Original Article

Evaluation of Antioxidant Effects of Coenzyme Q10 against Hyperglycemia-Mediated Oxidative Stress by Focusing on *Nrf2/Keap1/HO-1* Signaling Pathway in the Liver of Diabetic Rats

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What's Known

- Nuclear factor erythroid 2–related factor 2 (*Nrf2*) signaling pathway is suppressed in the context of diabetes mellitus, leading to the onset of oxidative stress. The *Nrf2* activation by antioxidant compounds (natural products) decreases oxidative stress and alleviates diabetes mellitus.

What's New

- Coenzyme Q10, known for its antioxidant properties, facilitates the induction of the *Nrf2/Keap1/HO-1/NQO1* signaling pathway in the livers of diabetic rats. This induction efficiently reduces oxidative stress and hinders the progression of diabetes.

Abstract

Background: Hyperglycemia-induced oxidative stress can damage the liver and lead to diabetes complications. Coenzyme Q10 (CoQ-10) reduces diabetes-related oxidative stress. However, its molecular mechanisms are still unclear. This study aimed to examine CoQ-10's antioxidant capabilities against hyperglycemia-induced oxidative stress in the livers of diabetic rats, specifically targeting the *Nrf2/Keap1/ARE* signaling pathway.

Methods: This study was conducted between 2020-2021 at Arak University of Medical Sciences. A total of 30 male adult Wistar rats (8 weeks old) weighing 220-250 g were randomly assigned to five groups (n=6 in each group): control healthy, sesame oil (CoQ-10 solvent), CoQ-10 (10 mg/Kg), diabetic, and diabetic+CoQ-10. Liver oxidative stress indicators, including malondialdehyde, catalase, glutathione peroxidase, and glutathione, were estimated using the spectrophotometry method. *Nrf2, Keap1, HO-1, and NQO1* gene expressions were measured using real-time PCR tests in the liver tissue. All treatments were conducted for 6 weeks. Statistical analysis was performed using SPSS software. One-way ANOVA followed by LSD's or Tukey's *post hoc* tests were used to compare the results of different groups. $P<0.05$ was considered statistically significant.

Results: The findings showed that induction of diabetes significantly increased *Keap1* expression (2.1±0.9 folds, P=0.01), and significantly inhibited the mRNAs expression of *Nrf2* (0.38±0.2 folds, P=0.009), *HO-1* (0.27±0.1 folds, P=0.02), and *NQO1* (0.26±0.1 folds P=0.01), compared with the healthy group. In the diabetic group, the activity of glutathione peroxidase, catalase enzymes, and glutathione levels was decreased with an increase in malondialdehyde level. CoQ-10 supplementation significantly up-regulated the expressions of *Nrf2* (0.85±0.3, P=0.04), *HO-1* (0.94±0.2, P=0.04), *NQO1* (0.88±0.5, P=0.03) genes, and inhibited *Keap1* expression (1.1±0.6, P=0.02). Furthermore, as compared to control diabetic rats, CoQ-10 ameliorated oxidative stress by decreasing malondialdehyde levels and increasing catalase, glutathione peroxidase activities, and glutathione levels in the liver tissues of the treated rats in the treatment group.

Conclusion: The findings of this study revealed that CoQ-10 could increase the antioxidant capacity of the liver tissue in diabetic rats by modulating the *Nrf2/Keap1/HO-1/NQO1* signaling pathway.

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Keywords ● Coenzyme Q10 ● Diabetes mellitus ● *Nrf2/Keap1/ARE* pathway ● Oxidative stress ● Rats

Introduction

One of the major complications that occur following diabetes mellitus, as the seventh leading cause of death, is excessive oxidant production and impaired antioxidant defense, which is characterized by elevated blood glucose levels and impaired carbohydrates, lipids, and protein metabolism, as well as changes in various gene expressions.[1] Hyperglycemia in diabetes continuously increases the mitochondria formation of free radicals, especially reactive oxygen species (ROS), in several tissues such as the liver, kidney, testis, and heart.[2]

