



An Investigation into the Effects of Ubiquinone on Inflammation, Diabetic Myopathy and Endotheliopathy, and CBC Parameters in Diabetic Rats

Yousif Jameel Jbrael

Department of Pharmacology and Toxicology, College of Pharmacy, Hawler medical university, Erbil, Iraq.

Yousifjbrael@gmail.com

Badraddin Kareem Hamad

University of Kurdistan Hawler (UKH), School of Medicine, Erbil, Iraq.

Badraddin.hamad@hmu.edu.krd

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Abstract

Oxidative stress and inflammation are connected to the development of metabolic disorders, such as diabetes. Diabetic-related oxidative stress is caused by the overproduction of oxidative-free radicals, which have been implicated in the mechanism of inflammation and damage to tissues. Our study aimed to investigate the effects of ubiquinone treatment on serum indicators of oxidative stress (malondialdehyde (MDA)), inflammation (interleukin 6 (IL-6)), vascular homeostasis (nitric oxide (NO)), and myopathy (myoglobin (MB)) in addition to measuring blood components parameters in streptozotocin-induced diabetic rats. Rats were separated into three groups; negative control group (N), diabetic control group (D), and ubiquinone-treated diabetic group (T). After 21 days, the blood and serum samples were taken to evaluate fasting blood glucose (FBG), MDA, IL-6, NO, MB, and hematological parameters. In hyperglycemic rats, the levels of FBG and serum levels of IL-6, MDA, and MB significantly increased, while NO levels decreased. Hyperglycemic condition significantly lowered the count of WBC ($P = 0.0098$) but insignificantly decreased values of platelets and RBC. Ubiquinone treatment significantly reduced blood glucose, IL-6, MDA, and MB levels in diabetic rats and raised NO levels. The effects of ubiquinone on WBC ($P = 0.648$), RBC ($P = 0.001$), and ($P = 0.398$) were insignificant, and only minor variations in WBC and platelet levels were observed. Our data support that ubiquinone supplementation could reduce proinflammation, oxidative stress, and myopathy markers and elevate NO levels in diabetic rats. The current study indicates ubiquinone may positively impact diabetic complications; however, additional research is required to determine its therapeutic benefit when added to standard diabetes treatment.

Keywords: Diabetes mellitus, Hyperglycemia, Oxidative stress, Antioxidant, Ubiquinone supplementation.

1. Introduction

Diabetes mellitus (DM) is a chronic disease of significant concern that is predicted to be the seventh worldwide leading cause of death by 2030, and diabetes-related global health expenditures

will increase dramatically over the next decade [1, 2]. It is a complex metabolic disorder and elevated blood glucose – hyperglycemia - is the major clinical hallmark of unregulated diabetes. Hyperglycemia is believed to generate reactive oxygen species (ROS) via activation of the hexosamine pathway, stimulation of protein kinase C signaling, and formation of advanced glycation end products (AGEs), resulting in oxidative stress in various organs [3, 4].

The oxidative stress role has been included as a crucial factor in unraveling the complex mechanism behind diabetes complications. The physiological and pathophysiological role of oxidative stress has been investigated in cardiomyocyte metabolism and coronary blood circulation in diabetic patients [5, 6]. Heightened antioxidant defense system consumption, overproduction of free radicals, and lipid peroxidation have been reported in diabetic rats. In recent years, the pivotal and crucial role of oxidative stress in the pathogenesis of several diabetes-related complications has attracted considerable attention [7, 8]. Hyperglycemia increases the production of AGEs by increasing nonenzymatic glycation and binding AGEs to their receptors, leading to an elevated formation of intracellular ROS through nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Correspondingly, the synthesis of diacylglycerol (which is elevated in diabetes) activates protein kinase C, leading to the formation of ROS via NADPH oxidase [9, 10]. They can oxidize essential cell constituents (nucleic acids, proteins, and lipids) and produce toxic byproducts that result in tissue damage. In addition, they alter the structure of biological molecules, even destroying them [11, 12]. Various study articles investigated the effect of antioxidant defense and oxidative stress on the onset of diabetes and its related conditions [9, 13-15]. It has been reported frequently that the development and progression of diabetes are associated with insufficient biological antioxidants which resulting oxidative stress [16]. Analyzing and exploring the extraction of natural antioxidants to replace synthetic antioxidants has recently caught the attention of investigators [17, 18]. It was suggested that emerging evidence supports the protective impact of dietary antioxidants as a potential supplementary treatment for delaying or preventing diabetic consequences [19]. Accordingly, further research is required to specify the natural source of antioxidants in order to improve the public health of diabetic patients.

Ubiquinone (Coenzyme Q10) is a potent lipophilic antioxidant found in the inner membrane of mitochondria. It is an endogenously synthesized constituent that functions as a crucial cofactor in the mitochondrial electron transport system [20, 21]. Ubiquinone is also involved in the recycling and regenerating of other antioxidants, including tocopherol, β -carotene, and ascorbate [22, 23]. Furthermore, like other antioxidants, ubiquinone hinders certain enzymes that catalyze the free radicals formation, thus attenuating oxidative stress and preventing the initiation and spread of lipid peroxidation in cell membranes. Enhancing the enzyme's potential to scavenge free radicals is an additional protective effect of ubiquinone [24, 25].

