Egy. J. Pure & Appl. Sci. 2015; 53(1):35-43

Egyptian Journal of Pure and Applied Science

Attenuation of Hepatorenal Toxicity Induced By Paracetamol and Gama Irradiation with Coenzyme Q10 Co-Supplementation in Male Albino Rats

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ARTICLE INFO

Article history:
Received 03 May 2015
Accepted 13 June 2015

Keywords:
Paracetamol;
Gamma Irradiation;
Coenzyme Q10;
Oxidative Stress.

ABSTRACT

Evaluation the protective role of coenzyme Q10 (CoQ10) on hepatorenal toxicity induced by paracetamol (PC) and/or γ-radiation exposure in male Swiss albino rats was undertaken in the present study. Rats categorized into eight treatment groups gavaged with CoQ10 (40 mg/kg BW) for two weeks concomitant with PC (200 mg/kg BW) and/or whole body γ-irradiation (3Gy/Week). Histopathological changes for liver and kidney tissues were examined. The results showed that PC as well as γ-irradiation induced a significant increase in ALT, AST, urea, creatinine, NO and MDA levels as an indication of liver and kidney damage compared with control animals. Hepatic levels of GSH and SOD were significantly reduced compared to normal rats. Supplementation of CoQ10 concomitant with PC and / or γ-radiation exposure normalized plasma levels of liver and kidney biomarkers and abrogated the enhanced oxidative stress as well as ameliorated the histopathological tissue damage. In conclusion, CoQ10 supplementation provided a potential protective effect against liver and kidney damage encountered with PC and γ-irradiation due to its free radical scavenging property.

Introduction

Many chemical compounds and clinically used drugs can cause cellular damage after metabolic activation to highly reactive compounds. One of the commonly used over-the-counter analgesics is paracetamol. The main problem with this medication is misuse through intentional or unintentional ingestion of supratherapeutic dosages, which usually lead to hepatic necrosis [1]. Paracetamol (acetaminophen, N-acetyl-*p*-aminophenol) is one of the most extensively used drugs worldwide for its analgesic, antipyretic and anti-inflammatory effects [2,3]. Although it is safe at therapeutic levels (10-15 mg/kg BW), an overdose can cause liver and kidney injury in humans [4,5]. PC is metabolized in liver by the cytochrome P450 system to form the highly reactive intermediate, N-acetyl-p-benzoquinoneimine (NAPQI). The NAPQI either binds irreversibly to cellular macromolecules resulting to cytotoxicity or can react with GSH that leads GSH depletion since GSH is essential for the protection of thiol and other nucleophilic groups in proteins from toxic metabolites that induced hepatic injury [6]. The binding of NAPQI with cellular proteins leads to mitochondrial dysfunction, oxidative and nitrative stress, and inflammatory reactions that eventually result in massive hepatocellular necrosis [7,8]. Along with the deleterious effects of ionizing radiation on biological system throughout the generation of reactive oxygen species (ROS) in cells [9,10], patients subjected to radiotherapy and consuming analgesic drugs such as PC were subjected to higher risk of oxidative stress toxicity during treatment course. Therefore, many compounds had been tested for their capability to protect against oxidative stress toxicity, and those possessing antioxidant properties contribute to the protection of cells and tissues against deleterious effects of ROS [11].

CoQ10 is a vitamin-like substance found throughout the body, especially in the heart, liver, kidney, and pancreas playing a key role in mitochondrial bioenergetics, prevention of DNA damage and lipid peroxidation [11,12]. CoQ10 has shown its potential in promoting cardiovascular health, combating aging, supporting healthy blood glucose levels and improving neurodegenerative diseases [13,14]. Thus our study was designed to evaluate the potential protective antioxidant

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activity of CoQ10 co-supplementation against the possible hepatorenal toxicity induced by PC and/or γ-radiation exposure in rats.

Materials and Methods

Chemicals

PC and CoQ10 were purchased from Sigma Chemical Company (USA). PC was suspended in warm distilled water and administered i.p daily for two weeks at a dose 200 mg/kg BW according to van der Kraan et al. [15]. CoQ10 suspended in distilled water gavaged daily for two weeks at a dose 40 mg/Kg BW according to Lund et al. [16]. All other chemicals and reagents used were of analytical grade and highest purity.

