Evaluation of brain monoamines in diabetic rats treated with quercetin

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ABSTRACT

Food derived antioxidants have a strong potential effect on the long term as chemo preventive agents in disease states involving oxidative stress. This study was done to evaluate the brain oxidative stress induced by streptozotocin (STZ) in rats with consequent changes in brain neurotransmitters and examined the potential protective role of quercetin (QCT) against the changes STZ induced. Forty male albino rats were used in this study and divided into four groups (10 rats in each group) as follow: Group I (control group) received a vehicle, group II (quercetin group) received daily intra peritoneal injection of QCT (15 mg/kg body weight/day), group III (diabetic group) diabetic rats received a vehicle, group IV (treated group) diabetic rats received a daily intra peritoneal injection of QCT (15 mg/kg body weight/day). After 8 weeks, blood, urine and brain tissues samples were collected. Oxidant/antioxidant parameters were determined included DNA damage by measuring urinary 8-hydroxyguanosine (8-OHdG) by Reverse-phase high-performance liquid chromatography (RP-HPLC) analysis using electrochemical detector. Brain neurotransmitters were estimated by RP-HPLC and UV detection. The current data appeared that, STZ effectively increased oxidative stress parameters along with disturbances in brain monoamines. Quercetin injection attenuated these elevation, thus a significant decreased was observed in oxidative stress parameters and brain neurotransmitters in treated group compared to diabetic one. We concluded that quercetin seems to be a highly promising agent in protecting the diabetic rats against oxidative damage and in preventing disturbances in brain monoamines due to STZ induced diabetes.

Keywords: diabetes, oxidative stress, quercetin, 8-hydroxyguanosine, neurotransmitters

INTRODUCTION

Diabetes mellitus, the high incidence of microvascular and atherosclerotic disorders have been associated with increased oxidative stress [1] which is generally attributed to the formation of the highly reactive hydroxyl radical (OH-) [2].

Streptozotocin (STZ) induces its diabetogenic activity mainly by inducing oxygen free radical and causing necrosis of the pancreas. Both the radical and non-radical oxidants can induce lipid peroxidation particularly of those lipoproteins that contain unsaturated fatty acids, which in turn stimulates glycation of protein [3], and DNA damage which was estimated by several ways [4], [5].

A potentially mutagenic DNA base, 8-hydroxyguanosine (8-OHguanine or 8-oxo guanine) is repaired, released from the cell, and eventually excreted via the urine as the base (8- OH-guanine) or the nucleoside, 8-hydroxy-2-deoxyguanosine (8–OH–dG, 8–oxo–dG). The urinary content of 8–OH–Dg represents an average rate of oxidative damage to guanine in the form of the free nucleotide (dGTP) and in DNA [6].
Some tissues, especially the brain, are much more vulnerable to oxidative stress because of their elevated consumption of oxygen and the consequent generation of large amounts of ROS, which are closely implicated in several diseases of the nervous system including Parkinson’s disease, schizophrenia and Alzheimer’s disease [7].

Chronic antioxidant therapies may be useful in decreasing the risk of diabetic complications and have been reported to attenuate inflammatory response, insulin resistance, and diabetes development [8].

Dietary supplement of antioxidants such as vitamins, flavonoids has been used to prevent the occurrence of many chronic diseases [9]. Flavonoids are a large group of natural polyphenolic substances widely distributed in the plant kingdom [10]. Quercetin (QCT) is one of the most widely distributed flavonoids, present in foods, including vegetables, especially onions, fruits, tea, and many other dietary sources [11].

Thus, from this point of view, we aimed to evaluate the brain monoamines levels and oxidative stress in experimental diabetes induced by STZ in rats and examined the potential protective role of quercetin against the changes STZ induced.

EXPERIMENTAL SECTION

Materials
Chemicals
Noradrenalin, dopamine, serotonin and 8-hydroxyguanosine HPLC standards, streptozotocin (STZ) and quercetin were purchased from Sigma Aldrich Chemicals Company St.Louis USA. All other chemicals were HPLC grade and purchased from Sigma.

Experimental animals:
Sixty male albino rats weighing 180-200 g were obtained from the animal house of National Research Center, Giza, Egypt, and fed a standard commercial diet (control diet) purchased from the Egyptian company of oils and soaps. Water was available ad-libitum for acclimatization before starting the experiment, kept under constant environmental conditions at room temperature.

The guidelines of the ethical care and treatment of the animals followed the regulations of the ethical committee of the National Research Centre (NRC).

