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Modulatory Effects of Coenzyme Q10 on Cyclophosphamide - Induced Hepatotoxicity in Adult Male Albino Rats via Regulation of Oxidative Stress, Inflammation and Apoptosis

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Abstract

Cancer and autoimmune diseases are both treated with the chemotherapy drug cyclophosphamide (CP). However, because of its harmful side effects, particularly hepatotoxicity, its use is restricted. The aim of this experiment was to evaluate the effect of coenzyme q10 (CoQ10) on CP induced hepatotoxicity in adult male albino rats. Sixty adult male albino rats (180-200 g) were divided into 6 groups, 3 control groups and 3 experimental groups {CoQ10-treated group, CP-treated group and CP+CoQ10-treated group}. After 4 weeks of treatment, blood samples were collected for biochemical studies, while tissues were taken for oxidative stress markers, qRT-PCR, histopathological and immunohistochemical studies. Our outcomes clarified that CP provoked an increase in liver enzymes (ALT, AST, ALP and γGT) along with severe hepatic histopathological changes. Also, CP showed a subsequent decrease in the antioxidant enzyme activities (SOD, CAT, GSH), and conversely, (MDA) levels were markedly elevated. CP activated the inflammatory pathway demonstrated by elevated serum TNF-α and IL-1β, and up-regulated hepatic NF-kB p65 gene expression. CP affected PPAR-γ and Nrf2 pathway by down-regulating their gene expression in hepatic tissues. It also activated apoptotic pathway by up-regulation of Bax and down regulation of Bcl-2 leading to elevated apoptotic index (Bax/Bcl-2) ratio in hepatic tissues. However, treatment with CoQ10 ameliorated liver enzymes, anti-oxidant enzymes, up-regulated PPAR-γ and Nrf2 gene expression besides regulated inflammation and apoptosis. Histological hepatic improvement also fortified the defensive effects of CoQ10. These outcomes advocated CoQ10 as a potent natural medication that reduces the damaging effects of CP on hepatic tissues.

Introduction

Cyclophosphamide (CP) is an anticancer drug and immunosuppressive agent extensively applied for the treatment of both neoplastic and non-neoplastic diseases (Singh et al., 2018). Regardless of CP efficacy, its use is often restricted by related dangerous lethal outcomes, including hepatotoxicity, nephrotoxicity, reproductive toxicity and genotoxicity (Caglayan et al., 2018, Ekeleme-Egedigwe et al., 2019, Lin et al., 2017).

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Cyclophosphamide application can induce hepatotoxicity provoked by oxidative stress, activation of inflammatory cascade reactions, DNA damage and apoptosis resulting in biochemical and histological hepatic alterations (Mansour et al., 2017; Hamzeh et al., 2018).

The exact mechanism of CP toxicity resulting from acrolein metabolite as CP is converted into acrolein and phosphoramide mustard in the liver by hepatic microsomal cytochrome p-450 (Tong et al., 2017). Acrolein metabolite triggers reactive oxygen (ROS) species generation (Moghe et al., 2015). In turn, ROS provoke lipid peroxidation, inflammation and oxidative DNA damage that trigger the apoptotic signaling pathways and therefore implicated in CP organ toxicity (Hamzeh et al., 2018).

Enhancing the body's antioxidant defenses with natural antioxidants is fundamental for reducing the harmful effects of CP and its reactive metabolites. Because of this, effective drugs are required to protect healthy organs from the damaging adverse effects of chemotherapy (Caglayan et al., 2018).

Coenzyme Q10 (CoQ10), also known as ubiquinone, is the only naturally occurring, lipid-soluble vitamin-like substance that is produced endogenously (Littarru and Tiano, 2007). CoQ10 is abundant in meat, fish, nuts, broccoli, cauliflower, and certain oils so it can be taken exogenously with foods (Kubo et al., 2008).

Coenzyme Q10 is a strong lipophilic antioxidant for reducing free radicals levels in the body (Hussein et al., 2021) as it has the ability to reduce lipid, protein, and DNA oxidation (Bentov et al., 2014).

Coenzyme Q10 has anti-inflammatory and anti-apoptotic effects as many findings verified the preventive effects of CoQ10 in various forms of inflammatory and apoptotic tissue injury (Kabel and Elkhoely, 2017; El-Khadragy et al., 2020).

It is used as a dietary supplement and as a co-therapy for a wide range of illnesses, such as diabetes, cancer, cardio vascular diseases, and neurological disorders (Hernández-Camacho et al., 2018). Therefore, the goal of the current investigation was to evaluate coenzyme q10's protective outcomes against cyclophosphamide-induced hepatotoxicity in adult albino rats.

Materials and methods

Chemicals

Cyclophosphamide (C7H15Cl2N2O2P) is available as pale yellow crystalline odorless powder with ≥ 97% HPLC and CAS number 50-18-0 and coenzyme Q10 (C59H90O4) in the form of yellow to dark orange crystalline odorless powder with ≥ 98% HPLC and CAS number 303-98-0 were obtained from Sigma Aldrich Pharmaceutical Co., Steinheim, Germany. Distilled water as a solvent agent for cyclophosphamide and corn oil as a solvent agent for coenzyme Q10 (in the form of oily solution) obtained from El Gomhoria Pharmaceutical Co., Zagazig, Egypt.

Experimental protocol

Sixty adult male albino rats, weighing 180–200 g, were used in the study. The rat species were obtained from Zagazig University's Faculty of Medicine's Animal House. Prior to the trial, all rats had a two-week acclimatization phase. Rats were kept under standard environmental conditions (20–25 °C, 40–55% humidity, and a 12 h light/dark cycle) with free access to standard diet and water. The local committee authorized the experiment's design, and all animals were treated humanely in accordance with the National Institutes of Health's (NIH) guidelines for animal care **(Approval number: ZU-IACUC/3/F/3/202).**

This study design included six equal experimental groups, each containing ten rats. Group I was used as control (received regular diet and tap water). Group II: each rat was given distilled water (CP solvent) in a dose of 1ml once daily by oral gavage for 4 weeks. Group III: each rat was given corn oil (CoQ10 solvent) in a dose of 1 ml once daily by oral gavage for 4 weeks. Group IV: each rat was given

CoQ10 in a dose of 10 mg/kg body weight in 1ml corn oil once daily by oral gavage for 4 weeks (El-Sheikh et al., 2014). Group V: each rat was given CP in a dose of 8.2 mg/kg body weight {1/20 of LD50} in 1ml distilled water once daily by oral gavage for 4 weeks (Hagenbeek and Martens, 1982). Group VI: was given both CP and CoQ10 with the same doses for the same duration.

Sample collection

At the end of the experiment, intraperitoneal injection of pentobarbital l50 mg/kg was applied to induce anesthesia, then venous blood samples were collected by capillary tubes from the retro-orbital plexus with an orifice of 0.6 mm that was introduced into the orbit of the eye at an anterior angle, then revolved to drill through the conjunctiva in the direction of the site of the optic nerve and the blood spontaneously discharge into the capillary tube (Johnson, 2007), the separation of the serum was finished by blood centrifugation at 664 × g for 10 minutes, serum was used for the analysis of liver enzymes {alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyle transferase (γGT)}and inflammatory markers {tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β)}. After that, all rats were sacrificed by cervical dislocation, liver tissues were removed from each rat, and one portion of the tissue was immediately fresh frozen at −20 °C, transported on dry ice, and stored at −80 °C to obtain homogenates for the analysis of oxidative stress markers{malondialdhyde (MDA), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH)} and RT-PCR. The remaining components were quickly fixed in 10 % formal saline for histopathological and immunohistochemical studies.