To guard against or respond to the destructive effects of free radicals, various antioxidant defense systems including anti-oxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx), NADPH dehydrogenase quinone (*NQO1*), and Heme oxygenase 1 (*HO-1*) are induced in cells. On the other hand, antioxidant factors such as vitamins C and E act as free radical scavengers.[3] Coenzyme Q10 (CoQ-10) is a fat-soluble molecule made naturally in the body. It plays an important role as a carrier for ATP synthesis.[4] In recent years, CoQ-10 due to its anti-inflammatory, antioxidant, and antidiabetic effects has received much attention in various therapeutic applications. As a powerful antioxidant, CoQ-10 is involved in neutralizing free radicals and inhibiting lipid peroxidation.[5] Recently, CoQ-10 has been used to treat prevalent cardiac problems, diabetes, neurological diseases such as Alzheimer's and Parkinson's, and metabolic disorders including hyperlipidemia, hypertension, and mitochondrial diseases.[6] Several studies demonstrated that CoQ-10 improved oxidative stress and clinical manifestations of diabetes mellitus, whereas its exact molecular mechanisms remained fully unknown.[7] In this regard, for identification of the possible mechanisms involved in the antioxidant property of CoQ-10, the genes involved in this pathway must be determined to achieve new supplementary therapeutic alternatives for the treatment of diabetes.

One of the fundamental signaling pathways that play a key role in regulating the expression of various antioxidant genes, including *NQO1, HO-1, CAT, and GPx*, is nuclear factor erythroid 2–related factor 2 /Kelch-like ECH-associated protein1(*Nrf2/Keap1*). Finally, the product of these main genes improves cellular antioxidant defenses against oxidative stress and restores cellular homeostasis.[8]

HO-1, which has antioxidant, anti-apoptotic, and anti-inflammatory properties, is activated in response to oxidative stress and degrades heme into free ferrous iron, carbon monoxide, and biliverdin.[9] *NQO1* is an enzyme encoded by the *NQO1* gene, which encodes an oxidoreductase enzyme that plays an important role in the detoxification of quinones and their derivatives.[10] It was found that expression of *Nrf2* declined under chronic oxidative stress conditions, whereas *Nrf2* activators such as vitamin D, curcumin, and resveratrol significantly improved the *Nrf2* gene expression in combating chronic oxidative stress-induced illnesses such as diabetes, as well as reducing the expression of *Keap-1*, and ultimately stimulating the antioxidant enzymes expression.[11, 12]

Considering the stated findings, it was suggested that CoQ-10 could reduce the excessive generation of ROS while also alleviating the symptoms of diabetes mellitus. Nevertheless, the precise molecular mechanism by which CoQ-10 exhibits its antioxidant effects through the *Keap1/Nrf2/ARE* signaling pathway linked to diabetes mellitus has yet to be identified. Therefore, the present study was designed to investigate the effect of CoQ-10 supplementation on the expression of *Nrf2, Keap-1*, *HO-1*, and *NQO1* genes in the diabetic rat liver, as the main peripheral organ involved in diabetes. The findings of this study would shed light on a potential molecular mechanism through which CoQ-10 could act as an antioxidant, protecting liver cells in diabetes mellitus, which is characterized by hyperglycemia. This finding also suggested that further research into the manipulation of this signaling pathway could lead to the development of innovative therapeutic approaches.

Materials and Methods

Animals

A total of 30 male adult Wistar rats (8 weeks old), weighing 220-250 g, were used in the present research. The rats were housed in a temperature-controlled room (22±2 ºC) with a 12-h light/dark cycle and fed *ad libitum*. The study protocol was approved by the Ethics Committee of Arak University of Medical Sciences (code: IR.ARAKMU.REC.1399.186). The animal care and experimental procedures were performed according to the national guidelines, in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Animal Model for Diabetes Mellitus

To induce diabetes, the rats were given a single dose of Streptozotocin (STZ) (Sigma, St. Louis, MO, USA) injection, 55 mg/Kg dissolved in ice-cold citrate buffer pH 4.5 intraperitoneally (IP).[13]

To evaluate the fasting blood sugar (FBS), after 72 h and 7 days of injection of STZ, the blood samples were taken from the tails of the rats, using a glucometer. To confirm the induction of diabetes, the rats with blood glucose levels above 250 mg/dL were chosen for the rest of the experiments. On the first day of diabetic induction, the treatment was started.