Animals

Adult male Swiss albino rats weighing 200±20g were obtained from the Egyptian Organization for Biological Products and Vaccines at Giza (Egypt) and housed for treatment in National Centre for Radiation Research and Technology, Cairo. Experimental animals were maintained with free access to standard laboratory pellet chow and water *ad libitum*. Rats were kept in the laboratory under controlled conditions of temperature (27 ± 2^0C) and humidity (60±5%) with 12h light/12h dark cycles in well ventilated cages during acclimatization period of 10 days. Rats were allowed free access to water but not food for 10 hours before the experiment.

Radiation Facility

Whole body gamma irradiation carried out at the National Centre for Radiation Research and Technology (Atomic Energy Authority, Cairo) using Caesium-137 in a Gamma Cell-40 Irradiator. Animals were exposed 3 Gy/week for two weeks at a dose rate of 0.769 Gy/min.

Experimental Design

The rats were randomly divided into eight equal groups (n = 6). First group rats received a single oral dose of distilled water and served as control untreated animals. Second group rats gavaged with CoQ10 (40 mg/Kg BW) daily for two weeks. Hepatorenal toxicity was induced in animals of the third, fourth and fifth groups by i.p. PC injection (200 mg/kg BW) and / or fractionated dose of whole body γ-irradiation (3Gy / week) for a period of two weeks. The rats of the sixth, seventh and eighth groups received CoQ10 (40 mg/Kg BW) daily for two weeks concomitant with i.p. PC injection (200 mg/kg BW) and/or fractionated dose of whole body γ-irradiation (3Gy / week). At the end of the experimental period, all animals were sacrificed 24 hour after the last treatments under urethane anesthesia (1g/Kg BW). Blood was drawn from the vena cava into heparinized syringe and plasma were separated by centrifugation at 3000 g for 10 minutes at 4^0C and stored at -20^0C pending analyses. A midline abdominal incision was performed in each animal and their livers and kidneys were harvested, perfused with cold isotonic saline and dried carefully. Washed tissues were sliced and homogenized in isotonic ice cold 0.25M sucrose. The crude homogenate was centrifuged at 5000 rpm for 10 min at 4°C and the cytosolic supernatant fraction was separated and frozen at -20^0C for further measurements.

Biochemical Analyses

Assay of plasma ALT, AST, creatinine and urea were carried out using colorimetric assay kits according to the manufacturer's instructions (Biodiagnostic, Egypt). Crude tissues homogenates were used for determination of NO levels according to Miranda et al. [17], MDA levels following Yoshioka et al. [18], GSH concentration according to Beutler et al. [19] and SOD activity following Minami and Yoshikawa [20].

Histopathological Examinations

Portions of livers and kidneys from all animals were fixed in 10% neutral phosphate-buffered formalin for 24 hours. Dehydration of tissue specimens in ascending grades of alcohol was performed. Tissues were embedded with melted hard paraffin, then left to solidify at room temperature to form blocks. Cutting of paraffin blocks using a microtome was done giving 4 µm thick sections. Mounting of sections on clean glass slides took place. Staining of sections with conventional haematoxylin and eosin (H&E) stain was performed. Examination of sections from all groups using microscope and assessment of various groups was performed [21].

Statistical Analyses

One-way analysis of variance (ANOVA) with Protected Least Significant Difference (PLSD) was used to test differences in means of variables between groups. A probability of $P<0.05$ was considered significant. All data were analyzed by SPSS 13 software for Windows.

Results

Liver

1. Biochemical Analyses

Treatment of animals with PC and / or γ-irradiation (groups III, IV and V) induced hepatic toxicity throughout the significant ($P<0.01$) increase in plasma ALT and AST activities (**Fig. 1A&B**) with concomitant significant increase in NO and MDA levels in comparison with normal control levels. GSH contents and SOD activities were reduced significantly compared to normal control group (**Table 1**). The co-administration of CoQ10 in groups VI, VII and VIII showed statistically significant ($P<0.01$) improvement versus γ-radiation and/or PC groups (III, IV and V) throughout the exhibited significant modulation in studied parameters that augment the hepatic toxicity.

