Diabetes-Induced Cortical Neural Damage in Male Wistar Rats: Ameliorative Role of a Flavonoid

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Abstract

Diabetes mellitus (DM) is a multifactorial metabolic disease characterized by persistent hyperglycaemia associated with numerous toxic outcomes including neuropathy amongst others. Naringenin (NG) is a flavonoid with diverse pharmacological spectrum. This study evaluated the ameliorative role of naringenin against streptozotocin–induced diabetic cortical neural damage in rats. Post induction of diabetes, 30 Wistar rats were randomly allotted to five groups of six each and orally treated daily thus: Group-1 (vehicle control) received 0.1 mol/L citrate buffer, group 2 (negative control), groups 3-5 received 25, 50 and 100 mg/kg body weight of NG respectively for 35 days during which their fasting blood glucose (FBG) levels were measured. On the 37th day, all animals were sacrificed under diethyl ether anaesthesia, cranial vaults were opened and cerebral cortex eviscerated and suspended in liquid nitrogen for 5 min until all particles were frozen. Samples were stored in freezer at -80 ^{0}C for tissue enzymatic studies. At 50 and 100 mg/kg NG, significant (P<0.05) dose-dependent reduction in FBG as well as elevated serum insulin levels at 100 mg/kg NG only were recorded. Increased activities superoxide dismutase, catalase and glutathione but decreases in malondialdehyde levels were recorded at 50 and 100 mg/kg NG compared to control. Findings reveal that NG strengthens the intrinsic antioxidant defence system against streptozotocin-induced diabetic cortical neural damage in lower animals and thus could be exploited as an adjuvant in the management of diabetes and neuropathies especially after validation via clinical trials.

Keywords*: cortical neural damage, diabetes mellitus, diabetic neuropathy, polyphenols, oxidative stress, antioxidant*

Introduction

Diabetes mellitus is a chronic, metabolic and debilitating disease usually characterized by persistent high blood glucose levels (hyperglycaemia), reduced insulin action or insulin deficiency (Akah *et al.*, 2002). The disease aetiology is multifactorial, its diagnosis and progression are associated with pathological outcomes potentiating reduced life expectancy. The hyperglycaemic condition in turn, induces oxidative stress through several mechanisms (Fig. 1) such as glucose autoxidation, formation of excess advanced glycation end products (AGE), enhancement of the polyol pathway and activation of diacylglycerol-protein kinase C (DAG-PKC) pathway (Brownlee, 2005) with consequential diabetic neurovascular complications. In the pathogenesis of type 2 diabetes mellitus (T2DM), the development of insulin resistance (IR) is one of the major hallmarks. There is an established relationship between diabetes as well as other diseases with oxidative stress, with the latter being implicated as a common pathogenic factor that leads to the development of tissues-specific insulin resistance (IR) in diabetes (Arcaro *et al.*, 2002). Thus, the reduced insulin action or lack thereof is pivotal to the metabolic derangements linked to hyperglycaemia and diabetes, thus potentiating diabetic pathological endpoints and complications especially when managed poorly (WHO, 2014; 2015).

