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Potential efficacy of Coenzyme Q10 against oxytetracycline-induced hepatorenal and reproductive toxicity in male rats

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ABSTRACT

This study was performed to assess potential efficacy of Coenzyme Q10 (CoQ10) to restore oxytetracycline (OXT)-induced toxicity. Rats were distributed into four equal groups (6 each). The Control group received propylene glycol (0.5 ml p.o). CoQ10 group received Coenzyme Q10 (10 mg/kg BW p.o). OXT group (rats were injected intraperitoneally with 200 mg/kg BW oxytetracycline hydrochloride). OXT+CoQ10 group (rats were given the combined treatments). Treatments were used daily for seven days. Results indicated that OXT induced significant changes in serum biochemical parameters, significant increase of lipid peroxidation and exhaustion of reduced glutathione and catalase in liver, kidney and testis. Also, OXT reduced serum testosterone levels besides sperm motility and viability. Histopathological changes were marked in liver, kidney and to some extent in testis and epididymis. CoQ10 treatment restored oxidative stress in liver, kidney and testis, improved serum testosterone levels, sperm motility/viability as well as histopathological alterations. In conclusion, short-term administration of OXT induced hepatorenal and male reproductive toxicity through oxidative stress. This study is the first to suggest that CoQ10 could provide partial protection against OXT-induced hepatorenal/reproductive toxicity in male rats.

INTRODUCTION

Oxytetracycline (OXT) is a broad-spectrum antibiotic that is widely used in controlling respiratory diseases and other genital and skin infections in human and animals. Large doses of OXT without medical supervision have deleterious effects on kidney and liver (Abdel-Daim and Ghazy, 2015). While some studies reported no adverse effects of OXT on spermatogenesis (Abbitt *et al.*, 1984), other studies have shown that repeated doses of OXT have toxic effect on mature sperm in-vivo (Oyedeji *et al.*, 2013) and in-vitro (Hargreaves *et al.*, 1998). OXT was shown to exert its toxic effect via peroxidation of membrane lipids, mitochondrial damage, and impairment of the respiratory chain (Fromenty and Pessayre, 1997). Also, reduced tissue antioxidants biomarkers are associated with OXT overdose (Gnanasoundari and Pari, 2006).

Several studies investigated the effect of antioxidants to counteract OXT-induced oxidative stress and to alleviate its side effects in experimental animal models. For example, supplementation of ascorbic acid (Naseer and Alam, 1987), Naringenin "natural flavonoid" (Gnanasoundari and Pari, 2006), and Curcumin (Helal *et al.*, 2011) showed protective action against OXT detrimental effects on liver and kidney.

Coenzyme Q10 (CoQ10) is a fat-soluble substance that is primarily present in mitochondria where it plays a role in electron transport chain and plays a part in aerobic cellular respiration and ATP production. Therefore, organs of high energy requirement such as liver, kidney, and heart have higher concentrations of CoQ10 (Aberg *et al.*, 1992). Seminal plasma and spermatozoa also contain considerable amount of CoQ10 for protection against oxidative stress and preserving sperm integrity (Mancini and Balercia, 2011). Levels of CoQ10 in seminal plasma correlate significantly with sperm concentration and motility (Alleva *et al.*, 1997).

To the best of the authors' knowledge, the protective efficacy of CoQ10 against OXT-induced toxicity has not been

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investigated previously. Therefore, the present study was planned to assess efficacy of supplementing CoQ10 on oxidative stress status, liver/kidney function, semen quality and histopathology in OXT-intoxicated rats.