Experimental Protocol

Thirty rats were randomly allocated into five equal groups as follows: Group I: control healthy, Group II: sesame oil (solvent of CoQ-10), Group III: CoQ-10, Group IV: diabetic, and Group V: diabetic+CoQ-10. Groups III and V received CoQ-10 at a dose of 10 mg/Kg.[14] All groups were treated orally once daily for six weeks. At the end of the treatment period, the fasting animals were anesthetized with ketamine (50 mg/Kg, intraperitoneally)/xylazine (20 mg/Kg, intraperitoneally).[15] Afterwards, 5 mL blood was taken from their heart and centrifuged at 3000 rpm for 10 min to separate the serum for further biochemical analysis. Eventually, the liver tissues were removed and immediately frozen in liquid nitrogen. Then, they were stored at -80 °C for further experiments. The experimental protocol of the present study is summarized in figure 1.

Measurement of FBS and Oxidative Stress Markers

At the end of the six-week of study time, the diagnostic colorimetric kits (Pars Azmun Co., Iran) were used to measure fasting blood glucose (mg/dL) using an enzymatic colorimetric assay with an auto-analyzer, (Hitachi model 704; Tokyo, Japan).

Various markers of oxidative stress in the livers of the rats were analyzed to determine the effects of CoQ-10. To begin, 100 mg of wet liver tissues were finely minced and homogenized in 1 mL of cold potassium phosphate buffer (pH 7.4) (1:10, wt/v) on ice. Then, they were centrifuged at 10,000 rpm for 15 min at 4 °C.[15] The supernatant was used to measure the reduced glutathione (GSH µmol/g tissue), malondialdehyde (MDA, µmol/g tissue) levels, and antioxidant enzymes activity, including GPx (µmol/min/g tissue), and CAT (U/g tissue).

As previously described, the MDA level, as a lipid peroxidation breakdown product, was measured by the thiobarbituric acid (TBA) method.[16] GSH, a thiol-containing tripeptide (γ-glutamyl-cysteinyl-glycine), as a key intracellular antioxidant, and GPX activity, as the main antioxidant enzymes, were evaluated with an assay kit (Arsam Fara Zist Co., Iran) based on an enzymatic cycling method according to the manufacturer's protocol. CAT activity was evaluated using the protocol described in a previous study.[16] In general, the activity of CAT was measured by incubating liver supernatants with hydrogen peroxide as a substrate. The enzymatic reaction was stopped by adding ammonium molybdate. The absorbance of the yellow complex was measured at 374 nm.

RNA Extraction and cDNA Synthesis

Total RNA was extracted from 100 mg of liver tissues using the RNX-plus reagent (Sinaclon Co., Iran) according to the manufacturer's instructions. The concentration and purity of RNA were evaluated by Nano-drop (Biotech, USA). Complementary DNA (cDNA) was synthesized from 2 µg RNA using the First Strand cDNA Synthesis Kit according to the manufacturer's instructions (Yekta Tajhiz Azma Co., Iran). Synthesized cDNA was stored at -70 °C until usage.

Real-time qRT-PCR

Real-time qRT-PCR was performed using the light cycler real-time PCR system (Roche Diagnostics, Mannheim, Germany).