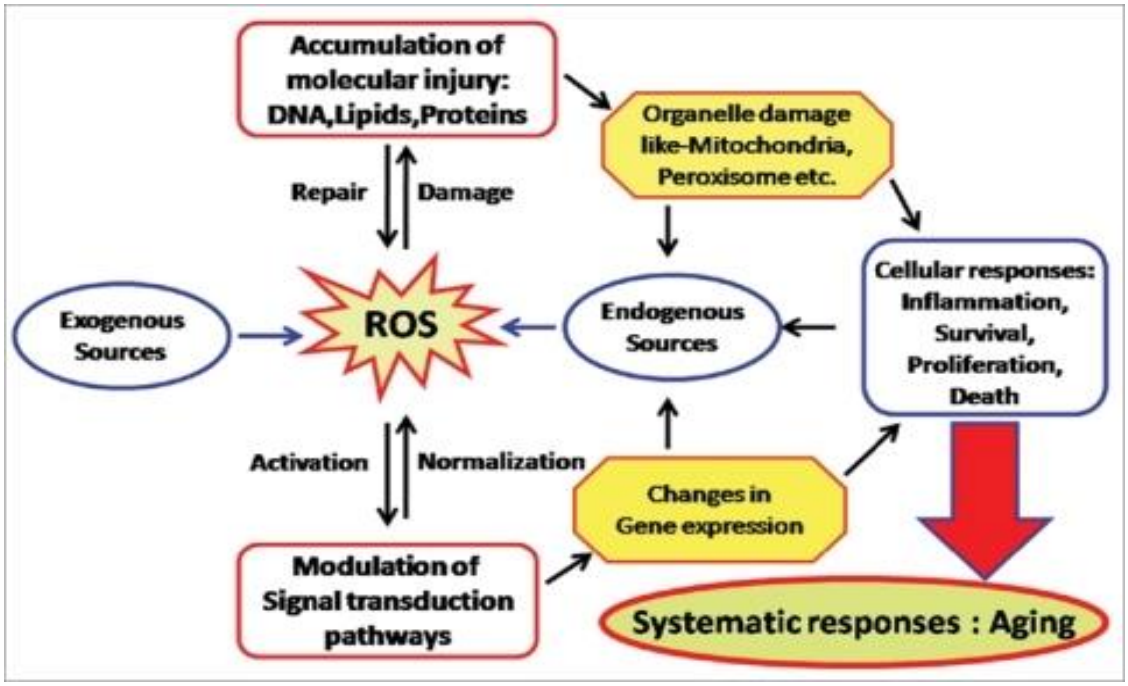

Fig. 1. Schematic representation of the possible pathway of oxidative stress damage
Source: www.ijmrs.org/oxidative_damage

Till date, numerous plant chemicals have been evaluated for their medicinal profile, and due to the far-reaching debilitating effects of diabetes, the quest for newer antidiabetic agents from plant sources has intensified. Thus, purified natural compounds from plants as well as their derivatives serve as templates for the synthesis of new generation antidiabetic drugs and nutraceuticals with heighten therapeutic but lower toxicity profile. Free radical scavengers like melatonin, lutein (Muriach *et al*., 2006), vitamin E (Tuzcu and Baydas, 2006) amongst others have been reported to ameliorate the free radical-mediated neural damage in a number of experimental diabetic models (Rask-Madsen and King, 2013; Al-Rejaie *et al.*, 2015). Naringenin (NG) is a known flavonoid with a diverse pharmacological spectrum. However, its neuroprotective role against diabetes-induced central neuropathy is yet to be evaluated. Therefore, the present study sought to investigate and evaluate the attenuating potential of naringenin on cortical neural damage in type 2 diabetes induced by streptozotocin in male Wistar albino rats.

Materials and Methods

Experimental Animals

Thirty-sixx (36) male Wistar albino rats weighing between 250 – 290 g were obtained from the Experimental Animal Care Centre of the College of Pharmacy, King Saud University (KSU), Riyadh, Saudi Arabia and were housed under air-conditioned room at regulated 25ºC ± 1ºC with 12 h light/dark cycle. The experimental animals were allowed to acclimatize to the room and were fed with standard mash (Grain Silos and Flour Mills Organization, Riyadh, Saudi Arabia) and water *ad libitum*. All experimental procedures were conducted in conformity with the institution's ethics (KSU) and National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH, 1996).

Induction of Diabetes

Thirty (30) rats were administered 60 mg/kg body weight of nicotinamide intraperitoneally (i.p), and 1 h thereafter, a freshly prepared streptozotocin (Cayman Chemical, Inc., USA) in 0.1 mol/L citrate buffered solution (pH 4.5) was administered to the animals. The control group was treated with 0.1 mol/L citrate buffer. Diabetes was confirmed 72 h thereafter via the fasting sugar test procedure using the tail blood read on ACCU-CHEK and compact plus glucometer (Roche Diagnostics, France). Experimental rats with blood glucose level of 250mg/dL and above were considered diabetic.

