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Role of N-Acetylcysteine and Coenzyme Q10 in the Amelioration of Myocardial Energy Expenditure and Oxidative Stress, Induced by Carbon Tetrachloride Intoxication in Rats

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Abstract

This study is designed to evaluate the potential impact of N-acetyl cysteine (NAC) and coenzyme Q10 (CoQ10) each alone or in combination against carbon tetrachloride (CCl_4)-induced cardiac damage in rats. Animals were treated with CCl_4 in single intraperitoneal dose of 1 mL/Kg body weight; CCl_4-intoxicated animals were pretreated with 20 mg/kg/d NAC or pretreated with 200 mg/kg/d CoQ10 or NAC and CoQ10 with the same previously mentioned doses. Carbon tetrachloride–intoxicated rats showed a significant elevation in nitric oxide and lipid peroxides and downregulation in reduced glutathione level and calcium adenosine triphosphatase. Cardiac glycolytic enzymes levels such as lactate dehydrogenase, phosphofructokinase, and hexokinase were declined coupled with a reduction in glucose content after CCl_4 treatment. Moreover, myocardial hydroxyproline level was significantly increased after CCl_4-treatment indicating accumulation of interstitial collagen. N-acetyl cysteine and/or CoQ10 effectively alleviated the disturbances in myocardial oxidative stress and antioxidant markers. These antioxidants effectively upregulated the reduction in cardiac energetic biomarkers due to CCl_4 treatment. N-acetyl cysteine and/or CoQ10 significantly decreased hydroxyproline level compared to that of CCl_4-treated rats. The current data showed that the aforementioned antioxidants have a remarkable cardioprotective effect, suggesting that they may be useful as prophylactic agents against the detrimental effects of cardiotoxins.

Keywords

calcium adenosine triphosphatase, glycolytic enzymes, hexokinase, phosphofructokinase, lactate dehydrogenase

Introduction

Oxidative stress arising from the imbalance between augmented free radical production and the inadequate antioxidant defense have been implicated by a variety of factors such as ionizing radiation or exposure to drugs and xenobiotics such as carbon tetrachloride (CCl_4).[1] Carbon tetrachloride acts as a strong hepatotoxic through the generation of reactive oxygen species (ROS).[2] It also causes a variety of pathophysiological disorders in other organs than liver including the heart.[3,4] Carbon tetrachloride toxicity results from its metabolism to trichloromethyl ($CCl_3^{\bullet}$) free radical by cytochrome P450.[5] Trichloromethyl and related reactive species participate in the initiation of oxidative stress that result in elevation in intracellular free calcium, lipid peroxidation, and alter membrane

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permeability and transport.[6] Different body tissues have antioxidant defense mechanisms to scavenge different free radicals. However, these mechanisms sometimes are altered by the increased liberation of reactive substances, resulting in oxidative stress.[4] A major mechanism for preventing damage by oxidative stress is the modulation of ROS induced by various toxic agents and tissues' antioxidants level. In view of these facts, the use of antioxidant compounds could be useful to reduce oxidative damage produced by such xenobiotic.

Several antioxidants including N-acetylcysteine, black tea extracts, vitamins, and melatonin were demonstrated to reduce CCl_4-induced toxicity.[7]

N-acetylcysteine (NAC) is derived from the sulfur-containing amino acid, cysteine. Along with glutamic acid and glycine, NAC is a precursor of glutathione (GSH), the body's most important cellular antioxidant and a direct ROS scavenger that has been successfully used in various experimental studies and clinical trials.[8] N-acetylcysteine has been used in clinical practice for several decades, where it had beneficial effects in reducing many pathological events including acetaminophen intoxication, respiratory distress syndrome, heavy metal toxicity, chemotherapy-induced toxicity, and psychiatric disorders.[9] Additionally, NAC was utilized as a therapeutic agent in many cardiovascular disorders such as doxorubicin-induced cardiotoxicity, ischemic heart diseases, and ischemia–reperfusion myocardial injury.[10]

