ONION PEEL EXTRACTS AMELIORATE OXIDATIVE STRESS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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EKSTRAKTI KORE CRNOG LUKA SNIŽAVAJU OKSIDATIVNI STRES KOD PACOVA SA STREPTOZOTOCINOM INDUKOVANIM DIJABETESOM

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ABSTRACT

Background: *Onion peel extracts contain quercetin derivatives, flavonoids that have been shown to significantly improve diabetic status and to exhibit antioxidant properties in animal models. Vitamin E is an important antioxidant that is present within the cell membrane and acts as a lipid-soluble scavenger. This study aimed to compare the efficacy of an onion peel extract and vitamin E to alleviate the altered redox balance of diabetic rats.*

Methods: *Streptozotocin-induced diabetic male Wistar rats (n = 34) were randomly divided into three treatment groups. For 45 days, the first group was fed a normal diet (diabetic control group), the second group was fed a normal diet plus 20 mg/kg body weight vitamin E and the third group was fed a normal diet plus 1% OPE .*

Results: *The formation of malondialdehyde and protein carbonyls was significantly suppressed and the activity of superoxide dismutase was increased in different areas of the brain upon onion peel extract administration (P < 0.001) compared to the diabetic control group. Furthermore, vitamin E did not significantly decrease the level of oxidative stress or the blood glucose concentration in these rats.*

Conclusion: *OPE is better able to ameliorate oxidative stress and hyperglycaemia than vitamin E in a diabetic rat model.*

Keywords: *Diabetes mellitus, Streptozotocin, Onion peel extract, Vitamin E, Oxidative stress.*

SAŽETAK

Uvod: *Ekstrakt kore crnog luka sadrži derivate kvercetina, najznačajnijeg flavonoida, koji poboljšavaju dijabetički status i imaju antioksidativna svojstva na animalnim modelima. Vitamin E je važan liposolubilni antioksidant koji je prisutan u ćelijskim membranama, koji može da bude liposolubilni skevendžer. Cilj ove studije je da uporedi efikasnost regulisanja poremećene redoks ravnoteže ekstraktom kore crnog luka i vitamonom E kod pacova sa dijabetesom.*

Metode*: Mužjaci Wister pacova sa streptozotocin-inukovanim dijabetesom (n = 34) su randomizacijom podeljeni u tri grupe. Jedna grupa je bila na normalnoj ishrani (dijabetična kontrolna grupa), druga grupa je dobijala vitamin E u dozi od 20 mg/kg telesne mase tokom 45 dana, i treća grupa je dobijala 1% ekstrakt kore crnog luka tokom istog vremenskog perioda.*

Rezultati: *Malondialdehid i proizvodi stvaranja karbonilnih proteina su bili značanjno smanjeni, a aktivnost superoksid dizmutaze je bila značajno povišena u različitim delovima mozga usled primene ektrakta kore crnog luka (P < 0.001) u odnosu na dijabitičnu kontrolnu grupu, dok primena vitamina E nije dovela do značajnog smanjenja oksidativnog stresa ni koncentracije glukoze u krvi.*

Zaključak: *Regulacija oksidativnog stresa, kao i hipoglikemijski uticaj ekstrakta kore crnog luka, je bila mnogo više izražena u odnosu na vitamin E u dijabetičnom eksperimentalnom modelu pacova.*

Ključne reči: *diabetes mellitus, streptozotocin, ekstrakt crnog luka, vitamin E, oxidativni stres*

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INTRODUCTION

Diabetes is characterised by hyperglycaemia and metabolic abnormalities due to decreased insulin levels or activity, which cause metabolic and physiological changes in various organs, including the brain [1]. In diabetes mellitus, 60-70% of deaths are due to diabetic neuropathy. Diabetic neuropathy is a complication of long-term diabetes that is mainly caused by hyperglycaemia. This complication produces oxidative stress in the central nervous system, which causes an imbalance in the oxidative status of the nervous tissue and leads to microvascular cerebral diseases [2,3] .