This research intends to investigate- the ubiquinone effect on hyperglycemia consequences that could be deduced from the interpretation of markers of oxidative stress, inflammation, myopathy, and endotheliopathy. Lipid oxidation products are the major damaging influence of ROS formation, as they consider irreversible oxidative modifications in membranes [26]. MDA is one of the most extensively researched end-products of lipid oxidation and is frequently used as an indicator of free radical-induced injuries or oxidative stress [27, 28]. Individuals with diabetes have elevated blood levels of interleukin-6 (IL-6), which is known as an inflammatory biomarker, and raising its level is regarded as the development of atherosclerosis and vascular disease [29]. Furthermore, free radicals, particularly superoxide anions, potentially interact with endothelium-derived Nitric oxide (NO) and suppress endothelial nitric oxide synthase (eNOS). This interaction

is one of the most influential pathways behind diabetic endothelial dysfunction [30]. The main regulator of vascular homeostasis, NO, is produced by eNOS in endothelial cells from L-arginine. It has a substantial role in protecting against the onset and development of cardiovascular disease [31]. Reduced NO bioavailability is characteristic of hyperglycemia-induced endothelial dysfunction [32].

This study aimed to examine ubiquinone impact on glycemic status and hyperglycemia-induced complications in STZ-induced diabetic rats. By assessing MDA, IL-6, NO, and MB levels in hyperglycemic rodents, the current study will evaluate the preventive effect of ubiquinone against oxidative stress, inflammation, and myopathy. Another objective is to investigate the antioxidant potential of ubiquinone on blood component parameters to gain a deeper understanding of the role of ubiquinone in mitigating diabetic complications.

2. Materials and methods

Chemicals

The Streptozotocin (STZ), with high purity at 97.5 percent, was purchased from Shanghai Macklin Biochemical Co., Ltd. Ubiquinone 200mg which had been purchased was in the gel form manufactured by America (Nature's bounty Pharmaceutical Inc., USA.). Rat malondialdehyde (MDA) enzyme linking immune-absorbent assay (ELISA) Kit (Catalogue Number: 201-11-0157, 48 tests), interleukin 6 (IL-6) ELISA Kit (Catalogue Number: 201-11-0136, 48 tests), and nitric oxide (NO) ELISA Kit (Catalogue Number: 201-11-0704, 48 tests) were purchased from Sunred Biotechnology Company, Shanghai, China. The myoglobin (MB) kit was obtained from Hotgen Biotech. Blood component parameters were measured using the Swelab Alfa Standard (Boule Medical AB, Sweden).

Experimental Animals

A total of 24 mature Sprague-Dawley albino rats weighing 250-350 g were purchased from the animal house of the University of Soran, and allowed to adapt to the new location for one week. Under standard laboratory settings, the animals were kept at a temperature range of 22 to 26 °C with a 12-hs. light:12-hs. dark cycle. Before and during the experiments, rats were fed with rat chow (according to the National Center for Drug Research and Quality Control, Baghdad) and tap water. The rats were randomly assigned into three groups of 8 animals per group; negative control, diabetic control, and diabetic rats treated with ubiquinone. All study protocols, including diabetes induction and sacrifice operations, were approved by the institutional ethics committee at Hawler medical university.

Experimental design

After acclimatizing for 7 days, the rodents were randomly allocated into three groups:

Group N: Negative control group (n = 7)

Group D: Diabetic control group (n = 7)

Group T: Ubiquinone treatment group (treat with ubiquinone, 5 gm per kg of food) (n = 7)

In the second and third groups (16 rats), we induced diabetes. Rats from the negative control group (Group N) and the diabetic control group (Group D) received the rat's standard diet without any supplementation. The third group (Group T) was provided with the ubiquinone-enriched diet, which was the basic diet enriched with 5 g of ubiquinone per kilogram of food [33-37]. All groups had unrestricted access to their respective diets for a period of three weeks.

Diabetes induction

The administered dose of STZ, a diabetogenic drug that damages pancreatic β -cells, specifies the establishment of a diabetic state in rats [38, 39]. The most typical method is to administer a single dose of STZ (40–70 mg/kg) intraperitoneally to rats between 8 and 10 weeks of age [40]. -Diabetes induction procedure was followed to the protocol written by Brian L. Furman [41]. Induction of experimental diabetes was performed following an overnight fast (8 hrs.) with a single intraperitoneal injection of STZ which was dissolved in citrate buffer (pH 4.4 to 4.5, 0.1 mol/L citric acid, 0.1 mol/L trisodium citrate) at dose 45 mg/kg of body weight. Seventy-two hours after STZ injection, hyperglycemia was validated by applying a small volume of peripheral blood from the animals' tails to test strips. Bayer Contour® TS Blood Glucose Monitoring System was used for the measurement of rat's blood sugar. Rodents with fasting blood glucose levels of more than 250 mg/dL were considered diabetics [42], and were separated into various experimental groups (diabetic control group and ubi quinone-treated diabetic group). Another group of rats received a single intraperitoneal administration of the citrate buffer and were deemed non-diabetic.