MATERIALS AND METHODS

Animals and experimental design

Adult male albino rats (170-200 g) were obtained from a closed colony at Faculty of Agriculture, Alexandria University, Egypt. Rats were maintained in metal wire cages with free access to food and water. Twenty-four rats were allocated into four identical groups, six each. Control group, rats were administered 0.5 ml of 20% propylene glycol using stomach tube. CoQ10 group, rats were administered Coenzyme Q10 that was dissolved in 20% propylene glycol (10 mg/kg BW) using stomach tube (El-Sheikh *et al.*, 2012). OXT group, rats were injected intraperitoneally with 200 mg/kg BW oxytetracycline hydrochloride solution (delta oxy® 5%, Delta Pharma Company, Egypt) (Saraswat *et al.*, 1997). OXT+CoQ10 group, rats were given the combined treatments with 1 hr interval. Coenzyme Q10 was obtained from Mepaco-Medifood Company, Egypt. Treatments were used daily for seven days. This study was permitted by the committee of scientific research ethics, Faculty of Veterinary Medicine, Alexandria University.

Body and relative organs weights

Rats' body weight was recorded just prior start of the experiment with insignificant difference between all groups. On the 8th day of the experiment, every rat was weighed, euthanized and dissected. Liver, kidneys, heart, testis, epididymis and accessory sex glands (prostate and seminal vesicle) were grossly examined and weighed. Index weight (IW) of each organ was calculated as follow: IW = organ weight (g)/body weight (g) × 100.

Hematological, biochemical and testosterone level evaluation

On the 8th day of the experiment, two blood samples were drained just before euthanasia from every rat. One blood sample was collected in heparinized tubes for hematological analysis and the other one was collected in plain tubes for serum separation. Serum was kept frozen at –20°C until used for biochemical and hormonal analysis. Hematological parameters were automatically measured by Mindray BC-30S Auto CBC Blood Hematology Analyzer Device. The evaluated hematological parameters were packed cell volume (PCV%), red blood cells (RBCs), white blood cells (WBCs) counts and hemoglobin (HB) concentration. All biochemical parameters were tested colourimetrically using commercial kits (Biodiagnostic, Chemical Co., Egypt). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were estimated according to Reitman and Frankel (1957) technique. Alkaline phosphatase (ALP) and gamma glutamyle transferase (GGT) were measured as the methods described by Kind and King (1954) and Szasz (1974), respectively. Serum total protein and albumin were estimated by using the method of Doumas et al. (1971). Serum globulin was calculated by subtracting the albumin value from total protein value of the same sample (Coles, 1974). Serum total bilirubin was analyzed according to Walter and Gerade (1970) method. Serum total cholesterol and triglycerides activities were estimated according to Johnson *et al.* (1999) and Fassati and Prencipe (1982), respectively. Also, serum urea was evaluated by enzymatic colourimetric technique of Coulomb and Farreau (1963). Serum creatinine was assessed colourimetrically according to Husdan and Rapoport (1968) technique. Testosterone was measured in serum by enzyme immunoassay kit (Immunometrics Ltd., London, UK) as described method of Demetrious (1987).

Lipid peroxidation and antioxidant status

Hepatic, renal and testicular specimens were collected and preserved frozen at –20°C for assessment of lipid peroxidation (LPO), reduced glutathione (GSH) and catalase enzyme. LPO was evaluated as malondialdehyde (MDA) according to the technique of Ohkawa *et al.* (1979). MDA was estimated after thiobarbituric acid reaction in acidic medium at 95°C for 30 minutes forming thiobarbituric acid reactive product, the resultant pink product (absorbance was estimated spectrophotometrically at 534 nm). GSH content was measured by the method of Sedlak and Lindsay (1968). The method is based on the reductive cleavage of 5,5-dithiobis-(2-nitrobenzoic acid) by sulfhydryl (-SH) group giving a yellow color with absorbance at 412 nm. Catalase enzyme (CAT) activity was assayed according to Aebi (1974). Catalase activity was valued on the basis of the reaction of the enzyme with a recognized amount of H_2O_2. The reaction is stopped after one minute with catalase inhibitor, in the existence of peroxidase (HRP) the remaining H_2O_2 reacts with 3,5-Dichloro-2-hydroxybenzene sulforic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity (measured spectrophotometrically at 500-520 nm) inversely proportional to the amount of the catalase in the original sample.