The brain was previously considered to be an insulin-insensitive tissue. However, recent molecular studies have indicated that insulin is present in several regions of the central nervous system, where it acts as a neuromodulator, inhibiting food intake and stimulating fat oxidation [4]. Cerebral glucose is increased after the onset of diabetes in rats [5]. This increase in the intracellular glucose load leads to the autoxidation of glucose, the generation of free radicals, enhanced lipid peroxidation and non-enzymatic protein glycation, and increased activation of the polyol pathway. The central nervous system is highly susceptible to oxidative stress. The vulnerability of the brain to oxidative stress induced by oxygen free radicals seems to be because the brain utilises about one-fifth of the total oxygen demand of the body for oxidative phosphorylation to acquire energy and that as it has a relatively small antioxidant capacity [6], the brain cannot neutralise the toxic effects of free radicals. Furthermore, the brain contains a high concentration of easily peroxidisable fatty acids [7], and it is known that certain regions of the brain are highly enriched in iron, a metal that is catalytically involved in the production of damaging oxygen free radical species when it is in free form [3,8]. ROS overload damages many cellular components, including proteins, DNA and membrane phospholipids [9-15]. Lipid peroxidation is the consequence of ROS, the role of which is well established in the pathogenesis of a wide range of diseases, such as Alzheimer's disease and Parkinson's disease [16-18], acute brain injuries, such as ischemia and head trauma [19-21], and some major metabolic diseases, such as diabetes mellitus (DM) [22]. Lipid peroxidation and the secondary and end products of non-enzymatic (autoxidative) fatty peroxide formation and decomposition can produce a large variety of aldehydes, including hexanal, malondialdehyde (MDA) and 5-hydroxynonenal [15]. Conceptually, these three facts indicate that MDA is an excellent index of lipid peroxidation. Protein carbonyls (PCs) are generated from oxidatively modified cellular proteins through a variety of mechanisms, including the direct oxidation of amino acid side chains and oxidation-induced peptide cleavage.

There are several proposed methods to increase insulin sensitivity and to combat against oxidative stress. Early diagnosis and prompt initiation of therapy are the main factors in reducing the population burden of diabetes. Although changes in lifestyle (weight loss, exercise, restricted diet, etc.) is always recommended to fight diabetes, the compliance rate is not at all satisfactory. In addition, several medicines are available to treat this disorder, but the aggressive use of medicine is restricted due to unwanted side effects. Therefore, the recent research trend involves the identification of a treatment with minimal side effects and maximum disease prevention. Currently, the main focus of research is on herbal remedies [23]. Many studies have indicated that diabetes can be delayed or prevented with dietary flavonoids. Flavonoids are naturally found in plant foods, and the flavonoid quercetin is one of the most common flavonoids present in foods. Some recent studies have suggested that quercetin improves diabetic status by either decreasing oxidative stress [24-26] or correcting altered hepatic gene expression [27].

Onion bulbs are the richest source of dietary flavonoids. At least 25 different flavonoids have been identified in onion bulbs, and quercetin and its glycosides are the most important ones [28]. Quercetin is present at a high concentration in the outer dry layers of the onion bulb [29]. These layers show strong antioxidant activity, and it has been proposed that quercetin is the main factor for this activity [30].

Vitamin E is a lipid-soluble vitamin with chain-breaking antioxidant activity. The major function of vitamin E is its role as a physiological membrane-bound antioxidant, protecting all cell membrane lipids from oxidative damage induced by reactive oxygen species [31].

Thus, the present study was conducted to investigate the potency of onion peel extract (OPE) and vitamin E to ameliorate oxidative stress in streptozotocin (STZ)-induced diabetes in a rat model. Accordingly, we conducted this study using an experimental rat model, assuming that the results would have similar implications in humans.

MATERIAL AND METHODS

Study location

The present study was an animal model-based case-control study that was undertaken in the department of Biochemistry with the collaboration of the department of Pharmacology at Burdwan Medical College (Burdwan, West Bengal, India).

Animals

Male Wistar albino rats (*Rattus norvegicus albinus*), between 1 to 2 months of age and weighing 150 ± 12 g (n = 34), were obtained from the appropriately maintained institutional animal house. The rats had free access to drinking water and rat food pellets. The light source in the animal room was regulated with a 12:12 hr light-dark cycle, a temperature of 22 ± 2°C and 45-50% relative humidity. All rats were acclimatised for at least 7 days before the induction of diabetes. All procedures involving animals were performed in accordance with the 'Guide for the Care and

Use of Laboratory Animals (1985)' by the NIH (Bethesda, MD, USA) and the 'Guidelines for Care and Use of Animals in Scientific Research' by the Indian National Science Academy (INSA; New Delhi, India). The study was approved by the institutional ethics committee for the care and use of laboratory animals and started after obtaining written consent [Memo No. BMC/2179/1 (3)].