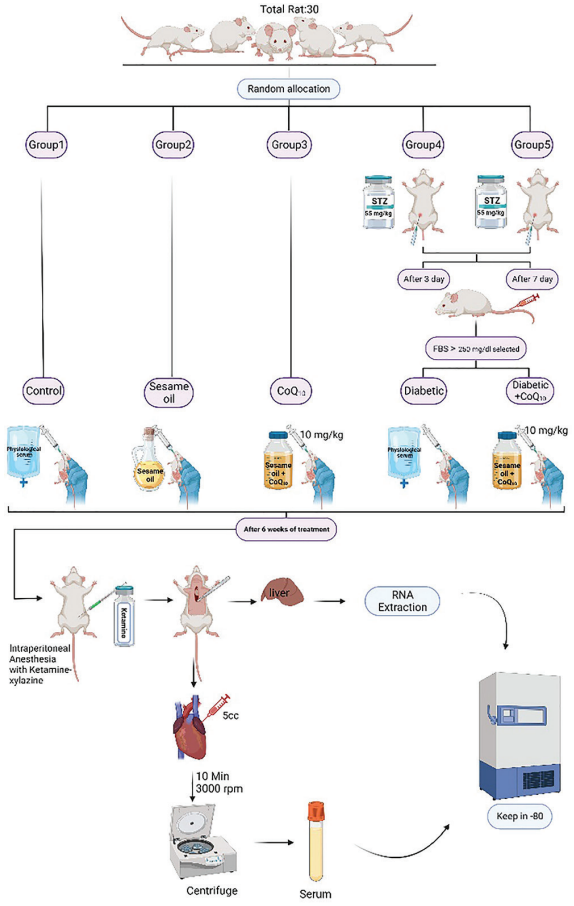

Figure 1: This graphic represents the experimental protocol of this study in the different groups and treatments.

Primers for each gene were designed using Allel ID software (Premier Biosoft, CA, USA) and confirmed by PubMed/Blast. The primers' characteristics, including sequence, accession number, annealing temperature (Ta), and product length, are shown in table 1. Each qRT-PCR reaction mixture contained 2 µL of cDNA (8-fold diluted), 0.5 µL of 5 mmol/L solutions of each of the forward and reverse primers, and 7.5 µL of 2x SYBR green DNA PCR Master Mix (Yekta Tajhis Azma Co., Iran) in a total volume of 15 µL. The relative expression of the target genes *Nrf2, HO-1, NQO1, and Keap1* was calculated using the $2^{-\Delta\Delta CT}$ formula. The CycloA gene was also regarded as a housekeeping gene and internal control. [17]

Statistical Analysis

All analyses were performed using the SPSS software version 23.0 (SPSS, Chicago, IL, USA). The obtained data were expressed as mean±SD. Statistical analysis was performed using One-way ANOVA followed by LSD's or Tukey's *post hoc* tests for comparison of the means between the groups. P<0.05 was considered statistically significant.

Results

Evaluation of the Effects of Coenzyme Q10 on FBS

As indicated in table 2, intraperitoneal injection of STZ (55 mg/Kg) significantly increased the FBS levels in diabetic rats (P<0.001) compared to the control healthy group. After 6 weeks of administration of CoQ-10 (10 mg/Kg) to diabetic rats, there were unexpected significant changes in the blood glucose levels (P<0.001). It is worth mentioning that treatment with CoQ-10 had no significant effects on FBS levels in healthy rats.

Evaluation of the Effects of Coenzyme Q10 on the Hepatic Oxidative Stress Parameters

As presented in table 2, MDA, as a main indicator of lipid peroxidation, was significantly increased in the liver tissue of the diabetic group compared to the control healthy controls (P<0.001). The MDA level decreased significantly in the diabetic rat receiving 10 mg/Kg CoQ-10 (P=0.005). The level of MDA decreased in the healthy rats treated by CoQ-10. However, no statistically significant differences were observed. Diabetic rats showed significantly lower levels of GSH (P<0.001), as well as antioxidant enzyme activities such as GPx (P=0.01) and CAT (P<0.001) in their liver tissues than the healthy controls.

Diabetic rats treated with CoQ-10 had significantly higher levels of GSH (P=0.01), GPX (P=0.02), and CAT than the diabetic control group (P=0.02). In the healthy groups, treatment with 10 mg/Kg CoQ-10 resulted in an increasing trend in the GSH and GPX levels, and CAT activities. However, but there was no statistically significant difference.

