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Original Article

Hepatoprotective Effect of Coenzyme Q10 in Rats with Diclofenac Toxicity

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Abstract

The liver and kidney are the most important organs in the body, and they both act as target structures for drug-induced injury as a consequence of their functions in metabolisms, detoxifications, storage, elimination of medications, and their metabolites. The present study aimed to examine the role of the natural and free radical scavenger "CoQ10" against diclofenac-induced hepatic and renal tissue injury. In total, 36 adult Wistar rats were randomly divided into three equal groups (n=12). The animals in the control group did not receive any medication or treatments, and the second group included animals that received intramuscular (IM) injection of Diclofenac (DF) (at a dose of 10 mg/kg once daily for 14 days). Moreover, the third group was given the IM injection of DF (at a dose of 10 mg/kg once daily for 14 days) +CoQ10. After 14 days, DF prompted signified hepatic and renal injury indicated by elevated biochemical parameters, such as total serum bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen, creatinine, and uric acid, compared to the control and the third group. However, the group that received Diclofenac+CoQ10 had significantly lower hepatic and renal dysfunctions, compared to the second treated group. DF toxic effects could be the consequences of mitochondrial dysfunction and free radical effects. Remarkably, therapeutic supplementation of CoQ10 diminished the DF-induced toxic oxidative injury and apoptotic cell death. The protective effects of CoQ10 were attributed to its antioxidants and free radical scavenger activity.

Keywords: Antioxidant, CoQ10, Diclofenac, Hepatotoxicity, Nephrotoxicity

1. Introduction

The liver and kidney are the most important organs in the body, and they both act as target structures for the drug-induced injury as a consequence of their functions in metabolisms, detoxifications, storage, elimination of medications, and their metabolites (1). The toxicity of these organs "based upon individual drug mechanisms" has a wide series of effects arising from cells damages and causing acute or chronic functional alterations to the corresponding tissues (2). Drug-induced liver and kidney injury is a recurrent adverse episode leading to morbidity and high healthcare utilization; moreover, its frequency in the general population ranges from 2.4/100,000 to 19/100,000 (1).

Diclofenac (DF) is a member of the nonsteroidal anti-inflammatory drugs (NSAIDs) and is generally taken for pain-relieving in addition to inflammation and fever treatment (3). Despite its healing benefits, DF is reported as one of the agents which trigger liver and kidney cell injury. The toxic effects of DF and its metabolites (4′, 5-hydroxydiclofenac) have been linked to mitochondrial injury and the disruption of immune-mediated protective systems according to several investigations and research (4). Evidence also showed

that DF causing cell necrosis is accompanied by a formation of the reactive oxygen species (ROS), as well as the inhibition of the enzymatic and non-enzymatic antioxidants activity in the kidney and liver cells. For that reason, any therapeutic reagent with antioxidant activity may decrease the cellular damages caused by the ROS and can be improved to be a medicated attitude to arise the DF toxic effects (5).

Coenzyme Q10 (CoQ10) is an important fat-soluble vitamin-like molecule naturally occurring in every cellular membrane. It is a normal part of the diet but most of the CoQ10 body's daily needing is synthesized in the body, especially in the liver because of its physical size and high metabolic capability (6). Researchers have discovered several biological activities, including free radical-scavenger properties, hepatic injury lowering effects, and reduced endothelial cell impairments. It also has an anti-oxidant, anti-fibrosis, and anti-inflammatory properties (7). Several studies were carried out based on the promise that elevated CoQ10 levels in disorders associated with low systemic CoQ10, such as neurodegenerative disease, liver and kidney cellular injury, diabetes mellitus, cancers, and heart failure, might improve the functions of the processes that need CoQ10 and deteriorate disease intensity (6). CoQ10 structure and its redox reactions are displayed in figure 1. Hepatotoxicity and nephrotoxicity are among the main reasons of morbidity and mortality around the world and the drug is one of the main causes of this toxicity (8). Moreover, the hepatotoxicity and nephrotoxicity of drugs are the major reasons for drug withdrawal from the pharmaceutical markets and the disturbance of the improvement of novel molecules (9). Therefore, based on these principles and the fact that there have been relatively few clinical investigations involving the CoQ10 with liver and kidney diseases, this study aimed to evaluate the protecting influence of CoQ10 against the hepatic and renal injury induced by DF and introduce the acquired data to the clinical applications.