Biochemical Study

Liver enzymes assay

Serum ALT (U/L) and AST (U/L) were determined using colorimetric kits and performed according to the procedure described by Reitman and Frankel (1957). Serum ALP (IU/L) was assayed using colorimetric kits and performed following the method proposed by Belfield and Goldberg (1971). Serum γGT (U/L) was assayed using colorimetric kits and performed according the method proposed by Persijn and van der Slik (1976). All kits used were obtained from Biodiagnostic Co., Egypt.

Pro-inflammatory markers assay

Serum TNF- α and IL-1β was calculated using rat TNF- α ELISA Kit with Cat. No. (MBS2507393) and rat IL-1β ELISA Kit with Cat. No. (ab255730) respectively acquired from MyBiosource (San Diego, California, United States) depend on Sandwich-ELISA principle. OD was estimated spectrophotometrically at a wavelength of 450 nm, where the values were correlated to the rat TNF-α and IL-1β concentration.

Antioxidant enzymes assay

Tissue superoxide dismutase (SOD) (U/mg) was determined using (SOD Kit Cus-abio Biotech Co., Ltd. Cat No. CSB-E08555r) and performed following the method suggested by Sun et al (1988), based on inhibition of nitroblue tetrazolium reduction. Tissue catalase (CAT) (U/g) was tested using (CAT MyBiosource kit, Inc. Cat No. MBS2600683) and performed according to Beers and Jr, Sizer (1952), based on peroxide removal method. Tissue reduced glutathione (GSH) (umol/mg) was tested spectrophotometrically according to the method suggested by Mannervik, B. (1999) based on reduction of glutathione disulfide to reduced glutathione by monitoring the oxidation of NADPH as visualized by a decrease in absorbance at 340 nm

Lipid peroxidation assay

Tissue malondialdehyde (MDA) (umol/g) was assessed at a wave length of 534 nm using MDA

colorimetric kits achieved from Biodiagnostic Co., Egypt, according to the method suggested by Ohkawa et al. (1979).

Reverse transcription-polymerase chain reaction (RT–PCR) Analysis:

Total RNA was extracted from hepatic tissue using Trizol. We used (Invitrogen; Thermo Fisher Scientific, Waltham, MA, USA) and HiSenScript™ RH (−) cDNA synthesis kit (iNtRON Biotechnology Co., South Korea) for cDNA synthesis according to the manufacturer's instruction. We achieved RT-PCR in Mx3005P real-time PCR system (Agilent Stratagene, USA) using TOPreal™ qPCR 2X PreMIX following manufacturer's instructions (Arisha et al., 2019). The PCR-cycling condition involves an initial denaturation at 95 ∘C/12 min, followed by 40 cycles of denaturation for 20 s/95 ∘C, hardening for 30 s/60 ∘C, and extension at 72 ∘C/30 s. The oligonucleotide-specific primers were manufactured by Sangon Biotech (Beijing, China) according to Khamis et al. (2020). Gapdh was applied as an internal reference gene to standardize the apoptotic genes expression. The outcomes were expressed as the ratio of reference gene to target gene by using the following formula: ΔCt = Ct (apoptotic genes)—Ct (Gapdh). The following formula was used: ΔΔCt = ΔCt (Treated)—ΔCt (control) to detect the relative expression levels. Thus, the expression levels were expressed as n-fold differences relative to the calibrator. The assessment was used to plot the expression of apoptotic genes using the expression of 2-ΔΔCt (Livak and Schmittgen 2001).

The primers being used:

Gene	Forward sequence	Reverse sequence	LOT
PPAR-γ	CCTGAAGCTCCAAGAATACC	GATGCTTTATCCCCACAGAC	NM_013124.3
Nrf2	CACATCCAGACAGACACCAG	CTACAAATGGGAATGTCTCTGC	NM_031789.2
NF-kB p65	TCTCAGCTGCGACCCCG	TGGGCTGCTCAATGATCTCC	AF_079314
Gapdh	GGCACAGTCAAGGCTGAGAATG	ATGGTGGTGAAGACGCCAGTA	NM_017008.4

II- Histopathological study

Hepatic tissues were sectioned into 5 um thick successive sections using a Leica RM 2135 Bio Cut Rotary Microtome, mounted on glass slides, stained with hematoxylin and eosin (H&E), and then checked under a light microscope. Hepatic tissues were fixed in 10% formal saline for 48 hours before use (Bancroft and Gamble, 2008).

III- Immunohistochemical study

Immunohistochemical examination was prepared in consistent with the phases demonstrated by Ramos-Vara et al. (2008). Hepatic sections were microwave-irradiated in 0.1 mol/l sodium citrate buffer (pH 6.0) for 20 minutes after deparaffinization and rehydration. Endogenous peroxidases were blanched with 3% H2O2, followed by a 10-minute rinse in Trisbuffer (pH 7.4). Rabbit monoclonal anti-Bax antibody (1:250; ab32503; Abcam, Cambridge, UK) and rabbit polyclonal anti-Bcl-2 antibody (1:50; ab59348; Abcam, Cambridge, UK) were incubated overnight on hepatic tissue before being washed in Tris buffer. By leaving out the primary antibodies, the specificity of the antibodies was observed. Hematoxylin was consumed as a counterstain after tissue visualization with 3.30-diaminobenzidine (DAB). Hepatic sections were then fixed with DPX and cover-slipped after being dehydrated in xylene.

IV- Morphometric study

The interactive measure menu on the image analyzer computer system Leica Qwin 500 (Leica Ltd, Cambridge, UK) at the Pathology Department, Faculty of Dentistry, Cairo University, Egypt, was used to estimate the area percent of Bax and Bcl-2 proteins. The measuring frame was selected to have a standard area of 118476.6 m2 so that the brown positive immune reaction could be noticed and evaluated. Ten readings from five non-overlapping sections were selected from each rat in all groups.

V-Statistical analysis

Data were shown as a mean and standard deviation (X±SD) for all groups. The Chicago, USA-based SPSS application, version 21, was employed. One-way analysis of variance (ANOVA), followed by the LSD test for multiple comparisons between various groups, was applied to statistically assess whether there was a significant difference. When the probability values (P) were less than 0.05, they were considered significant, and when they were less than 0.001, they were considered extremely significant (Petrie and Sabin, 2005).

Results:

There was no observed significant difference between the groups receiving distilled water, corn oil, and CoQ10 and the control group as regard all the measures parameters.

Liver enzymes

There was a highly significant elevation in serum liver enzymes levels in CP treated group when compared to the control group (P<0.001). While, there was a highly significant reduction in serum liver enzymes levels in CP+CoQ10 treated group towards the control when compared to CP treated group (P<0.001) as shown in fig -1

Pro-inflammatory markers

There was a highly significant elevation in serum TNF- α and IL-1β levels in CP treated group in comparison with the control group (P<0.001). While, there was a highly significant reduction in serum TNF- α and IL-1β levels in CP+CoQ10 treated group towards the control when compared to CP treated group (P<0.001) as shown in Fig -2.

Oxidative stress markers

There was a highly significant elevation in MDA level and a highly significant reduction in SOD, CAT and GSH levels of hepatic tissue in CP treated group in comparison with the control group (P<0.001). While,

there was a highly significant reduction in MDA level and a highly significant elevation in SOD, CAT and GSH levels of hepatic tissue in CP+CoQ10 treated group towards the control when compared to CP treated group (P<0.001) as shown in Fig -3.