Table 1: Primers characteristics used in this study

Gene	Accession number	Primer Sequence (5′ to 3′)	Ta	Products length
Nrf2	NM_001399173	Forward: ACAACTGGATGAAGAGACCG Reverse: TGTGGGCAACCTGGGAGTAG	56	101
HO-1	XM_039097470	Forward: CTAGCCTGGTTCAAGATACTAC Reverse: GGAAACTGAGTGTGAGGAC	58	111
NQO1	NM_017000.3	Forward: GTCATCTCTGGCGTATAAGG Reverse: CAATGGGAACTGAAATATCACC	55	100
Keap-1	NM_057152.2	Forward: CAGCGTGGAGAGATATGAG Reverse: AGTAACATTCTGCCGAGTT	52	158
Cyclo A	XM_006250801.4	Forward: GGCAAATGCTGGACCAAACAC Reverse: TTAGAGTTGTCCACAGTCGGAGATG	62	196

Table 2: Effect of coenzyme Q10 (CoQ-10) on fasting blood glucose and hepatic amount of antioxidant parameters in different experimental groups

Parameters	Groups					P value*	P value#
	Healthy control	Sesame oil	CoQ-10	Diabetic control	Diabetic+ CoQ-10		
FBS (mg/dL)	95.16±7.25	88.5±6.52	89.2±5.21	505.8±32.9*	440.8±36.23##	<0.001	<0.001
MDA (µmol/g tissue)	28.7±12.31	27.5±8.36	24.16±9.17	83.02±18.37*	46.9±22.22#	<0.001	0.005
Catalase (U/g tissue)	35.15±0.82	35.99±2.29	36.19±1.78	29.53±2.18*	33.12±1.94#	<0.001	0.02
GSH (µmol/g tissue)	16.17±2.28	16.57±2.15	17.70±3.33	8.68±1.38*	13.79±3.47#	<0.001	0.01
GPx (U/g tissue)	70.01±24.93	73.54±17.37	78.07±23.73	28.29±12.09*	66.47±21.07#	0.01	0.02

Values are expressed as mean±SD (n=6). *P<0.05 represents significant differences with the control group, #P<0.05 represents significant differences between the diabetic groups, and ##P<0.05 represents significant differences between the control and the diabetic groups.