Preparation of OPE

The outer dry layers of onion bulbs (*Allium cepa* L.) were extracted with 60% ethanol adjusted to pH 5.5 at 50°C for 3 hours. The extract was concentrated and then freeze dried. The amount of total polyphenol and quercetin were 616.08 ± 13.82 mg/g and 104.52 ± 7.81 mg/g as determined by the methods of Folin-Ciocalteu [32] and Hertog *et al.* [33], respectively.

Study design

STZ was dissolved in saline sodium citrate buffer (50 mM sodium citrate, 0.9% NaCl, pH 4.5) . Diabetes was induced in male neonatal Wistar rats at birth by a single intravenous injection of freshly prepared STZ at a dose of 100 mg/kg body weight. Forty-two days after STZ administration, the plasma glucose level of each rat was determined to confirm the induction of diabetes[171]. Rats with fasting plasma glucose levels that were greater than 16.65 mmol/L were considered to be diabetic and used for further studies.

Then, the rats with fasting blood glucose levels above 300 mg/dl were randomly divided into 3 groups and treated for 45 days. The first group was fed a normal diet only (diabetic control group), the second group was fed a normal diet containing 20 mg/kg vitamin E and the third group was fed a normal diet containing 1% OPE. At the end of the treatment period, all the rats were sacrificed by cervical dislocation.

Tissue sample preparation

Preparation of brain extracts

To determine the ability of OPE and vitamin E to reduce oxidative stress in different areas of the brain, rat brains were dissected and segregated in the following order: cortex, cerebellum, midbrain and basal ganglia. The brain tissues were washed gently in saline to remove any blood and then immediately frozen, first at -20 °C and then at -70 °C, and kept under these conditions (-70 °C) until the chemical analysis was performed. All assays were completed on the same day of sample collection. For homogenisation, the samples were washed and then minced with a sharp surgical blade in small volumes of ice-cold (not frozen) homogenisation buffer [0.1 M Tris-HCl (pH 7.35) and 100 µM ethylenediaminetetraacetic acid (EDTA)]. Immediately, the samples were homogenised in 10 volumes of the ice-cold buffer solution in a motor-driven glass tissue homogeniser in presence of a few properly washed particles of sand. During the whole homogenisation procedure, the homogeniser was kept on ice to dissipate any heat. Thereafter, the samples were centrifuged at 10,000 × g for 10 min at 4°C. The supernatants from the homogenates were collected and were immediately analysed for MDA content, PC product content, cytosolic superoxide dismutase (Cu^{2+}-Zn^{2+}-SOD) activity and tissue protein concentration.

Collection and processing of blood

Blood was withdrawn from tail of each rat to determine the blood glucose level. Some of the blood was separated into a heparinised vial to obtain plasma.

Biochemical assays

Blood was separated into a heparinised vial to obtain plasma and a plain vial to obtain serum . Plasma glucose was assayed photometrically using the glucose oxidase peroxidase (GOD-POD) method [35]. MDA, a marker of lipid peroxidation due to oxidative stress, was measured via its reaction with thiobarbituric acid at 532 nm [36]. The brain tissue levels of MDA were calculated using a calibration curve that was derived using 1,1,3,3- tetraethoxypropane (Fluka, Germany) as an external calibration standard. The calibration curve was linear from 1.25-2.5 nmol/ml (r^2=0.997). Oxidation-induced changes in the tissue proteins were estimated by measuring the protein carbonyl product content. The method used is based on the reaction of carbonyl groups with 2,4-dinitrophenylhydrazine to form a 2,4-dinitrophenylhydrazone-reactive carbonyl derivative that can be measured at 370 nm. [37]. The cytosolic superoxide dismutase (SOD) activity was estimated using the method of Kakkar et al. [38], where one unit of SOD was defined as the amount of enzyme that inhibits the rate of electron transfer from NADH to nitroblue tetrazolium (NBT) by 50 % under specified conditions. The protein concentration was measured using the method of Lowry et al. [39], in which the proteins in the tissue homogenates react with alkaline copper sulphate, followed by Folin's phenol reagent (SRL, India). The absorbance values of the samples were then compared to a standard curve that was prepared using known concentrations of bovine serum albumin (Merck, Germany). All photometric measurements were performed with a dual-beam spectrophotometer (UV 5704SS). The blood glucose levels are expressed in units of mmol/L, while the other parameters are expressed in their corresponding units per mg of tissue protein.

