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ORIGINAL ARTICLE

Effects of *Nigella sativa* and thymoquinone on biochemical and subcellular changes in pancreatic β-cells of streptozotocin-induced diabetic rats

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Abstract

Background: The present study investigated the effects of *Nigella sativa* aqueous extract and oil, as well as thymoquinone, on serum insulin and glucose concentrations in streptozotocin (STZ) diabetic rats.

Methods: Rats were divided into five experimental groups (control, untreated STZ-diabetic, and aqueous extract-, oil-, or thymoquinone-treated diabetic rats). Treated rats received 2 mL/kg, i.p., 5% *N. sativa* extract, 0.2 mL/kg, i.p., *N. sativa* oil, or 3 mg/mL, i.p., thymoquinone 6 days/week for 30 days. Serum insulin and glucose concentrations, superoxide dismutase (SOD) levels, and pancreatic tissue malondialdehyde (MDA) were determined. Electron microscopy was used to identify any subcellular changes.

Results: Diabetes increased tissue MDA and serum glucose levels and decreased insulin and SOD levels. Treatment of rats with *N. sativa* extract and oil, as well as thymoquinone, significantly decreased the diabetes-induced increases in tissue MDA and serum glucose and significantly increased serum insulin and tissue SOD. Ultrastructurally, thymoquinone ameliorated most of the toxic effects of STZ, including segregated nucleoli, heterochromatin aggregates (indicating DNA damage), and mitochondrial vacuolization and fragmentation. The aqueous extract of *N. sativa* also reversed these effects of STZ, but to a lesser extent. The *N. sativa* oil restored normal insulin levels, but failed to decrease serum glucose concentrations to normal.

Conclusions: The biochemical and ultrastructural findings suggest that *N. sativa* extract and thymoquinone have therapeutic and protect against STZ-diabetes by decreasing oxidative stress, thus preserving pancreatic β-cell integrity. The hypoglycemic effect observed could be due to amelioration of β-cell ultrastructure, thus leading to increased insulin levels. Consequently, *N. sativa* and thymoquinone may prove clinically useful in the treatment of diabetics and in the protection of β-cells against oxidative stress.

Keywords: *Nigella sativa*, streptozotocin diabetes, thymoquinone.

Introduction

Diabetes mellitus is a common metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1] The increased extra- and intracellular glucose concentrations result in oxidative stress, which seems to be due mainly to increased production of reactive oxygen

species (ROS) and free radicals, and a sharp reduction in antioxidant defenses.[2] Several mechanisms seem to be involved in the generation of this oxidative stress in experimental animal models, as well as in patients with Type 1 and Type 2 diabetes, including glucose auto-oxidation, protein glycation, and the formation of advanced glycation endproducts.[3] Streptozotocin (STZ), an antibiotic produced by *Streptomyces achromagens*, is the most commonly used agent to establish experimental diabetes[4] because of its ability to selectively target and destroy insulin-producing pancreatic islet β-cells. Its diabetogenic action has been ascribed to an increase in the intracellular methylation reaction,[5] DNA strand breaks, and the production of nitric oxide (NO)[6] and free radicals.[7] NO is involved in pancreatic destruction, where the interaction between NO and ROS modulates oxidative damage.[8] STZ can be used to induce different types of diabetes.[9] For example, to produce experimental models of Type 1 diabetes, mice are treated with high doses of STZ, which depletes β-cells.[10]

Traditional plant remedies have been used to relieve diabetes, with more than 1000 different plants described as being useful.[11] *Nigella sativa* L., commonly known as black seed, is a plant spices belonging to the Ranunculaceae family.[12] *Nigella sativa* extract has been shown to possess immunopotentiating,[13] antioxidant,[14] antitumoral,[15] and antidiabetic[16] properties. The oil of *N. sativa* exhibits analgesic and anti-inflammatory effects in rats.[17] Most of these properties have been attributed mainly to the quinone constituents of *N. sativa*, of which thymoquinone is the main active ingredient of the volatile oil isolated from the black seeds.[18] Thymoquinone has been shown to possess strong antioxidant properties[17] and to suppress the expression of inducible NO synthase in rat macrophages.[19] The aim of the present study was to investigate the antidiabetic and antioxidant effects of *N. sativa* aqueous extract and oil, as well as thymoquinone, in STZ-diabetic rats. In addition, the subcellular structure of β-cells was investigated to detect any pathological changes.

Methods

Animals

One hundred and fifty male Sprague-Dawley rats (weight 150–250 g; mean age 16 weeks) were used. A stock of inbred rats was obtained by brother–sister mating in the animal house of Beirut Arab University, Beirut, Lebanon. Rats were maintained under standard laboratory conditions (temperature 22 ± 2°C; 12-h light–dark cycle) and were fed daily with standard rat pellets and tap water available *ad libitum*. All rats received human care according to the criteria outlined in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health.[20]

Chemicals

The STZ used in the present study was purchased from Fluka (St Louis, MO, USA); thymoquinone (TQ) was purchased from Sigma-Aldrich (St Louis, MO, USA); the *N. sativa* seeds were purchased from a local herb store in Beirut. The aqueous extract was prepared daily by boiling 2.5 g seeds in drinking water for 10 min and adjusting the final volume to 50 mL to obtain a 5% extract of *N. sativa* seeds. The *N. sativa* oil was purchased from a local pharmacy in Beirut.