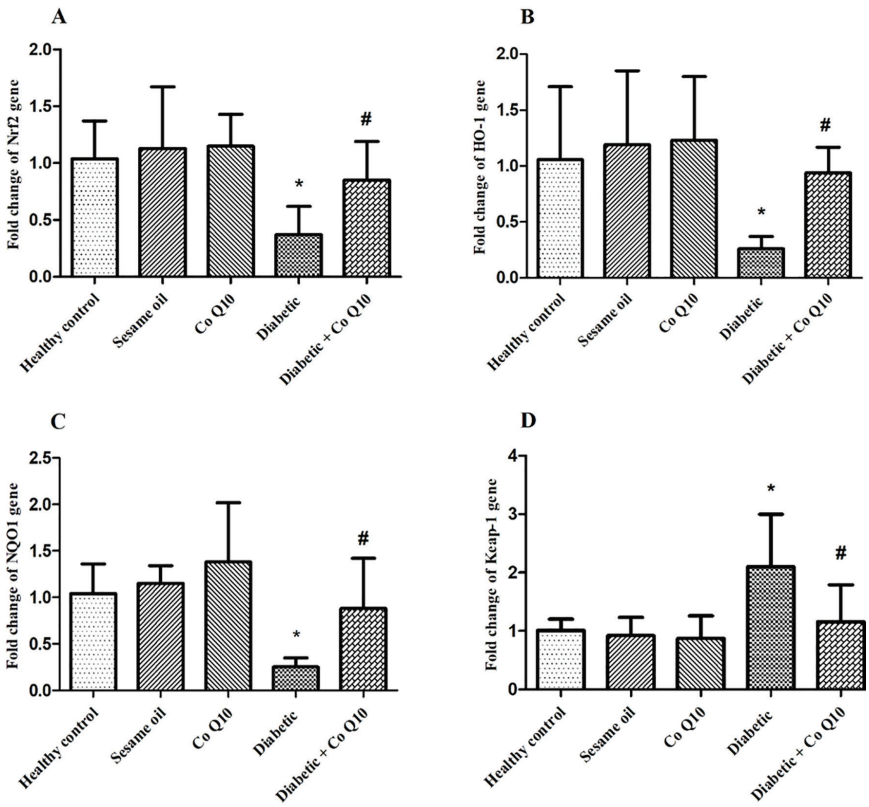

Figure 2: The figure shows the comparison of the levels of gene expression of *Nrf2* (A), *HO-1* (B), *NQO1* (C), and *Keap1* (D) in the liver tissue of different treated groups. Values are presented as mean±SD (n=6). *P<0.05 represents significant differences with the control group, and #P<0.05 represents significant differences between the diabetic and control groups.

Evaluation of the Effect of Coenzyme Q10 on the eExpression of Nrf2, HO-1, NQO1, and Keap1 Genes in Homogenate of Liver Tissue

Figure 2 shows the results obtained by quantitative real-time reverse transcription–polymerase chain reaction (RT-PCR) assay of the expression level of hepatic genes including *Nrf2, HO-1, NQO1, and Keap1*. Based on the analysis of the data, the expression levels of *Nrf2* (0.38±0.2 *vs* 1±0.3 folds, P=0.009), *HO-1* (0.27±0.1 *vs* 1.06±0.6 folds, P=0.02), and *NQO1* (0.26±0.1 *vs* 1.04±0.3 folds, P=0.01) were significantly lower in the diabetic control rats than the healthy ones. However, the expression level of *Keap1* increased significantly (2.1±0.9 *vs* 1±0.19 folds, P=0.01). Moreover, the treatment with CoQ-10 indicated a significant increase in the levels of *Nrf2* (0.85±0.3 *vs* 0.38±0.2 folds, P=0.04), *HO-1*(0.94±0.2 *vs* 0.27±0.1 folds, P=0.04), and *NQO1* (0.88±0.5 *vs* 1.04±0.3fold, P=0.03) mRNA expression compared with the diabetic controls. The results demonstrated that diabetic rats treated with CoQ-10 had significantly lower levels of *Keap1* mRNA expression than the diabetic control group (1.1±0.6 *vs* 2.1±0.9 folds, P=0.02).

Discussion

The primary findings of the present study were the significant enhancement of *Nrf2*, *HO-1*, and *NQO1* gene expressions, along with the suppression of *Keap1* expression, due to CoQ-10 supplementation. Moreover, when compared to the diabetic control group, CoQ-10 significantly improved oxidative stress by reducing MDA levels and increasing the activity of CAT and GPX, as well as elevating GSH levels in the liver tissues of the treated rats.

It was suggested that oxidative stress is a fundamental mechanism contributing to the development of diabetes mellitus.[18] It is widely recognized that the elevation of blood sugar levels, through the generation of ROS and a weakening of the body's antioxidant defense mechanisms, could result in increased oxidative stress, which ultimately leads to cellular and tissue damage.[19] CoQ-10, known for its potent antioxidant properties, could neutralize free radicals, inhibit lipid peroxidation, and provide protection to various tissues, including pancreatic β-cells and liver cells. It also plays a role in protecting cell membranes against damage caused by oxidative stress.[5] In the present study, a significant increase in serum glucose levels in rats with STZ-induced diabetes was observed. The STZ affected β-cells through by rapidly depleting ATP and causing cytotoxic effects through increased oxidative stress,

alterations in cellular metabolism, and disruption of mitochondrial function.[20] When CoQ-10 was administered to diabetic rats, it led to a significant decrease in serum glucose levels. However, they did not return to the levels found in healthy rats. It is worth noting that a previous study found that a two-week treatment with CoQ-10, combined with niacin, significantly reduced FBS in rats induced with diabetes using 60 mg/Kg of STZ.[21] Additionally, a systematic review and meta-analysis of 297 articles reported that long-term CoQ-10 consumption, typically over about 12 weeks, enhanced glycemic control, reduced triglycerides (TG), and increased high-density lipoprotein cholesterol (HDL-C) levels in patients with type 2 diabetes mellitus (T2DM).[7]