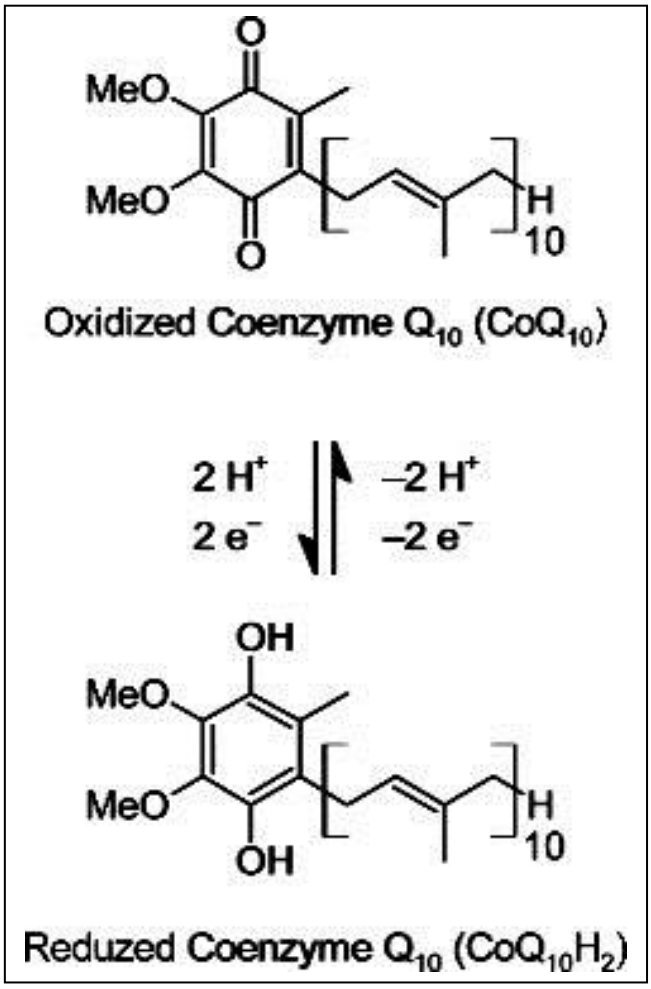

Figure 1. CoQ10 structure and redox reaction (6)

2. Materials and Methods

2.1. Experimental Design

In total, 36 adult Wistar rats of six weeks old with approximately 250-300 g were obtained from the animal house of the Pharmacology Department, University of Kerbala, Kerbala, Iraq. All the rats were housed in a normal light\dark cycle (12:12 h) and fed with a standard laboratory diet with an *add libitum* access to water to certify standard rat growth and performance. The rats were randomly divided into three groups (n=12). The animals in the control group did not receive any medication or treatments, and the second group included animals that received DF. Moreover, the third group was given DF+ CoQ10 for two weeks (Table 1).

Table 1. Experimental design

Criteria	NO.	Name	Number of rats	Properties
Control group	1st	Control	12	Received no medications
Treatment groups	2nd	Diclofenac group	12	Received diclofenac only
	3rd	Diclofenac+CoQ10 group	12	Received diclofenac and CoQ10

2.2. Chemicals

2.2.1. Diclofenac

Each rat in the 2nd and 3rd groups received DF at a dose of 10 mg/kg once daily for 14 days by intramuscular injection (IM) using an insulin syringe (10). DF ampoules (Mepha Pharma AG, Switzerland) were used for the preparation of each dose by direct mixing with normal saline.

