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I.C. CHIS[1], A. CLICHICI[2], A.L. NAGY[3], A. OROS[3], C. CATOI[4], S. CLICHICI[1]

QUERCETIN IN ASSOCIATION WITH MODERATE EXERCISE TRAINING ATTENUATES INJURIES INDUCED BY EXPERIMENTAL DIABETES IN SCIATIC NERVES

[1]Department of Physiology, 'Iuliu Hatieganu' University of Medicine and Pharmacy, Cluj-Napoca, Romania; [2]Student, University of Medicine and Pharmacy 'Iuliu Hatieganu', Cluj-Napoca, Romania; [3]Department of Veterinary Toxicology, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania; [4]Department of Veterinary Pathology, University of Agricultural Sciences and Veterinary Medicine, Cluj Napoca, Romania

Diabetic neuropathy (DN) is a frequent, serious and debilitating chronic complication of diabetes mellitus (DM). Hyperglycemia, oxidative and nitrosative stress are involved in causing nerve damage in several animals, humans and experimental models of diabetes. This study was designed to investigate the synergistic cumulative neuroprotective effect of quercetin administration and moderate exercise training on sciatic nerves injuries in streptozotocin (STZ)-diabetic rats. DM was induced in Wistar rats by intraperitoneal administration of STZ (60 mg/kg). The diabetic rats received quercetin (30 mg/kg body weight/day) and performed an exercise training program (30 minutes/day, 5 days/week) for 5 weeks. Various biochemical parameters of oxidative and nitrosative stress were determined and histopathological evaluations were performed from sciatic nerves. Diabetic rats showed significantly increased oxidative and nitrosative stress parameter levels in the sciatic nerves. Diabetic trained rats treated with quercetin exhibited a significantly reduced hyperglycemia and its metabolic abnormalities induced by intraperitoneal administration of STZ. Histological alterations of the sciatic nerves induced after STZ administration were restored by administration of quercetin. Quercetin administration in association with moderate exercise training not only attenuated the diabetic condition but also restored sciatic nerves injuries by controlling hyperglycemia to down-regulate the generation of free radicals, as well as the elevation of antioxidant enzymes.

Key words: *diabetes mellitus, sciatic nerves, diabetic neuropathy, oxidative stress, quercetin, exercise training, reactive oxygen/nitrogen species, lipid peroxidation*

INTRODUCTION

The oxidative and nitrosative stress, hyperglycemia, hyperlipidemia, inflammation and impaired insulin signaling during the development of diabetes mellitus (DM), a complex chronic endocrine-metabolic disorder, can cause diabetic complications (1,2). Among these complications we can enumerate chronic tissue dysfunction and organ failure such as retinopathy, nephropathy, cardiomyopathy and neuropathy, complications conditions considered as risk factors for morbidity and mortality in patients with DM (3, 4).

Diabetic peripheral neuropathy (DPN) is an important complication of DM that affects approximately 60% of diabetic patients. Dyslipidemia, persistent hyperglycemia, inflammation, over production of reactive oxygen/nitrogen species and lowering the antioxidant defenses are mechanisms which play an important role in the development of DPN (5-8). In DPN the oxidative stress results from an over production of reactive oxygen/nitrogen species (ROS/RNS) generated by glucose autoxidation, mitochondria dysfunction, protein glycation and from decreased antioxidant defenses (8). Oxidative stress causes accumulation of ROS and RNS in cells, from either excessive production or insufficient neutralization, causing damage to proteins, lipids and DNA.

Various therapeutics are available for the treatment of diabetic neuropathy but one absolute cure does not exist and additional research is necessary to comprehensively understand the underlying pathophysiological pathways. A number of recent studies have demonstrated the potential health benefits of natural compounds, flavonoids such as quercetin, which may be effective as supplementary treatment for diabetes and its complications (2, 8-12).

The flavonol quercetin (3,3',4',5,7-pentahydroxyflavone) is present in many fruits and vegetables such as onions, apples, berries, red grapes, red wine, broccoli, tea, flowers, *etc*. (9, 11, 13). The quercetin exhibits varied biological actions such as antioxidant (14), anti-inflammatory, antibacterial, antiviral, gastroprotective, immune-modulator, antiatherogenic, antihypertensive, inhibition of angiogenesis and carcinogenesis. Recent researches have shown its hypoglycemic and neuroprotective effects in DM (9, 11, 12, 15-17).

The therapeutic and protective effects of exercise training on DPN have been established in many previous studies (5, 6, 18, 19). Regular physical activity exerts health-beneficial effects, including cognitive and mental functions (20, 21). Recent researches have shown that the early aerobic exercise program can improve the symptoms of neuropathy and restore morphometric alterations in the injured nerves, especially those

in small diameter fibers (5, 6, 18). After treadmill exercise (for a period of four consecutive weeks), a significant reduction in myelin breakdown and remarkable improvement in degenerated sciatic nerve was observed in experimental diabetic neuropathy (19). The effects of training in DPN depend on the duration and the moment of its start. Thus, if the duration of the exercise program is too long, stressful conditions can cause hyperglycemia or hypoglycemia, as well as they can possibly increase the risk of musculoskeletal injuries and can aggravate DPN. Also, for retarding the progression of DPN, an early exercise program is considered better than a late exercise program (5, 6, 18).

In the present study, we wanted to evaluate the effects of quercetin in association with moderate swimming exercise on sciatic nerves injuries in streptozotocin (STZ)-induced diabetic rats. We hypothesized that quercetin and moderate exercise training might reduce sciatic nerve (histopathological) damage induced by DM by attenuating fasting blood glucose levels and by suppressing oxidative and nitrosative stress.

MATERIAL AND METHODS

Animals

Eighty healthy Wistar albino male rats (three months old) (body weight = 270 ± 30 g) were randomly divided into control and a diabetic model group. The rats were purchased from the Experimental Animal House of the Faculty of Medicine within 'Iuliu Hatieganu' University of Medicine and Pharmacy of Cluj-Napoca, Romania. All the animals used in the experiment were kept for ten days to acclimatize to the conditions of the Animal House Laboratory at the Physiology Department before being introduced in the study. Throughout the entire period of the experiment, all the rats were maintained in special cages, which were kept under control, artificially illuminated (12 h dark/12 h light cycle), at a temperature of 21 – 23°C and at 50% – 60% humidity in an animal room. The animals were given standard rat pellets diet and water *ad libitum*.

