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Original Article

CoQ10 dampens the deleterious impact of doxorubicin-induced liver and spleen injury in white Albino rats

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ABSTRACT

The liver and spleen are vital organs involved in drug clearance and maintenance of the hemostasis. Doxorubicin is an important chemotherapeutic agent used in the treatment of hematological cancers and solid tumors. We aimed to identify the protective impact of Coenzyme Q10 (CoQ10) against doxorubicin-induced normal tissue damage using the liver and spleen as a model. To do so, a total of 27 rats were used in this study and they were divided into three groups of 9 each. Group 1 received CoQ10 over 17 days with a single injection dose of normal saline at day 13. Group 2 received normal saline over 17 days with a single injection dose of doxorubicin at day 13. Group 3 received CoQ10 over 17 days with a single injection dose of doxorubicin at day 13. Spleen and liver were harvested and processed for slide preparation to be examined under a light microscope. The results confirmed that doxorubicin induced liver and spleen damage. The liver damage was evidenced by hepatic coagulative necrosis of hepatocytes, dilatation of sinusoids and congestion of portal vein, while spleen damage was evidenced by loss of white pulp with atrophy, hyaline degeneration of splenic artery, and necrosis of red pulp. CoQ10 reduced these damaging effects in both the spleen and liver to a large extent restoring the normal tissue architecture of liver and spleen. The present study concluded that CoQ10 can counteract the harmful effects of Doxorubicin and preserve the liver and spleen.

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KEYWORDS: doxorubicin, liver histology, CoQ10, ubiquinone, mitochondria, respiratory chain.

INTRODUCTION

Chemotherapy kills cancer cells beside damaging the normal cells, inducing cellular damage in vital organs, which includes highly proliferating and soft tissues (e.g. bone marrow, hair follicles, intestinal epithelial cells, liver, kidney, and spleen) [1]. The liver is the target organ for drug metabolites and toxins due to its role in metabolism of food, chemicals, xenobiotics, and drugs. Anticancer metabolism usually takes place in the liver and the metabolites usually are eliminated through the liver and/or kidneys, and hence these are highly susceptible organs to toxicity [2].

Doxorubicin is used in the treatment of hematological cancers and solid tumors. Dox mode of action is based on inhibition of topoisomerase II (TopoII) resulting in breakdown of DNA double-strand [3]. The DNA damage response (DDR) signalling cascade is then activated, directing the repair machinery to restore this damage. If this fails, apoptosis is triggered by the DNA repair program [4]. Actively multiplying cells, such as tumor cells, are thought to be more vulnerable to the ensuing DNA damage than normal cells, forming a chemotherapeutic window. Other TopoII inhibitors, such as the Doxo analogues daunorubicin, idarubicin, epirubicin, and aclarubicin, as well as structurally unrelated medicines like etoposide, have been produced [3]. TopoIIa is released from nucleoli and accumulates on chromatin as a result of exposure to these medicines [5].

CoQ10 is a part of the electron-transport chain that produces ATP molecules during aerobic cellular respiration. Aerobic respiration is responsible for the production of 95% of the ATP in the human body. As a result, the heart, kidney, and liver all have a lot of CoQ10 [6].

CoQ10 is an antioxidant that is naturally produced by living organisms [7]. CoQ10 has a stronger antioxidant effect than vitamin E [8]. Natural coenzyme Q (ubiquinone) is a 2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone with a polyprenylated side chain ranging from 6 to 10 units in length. Only CoQ9 and CoQ10 are found in the mitochondrial respiratory chain that serve as a diffusible electron carrier in mammals, with CoQ9 being found only in rodents [9]. CoQ exists in three redox states: fully oxidized (ubiquinone), semiquinone (ubisemiquinone), and fully reduced (ubiquinol). However, the presence of varying numbers of protons expands the number of potential quinone ring redox forms [10].

