Original Research Article

The potential protective role of coenzyme q10 on the cyclophosphamide-induced lung toxicity in adult male albino rats: a histological and ultrastructural study

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ABSTRACT

Background: Cyclophosphamide is anticancer and immunosuppressant agent used to treat malignant and autoimmune diseases. Its long-term use causes side effects, as infertility and pulmonary toxicity. Coenzyme Q10; the only synthesized antioxidant in human body, acts as powerful antioxidant, scavenging free radicals, and inhibiting lipid peroxidation. Aim of present study was to examine effect of coenzyme Q10 on blood biochemical profiles, histopathological changes in lungs of adult rats exposed to cyclophosphamide-induced toxicity.

Methods: 36 adult male albino rats divided into four groups; control and three experimental each having 9 rats. First experimental group received coenzyme Q10, second received cyclophosphamide while third group received coenzyme Q10 along with cyclophosphamide. Experiment lasted for 7 days. On 8th day, animals were sacrificed by decapitation. Lung tissue samples were collected for histopathological examination. SOD (superoxide dismutase) and MDA (malondialdehyde) levels were determined and used for statistical analysis.

Results: In coenzyme Q10 treated group, H&E stained sections revealed normal respiratory alveoli. Ultrathin sections revealed normal alveolar septa, pneumocyte and blood capillaries contain erythrocytes. In cyclophosphamide treated group, H&E stained sections revealed peribronchial and interstitial fibrosis. Ultrathin sections revealed alveoli having apparent free lumen with extravasated erythrocytes. Alveolar septa revealed collagen fibrils deposits, and proliferated fibroblasts. In combined coenzyme Q10 and cyclophosphamide treated group, H&E stained sections revealed marked decrease of inter-alveolar tissue thickening. Ultrathin sections revealed destructed alveolar septa with dissociated remnants of collagen fibrils. Blood capillaries appeared wide, containing monocytes and erythrocytes.

Conclusions: Administration of coenzyme Q10 with cyclophosphamide is advised to alleviate cyclophosphamide-induced lung toxicity.

Keywords: Cyclophosphamide, Coenzyme Q10, Lung toxicity, SOD, MDA

INTRODUCTION

Cyclophosphamide is an anticancer agent that is used in combination with other chemotherapeutic agents for the treatment of a variety of malignant diseases. It has also been reported as an immunosuppressant agent, increasingly used for the treatment of certain autoimmune diseases, either as a sole agent or in combination with corticosteroids.1 Cyclophosphamide is used in the treatment of multiple myeloma, acute and chronic leukemia, lymphoma and rheumatoid arthritis.2

Cyclophosphamide is metabolized by a microsomal cytochrome P450 enzyme in the liver, to the toxic metabolites 4-hydroxycyclophosphamide, acrolein, and phosphoramidine mustard. Acrolein is related to...
cyclophosphamide side effects by inhibiting the antioxidant system, causing reactive oxygen species production in the cells. On the other hand, phosphoramidate mustard is behind cyclophosphamide therapeutic effects.\(^3\)

Unfortunately, long-term use of cyclophosphamide is associated with a multitude of significant side effects, such as leukopenia, hemorrhagic cystitis, infertility, the development of secondary malignancies, and pulmonary toxicity. Two distinct clinical patterns of pulmonary toxicity were reported associating the use of cyclophosphamide; an acute interstitial pneumonia that occurs early during treatment, and a chronic, progressive, fibrotic process that may occur after prolonged therapy. The risk for pulmonary toxicity may be increased by the associated use of radiation, oxygen therapy, or other drugs with potential pulmonary toxicity.\(^4,5\)

Coenzyme Q10 (CoQ10) also known as ubiquinone is a fat-soluble, vitamin-like substance.\(^6\) Coenzyme Q10, is the only synthesized antioxidant in human body.\(^7\) It is found everywhere in the body. The biosynthesis of CoQ10 takes place in the mitochondria of vital organs with high-energy turnover such as brain, heart, liver, muscle, and kidney.\(^8\) CoQ10 is a naturally occurring lipid soluble benzoquinone can be obtained from consumption of meat, poultry, fish, vegetables and fruits.\(^9\)