All the experiments were performed according (No. 78/20.02.2014) to the approved animal protocols of the Ethical Committee on Animal Welfare of 'Iuliu Hatieganu' University in accordance with the Romanian Ministry of Health and complying with Guidelines in the Use of Animals in Toxicology.

Induction of experimental diabetes mellitus by streptozotocin

Type 1 diabetes mellitus was induced in rats by a single intraperitoneal (i.p.) injection of streptozotocin (STZ) (Sigma-Aldrich Chemical Company Inc., Gillingham, Dorset, UK) at a dose of 60 mg/kg body weight in 0.1 mol/L sodium citrate buffer, pH 4.5, as described previously (12, 22-24). Streptozotocin injected animals were given an i.p. injection with 1 mL of 10% glucose saline, 30 minutes after STZ administration to prevent initial drug-induced hypoglycemic mortality (25, 26). The control group who received a single i.p. injection with an equal volume of citrate sodium buffer used to dissolve STZ. Afterwards, 96 h after the STZ administration, diabetes was confirmed by measuring the fasting blood glucose (FBG) concentration. Rats that had a higher FBG level than 250 mg/dL (13.89 mmol/L) were included in the study as diabetic rats. One week after the STZ administration (the 8th day), the FBG level was measured and the treatment and exercise training started. Blood glucose was measured from the retro-orbital venous plexus of overnight fasting animals using the ACCU-CHEK Sensor System from Roche Diagnostics GmbH (Mannheim, Germany). The monitoring of FBG level was made through a quick method, using a single drop of capillary blood, sampled from the level of the tail, applied on a glucose test strip (Glucometer ACCU-CHEK Go, Roche).

Moderate exercise training protocol

The rats went through a regular moderate exercise program by swimming exercises for 30 minutes/day, 5 days/week, for 5 weeks. According to the exercise training program used in the reports by Pyun *et al*. (27) and Teixeira de Lemos *et al*. (28) the swimming exercise program was described in the previous article (12). The sedentary rats were subjected to the same sampling and handling procedures as the exercise training animals, but they remained in their cages without food and water for the duration of the swimming exercise period.

Animals groups and treatment

The rats were randomly divided into eight experimental groups (n = 10): the first group (control + sedentary, CS)-non-diabetic, sedentary untreated control rats; the second group (control + exercise, CE)-non-diabetic, trained untreated control rats; the third group (control + sedentary + quercetin, CSQ)-non-diabetic, sedentary control rats treated with quercetin; the fourth group (control + exercise + quercetin, CEQ)-non-diabetic, trained control rats treated with quercetin; the fifth group (diabetes + sedentary, DS)-diabetic, sedentary untreated control rats; the sixth group (diabetes + exercise, DE)-diabetic, trained untreated control rats; the seventh group (diabetes + sedentary + quercetin, DSQ)-diabetic, sedentary rats treated with quercetin; the eighth group (diabetes + exercise + quercetin, DEQ)-diabetic, trained rats treated with quercetin.

The quercetin (Sigma-Aldrich Chemical Company Inc., Gillingham, Dorset, UK) was orally administered *via* an intragastric tube (0.6 mL/rat), in a dose of 30 mg/kg body weight once a day for 5 weeks. Quercetin was suspended in 0.5% carboxymethylcellulose (CMC) solution as a vehicle. The rats from the groups: CS, CE, DS and DE were treated with CMC (0.6 mL/rat) administered orally once a day for 5 weeks. The quercetin and CMC administration in diabetic rats started one week after the STZ administration.

Then the animals were included in a long-term study for 6 weeks. For the entire duration of the experiment, the following features were monitored in each animal: the water consumption (daily), food consumption (weekly), body weight (BW) (weekly), mortality (daily) and diuresis (daily). The FBG level was measured in all the experimental animals at the beginning of the experiment, 96 hours after the STZ administration, 7 days after the STZ administration and at the end of the experiment.

Six weeks after the STZ administration, after overnight food deprivation, the blood glucose of the all experimental rats was measured using a glucometer and then the animals were anesthetized with an i.p. injection of sodium pentobarbital (60 mg/rat) and sacrificed by cervical dislocation. The sciatic nerve of the left front paw was isolated and rinsed in cold saline.

Sampling and tissue processing

The sciatic nerve tissue was divided into two parts: one part was frozen with liquid nitrogen and stored in a –80°C refrigerator until biochemical assays and the other part was divided into two sections of 1 mm. The sections were immediately immersed in 10% formalin for histopathological evaluation.

Preparation of sciatic nerve homogenates

The sciatic nerve samples were homogenized in 0.1 M Tris HCl buffer at pH 7.4 with a Brinkman Polytron Kinematica homogenizer (Lucerne, Switzerland). Then, the nerve homogenate was centrifuged at 2500 rpm for 10 min at 4°C. The supernatant was removed from the homogenates and quickly frozen at –80°C until determination of biochemical parameters.

Measurement of biochemical parameters of oxidative and nitrosative stress

By measuring the free radical production, the levels of oxidative and nitrosative stress in the sciatic nerve homogenate were indirectly assessed.

Estimation of lipid peroxidation

The lipids are one of the primary targets of ROS. The peroxidation of lipids produces highly reactive aldehydes, including malondialdehyde (MDA). The MDA is a primary biomarker of free radical mediated lipid damage and oxidative stress. The MDA levels were measured from the sciatic nerve homogenate using the fluorimetric method with 2-thiobarbituric acid described by Conti method (29). The MDA was spectrofluorimetrically determined in the organic phase using a synchronous technique with excitation at 534 nm and emission at 548 nm. The MDA levels were expressed as nanomole per milligram protein (nmol MDA/mg protein).

Estimation of protein carbonylation

The protein carbonylation under ROS action was estimated from the sciatic nerve homogenate by measuring protein carbonyl (PC) group levels. The protein carbonyl derivatives that are produced through the protein oxidative damage were determined using the fluorimetric method with 2,4-dinitrophenyl-hydrazine (DNPH) (30). The readings were performed using a spectrophotometer at 355 – 390 nm and in order to calculate the remaining carbonyl fragments, the molar extinction coefficient with a value of 22,000/M/cm was used. The levels of the carbonyl-derivative groups were expressed as nanomole per milligram of protein (nmol/mg protein).

