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# COENZYME Q10 AMELORATES CISPLATIN-INDUCED NEPHROTOXICITY IN RATS

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## Abstract

Cisplatin, a platinum based antineoplastic drug, is gold therapy for the treatment of solid tumors, but its clinical uses are limited by dose-dependent nephrotoxicity. drugs with antioxidants, anti-inflammatory and/or antiapoptotic activity may thus represent potential therapeutic options to prevent cisplatin-associated nephrotoxicity. Among these, Coenzyme Q10 has multiple pharmacological properties including antioxidants, anti-inflammatory and antiapoptotic effects.

The current study aimed to investigate whether Coenzyme Q10 could ameliorate nephrotoxicity induced by cisplatin or not.

Twenty-four adult Wistar albino rats were randomly divided into three groups (8 animal in each group). The first group (control group) receives olive oil intraperitoneally; the second group (cisplatin treated group) receives a single intraperitoneal injection of Cisplatin and the third group (coenzyme Q10 + cisplatin treated group) receives coenzyme Q10 and a cisplatin dose equivalent to that of the cisplatin only group.

Cisplatin-induced nephrotoxicity was indicated by significant increases in both serum urea nitrogen and renal tissue interleukin-18 (a marker of inflammation). coenzyme Q10 significantly attenuated, but not completely reversed cisplatin-induced nephrotoxicity through lowering both serum urea nitrogen and renal concentration of interleukin-18 relative to cisplatin group.

CoQ10 has a protective effect against Cis-induced nephrotoxicity that was proven by biochemical analysis.

**Keywords**: *Cisplatin, Renal toxicity, CoQ10, IL-18.*

## Introduction

Cisplatin (Cis) is a powerful anticancer agent employed in the treatment of malignant tumors (1). The highly incidence of dose-related nephrotoxicity can restrict its clinical uses (2). Indeed, different complex events are implicated in the mechanism of Cis-induced nephrotoxicity including inflammation, oxidative stress and cell apoptosis (3). The free radicals releasing, production of reactive oxygen species (ROS), antioxidant enzymes inhibition and mitochondrial oxidative harm provoking have been shown to mediate the Cis-induced toxicity (4). The consequence of ROS generated is the activation of a transcriptional factor (NF-κB). NF-κB up-regulated the expression of pro-inflammatory cytokines like TNF-α IL-18 and IL-6 (5). Other studies have demonstrated that Cis promotes the intrinsic apoptotic pathway through activation of the pro-apoptotic gene Bax which binds to the mitochondrial membrane. The consequences of Bax activation are releasing of cytochrome-c from the mitochondria thereby activating cell death (5)(6). Moreover, Cis induces several signal transduction pathways, such as those involving the p53 protein, that ends in the activation of renal tubular cell apoptosis (3).

In clinical settings, nephrotoxicity of Cis displays a marked challenge in the ward of its proven chemotherapeutic effectiveness, which therefore demands confirming more rigorous approaches to protect the kidney while not reducing its tumor-cidal effects. Recently, it has been demonstrated that nephrotoxic effects of Cis were attenuated by regular Coenzyme Q10 (CoQ10) treatment and that effects were mediated through reductions in oxidative stress, inflammation, and apoptosis.

CoQ10 as a redox-active lipophilic ROS scavenger has found in various cellular organelles such as mitochondria, lysosomes and Golgi vesicles (7). It participates in electron transfer in the mitochondrial oxidative respiratory chain, producing adenosine triphosphate (ATP) (8). It is used as a dietary supplementation and as a co therapy in conjunction with medication in a number of conditions, including cardiovascular diseases, cancer, muscular neurodegenerative disorders, and diabetes (9). CoQ10 has antioxidant activity and prevents membrane lipid peroxidation by both direct ROS scavenging and indirect activity through cellular vitamin E and C regeneration and increment of GSH reductase and superoxide dismutase levels (8). CoQ10 is a therapeutic choice to attenuate acute nephrotoxicity through decreased the expression of inducible nitric oxide synthase (iNOS), NF-κB, caspase-3 and p53 in renal tissue (10). The CoQ10 quenches ROS production and accumulation by interfering with the production of lipid peroxyl radicals, and by lowering the expression of NADPH oxidase and NO production excess (11). The consequences of scavenging ROS are inhibition of activation of NF-κB, IL-18 with subsequent improvement in the expression of iNOS which lead to prevent the inflammation in renal tissue (12). CoQ10 by preventing the release and activation of degenerative pro- apoptotic molecules including cytochrome c and caspases 3 and 9, ameliorates the apoptosis (13).