Coenzyme Q10 can provide protection for membrane phospholipids, mitochondrial membrane protein, and low-density lipoprotein against oxidative damage. It acts as essential and powerful antioxidant, scavenging free radicals, and inhibiting lipid peroxidation.\(^10\) The antioxidant effect of CoQ10 also enhance the availability of other antioxidants such as vitamin C, vitamin E, and beta-carotene.\(^11\) The free radical scavenging properties of CoQ10 have been known to serve as a crucial antioxidant activity to prevent lipid and membrane oxidation against pro-oxidative damage in tissues.\(^12,13\) Hence, dietary supplementation with CoQ10 has an effective tool of scavenging reactive oxygen species (ROS) due to its ability to quench singlet oxygen and peroxo radicals.\(^14\)

In addition to its antioxidant activity, CoQ10 is a key element for intracellular energy production through the generation of adenosine triphosphate (ATP) via oxidative phosphorylation by transferring electrons of the mitochondrial respiratory chain, responsible for improvement of endothelial dysfunction as well as important for activating mitochondrial uncoupling proteins.\(^15,16\)

The beneficial effects of dietary CoQ10 on clinical applications have been reported to ameliorate cardiovascular disease such as congestive heart failure, cardiomyopathy and mitochondrial disorder in humans.\(^16,17,18\) CoQ10 treatment is helpful for lowering pro-inflammatory cytokines and blood viscosity in patients with heart failure and coronary artery disease. It can also improve ischemia and reperfusion injury of coronary revascularization.\(^18\)

The anti-oxidative and anti-inflammatory potential of CoQ10 was reported to be beneficial for the nephropathy induced by cisplatin or cyclosporine.\(^19,20\)

It has been suggested that some types of antioxidants have health benefits that fight cyclophosphamide-induced toxicity in lung tissue. However, the effectiveness of coenzyme Q10 as a protective role against oxidative injury varies greatly according to the physiological status of animals, health and disease of animals, concentration and period of dietary CoQ10 supplementation, interaction with other nutrients, etc. Thus, there is still a lack of evidence about whether the administration of CoQ10 to animals during oxidative stress can affect the antioxidant system, despite the rather well recognized antioxidant effects of CoQ10 in vitro.\(^21\)

In the above context, the aim of this study was designed to examine the effects of dietary CoQ10 on blood biochemical profiles, the histopathological changes in lungs of adult rats exposed to early cyclophosphamide-induced lung toxicity.

**METHODS**

**Experimental animals**

After obtaining the approval of Ethical Committee of Animal Research Ethics (CARE), Faculty of Medicine, Ain Shams University. Thirty –six healthy Wistar adult male albino rats (weighing 220-250 gm) were obtained and locally bred at the animal house of the Medical Research Center (MRC), Faculty of Medicine, Ain Shams University. Rats were housed in plastic cages, two rats per cage, and were left one week before the start of the experiment to acclimatize to experimental conditions. The rats were exposed to 12 hours light/dark cycle and allowed daily diet and free water access (ad libitum) with suitable environmental conditions and good ventilation.

**Experimental design**

The rats were divided into four groups (9 rats per group)

**Group I: Control group**

Rats (9 rats) were housed under normal (pathogen-free) conditions and were given only intragastric (i.g.) solvent (corn oil) for 7 days.

**Group II: Co enzyme Q10**

Rats (9 rats) were given i.g. Co enzyme Q10 (300 mg/kg) dissolved in corn oil for 7 days.

On the 8th day of the experiment, the animals were sacrificed by decapitation. The lung tissue samples were collected for histopathological examination.
**Group III: CYP group**

Rats (9 rats) were given i.g. corn oil for 7 days as a placebo, and single-dose intraperitoneal (i.p.) CYP (200mg/kg) was given on the 7th day of the study.

**Group IV: Co enzyme Q10 + CYP group**

Rats (9 rats) were given i.g. Co enzyme Q10 (300 mg/kg) dissolved in corn oil for 7 days, and a single injection of i.p. CYP (200 mg/kg) was administered on the 7th day.