The activity of some antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) in the sciatic nerve homogenate was determined.

Estimation of superoxide dismutase activity

The SOD activity was determined by enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minneapolis, MN, USA). The SOD activity was expressed as units per milligram of protein (ng/mg protein).

Estimation of catalase activity

The CAT activity was assayed using the method proposed by Pippenger *et al*. (31). The method consists in following the change in absorbance of a solution of H_2O_2 10 mM in potassium phosphate buffer 0.05 M, pH = 7.4 to 240 nm. One unit of CAT is defined as the amount of enzymes which induces reduction in the absorbance of 0.43 at 25°C for 3 min. The CAT activity was also expressed as units per milligram of protein (U/mg protein).

Estimation of nitrite concentration

The accumulation of nitrite in the supernatant, an indicator of nitric oxide (NO) production, was determined by colorimetric assay with Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) (Titherage method) (32). The supernatant was mixed with an equal volume of Griess reagent followed by spectrophotometric measurement at 543 nm. The concentration of nitrite in the supernatant was determined by comparison with a sodium nitrite standard curve. The nitrite levels are presented as nanomole per milligram of protein (nmol/mg protein).

Estimation of inducible nitric oxide synthase (iNOS)

The level of iNOS in the sciatic nerve homogenate was measured using the commercially available ELISA kit (R&D Systems). The iNOS levels are expressed in nanogram per milligram of protein (ng/mg protein).

Light microscopy studies of the sciatic nerve

Sections of the sciatic nerve were taken during necropsy and fixed in 10% neutral buffered formalin, embedded in paraffin and cut at 4 micrometers. Serial sections were stained using hematoxylin and eosin (H&E) stain and Goldner's Trichrome stain. Then the slides were examined under a BX51 Olympus microscope (2 Corporate Center Drive, Melville, NY 11747-3157, U.S.A.). The images were taken with an Olympus UC 30 digital camera and processed by a special image acquisition and processing program (Olympus Stream Basic). The neurohistopathological study aimed to evidence inflammation in all nerve compartments, nerve fiber injury and vascular injury (33). The nerve fiber injury was assessed according to Lirk *et al*. (33) using a simple, semi-quantitative four-point score, where 0 represents a normal morphology nerve and 4 represents extreme injury with inflammation and destruction of all components of the nerve, including axons and myelin, extending throughout the nerve bundle.

Statistical analysis

The statistical analysis was performed using the SPSS software package (version 17.0, SPSS Inc., Chicago, IL 60606-6412, USA). The data were reported as mean ± S.D. One-way analysis of variance (ANOVA) was used to compare differences between groups, and two-way ANOVA for repeated measurements, followed by Tukey's multiple posttest comparisons, to compare the responses to quercetin and exercise. Three-way ANOVA was used to compare cumulative response to quercetin and exercise in diabetes. Differences were considered significant if $P < 0.05$.

RESULTS

Changes in the blood glucose level in the experimental groups

The fasting blood glucose (FBG) levels of all experimental rats were measured at the beginning of the experiment (day 1), 96 h (day 5) and 1 week (day 8) after the administration of streptozotocin (STZ) and at the end of the experiment (day 42) (*Table 1*). The diabetic rats in all groups (DS, DE, DSQ and DEQ) showed very significant increase ($P < 0.0001$) in FBG levels as compared with control non-diabetics animals (CS, CE, CSQ and CEQ). The diabetic rats of DS group showed very significant increase ($P < 0.0001$) in FBG levels until the end of the experiment as compared with all experimental groups. Significantly decreased ($P < 0.05$) FBG levels were observed in the DE group after 5 weeks of moderate exercise training. Quercetin-treated diabetic animals (DSQ and DEQ) showed a significant ($P < 0.0001$) reduction in the FBG levels after 5 weeks as compared with control groups and

Table 1. The effects of quercetin and moderate exercise training on fasting blood glucose (FBG) (mg/dL) in control and diabetic rats.

	CS	CE	CSQ	CEQ	DS	DE	DSQ	DEQ
Initial FBG (mg/dL)	82.8±5.18	85±5.17	83.9±1.79	84±2.90	86.1±3.14	86.6±2.75	87.2±2.86	88.4±2.54
The FBG (mg/dL) on the 5th day	83.3±4.54	85.8±3.61	84.1±1.79	84.4±2.71	292±31.28^{aaa}	296.8±27.97^{aaa}	297.8±26.65^{aaa}	294±29.6^{aaa}
The FBG (mg/dL) on the 8th day	82.8±5.18	85±5.45	83.9±1.79	84±2.90	354.3±9.15^{aaa}	350.6±10.82^{aaa}	356.5±7.89^{aaa}	358.8±5.07^{aaa}
Final FBG (mg/dL)	82.9±5.15	84.7±5.29	83.8±1.54	84.1±2.76	489.6±23.66^{aaa}	347.4±9.69^{b}	197.2±11.06^{bbb}	167.5±54.07^{bbb}

Results are the means ± S.D. for ten animals in each group in the CS (non-diabetic sedentary untreated control rats), CE (non-diabetic trained untreated control rats), CSQ (non-diabetic sedentary control rats treated with quercetin), CEQ (non-diabetic trained control rats treated with quercetin), DS (diabetic sedentary control rats), DE (diabetic trained untreated control rats), DSQ (diabetic sedentary rats treated with quercetin), DEQ (diabetic trained rats treated with quercetin) groups. Statistically significant differences are indicated by the symbols: $^{aaa}P < 0.001$ vs. CS group; $^{b}P < 0.05$, $^{bbb}P < 0.001$ vs. DS group.