Because this multiple pharmacological effects of CoQ10 as an antioxidant, anti-inflammatory, and anti-apoptotic effects, this will be encouraging to conduct the present study in order to assess the defensive effect of CoQ10 in rats exposed to Cis-induced nephrotoxicity.

## Methods

### Animals

A total of 24 adult Wistar albino rats, weighing 200-250 g, were obtained from Faculty of Science, University Of Kufa. Sample size was used according to the previous study (14)(15). The animals were placed in the animal house facility of the Faculty of Pharmacy, University Of Kufa. The animals were kept in a room at a controlled temperature (20 ± 2 °C) and humidity (50%) with equal day/night cycle (12 light/12 dark). The animals were adjusted for 1 week before the study and had free access to standard laboratory chow and water ad libitum. All experimental procedures were conducted in accordance with the principles for the care and use of laboratory animals in research and approved by the High Committee for Review and Approval of Research Proposals of the Faculty of Pharmacy of the University Of Kufa. (Ref.#2047, date:16/04/2018).

**Study design**

Induction of acute nephrotoxicity was carried out on rats fed on standard chow diet and injected with a single intraperitoneal (i.p.) dose of Cis (Ebewe pharma, Australia) in a dose of 13 mg/kg body weight (16). After 1-week adaptation period, the animals were randomly separated into three groups (eight rats in each ):

1- Control group, which received i.p. injection of olive oil (2ml/kg body weight) once daily for six consecutive days.

2- Cis-treated group, which received a single i.p. injection of Cis at a dose of (13 mg/kg body weight) on the third day (16).

3- CoQ10 plus Cis group, which received Coenzyme Q10 (Swapnroop Drugs and Pharmaceuticals, India) 10 mg/kg body weight i.p. once daily for six consecutive days starting two days before Cis administration, and on the third day Cis (13 mg/kg) is administrated i.p.

During the treatment period, one rat died from both Cis and CoQ10 plus Cis groups, and therefore data from these groups were collected from 7 animal per group. No death was observed within the control group. Olive oil had been gently heated to about 49°C and coenzyme Q10 dissolved in it.(50mg of COQ10 dissolved in 5ml olive oil) (17). After 24hr from the last injection, the rats were anesthetized with 100 mg/kg of ketamine (Duopharma, Malaysia) and 10 mg/kg of xylazine (Alfasan woerden, Holland) (i.p.) (18), and the blood samples were obtained and then the animals were sacrificed by phenobarbital overdose. About 3ml of blood was obtained from each rat by cardiac puncture using a disposable syringe. Then the abdomen was opened through a midline incision and the kidneys were quickly removed. One kidney was isolated, kept at −70°C and subsequently homogenized.

**Biochemical measurement**

Each blood sample was placed in a plain tube and left for 15 - 20 minutes at room temperature and used to obtain serum via centrifugation for 10 minutes at 5000 rpm to obtain clear sera which were stored at−20◦C for subsequent measurement of serum urea nitrogen level by using Refltron instrument (Roche, USA) using colorimetric assay kits (19). One kidney was minced to small pieces and rinsed in ice-cold potassium phosphate saline (PBS) (0.01 M, pH 7.4) to remove excess blood thoroughly. Tissue pieces were weighed and then homogenized in PBS (tissue weight(g):PBS (mL) volume = 1:9) with glass homogenizer on ice. To further break the cells, the suspension was sonicated with ultrasonic cell disrupter. The homogenates were centrifuged at 5000 rpm for 5 min (by use Universal320 R) to get the supernatant (Elabscience, USA) (20). The resulting supernatant was used for determination of IL-18 by enzyme-linked immunosorbent assay (ELISA) using rat IL-18 immunoassay kit at 450 nm ± 2 nm according to the recommendations of the manufacturer (Elabscience, USA).

**Statistical Analyses:**

Statistical analysis was done by using the SPSS (statistical package for social sciences) version 24. The data were expressed as mean ± standard error mean (SEM).Independent t-test was used and $P \leq 0.05$ was considered to be statistically significant.

## Results

**The effect of Coenzyme Q10 on rat kidney function test (serum urea nitrogen concentration) after cisplatin administration**

The injection of 13mg/kg of Cis significantly increased the serum levels of urea nitrogen ($P< 0.0001$) of treated rats when compared with the control group. Pretreatment with CoQ10 significantly reduced serum urea nitrogen ($P< 0.0001$) of treated rats when compared to Cis group. However, CoQ10 administration did not completely revert rat kidney function test back to control levels ($P< 0.0001$) (Table1) and (Fig. 1a).