Statistical analysis

The mean values were analysed for significant differences between the diabetic control group (I) and the treatment groups (II and III) using independent t-tests. For all tests, the p-value was considered to be significant if it was less than 0.05 at a confidence level of 95 %. All statistical analyses were performed with the SPSS statistical software package (version 11.5 for Windows).

Parameters	Parameters	Group I (STZ) n = 12	Group II (STZ + vit E) n = 10	Group III (STZ + OPE) n = 12	Group I vs. Group II	Group I vs. Group III	Group II vs. Group III
Blood glucose (mmol/L)	Plasma	22.5 ± 3.49	22.89 ± 0.48	8.98 ± 1.68	p> 0.05	p<0.001	p<0.001
Tissue MDA (nmol/mg protein)	Cortex	0.95 ± 0.06	0.89 ± 0.08	0.53 ± 0.04	p> 0.05	p<0.001	p<0.001
	Cerebellum	0.67 ± 0.06	0.64 ± 0.06	0.35 ± 0.06	p> 0.05	p<0.001	p<0.001
	Midbrain	0.79 ± 0.01	0.76 ± 0.04	0.33 ± 0.05	p> 0.05	p<0.001	p<0.001
	Basal ganglia	1.39 ± 0.28	1.27 ± 0.25	0.47 ± 0.04	p> 0.05	p<0.001	p<0.001
Tissue PC (mM/mg protein)	Cortex	0.31 ± 0.02	0.29 ± 0.02	0.18 ± 0.02	p> 0.05	p<0.001	p<0.001
	Cerebellum	0.26 ± 0.04	0.23 ± 0.03	0.11± 0.02	p> 0.05	p<0.001	p<0.001
	Midbrain	0.28 ± 0.02	0.26 ± 0.02	0.14 ± 0.03	p> 0.05	p<0.001	p<0.001
	Basal ganglia	0.56 ± 0.10	0.49± 0.11	0.25 ± 0.04	p> 0.05	p<0.001	p<0.001
Cytosolic SOD (IU/mg protein)	Cortex	0.66 ± 0.05	0.75± 0.05	1.31 ± 0.04	p = 0.032	p<0.001	p> 0.05
	Cerebellum	0.59 ± 0.05	0.71 ± 0.11	0.97 ± 0.11	p = 0.026	p<0.001	p> 0.05
	Midbrain	0.44 ± 0.02	0.52 ± 0.23	0.88 ± 0.15	p = 0.017	p<0.001	p> 0.05
	Basal ganglia	0.79 ± 0.12	0.98 ± 0.17	1.29 ± 0.20	p>0.05	p<0.001	p> 0.05

Table 1. Differences between the mean values of the studied parameters in the rats of the three treatment groups.

Values are means ± SD; $p < 0.05$ was considered to be statistically significant.

RESULTS

To compare the efficiencies of OPE and vitamin E in reducing oxidative stress, an independent sample t-test was performed between the Group I rats and the Group II rats as well as between the Group I rats and the Group III rats (Table 1). The MDA and PC product contents were found to be significantly suppressed and SOD activity was improved in different areas of the brain upon OPE administration ($P < 0.001$, Figure 2). In addition, the Group III rats showed a lower mean plasma glucose level (8.98 (range 7.3 to 10.66) mmol/L) than the diabetic control group (22.5 ± 3.49 (range 18.97 to 26.02) mmol/L). Diet supplementation with vitamin E failed to significantly decrease both the level of oxidative stress and the blood glucose concentration (Figure 2). In fact, there was significant decrease in the oxidative parameters of the OPE-treated group (III) compared to the vitamin E-treated group (II).