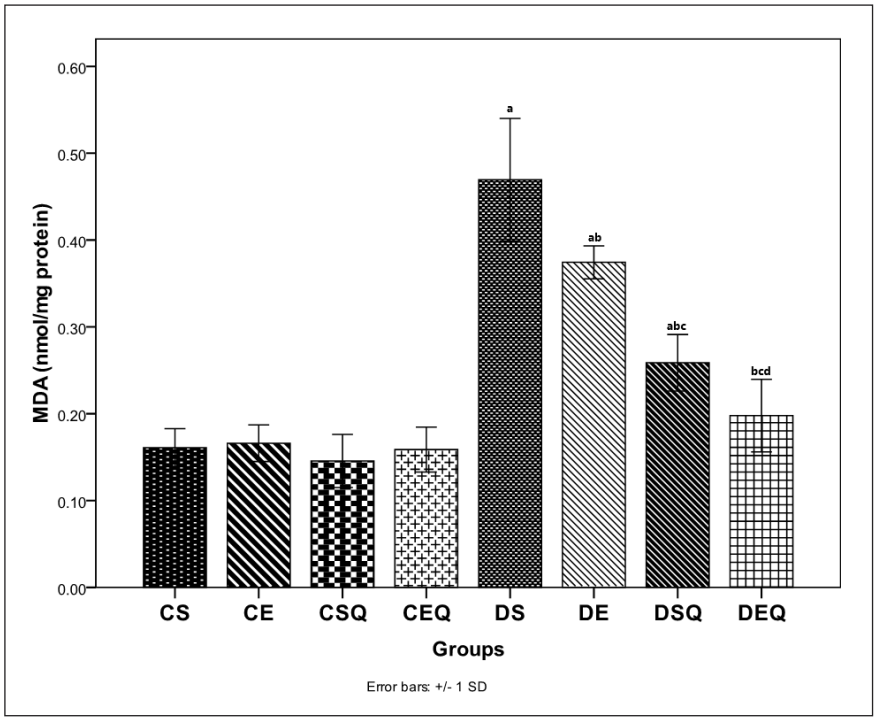

Fig. 1. The effects of quercetin and moderate exercise training on lipid peroxidation (MDA) (nmol/mg protein) levels in the sciatic nerve tissue homogenates of control and diabetic rats. Results are the means ± S.D. for ten animals each group defined as in the caption of *Fig. 1*. Statistically significant differences are indicated by the symbols: $^{a}P < 0.05$ vs. CS group, $^{b}P < 0.05$ vs. DS group, $^{c}P < 0.05$ vs. DE group and $^{d}P < 0.05$ vs. DSQ group.

quercetin treatment in association with moderate exercise training had cumulative effects in significantly reducing the FBG levels in diabetic rats (DEQ group).

Oxidative stress in sciatic nerve of the rats of all experimental groups

As a measurement of oxidative stress we determined the MDA and PC levels in the sciatic nerve of STZ-induced diabetic rats after 5 weeks of diabetes (*Fig. 1* and *Fig. 2*). The data in *Figs. 1* and *2* demonstrate that the MDA and PC levels in the sciatic nerve of diabetic rats are significantly increased ($P < 0.05$) after 5 weeks of diabetes (DS group). The moderate exercise training for 5 weeks significantly decreased the MDA and PC levels ($P < 0.05$) in the sciatic nerve of the diabetic rats (DE group). The quercetin treatment of diabetic rats (DSQ group) significantly decreased the MDA and PC levels ($P < 0.05$) in the sciatic nerve. The diabetic rats treated with quercetin and subjected to moderate exercise training for 5 weeks (DEQ group) showed significantly reduced MDA and PC levels ($P < 0.05$) in the sciatic nerve as compared with the control diabetic rats (DS, DE and DSQ groups).

Figs. 3 and *4* show the effects of quercetin and moderate exercise training on the activities of SOD and CAT in the sciatic nerve of the control normal rats and diabetic rats. The activities of SOD (*Fig. 3*) and CAT (*Fig. 4*) in the sciatic nerve were significantly lowered ($P < 0.05$) 5 weeks after the STZ administration. Moderate exercise training for 5 weeks significantly increased the SOD and CAT activities in the sciatic nerve of diabetic rats (DE group). The SOD (*Fig. 3*) and CAT (*Fig. 4*) activities in the sciatic nerve significantly increased ($P < 0.05$) in the diabetic rats treated with quercetin and subjected to moderate exercise training (DEQ group) as compared with the diabetic control rats (DS, DE and DSQ groups). Quercetin treatment and the moderate exercise training had cumulative effects ($P < 0.05$) on the increase of antioxidant enzymes (SOD and CAT) in the sciatic nerve of diabetic rats.

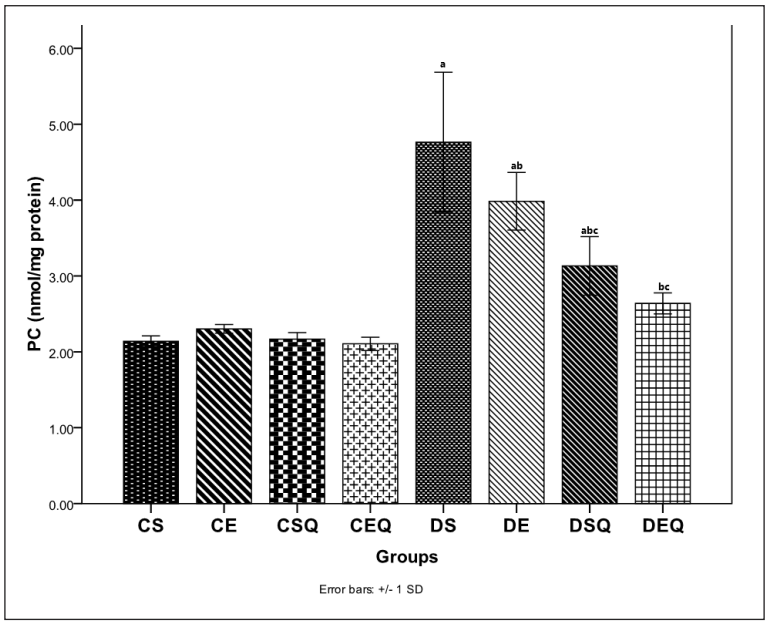

Fig. 2. The effects of quercetin and moderate exercise training on protein carbonyl (PC) groups (nmol/mg protein) in the sciatic nerve tissue homogenates of control and diabetic rats. Results are the means ± S.D. for ten animals each group defined as in the caption of *Fig. 1*. Statistically significant differences are indicated by the symbols: $^{a}P < 0.05$ vs. CS group, $^{b}P < 0.05$ vs. DS group and $^{c}P < 0.05$ vs. DE group.