Experimental design

Rats were divided into five groups (n = 30 in each group). Diabetes was induced in all groups except the control group by a single intraperitoneal (i.p.) injection of STZ (50 mg/kg) freshly dissolved in 5 mmol/L citrate buffer (pH 4.5), as described previously.[21] One day after STZ injection, diabetes was confirmed by measuring blood glucose levels in blood samples from the tail vein with a One Touch Glucometer (Life scan; Johnson & Johnso, New Brunswick, NJ, USA). Rats with blood glucose levels ≥250mg/dL were considered diabetic. Detailed descriptions of the five groups are as follows: Group A (control group), rats were injected with an equal volume of vehicle (citrate buffer); Group B, untreated STZ-diabetic rats; Group C, STZ-diabetic rats treated with 2 mL/kg, i.p., of a 5% *N. sativa* aqueous extract 6 days/week; Group D, STZ-diabetic rats treated with 0.2 mL/kg, i.p., *N. sativa* oil 6 days/week; Group E, STZ-diabetic rats treated with 3 mg/mL, i.p., TQ 6 days/week. None of the rats was treated with insulin at any time during the experiment, which lasted for 30 days. Rats received their last injection 24 h before being killed. Five randomly selected animals from the different groups were killed under anesthesia before and then 1, 10, 20, and 30 days after STZ injection.

Biochemical investigations

Blood samples were collected by cardiac puncture and were separated to obtain serum samples. Serum glucose levels were determined by the glucose oxidase method using a Biosub Glucose kit (Biocon Diagnostics, Bangalore, India). Serum insulin levels were

determined by ELISA using a commercially available kit (Millipore, Carlsbad, CA, USA). Tissue homogenates were prepared by homogenizing pancreatic tissue in protein extraction buffer containing 0.1 mol/L Tris-HCl, 0.1 mol/L KCl, 0.5% sodium dodecyl sulfate and protease inhibitor; superoxide dismutase (SOD) and malondialdehyde (MDA) levels were measured in tissue homogenate. MDA levels were determined using a lipid peroxide (malondialdehyde) kit from Bio-Diagnostic (Beirut, Lebanon), using thiobarbituric acid (TBA) to produce thiobarbituric acid-reactive substances. SOD levels were assayed using a commercially available kit (Fluka).

Histopathological and ultrastructural procedures

Small blocks ($\sim$1 mm^3) from pancreatic tissues of different groups were removed after 30 days of treatment and dropped as quickly as possible into formalin glutaraldehyde fixative buffered with 0.1 mol/L phosphate buffer (pH 7.4). Samples were post-fixed in 1% buffered osmium tetraoxide for 2 h at 4°C and then dehydrated using an ascending grade of ethanol concentrations, transferred to propylene oxide, and then embedded in a mixture of 1:1 of Epon:Araldite. Polymerization was achieved by incubation of the samples in an oven at 60°C for 24 h. Ultrathin sections (50 nm) were obtained from these blocks using an LKB ultratome (Holly, MI, USA). The sections were then mounted on copper grids, double stained with uranyl acetate and lead citrate, and examined under a JEOL 100CX transmission electron microscope (JEOL, Mitaka, Tokyo, Japan). Semithin sections (1 μm) were also cut from the blocks, stained with Toluidine blue, and examined using light microscopy.

Statistical analysis

Data are expressed as the mean ± SE. Two-way analysis of variance (ANOVA) was performed using SPSS v. 16 software (SPSS, Chicago, IL, USA) to assess the overall effects of treatment and interactions between treatment and time on the physiological parameters. If significant differences were found, repeated one-way ANOVA with a *post hoc* least significant difference (LSD) test was used to determine the effects of individual treatments.

Results

Biochemical findings

Serum glucose and insulin levels for all experimental groups are given in Tables 1 and 2. The diabetic group (Group B) exhibited hyperglycemia, with significantly increased serum glucose levels ($P < 0.05$; Table 1) and significantly decreased serum insulin levels ($P < 0.05$; Table 2) compared with control (Group A) at different time points. However, treatment of diabetic rats with the aqueous extract of *N. sativa* (Group C) resulted in a significant decrease in serum glucose levels compared with the untreated diabetic group (Group B) after 10 days treatment ($P < 0.05$; Table 1). After 30 days, serum glucose levels had decreased to levels that did not differ significantly from basal levels seen in the control group (Group A). However, treatment with *N. sativa* aqueous extract for 30 days had no significant effect on insulin levels compared with those in the untreated diabetic group (Group B); levels in both Groups B and C remained well below those in the control group (Group A; $P < 0.05$; Table 2).

After 10 days treatment with *N. sativa* oil (Group D), there was a slight, but non-significant ($P > 0.05$), decrease in serum glucose levels compared with those in the untreated diabetic group (Group B). After 30 days, glucose levels in Group D remained significantly higher than those in the control group (Group A; $P < 0.05$; Table 1). Although after 20 days treatment with *N. sativa* oil tended to increase insulin levels compared with the untreated diabetic group (Group B), the differences failed to reach statistical significance ($P > 0.05$; Table 2). By the end of the experimental period, serum insulin levels in Group D were 2.13 ± 0.54 ng/mL, which did not differ significantly from that in the control group ($P > 0.05$; Table 2).