2.2.2. CoQ10

The rats in the third group received 1 mL of CoQ10 daily by oral gavage during the 14 days of the experiment (11). CoQ10 solution (100 mg\125 ml) (America Mediac and Science Co., USA) was used in the dose preparation.

2.3. Biochemical Assays

The research continued for 14 days, and 24 h after the last dose, the rats were anesthetized with the aid of chloroform. Afterward, the blood was drawn for the biochemical parameter analysis. Thereafter, the animals were sacrificed by cervical dislocation. Total serum bilirubin (TSB) (mg/dL), alkaline phosphatase (ALP), aspartate-aminotransferase (AST) (U/L), and alanine-aminotransferase (ALT) (U/L) have been measured as biomarkers for liver function, whereas blood urea (BU) (mg/dL), creatinine, and uric acid (UA) were measured as biomarkers for kidney functions. All the biochemical analyses were measured in the blood samples using a Dri-Chem-NX500 auto-analyzer (Fujifilm Corporations, Japan).

2.4. Statistical Analysis

The data were statistically analyzed by one-way analysis of variance, followed by the least significant difference test. The level of significance was regarded as $P<0.05$.

3. Results

The present study assessed if CoQ10 could ameliorate the DF-induced hepatic and renal injury. The baseline values for the analyzed biomarkers are presented in tables 2 and 3. ALT, AST, ALP, TSB, BU, creatinine, and UA levels tended to show normal amounts in the control group. In addition, these parameters showed upper levels in the 2nd group, compared to the 1st and 3rd groups. The responses to the CoQ10 cure of each measured marker were significant (P<0.05) (Tables 1 and 2). The DF-exposed group showed hepatic and renal toxicity, as directed by raised values of ALT, AST, ALP, creatinine, and urea serum biomarkers in a significant manner. In addition, a reduction was observed in the albumin and total protein levels, compared to the control rats. On the other hand, there was an improvement in the hepatic and renal parameters when DF was co-administrated with CoQ10. Moreover, the kidney biomarkers were measured and expressed in mean and compared with the control group results as shown in table 3 and figure 2.

Table 2. Effects of CoQ10 on total serum bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase

Parameters / Treatments	TSB	ALP	ALT	AST
Control	0.15±0.034^{a}	222±9.76^{a}	45.6±3.42^{a}	149.8±23.9^{a}
Diclofenac acid	0.28±0.030^{b}	287.8±35.08^{b}	65.83±3.17^{b}	378±47.5^{b}
Diclofenac acid+Q10	0.23±0.021^{a}	269.5±6.33^{ab}	59.1±7.76^{ab}	341±16.6^{b}

Values were expressed as mean±SEM, and different small letters show a significant difference ($P\leq0.05$)

Table 3. Effects of CoQ10 on creatinine, uric acid, and blood urea in the rat model of kidney injury caused by Diclofenac

Parameters / Treatments	Creatinine	UA	BU
Control	0.683±0.08[a]	1.03±0.08[a]	29.71±2.18[a]
Diclofenac acid	0.8±0.01[a]	1.57±0.19[b]	38.38±1.49[b]
Diclofenac acid+Q10	0.7±0.025[a]	1.04±0.12[a]	34.58±1.11[ab]