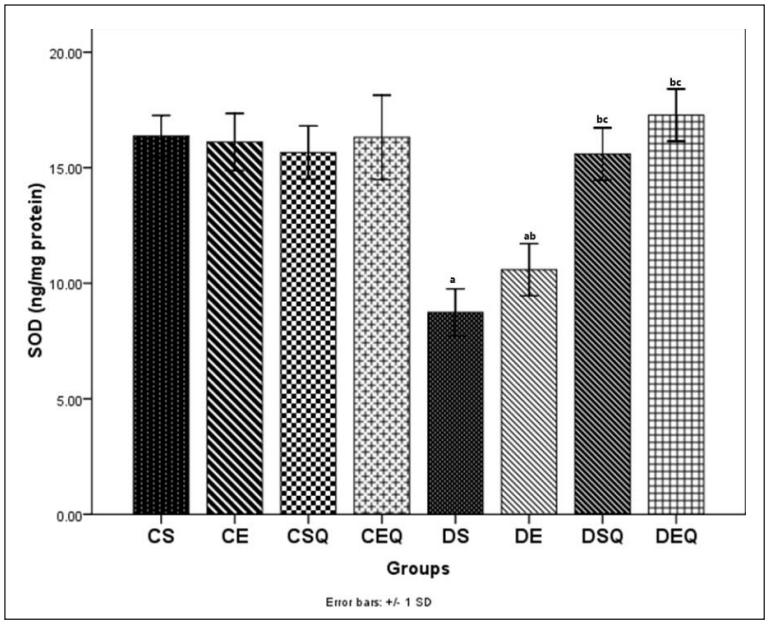

Fig. 3. The effects of quercetin and moderate exercise training on the level of superoxide dismutase (SOD) (ng/mg protein) activity in the sciatic nerve tissue homogenates of control and diabetic rats. Results are the means ± S.D. for ten animals each group, defined as in the caption of *Fig. 1*. Statistically significant differences are indicated by the symbols: $^{a}P < 0.05$ vs. CS group, $^{b}P < 0.05$ vs. DS group and $^{c}P < 0.05$ vs. DE group.

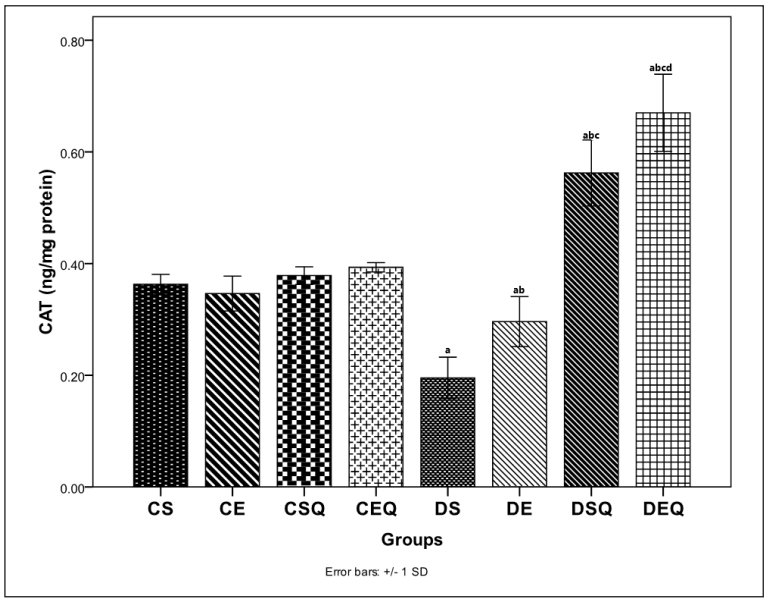

Fig. 4. The effects of quercetin and moderate exercise training on the level of catalase (CAT) (ng/mg protein) activity in the sciatic nerve tissue homogenates of control and diabetic rats. Results are the means ± S.D. for ten animals each group, defined as in the caption of *Fig. 1*. Statistically significant differences are indicated by the symbols: $^{a}P < 0.05$ vs. CS group, $^{b}P < 0.05$ vs. DS group, $^{c}P < 0.05$ vs. DE group and $^{d}P < 0.05$ vs. DSQ group.

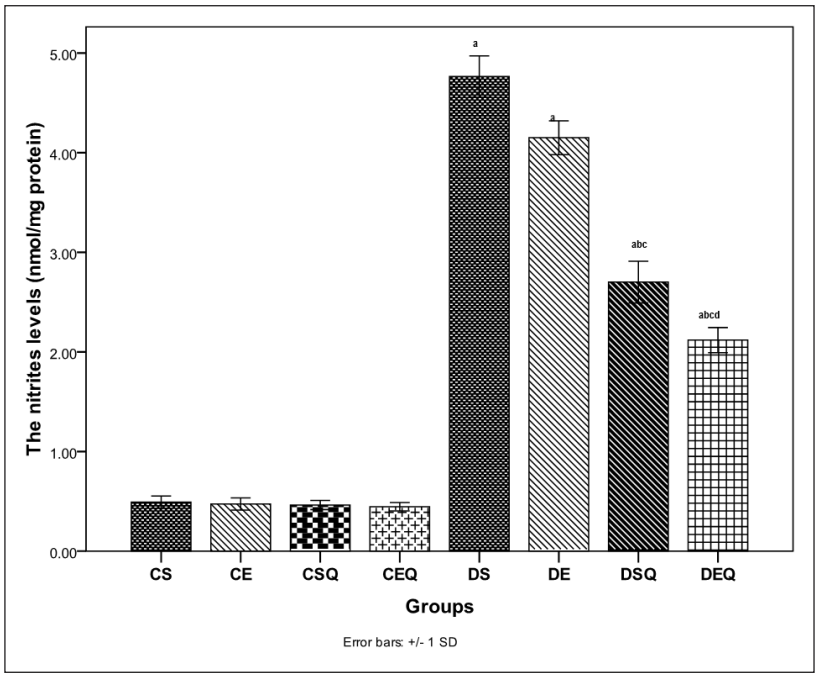

Fig. 5. The effects of quercetin and moderate exercise training on the level of nitrites (nmol/mg protein) in the sciatic nerve tissue homogenates of control and diabetic rats. Results are the means ± S.D. for ten animals each group, defined as in the caption of *Fig. 1*. Statistically significant differences are indicated by the symbols: $^{a}P < 0.05$ vs. CS group, $^{b}P < 0.05$ vs. DS group, $^{c}P < 0.05$ vs. DE group and $^{d}P < 0.05$ vs. DSQ group.