Table 1 Serum glucose concentrations (mg/dL) in the different groups over time

	Group A	Group B	Group C	Group D	Group E
Duration of treatment (days)					
0‡	163 ± 20	164 ± 17	173 ± 10	139 ± 17	139 ± 17
1	169 ± 16	573 ± 61*	598 ± 13*	539 ± 31*	530 ± 25*
10	169 ± 22	655 ± 5*	469 ± 42*†	487 ± 88*	432 ± 5*†
20	152 ± 29	323 ± 30*	341 ± 47*	371 ± 27*	378 ± 49*
30	154 ± 18	324 ± 21*	278 ± 43	327 ± 29*	289 ± 49

Data are the mean ± SE ($n = 6$). *$P < 0.05$ compared with control (Group A); †$P < 0.05$ compared with untreated diabetic rats (Group B; repeated one-way analysis of variance followed by *post hoc* least significant difference test). ‡A significant interaction was observed between time and treatment by two-way analysis of variance ($F = 5.757$; $P < 0.05$).
Group A, control group; Group B, untreated streptozotocin (STZ)-diabetic rats; Group C, STZ-diabetic rats treated with 2 mL/kg, i.p., of a 5% *N. sativa* aqueous extract 6 days/week; Group D, STZ-diabetic rats treated with 0.2 mL/kg, i.p., *N. sativa* oil 6 days/week; Group E, STZ-diabetic rats treated with 3 mg/mL, i.p., TQ 6 days/week.

Table 2 Serum insulin concentrations in the different groups

	Serum insulin (ng/mL)				
	Group A	Group B	Group C	Group D	Group E
Duration of treatment (days)					
0†	2.87 ± 0.17	2.83 ± 0.17	2.95 ± 0.16	2.78 ± 0.12	2.86 ± 0.19
1	2.89 ± 0.15	0.95 ± 0.08*	1.03 ± 0.05*	1.47 ± 0.21*	1.10 ± 0.04*
10	2.81 ± 0.14	1.05 ± 0.02*	1.05 ± 0.11*	1.45 ± 0.18*	1.38 ± 0.10*
20	2.83 ± 0.19	1.12 ± 0.09*	1.50 ± 0.42*	1.82 ± 0.27*	1.23 ± 0.15*
30	2.86 ± 0.17	1.40 ± 0.09*	1.50 ± 0.23*	2.13 ± 0.54	2.31 ± 0.36

Data are the mean ± SE ($n = 6$). *$P < 0.05$ compared with control (Group A; repeated one-way analysis of variance followed by *post hoc* least significant difference test). †A significant interaction was observed between time and treatment two-way analysis of variance ($F = 3.039$; $P < 0.05$).

Group A, control group; Group B, untreated streptozotocin (STZ)-diabetic rats; Group C, STZ-diabetic rats treated with 2 mL/kg, i.p., of a 5% *N. sativa* aqueous extract 6 days/week; Group D, STZ-diabetic rats treated with 0.2 mL/kg, i.p., *N. sativa* oil 6 days/week; Group E, STZ-diabetic rats treated with 3 mg/mL, i.p., TQ 6 days/week.

After 30 days, treatment of rats with TQ (Group E) lowered serum glucose levels that did not differ significantly from those in the control group ($P > 0.05$). A significant decrease ($P < 0.05$) in serum glucose levels compared with the untreated diabetic group (Group B) was first noted after 10 days TQ treatment. In addition, TQ treatment restored serum insulin levels, which were 2.31 ± 0.36 ng/mL after 30 days treatment ($P < 0.05$; Table 2).

Tissue SOD and MDA levels for all experimental groups are given in Tables 3 and 4. There were no changes in SOD and MDA levels in the control group throughout the experimental period. Conversely, STZ significantly decreased SOD levels threefold and significantly increased tissue MDA levels up to twofold compared with the control group starting 10 days after STZ injection (Tables 3 and 4).

In Group C rats, tissue SOD levels were restored after 30 days treatment with the aqueous extract of *N. sativa* to 15.01 ± 2.90 U/mg protein, which did not differ significantly from SOD levels in the control group (Group A; $P < 0.05$; Table 3). In addition, 20 days treatment with the aqueous extract of *N. sativa* significantly decreased tissue MDA compared with that in untreated diabetic rats (Group B) to levels that did not differ significantly from control (Table 4).

In Group D, 20 days treatment with *N. sativa* oil significantly decreased SOD levels compared with the untreated diabetic group (Group B). However, tissue SOD levels after 20 and 30 days treatment with the oil remained significantly lower than those in the control group (Group A; Table 3). Although a significant twofold decrease in MDA levels was seen in Group D compared with Group B after 30 days treatment, there were no significant differences between Groups D and A at any time point (Table 4).

In Group E, treatment with TQ restored SOD levels to normal after 30 days, with significant increases in

Table 3 Tissue superoxide dismutase levels in the different groups

	Superoxide dismutase (U/mg protein)				
	Group A	Group B	Group C	Group D	Group E
Duration of treatment (days)					
0‡	17.23 ± 1.77	17.88 ± 1.48	17.18 ± 1.79	17.10 ± 1.88	17.06 ± 2.77
1	16.93 ± 2.39	13.99 ± 2.74	13.00 ± 2.52	13.36 ± 2.40	13.46 ± 2.94
10	16.74 ± 1.05	5.20 ± 0.34*	8.24 ± 2.19*	5.33 ± 1.00*	5.00 ± 0.16*
20	17.33 ± 0.99	4.61 ± 0.91*	6.29 ± 1.20*	8.85 ± 0.60*†	8.17 ± 1.11*†
30	17.11 ± 2.01	10.83 ± 0.46*	15.01 ± 2.90	8.02 ± 1.42*	13.59 ± 2.81

Data are the mean ± SE ($n = 6$). *$P < 0.05$ compared with control (Group A); †$P < 0.05$ compared with untreated diabetic rats (Group B; repeated one-way analysis of variance followed by *post hoc* least significant difference test). ‡A significant interaction was observed between time and treatment by two-way analysis of variance ($F = 1.926$; $P = 0.024$).