With its role in accepting and donating electrons during the transit of electrons from nicotinamide adenine dinucleotide hydrogen (NADH) to oxygen, CoQ10 is an essential part of the electron transport chain in mitochondria. As a result, it is essential for the oxidative phosphorylation process that produces ATP. CoQ10 exists as ubiquinol in its completely reduced state and ubiquinone in its oxidised state. It can also exist as a partly reduced semiquinone intermediate, and in this condition, it is a free radical. CoQ10 may provide its electrons to free radicals like the hydroxyl radical in its reduced state, making it a good antioxidant. It can absorb electrons from free radicals like the superoxide radical in its oxidised form but cannot contribute to them [11]. Therefore, CoQ10 only functions as a conventional antioxidant when it is oxidised, giving free radical electrons and halting the free radical cascade. CoQ10 may operate normally in the mitochondria in both the reduced and oxidised forms, where it is both stable and active [12]. Numerous clinical studies have been conducted to evaluate CoQ10's effectiveness in treating various disease states as a result of its special free radical scavenging abilities. Oral administration of CoQ10 (ubiquinone) is effective in treating or preventing a wide range of medical conditions, including atherosclerosis [13], failure of heart muscle contractility [14], chemotherapy-induced toxicity [15], Parkinson's, neurological diseases [16], and inflammation [17].

Although CoQ10 has been identified as the main endogenous lipophilic antioxidant molecule generated by cells, its physiological significance outside of mitochondria is still up for discussion. Fibroblast Specific Protein 1 (FSP1) has recently been recognized as a unique CoQ10 plasma membrane oxidoreductase that guards cells against glutathione-independent ferroptosis [18, 19].

The only one of its type, CoQ10 is a mobile lipophilic electron transporter that also produces lipid-soluble antioxidants on its own [20]. The respiratory chain function in the mitochondrial membrane as well as the defense of lipids against oxidation in the Golgi and plasma membranes depend on CoQ10 for electron transport [21, 22]. The redox cycle that CoQ10 goes through in cells (completely oxidized ubiquinone vs. fully reduced ubiquinol) is crucial to its function in the mitochondrial electron transport chain. In the plasma membrane, CoQ10 also functions as a lipophilic antioxidant that scavenges free radicals [23]. While the mitochondrial redox cycle in mitochondria has a well-defined function, what controls the redox cycle of the CoQ10 pool outside of the mitochondria (such as the plasma membrane) is less apparent. The oxidoreductase that lowers CoQ10 at the plasma membrane is FSP1. Thus, the suppression of ferroptosis and lipid peroxidation is achieved by the FSP1/CoQ10/NADH system alone. CoQ10 functions as a protective agent in the cell's plasma membrane. CoQ10 plays a crucial role as a protective agent in the cell's plasma membrane, preventing the oxidant potential of metabolic waste products from triggering ferroptosis [18].

Antioxidant capabilities are thought to exist in CoQ10. It could be because ubiquinone, the oxidized form of CoQ10, is utilized as a dietary supplement even though ubiquinol, the reduced form of CoQ10, possesses antioxidant properties [24]. CoQ10 is also a potent antioxidant that neutralizes free radicals, prevents lipid peroxidation in cellular biomembranes from starting and spreading, and stimulates tocopherol regeneration [12, 23]. The binding and enzymatic activity of proteins and enzymes can be inhibited

by oxidative alterations caused by ROS-induced denaturation [25-28].

While some of the reactive species are capable of directly triggering an inflammatory response, others can operate as signaling molecules in the cellular response to triggers like tumor necrosis factor α. In addition to removing reactive species, antioxidants may help lessen inflammatory reactions and the harm they produce. Anti-inflammatory drug therapy can thereby reduce the load of oxidative stress [29-31]. Although its therapeutic effects are limited CoQ10 also possesses anti-inflammatory characteristics that inhibit the synthesis of pro-inflammatory cytokines such as tumour necrosis factor-α [32-34].

MATERIALS AND METHODS

Study settings: The study was approved by the Animal Welfare Committee in the College of Veterinary Medicine/University of Mosul (Approval Letter UM.VET.2021.33).

Animal housing: The animals (Male rats, aged 10-12 weeks, and weighing 250-300 g) were kindly provided by the animal house and care of the College of Veterinary Medicine/University of Mosul. The animals were kept under close monitoring with proper laboratory animal standard conditions of fresh air, dark-light cycle, and free access to water and food.

Study design: A total of 27 white male albino rats were enrolled in the present study and they were divided into 3 groups (9 each) as follows (Figure 1):

CoQ10-group: received 10 mg/kg CoQ10 orally for 17 days and intraperitoneal normal saline on day 13.

Dox-group: received distilled water orally for 17 days and 15mg/kg intraperitoneal Dox on day 13.

Dox/CoQ10-group: received 10 mg/kg CoQ10 orally for 17 days and 15mg/kg intraperitoneal Dox on day 13.

The used drugs doxorubicin (manufactured by Saba-Turky), and CoQ10 (manufactured by 21st Century®-USA) were purchased from a local private pharmacy. CoQ10 was prepared in a normal saline solution (0.9 per cent NaCl) with 1 percent Tween 80 (v: v) by mixing it overnight at 25 °C and putting it in a dark container to prevent it from decomposition.

Euthanasia and histological analysis

Euthanasia: The animals were killed *via* cervical spine dislocation once the experiment trial was finished.

Tissue processing: Spleen and liver bodies were processed to create microscopic slides.

A mid-ventral incision was made using scissors and forceps, running from the centre of the neck

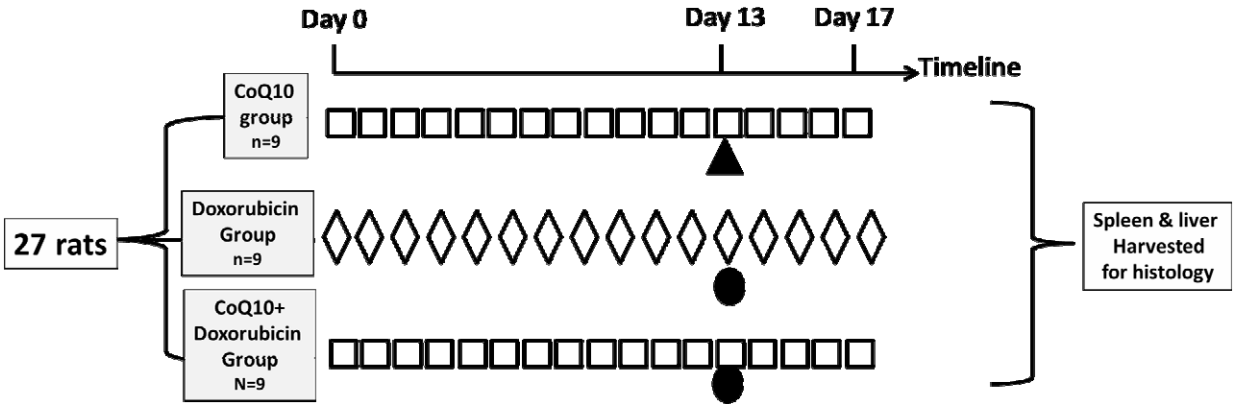

Figure 1. Schematic diagram describing workflow of the study design involving 27 rats (9 each group). Group 1 represented the CoQ10 group. Group 2 represented the Dox group. Group 3 represented CoQ10+Dox group. Each square and diamond shape represents a day in the study period. Square = CoQ10, Diamond = Normal Saline, black Circle = Dox, Black triangle = injected normal saline.

to the scrotal sac. The liver and spleen were taken from the abdominal cavity and cleaned in the sink before being transferred to a petri dish containing distilled water solution and the spleen and liver were separated from the associated fat connective tissue, which was then dried on filter paper. Liver and spleen specimens were collected and preserved in a 10% neutral buffered formalin solution. All specimens were fixed in the fixative for around 48 hours before further processing. To remove the fixative, the previously fixed tissues were washed under running water for 6 to 8 hours overnight. The length of time the tissues spend in the stabilizer is directly proportional to the washing time. The tissues were dried by removing all of the water from the tissue that had been fixed.

Dehydration: In the processing of tissue, a variety of dehydrating agents were employed. Alcohol was used in place of water leading to tissue dehydration. Capsules were filled with tissue blocks (cassettes). These tissues were subjected to a series of baths with increasing alcohol content, including two in 80% alcohol for an hour, one in 90% alcohol for an hour, and three in 100% alcohol for an hour.

Clearing: Paraffin wax was used in place of alcohol. Because paraffin wax is not soluble in alcohol, a solvent was instead used, such as xylene, chloroform, benzene, carbon tetrachloride, or cedarwood oil, in which the wax is soluble. The clearing agent was applied for an hour in two baths.