Research Design

Post-induction of diabetes, rats were randomly divided into five (5) groups, consisting of six (6) rats each. Group 1 (non-diabetic) served as the vehicle control and was treated orally daily with 0.1 mol/L citrate buffered solution (pH 4.5) only. Group 2 (diabetic) served as the negative control and received no treatment except food and water *ad libitum*. While groups 3, 4 and 5 were treated daily with low, middle and high doses of NG (25, 50 and 100 mg/kg body weight) respectively for 35 days, during which fasting blood glucose (FBG) levels were measured weekly and recorded. The administration of NG was done orally using gavages and all experimental subjects were closely monitored for weekly weight change, feeding and drinking habit as well as general morphological changes. On the 36th day, all animals were weighed and fasted overnight, and were euthanized under diethyl ether anaesthesia and sacrificed. From each euthanized animal, the cranial vault was opened and the cerebral cortex was eviscerated. The eviscerated cerebral cortexes were suspended in liquid nitrogen for 3 – 5 minutes until all particles were frozen. These samples were stored in a freezer regulated at -80 ^{0}C until when used for tissue enzymatic antioxidative stress studies.

Tissue Preparation for Enzyme Assay

The cerebral cortex of each rat was homogenized in 10 mL of 100 mMol KH_2PO_4 buffer containing 1 mMol EDTA (pH 7.4). It was centrifuged at 12,000 rpm for 30 min at 4 ^{0}C. The post-mitochondrial supernatant was then collected for enzymatic studies. The protein concentration of the tissue was determined by the method of Lowry *et al*. (1951), using crystalline Bovine Serum Albumin (BSA) as standard.

Estimation of Catalase (CAT) Activities in the Cerebral Cortex

The catalase (CAT) enzymatic activity was measured according to method of Aebi (1984). The post-mitochondrial supernatant of the cerebral cortex homogenate was mixed with 50 mMol/L phosphate buffer (pH 7.0) and 20 mMol/L H_2O_2 and read spectrophotometrically, thus following the decrease in absorbance at 240 nm, CAT activity was determined and expressed in terms of units/mg protein.

Estimation of Superoxide Dismutase (SOD) Activities in the Cerebral Cortex

The enzymatic activity of SOD was assayed according to the method earlier reported by Kono (1978). Superoxide anions generated hydroxylamine hydrochloride oxidation

mediated nitro-blue-tetrazolium reduction to a blue formazon, which was then measured at 560 nm under aerobic conditions. Superoxide dismutase inhibits nitro-blue-tetrazolium reduction. The extent of the inhibition was taken as a measure of SOD activity. This was expressed as units/mg protein.

Estimation of Glutathione (GSH) Level in the Cerebral Cortex

Glutathione level was estimated using the method earlier described by Sedlak and Lindsay (1968). This involved the mixing of the cerebral cortex homogenate with 0.2M Tris buffer (pH 8.2) and 0.1 mL of 0.01 M Ellman's reagent, [5,5'-dithiobis-(2-nitro-benzoic acid)] (DTNB). The mixtures were centrifuged at 3000 rpm at room temperature for 15 min and the absorbance of the clear supernatant was read at 412 nm to measure the concentration of glutathione using spectrophotometer (LKB-Pharmacia, Ireland). GSH was expressed as U/mg protein.

Estimation of Malondialdehyde (MDA) Level in the Cerebral Cortex

The level of MDA, a known product of lipid peroxidation (LPO) in each homogenized cerebral cortex was estimated using TBARS assay kit (ZeptoMetrix Corporation, USA) following the method of Colado *et al.* (1997). Exactly 100 mL of the homogenate was mixed with 2.5 mL of the kit's reaction buffer and was heated for 1 h at 95 ^{0}C. After cooling, the absorbance of the supernatant was measured at 532 nm using spectrophotometer (LKB-Pharmacia, Ireland). This estimation was done in strict adherence to manufacturer's protocols. MDA was expressed as nmol/mg protein.

Statistical Analysis

Using Graph Pad Prism software (version 6), the results were statistically analysed by one-way of variance (ANOVA) followed by Newman–Keuls as post hoc test and $p<0.05$ was considered statistically significant. The results were expressed as mean ± SD.