Lipids are the primary biomolecules affected by oxidative stress, and MDA is the predominant product resulting from the peroxidation of polyunsaturated fatty acids. MDA plays a crucial role in the development of various chronic diseases, including diabetes, making it a key focus of study in this context. The findings of the present study were in line with previous studies, which indicated that rats with STZ-induced diabetes had increased MDA levels and decreased GSH levels compared to healthy controls.[22] However, CoQ-10 treatment significantly reduced MDA levels and increased GSH levels. In diabetic animals, CAT and GPX activity was significantly lower than in the healthy control group, indicating an inadequate response to hyperglycemia-induced oxidative stress.[19] CoQ-10 supplementation in diabetic rats enhanced the activity of CAT and GPX activity, as two crucial antioxidant enzymes, combating oxidative stress.[23] Elevated ROS levels and reduced antioxidant defenses might lead to lipid peroxidation. Initially, cells attempt to counteract free radical production by increasing antioxidant enzyme levels. However, when these enzymes are depleted, cells become vulnerable to oxidative damage. CoQ-10, a component of the electron transport chain, significantly enhances cellular antioxidant capacity by influencing antioxidant enzyme production.[24] Additionally, CoQ-10 reacts with free oxygen radicals, inhibiting lipid peroxidation and protecting biological membranes from oxidative stress.[25] Moreover, CoQ-10 contributes to a reduction in ROS levels in the liver by increasing the ratio of GSH to oxidized glutathione (GSSG), minimizing the consequences of oxidative stress.[26] Under normal or non-stressful conditions, *Nrf2*, as a specific transcription factor, remains in the cytoplasm and bounds to *Keap1*, which facilitates its rapid degradation.[27] However, when oxidative stress occurs, *Nrf2* trans locates into the nucleus and binds to genes containing ARE sequences, including *NQO1*, *HO-1*, glutamyl cysteine ligase, Glutathione-S-transferase, GPX, and CAT. These genes play a crucial role in defending against harmful free radicals by acting as antioxidants. It is widely recognized that the *Nrf2/Keap-1* pathway serves cytoprotective, redox regulation, and metabolic functions in various cell types. In diabetes, this pathway becomes disrupted due to oxidative stress.[28] A recent research finding indicated that diabetes reduced the expression of *Nrf2, HO-1,* and *NQO1* mRNA while increasing the expression of *Keap1* mRNA. This implied that *Keap1* sequestered and degraded *Nrf2* through the proteasomal pathway, preventing it from activating its target genes.[29] Abdelsamia EM and others demonstrated that in the later stages of diabetes, cardiac *Nrf2* expression decreased, which was associated with the development of diabetic cardiomyopathy.[30] The findings of the present study were consistent with previous studies indicating that oxidative stress impaired cellular antioxidant defenses by affecting various transcription factors, such as *Nrf2*, *HO-1*, and *NQO1*.[31, 32] Previous studies highlighted the crucial role of various antioxidant compounds in restoring balance during stressful conditions by activating this pathway. For instance, pelargonidin chloride, a major anthocyanin found in berries, was shown to protect HepG2 cells from citrinin-induced oxidative stress by modulating the *Keap1/Nrf2* pathway.[33] Additionally, the extraction of strawberry leaves influenced the *Nrf2/HO-1* signaling pathway and enhanced the activity of CAT and superoxide dismutase (SOD) enzymes. This could help mitigate oxidative stress and reduce inflammatory responses in a rat model with STZ-induced diabetes.[34] In another study, Bhakkiyalakshmi and others observed significantly reduced *Nrf2* expression levels in diabetic mice. However, treatment with pterostilbene led to an upregulation of *Nrf2* expression.[35] Conversely, natural products, such as phloretin (PHL), demonstrated inhibitory effects on the expression of pro-oxidant, pro-inflammatory, hypertrophy, and fibrosis-related cytokines in cardiac H9c2 cells exposed to high glucose by targeting the *Keap1/Nrf2* pathway. Furthermore, quercetin, a bioflavonoid found in various foods, is known for its potent antioxidant properties, was demonstrated to enhance neural function by triggering the *Nrf2*/ARE signaling pathway in SH-SY5Y cells under high glucose conditions.[36] In the present research, it was observed that treating diabetic rats with 10 mg/Kg of CoQ-10 led to increased expression of liver genes associated with *Nrf2*,