2. Histopathology

Liver tissues either for control or CoQ10 co-supplementation groups showed normal hepatic architecture of normal parenchyma, vascular and stroma with no inflammatory infiltrate or fibrosis in the portal tracts and no degenerative changes (**Fig. 2 A**). Liver tissues from PC treated animals showed highly degenerative effects such fragmented tissue (curved arrow) and vacuolated aeries (↕) extending into hepatic lobules, leading to their complete separation with more necrotic cells, pyknotic nuclei and fatty degenerated liver cells than in the controls (**Fig. 2 B and B***). Liver steatosis was apparently detected after exposure to whole body γ-irradiation (**Fig. 2 C**). Permanent

observations of liver steatosis (▲) with infiltration of inflammatory cells (↓) were detected after treatment of animals with PC and whole body γ-irradiation (**Fig. 2 D and D***). Co-supplementation of CoQ10 with PC showed the presence of some liver steatosis (→) with the presence of foamy cytoplasmic structure (**Fig. 2 E**). Rats supplemented by CoQ10 and treated by γ-radiation showed normal structure in liver tissue (**Fig. 2 F**). Rats supplemented by CoQ10 and treated by PC and γ-irradiation characterized by recurrence of normal liver structure appearance (**Fig. 2 G**).

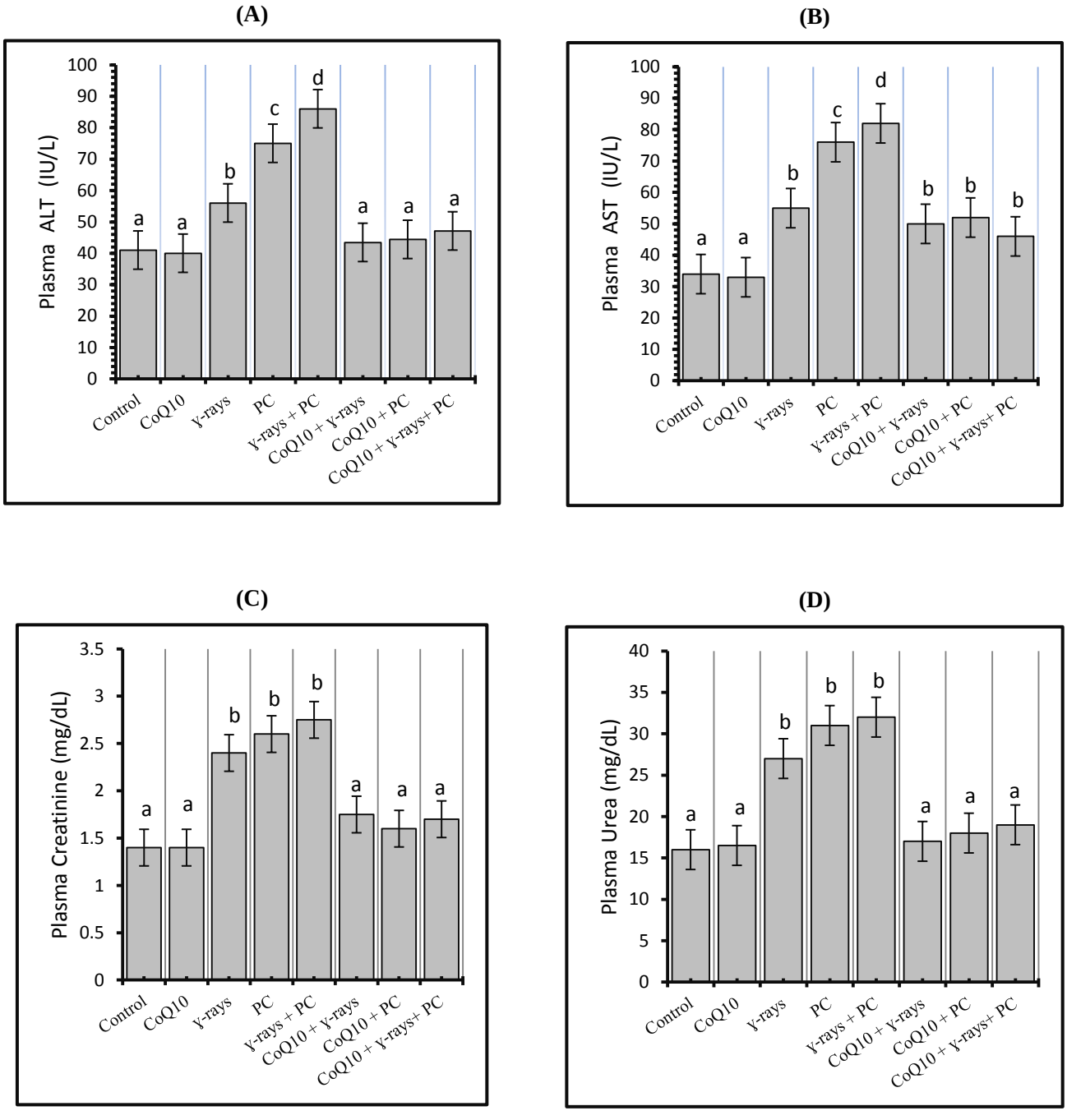

Fig. 1: Effects of the co-administration of CoQ10 upon rats plasma liver function markers (**A**) ALT, (**B**) AST and kidney function markers (**C**) creatinine, (**D**) urea exposed to PC and / or γ-radiation (ɣ-rays) for two weeks. Values are presented as means ± SEM of six observations.Bars sharing the same superscript are not significantly different (p>0.05).