RT-PCR

There was a highly significant reduction in PPAR-γ and Nrf2 expression levels and a highly significant elevation in NF-kB p65 expression level of hepatic tissue in CP treated group in comparison with the control group (P<0.001). While, there was a highly significant elevation in PPAR-γ and Nrf2 expression levels and a highly significant reduction in NF-kB p65 expression level of hepatic tissue in CP+CoQ10 treated group toward the control when compared to CP treated group (P<0.001) as shown in Fig -4.

Histopathological result

Histological examination of the control groups and CoQ10 group showed the same histological results. Slides showed the normal histological structure of the polygonal classic hepatic lobules that had tightly packed cords of hepatocytes radiating from central vein toward the periphery of the lobule and had rounded pale vesicular nuclei and eosinophilic cytoplasm. Some cells were binucleated. The blood sinusoids with their kupffer and endothelial cells lining were seen separating the hepatocytes cords. Portal areas were found at the corners of each adjacent hepatic lobule that contained branches of hepatic artery, portal vein and bile ducts (Fig. 5.a & 5.b &5.c & 5.d & 5.e). Sections of the CP-treated hepatic slides stained with H&E revealed loss of the normal organization of the hepatic lobules with dilated congested central and portal veins, increased perivascular connective tissue. The most affected lobules showed hepatocytes that had darkly stained pyknotic nuclei and vacuolated cytoplasm and others had marked vacuolated cytoplasm without nuclei. Congested and dilated blood sinusoids lined by distorted endothelium were also seen. Bile duct proliferations were noticed with marked periportal stroma (Fig. 5.f & 5.g & 5.h). H&E-stained sections of CP+CoQ10-treated hepatic slides

revealed improvement of the hepatic lobules organization with apparent normal central vein. Normal hepatocytes that had vesicular nuclei, some of them were binucleated and the others had pyknotic nuclei with vacuolated cytoplasm. Few dilated congested blood sinusoids were also seen. The portal area appears normal that contained branches of hepatic artery, portal vein and bile ducts (Fig. 5.i).

Immunohistochemical result:

Immunohistochemical analysis of the control groups, CoQ10 group revealed the same findings. Immunohistochemical reaction for Bcl-2 stained hepatic sections of the control groups and CoQ10 group revealed positive Bcl-2 immunoreaction in the cytoplasm of hepatocytes and endothelial cells lining blood sinusoids (Fig. 6.a & 6.b). In CP treated group, negative Bcl-2 immunoreaction was noticed in the cytoplasm of hepatocytes and endothelial cells lining blood sinusoids (Fig. 6.c). In CP+CoQ10 treated group, moderate improvement with moderate positive Bcl-2 immunoreaction was detected in the cytoplasm of most of hepatocytes and endothelial cells lining blood sinusoids (Fig. 6.d). Immunohistochemically stained sections for Bax in hepatic tissue of the control groups and CoQ10 group revealed negative Bax immunoreaction in the cytoplasm of hepatocytes and faint positive immunoreaction in the cytoplasm of some endothelial cells lining blood sinusoids (Figure 7.a & 7.b). In CP treated group, strong positive Bax immunoreaction in the cytoplasm of hepatocytes (Fig. 7.c). In CP+CoQ10 treated group, marked improvement with faint positive Bax immunoreaction in the cytoplasm of hepatocytes and moderate positive reaction in endothelial cells lining blood sinusoids (Fig. 7.d).

Morphometric result

There was a highly significant increase in Bax and a highly significant decrease in Bcl-2 expression levels with significant elevation in apoptotic index (Bax/Bcl-2 ratio) in CP treated group in comparison with the control groups (P<0.001). While, there was

a highly significant decrease in Bax and a highly significant increase in Bcl-2 expression levels with significant reduction in apoptotic index (Bax/Bcl-2 ratio) in CP+CoQ10 treated group towards the control when compared to CP treated group (P<0.001) as shown in fig-8.
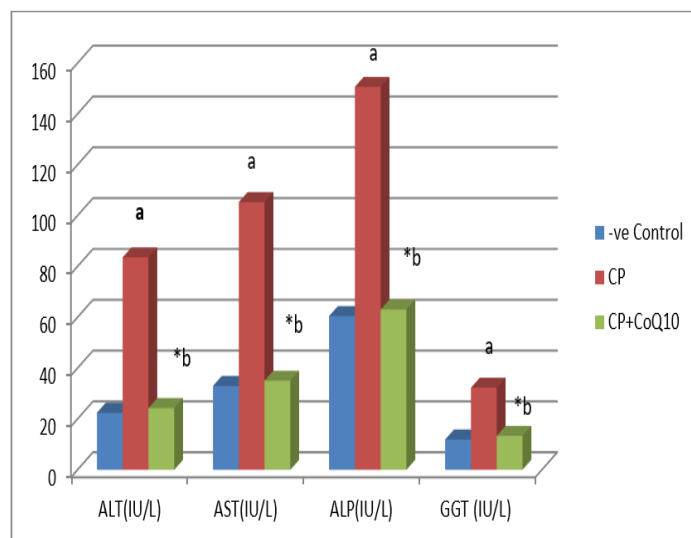
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Fig. (1): Bar chart showing comparative magnitude of mean values of serum ALT & AST & ALP & GGT (IU/L) in group I (–ve control), group V (CP) and group VI (CP+CoQ10) after 4 weeks of administration. *p<0.05, ap <0.001 when values are compared to control group, bp<0.001 when values are compared to CP group.
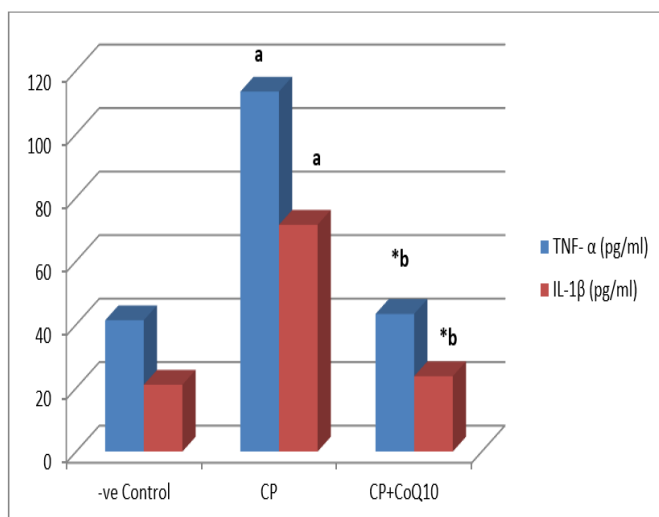

