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Original Article

Antihyperglycemic and antilipidperoxidative effects of flavanoid naringin in streptozotocin-nicotinamide induced diabetic rats

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ABSTRACT

In light of evidence that some complications of diabetic mellitus may be caused or exacerbated by an oxidative stress, the putative protective effect of naringin was investigated in streptozotocin-nicotinamide induced diabetic rats. Naringin was given for 42 days at a daily dose equivalent to 80 mg/kg of body weight. Lipid peroxidation levels and non-enzymic antioxidants such as vitamin C, vitamin E and glutathione were than measured in plasma. Under our experimental conditions, naringin was found to significantly reduce the blood glucose and increase the plasma insulin level in diabetic rats. Our results suggest that the antihyperglycemic action of naringin is exerted via the stimulation of insulin secretion. In addition, naringin appears to exert an antioxidative property demonstrated by the increase of vitamin C, vitamin E and glutathione levels in plasma and a lowering of lipid peroxidation levels in plasma. In conclusion, naringin possess a potential hypoglycemic effect in streptozotocin-nicotinamide induced diabetic rats.

1. Introduction

Diabetes mellitus is the name given to a group of disorders with different etiologies. It is characterized by disarrangements in carbohydrates, proteins and fat metabolism caused by the complete or relative insufficiency of insulin secretion and/or insulin action [1]. The World Health Organization (WHO) estimates that more than 220 million people worldwide have diabetes and this number is likely to more than double by 2030 [2]. The occurrence and consequences associated with diabetes are found to be high in countries like India (31.7%), China (20.8%) and USA (17.7%). The rate is expected to rise to 79.4%, 42.3% and 30.3%, respectively, by 2030 in the above countries [3]. Oxidative stress has been implicated in a host of diabetes related complications including premature atherosclerosis [4]. However, despite robust experimental evidence suggesting that increased oxidative load has a significant role in the pathogenesis of diabetes [5, 6, 7]. Plants used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the control of this disease. Despite the presence of known antidiabetic medicines in the pharmaceutical market, screening for new sources from natural plants is still attractive because they contain substances that are effective and safe in diabetes mellitus [8].

The plant kingdom has become a target for the search for new drugs and biologically active compounds [9]. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes, but only a few have received scientific scrutiny. Phytochemicals isolated from plant sources are used for the prevention and treatment of cancer, heart disease, diabetes and high blood pressure. A number of substances have been identified, which protect against experimental diabetes [10]. Any substance that can prevent an attack or accelerate the process of recovery will have considerable clinical application

Flavonoids are a group of naturally occurring compounds ubiquitous in the plant kingdom and known to have strong antioxidant effects. They are widely distributed in foods of plant origin such as vegetables, fruits, tea and wine. Naringin (4′,5,7-trihydroxy flavonone 7-rhamnoglucoside) is the predominant flavonone found in grape fruit and related citrus species [11]. Currently, there is much interest in the usefulness of citrus fruits because of their intake appears to be associated with reduced risk of certain chronic diseases and increased survival [12]. Naringin is responsible for the characteristic sour flavor of the fruits [13]. Among the naturally occurring flavonoids, naringin has been empirically proven to have no side effects, as humans have been ingesting grapes and citrus fruits for a long time [14]. The role of

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naringin had recently received considerable attention as dietary antioxidant. Naringin exhibits various pharmacological and therapeutic properties: antimicrobial, antimutagenic, anticancer, anti-inflammatory, cholesterol lowering, free radical scavenging and antioxidant effects [15, 16, 17].

To our knowledge, a biochemical investigation has not been carried out on the effect of naringin on the plasma antioxidant status of experimental diabetic rats. The present study was undertaken in streptozotocin-nicotinamide diabetic rats to verify the antihyperglycemic effect, to investigate the effect on lipid peroxidation and to evaluate the potential of nonenzymatic antioxidant in plasma, of naringin.

2. Materials and methods

2.1. Animals

Adult male albino Wistar rats (8 weeks), weighing 200 to 200 g bred in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, were used. All animal experiments were approved by the ethical committee (Vide. No: 582, 2008), Annamalai University and were in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. The animals were housed in polycarbonate cages in a room with a 12 h daynight cycle, temperature of 24±2°C, humidity of 45% to 64%. During the whole experimental period, animals were fed with a balanced commercial diet (Hindustan Lever Ltd., Mumbai, India) and water ad libitum.

2.2. Drug and Chemicals

Naringin, Streptozotocin (STZ) and all other biochemicals were purchased from Sigma Chemical Company, St Louis, MO, USA. All chemicals were of analytical grade.

2.3. Experimental induction of type 2 diabetes

Non-insulin dependent diabetes mellitus was induced [18] in overnight fasted rats by a single intraperitoneal injection (i.p) of 45 mg/kg body weight STZ, 15 min after the i.p administration of 110 mg/kg body weight of nicotinamide. STZ was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. The elevated glucose levels in plasma determined at 72 h, confirmed hyperglycemia. The animals with blood glucose concentration more than 250 mg/dl will be used for the study.