**The effect of Coenzyme Q10 on inflammatory markers (IL-18) in renal tissue after cisplatin administration**

the renal concentrations of IL-18 of Cis treated rats were significantly ($P<0.0001$) increased when compared with the control group. The administration of CoQ10 (10 mg/kg) intraperitoneally once daily for 6 consecutive days starting 2 days before Cis administration significantly reduced the concentrations of IL-18 in renal tissue ($P< 0.0001$) of treated rats when compared to Cis group. (Table 2) and (Fig. 1b).

## Discussion

The current study has tried to investigate the nephron-protective effect of CoQ10 in Cis-induced nephrotoxicity in the rat model. The renal protective effect of CoQ10 was manifested by a significant reduction of the levels of serum urea nitrogen and a significant decrease of the levels of IL-18 in renal tissue of Cis treated rats. This protective effect of CoQ10 was attributed to its potential antioxidant activity and thereby attenuation of inflammation in the kidney. The nephrotoxicity represent a major limiting factor in the uses of Cis (12). Present study demonstrated significant elevation in serum urea nitrogen following Cis administration in rats as compared to the control group. The elevation of serum urea nitrogen probably as consequences of renal tubular and vasculature injuries which lead to reduction of glomerular filtration rate as indicated by previous studies which showed that Cis has harmful effect on renal tubules and renal vasculature (5)(21). However, these effects of Cis reversed by CoQ10 treatment. One of the mechanism through which Cis causes nephrotoxicity is oxidative stress. Cis induces ROS production and reduction of the antioxidant defense system (23). Cis, through ROS generation, stimulates nuclear translocation of Nrf2, and activate NF-κB. NF-κB was involved in the mechanism of inflammation and apoptosis (24). Studies have demonstrated that Cis promotes the intrinsic apoptotic pathway through activation of the pro-apoptotic gene Bax which binds to the mitochondrial membrane. The consequences of Bax activation are releasing of cytochrome-c from the mitochondria thereby activating cell death (6). Cis treatment causes phosphorylation of p53 which is a tumor suppressor that leads to cell cycle arrest or apoptosis (25). Many studies indicated the anti-apoptotic effect of CoQ10 by reducing the expression of inducible nitric oxide synthase (iNOS), caspase-3, NF-κB and p53 in renal tissue (13). In the pathogenesis of Cis nephrotoxicity, inflammation has played an important role (26). Accumulating evidence supports that Cis up-regulated the expression of inflammatory cytokines and chemokines, such as interleukin (IL-6 and IL-18), interferon-c and TNF-α (22)(25). The consequence of ROS generated as a result of Cis administration and activation of MAPK is the activation of a transcriptional factor (NF-κB). NF-κB up-regulated the expression of pro-inflammatory cytokines like IL-18 (5). After nuclear translocation, Nrf2 regulates the transcription of many genes including HO-1, c-glutamylcysteine synthase and glutathione S-transferase (27). In addition, Cis activates iNOS which synthesize NO that play role in renal pathophysiology (28). In parallel, the results of the present study further supporting the inflammatory pathway accompanying with Cis-induced nephrotoxicity as proof by the significant elevation in renal level of IL-18 in Cis treated group as compared to control group. Moreover, the recent studies revealed that Cis causes intracellularly injury resulting in the release of DAMPS. As a consequence of action of DAMPs on Toll-like receptors (TLRs), are the release of cytokines like IL-18 (29). In conclusion, the present study indicates that inflammation is the potential factors involved in the Cis-induced pathogenesis. CoQ10 pretreatment attenuates Cis induced nephrotoxicity through preventing inflammation in the kidney. Therefore, CoQ10 may protect the kidneys from the toxic effects of Cis.

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**Table 1.** Effect of Cis and $CoQ_{10}$ on serum urea nitrogen concentration

| Group | Serum urea nitrogen concentration (mg/dl) |
|---|---|
| Control (n=8) | 33.94 ± 0.54998 |
| Cis (n=7) | 316.5714 ± 4.48201 |
| $CoQ_{10}$ plus Cis (n=7) | 205.0000 ± 1.55839 |
| Data are expressed as mean ± SEM. *P* ≤ 0.05 for Cis vs. control | |

**Table 2** Effect of Cis and $CoQ_{10}$ on renal IL-18 concentration (pg/ml)

| Group | Renal IL-18 concentration (pg/ml) |
|---|---|
| **Control (n=8)** | 219.04 ± 0.5295699 |
| **Cis (n=7)** | 357.773186 ± 8.9033707 |
| **$CoQ_{10}$ plus Cis (n=7)** | 231.760886 ± 1.8364756 |
| Data is expressed as mean ± SEM. *P* ≤ 0.05 for Cis vs. control | |