Impregnation or Infiltration: The removal of the cleansing agent from the tissues was accomplished by diffusion into the surrounding melted wax (infiltration). It diffuses into tissues by replacing xylene (impregnating). The steps include changing xylene with paraffin, taking two baths in molten paraffin for one hour, setting the paraffin oven to a temperature of 50 to 56 ℃, and getting rid of any air bubbles.

Embedding: Casting the tissue block that has been penetrated by paraffin and impregnated in warm liquid paraffin, which cools and solidifies into a block. In this method paraffin wax is first melted at 56 to 58 ℃, filtered through coarse filter paper, and then poured into a mould with tissue inside. The mould is then submerged in cold water or refrigerated for 10 to 20 minutes.

Labeling: To identify a block for indefinite storage, a little piece of paper with an identifying number written in pencil or ink was fastened to one side of the block.

Trimming: Once the wax blocks have been prepared, it is advisable to cut them using a sharp blade till the specimen is in an ideal cutting position, with its edges parallel and ready to be placed on the microtome knife's edge.

Sectioning Microtome: This tool is used to cut biological specimens into ultra-thin slices. The steps involve trimming the block with a knife until 1 to 3 mm of paraffin is left on all sides of the tissue, fixing the block in the microtome's block holder, placing a knife firmly in the appropriate position on the knife holder, and cutting enough sections for microscopic examination. After being divided into sections, the tissues were allowed to float in a warm water bath, which helped to remove the wrinkles. The slide was then dried for 5 minutes on the hot plate.

Histological stains (hematoxylin and eosin stain): Hematoxyline and eosin stain (China) was used to examine the overall histological characteristics of liver and spleen tissue. Before the beginning of tissue staining, the paraffin wax was removed. To successfully eliminate the paraffin, xylene and xylene equivalents, which are commonly used in tissue processing, were used. Three clearing steps, each lasting three minutes, are generally sufficient to prepare the tissue for hydration and staining with aqueous hematoxylin. Following that, anhydrous alcohol was used, followed by diluted alcohol (95%) and water.

RESULTS

CoQ10 hepatoprotection against doxorubicin

Liver sections of rats treated with CoQ10: The liver sections of rats treated with CoQ10 showed normal tissue architecture characterized by normal central vein, intact portal area, and normal-shaped sinusoids (Figure 2).

Liver sections of rats treated with doxorubicin: The liver sections of rats treated with doxorubicin showed abnormal tissue architecture characterized by coagulative necrosis of hepatocytes, dilatation of sinusoids and congestion of portal vein (Figure 3).

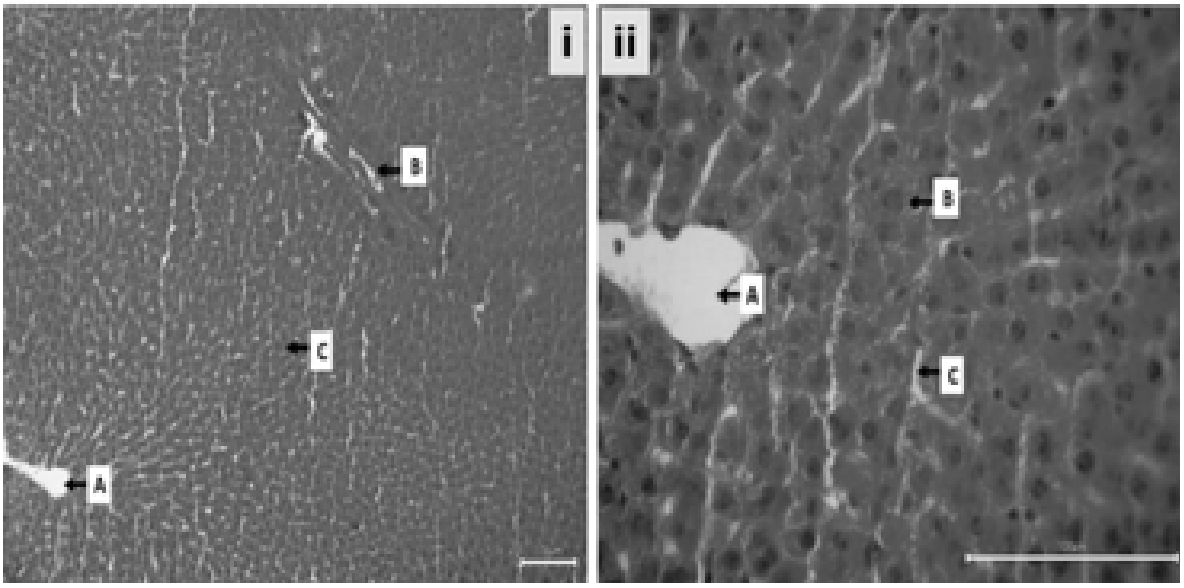

Figure 2. A representative image of rat liver sections treated with CoQ10 showing normal structural architecture. Central vein (A), hepatocytes (B) and sinusoids (C). H&E stain, Scale bar = 100µm (i), 400µm (ii).

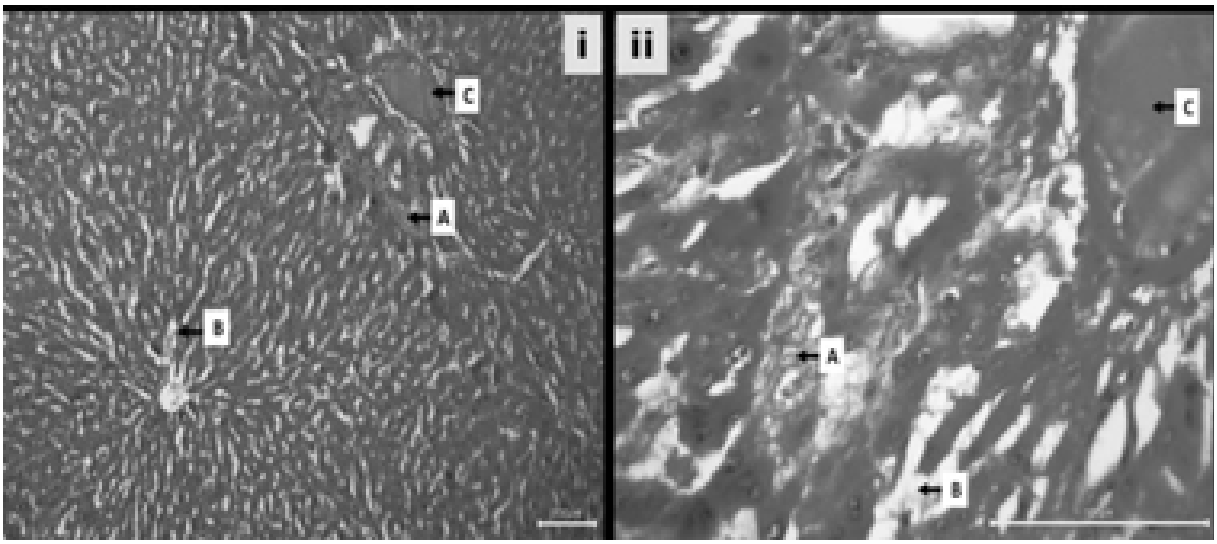

Figure 3. Representative images of rat liver sections treated with doxorubicin showing abnormal structural architecture characterized by coagulative necrosis of hepatocytes (A), dilatation of sinusoids (B), and congestion of portal vein (C). H&E stain, Scale bar = 100 µm (i), 400 µm (ii).

Liver sections of rats treated with doxorubicin+ CoQ10: The liver sections of rats treated with doxorubicin and CoQ10 showed restoration of normal hepatic structural architecture with intact hepatocytes, intact central vein, and mild congestion of portal vein (Figure 4).

Spleen protection by CoQ10 against doxorubicin

Spleen sections of rats treated with CoQ10: The spleen sections of rats treated with CoQ10 showed normal tissue architecture characterized by normal white pulp, normal central artery, and intact red pulp (Figure 5).

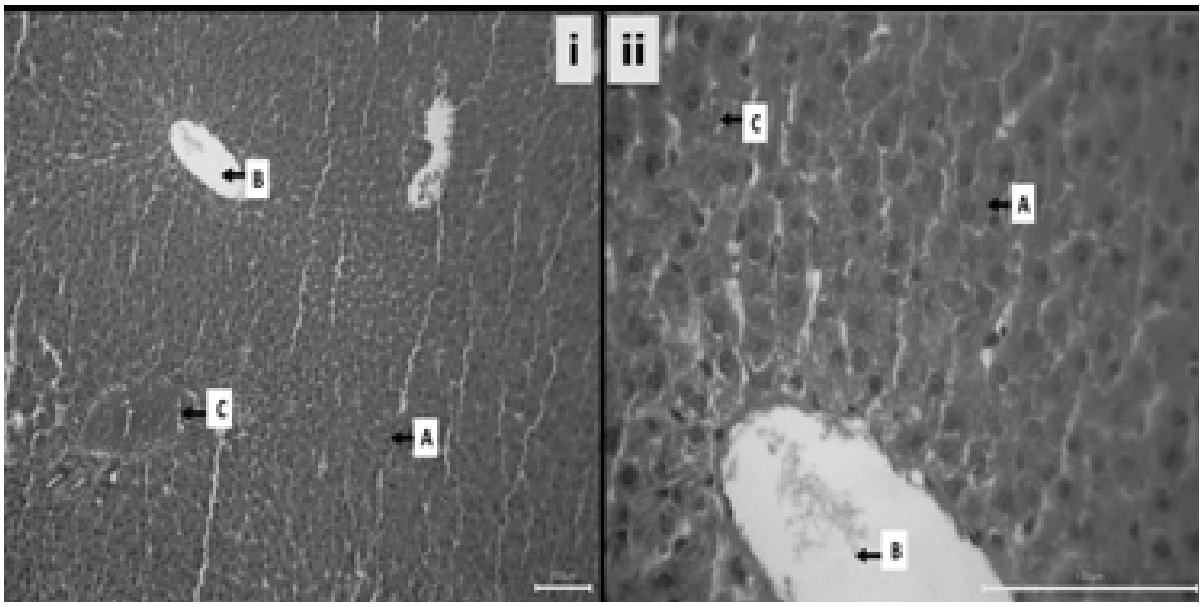

Figure 4. A representative image of rat liver sections treated with doxorubicin+CoQ10 showing intact hepatocytes (A) and central vein (B) with congestion of portal vein (C). H&E stain, Scale bar = 100 µm (i), 400 µm (ii).

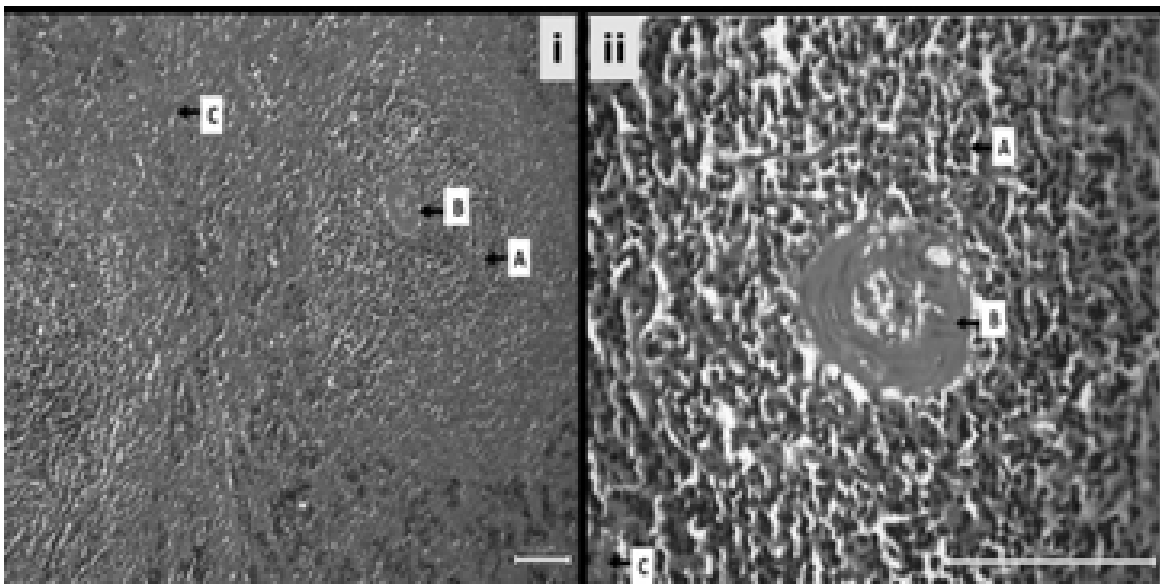

Figure 5. A representative image of rat spleen sections treated with CoQ10 showing normal structural architecture characterized by white pulp (A) with central artery (B) and red pulp (C). H&E stain, Scale bar = 100 µm (i), 400 µm (ii).

Spleen sections of rats treated with doxorubicin: The spleen sections of rats treated with doxorubicin showed loss of white pulp with atrophy, hyaline degeneration of splenic artery, and necrosis of red pulp (Figure 6).

Spleen sections of rats treated with doxorubicin+ CoQ10: The spleen sections of rats treated with doxorubicin and CoQ10 showed restoration of normal spleen structural architecture with intact white pulp and the presence of intact

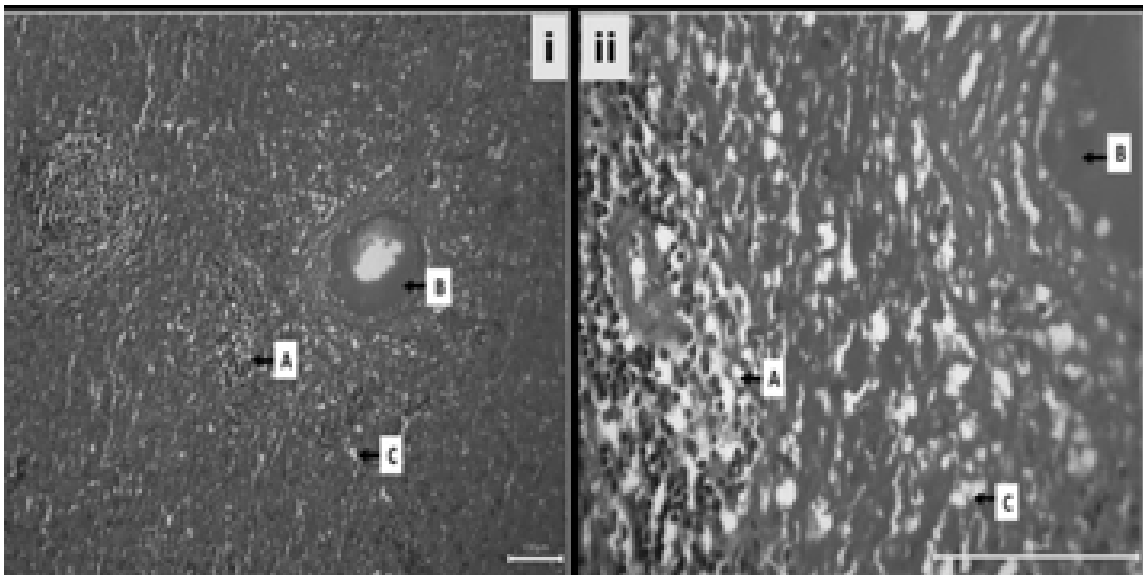

Figure 6. A representative image of rat spleen sections treated with doxorubicin showing abnormal structural architecture characterized by loss of white pulp with atrophy (A), hyaline degeneration of splenic artery (B) and necrosis of red pulp (C). H&E stain, Scale bar = 100 µm (i), 400 µm (ii).