Group A, control group; Group B, untreated streptozotocin (STZ)-diabetic rats; Group C, STZ-diabetic rats treated with 2 mL/kg, i.p., of a 5% *N. sativa* aqueous extract 6 days/week; Group D, STZ-diabetic rats treated with 0.2 mL/kg, i.p., *N. sativa* oil 6 days/week; Group E, STZ-diabetic rats treated with 3 mg/mL, i.p., TQ 6 days/week.

Table 4 Tissue malondialdehyde levels (μmol/g protein) in the different groups

	Malondialdehyde (μmol/g protein)				
	Group A	Group B	Group C	Group D	Group E
Duration of treatment (days)					
0‡	3.14 ± 0.37	3.22 ± 0.41	3.25 ± 0.37	3.22 ± 0.41	3.13 ± 0.41
1	3.19 ± 0.42	3.34 ± 0.48	3.35 ± 0.50	3.66 ± 0.49	3.51 ± 0.36
10	3.28 ± 0.41	6.86 ± 0.40*	5.34 ± 1.15	3.09 ± 0.26†	3.24 ± 0.44†
20	3.24 ± 0.41	5.11 ± 0.48*	2.80 ± 0.06†	4.14 ± 0.30	4.17 ± 0.91
30	3.29 ± 0.39	6.73 ± 1.07*	5.12 ± 0.45	3.37 ± 0.54†	3.85 ± 0.29†

Data are the mean ± SE (n = 6). *P < 0.05 compared with control (Group A); †P < 0.05 compared with untreated diabetic rats (Group B; repeated one-way analysis of variance followed by *post hoc* least significant difference test). ‡A significant interaction was observed between time and treatment by two-way analysis of variance (F = 2.929; P < 0.01).
Group A, control group; Group B, untreated streptozotocin (STZ)-diabetic rats; Group C, STZ-diabetic rats treated with 2 mL/kg, i.p., of a 5% *N. sativa* aqueous extract 6 days/week; Group D, STZ-diabetic rats treated with 0.2 mL/kg, i.p., *N. sativa* oil 6 days/week; Group E, STZ-diabetic rats treated with 3 mg/mL, i.p., TQ 6 days/week.

SOD levels compared with the diabetic group (Group B) seen after 10 days treatment (Table 3). In addition, significant decreases in tissue MDA levels were seen in Group E compared with Group B after 10 days of treatment. It is important to note that there were no significant differences in MDA levels between Groups E and A (control group) at any time point (Table 4).

Histopathological and ultrastructural findings

Light and electron microscopy investigation of the pancreas of control rats (Group A) demonstrated normal pancreatic architecture (Fig. 1). In contrast, semithin sections of the pancreas from untreated diabetic rats (Group B) revealed that the islets were relatively small, atrophied, and showed a reduction in the number of polygonal islet cells. Extensive fibrosis was noted in connective tissue areas surrounding blood vessels. Insulitis was noted in several islets. Ultrastructurally, β-cells appeared polygonal in shape with oval nuclei and displayed several pathological findings. Some cells showed a slightly irregular outline around the nuclei, increased numbers of dilated nuclear pores, decreased heterochromatin, and nuclear inclusions (Fig. 2a). The nucleoli appeared segregated in most cells. An increased number of small, elongated mitochondria was noted, with most displaying signs of fragmentation. The Golgi and rough endoplasmic reticulum (rER) cisternea appeared dilated compared with normal. Ultrastructural observation also revealed the presence of autophagosomes in some β-cells. The cytoplasm appeared vacuolated and degranulated, with an increased number of empty granules (Fig. 2b).

In the diabetic group treated with *N. sativa* aqueous extract (Group C), light micrographs revealed lightly stained, small, round islets with a reduced number of polygonal cells compared with the control group. Electron micrography revealed that some β-cells had irregular-shaped nuclei and terminalized and segregated nucleoli, but with a normal distribution of heterochromatin both centrally and peripherally (Fig. 3a). The nuclear envelope showed distinct membranes, a dilated perinuclear space, and nuclear blebbing at certain sites. The number and distribution of mitochondria were similar to control and only a few mitochondria showed signs of fragmentation. Most secretory granules contained a dense core, a few displayed less dense cores, and very few appeared empty (Fig. 3b).

Semithin sections of animals treated with *N. sativa* oil (Group D) revealed islets of a relatively small size and irregular in shape compared with control. In addition, an increased number of polygonal islet cells was observed compared with that seen in Group B. Ultrastructurally, the β-cells of Group D appeared polygonal in shape, had pleomorphic nuclei and displayed cellular damage. A slightly irregular outline around the nuclei, with a distinct nuclear envelope, dilated perinuclear space, and even chromatin distribution were noted (Fig. 4a). Very few mitochondria were observed in β-cells after *N. sativa* oil treatment compared with control. Most of the secretory vesicles in the cytoplasm were either empty or had less dense cores (Fig. 4b).

