; DMSO: 1 ml/kg b.w.);
- group II: normal + chrysin (100 mg/kg b.w.);
- group III: diabetic control;
- group IV: diabetic + chrysin (25 mg/kg b.w.);
- group V: diabetic + chrysin (50 mg/kg b.w.);
- group VI: diabetic + chrysin (100 mg/kg b.w.).

Chrysin was dissolved in 0.2% dimethyl sulfoxide (DMSO) and administrated to rats orally using an intragastric tube. At the end of the experimental period, all the animals were fasted overnight, anaesthetized using pentobarbital sodium (35 mg/kg b.w., intramuscular injection), and sacrificed by cervical decapitation. The blood samples were collected in the test tubes containing potassium oxalate and sodium fluoride (3:1) as anticoagulant for the separation of plasma. The liver and kidney were immediately dissected out, washed in ice-cold saline to remove the blood, and pat dried and weighed. The liver and kidney were weighed and 10% of tissue homogenate was used for the analysis of lipid peroxidation, enzymatic and non-enzymatic antioxidants.

2.5. Biochemical estimations

Plasma thiobarbituric acid reactive substances (TBARS) were estimated by the method of Yagi et al. [19]. The concentration of TBARS in the tissue was estimated by the method of Fraga et al. [20]. The levels of lipid hydroperoxides (HP) were estimated by the method of Jiang et al. [21].

The activity of SOD was assayed according to the procedure of Kakkar et al. [22]. The activity of catalase was assayed by the method of Sinha et al. [23]. The activity of GPx was assayed by the method of Rotruck et al. [24]. The level of GSH was estimated by the method of Ellman et al. [25]. The levels of vitamin C were estimated by the method of Omaye et al. [26]. The levels of vitamin E were estimated by the method of Baker et al. [27].

2.6. Histopathology

Tissues (liver and kidney) obtained from all experimental groups were washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the tissue was processed by embedding in paraffin. Then, the tissue was sectioned and stained with Hematoxylin and Eosin (H–E) and examined under high power microscope and photomicrographs were taken.

2.7. Statistical analysis

Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT)