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Isfahan University of Medical Sciences

Original Article

Biochemical, histological, and immunohistochemical study on the therapeutic effects and mechanism of coenzyme Q_{10} in type 2 diabetes mellitus

Manal Ismaeil Khalil[1], Ali Louei Monfared[1,*], and Hussein Bashar Mahmood[2]

[1]*Department of Histology, Faculty of Veterinary Sciences, Ilam University, Ilam, Iran.*
[2]*Anatomy and Histology Department, College of Veterinary Medicine Sciences, University of Kerbala, Kerbala, Iraq.*

Abstract

Background and purpose: Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by β-cell dysfunction, insulin resistance, and elevated blood sugar levels. Several studies have explored the therapeutic potential of coenzyme Q_{10} (CoQ_{10}) in managing diabetes, but no reports have examined the possible mechanism of CoQ_{10} in T2DM. Here, we reported that CoQ_{10} protects pancreatic β-cell structure and function by modulating the expression of mir-33a/ mir-21/SREBP1 and described more detailed tissue alterations.
Experimental approach: The study randomly divided rats into three groups (n = 10): control, diabetic, and diabetic + CoQ_{10}. The diabetic + CoQ_{10} group consisted of diabetic rats that were concurrently administered CoQ_{10} (20 mg/kg/i.p.) three days/week for eight weeks. In addition to microscopic examination, the study involved evaluating glucose, insulin, and oxidative profiles in the serum and analyzing the levels of cholesterol, mir-33a, mir-21, and SREBP1 in pancreatic tissue.
Findings/Results: Our results revealed that CoQ_{10} restores glucose/insulin homeostasis, oxidative parameters, cholesterol levels, and the expressions of mir-33a, mir-21, and SREBP1. In addition, the CoQ_{10}-treated diabetic rats showed increased active β-cells compared to the diabetic group. The immunohistochemical examination of insulin revealed a higher quantity and larger size of pancreatic islets in the experimental group.
Conclusion and implications: The restoration of β-cell integrity following treatment with CoQ_{10} may elucidate the therapeutic benefits of this compound in diabetes management, potentially through its influence on the pancreatic expression of mir-33a/mir-21/SREBP1, subsequently maintaining healthy tissue.

Keywords: Coenzyme Q_{10}; Diabetes mellitus; MicroRNAs; Rat; SREBP1; Tissue.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a prevalent metabolic disorder characterized by persistent high blood sugar levels, often accompanied by varying degrees of dysfunction in the metabolism of carbohydrates, lipids, and proteins. The development of T2DM can have diverse causes but consistently involves a lack of insulin secretion by pancreatic islet β-cells as the disease progresses. Common symptoms of T2DM include increased urination, excessive thirst, heightened appetite, and weight loss (1,2). Studies have demonstrated that maintaining optimal glycemic control can help reduce the likelihood of developing T2DM and its associated complications, especially cardiovascular outcomes, to a certain degree (3).

MicroRNAs (miRNAs) are short RNA molecules that modulate gene expression post-transcriptionally by binding to the 3′untranslated regions of target genes. This leads to mRNA degradation or translational inhibition, ultimately resulting in reduced protein levels (4-6).

*Corresponding author: A. Louei Monfared
Tel: +98-9183419098, Fax: +98-8432224308
Email: a.loueimonfared@ilam.ac.ir

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Previous research has identified associations between specific miRNAs and the pathogenesis of T2DM. For example, in models of inflammation and diabetes, there is an observed increase in microRNA-21 (miR-21) levels within pancreatic β-cells (7). Conversely, microRNA-33a (miR-33a) plays a crucial role in regulating glucose homeostasis by upregulating the expression of genes involved in hepatic glucose synthesis (8).

Sterol regulatory element binding proteins (SREBPs) comprise three isoforms, namely SREBP1a and 1c, encoded by *SREBP1*, and SREBP2, encoded by *SREBP2* (9). These molecules play a role in the development of various diseases, including diabetes mellitus (10,11). SREBP1 serves as a crucial gene transcription factor that activates the genes necessary for hepatic lipogenesis (12), which can lead to pancreatic fat accumulation (13). Moreover, abnormal hepatic lipid metabolism is linked to β-cell dysfunction in T2DM (14). Studies suggest that hyperglycemia can directly increase renal expression of SREBP1 in diabetes, resulting in elevated triglyceride accumulation in the kidney (15). Therefore, SREBPs are implicated in the progression of diabetes through lipid-dependent mechanisms. Furthermore, hepatic steatosis induced by SREBP may exacerbate disease progression in various organs beyond diabetes (11).

Coenzyme Q_{10} (CoQ_{10}) acts as an electron carrier in the mitochondrial respiratory chain and acts as an intracellular antioxidant. Additionally, CoQ_{10} has demonstrated efficacy in the management of neurodegenerative, metabolic, and mitochondrial disorders (16,17). There is evidence suggesting CoQ_{10} may offer protection to pancreatic β-cells by exerting anti-apoptotic effects against mitochondrial stress induced by staurosporine (18). Previous research has also explored the clinical implications of CoQ_{10} in individuals with T2DM (19-21). In addition, the protective activity of CoQ10 and L-carnitine against statin-induced pancreatic mitochondrial damage has attracted considerable research activity in recent years (22). However, the mechanism by which CoQ_{10} reverses diabetic side effects remains uncertain. Therefore, as a putative mechanism, the involvement of miR-33a, miR-21, and SREBP1 in the ameliorative effect of CoQ_{10} in diabetes was investigated. The histological and immunohistochemical methods were applied to examine the structure and function of the pancreatic β-cell. Also, the pancreatic expression of miR-33a, miR-21, and SREBP1 as fundamental macromolecules in the pathogenesis of diabetes mellitus were investigated by real-time polymerase chain reaction (RT-PCR) and western blotting techniques, respectively.

MATERIALS AND METHODS

Chemicals

CoQ_{10} and streptozotocin (STZ) were procured from Sigma Chemical Company, while other chemicals were acquired from Sigma Chemical Co. or Merck Corporation (Germany).

Animals and study design

Thirty adult male Wistar rats (7-8 weeks old and weighing 230-250 g) were included in the study. The rats were housed in a standard environment with *ad libitum* access to food and water. They were maintained under typical laboratory conditions (12/12-h light/dark cycle, temperature 23 ± 3 ℃, and relative humidity of 45-65%). After one week of acclimatization, the experiment plan was made by randomly dividing rats into three groups: control, diabetic, and diabetic + CoQ_{10}.

The animal ethics committee of Ilam University, Ilam, Iran approved the entire experimental work through Ethic No. IR.ILAM.REC.1401.002.

Induction of T2DM

T2DM was induced in rats by injection of low-dose STZ (30 mg/kg b.w.) intraperitoneally in a 0.1 M citrate buffer (pH 4.5). Rats with a baseline blood glucose level ≥ 300 mg/dL two days post-injection were considered diabetic and included in the study. The diabetic + CoQ_{10} group was comprised of diabetic rats concurrently administered with CoQ_{10} (20 mg/kg/i.p.) 3 days/week over eight weeks. The selection of the test duration and drug dosage were according to prior research findings (23-25).

Measurement of serum glucose and insulin

The animals fasted overnight with free access to water on the last day of the experimental model before the induction of anesthesia for the collection of blood or tissue samples. Then, blood samples were collected and serum was separated. The serum level of glucose was determined using the glucose oxidase system. In addition, the blood level of insulin was measured using a rat-specific enzyme-linked immunosorbent assay (ELISA) (26).

Tissue homogenizing and measurement of pancreatic cholesterol content

Pancreas specimens were obtained from five rats in each experimental group and subsequently homogenized in phosphate-buffered saline (PBS, pH 7.4) to facilitate tissue homogenization. The resulting supernatants were obtained by centrifugation at 3,000 rpm for 20 min and then stored in liquid nitrogen for subsequent analysis using an ELISA test. Pancreas tissue cholesterol content was measured according to the method proposed by Wang *et al*. (27).

Measurement of the activity of antioxidant enzymes and the concentration of the malondialdehyde

Catalase (CAT) activity was assessed by the procedure outlined by *Aebi* (28). Superoxide dismutase (SOD) activity was determined following the methodology described by Beyer and Fridovich (29). Glutathione peroxidase (GPx) activity was measured based on the protocol established by Paglia and Valentine (30). Malondialdehyde (MDA) levels were quantified using the method introduced by Pilz *et al*. (31).

Measurement of pancreatic expression of miR-33a and miR-21 by RT-PCR assay

The pancreatic expression levels of miR-33a and miR-21 were assessed through an RT-PCR assay. Initially, total miRNA was extracted from 50 mg of pancreatic tissue using a miRcute miRNA isolation kit (TIANGEN, China) as per the manufacturer's guidelines. The purity of RNA was determined by measuring the optical density (OD) at a 260/280 OD ratio using an Eppendorf µCuvette G1.0 micro-volume measuring cell (Eppendorf, Germany). The synthesis of the first complementary DNA (cDNA) strand from total RNA was carried out using the miRcute miRNA first-strand Cdna synthesis kit (TIANGEN, China), employing a polyadenylation method recommended by the manufacturer. RT-PCR was performed using the ABI Stepone Plus detection system (ABI, USA) by the miRcute miRNA qPCR detection kit, SYBR Green (TIANGEN, China).

RT-PCR was conducted under the following reaction conditions: 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 30 s. The U_6 small nuclear ribonucleic acid (snRNA) was used as the housekeeping gene. All RT-PCR reactions were performed using three technical replicates. Two separate reactions without cDNA or with RNA were performed in parallel as controls. Relative quantification was performed according to the comparative $2^{-\Delta\Delta Ct}$ method, using StepOne software 2.3 (32). The primer details are provided in Table 1.

Table 1. The list of primers used in this study.

Gene name	Sequence
miR-33a	F: GTGCATTGTAGTTGCATTG
miR-21	F: AGCTTATCAGACTGATGTTG
cDNA adapter	R: GAACATGTCTGCGTATCTC
U_6	F: CTCGCTTCGGCAGCACA
U_6	R: AACGCTTCACGAATTTGCGT

Measurement of pancreatic expression of SREBP1 by western blot assay

The pancreatic expression of SREBP1 was assessed through western blotting analysis. Tissue homogenate was prepared from frozen samples and total protein from pancreatic tissue was quantified using a Bradford protein assay kit (Bio-Rad Laboratories Inc., California, USA). Subsequently, 20 µg of protein was separated on a 10% sodium dodecyl sulfate (SDS)/polyacrylamide gel and transferred onto polyvinylidene difluoride (PVDF) membranes (Amersham Protran 0.45 µm).

The membranes were then blocked with tris-buffered saline with Tween® 20 (TBS-T) buffer and 5% bovine serum albumin at 4 °C for 2 h.

The membranes were incubated overnight at 4 °C with primary antibodies targeting SREBP1 (1:1000) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:500; Santa Cruz Biotechnology Inc., Dallas, TX, USA). The next day, the blots were washed three times with TBS-T and then exposed to a horseradish peroxidase-conjugated anti-mouse secondary antibody (m-IgG κBP-HRP: sc-516102, 1:1000) for 1 h at room temperature. Subsequently, the blots were treated with an enhanced chemiluminescence (ECL) solution (Abcam, 133408, USA) and the bands were visualized and analyzed using a chemiluminescence detection system (Fusion FX; Vilber, USA). Densitometry was used to quantify the signals and semi-quantitative analysis was performed by evaluating the protein bands with a JS 2000 scanner (BonninTech, China). The density of the bands was calculated and the expression of SREBP1 was normalized to GAPDH as a housekeeping protein (33). The results presented are from three independent experiments.

Histological and immunohistochemical studies

The pancreas was excised and histologic alterations in pancreatic cells were investigated by microscopic examination after hematoxylin-eosin (H&E) or Gomori staining (34,35). Immunostaining for insulin was conducted using the Dako EnVision detection immunohistochemistry kit (Envision FLEX, Dako, K8000, Denmark). Insulin polyclonal-antibody (INS Polyclonal Antibody, E-AB-67409, Elabscience, China) was diluted 100-fold with antibody diluent (EnVision FLEX Antibody Diluent, Dako, K8006, Denmark) and applied overnight to detect insulin in the pancreatic tissue.

Subsequently, the tissue sections were treated with a secondary antibody labeled with horseradish peroxidase (EnVision FLEX /HRP, SM802) and incubated at room temperature in a humidity chamber for 30 min. After rinsing, the sections were immersed in a TBS buffer bath (EnVision FLEX Wash Buffer, SM831) for 5 min each. Immunohistochemical staining was carried out using hematoxylin as a blue background color and diaminobenzidine (DAB) as a brown stain to visualize positively stained tissue regions. The sections were counter-stained with Mayer hematoxylin (Mayer hematoxylin, Bio-Optica, 05-06002/L, Italy) for 3 min followed by tap water rinsing. Subsequently, the tissue sections were dehydrated in a series of ethanol alcohol solutions (70%, 90%, and 100%) for 2 min each, then immersed in xylene, mounted with dibutyl phthalate polystyrene xylene mounting media, and covered with coverslips. Finally, the tissue sections were examined under a light microscope at magnifications of 100× and 400× (35).

Statistical analysis

The statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL). The data underwent analysis of variance (ANOVA), followed by the Tukey post hoc test. *P*-values ≤ 0.05 were considered statistically significant.

RESULTS

CoQ_{10} effects on serum insulin, serum glucose, and pancreatic cholesterol level

As shown in Fig. 1, diabetic rats exhibited a notable reduction in serum insulin levels while displaying elevated glucose levels in comparison to the control group. Conversely, the administration of CoQ_{10} to diabetic rats resulted in a substantial increase in serum insulin levels and a marked decrease in serum glucose levels compared to diabetic rats (Fig. 1A and B). Furthermore, pancreatic cholesterol content was significantly higher in the diabetic group as opposed to the normal controls. Notably, the administration of CoQ_{10} to diabetic rats led to a significant reduction in pancreatic cholesterol levels (Fig. 1C).

CoQ_{10} effects on the activity of antioxidant enzymes in pancreas tissue

To explore the antioxidative function of CoQ_{10} in T2DM, the levels of oxidative stress markers in the pancreas were assessed. The results depicted in Fig. 2A-D indicate a notable reduction in the activities of CAT, SOD, and GPX in pancreatic tissues of diabetic rats in

comparison with the control group. Furthermore, there was a significant elevation in the concentration of MDA, a byproduct of lipid peroxidation, in the pancreatic tissue of diabetic rats as opposed to the control counterparts. Administration of CoQ_{10} to diabetic rats led to a significant enhancement in CAT and GPX levels compared to diabetic rats without CoQ_{10} treatment. Although there were increases in SOD activity in the diabetic rats receiving CoQ_{10}, these changes did not reach statistical significance. Moreover, CoQ_{10} supplementation in diabetic rats resulted in a significant reduction in MDA levels compared to the diabetic group.

CoQ_{10} effects on the expression of miR-33a and miR-21 in pancreas tissue

The RT-PCR analysis depicted in Fig. 3 illustrates the expression patterns of miRNAs in the tissue context of diabetes. The findings indicate a substantial increase in the levels of miR-33a and miR-21 in the pancreatic tissue of diabetic rats compared to the control group. Conversely, diabetic rats treated with CoQ_{10} exhibited a notable decrease in the expression of miR-33a and miR-21 in the pancreas. While CoQ_{10} was effective in reducing the expression of miR-21 in diabetic rats, there was a significant rise in miR-21 levels compared to the control group (Fig. 3A and B).

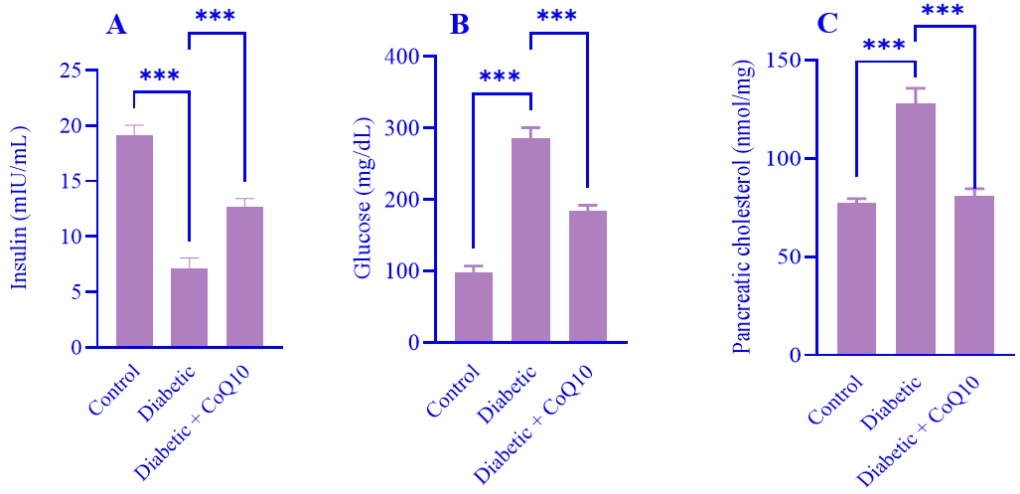

Fig. 1. The improving effect of CoQ_{10} (20 mg/kg/i.p., 3 days/week for eight weeks) on serum levels of (A) insulin, (B) glucose, and (C) pancreatic levels of cholesterol in diabetic rats. Data are presented as mean ± SEM, n = 10. ***$P \leq 0.001$ indicates significant differences between the designated groups. CoQ_{10}, coenzyme Q_{10}.

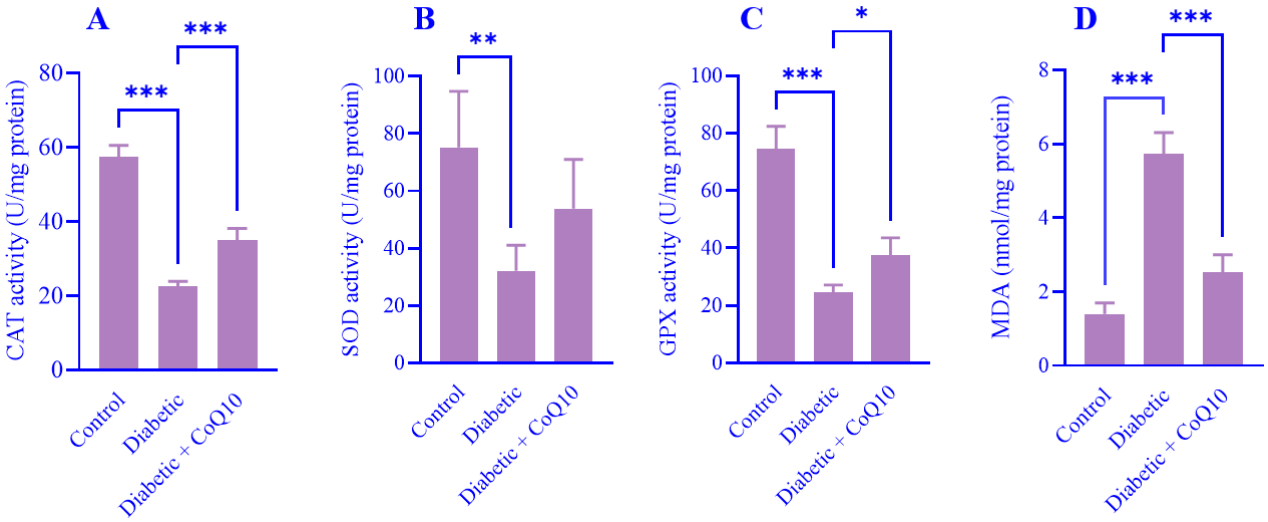

Fig. 2. The improving effect of CoQ_{10} (20 mg/kg/i.p., 3 days/week for eight weeks) on the oxidative stress markers in diabetic rats. Data are presented as mean ± SEM; n = 10. *$P \leq 0.05$, **$P \leq 0.01$, and ***$P \leq 0.001$ indicate significant differences between the designated groups. CoQ_{10}, coenzyme Q_{10}; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; MDA, malondialdehyde.

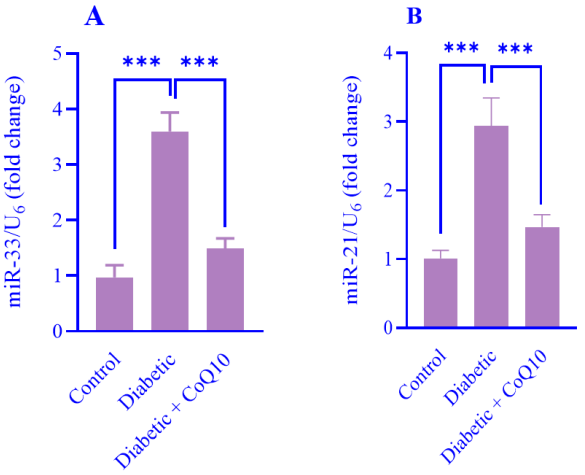

Fig. 3. The improving effect of CoQ_{10} (20 mg/kg/i.p., 3 days/week for eight weeks) on the expression of (A) miR-33a and (B) miR-21 in diabetic rats. Data are expressed as mean ± SEM; n = 10. ***$P \leq 0.001$ indicates significant differences between the designated groups. CoQ_{10}, coenzyme Q_{10}; miR-21, microRNA-21; miR-33a, microRNA-33a.

CoQ_{10} effects on the expression of SREBP1 in pancreas tissue

To explore the potential involvement of SREBP1 in the protective mechanism of CoQ_{10} against T2DM, the expression level of SREBP1 in pancreatic tissue was assessed through western blot analysis. The findings revealed an elevation in SREBP1 levels in the pancreatic tissue of diabetic rats in comparison to control groups. Notably, a marked reduction in the relative expression of SREBP1 was observed in diabetic animals following CoQ_{10} supplementation compared to the diabetic group (Fig. 4A and B).

CoQ_{10} effects on histological parameters

The histological characteristics of pancreatic islets from various experimental groups are depicted in Fig. 5. Control rats exhibited no discernible alterations, with β-cells displaying robust insulin immuno-reactivity and occupying the majority of the islet area. Conversely, diabetic animals displayed marked necrosis of β-cells within the islets, resulting in a reduction in anti-insulin antibody expression and the formation of cell-deficient spaces encompassing over 50% of the islet area. Additionally, a noticeable decrease in both the size and quantity of islets was observed in the diabetic group. Conversely, histological analysis of diabetic rats treated with CoQ_{10} revealed no significant changes, although a few sections exhibited minor β-cell necrosis leading to the formation of limited spaces within the islet area. Notably, in this group, approximately 75% of the islet area was occupied by anti-insulin antibodies, indicating the presence of active β-cells (Fig. 5).

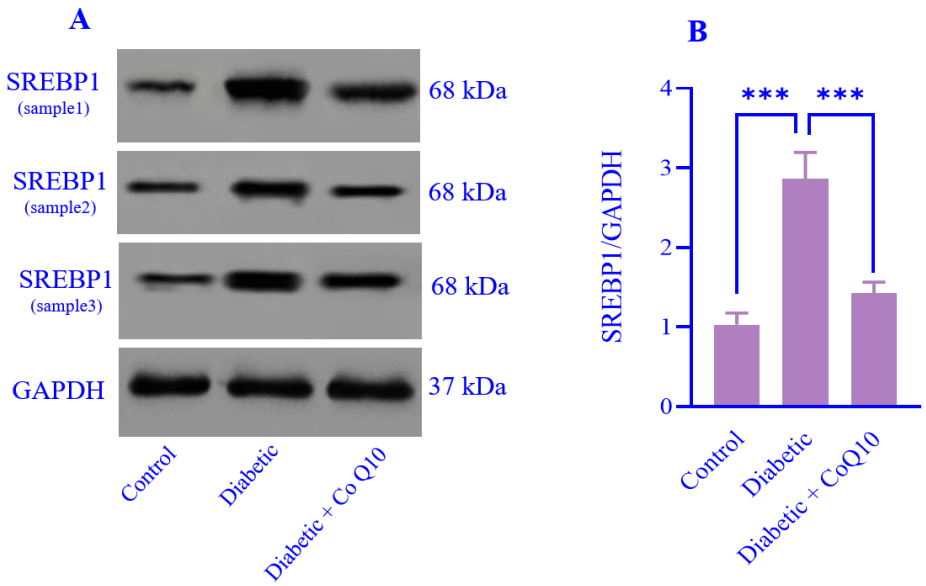

Fig. 4. The improving effect of CoQ_{10} (20 mg/kg/i.p., 3 days/week for eight weeks) on the expression of SREBP1 in the pancreas of diabetic rats. (A) Western blot analysis; (B) Semi-quantification of SREBP1 expression. GAPDH was used as an internal reference. Data are expressed as mean ± SEM; n = 10. ***$P \leq 0.001$. CoQ_{10}, coenzyme Q_{10}; SREBP1, sterol regulatory element-binding protein-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

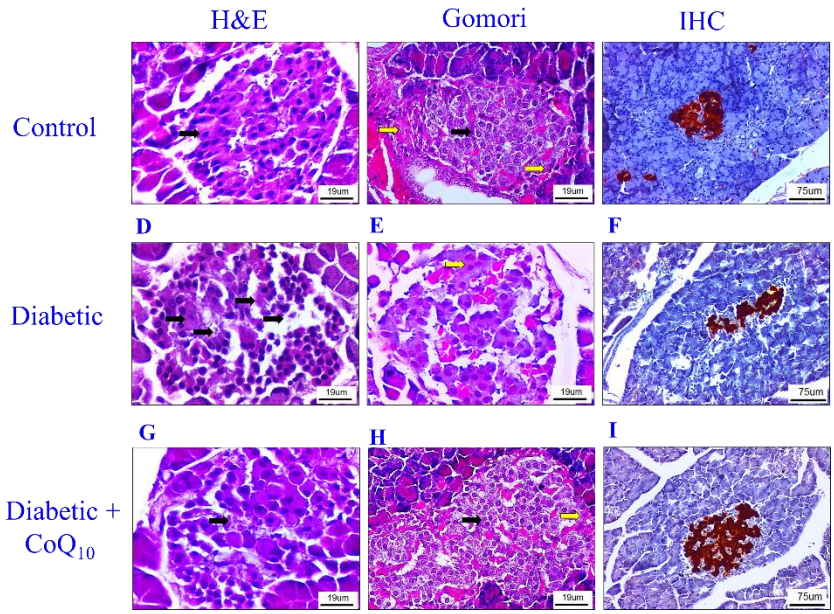

Fig. 5. Effect of CoQ_{10} (20 mg/kg/i.p., 3 days/week for eight weeks) on histological and insulin immune-histochemical changes on the pancreas in STZ-induced T2DM. (A-C) Control groups: normal islet's architecture showing granulated cytoplasm of islet cells with light and large nucleated beta-cells (black arrow) and dark and small nucleated-alpha cells (yellow arrow). Note the strong immuno-reactivity of insulin in beta-cells (brown stain), which occupy most of the islet area. (D-F) Diabetic groups: massive necrotic β-cells, along with pyknotic nuclei (black arrow) are clear. Note the expression of anti-insulin antibody, which occupied less than 50% of the islet's area, also the spaces with no expression of anti-insulin antibody indicate necrotic beta-cells in the affected islet area. (G-I) Diabetic + CoQ_{10} groups: the islets still have few local necrosis of beta cells (black arrow). Normal alpha and beta cells are noticeable. Note that the expression of anti-insulin antibodies occupied about 75% of the islet's area, indicating the presence of active β-cells. A, B, D, E, G, H: 400× and C, F, I: 100x. CoQ_{10}, coenzyme Q_{10}; STZ, streptozotocin; T2DM, type 2 diabetes mellitus; H&E, hematoxylin and eosin staining; IHC, immunohistochemistry.

DISCUSSION

T2DM is a metabolic disorder characterized by hyperglycemia resulting from the dysfunction of pancreatic β-cells (36). Despite extensive research efforts, the absence of an effective pharmaceutical treatment for diabetes remains a significant challenge in the medical field. Accordingly, there is an increasing desire to discover new substances that could function as potential medicinal remedies for diabetes and its associated issues (37). The findings of the present study indicated that treatment with CoQ_{10} in diabetic rats led to an increase in insulin levels and a decrease in blood glucose levels. Moreover, CoQ_{10} was found to effectively restore cholesterol levels in the pancreas. Additionally, CoQ_{10} was observed to reduce the levels of miR-33a, miR-21, and SREBP1 in the pancreas while enhancing components of the antioxidant defense system, ultimately contributing to the restoration of pancreatic tissue structure. These pathways have the potential to significantly impact the modification of pancreatic lesions and reverse the histological alterations associated with diabetes.

The current histological results indicated extensive necrosis of β-cells within the diabetic group. Furthermore, there was a reduction in the presence of anti-insulin antibodies, resulting in insulin-expressing β-cells occupying less than half of the islet's area. These observations validate the outcomes documented in previous research studies (38,39). Alternatively, treatment with CoQ_{10} may lead to the restoration of the pancreatic structure in diabetic rats, which includes the size of islets and the number of β-cells. This indicates that CoQ_{10} could potentially work as an insulin sensitizer in mitigating T2DM. These results support earlier studies that demonstrated the ability of CoQ_{10} to reduce the significant damage to pancreatic islets caused by diabetes (40).

The results of our biochemical investigation into the regulatory impact of CoQ_{10} on the insulin/glucose profile in diabetic rats align

with the histological observations. Our study demonstrated that the degradation of β-cells leads to pancreatic dysfunction, as evidenced by the reduction in serum insulin levels. This parallels previous research that has also highlighted the detrimental effects of diabetes on pancreatic function (41,42). Furthermore, the current research illustrated the protective impact of CoQ_{10} supplementation on the pancreas and its role in insulin production to regulate blood glucose levels. Consistent with our findings, previous studies have also shown the beneficial effects of CoQ_{10} on the function of pancreatic β-cells by maintaining mitochondrial respiration in rat pancreas (43,44).

Numerous studies have focused on miRNAs as potential therapeutic targets for diabetes. In addition, diabetes is known to alter the expression of miRNAs in various tissues, including the pancreas (45). On the other hand, research has shown that CoQ_{10} can enhance miRNA control over cholesterol metabolism and inhibit atherosclerosis (46). The present investigation demonstrated, for the first time, a significant elevation in the levels of miR-33a and miR-21 in diabetic rats, a phenomenon that was substantially mitigated by the administration of CoQ_{10} supplementation. These results suggest a potential mechanistic role for CoQ_{10} in managing oxidative stress associated with diabetes by regulating miR-33a and miR-21 levels in the pancreas. miR-33, a well-studied cholesterol-regulating miRNA (47) is of particular interest due to its role in cholesterol homeostasis and modulation of lipid oxidation-related genes (48-50). T2DM can disrupt lipid metabolism and pancreatic β-cell mass, leading to increased β-cell apoptosis and necrosis (51,52). The current study also revealed a significant increase in pancreatic cholesterol content in the diabetic group. This finding aligns with previous research (53) suggesting that elevated pancreatic cholesterol levels may exacerbate islet amyloidogenesis, β-cell dysfunction, and glucose homeostasis. The study also revealed that CoQ_{10} effectively decreased pancreatic cholesterol levels, which is in line with its known anti-hyperlipidemic properties when combined with omega-3 in obese individuals (54). miR-33a, located within intron 16 of the human SREBP gene (55), was associated with increased SREBP1 expression and cholesterol accumulation in the pancreas. In this study, treatment with CoQ_{10} in diabetic rats regulated SREBP1 expression and reduced cholesterol accumulation. Glucose is essential for the regulation of SREBP1 expression, as indicated by previous research (56). Also, CoQ_{10} has the potential to influence the SREBP1 gene, resulting in reduced levels of SREBP1 protein in the pancreas. This modulation leads to the down-regulation of lipogenic genes and a decrease in cholesterol levels. In individuals with diabetes, the elevated miR-33 levels can be mitigated by CoQ_{10}, potentially enhancing insulin production and secretion in β-cells. Studies have demonstrated that the expression of miR-33 and miR-370 can ameliorate the lipotoxic oxidative stress experienced by the pancreas (57). Furthermore, an investigation was conducted into the association between miR-33a, miR-122, erythrocyte membrane fatty acids profile, and serum lipids with components of metabolic syndrome in an Iranian population affected by T2DM. The researchers concluded that assessing their levels could serve as a valuable extra indicator for cardiometabolic issues in patients with T2DM (58).

Patients suffering from T2DM and its associated complications commonly experience reduced beta-cell mass and persistent oxidative stress, a condition that has been linked to hyperglycemia (59-61). It has been suggested that boosting intracellular antioxidants could be a targeted approach to mitigate oxidative stress and enhance the functioning of β-cells in addressing diabetes (62). The present study revealed a notable decline in the activities of CAT, SOD, and GPX, along with a significant rise in MDA levels in pancreatic tissues of diabetic rats compared to the control groups. Conversely, supplementation of CoQ_{10} in diabetic rats led to a marked reduction in MDA levels and an increase in CAT and GPX levels compared to the diabetic group. Furthermore, research has indicated that CoQ_{10} plays a crucial role in diminishing oxidative stress induced by tacrolimus and safeguarding the mitochondria in pancreatic β-cells (63).

In the present study, the administration of CoQ_{10} (20 mg/kg/i.p.) to diabetic rats led to a notable enhancement in the pancreas' structure and performance. Nonetheless, previous research indicates that the optimal concentration of CoQ_{10} for experiencing its beneficial effects falls within the range of 100 to 200 mg/kg (17). The disparity in outcomes between these studies appears to be linked to the method of CoQ_{10} administration, with lower concentrations proving more effective when administered through injections.

As illustrated in Fig. 3, the possible mechanism underlying the advantageous impacts of CoQ_{10} in diabetes involves the downregulation of miR-21 and miR-33 expressions, consequently leading to decreased expression of the SREBP1 protein within the pancreatic tissue. These modifications may contribute to the normalization of the pancreatic antioxidant system, the reversal of structural abnormalities in the pancreatic tissue, and, ultimately, the enhancement of serum insulin levels.

Importantly, it is noteworthy to mention that our study reveals, for the first time, the significant hypoglycemic, anti-cholesterolemic, and antioxidative properties of CoQ_{10} in diabetic rats, probably through the suppression of miR-21, miR-33, and SREBP1 protein expressions. Furthermore, tissue analyses revealed the beneficial effects of CoQ_{10} in diabetic rats. Consequently, it is inferred that CoQ_{10} may serve as a supplementary therapy in diabetes management and aid in the mitigation of associated complications.

One constraint of this research was the lack of electron microscopy analysis to provide a more comprehensive understanding of tissue regeneration in instances of diabetes or CoQ_{10} supplementation. Consequently, the specific organelles influenced by CoQ_{10} and the mechanisms involved remain ambiguous. Another limitation of the current study is the absence of data exploring the impact of CoQ_{10} on non-diabetic rodents. The inclusion of such subjects could elucidate the distinct effects of CoQ_{10} on non-diabetic animals, thereby enhancing our comprehension of the advantageous properties of CoQ_{10} in the context of diabetes. So, further research is needed to elucidate the exact mechanism(s) through which CoQ_{10} impacts pancreatic β-cell function and structure.

CONCLUSION

The restoration of β-cell integrity following treatment with CoQ_{10} may elucidate the therapeutic benefits of this compound in diabetes management, potentially through its influence on the pancreatic expression of the miR-33a/miR-21/SREBP1, subsequently maintaining healthy tissue. However, further studies are needed to elucidate the hypoglycemic and pancreas-protective effects of CoQ_{10}.

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

M. Ismaeil Khalil conceived the study, conducted the work, and performed statistical analysis; A. Louei Monfared and H. Bashar Mahmood performed the experiments and wrote the manuscript. All authors read and approved the finalized article.

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