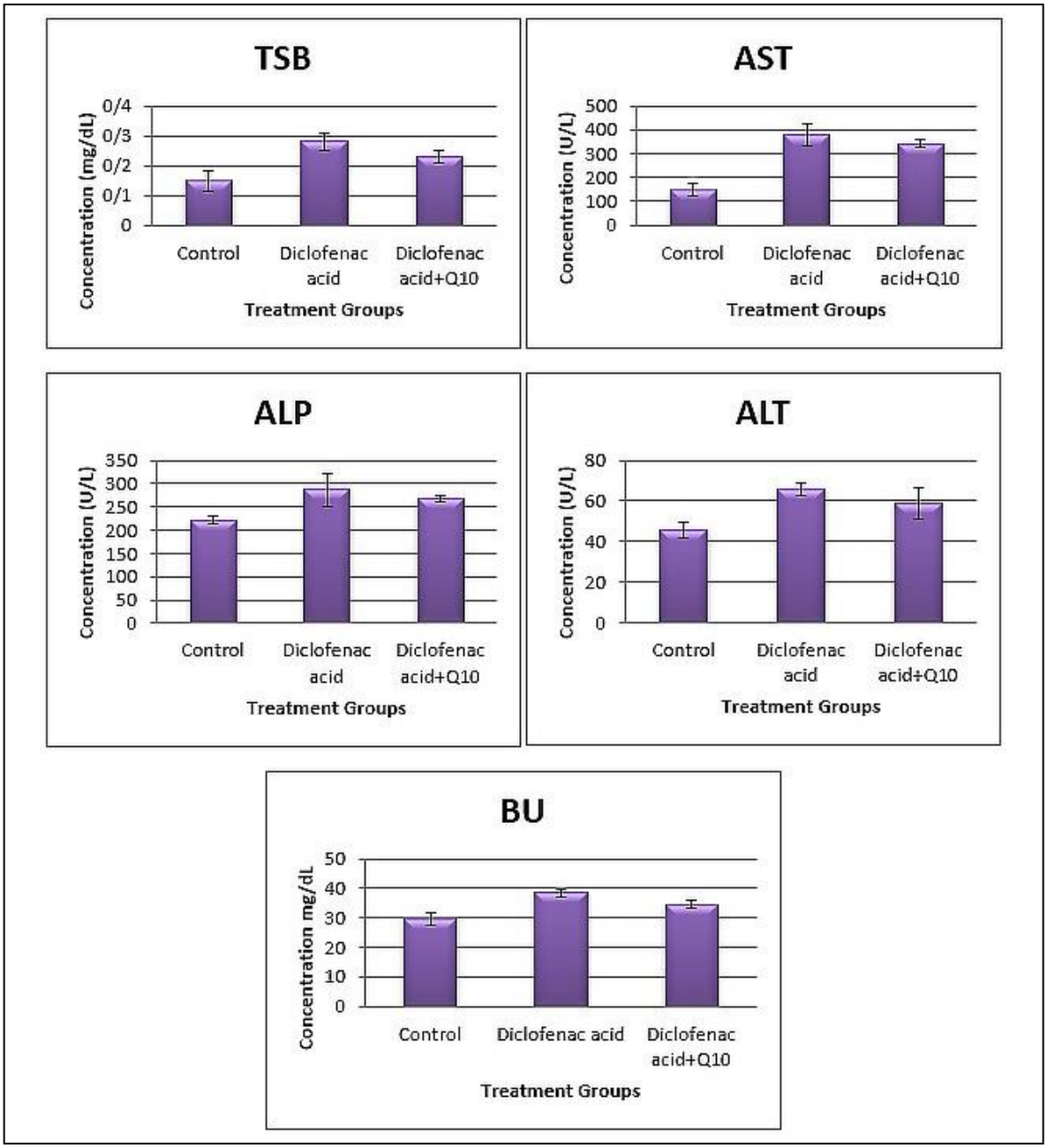

Figure 2. Liver biomarkers. A: TSB, B: ALP, C: AST, D: ALT

4. Discussion

Biomarkers are useful diagnostic criteria in cases of liver and kidney dysfunction induced by the drug. In such situations of organ injury, biomarkers could be used to check the cell damage, impairment severity, and disorder prognosis (12). NSAIDs have several therapeutic effects and are mainly used for their pain-relieving, anti-fever, and anti-inflammation actions. They are mostly obtainable as over-the-counter preparations without considering their contraindicated cases and toxic effects (13). DF treatment is reported to be responsible for several pathological cases, especially within the long-term administration (14). Peptic ulcers, gastrointestinal hemorrhage, liver cells necrosis, and renal failure have been notified to be of the major DF toxic effects. Consequently, great worry and efforts should be paid against the toxicity of DF, and any agents and mechanisms that may reduce these pathological effects should be examined (15).