Methods
Induction of diabetes
STZ was dissolved in 50 mM sodium citrate solution (pH adjusted at 4.5) containing 150 mM NaCl. The solution containing (6.0 mg/100g body weight) was subcutaneously administrated in rats; fasting blood sugar was estimated after 3 days to confirm the development of diabetes mellitus [12].

Experimental design
Forty male albino rats were used in this study and divided into four groups (ten rats in each group) as follows:

Group I (control group): healthy rats received a vehicle.

Group II (quercetin group): healthy rats were received a daily intra peritoneal injection of QCT (15 mg/kg body weight/day).

Group III (diabetic group): diabetic rats received a vehicle.

Group IV (treated group): diabetic rats were received a daily intra peritoneal injection of QCT (15 mg/kg body weight/day) [13].

After the experimental period (8 weeks) [14], animals were kept in metabolic individual cages for collection of 24 h urine samples, they were kept fasting for 12 h before blood sampling: blood was withdrawn from the retro-orbital venous plexus of the eye using capillary tubes and collected in tubes containing sodium fluoride for blood glucose estimation.

Biochemical assays
Fasting blood sugar was estimated by colorimetric method using commercial kit purchased from Vitro Scient, Egypt, based on the method described previously [15]. Oxidant/antioxidant parameters were determined by
commercial kits; brain malondialdehyde (MDA) [16], superoxide dismutase (SOD) [17] and nitric oxide (NO) [18] were estimated by colorimetric methods according to the methods described previously. All kits were purchased from BioMed. Diagnostics. Brain advanced oxidation protein products (AOPP) as a marker of oxidative stress was measured by ELISA kit [19].

**Determination of Brain Monoamines**

Determination of brain serotonin, noradrenaline, and dopamine was carried out using high performance liquid chromatography (HPLC) system, Agilent technologies 1100 series, equipped with a quaternary pump (Quat pump, G131A model). Separation was achieved on ODS-reversed phase column (C18, 25 x 0.46 cm i.d. 5 µm). The mobile phase consisted of potassium phosphate buffer/methanol 97/3 (v/v) and was delivered at a flow rate of 1 ml/min. UV detection was performed at 270 nm, and the injection volume was 20 µl. The concentration of both catecholamines and serotonin were determined by external standard method using peak areas. Serial dilutions of each standard were injected, and their peak areas were determined. A linear standard curve for each standard was constructed by plotting peak areas versus the corresponding concentrations. The concentration in samples was obtained from the curve.

**Estimation of urinary 8-hydroxyguanosine by HPLC:**

Protocol for urinary 8-hydroxyguanosine (8-OHdG) analysis was modified from the method described before [20]. 8-OHdG was extracted from 1 ml urine; eluents were dried under ultrapure N₂ stream and were reconstituted in 5 ml deionized water. 20 µl from each sample and also from the different concentrations of the standard were injected in HPLC, and the concentration of urinary 8-OHdG was calculated from the standard curve and then was divided on the urinary creatinine. Urinary creatinine was estimated by kinetic method as described by Larsen (1972) [21].

**HPLC condition:**

HPLC column C18 (260 x 4.6, particle size 5 µl) using mobile phase acetonitrile/methanol/phosphate buffer (25/10/965) v/v. Phosphate buffer was prepared by dissolving 8.8 g of potassium dihydrogen phosphate in 1000 ml deionized water and pH was adjusted at 3.5. The buffer was then filtered 2 times before being used at a flow rate of 1 ml/min using electrochemical detector with cell potential of 600 mV [4].

**RESULTS AND DISCUSSION**

Diabetes mellitus is a complex disease associated with peripheral and central complications. These complications include retinopathy, nephropathy and neuropathy [7].

Streptozotocin (STZ, 2-deoxy-2-(3-methyl-3-nitrosoureldo)-Dglucopyranose), is synthesized by Streptoinycetes achroniogens and has been long used to generate animal models of diabetes [22]. Streptozotocin-induced diabetic animals exhibit most of the diabetic complication [23].

![Fig. (1) : Fasting blood sugar in different studied groups](image-url)

Significant p value < 0.05, a* = significant difference compared to control group, b* = significant difference compared to diabetic group.
Fig. (2): MDA in different studied groups
Significant p value < 0.05, a* = significant difference compared to control group,
b* = significant difference compared to diabetic group.

Fig. (3): AOPP in different studied groups
Significant p value < 0.05, a* = significant difference compared to control group,
b* = significant difference compared to diabetic group.