Fig. (2): Bar chart showing comparative magnitude of mean values of serum TNF- α (pg/ml) & serum IL-1β (pg/ml) in group I (–ve control), group V (CP) and group VI (CP+CoQ10) after 4 weeks of administration. *p<0.05, ap <0.001 when values are compared to control group, bp<0.001 when values are compared to CP group
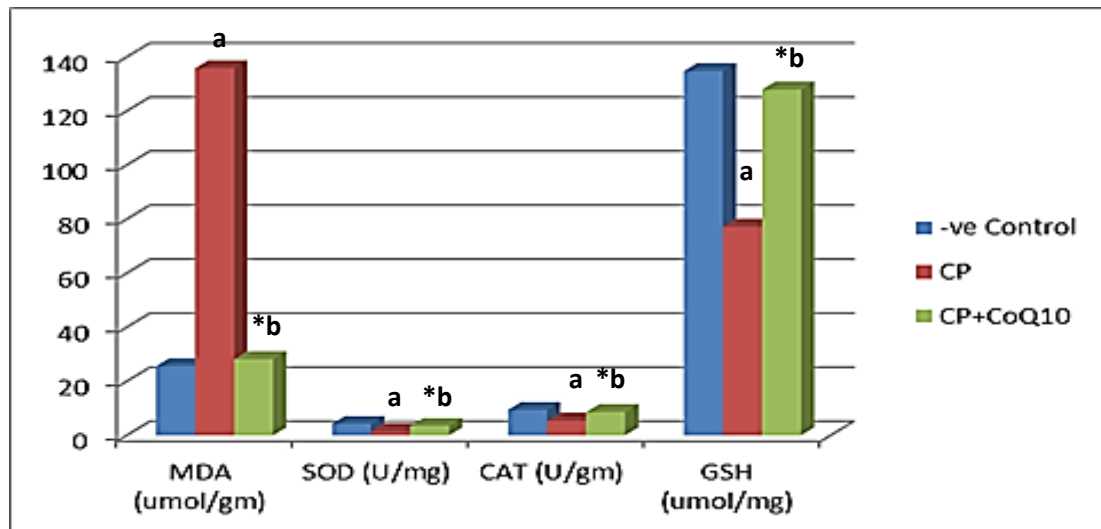
Fig. (3): Bar chart showing comparative magnitude of mean values of MDA (umol/gm) & SOD (U/mg) & CAT (U/gm) & GSH (umol/mg) in liver tissues in group I (–ve control), group V (CP) and group VI (CP+CoQ10) after 4 weeks of administration *p<0.05, ap<0.001 when values are compared to control group, bp<0.001 when values are compared to CP group.