Table 1: Effects of the co-supplementation of CoQ10 upon hepatic and renal oxidative stress indices in rats exposed to PC and / or γ-radiation for two weeks *.

Parameter / Group	NO (µmol/g tissue)		MDA (µmol/g tissue)		GSH (mg/g tissue)		SOD (U/g tissue)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Group I (Control)	769.6±13.7^{a}	775.2±30^{a}	106.6±10.8^{a}	72.6±4.5^{a}	10.5±2.8^{a}	7.7±0.7^{a}	9.3±2.2^{a}	8.4±1.1^{a}
Group II (CoQ10)	763.2±13.4^{a}	771.1±15^{a}	99.6±15.2^{a}	70.2±9.2^{a}	10.2±1.9^{a}	7.4±0.5^{a}	9.6±2.3^{a}	7.9±0.5^{a}
GroupIII (γ-rays)	855.2±14.5^{b}	863.7±19.3^{b}	146.6±10.1^{b}	97.9±6.5^{b}	8.1±1.97^{b}	5.6±0.2^{b}	7.2±1.7^{b}	4.3±0.4^{b}
Group IV (PC)	876.8±9.9^{b}	889.6±8.1^{b}	164.3±10.4^{b}	133.3±6.9^{c}	6.5±2.6^{c}	3.3±0.1^{c}	4.4±1.7^{c}	3.1±0.2^{b}
Group V (γ-rays+PC)	950.6±14.5^{c}	934.4±25.6^{c}	195.5±10.5^{c}	159.4±6.8^{d}	3.4±1.5^{d}	2.1±0.4^{d}	2.3±1.5^{d}	1.8±0.8^{c}
Group VI (CoQ10+γ-rays)	810.3±14.3^{d}	813.8±22.2^{d}	136.6±15.2^{b}	125.7±15^{c}	9.6±2.2^{a}	5.6±0.4^{b}	8.1±2.8^{b}	7.1±0.5^{a}
Group VII (CoQ10+PC)	825.2±14.5^{d}	815.6±14.4^{d}	116.2±10.8^{a}	81.2±10.3^{a}	9.2±2.1^{a}	5.4±0.2^{b}	7.9±2.2^{b}	7.4±0.7^{a}
Group VIII (CoQ10+γ-rays+PC)	817.6±13.5^{d}	813.3±25.1^{d}	110.2±4.8^{a}	78.2±8.5^{a}	9.3±2.3^{a}	6.3±0.3^{b}	9.2±2.1^{a}	7.7±0.3^{a}

* Each value represents mean of 6 records ± SD.

* Means±SD in the same **column** sharing the same superscript are not significantly different (p>0.05) (One-Way ANOVA-Protected Least Significance difference (PLSD) - Fisher post hoc test).