On the other hand, coenzyme Q10 (CoQ10) has been shown to be a safe supplement in humans with minimal side effects.[11] It is an important component of the mitochondria; thus it is especially vital to many body organs that have a high demand for oxygen, including the heart.[12] A principal function of CoQ10, in its quinone form, is to act as an electron carrier, transferring electrons in the mitochondrial electron transport chain between complexes I and III and complexes II and III. In its quinol form (the reduced form of quinone), it acts as the most abundant and efficient antioxidant in the body.[13] Coenzyme Q10 appears to be involved in maintaining cellular redox potential of cardiac tissue when the heart is exposed to oxidative stress in various pathogenic disorders.[14] It is crucial for the preservation of oxidative phosphorylation and improving cellular bioenergetics in the myocardium under conditions of metabolic stress with resultant reduction in myocardial damage and improvement in poststress contractile function.[15] It enhances mitochondrial activity related to the synthesis of adenosine triphosphate.[16] In addition, it has an antioxidant, a free radical scavenging, and a vasodilator effect which may be helpful in cardiopathy[17] and in reducing low-density lipoprotein oxidation, thus preventing the progression of atherosclerosis.[15]

From all of the previous studies, we hypothesized that CCl4-induced cardiotoxicity involves excessive generation of ROS and oxidative stress. This study was designed to evaluate the effects of supplementation with antioxidants such as NAC and/or CoQ10 on energy expenditure, myocardial energy metabolism, and oxidative stress biomarkers in rats' heart after CCl_4 intoxication.

Materials and Methods

Drugs and Chemicals

N-acetylcysteine, coenzyme Q10, and other chemicals utilized in this research are of high analytical grade and were isolated from Sigma-Aldrich Chemical Co, St. Louis, Missouri.

Animals

Fifty healthy male Wistar albino rats (180-200 g) were supplied by the Experimental Animal Center, College of Pharmacy, King Saud University. Animals were maintained under conditions of temperature at $23^\circ C \pm 2^\circ C$ with $50\% \pm 20\%$ relative humidity. Animals were kept on a 12:12 light–dark cycle and provided ad libitum access to water and compositionally balanced normal rat chow following protocols of the Animal Care and Use Committee, College of Pharmacy, King Saud University.

Experimental Protocol

After 1 week of acclimation, the rats fasted overnight before treatment. Then, the animals were randomized into 5 groups, 10 rats each group, (1) Control group: normal animals not receiving any medication, but vehicles include 4 days intraperitoneal (IP) injection of normal saline followed by single dose of corn oil fifth day; (2) CCl_4-intoxicated group: animals were treated with CCl_4 mixed with corn oil (80% v/v) in single IP dose of 1 mL/Kg body weight according to the method described by Botsoglou et al.[18]; (3) NAC + CCl_4 group: CCl_4-intoxicated animals pretreated with 20 mg/kg/d NAC[19]; (4) CoQ10 + CCl_4 group: CCl_4-intoxicated animals pretreated with 200 mg/kg/d CoQ10[20]; and (5) NAC + CoQ10 + CCl_4 group: CCl_4-intoxicated animals pretreated with NAC and CoQ10 with the same previously mentioned doses. N-acetylcysteine and/or CoQ 10 were given for 4 days before CCl_4 administration. All drugs were dissolved in saline except for CCl_4 which was suspended in corn oil. Drug solutions were prepared just before the start of the experiment and were given intraperitoneally. After the experimental period, the animals fasted overnight. Then, animals were euthanized while under ether anesthesia. Their hearts were isolated and weighed after washing with ice-cold saline and homogenized. The homogenates were centrifuged at 4000 rpm/4°C for 15 minutes, and the supernatants were kept at $-80^\circ C$ for further analysis.

Measurement of Biochemical Parameters

Determination of myocardial lactate dehydrogenase and creatine phosphokinase creatine phosphokinase activities. The activity of myocardial lactate dehydrogenase (LDH) was estimated using the protocol described previously by Bergmeyer.[21] The activity of myocardial creatine phosphokinase (CPK) was assayed with the technique described previously by Rosalki.[22]

Determination of myocardial total nitrate/nitrite concentrations. Total nitrate/nitrite, an indirect measure for nitric oxide (NO) synthesis, was measured according to the method described by Moshage et al [23] using Griess reagent (sulfanilamide and N-1-naphthylethenediamine dihydrochloride) in acid medium.