Results

Effect of Naringenin on Blood Glucose Levels

The diabetic rats had significantly (P <0.05) higher fasting blood glucose levels as well as reduced insulin levels compared to vehicle control animals. At 50 and 100 mg/kg body weight of NG, a significant dose-dependent decrease in fasting glucose levels were respectively recorded (Fig. 2). However, insulin level was significantly higher at 100 mg/kg body weight of NG only, compared to the negative control group (Fig. 3).

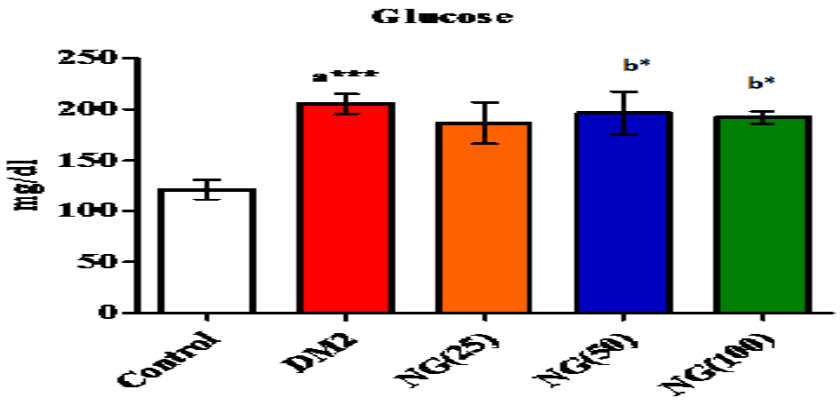

Figure 2: Effect of Naringenin (NG) on fasting blood glucose levels of diabetic rats. Data expressed as mean ± SD; n = 6; C = control group, DM2 = diabetic group; [a] = C versus DM2; [b] = DM2 versus NG – 50 & NG – 100. **P*< 0.05 and ***P*< 0.01 for b* and a*** respectively.

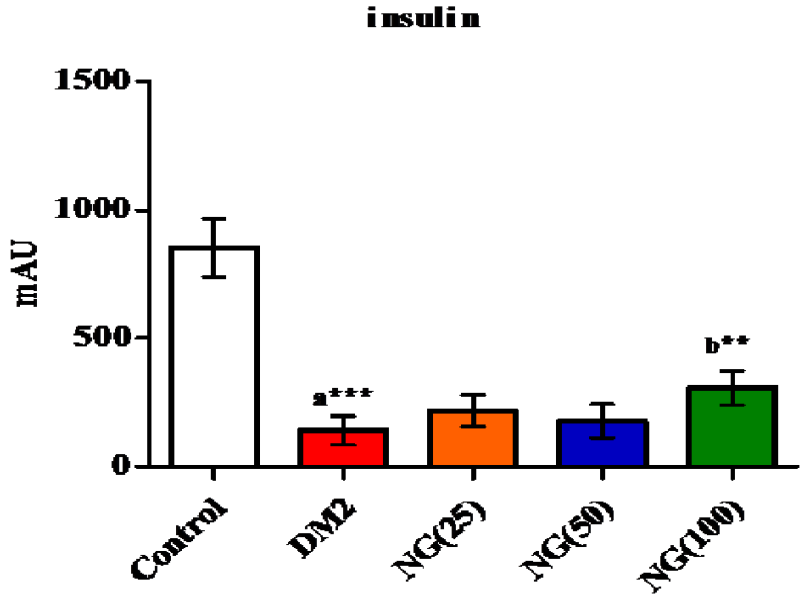

Fig. 3: Effect of Naringenin (NG) on insulin levels of diabetic rats. Data expressed as mean ± SD; n = 6; C = control group, DM2 = diabetic group; [a] = C versus DM2; [b] = DM2 versus NG – 50 & NG – 100. **P*< 0.05 and ***P*< 0.01 for b* and a*** respectively.

Effect of Naringenin on CAT Activities

The result revealed a significant (*P*<0.05, *P*<0.01) reduction in the enzymatic

activities of CAT in the eviscerated cerebral cortex (es) of the diabetic rats compared to the vehicle control group. At 50 and 100 mg/kg body weight of NG, significant increases in the activities of CAT were respectively recorded in a dose-dependent fashion compared to the negative control (Fig. 4).