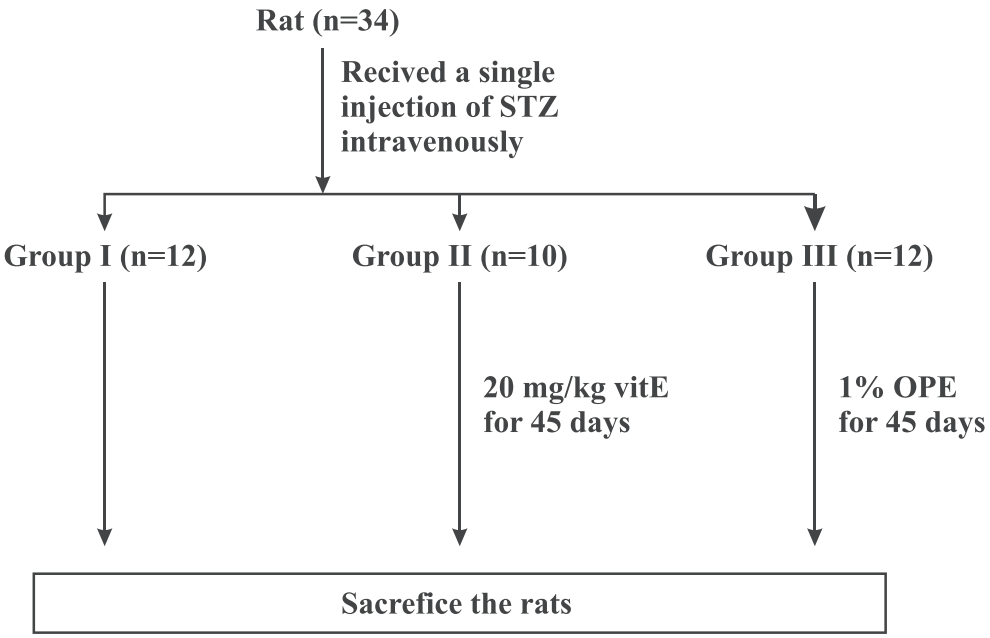

Figure 1. Study design.