Fig. (4): NO in different studied groups
Significant p value < 0.05, a* = significant difference compared to control group,
b* = significant difference compared to diabetic group.
Several investigations have confirmed the role of oxidative stress in development diabetic mediated disorders, possibly via the formation of free radicals [7]. Thus, we evaluated the brain oxidative stress induced by STZ in rats with consequent changes in brain neurotransmitters and examined the potential protective role of quercetin against the changes STZ induced.

In this study, the mean value of fasting blood sugar was significantly increased in diabetic rats compared to control (Figure 1).

Increased oxidative stress could be one of the common pathogenic factors of diabetic complications [24]. Oxidative damage is generally attributed to the formation of highly reactive OH which leads to severe oxidative damage of the cell's components like lipids, proteins and DNA [25]. These results were in agreement with our results, thus in this
study STZ significantly increased the oxidative stress parameters (brain MDA, AOPP and NO and urinary 8-OHdG), in addition to the reduction of brain SOD activity in diabetic group compared to control (Figures 2-6).

Measurement of brain MDA helps to assess the extent of tissues damage of diabetic rats and can be related to overproduction of lipid peroxidation and declined antioxidant enzyme (SOD) observed in diabetic rats can be attributed to increased membrane lipid peroxidation process and thereby contributing to alteration in antioxidant status [26]. The elevation of urinary excretion of 8-OHdG in this study reflects mitochondrial oxidative damage [24].

In recent years, the injuries of the central nervous system induced by diabetes has drawn more attention, and a lot of data show that, the patient may have damaged cognitive function, mainly the dysfunction in learning and memory [27].

In this study, STZ induction significantly increased brain monoamines in diabetic group compared to control. This result was in agreement with a previous study [28], which indicated that, diabetes alters the catecholaminergic system in a very specific manner.

Quercetin, a polyphenol of the flavonol group is found in abundance in green vegetables, green tea, citrus fruits, and red grape wine. Its protective role has been reported in animal models of diabetes. In the present study we have shown that quercetin works as an anti-diabetic agent via targeting both hyperglycemia and oxidative stress, this is shown by the reduction in fasting blood glucose levels in the quercetin supplemented group.

Concomitantly, Abdalla et al. [29] indicated that, quercetin prevents alterations in oxidative stress parameters as well as neurotransmitters parameters, consequently preventing memory impairment and anxiogenic-like behavior displayed by STZ. These results may contribute to a better understanding of the neuroprotective role of quercetin, emphasizing the influence of this flavonoid in the diet for human health, possibly preventing brain injury associated with diabetes.

It has been shown that quercetin scavenges reactive oxygen species and reduces oxidative DNA damage [30] as was found in our study. Various reports suggest that quercetin passes through the blood-brain-barrier and influences the neuronal cells directly. Indeed, a higher concentration of quercetin metabolites appears in the brain after several hours of administration of quercetin [31].

Quercetin inhibits lipid peroxidation and preserves brain antioxidant enzyme hence there is a significant elevation in brain SOD in the quercetin treated group in the current study. This proves the two important actions of quercetin in oxidative stress i.e., quenching of reactive oxygen species and enhancing the cellular antioxidant defense system. The antioxidant efficacy of quercetin is reported to be due to its higher diffusion into the membranes [32] allowing it to scavenge oxygen radicals at several sites throughout the lipid bilayer; and its pentahydroxy flavone structure allowing it to chelate metal ions via the ortho dihydroxy phenolic structure, thereby scavenging lipid alkoxyl and peroxyl radicals [33].

A novel mechanism for quercetin induced cytoprotection has been described involving the sterol regulatory element binding protein-2 (SREBP-2) mediated sterol synthesis that decreases lipid peroxidation by maintaining membrane integrity during oxidative stress [34]. In addition, quercetin metabolites can inhibit peroxynitrite-mediated oxidation, similar to free quercetin and confirming that flavonoids can protect against reactive oxygen species [35].

CONCLUSION

The current data appeared that, STZ causes an elevation of oxidative stress parameters in rat's brain as well as a depletion of antioxidant enzymes resulting in disturbances in brain monoamines level. Pharmacological agents like flavonoid quercetin, capable of scavenging free radicals and/or inhibiting lipid hydroperoxides, and thereby protecting neurons from oxidative injuries may provide useful therapeutic potentials for the prevention or treatment for the neurodegenerative disorders caused by stress.

Acknowledgement

The authors are thankful to the National Research Center (NRC) for the unlimited support to carry out this work.

REFERENCES


[14] AS Dias; M Porawski; M Alonso et al. **2005, J Nutr.** 135 (10); 2299-2304.