Fig. (4): Bar chart showing comparative magnitude of mean values of PPAR-γ, Nrf2 and NF-kB p65 expression in liver tissues in group I (–ve control), group V (CP) and group VI (CP+CoQ10) after 4 weeks of administration. *p<0.05, ap<0.001 when values are compared to control group, bp<0.001 when values are compared to CP group.
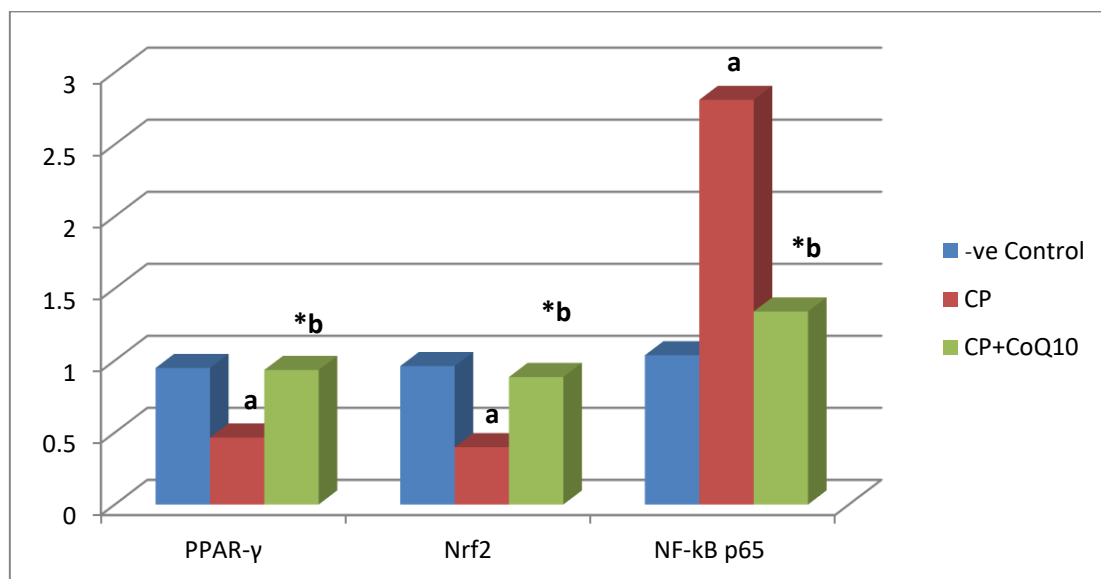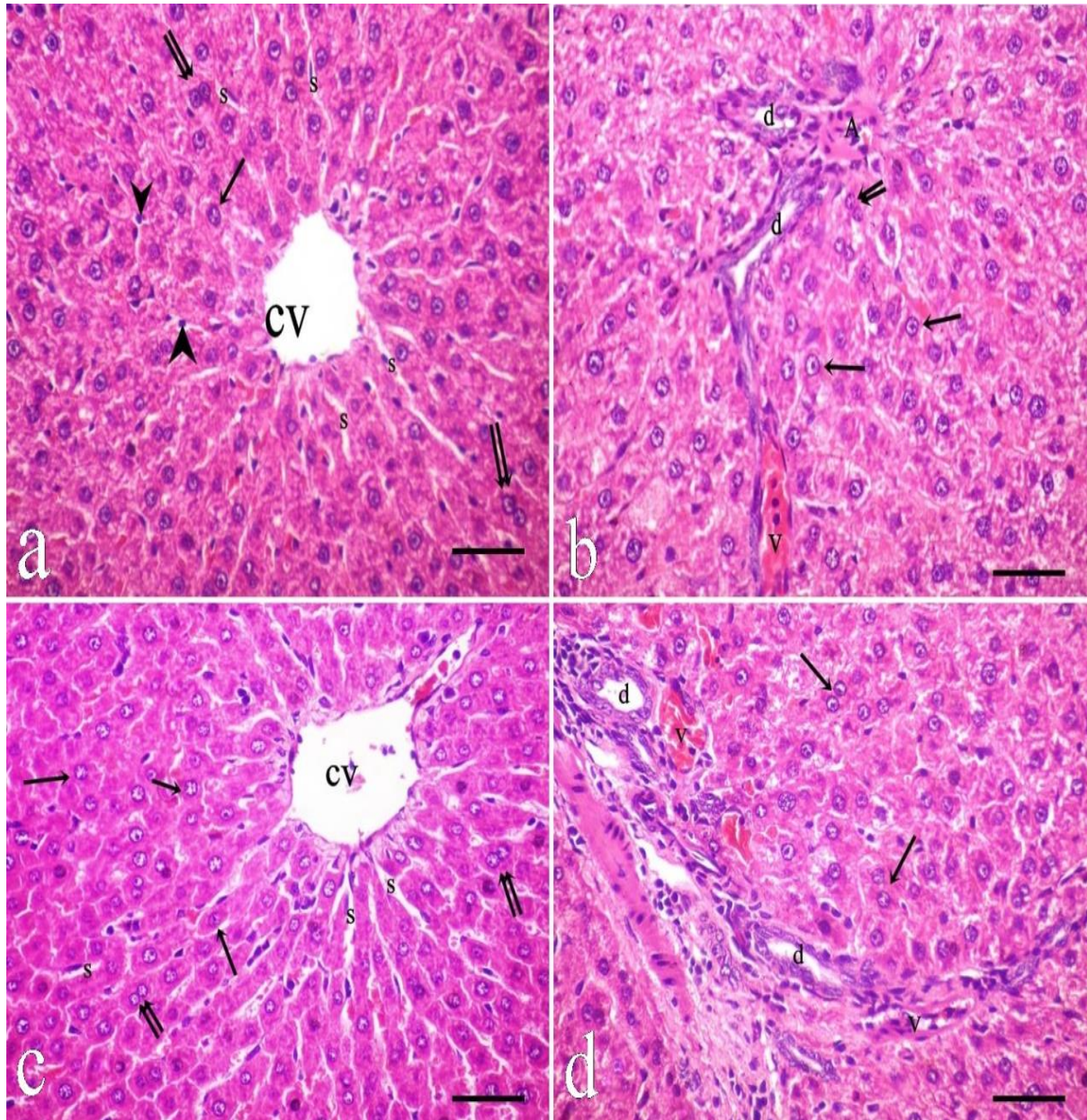
Fig (5). H&E-stained sections of **a:** control group showing the normal histological structure of the polygonal classic hepatic lobules with normal central vein (CV) and polygonal hepatocytes (arrow) with rounded vesicular nuclei and acidophilic cytoplasm. Some cells are binucleated (double arrow). Blood sinusoids (S) are observed inbetween hepatocytic cords with kuppfer cells (arrow head). **b:** control group showing normal portal area that contains branches of hepatic artery (A), portal vein (V) and bile ducts (d) surrounded by normal hepatocytes (arrow). Some cells are binucleated (double arrow). **C:** CoQ10 group showing the same normal hepatic histological structure with normal central vein (CV) and polygonal hepatocytes (arrow) with rounded vesicular nuclei and acidophilic cytoplasm. Some cells are binucleated (double arrow). Blood sinusoids (S) lined by endothelial cells are observed inbetween hepatocytic cords. **d:** CoQ10 group showing normal portal area that contains portal vein (V) and bile duct (d) that are surrounded by normal hepatocytes (arrow).
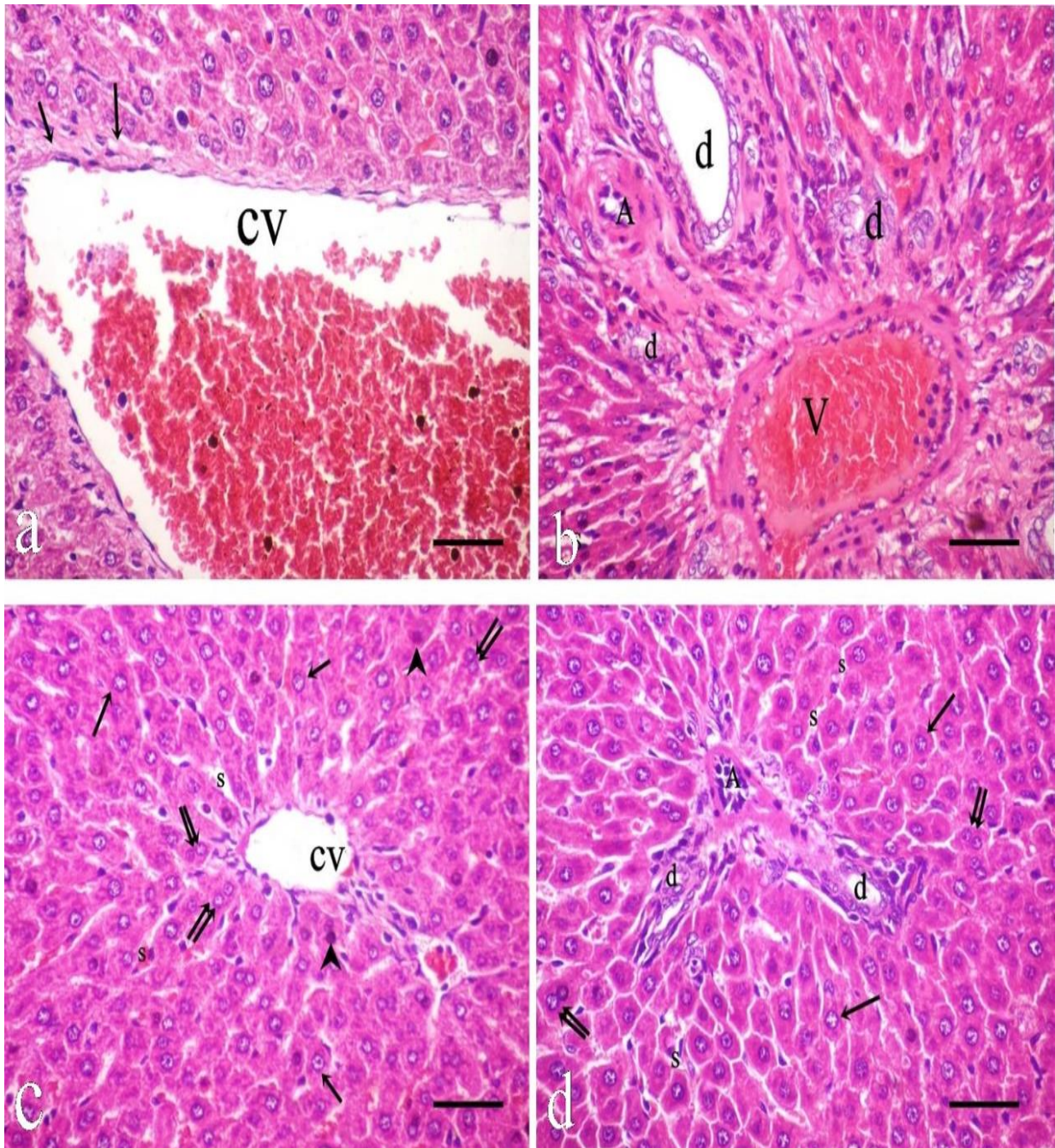
Fig. (6): **a:** CP group showing extremely dilated congested central vein (CV) and increased perivascular connective tissue (arrow). **b:** CP group showing a branch of hepatic artery (A) with thickened lumen, dilated congested portal vein (V) and bile duct proliferations (d).   **c:** CP+CoQ10 group showing improvement of the histological organization of the hepatic lobules with apparent normal hepatocytes (arrow) are radiating from normal central vein (CV). Some of hepatocytes are binucleated (double arrow) and others have pyknotic nuclei (arrow head). Few dilated blood sinusoids (S) are also seen. **d:** CP+CoQ10 group showing improvement of the portal area that contains a branche of hepatic artery (A) and bile ducts (d) with normal appearance. Normal hepatocytes (arrow), some of them are binucleated (double arrow). Slightly dilated blood sinusoids (S) are also noticed. All groups: H&Ex400 (scale bar 50 µm).