2.4. Experimental design

In the experiment, a total of 24 rats (12 diabetic surviving rats, 12 normal rats) were used. The rats were divided into four groups of six each, after the induction of STZ diabetes. Naringin was orally administered using an intragastric tube for a period of 42 days.

Group 1: Normal rats
Group 2: Normal rats + naringin (80 mg/kg b.w)
Group 3: Diabetic rats
Group 4: Diabetic rats + naringin (80 mg/kg b.w)

At the end of the experimental period, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride mixture for the estimation of blood glucose. Plasma was separated for the estimation of insulin and other biochemical parameters.

2.5.Biochemical assays

2.5.1 Estimation of blood glucose and plasma insulin

Blood glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India)[19]. Plasma insulin was assayed by ELISA using a BoehringerMannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany).

2.5.2.Estimation of lipid peroxidation and non-enzymic antioxidants

Lipid peroxidation in plasma were estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) and hydroperoxides using the methods of Fraga et al. [20] and Jiang et al. [21], respectively. Vitamin C, E and reduced glutathione (GSH) were estimated by the method of Omaye et al. [22], Baker et al. [23] and Ellman [24], respectively.

2.6. Statistical analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Duncan's multiple range test (DMRT) using a statistically software package (SPSS for Windows, V.13.0, Chicago, USA). Values were presented as mean ±S.D $p<0.05$ were considered as statistically significant [25].

3. Results

3.1. Changes in blood glucose and plasma insulin

The levels of blood glucose and plasma insulin in normal and experimental rats are shown in Table 1. The experimental rats showed significant increase in the level of blood glucose with significant decrease in the level of plasma insulin. Treatment with naringin (80 mg/kg bwt) for a period of 42 days to experimental rats significantly retained the above biochemical changes. In our previous study, we reported that naringin at 80 mg/kg bwt, showed a better effect than 20 and 40 mg/kg bwt, therefore the 80 mg/kg bwt was used in this study. The administration of naringin to normal rats did not show any significant changes in blood glucose and plasma insulin levels.

Table 1. Changes in the levels of blood glucose and plasma insulin in normal and experimental animals

Groups	Blood glucose (mg/dl)	Plasma insulin (µU/ml)
Normal	81.36 ± 7.12[a]	14.82 ±1.19[a]
Normal + naringin (80mg/kg)	78.43±7.05[a]	15.37 ± 1.21[a]
Diabetic	358.25 ± 31.94[b]	7.43 ± 0.45[b]
Diabetic + naringin (80mg/kg)	124.97 ± 10.82[c]	12.05 ± 0.74[c]

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

3.2. Effect of naringin on plasma lipid peroxidation

Table 2. Shows the levels of TBARS and lipid peroxide in plasma in normal and diabetic rats. Untreated diabetic rats showed a significant increase in plasma lipid peroxidation levels when compared to normal rats. Naringin treated with experimental rats had significantly lower plasma lipid peroxide and TBARS levels as compared with the untreated diabetic rats.

Table 2. Changes in the levels of plasma TBARS and hydroperoxides in normal and experimental animals

Groups	TBARS (mmoles/dl)	Hydroperoxides (x 10^{-5} mM/100ml)
Normal	0.16 ± 0.10[a]	10.36 ± 0.69[a]
Normal + naringin (80mg/kg)	0.15 ± 0.01[a]	10.18 ± 0.67[a]
Diabetic	0.38 ± 0.03[b]	21.73 ±1.52[b]
Diabetic + naringin (80mg/kg)	0.24 ± 0.01[c]	12.05 ± 0.84[c]

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

3.3. Effect of naringin on plasma antioxidants

Table 3. Shows the levels of plasma antioxidants in normal and experimental rats. Plasma GSH, vitamin C and vitamin E levels were significantly lower in diabetic rats than in normal rats. Experimental rats treated with naringin had near normal levels of plasma antioxidants.

Table 3. Changes in the levels of vitamin C, vitamin E, and reduced glutathione (GSH) in plasma of normal and experimental animals

Groups	Vitamin C (mg/dl)	Vitamin E (mg/dl)	Reduced glutathione (mg/dl)
Normal	1.83 ± 0.12[a]	1.64 ± 0.11[a]	24.35 ± 1.81[a]
Normal + naringin (80mg/kg)	1.92 ± 0.14[a]	1.71 ± 0.12[a]	25.48 ± 1.98[a]
Diabetic	0.79 ± 0.05[b]	0.63± 0.04[b]	11.85 ± 0.93[b]
Diabetic + naringin (80mg/kg)	1.64 ± 0.11[c]	1.47± 0.09[c]	21.19 ± 1.36[c]