A significant amount of research has proposed that oxidative stress is responsible for DF toxicity and damaging effects. ROS (superoxide anions, $O2^{--}$, hydrogen- peroxides, and hydroxyl radicals) and the reactive nitrogen species (nitric oxide, NO, peroxynitrite, and $ONOO^{-}$) usually initiate the oxidative stress process as the consequences of endogenous antioxidant molecule depletion (2). As a result, tissue damages are triggered through a number of reactions, including the stimulation of fat peroxidation, mitochondrial disturbance, nucleic acid destruction, peptide nitration, and apoptosis (16). CoQ10 acts mainly to transfer the electrons in the respiratory chain in the living organisms' mitochondrial membrane, which is a necessary step for ATP generation via oxidative phosphorylation (20). It is also revealed as a redox-active and antioxidant lipoprotein compound of the phospholipid layers of the tissue cell membranes (17, 18).

The recorded data from the treated animals in the 3rd group showed that CoQ10 supplementation lessened plasma concentrations of the liver (Table 2) and kidney biomarkers (Table 3). In support of this result, a number of research on different animal models had established the effect of CoQ10 to moderate or stop the progression of hepatocytes cirrhosis after exposure to different types of toxic reagents. A study conducted by Chen, Tang (19) revealed the CoQ10 protection effects on the rat's liver intoxication derived by acetaminophen, mainly due to the antioxidants, anti-inflammatory, and anti-apoptosis properties of CoQ10. Another study demonstrated a significant decline in the liver parameters in mice receiving CoQ10, compared to the sepsis-induced liver injury group (20). In Saudi Arabia, a study revealed that CoQ10 might initiate the antioxidant mechanism and prevented the inflammatory and apoptosis events in response to renal injury caused by acetate in the rat model (21).

The CoQ10 capability as an antioxidant, anti-fibrosis, and anti-inflammatory reagent against DF prompted that hepatic and renal injury could be indicated by the improvement of the liver and kidney functions that were signified after the CoQ10 administration. The mechanisms involved in the organ healing induced by the CoQ10 are related to the improvement in the cell antioxidant capacity, ROS level lowering, and the production of pro-inflammatory cytokines (22).

In addition, the meta-analyses of research have confirmed that CoQ10 administration may significantly decrease the inflammatory mediators, such as the C-reactive proteins, tumors necrosis factor-alpha, and interleukins 6 levels in a significant manner (23). It is established by the ongoing data that a high dose of DF sodium had several negative consequences on the hematological, biochemical, oxidative state, and histological parameters of the liver and kidney. This damaging impact is due to the drug-induced oxidative stress in these two vital organs.

5. Conclusion

In conclusion, the findings of the current study demonstrated that CoQ10 had a definite prophylactic influence against liver and kidney cell toxicity induced

by diverse agents, such as the NSAID. However, more studies are necessary to confirm the efficacy, therapeutic effects, side effects, and the exact dose in human beings. The proofing of the CoQ10 as a medication line for liver and kidney toxicity will have a huge impact on the patients waiting to receive liver and kidney transplantation by slowing the disease's progression, thereby saving lives and giving them a higher chance of survival.

Authors' Contribution

Study concept and design: M. A. Z.
Acquisition of data: M. A. Z.
Analysis and interpretation of data: Z. A. A.
Drafting of the manuscript: N. D. A.
Critical revision of the manuscript for important intellectual content: Z. A. A.
Statistical analysis: N. D. A.
Administrative, technical, and material support: M. A. Z.

Ethics

The current study was conducted during January 2021 in the Pharmacy College University of Kerbala, Kerbala, Iraq, and followed all the instructions of the Animal Ethics Committee.

Conflict of Interest

The authors declare that they have no conflict of interest.

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