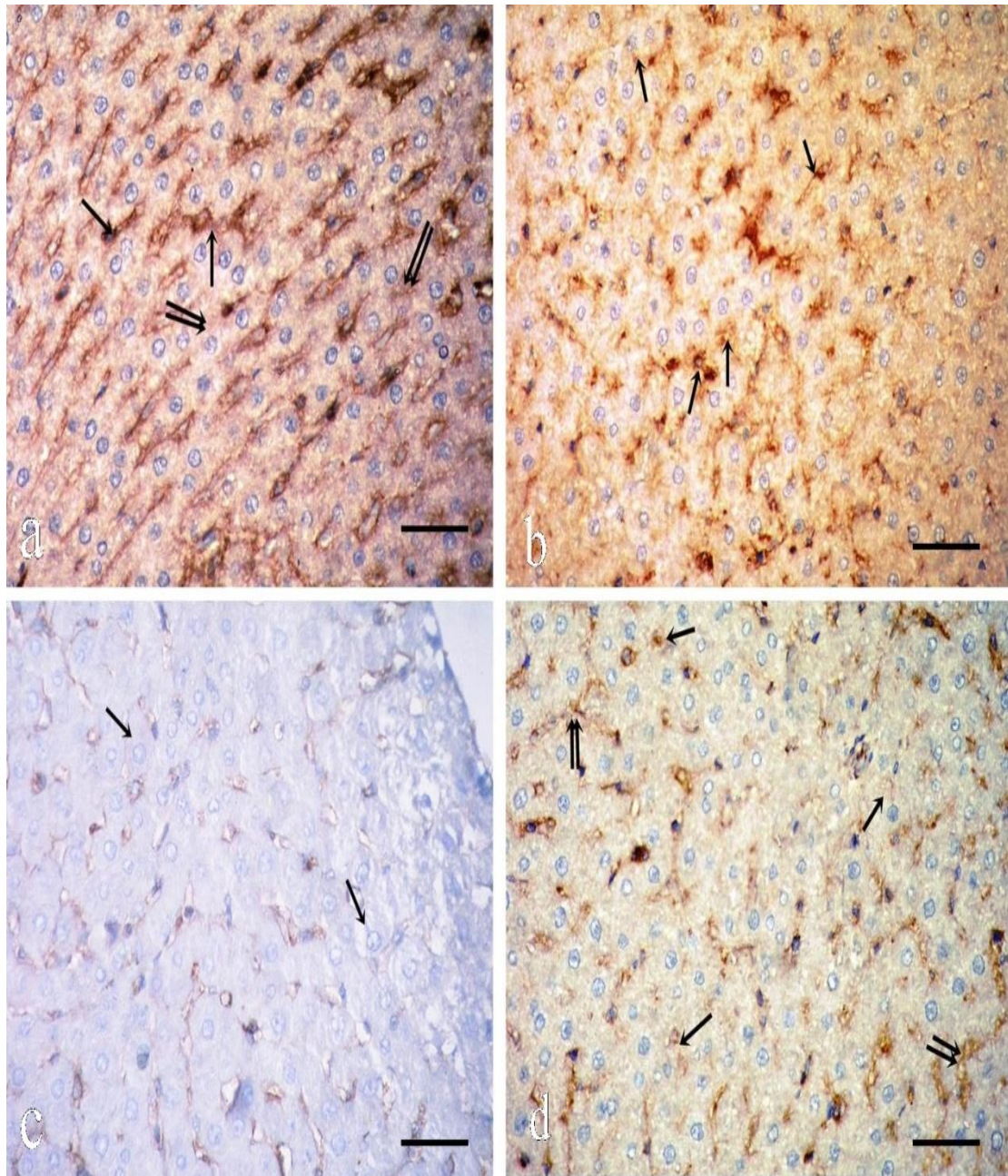
Fig. (7). **a**: Immunohistochemical reaction for Bcl-2 stained sections in the liver of the control groups showing strong positive immunoreaction for Bcl-2 in the cytoplasm of hepatocytes (arrow) and endothelial cells (double arrow) lining blood sinusoids. **b**: In CoQ10 treated group, strong positive immunoreaction for Bcl-2 in the cytoplasm of hepatocytes (arrow) and endothelial cells (double arrow) lining blood sinusoids. **c**: CP treated group showing negative immunoreaction for Bcl-2 in the cytoplasm of hepatocytes (arrow). **d**: CP+CoQ10 treated group reveals moderate positive immunoreaction for Bcl-2 in the cytoplasm of hepatocytes (arrow) and strong positive reaction in endothelial cells lining blood sinusoids (double arrows)., Bcl-2 ×400 (scale bar 50 mm).
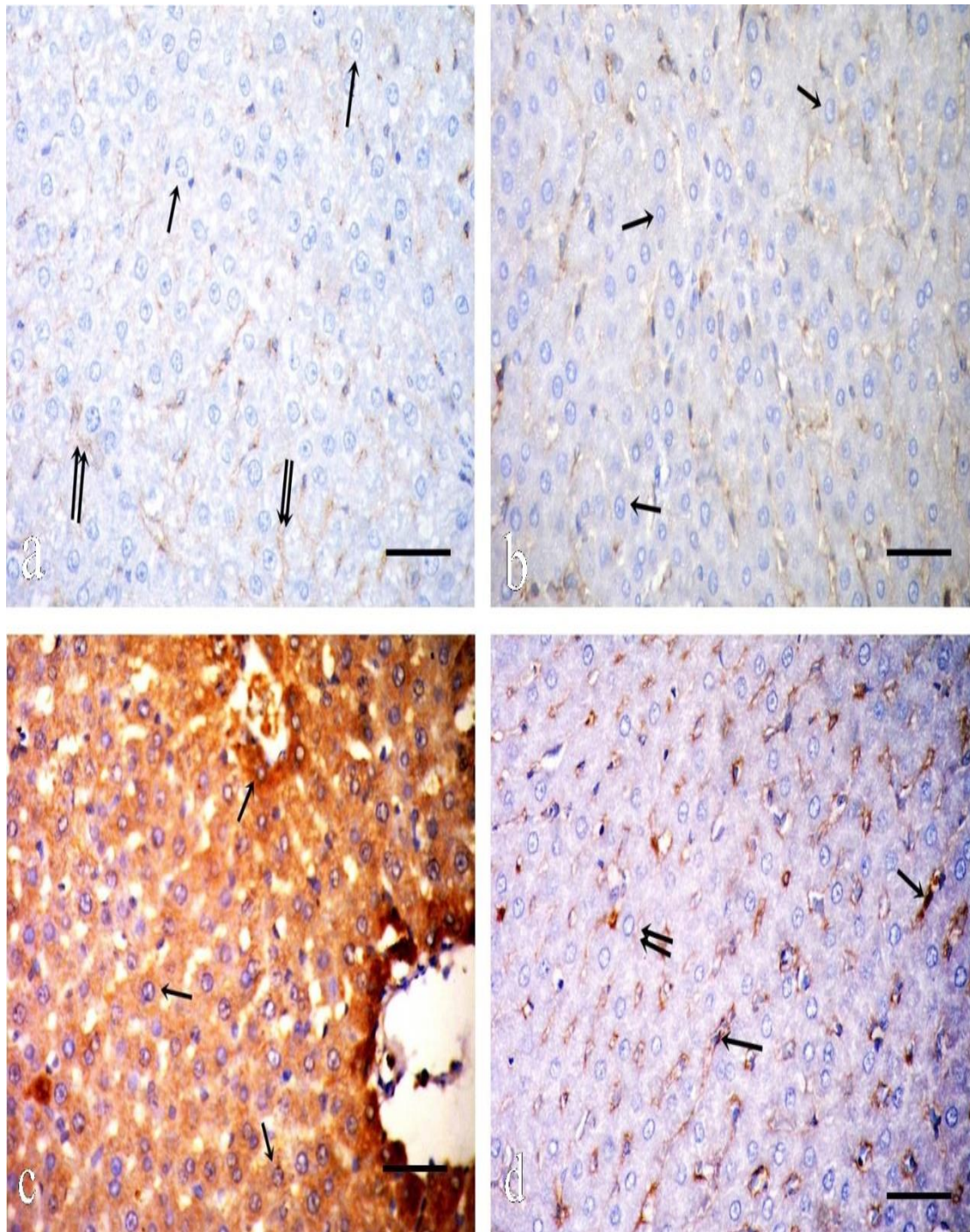
Fig. (8). **a**: Immunohistochemical reaction for Bax stained sections in the liver of the control groups showing negative immunoreaction for Bax in the cytoplasm of hepatocytes (arrow) and faint positive immunoreaction in the cytoplasm of some endothelial cells lining blood sinusoids (double arrows).   **b:** In CoQ10 treated group, showing negative immunoreaction for Bax in the cytoplasm of hepatocytes (arrow). **c**: CP treated group showing strong positive immunoreaction for Bax in the cytoplasm of hepatocytes (arrow). **d:** CP+CoQ10 treated group reveals showing faint positive immunoreaction for Bax in the cytoplasm of hepatocytes (double arrow) and moderate positive reaction in endothelial cells (arrow) lining blood sinusoids, Bax ×400 (scale bar 50 mm).
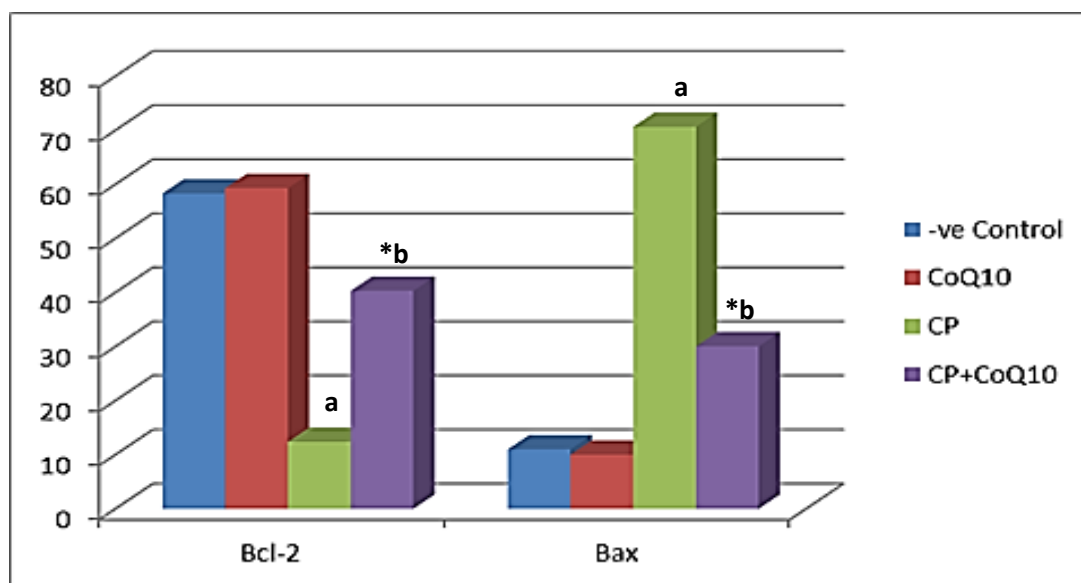
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Fig. (9): Bar chart showing area % of Bcl-2 and Bax immunoreaction in liver tissue in group I (–ve control), group IV (CoQ10), group V (CP) and group VI (CP+CoQ10) after 4 weeks of administration. *p<0.05, ap<0.001 when values are compared to control group, bp<0.001 when values are compared to CP group.