Semen collection and evaluation of sperm motility

After euthanasia of animals, epididymis of each rat was harvested and semen was squeezed from the cauda epididymis through an incision onto a pre-warmed microscope slide. Immediately, undiluted semen drop was assessed for mass motility using 0 - 5+ scale under bright field microscope, magnification X100. Total and progressive motility were determined in diluted semen drop using 2.9% of pre-warmed sodium citrate then examined under the microscope (X400) equipped with warm stage; motility was estimated on a continuous scale of 0 to 100% (Mondal *et al.*, 2013).

Sperm concentration

The concentration of spermatozoa (million/ml) was estimated by hemocytometer method using bright field optics (X400) (Sorensen, 1979).

Live sperm percentage

Alive spermatozoa percentage was evaluated using eosin-nigrosine stain in uniform smears using bright field optics (X400). At least 200 sperms were counted in 5 microscopic fields (Laing, 1979).

Pathological examination

After euthanasia and dissection of rats, all organs were examined grossly. Specimens of liver, kidney, testis and epididymis were harvested and rapidly fixed in formalin solution 10% for at least 24 hours for histopathology. Fixed specimens were dehydrated in ascending alcohol solutions then cleared in xylene, embedded in paraffin blocks and sectioned by a microtome. Five μm thick sections were stained using hematoxylin and eosin (HE). Histopathological lesions were recorded and scored (0 = absent, 1 = mild, 2 = moderate and 3 = severe).

Statistical analysis

Variances in body weight were assessed using a repeated measures analysis of variance (ANOVA). For variables measured once, we used one-way ANOVA with Duncan's Multiple Range post-hoc ranking of means. Arcsine and logarithmic transformations were performed on percentage data and sperm concentration, respectively before analysis to normalize their distribution. Ordinal scores of sperm mass motility and histopathologic lesions as well as non-normal and/or non-homogenous data were analyzed non-parametrically with Kruskal-Wallis test followed by Dunn's test for multiple comparisons. Data are expressed as mean ± standard error of mean. $P > 0.05$ was considered non-significant. SAS software (v9.1, SAS Institute Inc., Cary, NC, USA) was used to run all analyses.

RESULTS

Body and relative organs weights

Table 1 displays that final body weight of OXT and OXT+CoQ10 groups significantly ($P < 0.05$) decreased in comparison to control group. Liver and kidneys relative weights significantly increased in OXT and OXT+CoQ10 groups compared to control group. Testis relative weight significantly increased in OXT group. Combined treatment of OXT and CoQ10 showed no significant difference in testis relative weight. No significant difference was recorded in heart, epididymis and accessory sex glands relative weights in all treated groups compared to control group.

Table 1: Effect of oxytetracycline (OXT) and Coenzyme Q10 (CoQ10) on body weight (BW) and index organ weight (IW) of rats.

	Control	CoQ10	OXT	OXT+ CoQ10
Initial BW (g)	248 ± 10.1[a]	244 ± 11.4[a]	243 ± 7.5[a]	247 ± 8.2[a]
Final BW (g)	297 ± 8.0[a]	275 ± 17.2[a]	191 ± 15.6[b]	223 ± 22.3[b]
Liver IW	3.87 ± 0.13[b]	3.96 ± 0.11[ab]	5.26 ± 0.34[a]	4.90 ± 0.20[a]
Kidney IW	0.68 ± 0.01[b]	0.72 ± 0.02[ab]	1.70 ± 0.47[a]	1.52 ± 0.24[a]
Heart IW	0.38 ± 0.03[a]	0.41 ± 0.03[a]	0.47 ± 0.01[a]	0.46 ± 0.01[a]
Testis IW	1.10 ± 0.06[b]	1.17 ± 0.05[b]	1.51 ± 0.17[a]	1.36 ± 0.11[ab]
Epididymis IW	0.46 ± 0.02[a]	0.50 ± 0.03[a]	0.91 ± 0.29[a]	0.51 ± 0.05[a]
Accessory glands IW	0.72 ± 0.05[a]	0.77 ± 0.06[a]	0.52 ± 0.11[a]	0.54 ± 0.11[a]

Values are means ± standard errors. Means in the same row not sharing a common superscript letter are significantly different ($P < 0.05$). Index Weight (IW) = organ weight (g)/body weight (g) × 100.