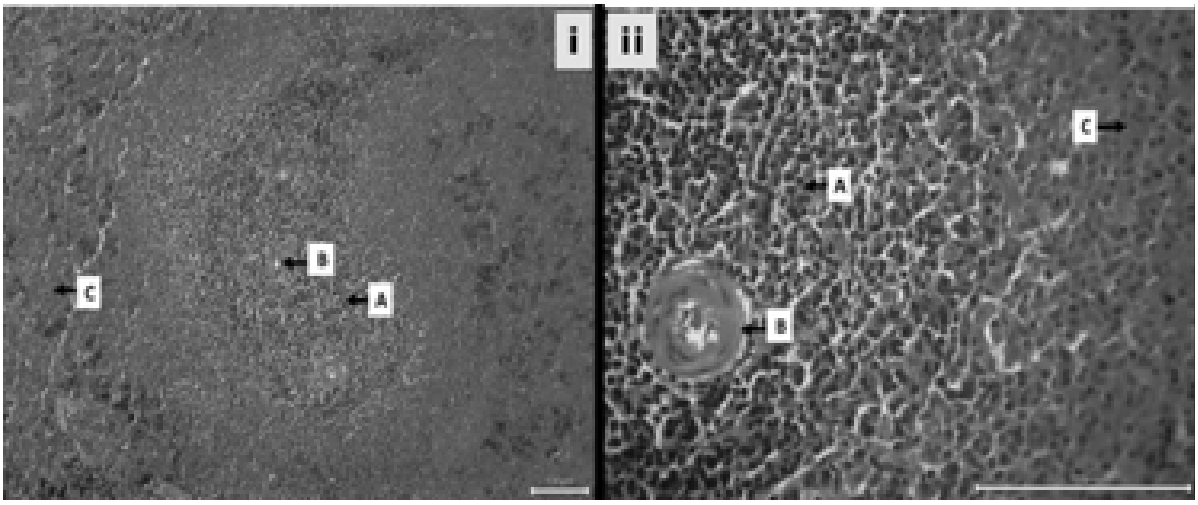

Figure 7. A representative image of rat spleen sections treated with doxorubicin+CoQ10 showing intact spleen characterized by white pulp (A) with central artery (B) and red pulp (C). H&E stain, Scale bar = 100 µm (i), 400 µm (ii).

central artery and normal red pulp hepatocytes (Figure 7).

DISCUSSION

In the present study, doxorubicin induced liver and spleen damage. The liver damage is characterized by hepatic coagulative necrosis of hepatocytes, dilatation of sinusoids and congestion of portal vein, while spleen damage is characterized by loss of white pulp with atrophy, hyaline degeneration of splenic artery, and necrosis of red pulp. CoQ10 reduced these damaging effects in both the spleen and liver to a large extent restoring the liver and spleen architecture to near normal.

The mechanism by which Dox induces tissue damage involves generation of reactive oxygen

species that induce oxidative stress with subsequent liver and spleen damage and metabolic derangements [35]. These reactive oxygen species invoke lipid peroxidation, prompting liver and spleen damage which has been confirmed by a previous study [36].

CoQ10 through its antioxidant activity [37, 38] maintained cellular quasi-equilibrium and reduced tissue damage, restoring normal architecture which has been confirmed by numerous previous studies [39-41].

In addition to Dox-induced oxidative stress, another proposed mechanism related to Dox leads to reduced hepatic Nrf2 protein expression which is important in the maintenance of liver functionality and architecture [42]. Tarry-Adkins *et al*. have mentioned that CoQ10 maintains the Nrf2 pathway and increases the Nrf2 protein production protecting the tissue [43]; moreover, CoQ10 has been reported as having anti-fibrotic activity preventing the architectural modulation of liver and spleen tissues [44].

It has been reported that Dox administration is associated with the upregulation of intrinsic CoQ10 gene expression and CoQ10 production; thus this compensatory protective mechanism was utilized by the administration of exogenous CoQ10 to prevent the damaging effects of Dox [45-47].

Alternative studies conducted on the protective role of CoQ10 in Dox-induced nephrotoxicity have found that CoQ10 saved the kidney architecture and function even better than other nephroprotective products [48-50].

The dose used in our study and the preconditioning of the rats with CoQ10 before Dox administration and the subsequent Dox-dose have been reported by previous studies [51-53]. In the present study, we used an accepted therapeutic dose in rats to avoid the prooxidant potential of high doses of CoQ10 because there is evidence that a high dose of CoQ10 causes prooxidant effects [51-53]. This property has been reported in similar flavonoids: quercetin, myricetin, kaempferol [54], and curcumin [55].

This claim has been supported in clinical settings when CoQ10 administered to renal hemodialysis patients improved the total antioxidant status but reduced oxygen radical absorbing capacity [56].

CONCLUSION

Doxorubicin is a commonly used chemotherapeutic agent and is characterized by adverse events on vital organs due to their oxidative and inflammatory reactions. The present study confirmed that CoQ10 possess a protective capacity against doxorubicin-induced liver and spleen toxicities. This finding could potentially enhance its application in the treatment of cancer disease as an adjuvant agent.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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