Discussion

The present findings were designed to evaluate the influence of CoQ10 on cyclophosphamide induced hepatotoxicity in adult male albino rats. According to the present study's findings, serum liver enzymes (ALT, AST, ALP and γGT) levels in the CP-treated group were significantly higher than those in the control group. These findings agreed with the results of Akamo et al. (2021). Cyclophosphamide toxicity induced marked reduction of serum liver enzymes levels due to induction of cytoplasmic cellular leakage, oxidative stress, inflammation, and disturbance in the functional integrity of hepatic membrane architecture (Kaur, 2019).

Our outcomes also revealed that CP caused a significant rise in hepatic MDA compared to control in accordance with the findings of Iqubal et al. (2020), confirming CP-induced lipid peroxidation. Caglayan et al. (2018) explained that lipid peroxidation is caused by CP attacks on the membrane phospholipids, which are extremely sensitive to ROS, resulting in MDA elevation. There was a highly significant decrease in SOD, CAT and GSH activities in hepatic tissues of CP-treated group

compared to control in accordance with the findings of Temel et al. (2020). Free radicals formed by CP can attack lipids and cause serious changes in membrane structure and function of anti-oxidant enzymes resulting in its depletion (Mahmoud et al. 2017).

The results of the current investigation revealed a highly significant increase in serum levels of TNF-α and IL-1β in CP-treated group in comparison with the control groups. These findings are in line with the study of Abd-ElRaouf et al. (2021). Inflammation is controlled and induced in part by oxidative stress (Reuter et al., 2010). Stress signaling and pro-inflammatory pathways can be activated by the sustained ROS and RNS production caused by CP application (Mahmoud, 2014). CP is converted into active metabolites (acrolein and phosphoramide mustard) by hepatic microsomal enzymes (Caglayan, 2019). Acrolein is cytotoxic and promotes intracellular ROS and NO production, which results in the generation of peroxide and the creation of oxidative stress (Sinanoglu et al., 2012). Kolios et al. (2006) explained that increased level of pro-inflammatory cytokines in liver tissues leads to the recruitment of neutrophils and other inflammatory

cells and activation of endothelial cells resulting in development of liver necrosis.

The results of the current investigation showed that there was a highly significant drop in PPAR-γ expression level in hepatic tissues of the CP-treated group compared to the control group. The current findings are along with the study directed by Mohammed et al. (2020). Zarei and Shivanandappa (2013) reported that CP administration also induced down-regulation of PPAR-γ gene expression that activated NF-κB signaling pathways which mediated triggering of pro-inflammatory markers (TNF- α and IL-1β). It was reported that PPAR-γ deficiency is associated with induction of oxidative stress, inflammation and apoptosis in liver of CP-treated rats but PPAR-γ activation protected against CP-induced hepatotoxicity through reduction of oxidative stress, inflammation and apoptosis (Mahmoud and Al Dera, 2015; Mahmoud et al., 2017). According to the present findings, there was a highly significant elevation in NF-kB p65 expression in hepatic tissues of CP-treated group compared to control. These findings are matched with Temel et al. (2020). It is widely known that CP activates NF-kB p65, which is transported into the nucleus and responsible for the transcription of inflammatory markers such as TNF-α, IL-1β, IL-6, COX-2, and iNOS (Cheng et al., 2019). Additionally, NF-kB p65 not only targets inflammation directly but also indirectly controls cell division, proliferation, and death (Liu et al., 2017). The main mechanism of CP-induced NF-κB signaling activation is CP-induced oxidative stress (Mahmoud et al., 2017). Oxidative stress activates NF-kB, which then produces pro-inflammatory cytokines that increase tissue damage (El-Kholy et al., 2017). Therefore, antioxidants may be able to guard against the harmful effects brought on by CP (Hamsa and Kuttan, 2010). In addition, present investigation revealed a highly significant decrease in Nrf2 expression level in hepatic tissues of CP-treated group compared to control. This finding is in a line with the study conducted by Sun et al. (2021). Iqubal et al. (2019) mentioned that Nrf2 inhibition may occur from the active CP metabolites' induction of ROS and suppression of the endogenous antioxidant defense system. Also, CP was discovered to disrupt the Nrf2/ARE/HO-1 antioxidant signaling pathway in rats by the same mechanism (Abd El-Twab et al., 2019).

The histological findings detected in the CP-treated group run in accordance with Iqubal et al. (2020) where they showed that CP exposure caused a significant morphological alterations in hepatic parenchyma, disorganization of hepatic structure, hepatocytes vacuolization with pyknotic nucleui, coagulation necrosis, central and portal vein dilatation with congestion, ductal proliferation, peripotal infiltration and sinusoidal dilatation with congestion. The mechanism by which CP induced hepatic histopathological alteration has been speculated to be associated with acrolein metabolite that triggers the ROS production (Aladaileh et al., 2019). ROS can provoke lipid peroxidation, protein carbonylation and DNA oxidation and subsequently disruption in the cell membrane structure and function. Acrolein can also activate pro-inflammatory mediators and multiple signalling pathways that can facilitate cell death (Hamzeh et al., 2018).