In the diabetic rats treated with TQ (Group E), light micrographs of the pancreas revealed lightly stained, elongated islets of similar size to those seen in the control group (Group A). Electron micrographs showed that the β-cells were polygonal in shape with pleomorphic nuclei. In some cells, slightly irregular and clefted nuclei were observed. In others, the nuclei appeared

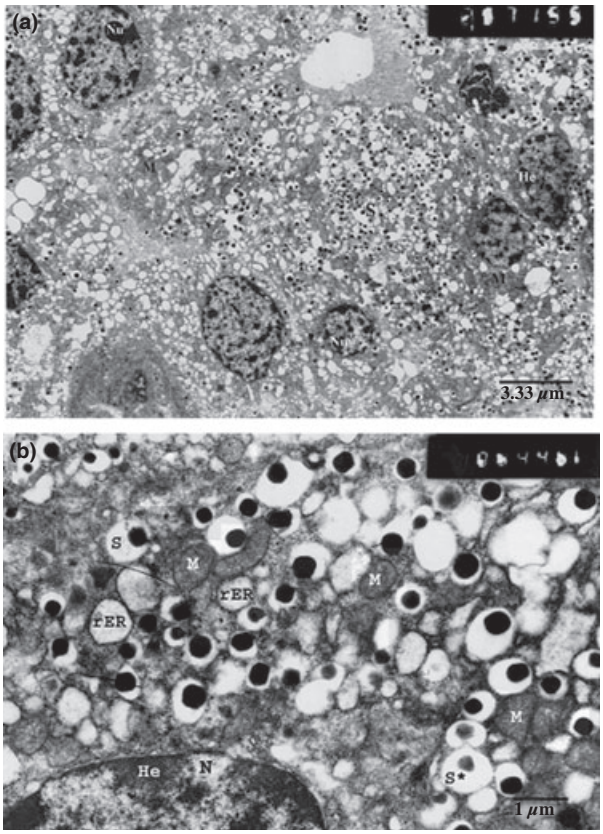

Figure 1 (a) Low-power electron micrograph (EM) of the Islets of Langerhans in control rats (Group A) showing polygonal β-cells with indistinct intercellular spaces scattered around a blood capillary (BC). Note the basally located oval nuclei (N), visible nucleoli (Nu), evenly distributed chromatin, and distinct nuclear envelope (Ne). Note also the numerous round (arrow) and elongated (arrowhead) mitochondria with dark matrices, and the characteristic β-secretory granules (s) occupying the apical portion of the β-cells. (b) High-power EM of the Islets of Langerhans showing part of the nucleus of a β-cell, with heterochromatin (He) along the inner nuclear membrane, a distinct nuclear envelope (Ne), and a visible perinuclear space. Note the few round and elongated mitochondria (M), cross-sections of rough endoplasmic reticulum (rER) lined with ribosomes, free ribosomes, polysomes, characteristic β-secretory granules (s), and secretory granules with less dense cores (S*).

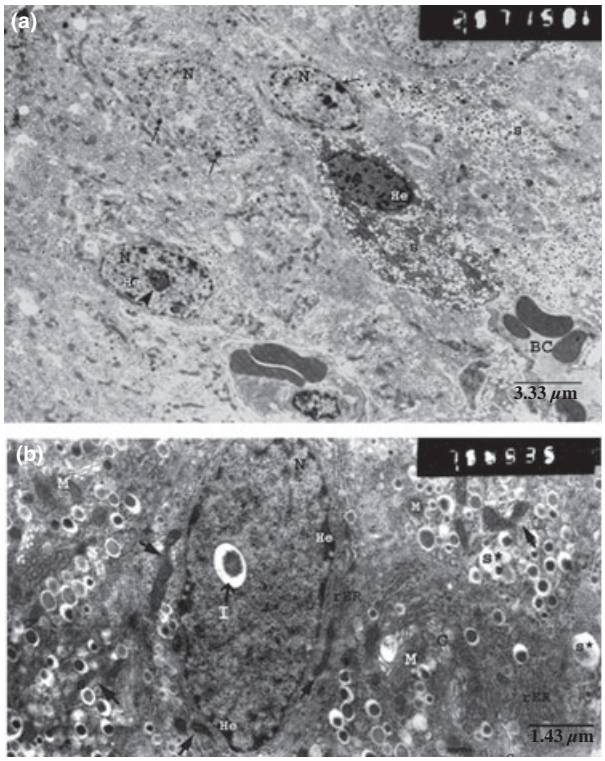

Figure 2 (a) Low-power electron micrograph (EM) of the Islets of Langerhans in untreated diabetic rats (Group B) showing polygonal β-cells with basally located oval nuclei (N), a central segregated nucleolus (arrowhead), decreased heterochromatin (arrow) in the form of clumps marginating or attached to the inner nuclear membrane of the indistinct nuclear envelope (Ne) as well as indistinct intercellular spaces. Note the small, round, and elongated mitochondria and the characteristic β-secretory granules with dense cores (s). Note also the blood capillaries (BC) filled with erythrocytes. (b) High-power EM of the Islets of Langerhans showing an oval shaped nucleus (N) housing an inclusion (I) in the nucleoplasm, abnormal distribution of chromatin, decreased heterochromatin (He), indistinct nuclear envelope (Ne), round and elongated electron-dense mitochondria (M), with arrows indicating elongated mitochondria showing signs of fragmentation, the Golgi apparatus (G), the distended cisternae, and β-secretory granules with less dense cores (s*).

round but with a segregated nucleolus and indistinct nuclear envelope with dilated pores (Fig. 5a). Small nuclei were also observed in other cells. In most nuclei, an even distribution of chromatin was noted, although reduced heterochromatin was observed in a few cells. Numerous elongated mitochondria with dark matrices were observed scattered throughout the cytoplasm. The Golgi cisternea and rER appeared dilated. Numerous secretory granules with dense cores and of different sizes were observed scattered throughout the cytoplasm of β-cells (Fig. 5b).