Determination of lipid peroxidation in myocardial tissues. The degree of lipid peroxidation in myocardial tissues was determined by measuring thiobarbituric acid reactive substances (TBARS) in the heart homogenate.[24] The absorbance was measured spectrophotometrically at 532 nm, and the concentrations were expressed as μmol TBARS/g wet tissue.

Determination of myocardial GSH. Reduced GSH was measured using the technique described by Ellman.[25]

Determination of heart collagen concentration. Myocardial collagen content was estimated by measuring hydroxyproline content according to the method of Jamall et al.[26]

Determination of myocardial glucose level. Glucose concentration in heart tissues was determined using Diamond Diagnostic Kits.[27]

Determination of myocardial calcium adenosine triphosphatase (Ca^{+2} ATPase). Myocardial Ca^{2+} ATPase was measured according to the technique of Hjerten and Pan.[28]

Determination of myocardial hexokinase and phosphofructokinase activities. The activity of myocardial hexokinase (HK) was measured by the method of Gumaa and McLean,[29] and activity of myocardial phosphofructokinase (PFK) was determined using the method described by Kemp.[30]

Statistical Analysis

Statistical analysis of the experimental data was performed using 1-way analysis of variance followed by Bonferroni test for multiple comparisons. Results were expressed as mean (Mean $\pm$ standard deviation). Differences were considered significant at P value of $\leq .05$.

Result

The current investigation revealed that CCl_4 intoxication significantly decreases myocardial CPK and LDH activities after CCl_4 intoxication (Figure 1). Moreover, significant elevation was noted in the levels of cardiac NO and lipid peroxides (TBARS) compared to normal control values ($P < .001$; Figure 2). This increase in oxidative stress biomarkers was confirmed by the depletion in myocardial nonenzymatic antioxidant GSH content (Figure 2). In addition, a concomitant decrease in Ca^{+2}-ATPase activity was determined in CCl_4-treated group (Table 1). Additionally, a significant reduction in cardiac contents of glucose (Figure 2), together with depletion in myocardial glycolytic enzyme activities (HK and PFK), were observed in CCl_4-treated rats revealing alteration in energy metabolism in intoxicated hearts (Table 1). Such effect could be due to the inhibitory action of CCl_4 on myocardial glucose uptake and glycolytic pathway in rats. Also, a significant rise in the myocardial level of hydroxyproline was observed in CCl_4-treated animals at $P < .001$ (Table 1), indicating interstitial collagen accumulation and a very severe myocardial damage.