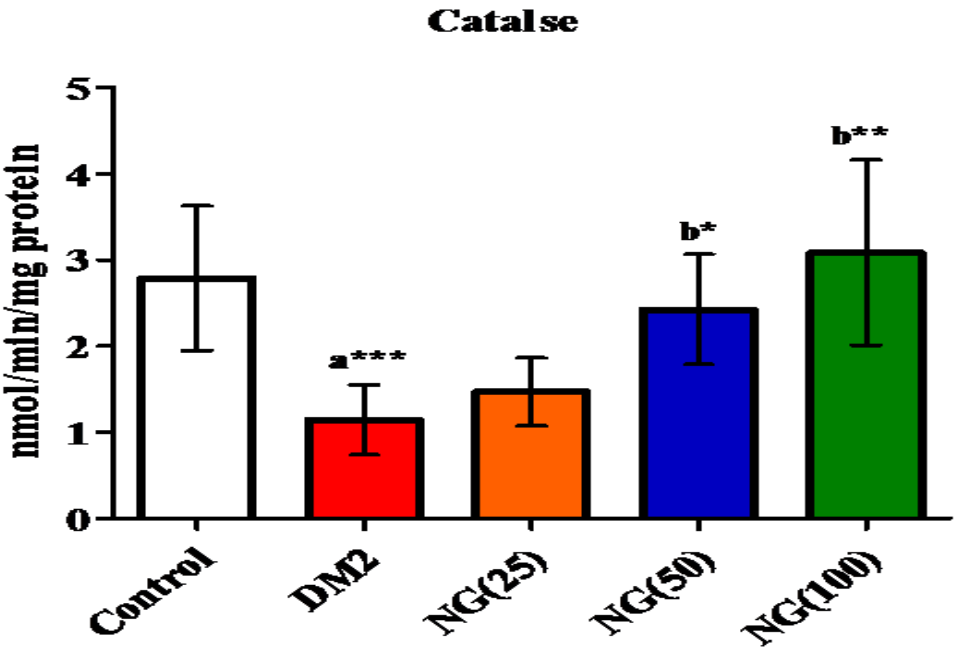

Fig. 4. Effect of Naringenin (NG) on CAT activities in STZ-induced diabetic rats. Data expressed as mean ± SD (n = 6); DM2 = diabetic group; [a] = control versus DM2; [b] = DM2 versus NG (50 mg/kg) and NG (100 mg/kg). *$p < 0.05$ and **$p < 0.01$ and ***$p < 0.001$ respectively.

Effect of Naringenin on SOD Activities

Superoxide Dismutase (SOD) activity was significantly reduced in the cerebral cortex of diabetic rats compared to vehicle control. But at 100 mg/kg body weight of NG, the activities of SOD were significantly enhanced compared to the negative control (Fig. 5).