Discussion

The effect of *N. sativa* extract and oil and TQ on diabetes, oxidative stress, and β-cell damage in STZ-diabetic rats was evaluated both ultrastructurally and biochemically. Diabetics and experimental animal models of diabetes exhibit high oxidative stress due to chronic hyperglycemia, which depletes the activity of the antioxidant defense mechanism, promoting the generation of free radicals.[22] It has been reported that the excessive availability of free radicals, accompanied by a reduction in the antioxidant capacity, leads to

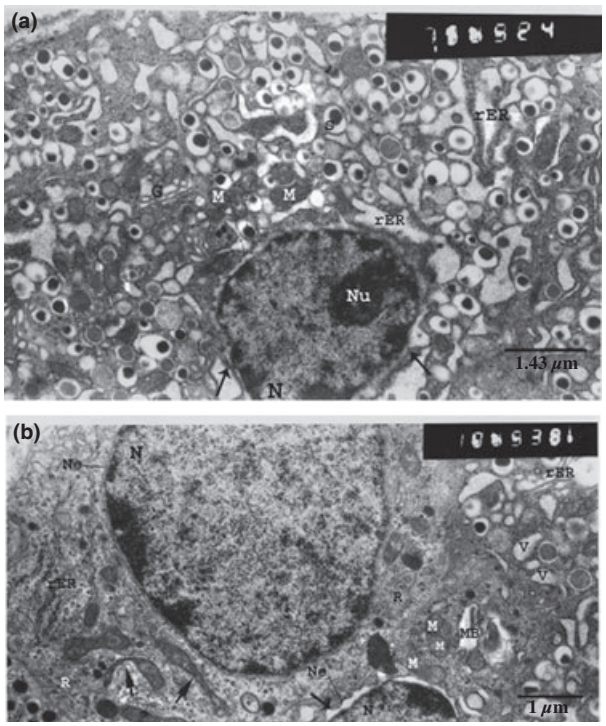

Figure 3 (a) High-power electron micrograph (EM) of the Islets of Langerhans in diabetic rats treated with an aqueous extract of *Nigella sativa* (Group C) showing a β-cell with a small round nucleus (N), a segregated terminalized nucleolus (Nu), dilated perinuclear space, slight blebbing (arrow) of the nuclear envelope (Ne), small, round, and elongated mitochondria (M) with dark matrices, dilated cross-sections of rough endoplasmic reticulum (rER) aligned with ribosomes, and small β-secretory granules (s). (b) High-power EM of the Islets of Langerhans showing an α-cell with a large nucleus (N), distinct nuclear envelope (Ne), black arrows pointing at elongated mitochondria showing signs of fragmentation, parallel stacks of membrane-bound rER cisternea, and free ribosomes (R). Note also the β-cell showing part of the nucleus (N) with a distinct nuclear envelope (Ne), blebbing at certain sites (arrow), a dilated perinuclear space, elongated and round mitochondria (M), dilated rER aligned with ribosomes, free ribosomes (R), characteristic β-secretory granules (s), the disrupted cytoplasm with small vacuoles, and myelenoid bodies (MB) in the area where numerous ribosomes are noted.

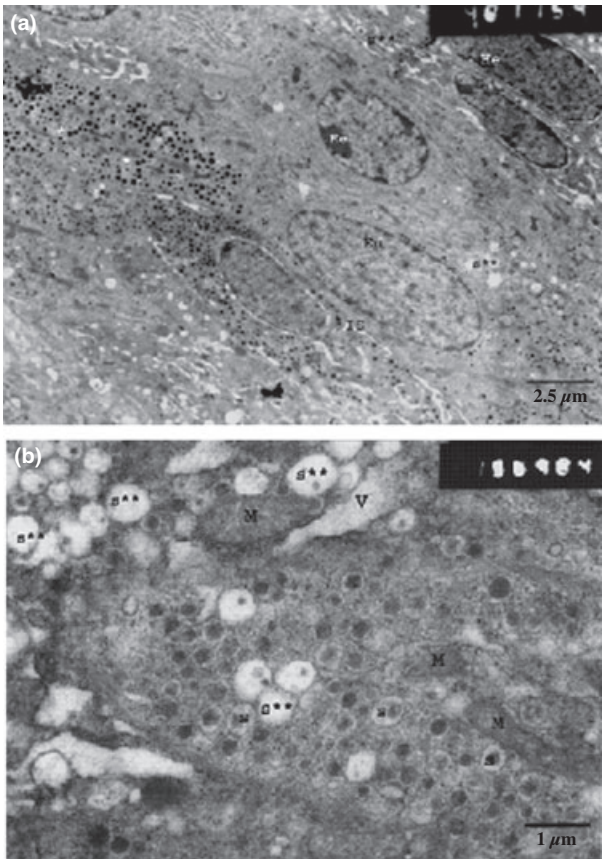

Figure 4 (a) Low-power electron micrograph (EM) of the Islets of Langerhans in rats treated with *Nigella sativa* oil (Group D) showing oval-shaped α- and β-cells with basally located oval nuclei. Note the euchromatic nuclei of the α-cells, dense α-secretory granules (*) in the cytoplasm, the heterochromatic nuclei in β-cells, empty characteristic β-secretory granules (s**), distinct intercellular spaces, and vacuolization of the cytoplasm. (b) High-power EM of the Islets of Langerhans showing part of the cytoplasm of a β-cell with elongated mitochondria (M), the small β-secretory granules possessing slightly dense cores (s), the large empty secretory granules (s**), and vacuolization of the cytoplasm at certain sites (V).