**Processing of samples**

**Preparation of paraffin blocks**

Samples of lungs from the rats were dissected and placed in 10 % formalin. The samples were dehydrated using a graded percentage of ethanol and then fixed in paraffin wax for 1 hour to form paraffin blocks. The blocks were trimmed and cut into 4-μm thick sections. Some sections were stained with hematoxylin and eosin (H&E), and Masson’s Trichrome and then mounted using Depex-Polystyrene dissolved in xylene mountant to examine the histological structure of the lung in the different groups.

**Ultrastructural study**

Specimens for electron microscope were fixed in 2.5% glutaraldehyde in phosphate buffer for 3 hours. Fixed tissue samples were washed with phosphate buffer and post fixed in 1% osmium tetraoxide. After dehydration in ascending grades of alcohol and embedding in Epon, ultrathin sections were cut and stained with uranyl acetate and lead citrate according to 22. The grids were examined and photographed with transmission electron microscope Sumy Electron Optics – Video Imaging System, PEM-100, and were photographed on Kodak film ASA 400.

**Immunohistochemical staining for Caspase III**

Immunohistochemical staining for Caspase III was performed on 5 microns thick paraffin sections. Lung sections were deparaffinized and hydrated in 3% H2O2 for 5 minutes and rinsed with phosphate buffered saline (PBS) and then incubated (5% min, room temperature) with rabbit polyclonal antihuman caspase-3 (0.5 ug/ml) in 1.5% normal goat serum in PBS. The sections then were incubated with biotin-conjugated goat anti-rabbit IgG (1:200, 1h, room temperature), avidin-biotin-peroxidase complex (Santa Cruz Biotechnology, Inc., rabbit peroxidase kit; 1 h) and DAB (3,3′-Diaminobenzidine) solution. Sections were counterstained with hematoxylin. For negative controls, rabbit IgG (1ug/ml) instead of primary antibody was added to the reaction. This study clarified the apoptotic cells which appeared brownish in colour 23.

All sections were examined and photographed using light microscope (Olympus 268 M microscope).

**Biochemical analysis**

The blood and lung tissue samples were collected for biochemical analysis of oxidative stress. The homogenates were centrifuged at 10,000 g for 20 min at 4°C, and the supernatants were obtained; superoxide dismutase (SOD) activity and malondialdehyde (MDA) levels were determined.

**Super oxide dismutase level detection (SOD) in the lung tissue**

Superoxide dismutases (SODs) are metalloenzymes that catalyze the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defense mechanism.

\[ 202^{−}+2H^{+} → H_{2}O_{2} + O_{2} \]

Three types of SODs have been characterized according to their metal content: copper zinc (Cu/Zn), manganese (Mn), and iron (Fe). The amount of SOD present in cellular and extracellular environments is crucial for the prevention of diseases linked to oxidative stress. This Assay of SODs relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. 24

**Malondialdehyde level detection (MDA) in the lung tissue**

The supernatant from lung homogenising buffer was used for determinations of the enzymatic activities of malondialdehyde (MDA). 25 The activity of MDA in the lung tissue was determined using assay kits (Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer’s instructions.

**Statistical analysis**

Statistical measures were done using SPSS (Statistical Package for Social Studies – Version 13.0). One-way analysis of variance (ANOVA) was employed to compare means between groups. Bonferroni Post Hoc test was used to detect significance between every two individual groups. The significance of the data was determined by the probability (P value). P>0.05 was considered nonsignificant, p<0.05 was considered significant, and p<0.01 was considered highly significant. Data were represented in tables and column charts. The column charts were prepared using Microsoft Excel 2017.

**RESULTS**

**Histopathological findings**

**Group I: Control group**

Examination of the sections of group I revealed the following:
H&E stained sections revealed normal respiratory alveoli lined with normal alveolar lining epithelial cells (Figure 1A). Masson Trichrome stained sections showed thin fibrous layer distributed within the interalveolar tissues (Figure 1B). Immunohistochemically stained sections with caspase 3 antibody showed mild expression of caspase 3 expression within the alveolar cells (Figure 1C). Ultrathin sections revealed apparently normal alveolar septa, pneumocyte type II (PII), and type I (Figure 1D).