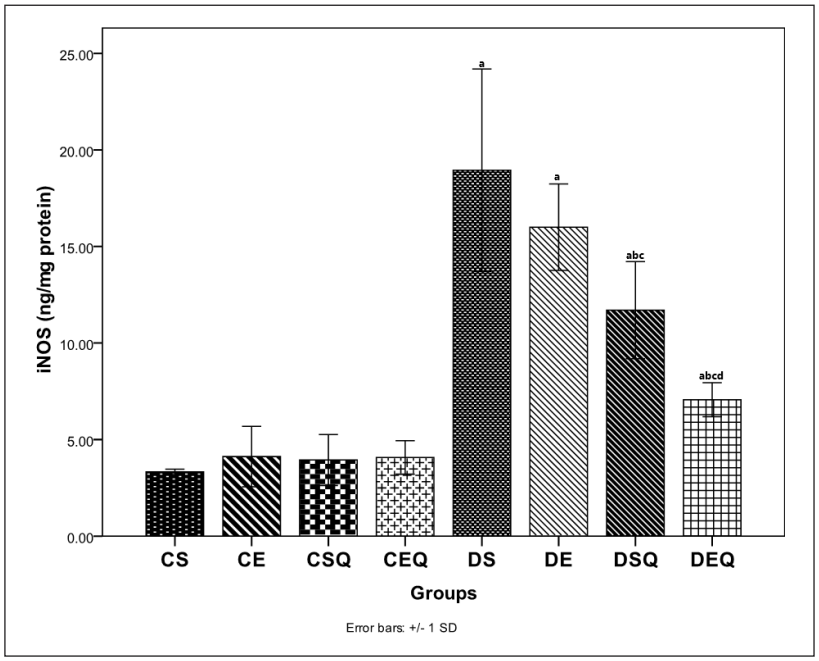

Fig. 6. The effects of quercetin and moderate exercise training on the level of inducible nitric oxide synthase (iNOS) (ng/mg protein) in the sciatic nerve tissue homogenates of control and diabetic rats. Results are the means ± S.D. for ten animals each group, defined as in the caption of *Fig. 1*. Statistically significant differences are indicated by the symbols: $^{a}P < 0.05$ vs. CS group, $^{b}P < 0.05$ vs. DS group, $^{c}P < 0.05$ *vs.* DE group and $^{d}P < 0.05$ vs. DSQ group.

The changes of the nitrite levels in the sciatic nerve tissue and iNOS production in experimental groups

The levels of nitrites (biomarker for NO production) were increased ($P < 0.05$) in the sciatic nerve in the diabetic rats (DS group) and significantly decreased ($P < 0.05$) after 5 weeks of moderate exercise training (DE group) and respectively after 5 weeks of quercetin treatment (DSQ group). Quercetin administration in association with moderate exercise training for 5 weeks exerted cumulative effects ($P < 0.05$) in reducing the levels of nitrites in diabetic rats (DEQ group) (*Fig. 5*).

The NO bioavailability in the sciatic nerve of the diabetic rats has been assessed by determining inducible nitric oxide synthase (iNOS) levels (*Fig. 6*). The iNOS levels in the sciatic nerve significantly increased ($P < 0.05$) 5 weeks after STZ administration (DS group). The diabetic rats subjected to moderate exercise training (DE group) exhibited significantly decreased iNOS levels ($P < 0.05$) in the sciatic nerve as compared with the diabetic control rats (DS group). The quercetin treatment on the diabetic sedentary rats (DSQ group) and on the diabetic trained rats (DEQ group) determined significantly decreased iNOS levels ($P < 0.05$) in the sciatic nerve as compared with the diabetic control rats (DS and DE groups).

Histopathological findings in the sciatic nerve

Under H&E and Goldner's Trichrome staining, there was no histological difference in the examined sections of the sciatic nerve in the non-diabetic control groups (CS, CE, CSQ and CEQ) (*Figs. 7* and *8A-8D*).

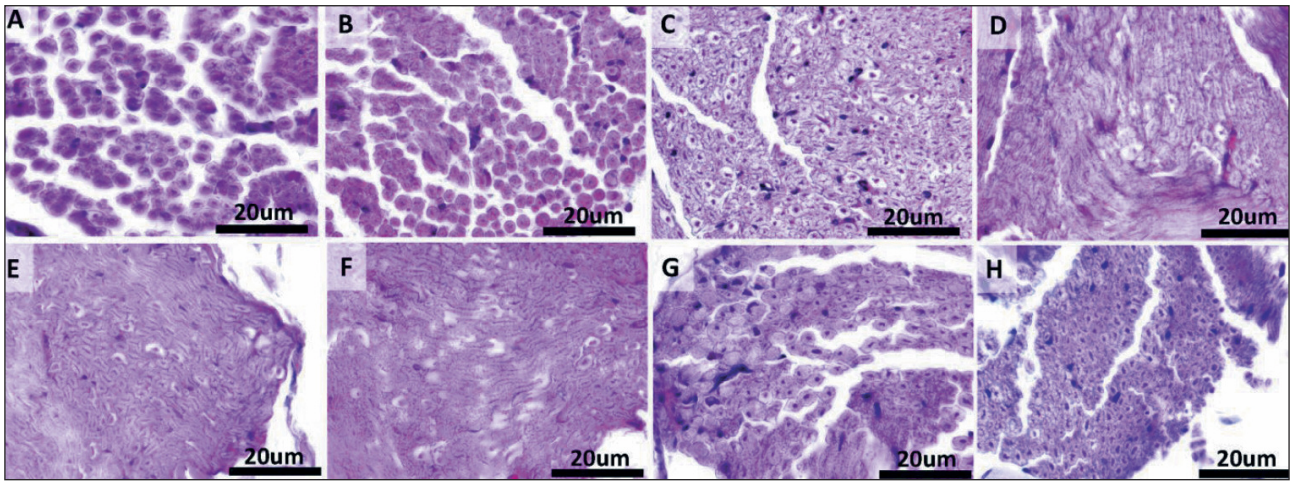

Fig. 7. Morphology of the sciatic nerve of rats in: CS group: non-diabetic sedentary untreated control rats (*A*); CE group: non-diabetic trained untreated control rats (*B*); CSQ group: non-diabetic sedentary control rats treated with quercetin (*C*); CEQ group: non-diabetic trained control rats treated with quercetin (*D*); DS group: diabetic sedentary control rats (*E*); DE group: diabetic trained untreated control rats (*F*) and DSQ group: diabetic sedentary rats treated with quercetin (*G*); DEQ group; diabetic trained rats treated with quercetin (*H*). H&E stain. Scale bar = 20 µm.