cellular dysfunction.[23] The β-cell cytotoxicity of STZ is thought to be mediated by inhibition of free radical scavenging, which enhances the production of superoxide radicals, resulting in lipid peroxidation, DNA damage, and sulfhydryl oxidation.[21]

In the present study, light micrographs revealed that STZ altered islet morphology. Degranulation and degeneration of β-cells was observed ultrastructurally. Moreover, mitochondrial vacuolization, fragmentation, dilation of the endoplasmic reticulum (ER) and Golgi apparatus, decreased secretory granules, and cytoplasmic vacuolation were noted. These results are in agreement with those reported previously.[24–28] This damage was accompanied by a significant increase in serum glucose levels and a significant decrease in insulin secretion. In addition, tissue MDA was significantly increased, with a significant reduction in the antioxidant enzyme activity of SOD compared with the control group, which may account for the tissue damage observed. The increased lipid peroxidation resulted in disruption of important lipid-containing membranes, including the nuclear envelope, ER membranes, and vacuoles, leading to the observed dilation. Vacuolation and fragmentation of the mitochondria led to disruption of the antioxidative mechanism, reflecting a limited capacity of the mitochondria to overcome the

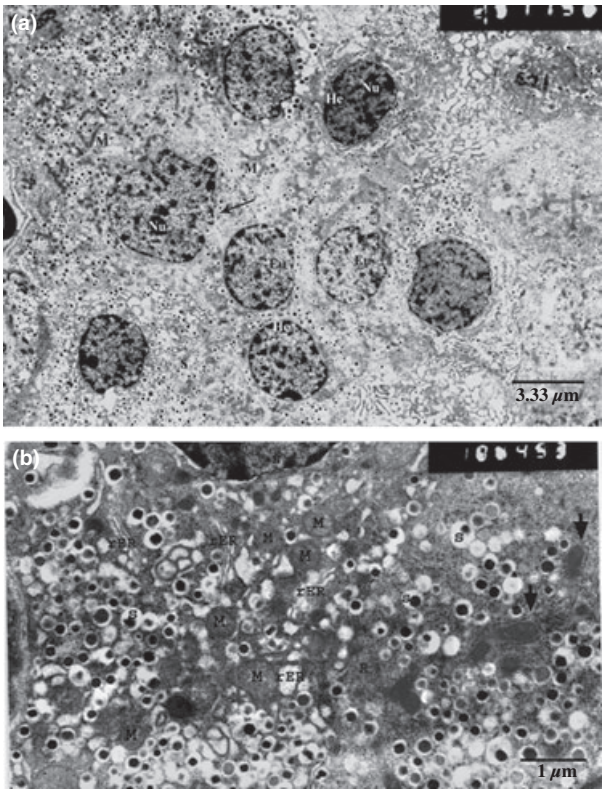

Figure 5 (a) Low-power electron micrograph (EM) of the Islets of Langerhans in rats treated with thymoquinone, the active ingredient of *Nigella sativa* (Group E), showing polygonal *β*-cells with pleomorphic nuclei (N) and prominent nucleoli (Nu), evenly distributed heterochromatin (He), small elongated mitochondria (M), and the characteristic *β*-secretory granules (s) in the apical portion of the *β*-cells. The arrow indicates the clefted nucleus. (b) High-power EM of the Islets of Langerhans showing in detail part of the *β*-cell nucleus (N) with a distinct nuclear envelope (Ne) and slight dilation of the perinuclear space. Note the round mitochondria (M), elongated mitochondria (black arrows) with dark matrices, dilated cross-sections of rough endoplasmic reticulum (rER), free ribosomes (R), and *β*-secretory granules (s).

oxidative stress. This observation is in agreement with the biochemical finding of decreased SOD activity in Group B.

The results of the present study are consistent with previous studies reported increased lipid peroxidation and decreased antioxidant enzymes in diabetes mellitus.[21,29,30] Kennedy and Baynes[31] suggested that this effect could be due to non-enzymatic glycosylation of proteins and enzymes. Schettler et al.[32] suggested that the reduced antioxidant capacity was due to increased oxygen metabolites, which cause a decrease in the antioxidant defense system.

Administration of *N. sativa* aqueous extract resulted in a significant lowering of elevated serum glucose levels after 10 days treatment and an increase in insulin concentrations after 20 days treatment. However, insulin levels did not return to normal. It is of interest to note that the glucose concentration decreased significantly compared with those in the untreated diabetic group (Group B) after 10 days, although insulin levels were not significantly increased at the same time point. This difference may indicate that the observed hypoglycemic effect is not related directly to insulin action and may be mediated by another mechanism. Recently, Meddah et al.[33] showed that chronic oral administration of *N. sativa* seeds to rats inhibited intestinal glucose absorption, which may contribute to the hypoglycemic effect of *N. sativa*. Conversely, the use of the aqueous extract prevented any significant changes in MDA levels compared with the control group at all time points evaluated, whereas SOD levels were restored to normal after 30 days treatment. These observations are in agreement with the results reported in other studies in experimental animals that also indicate that *N. sativa* treatment increased blood glucose and insulin, and decreases MDA and SOD levels.[12,16,21,30] However, there have been no morphological studies to date examining the pancreatic ultrastructure in STZ-diabetic rats treated with *N. sativa* extracts and TQ. Morphologically, treatment with the aqueous extract indicated partial regeneration of the islet and *β*-cells, in agreement with the results reported by Kanter et al.[21] Ultrastructurally, the aqueous extract prevented cytoplasmic vacuolation, fragmentation of mitochondria, and increased the number of secretory granules. These protective effects may be attributed to the antioxidant properties of the *N. sativa* aqueous extract, which inhibited lipid peroxidation, decreased the generation of ROS, and increased SOD activity. However, the distinct membranes, dilated perinuclear space, and nuclear membrane blebbing observed at certain sites of the nuclear envelope and dilated ER could be due to the non-significant increase in MDA levels accompanied by the decrease in SOD activity, which persisted for 20 days.