Hematological and serum biochemical results

In comparison to control group, Table 2 illustrates that HB concentration, RBCs and WBCs counts were significantly ($P < 0.05$) declined in OXT and OXT+CoQ10 groups. PCV% showed no significant alteration in OXT and OXT+CoQ10 groups. Also, there was a significant ($P < 0.05$) increase in serum activities of GGT, ALP, AST and total bilirubin, and a significant reduction in serum total proteins and albumin concentration in OXT and OXT+CoQ10 groups. CoQ10 treatment ameliorated serum AST levels in OXT+CoQ10 group compared to levels recorded in OXT group. No significant differences were recorded in serum ALT and serum cholesterol levels in all treated groups. Serum triglycerides levels were significantly reduced in CoQ10 treated groups, while it did not differ significantly in OXT group. Serum urea and creatinine values were significantly elevated in OXT and OXT+CoQ10 groups compared to control.

Table 2: Effect of oxytetracycline (OXT) and Coenzyme Q10 (CoQ10) on hematological and serum biochemical parameters of rats.

	Control	CoQ10	OXT	OXT+ CoQ10
PCV, %	37.9 ± 0.44[ab]	39.8 ± 0.75[a]	35.8 ± 1.54[b]	35.0 ± 1.54[b]
HB, g/dl	13.1 ± 0.18[a]	14.3 ± 0.12[a]	11.45 ± 0.34[b]	11.9 ± 0.28[b]
RBCs, 10⁶/ μl	6.4 ± 0.09[a]	6.77 ± 0.20[a]	5.73 ± 0.14[b]	5.84 ± 0.10[b]
WBCs, 10³/μl	13.4 ± 0.51[a]	13.7 ± 0.45[a]	10.5 ± 0.79[b]	11.1 ± 0.80[b]
GGT, U/L	0.56 ± 0.08[b]	0.56 ± 0.10[b]	1.86 ± 0.25[a]	1.77 ± 0.31[a]
ALP, U/L	201 ± 17.76[b]	197 ± 14.51[b]	425 ± 37.6[a]	410 ± 39.9[a]
ALT, U/L	41.3 ± 1.04[a]	40.70 ± 1.90[a]	38.9 ± 1.47[a]	39.7 ± 1.93[a]
AST, U/L	121 ± 1.85[c]	121 ± 3.31[c]	228 ± 9.68[a]	155 ± 7.07[b]
Total bilirubin, mg/dl	0.16 ± 0.02[b]	0.15 ± 0.02[b]	0.37 ± 0.06[a]	0.31 ± 0.06[a]
Total protein, g/dl	6.12 ± 0.03[a]	6.49 ± 0.07[a]	5.24 ± 0.22[b]	5.56 ± 0.12[b]
Albumin, g/dl	4.36 ± 0.07[a]	4.50 ± 0.19[a]	3.65 ± 0.08[b]	3.93 ± 0.13[b]
Globulin, g/dl	1.76 ± 0.09[a]	1.99 ± 0.26[a]	1.59 ± 0.15[a]	1.63 ± 0.08[a]
Triglycerides, mg/dl	162 ± 2.58[a]	126 ± 1.79[b]	150 ± 6.77[a]	111 ± 6.48[c]
Total cholesterol, mg/dl	79.6 ± 1.58[ab]	73.4 ± 4.67[b]	98.6 ± 3.96[ab]	104 ± 16.0[a]
Urea, mg/dl	20.7 ± 0.97[b]	20.3 ± 1.02[b]	43 ± 1.90[a]	41.5 ± 3.13[a]
Creatinine, mg/dl	0.77 ± 0.06[b]	0.76 ± 0.05[b]	1.35 ± 0.09[a]	1.35 ± 0.08[a]

Values are means ± standard errors. Means in the same row not sharing a common superscript letter are significantly different ($P < 0.05$). PCV: Packed cell volume, HB: Hemoglobin concentration, RBCs: Red blood cells count, WBCs: White blood cells count, GGT: Gamma glutamyle transferase, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: Aspartate aminotransferase.