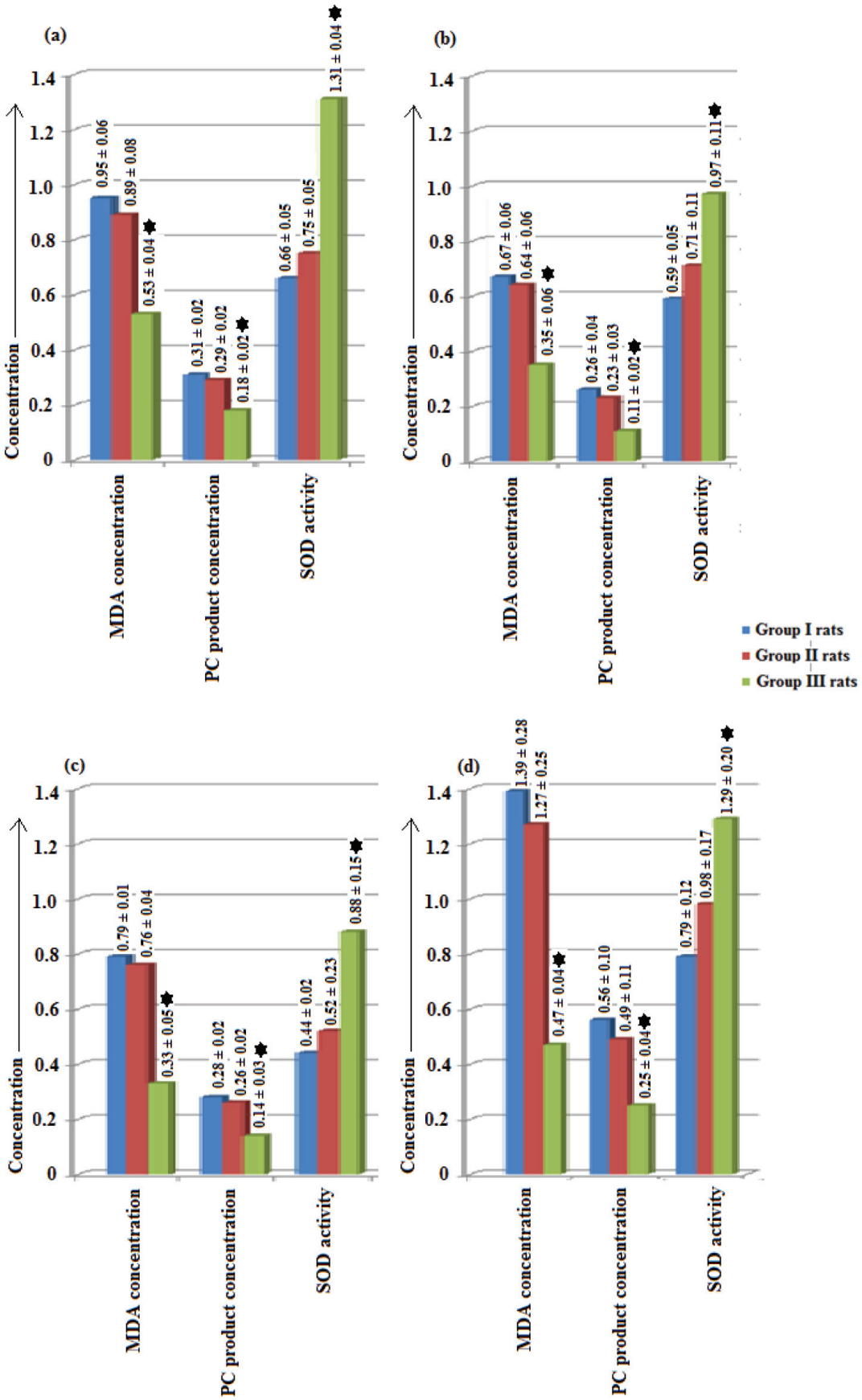

Figure 2. Histogram showing the distribution of MDA content, PC product content and SOD activity in the (a) cortex, (b) cerebellum, (c) mid-brain, and (d) basal ganglia of Group I (STZ), Group II (STZ + vit E) and Group III (STZ + OPE) rats. Asterisks indicate p<0.001.

DISCUSSION

Diabetes mellitus is a common but serious metabolic disorder that is associated with many functional and structural complications [40,41]. This disorder is associated with an increased production of reactive oxygen species (ROS) in both humans and animals. Experimental evidence has supported that ROS play roles in both pathogenesis and numerous pathophysiological mechanisms that trigger diabetic complications, which are primarily categorised as macroangiopathies or microangiopathies, the latter of which includes retinopathy, nephropathy, neuropathy, and microvascular damage to the cerebral artery [42,43]. Results of several previous studies have clearly indicated that STZ-induced hyperglycaemia causes oxidative stress, leading to compromised antioxidant activity in different areas of the brain [2,3,44-47]. Furthermore, hyperglycaemia-induced oxidative stress has been implicated in the development of diabetic neuropathy in the peripheral (PNS) and central nervous system (CNS) and nephropathy [48,49]. Free radical scavengers have been shown to protect neurons against a variety of experimental neurodegenerative conditions [50] and have been suggested to attenuate the oxidative stress and diabetic state induced by STZ [51,52]. The current study was designed to investigate the modulating effects of OPE and vitamin E on the altered redox balance observed in STZ-induced diabetic rats. We found that due to its own antioxidant activity as well as its insulin-sensitising effect, the administration 1% OPE alleviates oxidative stress more efficiently than vitamin E in all areas of the rat brain. Moreover, OPE improves hyperglycaemia, whereas vitamin E is ineffective in this area. This finding was well corroborated with a very recent work that demonstrated that OPE improves insulin action by up-regulating the expression of the insulin receptor and glucose transporters as well as by promoting glucose metabolism in peripheral tissues in diabetic rats.[53] In addition, several studies have suggested that impaired blood lipids are characteristic of subjects with insulin resistance, especially circulating FFAs. [54-56] FFAs directly activate macrophages to secrete pro-inflammatory cytokines that render muscle cells resistant to insulin [57,58]. FFAs also contribute to the increased production of reactive oxygen species and lead to the activation of stress-sensitive signalling pathways under hyperglycaemic status [59]. Upon the administration of OPE, the quercetin component of this extract acts as a strong antioxidant due to its ability to scavenge free radicals and bind to transition metal ions. By virtue of these properties, quercetin inhibits lipid peroxidation [60,61]. In the interaction of quercetin with free radicals, it can donate a proton and be converted to a radical itself, but the resulting unpaired electron is delocalised by resonance. Thus, the quercetin radical exists in a low energy state and, thus, is less reactive than its non-radical form [62]. Therefore, although the detailed mechanism awaits further investigation, we have shown that OPE improves insulin sensitivity and oxidative stress better than α-tocopherol in a rat brain model.

CONCLUSION

The present study demonstrated that OPE ameliorates oxidative stress and hyperglycaemia in diabetic rats better than vitamin E. Further studies should be performed to evaluate the use of onion peel extracts in the prevention and early treatment of type 2 diabetes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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