Treatment with *N. sativa* oil (Group D) for 30 days restored normal insulin levels, but failed to decrease serum glucose concentrations to normal, although serum glucose levels were decreased significantly compared with levels in untreated diabetic rats. This indicates that the glucose-lowering effect is not directly related to insulin action. Houcher et al.[12] showed that the use of the commercial oil at a dose of 2.5 mL/kg per day for 25 days significantly reduced blood glucose, especially during the first 10 days of treatment. Fararh et al.[34] demonstrated that *N. sativa* oil exhibited a significant hypoglycemic effect in STZ plus nicotinamide-induced diabetic hamsters after 4 weeks treatment,

indicating that the hypoglycemic effect of the oil is time dependant. In addition, Fararh et al.[34] showed a significant increase in serum insulin levels. Comparing the results of Houcher et al.[12] and Fararh et al.[34] with those of the present study indicates that the use of a higher dose of oil or for a longer period of time in the present study may have produced similar results. Fararh et al.[34] also suggested that the observed decrease in glucose after the first week of treatment with *N. sativa* oil may be due to decreased hepatic gluconeogenesis. However, after activation of β-cells in response to increased insulin levels, a significant decrease in glucose levels to normal was observed, which could be due to the combined action of decreased hepatic gluconeogenesis and activation of β-cells.[34]

Nigella sativa oil failed to restore tissue SOD levels to normal after 30 days of treatment and MDA levels did not differ significantly from those in the control group throughout the experimental period. This could be explained by a possible increase in the level and activity of other antioxidant enzymes. Histologically, the islets appeared relatively small in size and irregular in shape. Ultrastructurally, β-cells showed only few large mitochondria, indicating possible fusion of small mitochondria. In addition, most of the secretory granules appeared empty and the cytoplasm appeared disrupted, with wide intercellular spaces. These findings could be explained by decreased SOD activity and the non-significant increase in MDA detected in this group.

Treatment with TQ for 30 days restored both serum glucose and insulin levels to normal. Consistent with our results, Fararh et al.[35] showed that daily gastric administration of 50 mg/kg TQ for 30 days reduced both fasting glucose and glycated hemoglobin levels. They also stated that the glucose-lowering effect of TQ is not related directly to insulin action.[35] In diabetics, the increased gluconeogenesis is related to increased expression of gluconeogenic enzymes.[36] Fararh et al.[35] stated that TQ decreased the elevated gluconeogenesis by suppressing the synthesis of gluconeogenic enzymes. More recently, Peri and Sankaranarayanan[37] have shown that that daily gastric administration of 80 mg/kg TQ for 45 days produces a consistent, dose-dependent, and significant decrease in plasma glucose concentrations and an increase in insulin levels in STZ-diabetic rats. Peri and Sankaranarayanan[37] also showed that TQ decreased the activities of the gluconeogenic enzymes glucose-6-phosphtse and fructose-1,6-bisphosphatase in the diabetic rats, in agreement with the results reported by Fararh et al.[35] These observations provide an explanation for the significant decrease in serum glucose levels seen after 10 days treatment with TQ and *N. sativa* aqueous extract in the present study, even though insulin levels at the time were significantly decreased compared with the control values.

In addition, TQ treatment restored tissue SOD levels and inhibited the increase in tissue lipid peroxidation during the experimental period. Histologically, TQ ameliorated most of the toxic effects of STZ on pancreatic islets, with normal morphology observed in this group. Ultrastructurally, the β-cells retained their normal structure, even though an increased number of small mitochondria was observed compared with the control group. This may reflect a compensatory mechanism to adapt to metabolic changes by dividing to supply the energy for the synthesis and secretion of insulin and to increase the production of SOD and other antioxidant enzymes to protect against oxidative stress in β-cells. This is agreement with the biochemical findings of non-significant changes in MDA or lipid peroxidation and restoration of normal SOD levels. The increased insulin level noted may be due to the amelioration of the ultrastructure of β-cells, including the mitochondria and endoplasmic reticulum.

The results emphasize that *N. sativa* aqueous extract and TQ are effective in reducing hyperglycemia in STZ-diabetic rats and that the antidiabetic action is not related directly to insulin, but could be mediated, in part, by inhibition of gluconeogenesis. In addition to their antidiabetic effect, *N. sativa* and TQ exhibited potent antioxidant properties by inhibiting lipid peroxidation and increasing SOD enzyme activity. *Nigella sativa* and TQ protected and preserved β-cell integrity by decreasing oxidative stress. Thus, it may be concluded that the antidiabetic action of *N. sativa* and TQ could be due, in part, to amelioration of the cellular and subcellular structures of β-cells. It is important to note that TQ showed the most overall protective effect. Consequently, *N. sativa* and TQ may prove clinically useful for the treatment of diabetics and for protecting β-cells against oxidative stress.

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Disclosure

There are no conflicts of interest to declare.

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