Lipid peroxidation and antioxidant status

OXT induced significant increase in lipid peroxidation activity in liver, kidney and testis represented by amount of MDA produced (Table 3). In the OXT+CoQ10 treated rats, the level of MDA significantly reduced, however remained significantly higher than mean value recorded in control animals. MDA level in CoQ10 group did not show significant change when compared to control group. OXT induced significant decrease in the levels of both GSH and catalase as compared to control group. Concomitant administration of CoQ10 with OXT enhanced and raised the levels of those enzymes to statistically comparable values to that recorded in control group.

Table 3: Effect of oxytetracycline (OXT) and Coenzyme Q10 (CoQ10) on lipid peroxidation (MDA) (nmol/g), reduced glutathione (GSH) (mg/g) and catalase (mg/g) in rats' tissues.

		Control	CoQ10	OXT	OXT+CoQ10
Liver	MDA	5.34 ± 0.20^c	5.12 ± 0.10^c	11.9 ± 0.72^a	6.94 ± 0.30^b
	GSH	27.8 ± 1.20^a	30.7 ± 1.74^a	22.8 ± 1.68^b	31.9 ± 1.03^a
	Catalase	7.83 ± 0.32^a	7.47 ± 0.32^a	5.33 ± 0.27^b	7.23 ± 0.39^a
Kidney	MDA	3.97 ± 0.21^c	3.99 ± 0.19^c	9.68 ± 0.44^a	5.58 ± 0.55^b
	GSH	17.3 ± 1.18^a	18.4 ± 1.72^a	10.7 ± 0.60^b	16.3 ± 0.86^a
	Catalase	7.76 ± 0.41^a	7.65 ± 0.24^a	3.68 ± 0.13^b	7.43 ± 0.17^a
Testis	MDA	3.42 ± 0.18^c	3.17 ± 0.20^c	13.4 ± 0.32^a	7.02 ± 0.48^b
	GSH	29.4 ± 1.21^a	31.7 ± 1.57^a	14.2 ± 1.17^b	28.2 ± 0.77^a
	Catalase	5.83 ± 0.25^a	5.64 ± 0.33^a	3.32 ± 0.21^b	5.00 ± 0.40^a

Values are means ± standard errors. Means with different superscripts within the same row differ significantly ($P < 0.05$).

Sperm characteristics and serum testosterone levels

As depicted in Table 4, significant decrease in both mass and individual motility was observed in OXT group compared to control group. In addition, individual progressive motility showed a significant sharp decline in OXT-treated rats compared to control group. Percentage of live sperms decreased significantly in OXT group as well. Concomitant administration of CoQ10 with OXT enhanced all forms of motility and raised percentage of live sperms in this group as compared to rats received only OXT. There was no significant change in sperm concentration among all treated groups. On the other hand, semen parameters in CoQ10 group showed statistically comparable values to that recorded in control group. OXT induced significant drop in serum testosterone levels of treated rats compared to control group. Rats received both OXT and CoQ10 showed significant increase in levels of serum testosterone when compared to OXT group, however remained significantly lower than mean value recorded in control animals (Table 4).

Table 4: Effect of oxytetracycline (OXT) and Coenzyme Q10 (CoQ10) on sperm characteristics and serum testosterone levels of male rats.