**Figure 1:** Photomicrograph of a section of a rat’s lung of group I (control group).

(A): showing normal respiratory alveoli lined with normal alveolar lining epithelial cells (arrow head) (H&E X200); (B): showing thin fibrous layer distributed within the interalveolar tissues (arrowhead) (Masson Trichrome stain X200); (C): showing mild expression of caspase 3 expression within the alveolar cells (arrowhead) (Cleaved caspase 3 antibody X200); (D): an electron photomicrograph showing apparently normal alveolar septa (thick arrow), pneumocyte type II (PII), Type I (thin arrow) (Uranyl acetate and lead citrate X 1000).

**Figure 2:** Photomicrograph of a section of a rat’s lung of group II (coenzyme Q10 treated group).

(A): showing normal respiratory alveoli lined with normal alveolar lining epithelial cells (H&E X200); (B): showing thin fibrous layer distributed within the interalveolar and perivascular tissues (arrowhead) (Masson Trichrome stain X200); (C): showing mild expression of caspase 3 within the alveolar cells (arrowhead) (Cleaved caspase 3 antibody X 200); (D): an electron photomicrograph showing normal alveolar septa (arrow), pneumocyte (PII), and blood capillaries containing erythrocytes (ER) (Uranyl acetate and lead citrate X 1000).
Figure 3: Photomicrograph of a section of a rat’s lung of group III (cyclophosphamide treated group).

(A): showing features of interstitial pneumonia associated with peribronchial and interstitial fibrosis (arrow head) and mononuclear inflammatory cells infiltration (H&E X200); (B): showing features of marked perivascular and interstitial fibrosis (arrowhead) (Masson Trichrome stain X200); (C): showing marked increase of caspase 3 expression within the alveolar (arrowhead) and inter-alveolar tissues (arrow) (Cleaved caspase 3 antibody X200); (D): an electron photomicrograph showing intense collagen fibrils deposits in the alveolar septa (Coll), and fibroblasts (Fib). Note the alveolar epithelium (PII) beside extravasated erythrocytes in alveolar lumen (ER) (Uranyl acetate and lead citrate X 1000); (E): an electron photomicrograph showing free alveolar lumen (Lum), proliferated fibroblasts (Fib), pneumocyte type II (PII), interstitial macrophages (MC) and a few collagen deposits (Coll). Note the normal surface microvilli (arrow) of pneumocyte type II (PII) (Uranyl acetate and lead citrate X 1000); (F): an electron photomicrograph showing apoptotic pneumocyte (thick arrow) characterized by pyknotic nuclei. Few collagen fibrils deposits in the alveolar septa (Coll), and fibroblasts (Fib) are noticed. Pneumocyte type II (PII) with vacuolated cytoplasm and normal surface microvilli (thin arrow) can also be observed. Note the free alveolar lumen with little extravasated erythrocytes (ER) (Uranyl acetate and lead citrate X 1000).

Group II: Coenzyme Q10 treated group

Examination of the sections of group II revealed the following:

H&E stained sections revealed normal respiratory alveoli lined with normal alveolar lining epithelial cells (Figure 2A). Masson Trichrome stained sections showed thin fibrous layer distributed within the interalveolar and perivascular tissues (Figure 2B). Immunohistochemically stained sections with caspase 3 antibody showed mild expression of caspase 3 expressions within the alveolar cells (Figure 2C). Ultrathin sections revealed showing normal alveolar septa, pneumocyte (PII) and blood capillaries contain erythrocytes (Figure 2D).

Group III: Cyclophosphamide treated group

Examination of the sections of group III revealed the following:

H&E stained sections revealed features of interstitial pneumonia associated with peribronchial and interstitial fibrosis and mononuclear inflammatory cells infiltration (Figure 3A). Masson Trichrome stained sections showed features of marked perivascular and interstitial fibrosis (Figure 3B). Immunohistochemically stained sections with caspase 3 antibody showed marked increase of caspase 3 expression within the alveolar and inter-alveolar tissues (Figure 3C). Ultrathin sections revealed the alveoli to have apparent free lumen with little extravasated erythrocytes. The pneumocytes type II (PII) appeared having normal surface microvilli with vacuolated cytoplasm. However, some apoptotic pneumocytes type II (PII) with pyknotic nuclei could also be observed. The alveolar septa revealed collagen fibrils deposits, and proliferated fibroblasts. Also, interstitial macrophages could also be detected (Figures 3 D-F).

Group IV: combined Coenzyme Q10 + cyclophosphamide treated group

Examination of the sections of group IV revealed the following:

H&E stained sections revealed marked decrease of interalveolar tissue thickening (Figure 4A). Masson Trichrome stained sections showed marked decrease of interalveolar tissue fibrosis (Figure 4B). Immunohistochemically stained sections with caspase 3 expression (Figure 4C). Ultrathin sections revealed the alveoli to have apparent free lumen with little extravasated erythrocytes. The pneumocytes type II (PII) appeared having normal surface microvilli with vacuolated cytoplasm. However, some apoptotic pneumocytes type II (PII) with pyknotic nuclei could also be observed. The alveolar septa revealed collagen fibrils deposits, and proliferated fibroblasts. Also, interstitial macrophages could also be detected (Figures 4 D-F).
antibody showed marked decrease of caspase 3 expressions within the alveolar and inter-alveolar tissues (Figure 4C). Ultrathin sections revealed destructed alveolar septa with dissociated remnants of collagen fibrils (collagenolysis). Some macrophages nuclei could also be seen. The blood capillaries appeared wide, containing monocytes and erythrocytes. The blood air barrier appeared normal (Figure 4D and E).

Figure 4: Photomicrograph of a section of a rat’s lung of group IV (combined Q10 and cyclophosphamide treated group).
(A) showing marked decrease of interalveolar tissue thickening (arrowhead) (H&E X200). (B) showing marked decrease of interalveolar tissue fibrosis (arrowhead) (Masson Trichrome stain X200). (C) showing marked decrease of caspase 3 expression within the alveolar and inter-alveolar tissues (arrowhead) (Cleaved caspase 3 antibody X200). (D) an electron photomicrograph showing destructed alveolar septa (Coll) with dissociated collagen fibrils (collagenolysis). Note the macrophages nuclei (MC) (Uranyl acetate and lead citrate X 1000). (E) an electron photomicrograph showing remnant of collagen fibrils in the alveolar septa (Coll). Widening of blood capillaries containing monocytes (Mon) and erythrocytes (ER) can be seen. Note the apparent normal blood air barriers (thick arrows) (Uranyl acetate and lead citrate X 1000).

Figure 5: The lung SOD activity in different groups in (µl/ml).

![Graph showing lung SOD activity](image-url)
Morphometric findings

The lung superoxide dismutase (SOD) activity

Using the Post hoc test of Bonferroni, the lung superoxide dismutase (SOD) activity in group I (control) was 26.25±0.0089 µl/ml (mean±SD). In group II (coenzyme Q10), it was 26.24±0.012111 µl/ml (mean±SD) where it showed nonsignificant statistical difference when compared to the group I (control group) (p>0.05). In group III (cyclophosphamide), it was 13.18±0.030111 µl/ml (mean±SD) and it showed highly significant statistical decrease when compared to the group I (control group) (p<0.01). In group IV (combined coenzyme Q10+ cyclophosphamide), it was 26.23±0.005477 µl/ml (mean±SD) where it showed nonsignificant statistical difference when compared to the group I (control group) (p<0.05). Group IV also showed highly significant statistical increase when compared to the group III (cyclophosphamide group) (p<0.01) (Figure 5).

The lung malondialdehyde (MDA) levels

Using the Post hoc test of Bonferroni, the lung malondialdehyde (MDA) levels in group I (control) was 1.40±0.033116 mmol/ml (mean±SD). In group II (coenzyme Q10), it was 1.40±0.029439 mmol/ml (mean±SD) where it showed nonsignificant statistical difference when compared to the group I (control group) (p>0.05). In group III (cyclophosphamide), it was 2.90±0.030605 mmol/ml (mean±SD) and it showed highly significant statistical increase when compared to the group I (control group) (p<0.01). In group IV (combined coenzyme Q10+ cyclophosphamide), it was 1.42±0.019408 mmol/ml (mean±SD) and it showed nonsignificant statistical difference when compared to the group I (control group) (p<0.05). Group IV also showed highly significant statistical decrease when compared to the group III (cyclophosphamide group) (p<0.01) (Figure 6).