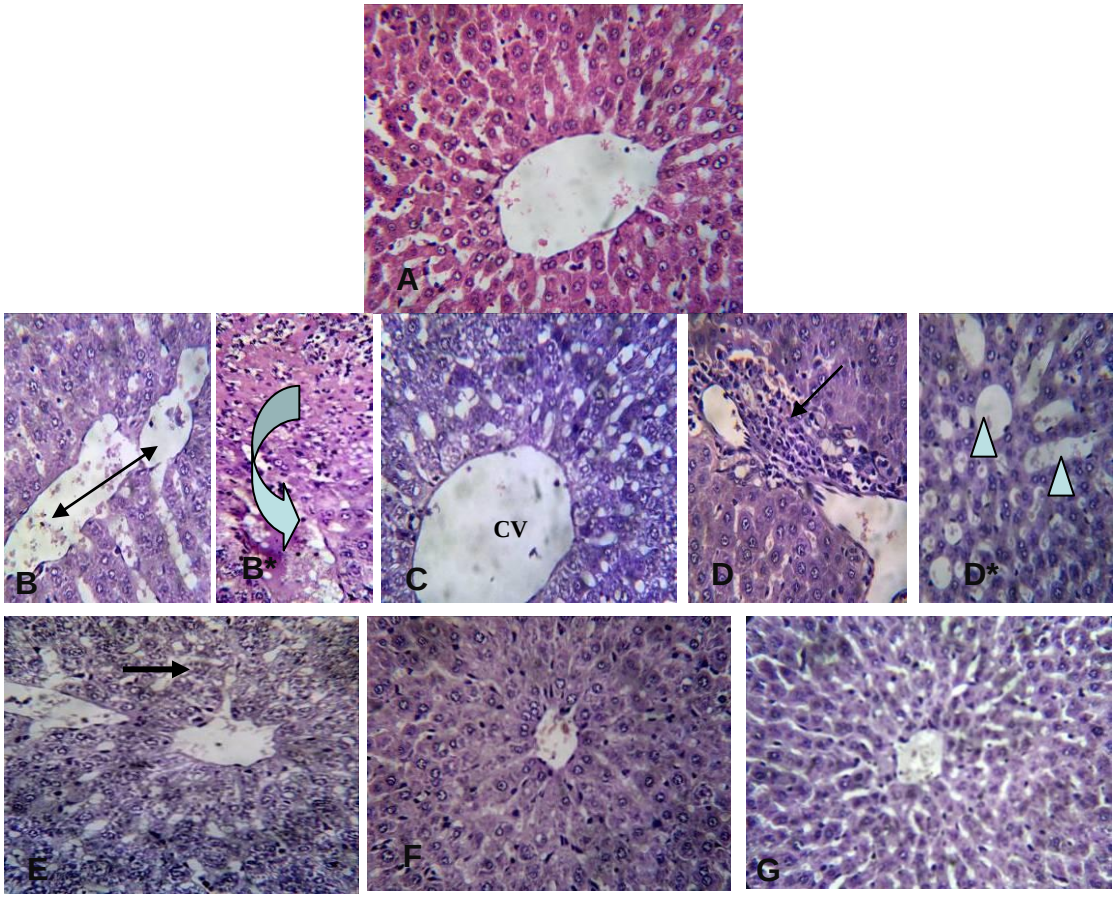

Fig. 2: Histopathological observations of the role of co- supplementation of CoQ10 in regenerating **liver** of albino rat treated by PC and γ- radiation exposure. **A:** Control liver, **B, B*:** Liver of rat treated by PC. C: Liver of rat exposed to γ- radiation. **D, D*:** Liver of rat treated by PC and exposed to γ- radiation. **E:** Liver of rat co-supplemented by CoQ10 and treated by PC. **F:** Liver of rat co-supplemented by CoQ10 and exposed to γ- radiation. **G:** Liver of rat supplemented by CoQ10, treated by PC and exposed to γ- radiation.