The highly significant increase in Bax with a highly significant decrease in Bcl-2 expression in cytoplasm of hepatocytes in CP-treated rats together with significant elevation of the apoptotic index (Bax/Bcl-2 ratio) runs in consistent with Cengiz et al. (2022) who observed that CP administration promotes hepatic apoptosis. CP induced imbalance of apoptosis-associated proteins such as pro-apoptotic protein Bax and anti-apoptotic protein Bcl-2 (Liu et al. 2016). This imbalance resulted in initiation of the fundamental apoptotic pathway with the release of

cytochrome c from the mitochondria, which started the basic apoptotic cascade and led to cell death (Kuzu et al., 2019). According to Mansour et al. (2017), CP-induced apoptosis is believed to be caused by increased levels of ROS/RNS in CP-treated rats, which then cause DNA damage and accelerate the rate of apoptosis.

Co-administration of coenzyme q10 with cyclophosphamide revealed a highly significant decrease in serum liver enzymes (ALT, AST, ALP, γGT) levels compared to CP-treated group. There are no previous reports described the outcome of coenzyme q10 on CP-induced hepatotoxicity, however, Khodir et al. (2021) described the hepato-protective role of CoQ10 on doxorubsin -induced hepatotoxicity by elevation of serum liver enzymes. According to Fouad and Jresat (2012) showed that CoQ10 protects rat's liver against acetaminophen-induced hepatotoxicity, most probably through its antioxidant, anti-inflammatory, and anti-apoptotic effects. As CoQ10 plays a critical role in the protection of membrane phospholipids and proteins present in the mitochondrial membrane from ROS-induced oxidative damage (Bentov et al., 2014).

Co-administration of CoQ10 with CP revealed a highly significant increase in SOD & CAT & GSH activities and a highly significant decrease in MDA activity in hepatic tissues compared to CP-treated group. These findings are in line with a study by Lamia et al. (2021). Hussein et al. (2021) reported that CoQ10 exhibits a prominent antioxidant effect and decreases the level of oxidative stress parameters. As it can scavenge free radicals and protected membrane phospholipids, mitochondrial membrane protein from oxidative stress and lipid peroxidation-induced damage (Fouad et al., 2013). Indeed, CoQ10 suppressed NADPH-oxidase expression, a great source of O2 • – (Ratliff et al., 2016), inhibits excess NO production (Abdel-Hady

and Abdel-Rahman, 2011) so can protect against lipid, protein and DNA oxidation (Pravst et al., 2010). Also, Co-administration of coenzyme q10 with cyclophosphamide revealed a highly significant reduction in serum levels of TNF-α and IL-1β compared to CP-treated group. Our findings are in accordance with the study directed by Saleh et al. (2017) who observed the protective effect of CoQ10 administration on acrylamide-induced hepatotoxicity in rats by marked decrease in serum levels of TNF-α and IL-1β. Coenzyme Q10 is convoluted in the prevention of inflammation in liver (Tarry-Adkins et al., 2016), kidneys (Kabel and Elkhoely, 2017) and testis (El-Khadragy et al., 2020). By hindering the release of pro-inflammatory cytokines like TNF-α and IL-1β, which are linked to organ damage, coenzyme q10 demonstrates anti-inflammatory properties (Mirmalek et al., 2016). Li et al. (2017) confirmed that CoQ10 decreases inflammatory cytokines levels resulting in inhibition of the matrix metalloproteinase which ultimately led to less inflammation and fibrosis.

Coenzyme Q10 significantly increased the PPAR-γ gene expression in hepatic tissues compared to CP-treated group. The present findings are in line with the report directed by Heidari et al. (2018) who reported the protective effect of CoQ10 administration on PPAR-γ gene expression in patients with diabetic nephropathy. Coenzyme Q10 may stimulate PPAR-γ gene expression via the calcium-mediated adenosine monophosphate activated protein kinase signal pathway. In addition, CoQ10 partially attenuated the influence of TNF-α on PPAR-γ suggesting the protective role of CoQ10 (Lee et al., 2012). Coenzyme Q10 is considered a novel PPAR-γ ligand with a potential for treatment of fatty liver diseases (Tiefenbach et al., 2018). When PPAR-γ is activated directly modulates the expression of several antioxidant genes, induces anti-inflammatory responses and the fibrogenic reaction

to liver injury is inhibited (Okuno et al., 2010; Yang et al., 2010). CoQ10 administration with CP revealed a highly significant increase in Nrf2 gene expression in hepatic tissues compared to CP-treated group. The current investigation supported by Mahmoud et al (2019) who demonstrated that CoQ10 pre-treatment safeguards hepatocytes against ischemia reperfusion-induced oxidative stress and apoptosis by increasing the expression of Nrf2 in rats. CoQ10 also significantly reduced NF-κB gene expression in hepatic tissues in accordance with Salama and Elgohary (2021) who found that CoQ10 co-treatment reduces NF-κB gene expression level in brain tissues following potassium dichromate exposure in rat models, which indicates the anti-inflammatory impact of CoQ10. According to Tarry-Adkins et al. (2016), CoQ10 activates the Nrf2/ARE pathway, which sequentially suppresses the production of the NF-kB p65 gene resulting in reduction of pro-inflammatory cytokines.

Co-administration of CoQ10 with CP showed marked improvement of the hepatic histological structure. The present investigation is along with the finding directed by Lamia et al. (2021) who stated that CoQ10 supplementation enhanced hepatic histological changes following carbon tetrachloride exposure, which is consistent with the findings of our investigation and suggests that CoQ10 can operate as a natural beneficial agent to prevent hepatic damage. Tarry-Adkins et al. (2016) found that CoQ10 significantly restored the apparent normal hepatic histoarchitecture by decreasing inflammatory cell infiltration and fibrous tissue deposition. Additionally, CoQ10 attenuated the hepatic oxidative stress and the intensity of inflammatory mediators which is the main cause of histological abnormalities (Mirmalek et al., 2016).

CoQ10 administration with CP revealed marked improvement with a significant reduction in Bax and rise in Bcl-2 expression. In addition, Apoptotic index (Bax/Bcl-2 ratio) revealed a significant reduction indicating the anti-apoptotic effect of CoQ10. The present study matched with the study demonstrated by Mahmoud et al. (2019) who stated that CoQ10 motivated regulation of apoptotic index (Bax/Bcl-2 ratio). Numerous studies have described CoQ10's anti-apoptotic properties (Kabel and Elkhoely, 2017). According to Papucci et al. (2003), CoQ10's anti-apoptotic properties are due to its ability to prevent DNA breakage, inhibit mitochondrial depolarization, and raise ATP level. Also, CoQ10 reduces the activity of the mitochondrial complex I, which inhibits the nuclear translocation of proteins that cause apoptosis and thus prevents cell death (Li et al., 2014). Moreover, Mahmoud and Al Dera (2015) reported that co-activation of PPAR-γ and Nrf2 (1) promoted expression of antioxidant proteins protecting cells from oxidative stress (2) down-regulated NF-kB and iNOS, and prevented the production of pro-inflammatory markers protecting cells from inflammation (3) protected against apoptosis by induction of Bcl-2 expression.

Conclusion:

Results of biochemical, histological, immunohistochemical, and morphometric examinations on adult male albino rats exposed to CP showed that this exposure caused apparent hepatic damage. Administration of CoQ10 may offer protection against these harmful consequences. It is recommended that clinicians should administrate CP with the proper dose and duration and need to consider possible side effects, interactions, and associated toxicities for patients before administering CP. For those patients, CoQ10 can be administered as a nutritional supplement to lessen the harmful effects of CP. It will take more research using various CoQ10 levels to evaluate the abilities of the antioxidant.

Disclosure statement

The author (s) did not disclose any potential conflicts of interest.

Data availability

Data can be available from corresponding author upon request.

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