**Figure 1.**

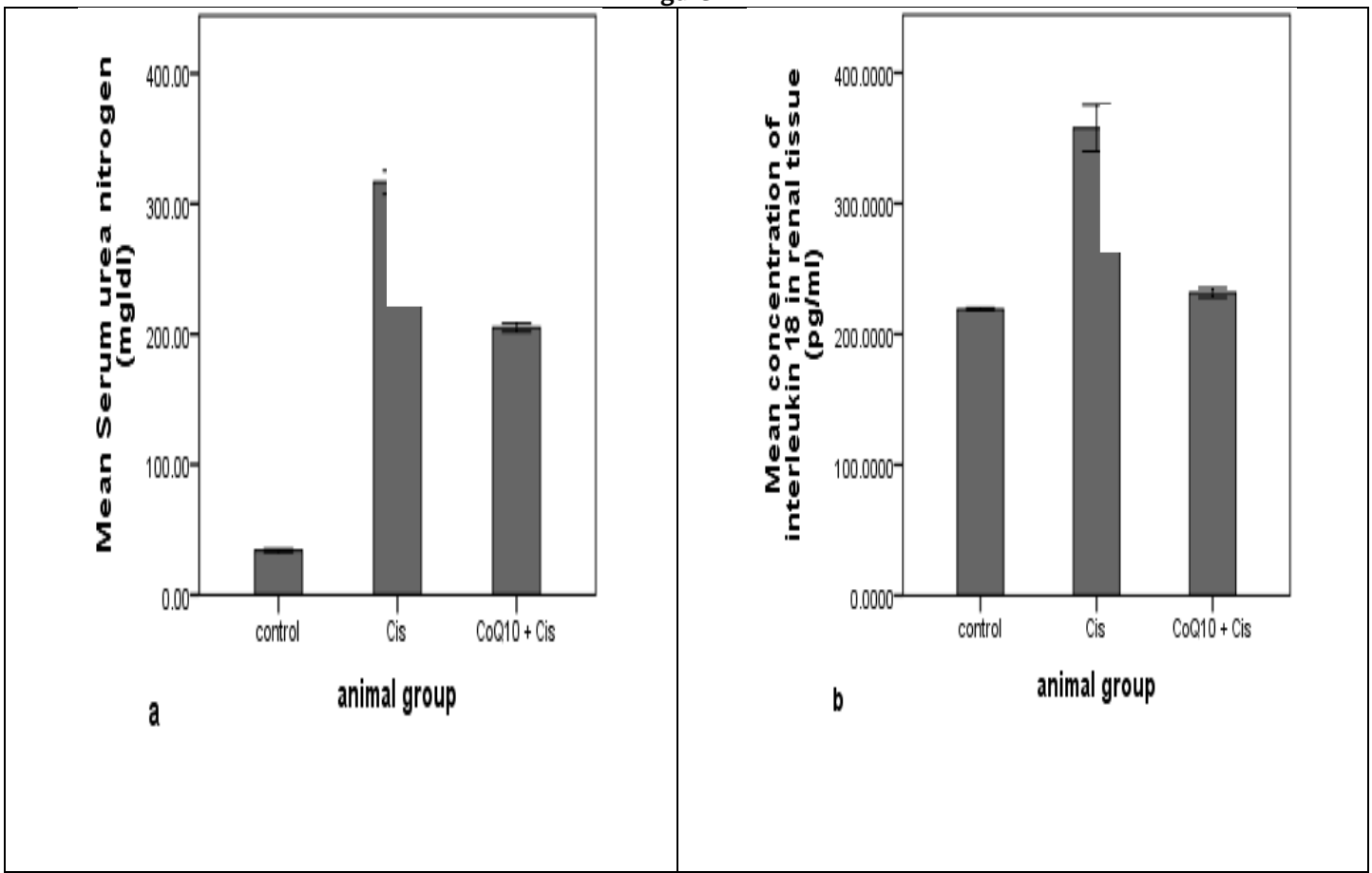


**Fig.1** Effects of Coenzyme $Q_{10}$ ($CoQ_{10}$) treatment on nephrotoxicity and inflammatory parameters on cisplatin-induced nephrotoxicity in adult Wistar albino rats. Graph demonstrated changes in (a) serum level of urea nitrogen, (b) renal tissue interleukin (IL-18) in control (n=8), Cis (n=7) and $CoQ_{10}$ plus Cis (n=7) experimental groups. All Data is expressed as mean ± SEM

* mean $P \leq 0.05$ Cis or $CoQ_{10}$ vs control

\# mean $P \leq 0.05$ $CoQ_{10}$ vs Cis