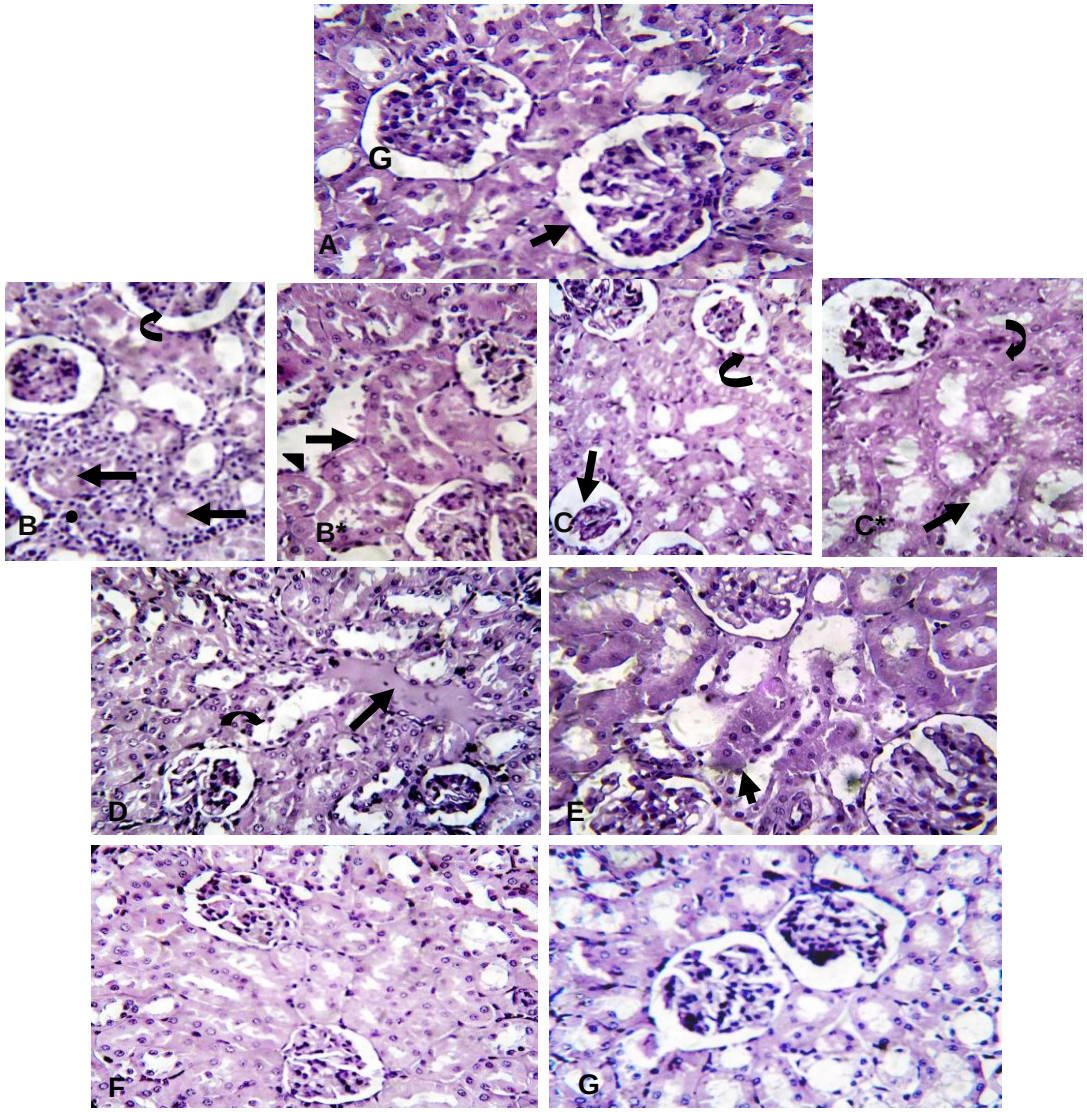

Fig. 3: Histopathological observations of the role of co- supplementation of CoQ10 in regenerating **kidney** of albino rat treated by PC and γ- radiation exposure. **A**: Control kidney. **B, B*:** kidney of rat treated by PC. **C, C*:** kidney of rat exposed to γ- radiation. **D:** kidney of rat treated by PC and exposed to γ- radiation. **E:** kidney of rat co-supplemented by CoQ10 and treated by PC. **F:** kidney of rat co-supplemented by CoQ10 and exposed to γ- radiation. **G:** kidney of rat supplemented by CoQ10, treated by PC and exposed to γ- radiation.

Kidney

1. Biochemical Analyses

Treatment of animals with PC and / or γ-irradiation (groups III, IV and V) induced renal toxicity throughout the significant (P<0.01) increase in plasma creatinine and urea levels (**Fig. 1 C&D**) with concomitant significant increase in NO and MDA levels in comparison with normal control levels while GSH contents and SOD activities were reduced significantly (P<0.01) compared to normal control group (Table 1). The co-administration of CoQ10 in groups VI, VII and VIII showed statistically significant (P<0.01) amelioration versus γ-radiation and / or PC groups (III, IV and V) throughout the exhibited significant modulation in studied parameters that augment the renal toxicity.