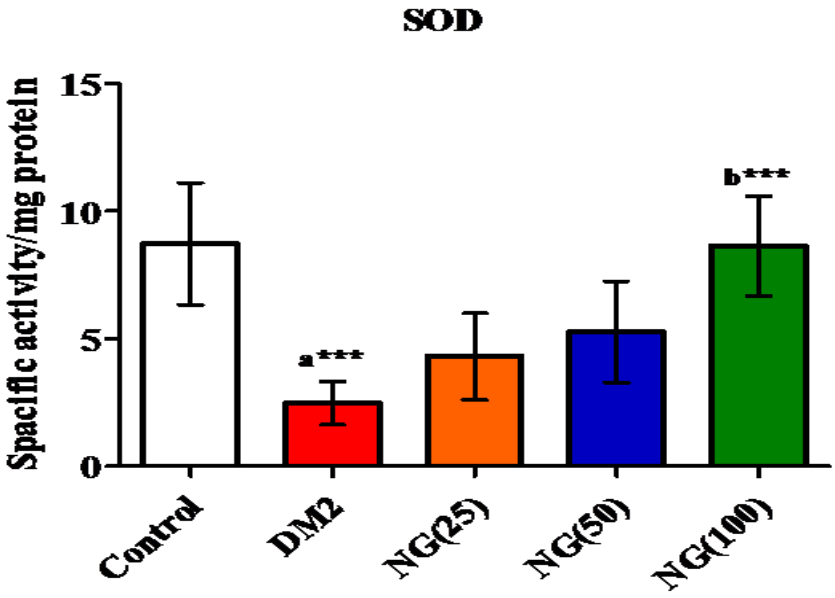

Fig. 5. Effect of Naringenin (NG) on SOD activities in STZ-induced diabetic rats. Data expressed as mean ± SD (n = 6); DM2 = diabetic group; [a] = control versus DM2; [b] = DM2 versus NG (50 mg/kg) and NG (100 mg/kg). .

Effect of Naringenin on GSH and GR Activities

The result showed significantly reduced activity of GSH and GR in the cerebral cortex of the diabetic animals compared to vehicle control. However, administration of varying doses of NG to the diabetic rats significantly elevated the glutathione levels as well as glutathione reductase (GR) activity in the cerebral cortex of the experimental animals compared to untreated diabetic rats (Fig. 6 and 7).

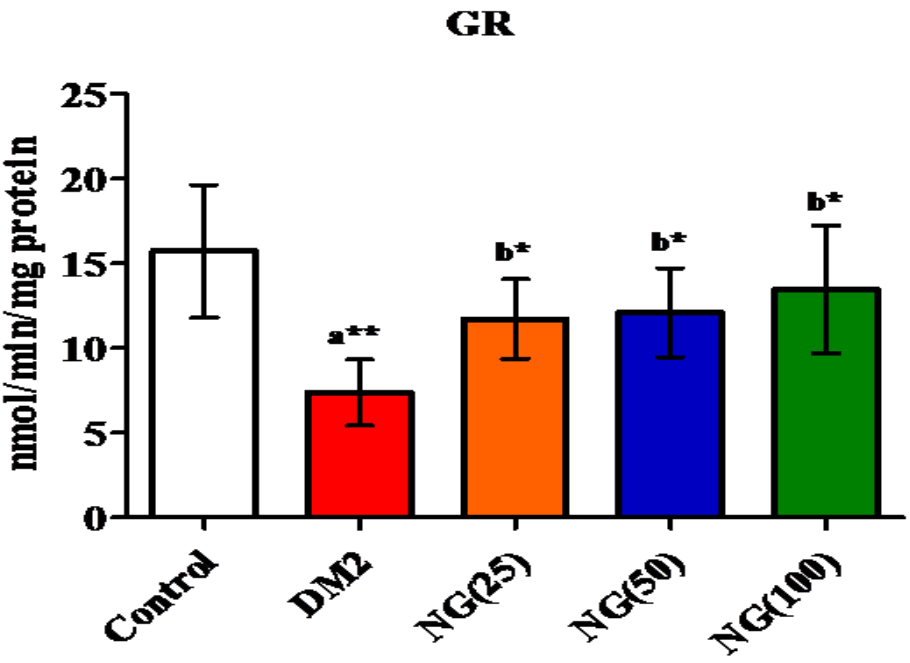

Fig. 6. Effect of Naringenin (NG) on GR in diabetic rats. Data expressed as mean ± SD; n = 6; C = control group, DM2 = diabetic group; [a] =C versus DM2; [b] = DM2 versus NG – 50 & NG – 100. *P< 0.05 and **P< 0.01 respectively.