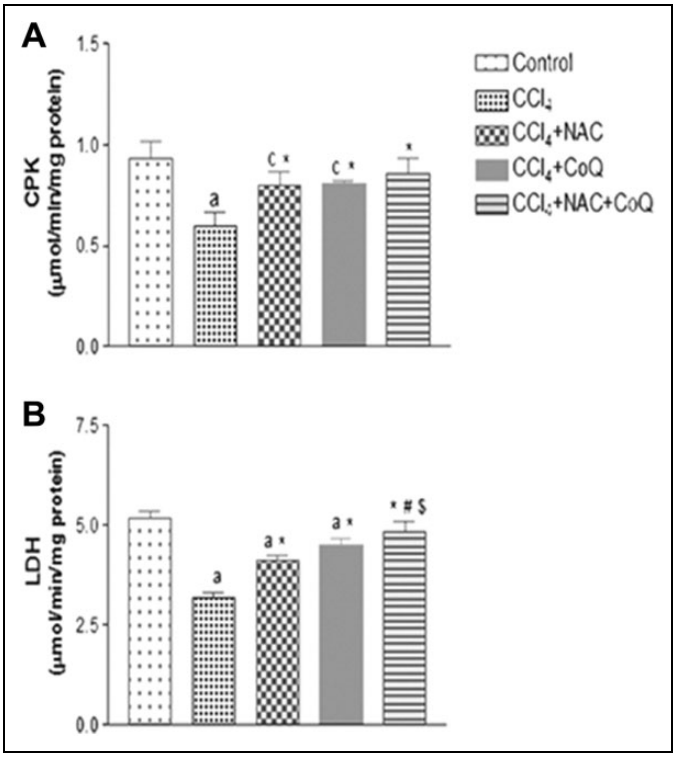

Figure 1. Effect of N-acetylcysteine and/or coenzyme Q10 pretreatment on creatine phosphokinasein (CPK) and lactate dehydrogenase (LDH) levels in myocardial tissue of CCl_4-intoxicated rats. Values are means of (6) data points (standard deviation). $^{a}P < .001$, $^{c}P < .05$ compared to control; $^{*}P < .001$ compared to CCl_4-intoxicated group; $^{\#}P < .001$ compared to NAC + CCl_4-treated group; and $^{\$}P < .05$ compared to CoQ10 + CCl_4-treated group respectively, Using analysis of variance (ANOVA) followed by Bonferroni as a post-ANOVA test

Treatments with NAC and/or CoQ10 effectively alleviated the changes in oxidative stress and antioxidant biomarkers (Figure 2). Also, they efficiently correct the decrease occurred in the myocardial energetic biomarkers of CCl_4-treated rats (Table 1). Additionally, the utilized agents restored the activity of cardiotoxicity enzymatic indices (Figure 1). In addition, NAC and/or CoQ10 significantly decreased myocardial hydroxyproline level in comparison to that of CCl_4-intoxicated rats, indicating their inhibitory effect on interstitial collagen accumulation (Table 1). Interestingly, the combination protocol synergistically prevented the alteration in most of the assessed biomarkers in response to CCl_4 intoxication and restored the level of these markers to nearly a normal value.

Discussion

Carbon tetrachloride induces the liberation of the ROS species that which damage mitochondria and cell membranes of the heart muscle cells.

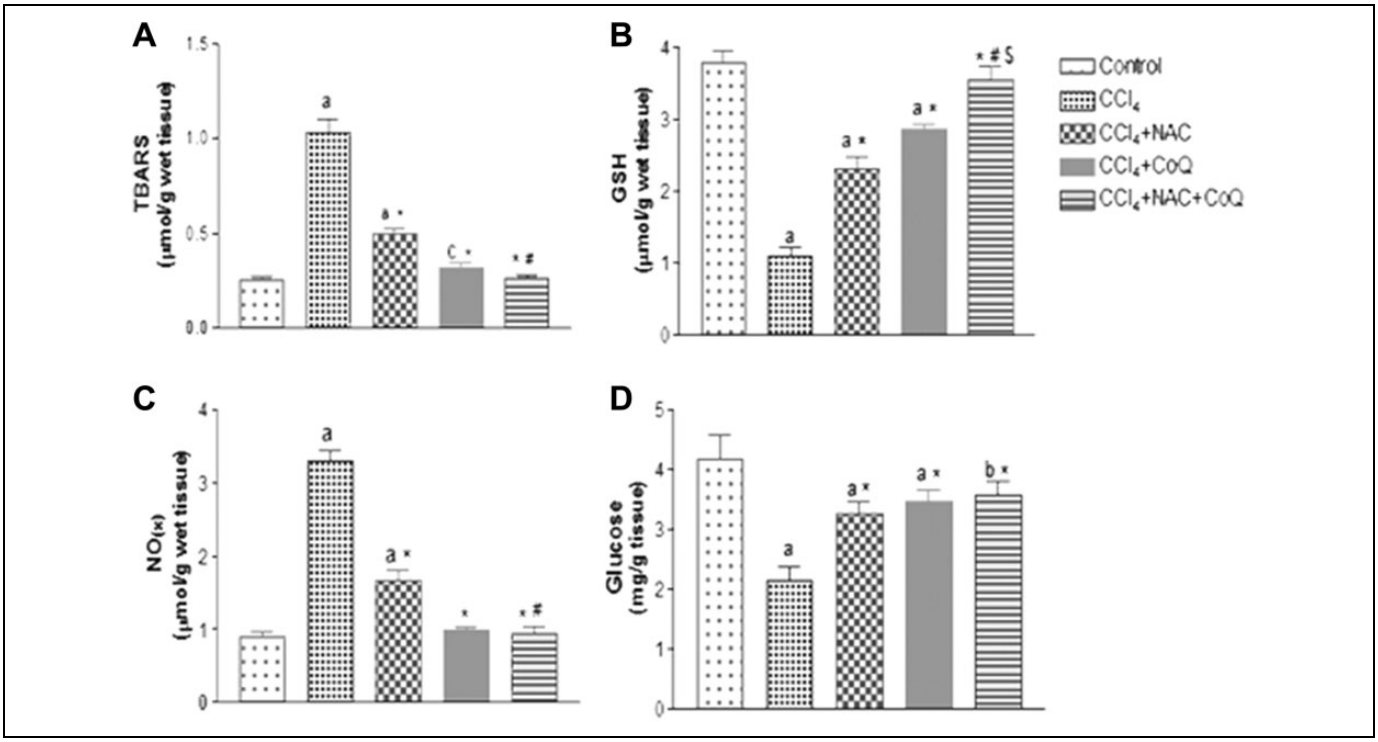

Figure 2. Effect of pretreatment with N-acetylcysteine and/or coenzyme Q10 on myocardial oxidative stress biomarkers in CCl_4-intoxicated rats. Values are means of (6) data points (standard deviation); $^{a}P < .001$, $^{b}P < .05$, $^{c}P < .05$ compared to control; $^{*}P < .001$ compared to CCl_4-intoxicated group; $^{\#}P < .001$ compared to NAC + CCl_4-treated group; and $^{\$}P < .001$ Compared to CoQ10 + CCl_4-treated group respectively using analysis of variance (ANOVA) followed by Bonferroni as a post-ANOVA test

Table 1. Effects of N-acetylcysteine and/or Coenzyme CQ10 Pretreatment on Energy Metabolism Biomarkers and Collagen Concentration in Heart Tissue of CCL_4-Intoxicated Rats.[a]

Groups	PFK, µmol/min/mg protein	HK, µmol/min/mg protein	Ca^{+2}ATPase, µmol Pi/mg protein	Hydroxypoline, mg/g tissue
Control	1.51 (0.12)	0.24 (0.013)	13.86 (1.84)	1.05 (0.10)
CCl_4	0.66 (0.09)[a]	0.09 (0.032)[a]	4.89 (0.46)[a]	4.65 (0.125)[a]
NAC + CCl_4	1.23 (0.11)[b]*	0.15 (0.02)[a]**	11.27 (1.22)[b]*	1.62 (0.08)[a]*
CoQ_{10} + CCl_4	1.43 (0.1)*	0.18 (0.02)[b]*	12.39 (0.60)*	1.44 (0.1)[a]*
CoQ_{10} + NAC + CCl_4	1.71 (0.11)[c]*#$$	0.21 (0.025)*#	12.59 (0.45)*	1.09 (0.16)*#$

Abbreviations: ANOVA, analysis of variance; ATPase, adinosine triphosphate hydrolizing enzyme; CCl4, carbon tetrachloride, CoQ10, Coenzyme CQ10; HK, hexokinase; NAC, N-acetyl cysteine; PFK, phosphofructokinase.
Values are means of (6) data points ± S.D.
[a]$P<0.001$.
[b]$P<0.05$.
[c]$P<0.05$ compared to Control.
*$P<0.001$, **$P< 0.01$ compared to CCl4 intoxicated group.
[#]$P <0.001$ compared to NAC+CCl4-treated group.
[$]$P <0.001$.
[$$]$P <0.01$ compared to CoQ10+ CCl4 treated group respectively, using ANOVA followed by Bonferroni as a post ANOVA test.