Blood and Serum collection

On the final day of the three-week treatment period, the animals were fasted overnight, sacrificed the next day, and collected blood samples. The blood collection procedure began with anesthetizing rats using a combination of ketamine (45 mg/kg) and xylazine (5 mg/kg), followed by a heart puncture with a sterile, disposable syringe. A portion of blood was obtained into EDTA-containing tubes and was immediately analyzed for blood component parameters and blood glucose levels. Another portion of the blood was poured into numbered gel tubes at room temperature and allowed to coagulate for 10 to 20 minutes. Then, the blood sample was centrifuged between 2000 and 3000 rpm for 20 minutes to separate the serum for biochemical analysis.

Measurement of Serum Biomarkers

Evaluation of MDA, IL-6 and NO

Oxidative stress (malondialdehyde (MDA)), inflammation (IL-6), and vascular homeostasis (nitric oxide (NO)) markers were evaluated using rat ELISA kits through microplate reader according to the absorbance principle. MDA, IL-6 and NO rat kits were purchased to assess MDA, IL-6 and NO according to the supplier's instructions and the protocol of the respective kits. Our ELISA kits for assessment of MDA, IL-6 and NO were designed to apply the double-antibody sandwich ELISA technique. Measurement of the optical density (OD) is spectrophotometrically accomplished at the wavelength of 450 nm. Calculate the standard curve linear regression equation based on the concentration of the standards and the respective OD values, and then enter the sample's OD values into the regression equation to determine the related sample's concentration.

Evaluation of serum levels of myoglobin

UP - Converting Phosphor Immunoassay Analyzer was employed for quantitative assessment of serum concentration of MB by using commercially available kits (Hotgen Biotech Co., Ltd). Either plasma or serum biomarker levels are evaluated using a technique that consists of a combination of Up-converting Phosphor Technology (UPT) employing immunochromatography. In comparison to other methods, the technical data of UPT are uncomplicated to comprehend, there is no background interference with a specified signal, and the luminescence is persistent without a

quench. Hotgen produced a POCT platform relying on UPT (UPT-3A) Series Immunoassay Analyzer and support reagents used in our study [43, 44].

Evaluation of blood component parameters

Blood collected via heart puncture was blended by a Blood Rolling Mixer machine and evaluated hematological values such as WBC, platelets (PLT), and RBC, employing the automatic hematology analyzer Swelab Alfa Standard (Boule Medical AB, Sweden) at Ala private laboratory in Soran city.

3. Statistical analysis

GraphPad Prism 9 (San CA, USA) was used to conduct statistical analysis. Before conducting the analysis, we conducted Normality and Lognormality tests to confirm that our data passed normality tests ($\alpha = 0.05$), relying on the Shapiro-Wilk tests. In order to compare the means of each parameter for the three groups (negative control = N, diabetic control = D, and ubiquinone-treated group = T), we conducted One-Way ANOVA. In relation to multiple comparisons, the mean of columns N and T has been compared with columns D. Dunnett is suggested when performing numerous comparisons using statistical hypothesis testing. All values were provided as mean \pm standard error of mean, and the results at $P < 0.05$ were considered as statistically significant.

4. Results

Effect of ubiquinone supplementation on blood glucose

As shown in **Table 1**. and **Figure 1**, the ubiquinone-treated group (5 g of ubiquinone per kilogram of diet) revealed a significant drop in blood glucose (from 4.685 ± 0.35 to 2.701 ± 0.3861 (mg/ml), $P = 0.0012$) compared to the diabetic group.

Table 1. The impact of ubiquinone supplement on hyperglycemia and oxidation markers in diabetic rats

Marker	Negative control (N)	Diabetic control (D)	Ubiquinone treatment (T)	P-value D vs. N	P-value D vs. T
Glucose (mg/ml)	0.9590 ± 0.09	4.685 ± 0.35	2.701 ± 0.3861	<0.0001	0.0012
MDA (nmol/ml)	4.32 ± 0.38	8.522 ± 0.22	5.083 ± 0.32	<0.0001	<0.0001
Values are expressed as mean \pm SEM, $P < 0.05$ indicates significant difference.					

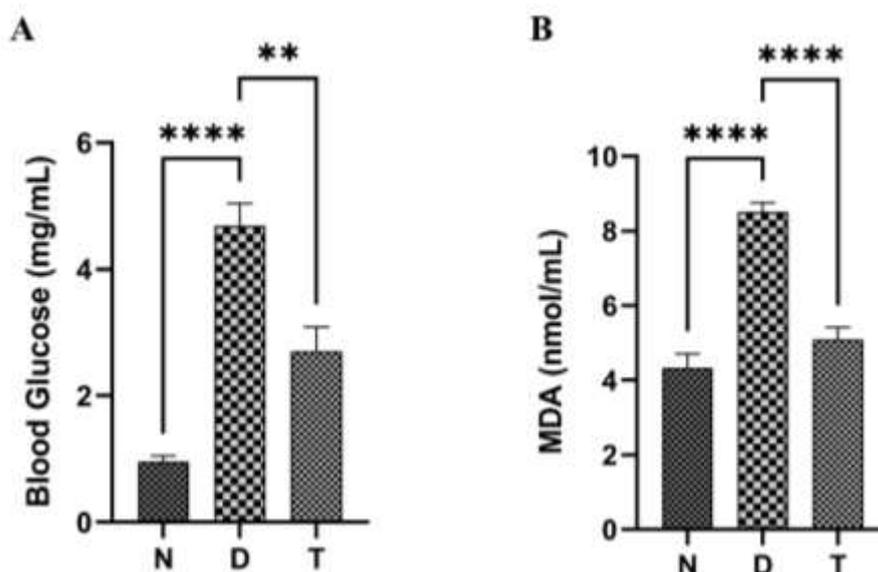


Figure 1. Effect of ubiquinone on blood glucose and serum MDA levels.

After three weeks, blood glucose levels (A) and serum levels of MDA (B) were measured in three groups of experimental rats; the Negative control group (N), the Diabetic control group (D), and the ubiquinone treatment group (T). Glucose levels were assessed by Bayer Contour® TS Blood Glucose Monitoring System. Serum levels of MDA were evaluated by ELISA. Data are shown as the mean \pm SE (n=7). During the same medication period, the bars were marked by different asterisks (*, **) differ significantly from each another ($P < 0.05$). The non-asterisk bars are not significantly different from one another ($P > 0.05$).

Effect of ubiquinone supplementation on MDA serum levels

The results were evaluated at the end of 21 days of the study in all groups of the experimental animals, and MDA serum levels values are presented in **Table 1.**; also, the changes in MDA serum levels are shown in **Figure 1.**

There was a significant difference between serum MDA levels of group D rats with group N and T. The concentration of MDA, as an end product of lipid peroxidation, increased dramatically in the serum of the diabetic rats as compared to the non-diabetic rats (from 4.32 ± 0.38 to 8.522 ± 0.22 nmol/ml, $P < 0.0001$). Treatment of diabetic rats with ubiquinone remarkably diminished MDA levels in serum as compared to diabetic controls (from 8.522 ± 0.22 to 5.083 ± 0.32 nmol/ml, $P < 0.0001$).

Effect of ubiquinone supplementation on IL-6 serum levels

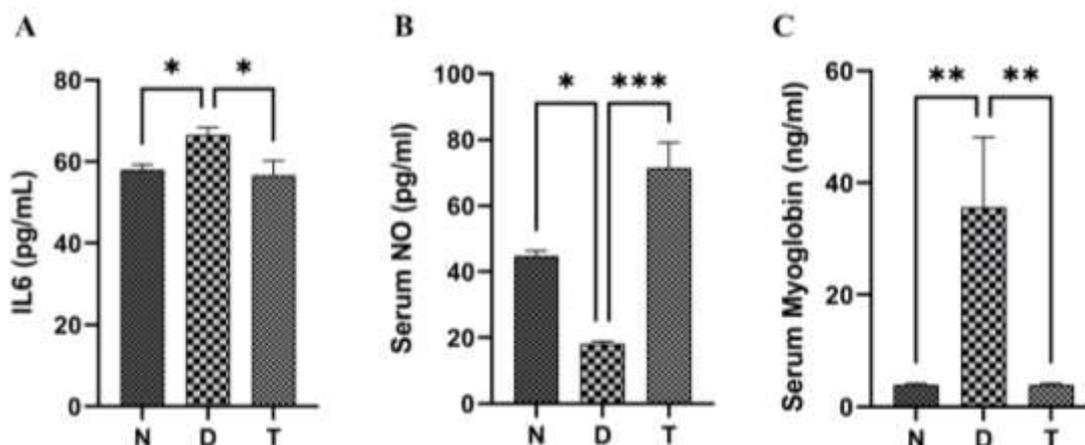
The results were assessed at the end of 21 days of the study in all groups, and the IL-6 values are shown in **Table 2.** Also, **Figure 2** shows the changes in serum levels of IL-6 in all groups of our experimental animals.

Group N rats had significantly lower levels of serum IL-6 than groups P and T animals. IL-6, a proinflammatory marker, was significantly increased in the serum of diabetic control rats relative to normal control rats ($P = 0.0493$). Compared to untreated diabetic rats, treatment with ubiquinone significantly decreased serum IL-6 levels (from 66.49 ± 1.87 to 56.71 ± 3.54 pg/ml, $P = 0.0233$).

Table 2. The impact of ubiquinone supplement on inflammation and muscle cell injury markers in diabetic rats

Marker	Negative control (N)	Diabetic control (D)	Ubiquinone treatment (T)	P-value D vs. N	P-value D vs. T
IL-6 (pg/ml)	58.04±1.35	66.49±1.87	56.71±3.54	0.0493	0.0233
NO (pg/ml)	44.20±1.91	18.19±0.79	71.00±7.78	0.0370	0.0005
MB (ng/ml)	3.90±0.30	29.65±11.42	3.900±0.33	0.0019	0.0031

Values are expressed as mean ±SEM, P<0.05 indicates significant difference

**Figure 2** Effect of ubiquinone on serum levels of IL-6, NO, and MB.

After three weeks, serum levels of IL-6 (A), NO (B), and MB (C) were measured in three groups of experimental rats; the Negative control group (N), the Diabetic control group (D), and the ubiquinone treatment group (T). Serum levels of IL-6 were evaluated by ELISA. Serum levels of MB were measured by UP - Converting Phosphor Immunoassay Analyzer. During the same medication period, the bars were marked by different asterisks (*, **) differ significantly from each another ($P < 0.05$). The non-asterisk bars are not significantly different from one another ($P > 0.05$).

Effect of ubiquinone supplementation on NO serum levels

In our study, the recorded nitric oxide values in the normal and ubiquinone-treated groups were significantly higher than in the diabetic group (**Table 2** and **Figure 2**). Compared to normal controls, there was a significant drop in serum NO levels in the STZ-treated group ($P = 0.0370$). Serum NO levels were changed toward normalcy in ubiquinone-treated groups and raised from 18.19 ± 0.79 in group D to 71.00 ± 7.78 pg/ml, $P = 0.0005$ in group T.

Effect of ubiquinone supplementation on serum levels of MB

As illustrated in **Figure 2** and documented parameters values in **Table 2**, the highest serum values of MB levels were witnessed in diabetic control rats (Group D). Serum MB levels were significantly decreased in the diabetic ubiquinone-treated group (Group T) compared with untreated rats in group D (from 29.65 ± 11.42 to 3.900 ± 0.33 ng/ml, $p = 0.0031$).

Effects of ubiquinone supplementation on blood component parameters

Table 3 and **Figure 3** demonstrated the results of total WBC, PLT, and RBC count in all groups of rats. The data from the **Table 3** showed a significantly ($P < 0.05$) decreased count of leukocytes

from 13.74 ± 0.79 ($10^9/L$) in normal control rats (group N) to 6.88 ± 1.8 ($10^9/L$) in diabetic control rats (group D). While values of thrombocytes in the diabetic control group did not significantly but slightly change when compared to normal rats. Erythrocyte values changes between the normal rat group and diabetic control group were insignificant.

Oral administration of ubiquinone did not significantly but slightly increase WBC and PLT parameters compared to diabetic control rats. RBC count in the ubiquinone-treated group (group T) decreased insignificantly compared to untreated diabetic rats.

Table 3. The impact of ubiquinone supplement on blood component parameters in diabetic rats

Parameters	Negative control (N)	Diabetic control (D)	Ubiquinone treatment (T)	P-value D vs. N	P-value D vs. T
WBC ($10^9/L$)	13.74 ± 0.79	6.88 ± 1.8	8.4 ± 1.32	0.0098	0.648
PLT ($10^9/L$)	586.8 ± 68.39	515.7 ± 78.64	624.4 ± 52.43	0.696	0.398
RBC ($10^{12}/L$)	8.47 ± 0.25	7.18 ± 1.52	7.05 ± 0.29	0.43	0.001

Values are expressed as mean \pm SEM, $P < 0.05$ indicates significant difference

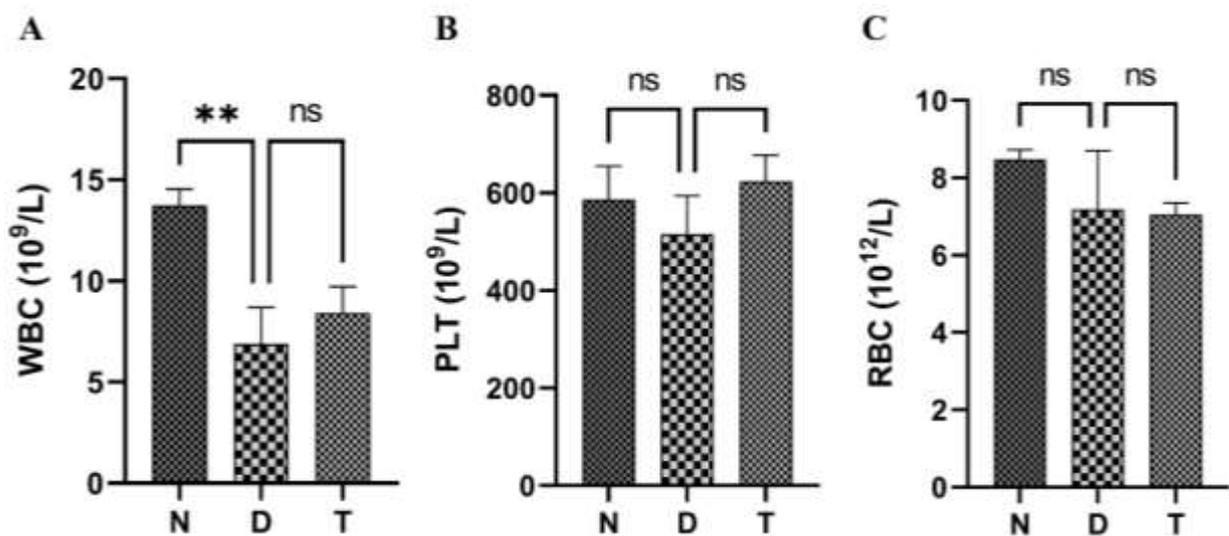


Figure 3. Effect of ubiquinone on blood levels of blood component parameters.

After three weeks, blood levels of WBC, PLT, and RBC were measured in three groups of experimental rats; the Negative control group (N), the Diabetic control group (D), and the ubiquinone treatment group (T). Blood component parameters were evaluated by the automatic hematology analyzer Swelab Alfa Standard (Boule Medical AB, Sweden). Data are presented as the mean \pm SE ($n=7$). During the same medication period, the bars were marked by different asterisks (*, **) differ significantly from each another ($P < 0.05$). ns: not significant.

5. Discussion

The current study was performed to investigate the modulating impacts of ubiquinone on fasting blood sugar (FBG), pro-inflammatory adipocytokine (IL-6), and the markers of diabetic myopathy, endotheliopathy and blood components in STZ-induced diabetic rats. The results of our study showed that ubiquinone attenuates hyperglycemia and ameliorates IL-6, MDA, NO and MB levels. The WBC level, however, has only been slightly altered.

Diabetic model validation and the Effect of ubiquinone on blood Glucose concentration

Diabetes was confirmed by the significant elevation of FBG in STZ-induced diabetic rats; this is caused by the destruction of pancreatic β -cells (β -cell failure) by a diabetogenic agent (STZ). As STZ toxicity progresses (**Figure 4**), some β -cells survive; however, hyperglycemic toxicity impairs these surviving β -cells further. A high glucose level increases nonenzymatic glycation of proteins, as well as glucose oxidation, all of which lead to oxidative stress [45]. A number of lines of research have described the consequences of persistent hyperglycemia - oxidative stress - which weakens antioxidant enzymes and other antioxidant molecules [46].

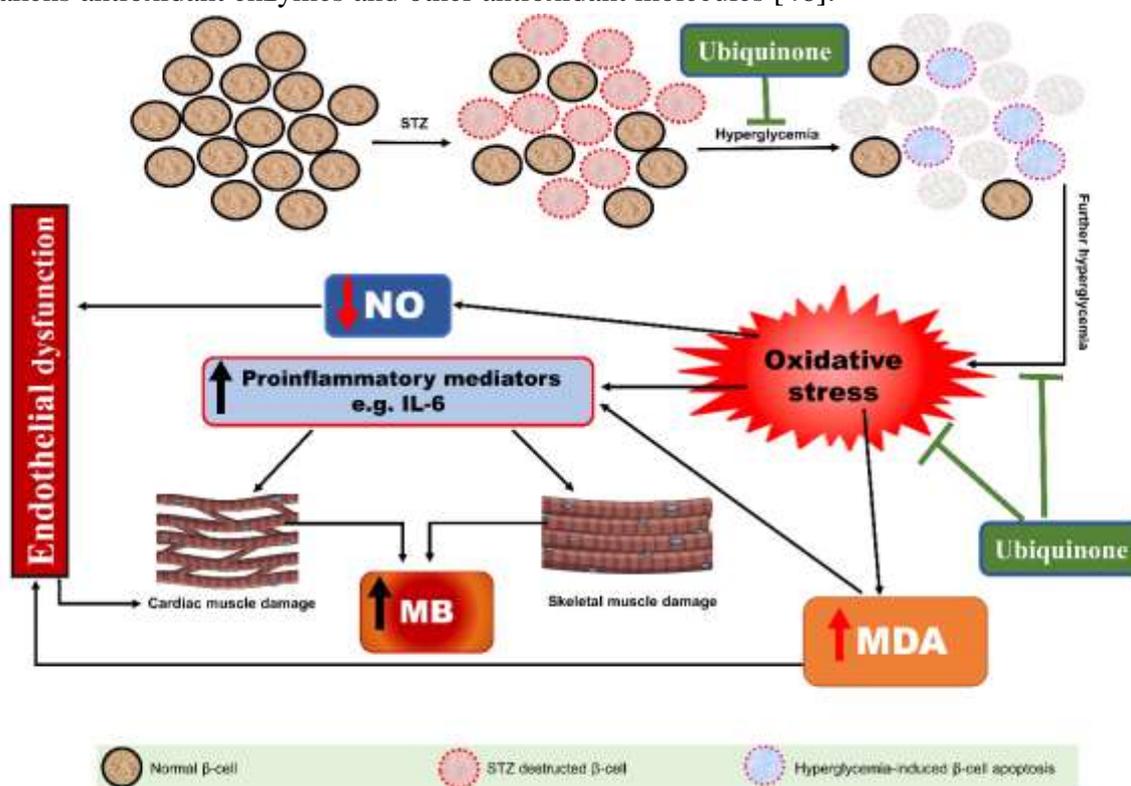


Figure 4. A schematic diagram demonstrating the mechanism of β -cell destruction by STZ and hyperglycemia and the proposed pathway wherein hyperglycemia triggers the release of IL-6 and MDA that causes muscle injury.

A glucose overload activates several metabolic pathways that produce more ROS and oxidative stress, leading to β -cells death (**Figure 4**). Combined with a low antioxidant capacity in cells, these pathways result in ROS production, causing secondary β -cell failure in diabetics [47]. In the presence of high levels of free radicals, pancreatic islet cells die and become dysfunctional (ROS-induced apoptosis).

Our data from analysis reveal that the administration of ubiquinone significantly lower blood glucose levels in the group of diabetic rats. As per the previous reports, it was found that ubiquinone has an anti-diabetic effect [48-51], while in contradictory some the literatures also revealed that ubiquinone did not have any effect on blood glucose level [52]. This reduction in the levels of FBG would be due to insulin release from survived β -cells which have been protected by the antioxidant impact of ubiquinone.