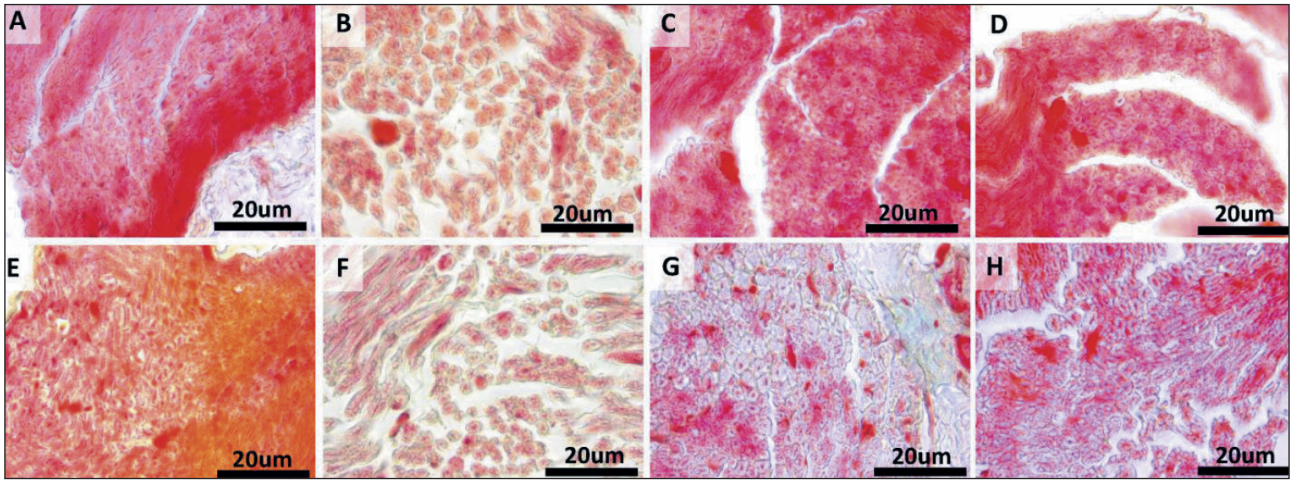

Fig. 8. Morphology of the sciatic nerve stained with Goldner's trichrome stain method in: CS group: non-diabetic sedentary untreated control rats (*A*); CE group: non-diabetic trained untreated control rats (*B*); CSQ group: non-diabetic sedentary control rats treated with quercetin (*C*); CEQ group: non-diabetic trained control rats treated with quercetin (*D*); DS group: diabetic sedentary control rats (*E*); DE group: diabetic trained untreated control rats (*F*) and DSQ group: diabetic sedentary rats treated with quercetin (*G*); DEQ group; diabetic trained rats treated with quercetin (*H*). Goldner's trichrome stain. Scale bar = 20 µm.

In H&E staining of the sciatic nerve of the animals from the non-diabetic groups (*Fig. 7A-7D*) showed normal morphology. The injury in the sciatic nerve of diabetic rats (DS group) was more severe compared with the non-diabetic control groups, consisting in axonal degeneration (atrophy), myelin sheet disruption, myelinic edema and endoneural edema (*Fig. 7E*). No signs of inflammation and vascular changes were observed in any of the animals.

In the diabetic rats, which were subjected to moderate exercise training (DE group) and received quercetin (DSQ group), the severity of histopathological alterations was relatively less than that of diabetic rats (DS group) (*Fig. 7F-7G*). The diabetic rats from the DEQ group (*Fig. 7H*) showed myelinic edema and axonal atrophy with partial regeneration (almost normal morphology).

Non-diabetic control groups (CS, CE, CSQ and CEQ) showed normal morphology in Goldner's trichrome staining of the sciatic nerve (*Fig. 8A-9D*). Under in Goldner's trichrome staining, the axonal degeneration (atrophy), myelin sheet disruption, myelinic edema and endoneural edema in the sciatic nerve were observed in the diabetic rats (DS group) compared with non-diabetic control groups (*Fig. 8E*). DE, DSQ and DEQ groups showed smaller myelinic edema and axonal atrophy in the sciatic nerve compared with the DS group with partial regeneration (almost normal morphology) in the DEQ group (*Fig. 8F-8H*).

In histopathological investigations, the rats from the non-diabetic control groups (CS, CE, CSQ and CEQ) didn't present a score '0' nerve injury. The diabetic rats (DS group) showed injury in the sciatic nerve which was more severe, namely score '3+' compared with non-diabetic control groups. In the DSQ and DEQ groups, the rats had a minimally elevated score '2+' nerve injury, compared with the DS group with an elevated score '3+' nerve injury. In the DEQ group, the rats had a minimally elevated score '1+' nerve injury (out of 4), compared with the DE group with an elevated score '3+' nerve injury.

DISCUSSION

Uncontrolled chronic hyperglycemia in diabetes mellitus (DM) can lead to severe complications including neuropathy. Chronic hyperglycemia is correlated with diabetes-induced deficits in motor and sensory nerve conduction velocities occurring in diabetic peripheral neuropathy (DPN) (8). Three important mechanisms are involved in the pathologic changes of DPN, inflammation, oxidative and nitrosative stress and mitochondrial dysfunction (34-36).