Nitric oxide exerts various influences on cardiac function and on the pathogenesis of cardiovascular diseases.[31] Nitric oxide modulates myocardium contractility via regulation of blood flow and its antiatherogenic effect.[32] High level of NO production induces several myocardial diseases.[33] The interaction between NO and other ROS such as superoxide radical ($O_2^{\bullet-}$) enhances its direct toxicity by forming secondary toxic oxidizing species, such as peroxynitrite (ONOO) which is able to oxidize many cellular targets such as lipid causing lipid peroxidation, a process leading to membrane damage.[34] Enhanced lipid peroxidation is associated with depletion of antioxidants, as GSH in myocardial tissues.[1] As the decline in GSH levels is an increase in oxidative stress, cellular damage can be noticed. Thus, the reduced levels of myocardial GSH observed in CCl_4-treated rats in this study might reflect oxidative damage. Data of the present study are in line with

all of the previous studies, where the significant increase in myocardial NO and MDA level was accompanied by a consequent reduction in the myocardial content of GSH and glucose, which finally end with oxidative myocardial damage. This increase in myocardial NO represent an additional explanation for myocardial lipid peroxidation and the increased myocardial MDA content and supporting the possibility of increased ROS production induced by CCl_4 treatment.

Prophylactic administration of NAC or CoQ10 either alone or in their combined form alleviated the alterations in the antioxidant biomarkers. This may be due to their ability to reduce NO level, suppress the level of lipid peroxidation of the myocardial membrane, and to increase GSH level. Our results are in accordance with previous studies which revealed that NAC was shown to reduce the level of NO, lipid peroxidation, and lung injury in rats treated with carrageenan.[35] Additionally, NAC is a precursor of GSH,[36] which is an important antioxidant involved in numerous physiological and pathological processes in the body including the heart. NAC may have the additional protective ability through upregulating antioxidant systems such as superoxide dismutase[37] or enhancing the catalytic activity of GSH peroxidase.[34] These effects aid in the reduction of lipid peroxidation and myocardial tissue injury. On the other hand, CoQ10 was found to suppress oxidative stress biomarkers as well as the inflammatory markers during cardiomyopathies.[38] Özdoğan et al[39] also reported that CoQ10 significantly decreased NO, MDA levels, and increased GSH content in an experimental model of metabolic syndrome. Moreover, CoQ_{10} afforded moderate protection against isoproterenol-induced cardiotoxicity and cardiac hypertrophy by preserving endogenous antioxidants (GSH) and reducing lipid peroxidation in rat heart.[40] Creatine kinase and LDH are crucial enzymes for high energy consuming tissues like the heart. These enzymes play an important role in energy metabolism.[41] In oxidative myocardial damage, cellular cardiac enzymes are released in correlation with variations in plasma membrane integrity and/or permeability.[42] In the current study, there is a significant decrease in myocardial l enzymes (CPK and LDH) after CCl_4 treatment. These effects could be secondary events following CCl_4-induced lipid peroxidation of cardiac membranes, with a consequent increase in enzyme leakage from cardiac myocytes. Our data also revealed that pretreatment with NAC, CoQ10, or their combination reversed the decrease in myocardial CPK and LDH induced by CCl_4 intoxication. This protective effect may be attributed to the antioxidant properties of these agents through which they preserved the integrity of myocardial cell membrane from oxidative injury and, consequently, decreased the cellular release of cardiac enzymes. In this context, additionally, Ghul et al[40] demonstrated that the pretreatment of isoproterenol-intoxicated rats with CoQ10 produced an inhibitory effect upon serum myocardial marker enzymes (CPK-MB, LDH, and aspartate aminotransferase activities) levels.