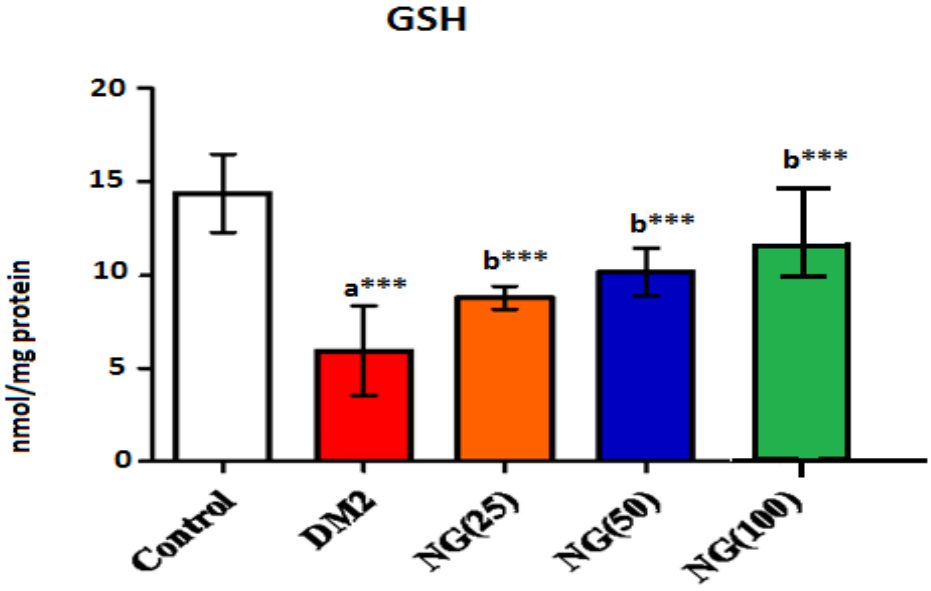

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Fig. 7. Effects of Naringenin (NG) on GSH in diabetic rats. Data expressed as mean ± SD; n = 6; C = control group, DM2 = diabetic group; [a] = C versus DM2; [b] = DM2 versus NG – 50 & NG – 100. *P< 0.05 and **P< 0.01 respectively.

Effect of Naringenin on MDA levels

The result presented a significantly elevated MDA levels in the cerebral cortex of the experimentally-induced diabetic rats compared to vehicle control. However, the administration of 50 and 100 mg/kg body weight of NG to diabetic rats showed significant reduction in MDA levels compared to negative control group (Fig. 8).

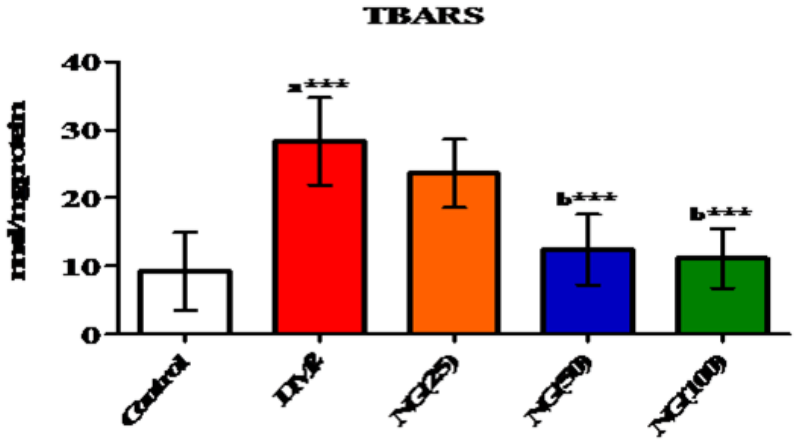

Fig. 8: Effect of Naringenin (NG) on the levels of TBARS and GSH in the cerebral cortex of diabetic rats. Data expressed as mean ± SD; n = 6; C = Control Group, DM2 = Diabetic Group; [a] = C versus DM2; [b] = DM2 versus NG – 50 or NG – 100. *P< 0.05; ***P< 0.01 respectively.

Discussion

Streptozotocin (STZ) is an alkylating antineoplastic agent and a naturally occurring glucosamine-nitrosourea substance with known toxicity profile to the pancreatic beta cells. Due to its identical nature to glucose, STZ transport into the cells is aided by the GLUT2 glucose transporter, thus explaining its relative toxicity to the pancreatic beta cells that are densely populated by this transporter (Schnedl *et al.*, 1994; Wang and Glenchmann, 1998). Its diabetogenic effect is believed to be through the activation of poly-ADP ribose polymerase (PARP) with a consequential pancreatic beta cells programmed cell death (Herceg and Wang, 2001). The programmed cell death of the beta cells depletes insulin production and levels, inducing hyperglycaemia that characterizes type 2 diabetes mellitus. Glucose metabolism is regulated chiefly in the liver, muscle and fat cells by insulin and glucagon and thus in diabetes, such intrinsic regulation is lost or hindered over time