HO-1, and *NQO1*. Simultaneously, it resulted in reduced levels of *Keap1* compared to the diabetic control group. Through activating the *Nrf2*/ARE signaling pathway, CoQ-10 effectively counteracted oxidative stress-induced diabetes, as was shown in our previous study on the liver cells of diabetic rats.[15] The protective effects of CoQ-10 enhanced the cell's antioxidant defenses by reducing oxidative stress markers. This was achieved through the inhibition of *Keap1*, leading to increased expression of *Nrf2*, *HO-1*, and *NQO1*. The findings of the present study supported those of an earlier study which reported that CoQ-10's activation of *Nrf2* might result from the sustained auto-oxidation of CoQ-10's quinone moiety.[37]

In a study by Pala R Ragip and others, CoQ-10 was found to induce the expression of NFκB, IκB, *Nrf2*, and *HO-1* in rats undergoing 6 weeks of chronic exercise training, highlighting its anti-inflammatory and antioxidant effects.[38] Similarly, Al Omar and others demonstrated that CoQ-10 exhibited anti-inflammatory, anti-apoptotic, and neuro-modulatory properties through the *Nrf2/HO-1* pathway. CoQ-10 also increased the activity of antioxidant enzymes in response to neurotoxicity induced by lead acetate (PbAc).[39] These findings suggested that CoQ-10 might modulate the *Keap1/Nrf2/ARE* pathway in diabetes, indicating its potential therapeutic role in conditions associated with oxidative stress.

While this study provided insightful information on the potential benefits of CoQ-10 in cells protecting against diabetes-induced oxidative stress, further research is essential to confirm these effects in humans. This study primarily focused on liver tissue, specifically the *Keap1/Nrf2/ARE* signaling pathway, and did not investigate the impact of CoQ-10 on other signaling pathways or organs related to diabetes. Analyzing all signaling elements through protein expression, using immunoblotting assays, would provide a more precise conclusion which should be considered in future studies. Moreover, the present study did not investigate liver histopathology, and the duration of the intervention was relatively short. Long-term studies are required to evaluate the sustained effects and safety of CoQ-10 supplementation in diabetes management.

Conclusion

This study indicated that CoQ-10 protected the liver tissues against diabetes-induced oxidative stress by regulating the *Nrf2/Keap1/HO-1/NQO1* signaling pathway, and subsequently stimulated the production of antioxidant enzymes. Therefore, CoQ-10 might be an appropriate choice for preventing and treating the complications of diabetes mellitus due to its therapeutic potential as antioxidant supplementation.

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Authors' Contribution

F.S: Study Conception and Design, Data collection and processing, Perform Experiment, Analysis and Interpretation of Results, Draft Manuscript Preparation, Visualization, Critical Revision, Editing of the Article and Supervision; M.B: Study Conception and Design, Analysis and Interpretation of Results, Draft Manuscript Preparation, Critical Revision and Editing of the Article; Z.N: Data collection and processing, Perform Experiment, Analysis and Interpretation of Results, Draft Manuscript Preparation, Visualization; S.D: Interpretation of Results, Draft Manuscript Preparation, Visualization, Critical Revision and Editing of the Article; M.R: Data collection and processing, Perform Experiment, Analysis and Interpretation of Results, Draft Manuscript Preparation; F.J.M: Study Conception and Design, Data collection and processing, Perform Experiment, Analysis and Interpretation of Results, Draft Manuscript Preparation, Visualization, Critical Revision and Editing of the Article, Funding Acquisition. All authors have read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

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