Ubiquinone's protective mechanism is due to its ability to neutralize free radicals. The ability of ubiquinone to scavenge ROS may be one of the reasons for its ability to inhibit NF- κ B activation and protection of pancreatic β -cells [49]. Hence, we focused on the antioxidative properties of

ubiquinone on pancreatic β -cell oxidative injury. ubiquinone neutralizes free radicals and prevents their cytotoxic effect on surviving β -cells after STZ injection (**Figure 4**). Due to its glucose lowering effect, ubiquinone may stimulate the synthesis of insulin from the residual β -cells or recovered β -cells.

Effect of ubiquinone on parameters of oxidative stress, MDA

To investigate the protective effects of ubiquinone against oxidative stress in diabetic animals, the levels of MDA in the serum samples were measured. After three weeks, our results showed that the MDA levels in the diabetic control group were considerably higher than in the normal control group. The diabetic group supplied with ubiquinone at a dose of 5 mg per kg of food had their MDA levels lower than the diabetic control group.

We noticed a significant increase in the MDA concentration in the diabetic rodents' serum, similar findings were reported by other researchers [53, 54]. Previous studies have also suggested that high level of free radicals could lead to the formation of lipid peroxidation in STZ-induced diabetes [55, 56]. In light of these findings, we hypothesized that ubiquinone prevents membrane lipid peroxidation by reacting with free radicals. Our research validated that ubiquinone supplementation substantially improved this oxidative status by reducing MDA concentration (**Figure 4**). The beneficial role of ubiquinone treatment was proved to reduce lipid peroxidation and serum MDA levels, according to the findings of earlier studies [27, 54, 57].

Effect of ubiquinone on the proinflammatory adipocytokine (IL-6)

Hyperglycemia and hyperlipidemia in diabetes and obesity are associated with diminished antioxidant enzymes and elevation of ROS levels [58]. Researchers demonstrated that elevated levels of ROS induced by a high glucose environment increases pancreatic expression of IL-6 and triggers mitochondrial dysfunction, inflammatory response, and apoptosis (especially pancreatic apoptosis) via the stress signaling pathway [59, 60]. In addition, MDA caused significant increases in the levels of several major proinflammatory biomarkers, including IL-6, IL-25, IL-8, ICAM-1, and primary peripheral blood lymphocytes (PLCs) [61]. Consistent with the previous findings, our study revealed an increase in the proinflammatory cytokine IL-6 in diabetics rats compared to the control group ($P = 0.0493$; **Table 2**). The schematic diagram (**Figure 4**) illustrated the heightening effect of hyperglycemia, oxidative stress, and MDA on IL-6 in.

Normally, there is a balance between oxidants and antioxidant defense, but hyperglycemia elevates ROS levels, leading to an increment of oxidation of proteins, lipids, and nucleic acids [62]. High glucose level also stimulates and enhances the production of reactive species via NADPH oxidase activity, which intensifies proinflammatory biomarkers, such as IL-1 β , IL-6, and TNF- α [63]. Impairment of endogenous antioxidant defense system results in the production of AGEs [64]. AGEs increase IL-6 secretion through ROS and NF- κ B activation [65]. In addition, ROS enhance the activation of NF- κ B in the cytoplasm and translocate the signal transduction pathway into the nucleus. In inflammation and apoptosis, NF- κ B acts as a key regulator. Phosphorylation of NF- κ B induces secretion of pro-inflammatory cytokines such as IL-6 [66]. NF- κ B, a transcription factor, is responsible for the upregulation of inflammatory proteins such as IL-6, iNOS, and TNF- α by attaching to their promoter [67]. Several clinical and experimental pieces of research have revealed the importance of cytokines in developing diabetes complications [68-70]. Consequently, antioxidant compounds could be a crucial defense to amelioration of the damaging effect of ROS.

In our data, we verified a substantial decrement in serum level of IL-6 in ubiquinone-treated diabetic rats compared with the normal rats, as shown in **Table 2**. Also, other studies showed the counteracting effect of antioxidants against inflammatory indicator, IL-6 [71, 72]. [73]. In support of our findings, a study found that the mRNA expression level of proinflammatory cytokines, IL-6, was significantly downregulated in the animals treated with ubiquinone compared to untreated diabetic rats [67]. Ubiquinone having antioxidant properties neutralizes and scavenges free radicals since we suggest that it elicits this beneficial effect via reducing the expression of NF- κ B and, subsequently, downregulating the levels of TNF- α and IL-6.

Effect of ubiquinone on the determinant of vascular homeostasis, NO

In the present study, we observed that hyperglycemia was accompanied by a reduction in NO levels. These findings are consistent with previous studies which reported that diabetes mellitus is correlated to reduced nitric oxide synthesis from endothelial cells [74, 75]. Hyperglycemia leads to depletion of the NADPH required to produce nitric oxide from L-arginine via nitric oxide synthase [76]. Conversely, a number of previous studies have revealed an increase in NO generation in diabetics [77, 78]. They postulated that the pro-inflammatory mediators IL-6 and TNF- α were the most significant contributors to the excessive generation of nitric oxide in hyperglycemic condition [78].