Intoxication with CCl_4 in the current study revealed a remarkable decline in the activity of Ca-ATPase compared to normal animals. This result was in line with other previous investigations in which CCl_4 intoxication inhibited Ca-ATPase activity in liver tissue.[43] The fall in the activity of Ca-ATPase is due to the ability of CCl_4 to increase myocardial lipid peroxidation, which is coupled with inactivation of membrane-bound enzymes.[44] Ca-ATPases of cardiac cells maintain normal calcium level. The inhibition of Ca-ATPase causes an alteration in cellular calcium homeostasis with an elevation in Ca^{2+} ions permeability.[45] The cytosolic calcium accumulation in the heart represents apoptosis and myocardial damage.[46] These data are in line with myocardial cell injury that was evidenced in the present study. Treatment with NAC significantly increased the activity of Ca-ATPase compared to its level in CCl_4-treated rats. In line with our data, Kamboj et al[47] demonstrated that NAC offers protection against carbofuran neurotoxic effects by restoring the activity of Na^+-K^+-ATPase and Ca^{2+}-ATPase. However, CoQ10 treatment either alone or with NAC proved to be more effective in the attenuation of the activity of this membrane-bound enzyme. The effect of the 2 aforementioned antioxidants is due to their inhibitory effect in membrane lipid peroxidation which has a vital role in the inactivation of membrane-bound enzymes.[48]

Excess ROS reacts with cellular proteins, fats, and carbohydrates, which leads to postoxidative molecular modification and triggers inflammatory processes, ultimately impairing efficient energy production and homeostatic cellular maintenance.[49] The current study revealed that CCl_4 intoxication significantly depleted glucose content in the heart with a significant decline in HK, PFK, and LDH activities. Nishitani et al[50] reported that CCl_4 downregulates glucose transporter and hence impairs glucose metabolism.[4] Pretreatment with either antioxidants or their combination increase the myocardial glucose level and its glycolytic enzymes and energetic biomarkers in relation to CCl_4-intoxicated animals. The combination group recorded the best result in restoring these bioenergetic markers to its normal level. Our results are in accordance with previous studies, in which CoQ10 was shown to improve glucose uptake by cardiomyocytes and enhance its metabolism. In line with this, many previous studies have shown that CoQ10 plays a vital role in the enhancement of cardiac bioenergetics.[12] Myocardial damage is a result of interstitial collagen accumulation, especially hydroxyproline.[51] The present work showed that CCl_4 exhibits significant increase in hydroxyproline level in heart tissues of CCl_4-intoxicated rats. The fibrogenic effect of CCl_4 on the heart may be attributed to tissue injury and hence the expression of the proinflammatory fibrogenic cytokines such as tumor necrosis factor α (TNF-α), interleukin-1β, and interleukin 6 which play a principle role in collagen accumulation.[52] When CCl_4-intoxicated rats were pretreated with either NAC or COQ10, the CCl_4-induced myocardial damage was attenuated by their significant reducing effect on cardiac hydroxyproline level. The hydroxyproline level was normalized leading to inhibition of interstitial collagen accumulation after treatment with a combination of the 2 drugs, which may indicate their potential antifibrotic effect. This was confirmed in a clinical study, which revealed that patients with heart failure showed a marked reduction in Interleukin 6 and TNF-α in CoQ10 to treated group.[53] Meanwhile,

NAC has been shown to abrogate fibrosis induced by transforming growth factor-β1 and to suppress the expression of the procollagen genes even in the absence of transforming growth factor-β1.[54]

Conclusion

It can be suggested that the alteration in myocardial levels of oxidative stress, antioxidants, and bioenergetics biomarkers, as well as the accumulation of interstitial collagen in cardiac tissue induced by CCl_4-intoxication, can be improved by administration of CoQ10 and/or NAC. The beneficial effects of these agents can be attributed to their antioxidant activity and suggest that they may be utilized as prophylactic agents against cardiotoxic agents.

Authors' Note

Naglaa F. El-Orabi and Laila M. Fadda contributed to study conception and design. Nayira A. Abd Elbaky, Naglaa F. El-Orabi, Laila M. Fadda, Omar H. Abd-Elkader, and Hanaa M. Ali contributed to acquisition of data. Nayira A. Abd Elbaky, Naglaa F. El-Orabi, Laila M. Fadda, and Omar H. Abd-Elkader contributed to analysis and interpretation of data. Naglaa F. El-Orabi and Laila M. Fadda helped in drafting the manuscript. Naglaa F. El-Orabi, Laila M. Fadda, and Hanaa M. Ali contributed in critical revision.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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