2. Histopathology

Kidney sections derived either from the control or CoQ10 co-supplementation groups showed the circular areas of the renal Malpighian corpuscle (↑) (**Fig. 3 A**). Rats given PC showed renal lesions included tubular necrosis and degeneration (↑) along with interstitial inflammatory cells (●) in addition of atrophied glomeruli (curved arrow). Some of the distal convoluted tubules cells appeared free from nuclei (arrow), some cells contained marginal chromatin (▼), and debris of ruptured cells could be detected (**Fig.3 B and B***). Kidney section of the experimental animals exposed to γ-radiation showed atrophied glomeruli, widened Bowman's space (↑) with high cellularity in the visceral layer of the Bowman's capsule (curved arrow). Some of the distal convoluted tubules cells appeared free from nuclei (curved arrow) and others showed marginal chromatin (↑) (**Fig. 3 C and C***). Treatment of animals with PC and γ-radiation exposure exhibited a highly obstruction of the convoluted tubules, presence of

fibrotic and bleeding lesion (↑) with the presence of some of the distal convoluted tubules cells contained marginal chromatin and debris of ruptured cells (curved arrow) (**Fig.3 D**). Co-supplementation of the experimental animals with CoQ10 and PC treatment recording some recurrence of normal appearance of tissue section structure however, some of convoluted tubules appeared obstructed and their cells showed hydropic degeneration (↑) (**Fig. 3 E**). Treatment of PC and γ-radiation groups by coenzyme Q10 predicts a return to normal structure of kidney tissue (**Fig.3 F and G**).

Discussion

The search for effective therapeutical agents for the treatment of drug- or chemical-induced injuries in liver and kidney is critical and of great interest [12, 22]. Thus, the primary objective of the present research was to determine the toxic versus reversible events with hepatic and renal dysfunction as result of γ-radiation and / or PC exposure with CoQ10 co-supplementation in male albino rats.

In our study, it was found that treatment of animals with PC and γ-irradiation increased ALT, AST, creatinine and urea in plasma. Concomitant elevations in the levels of NO and MDA contents with marked depletion of antioxidant markers GSH and SOD contents were observed. PC and whole body γ-irradiation induced hepatocytes lyses and cytosolic leakage resulted in elevated levels of ALT and AST since these enzymes are located in the cytosol and released into the blood following liver damage [23]. The oxidative stress mediated by PC metabolite, N-acetyl-p-benzoquinonimine (NAPQI) is considered as the main cause of hepatorenal toxicity [24]. In our study, a significant renal impairment in animals treated with PC and / or whole body γ-irradiation was demonstrated by an increase in plasma urea and creatinine. In renal diseases, urea accumulates because the rate of serum urea production exceeds the rate of clearance, while the elevation in plasma creatinine is observed only with marked damage to functioning nephrons. At doses within the therapeutic range, PC has been found to affect renal function by lowering renal blood flow, glomerular filtrationrate, sodium excretion and prostaglandin E2 excretion in both human and rat [25]. Elevation in NO levels was through the oxidative stress-associated mechanism involving nitric oxide synthase (NOS) due to superoxide anion overproduction. NO reacts with superoxide anion to form peroxynitrate that causes further cell damage by oxidizing and nitrating cellular macromolecules. Also, excess NO depletes intracellular GSH, increasing the susceptibility to oxidative stress [26]. MDA is a lipid peroxidation product that reflects the interaction between molecular oxygen and polyunsaturated fatty acids. Cell membranes have high polyunsaturated fatty acids content so they are particularly susceptible to peroxidation attack by ROS [27]. Our experimental data demonstrated that treatment of animals with PC and/or γ-irradiation increased the level of hepatic and renal lipid peroxidation due to increase production of reactive oxygen species which was in agreement with Ghosh and Sil [28] and Cigremis et al. [29] studies. Sena et al. [30] found that CoQ10 and/or α-tocopherol decrease glycated hemoglobin and pancreatic lipid peroxidation, concluding that these antioxidants increase some components of the antioxidant defense system. Moreover, GSH levels and SOD activities were decreased in livers and kidneys of PC and/or γ-radiation exposure treated animals. One of GSH key functions is to serve as reductant for toxic peroxides, and it also helps in keeping the enzymes in an active state by preventing the oxidation of –SH (sulfydryl) group to-S-S- (disulfide) group [31]. Extensive depletion of GSH leads to cell death [32].The decrease in GSH level and SOD activity in accordance with the data reported by Pradeep et al. [33] in case of γ- radiation exposure and with Babu et al. [34] in case of PC over dosage. Also, Irshad and Chaudhuri [35] indicated that there is a close relationship between depletion of GSH and antioxidant enzymes with increase in lipid peroxidation based on the excessive formation of ROS.