In the current investigation, NO concentration in the ubiquinone-treated diabetic rodents was significantly higher than in the diabetic group. Other investigators obtained the same results and stated that ubiquinone could recouple endothelial nitric oxide synthase and modify NO-associated signaling [75, 79]. As depicted in **Figure 4**, it appears that ubiquinone's antioxidant characteristics may protect NO from oxidative inactivation, allowing its concentration to increase in the treated group. Hyperglycemia lowers NO serum levels, while ubiquinone normalizes them, which suggests ubiquinone's protective role in cardiovascular endothelial cells.

Effect of ubiquinone on serum level of myopathy marker, MB

As to our investigation, we studied the protective role of ubiquinone against muscle cell injury in STZ-induced diabetic rats. We found higher MB levels in diabetic rats than in normal rats. Moreover, our data reveals that ubiquinone-treatment diabetic rats had lower MB levels compared to diabetic rats without treatment.

Cardiovascular biomarkers have a critical role in the early detection of acute myocardial infarction. Among numerous cardiac indicators, MB has been extensively recognized as one of the most acceptable potential biomarkers for the early detection of myocardial infarction, despite the fact that it is not cardiac-specific [80-82]. A study observed that MB levels of diabetic patients were higher than that of control subjects [83]. Similarly, in our work, we found higher serum level of MB in diabetics rats.

Focusing on skeletal muscle cell injury and ameliorating impact of ubiquinone in athletes, a study shows lower level of MB in the ubiquinone-treated group than in the subjects with placebo prescription. They presented that 300 mg/day of ubiquinone supplementation over two weeks raised ubiquinone levels in the blood and decreased MB concentrations and creatine kinase activity in 18 elite athletes included in a randomized clinical trial compared to the non-treated group [84]. The lowering impact of ubiquinone on the level of myopathy marker (MB) is attributed to its potential to attenuate oxidative stress and pro-inflammatory signaling and reduce lipid peroxidation [85].

As shown in the proposed diagram, hyperglycemia can stimulate the adipocytes to release proinflammatory cytokines, which cause inflammation and, finally, muscle cell apoptosis. Ubiquinone, a powerful antioxidant, would protect muscle tissues in humans and animals with hyperglycemia. First, it has an inhibitory impact on free radical production since it prevents indirect cytotoxicity by inhibiting NF- κ B activation, which stimulates the production of IL-6, and other proinflammatory proteins. Second, by neutralizing free radicals, ubiquinone prevents direct cytotoxicity, which occurs when free radicals react with vital cellular components like DNA and proteins and damage them. Third, ubiquinone protects pancreatic β -cells and has hypoglycemic properties since it lowers the influence of hyperglycemia on raising IL-6; consequently, ubiquinone reduces inflammation and apoptosis in cardiac and skeletal muscles tissue as illustrated in **Figure 4**.

Effect of ubiquinone on blood component parameters

Several studies have documented the effects of other antioxidants on hematological parameters, but none have studied ubiquinone's effects. WBC counts decreased in the diabetic control group according to our documented data (**Table 3** and **Figure 3**). The same results were obtained in diabetes subjects after alloxan-induced hyperglycemia was induced in rats [86]. In contrast to our findings, other researchers [87] found a significant rise in leukocyte counts in diabetic animals and they attributed that to STZ 's damaging effects. In our experiment, ubiquinone decreased WBC counts. In other studies, involving p-coumaric acid as an antioxidant, the same result was observed [86].

Like other studies' findings [88, 89], our results showed that rats with diabetes had slightly lower RBC count. The non-enzymatic glycosylation of RBC membrane proteins would increase under hyperglycemic conditions. As a result of oxidation of glycosylated membrane proteins and hyperglycemia in diabetes mellitus, lipid peroxides are produced, resulting in hemolysis of RBCs. Several pathological consequences of free radical-induced membrane lipid peroxidation include decreased cellular deformability, increased membrane rigidity, reduced deformability of erythrocyte membranes, and increased RBC death, leading to diabetic anemia. [90-93]. In our experiment, the administration of ubiquinone did not improve erythrocyte counts and survival. RBC count was found to be raised after antioxidant treatment by other researchers (Citrullus colocynthis pulp) therapy [88].

According to our data, hyperglycemia had a lowering effect on platelet count. A study shows that in children with proven T1DM, the platelet count is significantly reduced [94]. Diabetes patients are at higher risk of oxidative stress. Oxidative stress generates reactive oxygen species and promotes uncontrolled lipid peroxidation. The vast majority of cell membranes are composed of lipids, so unregulated lipid peroxidation could damage cells. One of the mechanisms underlying platelet dysfunction may involve glycosylation stress resulting in a rise in lipid peroxide levels. A rise of this magnitude could trigger the discharge of arachidonic acid from phospholipids and consequently enhance platelet activation. These findings indicate that increased lipid peroxidation and platelet activation are initial circumstances in T1DM that may be associated with an acute response to inflammation [95]. With respect of our data, Supplementation of diabetic rats' food with ubiquinone slightly increased PLT count.

6. Conclusions

In conclusion, the administration of ubiquinone had an attenuating impact on glucose levels in STZ-induced diabetic rats. In diabetic rats, ubiquinone supplementation modulated the level of markers correlated with oxidative stress (MDA), inflammation (IL-6), and diabetic myopathy (MB). Additionally, ubiquinone supplementation increases NO levels which could be reduced in diabetic endotheliopathy (NO). In this study, ubiquinone was found to be capable of alleviating diabetic complications as a therapeutic agent.

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