DISCUSSION

The present study clarified that cyclophosphamide exerted deleterious histopathological and biochemical effects on the lung of adult male albino rats. This was in agreement with Şengüla et al, who stated that cyclophosphamide is a widely used antineoplastic and immunosuppressive drug that causes multiple organ toxicity in humans and experimental animals. He added that biochemical and histological alterations induced by cyclophosphamide in experimental studies show similarity to those observed in humans.26

Histological examination of the sections of the lung tissues of the rats of group III (cyclophosphamide treated group) revealed features of interstitial pneumonia associated with peribronchial, perivascular, and interstitial fibrosis and mononuclear inflammatory cells infiltration. This was clarified in both the H&E and Masson Trichrome stained sections. In addition, the ultrastructural examination confirmed these findings where they revealed the alveolar septa to have collagen fibrils deposits, and proliferated fibroblasts. Also, interstitial macrophages could also be detected.

Similarly, Ashry et al and Suddek et al reported that cyclophosphamide toxicity in lung tissue is similar to interstitial pneumonia and pulmonary fibrosis. These
histopathologic patterns are often dose-limiting and even life-threatening.\(^4\)\(^5\)

Selman et al. reported that injured alveolar epithelial cells II synthesizes a variety of pro-fibrotic enzymes, cytokines and platelet derived growth factor leading to excessive connective tissue deposition, aberrant tissue remodeling and subsequent destruction of the lung parenchyma that forms fibrotic lesions resulting in progressive decline of lung function, and ultimately death.\(^27\)

Ott et al attributed the thickening of the interalveolar septa to the marked inflammatory cellular infiltration with lymphocytes, neutrophils, eosinophils, macrophages, proliferating fibroblasts with subsequent fibrosis.\(^28\)

According to Sharafkhaneh et al, the inflammatory process is accompanied by emphysema like reaction in the lung. Accumulation of these inflammatory components leads to release of destructive mediators as proteases and cytokines causing tissue destruction and remodeling.\(^29\)

Results of group III also showed marked increase of the apoptotic cells within the alveolar and inter-alveolar tissues which was proven through both the marked increase in the caspase 3 expression and through the ultrathin sections which showed some apoptotic pneumocytes type II (PII) with pyknotic nuclei. In addition, ultrathin sections also showed the alveoli to have apparent free lumen with little extravasated erythrocytes. The pneumocytes type II (PII) appeared having vacuolated cytoplasm.

Results of group III agree with Şengül et al who reported alveolar cell injuries, thickness in alveolar septa, polymorphonuclear cells, and erythrocytes in the alveolar lumen in the lung sections of the cyclophosphamide treated group.\(^26\)

In the present study, the superoxide dismutase (SOD) activity in lung homogenates was assessed statistically in the lung tissue of different groups. The SOD activity of the lungs of group III (cyclophosphamide treated group) showed highly significant decrease when compared with those of the other groups (p<0.05). Şengül et al stated similar results in the cyclophosphamide treated group.\(^26\) Moreover, Chakraborty et al and Choi et al reported that oxidative-stress-mediated cellular damages prevent free-radical scavenging enzymes, including SOD.\(^30\)\(^31\)

In addition, the malondialdehyde MDA levels as a marker of oxidative stress were also assessed statistically. The MDA levels of the group III (cyclophosphamide treated group) showed highly significant increase when compared with those of the other groups (p<0.05). This was also in agreement with Şengül et al who stated same results in the cyclophosphamide treated group.\(^26\)

Preiser stated that oxidative stress is an imbalance between the antioxidant and oxidant by excessive production of reactive oxygen species (ROS) and the ability of antioxidant systems to readily detoxify the ROS or repair the resulting damage.\(^32\) Moreover, Şengül et al added that oxidative enzymes are often used as indicators of oxidative stress in cells and can result in tissue damage. During oxidative stress, enhancement production of ROS with pro-inflammatory cytokines can lead to cellular injury in the lung following cyclophosphamide intoxication.\(^26\)