The histopathological examinations of liver tissues from PC treated animals showed a highly fragmented tissue, vacuolated areas with the presence of necrotic cells, pyknotic nuclei and fatty degenerated liver cells. These observations may be attributed to NO cytotoxicity and peroxynitrite which produced from the controlled reaction between NO and superoxide anion which trigger cellular responses ranging from subtle modulations of cell signaling to overwhelming oxidative injury, committing cells to necrosis or apoptosis [36]**.** Liver steatosis after exposure to fractionated dose of whole body γ-irradiation and / or PC was detected with the presence of inflammatory cells infiltration. Also, PC administration leads to renal lesions included tubular necrosis with the presence of interstitial inflammatory cells and atrophied glomeruli. Kidney sections of the experimental animals exposed to γ- radiation illustrated atrophied glomeruli, widened Bowman's space, high cellularity in the visceral layer of Bowman's capsule, and highly affected cytoplasm and nuclei of convoluted tubules. This confirm the findings of Agostino et al. [37] research which stated that the whole irradiated animals had severe renal damage involving glomeruli, tubules, interstitial tissue and blood vessels.

In our goal, the focus being oriented towards CoQ10 since it clearly pronounced in the amelioration of lipid peroxide content and antioxidant status in treated rats. The treatment revealed a significant ameliorated decrease in ALT, AST, creatinine, urea, and NO levels in comparison to PC and / or γ-radiation treated groups. It was reported that CoQ10 inhibits the generation of reactive oxygen species [38], scavenges lipid peroxidation products during free radical reactions [39], suppresses excess NO production and prevents nitrative tissue stress [40]. In addition, CoQ10 exhibits anti-inflammatory properties by reducing the release of proinflammatory cytokines during inflammatory injury [41,42]. A relatively

lower degree of hepatic damage in the CoQ10 treated groups is indicative of the hepatoprotective nature of PC [43]. Moreover, it may be due to the interference of CoQ10 in PC metabolism pathway or by inhibiting its conversion to its toxic metabolite NAPQI. Thus, supplementation of CoQ10 in diet is reported to help maintaining a favorable antioxidant / pro-oxidant balance so that decreasing ROS generation [44]. Ghule et al. [45] investigated the effect of pretreatment with CoQ10 (100 mg/kg) on isoproterenol (ISO)-induced cardiotoxicity and cardiac hypertrophy in rats. They found that pretreatment with CoQ10 (100 mg/kg) for 18 days was associated with moderate protection against ISO-induced cardiotoxicity and cardiac hypertrophy, and with lower myocardial injury by preserving endogenous antioxidants.

Treatment with CoQ10 recorded a development in liver structure for PC or whole body γ-irradiation treated group either alone or combined. All nuclei appeared to be rounded, oval containing one or two nucleoli and improvement of DNA patterns inside the nucleus was observed. Also, CoQ10 induce a return to normal structure of kidney tissue after final experimental treatments. Fouad and Jresat [26] observed throughout immunohistochemical analysis that coenzyme Q10 significantly decreased the PC-induced overexpression of inducible nitric oxide synthase, nuclear factor-$_K$B, caspase-3 and p53 in liver tissue. It was concluded that coenzyme Q10 protects rat liver against acute PC hepatotoxicity, most probably through its antioxidant, anti-inflammatory and antiapoptotic effects. Also, Fouad et al. [46] along with Phillipson [12] found that coenzyme Q10 significantly suppressed lipid peroxidation, prevented the depletion of reduced glutathione and superoxide dismutase activity, and decreased the elevations of tumor necrosis factor-α and nitric oxide in liver tissue of rats with hepatocellular carcinoma.

Conclusion

Our findings concluded that oral co-supplementation of CoQ10 reduced the hepatorenal toxicity induced by PC and / or whole body γ-irradiation by improving the antioxidant status which could support its potential use in antioxidant therapy.

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