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

4. Discussion

Diabetes is characterized by defects in both metabolic and vascular domains; this disease represents a privileged situation for oxidative stress exerting harmful effects [26]. The attachment of free radicals in diabetes and the responsibility of these toxic species in lipid peroxidation and the antioxidant defense system have been studied. Streptozotocin-induced diabetes is a well-documented model of experimental diabetes. Streptozotocin-diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia [27]. Hyperglycemia can stimulate oxidative stress is the autoxidation of glucose in the presence of transition metals as well as the generation of ROS during the process of glycation [28]. The roles of oxidative stress and antioxidants in organs and tissues damage have been studied extensively in experimental diabetes and diabetic patients [29]. STZ directly generates oxygen free radicals induced lipid peroxidation [30]. This study was therefore undertaken to assess antiperoxidative properties of naringin in STZ diabetic rats. We found that 42 days treatment with naringin at 80 mg/kg body weight reduces blood glucose levels in diabetic animals. The possible mechanism(s) by which naringin initiates antihyperglycemic action could be due to a possible pancreatic secretion of insulin from existing β-cells, with an intensification of glucose uptake by peripheral tissues or reduced glucose absorption from the gastrointestinal tract [31].

Hyperglycemia, the primary clinical manifestation of diabetes, is responsible for the development of diabetic complications. Several mechanisms may lead to increased oxidative stress in diabetes. Firstly, hyperglycemia may increase the generation of free radicals through the ability of glucose to enolize and yield oxidizing intermediates such as superoxide anion, hydroxyl radical, hydrogen peroxide and nitric oxide [32, 33]. Secondly, antioxidant defenses are reduced in diabetes [34]. Oxidative stress and the oxidative modification of biomolecules are involved in a number of physiological and pathophysiological processes. Oxidative stress occurs as a consequence of imbalance between prooxidants and antioxidants. If produced in excess, reactive oxygen species (ROS) lead to damage of macromolecules such as DNA, proteins and lipids.

The elevated level of lipid peroxides in the plasma of diabetic rats and lipid peroxidation is one of the characteristic features of chronic diabetes [35]. The increased level of plasma lipid peroxide in STZ-induced diabetes is generally through to be due to pathological changes to tissues that increase the production and liberation of lipid peroxides into the circulation [36]. Previous studies have reported increased levels of lipid peroxidation in plasma of diabetic rats [37]. Flavonoids have shown to inhibit lipid peroxidation formation in rat and also inhibit the free radical production in the cells at various stages. Our study clearly shows that the treatment of naringin significantly reduced the levels of TBARS and hydroperoxides in (80mg/kg) plasma of diabetic rats. In this context, Ali and Abd El Kadar have reported that naringin treatment reduce the levels of TBARS and hydroperoxides in STZ-induced lipid peroxidation [31].

Antioxidants play a major role in protecting biological systems against reactive oxygen species and reflect the antioxidant capacity of the system [38]. Vitamin E is the most ancient antioxidant in the lipid phase [39]. Apart from enzymic antioxidants, non-enzymic antioxidants play a vital role in protecting cells from oxidative changes. Vitamin E neutralizes the free radicals, preventing the chain reaction that contributes to oxidative damage [40]. It has the potential to quench lipid peroxidation and protects cellular structure from the attack of free radicals [41]. The decreased level of vitamin E observed in the plasma of diabetic rat [37].

Ascorbic acid is the most widely cited form of water-soluble antioxidant; it prevents oxidative damage to the cell membrane induced by aqueous radicals. Vitamin C can act as a co-oxidant by regenerating α-tocopherol from the alpha-tocopheroxyl radical produced during scavenging of free radicals. It is also possible that the correction of hyperglycemia has a sparing effect on vitamin C, and this increases the potential to recycle vitamin E [42, 43]. We also observed significant decrease in the levels of plasma vitamin C in diabetic rats could be due to increased utilization of vitamin C as an antioxidant defense against reactive oxygen species [44].

GSH, a non-enzymatic antioxidant is known to accord protection against ROS by effectively scavenging free radicals and other ROS directly and indirectly through enzymatic reactions [45]. GSH is an important antioxidant, which also maintains the

acid in their active reduced forms, and a synergistic interaction exists between α-tocopherol and ascorbic acid in suppressing the peroxidation. Decreased GSH levels in the circulation might be due to increased utilization in protecting 'SH' containing proteins from lipid peroxides [46]. The elevated level of GSH on treatment with naringin protects cellular proteins against oxidation through glutathione redox cycle and detoxifies the free radicals generated.

Naringin may exhibit antioxidant activity capacity based on increasing the upregulation of gene expressions in the antioxidants, with consequently enhancement the scavenging of reactive oxygen species accompanying with significantly lowering of lipid peroxidation suggests that administration of naringin increase the antioxidative potential. In addition like all the flavonoid compounds, presence of hydrogen donating substituents attached to aromatic rings may enable naringin to scavenge free radicals. [47]. Oral administration of naringin may reveal their action on glucose through reduction of oxidative stress intensity in hyperglycaemia. The protective effect of naringin may be connected with the normalization of hyperglycaemia, the inhibition of glucose autoxidation and, as a result, exhibited a potent significant amelioration of oxidative stress in the diabetic animals.

5. Conclusion

In conclusion, oral administration of naringin exhibits an antihyperglycemic and antilipidperoxidative effects on STZ-nicotinamide induced diabetic rats.

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