In the present study, the concomitant administration of coenzyme Q10 with cyclophosphamide had a notable protective effect against cyclophosphamide-induced histopathological and biochemical effects on the lung tissue of adult male rats. This was in agreement with Ashry et al and Suddek et al who suggested that some types of antioxidants have health benefits that fight cyclophosphamide toxicity in lung tissue.\(^25\)

Examination of the lung tissue of the rats of group IV (treated with concomitant administration of both coenzyme Q10 and cyclophosphamide) revealed marked decrease of interalveolar tissue fibrosis, with destructed alveolar septa and dissociated remnants of collagen fibrils (collagenolysis). In addition, it showed marked reduction of the apoptotic cells which was proved through the marked decrease of caspase 3 expressions within the alveolar and inter-alveolar tissues. Moreover, in ultrathin sections, the blood capillaries appeared wide, containing monocytes and erythrocytes, and the blood air barrier appeared normal.

Sime and O'Reilly also reported that dietary coenzyme Q10 supplementation has prevented alveolar damage and reduced the extent of pulmonary collagen deposition. This protective effect offered by coenzyme Q10 in vitro and in vivo may be the result of several effects including membrane stabilization, altered profibrotic gene expression, and ROS scavenging. Moreover, they had suggested that the protective effect of coenzyme Q10 against pulmonary fibrosis can be attributed to its ability to inhibit the release of profibrotic cytokines from macrophages including transforming growth factor-b1, which is responsible for extracellular matrix remodeling.\(^33\)

Gokce et al reported that coenzyme Q10 is the only antioxidant that is fat soluble, synthesized endogenously and is present in tissues in the active form.\(^34\) Moreover, Turunen et al stated that coenzyme Q10 is a mediator of lipid peroxidation and an essential cofactor in the electron transport chain and a component of the lipid membranes.\(^16\)

These effects might explain the notable reduction of the apoptotic cells expressed by marked decrease of caspase 3 expression within the alveolar and inter-alveolar tissues in this study in the lungs of rats of group IV (treated with
both coenzyme Q10 and cyclophosphamide) when compared with those of group III (cyclophosphamide treated group).

Gokce et al stated that the strong antioxidant coenzyme Q10 plays a role in membrane stabilization as it is a component of the mitochondrial electron transport chain. Also, it inhibits lipid peroxidation by preventing a peroxidation chain reaction and/or picking up ROS. In addition, Lim et al conceived that coenzyme Q10 might attenuate the release of tumor necrosis factor alpha (TNFα) through its ability to limit the membrane lipid peroxidation with its subsequent pro-inflammatory signaling. Also, being a vital component of the respiratory chain, it plays a key role in the synthesis of cellular ATP.

Khimenko et al stated that ROS production in lung injury results in high concentration of TNFα. In addition, Kant et al added that this TNFα is a cell signaling protein (cytokine) involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction. It is produced chiefly by activated macrophages, although it can be produced by many other inflammatory cells. It mediates the transcription of vast array of proteins involved in cell survival and proliferation, inflammatory response and is generally proapoptotic.

Bhagavan and Chopra had also identified that coenzyme Q10 is a modulator of gene expression, inflammatory processes and apoptosis.

The protective effect of coenzyme Q10 against cyclophosphamide-induced lung toxicity could explain the statistically highly significant increase in the SOD activity of the lungs of group IV (combined treatment with coenzyme Q10 and cyclophosphamide) when compared with those of group III (cyclophosphamide treated group). Moreover, there was statistically nonsignificant difference in the SOD activity of the lungs of group IV when compared with those of group I (control adult) and group II (coenzyme Q10).

In addition, there was statistically highly significant decrease in the lungs MDA levels of the lungs of group VI when compared to those of group III. However, they show statistically nonsignificant difference when compared with those of group I and group II.

CONCLUSION

From the previous results, it could be concluded that cyclophosphamide exerted deleterious histopathological and biochemical toxic effects on the lung of adult male albino rats. In this study, it was proven both histologically and biochemically that coenzyme Q10 had a notable protective effect against cyclophosphamide-induced lung toxicity. Therefore, concomitant administration of coenzyme Q10 with cyclophosphamide is advised to alleviate the cyclophosphamide-induced lung toxicity and prolong the usefulness of cyclophosphamide.

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