Streptozotocin (STZ)-induced diabetes is a reliable experimental model of DM type 1 accepted for the study of DM complications (37, 39). STZ once transported into the pancreatic β-cells through Glut2 glucose transporters fragments the deoxyribonucleic acid (DNA) and destroys the β-cells in the pancreas (37-39). Hyperglycemia and the significant increase of peroxynitrite formation, nitric oxide (NO) and reactive oxygen species (ROS) induced by STZ are responsible for the occurrence of DPN (1, 35-38).

In our present study we investigated the effect of quercetin treatment and moderate exercise training on diabetic-induced sciatic nerve injuries after induced DM type 1 in rats with STZ. All the rats developed signs of diabetes one week after the STZ administration, that is, hyperglycemia, glycosuria, polyuria, increased water consummation and weight loss.

Fasting blood glucose (FBG) levels were very high in DM groups 96 h after the STZ injection. The FBG levels grew by the end of the experiment, reaching the maximum level in day 42 in diabetic rats. After five weeks, the treatment with quercetin in a dose of 30 mg/kg daily showed a very significant fall of the FBG levels in the STZ-induced diabetic rats. These results are in accordance with our recent results (2, 12) and with other recent studies that have revealed the hypoglycemic effect of quercetin in diabetes (1, 4, 8-10). The diabetic rats subjected to regular moderate exercise program by swimming for 30 minutes/day, 5 days/week, for five weeks showed significantly decreased levels of FBG when compared with the sedentary diabetic rats. The FBG concentration of STZ-induced diabetic rats treated with quercetin and subjected to swimming exercise program was reduced significantly at the end of the study. The decrease in the FBG levels was interpreted in accordance with recent research as being due to the direct hypoglycemic effects of quercetin and of the reduction in insulin resistance to moderate physical effort. (10, 13, 15, 16, 40, 41).

ROS and reactive nitrogen species (RNS) are involved in causing nerve damage in several animals, humans and experimental models of diabetes (8). The mechanisms involved in oxidative and nitrosative stress-induced nerve dysfunctions include reduction in cellular antioxidants, DNA damage, lipid peroxidation, generation of ROS, mainly superoxide anion radicals and increased RNS, especially NO and peroxynitrite (8). Increases in the biomarkers of oxidative and nitrosative stress related to lipid peroxidation i.e. malondialdehyde (MDA), protein carbonylation (protein carbonyl group (PC)), the nitrite and iNOS levels together with inhibition of the synthesis of endogenous antioxidants (SOD and CAT) have been observed in the sciatic nerve tissue in our present *in vivo* experimental model of diabetes. The superproduction of ROS and NOS is responsible for damaging the lipids present in the myelinated structures of the nerves resulting in the loss of axons and disruption of the microvasculature in the peripheral nervous system (8, 35). Also, neuropathic pain associated with DPN is the consequence of oxidative damage to peripheral nerves which determine hyperexcitability in the afferent nociceptors and central neurons (8, 35). In our study the treatment of the STZ-diabetic rats with quercetin reduced MDA, PC, nitrite and iNOS levels and increased and antioxidant SOD and CAT activities in the sciatic nerve, suggesting that quercetin exerts neuroprotective effects in the peripheral nervous system. These results are in accordance with recent research which has shown that quercetin has neuroprotective effects through direct counteraction of oxidative and nitriosative stress by scavenging ROS, such as superoxide anion and RNS, such as NO and peroxynitrite and by increasing the activity of antioxidant enzymes (1, 9, 15, 36). Intermediate intensity aerobic exercise for 30 minutes is recommended for preventing diabetes complications (5). Moderate aerobic exercise training has beneficial effects in the DPN treatment (5, 6, 18, 19, 27, 28) by reducing hyperglycemia, hypertriglyceridemia, hypercholesterolemia, inflammation, oxidative and nitrosative stress. In our study, after five weeks of moderate exercise training by swimming exercises for 30 minutes/day, MDA, PC, the nitrite and iNOS levels significantly decreased while SOD and CAT activities significantly increased in the sciatic nerve of the diabetic rats. These results demonstrate the beneficial neuroprotective effects of intermediate intensity swimming exercise in the sciatic nerves injuries induced by diabetes.

In the present study, also the morphological changes of the sciatic nerve such as axonal degeneration (atrophy), myelin splitting, myelinic edema and endoneural edema were studied in STZ-diabetic rats six weeks after the STZ injection. The morphological observations showed partial regeneration with remyelination after five weeks of quercetin treatment in association with swimming exercise for 30 minutes. This finding shows the efficacy of quercetin in association with moderate exercise training in the improvement of axonal degeneration of nerve fibers in STZ-induced diabetic rats. These results are in accordance with the recent research which shows a significant reduction in myelin breakdown and a remarkable improvement in degenerated axons, found after treadmill exercise (once a day for 4 consecutive weeks) (5, 18, 19). The reduction of histological lesions in the sciatic nerve after five weeks of quercetin treatment was also observed by other researchers (9, 36) and we explained them as a consequence of the antioxidant effects of this flavonoid.

STZ-induced diabetes increased the tissue damage in the sciatic nerve, highlighted by marked increase of oxidative and nitrosative stress associated with axonal degeneration (atrophy) and myelin splitting. The quercetin administration in association with moderate exercise training significantly reduced the sciatic nerve pathological damage induced by diabetes and these effects were accompanied by increased antioxidant enzymes (SOD and CAT) activity level and reduced MDA, PC, nitrite and iNOS levels in the sciatic nerve tissue of STZ-diabetic rats. These findings suggest that treatment with quercetin and moderate exercise training restores the sciatic nerve structural integrity and the histopathological alterations in diabetes.

In conclusion, our findings suggest that treatment with quercetin in association with aerobic exercises of intermediate intensity for 30 minutes improved hyperglycemia and the antioxidant status in STZ-diabetic rats. Quercetin treatment and swimming exercise might improve the sciatic nerve function and reduce the sciatic nerve histopathological damage produced by STZ-induced diabetes through attenuating fasting blood glucose level and by suppressing oxidative and nitrosative stress, thereby increasing the NO bioavailability.

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Author's address: Dr. Irina Camelia Chis, Department of Physiology, 1-3, Clinicilor Street, 'Iuliu Hatiegan' University of Medicine and Pharmacy, RO 400023, Cluj-Napoca, Romania; E-Mail: irinnaus@yahoo.com; ichis@umfcluj.ro