resulting in hyperglycaemia. Therefore, the observed reduction in FBG as well as the increase in insulin levels recorded in this study suggests the ameliorative potentials of NG against the effect of STZ on the pancreas, perhaps by preventing the apoptosis of the beta cells via its antioxidant prowess. Earlier studies showed a link between oxidative stress and the pathogenesis of diabetes and its complications (Figueroa-Romero *et al.*, 2008; Ola *et al.,* 2014). Also, our findings demonstrate the involvement of oxidative stress as the activities of antioxidants enzymes in the cerebral cortex were grossly hindered. In this study, lipid peroxidation biomarker (MDA) in the cerebral cortex of diabetic animals was remarkable high, while GSH, GR, GPX, SOD and CAT activities were drastically inhibited. Several literatures have documented glutathione alterations – both in status and utilization, as an inevitable pathogenic hallmark in metabolic syndrome-associated oxidative stress. Thus, our findings corroborate such earlier studies (Zherebitskaya *et al.,* 2009; Al-Rejaie *et al.,* 2015) and recorded a significantly low GSH levels in the cerebral cortex of diabetic rats compared to that of non-diabetic animals. Glutathione, an endogenous antioxidant and coenzyme is known to mop up generated free radicals both from endogenous and exogenous sources, and thus is rightly considered an early and primary defence against oxidative stress and lipid peroxidation. The present study suggests that the neural cells of the cerebral cortex might be more vulnerable to be damaged by hyperglycaemia-induced oxidative stress.

The peroxisome proliferator activated receptor alpha (PPARα) has been reported to be involved in the regulation of specific amino acid found in the pathophysiological processes associated with metabolic disease. Thus, PPARα activators regulate the metabolism of cysteine – a rate-limiting substrate for the biosynthesis of GSH (Bella *et al.,* 1999; Kersten *et al.,* 2001). Also, phenolic and polyphenolic compounds like flavonoids and catechin have been strongly indicated to possess potent antioxidant properties and thus very functional in the prevention and therapy of disease (Decker, 1995; Fang, 2002). These plant chemicals are reported to be involved in the regulation of γ-glutamate cysteine ligase (γGCL) that catalyses the rate-limiting condensation of cysteine and glutamate to γ-glutamylcysteine in the synthesis of GSH (Guelzim *et al.,* 2011). Fang *et al.* (1998) showed a link between polyphenols and disease prevention and thus reported the pharmacological usefulness of tea polyphenols in the treatment of type 2 diabetes mellitus, hypertension and coronary heart disease. Naringenin is polyphenolic phytochemical capable of

activating the peroxisome proliferator activated receptors. Thus, the treatment of diabetic rats with varying doses of NG reversed the depleted GSH levels in diabetic rats. These observations strongly suggest that NG might be helpful in preventing diabetic neuropathy chiefly by reducing and/or ameliorating oxidative insults.

What is more, the treatment of experimentally induced diabetic rats with NG increased the activities of SOD, CAT, GPX and GR but decreased the level of MDA and this may be due to its ability to scavenge the free radical generated during diabetic-induced oxidative stress and Lipid peroxidation. As earlier reported, NG protects cells from ROS-mediated necrosis and apoptosis through its free radical scavenging actions (Miral *et al.*, 2002). The findings of the present study point towards the important role of antioxidant mechanism of NG in protection against cortical neural damage associated with diabetes in animal model.

Conclusion

Data generated from this study has shown NG to counteract the cortical neural damage associated with experimentally induced diabetes chiefly through its anti-diabetic and antioxidant properties, pointing toward the pharmacological usefulness of NG in the management of chronic diabetes associated with neuropathies. Thus, NG could be exploited as complementary and alternative therapy for diabetic neuropathy.

Conflict of Interest

The authors have no conflict of interest to declare.

Acknowledgement

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