	Control	CoQ10	OXT	OXT+CoQ10
Mass motility*	3.20 ± 0.37^a	2.80 ± 0.37^{ab}	0.60 ± 0.40^b	2.00 ± 0.55^{ab}
Total motility %	63.0 ± 4.36^a	63.0 ± 5.39^a	19.0 ± 5.34^c	41.0 ± 8.86^b
Progressive motility %	52.0 ± 5.83^a	50.0 ± 5.39^a	8.8 ± 3.32^c	28.0 ± 8.15^b
Sperm concentration (10^6/ml)	412 ± 54.63^a	350 ± 16.12^a	312 ± 39.3^a	308 ± 24.8^a
Alive sperm %	73.0 ± 5.39^a	69.0 ± 5.34^a	28.4 ± 4.80^c	48.0 ± 8.60^b
Testosterone, ng/ml	2.75 ± 0.07^a	3.16 ± 0.18^a	0.70 ± 0.11^c	1.65 ± 0.16^b

Values are means ± standard errors. Means in the same row not sharing a common superscript letter are significantly different ($P < 0.05$). *Scored at 0 to 5+.

Gross pathology

Liver, kidneys, testis and epididymis of control and CoQ10 groups appeared grossly normal. In OXT group, there was hepatomegaly with presence of greyish white areas on the surface of the livers. Also, kidneys were markedly swollen and pale. There were no significant changes detected in testis and epididymis. Similar changes of OXT group were detected in OXT+CoQ10 group but were less severe.

Histopathology

Table 5 illustrates the histopathological lesions and their intensity recorded in liver, kidneys, testis and epididymis of all experimental groups comparable to control group.

Table 5: Histopathological lesions of rats' organs and lesion scoring treated with oxytetracycline (OXT) and Coenzyme Q10 (CoQ10).

	Control	CoQ10	OXT	OXT+CoQ10
Liver				
Hepatocytic vacuolation	0 ± 0^b	0 ± 0^b	2.83 ± 0.17^a	1.33 ± 0.33^{ab}
Portal mononuclear infiltrates	0 ± 0^b	0 ± 0^b	2.83 ± 0.17^a	2.17 ± 0.40^a
Hepatic necrosis	0 ± 0^b	0 ± 0^b	1.83 ± 0.48^a	1.00 ± 0.26^{ab}
Kidneys				
Vacuolated renal tubular epithelium	0 ± 0^b	0 ± 0^b	2.50 ± 0.22^a	1.50 ± 0.22^{ab}
Interstitial mononuclear infiltrates	0 ± 0^b	0 ± 0^b	1.67 ± 0.21^a	1.17 ± 0.17^a
Tubulonecrosis	0 ± 0^b	0 ± 0^b	1.67 ± 0.21^a	0.33 ± 0.21^b
Testis				
Testicular degeneration	0 ± 0^b	0 ± 0^b	1.50 ± 0.50^a	0.67 ± 0.33^{ab}
Congestion/edema	0 ± 0^a	0 ± 0^a	0.67 ± 0.33^a	0 ± 0^a
Epididymis				
Desquamated germinal epithelium	0 ± 0^a	0 ± 0^a	0.67 ± 0.33^a	0 ± 0^a

Values are means ± standard errors. Groups in the same row not sharing a common superscript letter are significantly different ($P < 0.05$). Lesion scoring: (0) Absent, (1) Mild, (2) Moderate, (3) Severe.

Liver

Control (Fig. 1A) and CoQ10 (Fig. 1B) rats showed normal hepatic histoarchitecture. OXT rats exhibited diffuse hepatocytic vacuolation and mononuclear cell infiltrates in portal areas (Fig. 1C). Focal to multifocal hepatocytic necrosis with mononuclears infiltration (Fig. 1D) were recorded in OXT group. Also, centrilobular (Fig. 1E) and periportal hepatocytic necrosis concomitant with inflammatory cells infiltration were noticed. OXT+CoQ10 rats showed similar lesions of OXT group but were less severe in intensity and pattern (Fig. 1F). Otherwise, the hepatic parenchyma of OXT+CoQ10 rats appeared almost normal (Fig. 1G).

Kidneys

Kidneys of control (Fig. 2A) and CoQ10 (Fig. 2B) rats showed normal histologic structure. OXT treatment induced severe renal lesions such as marked vacuolation of renal tubular epithelium, perivascular lymphocytic infiltration (Fig. 2C) and detachment of renal epithelium into tubular lumina. Multifocal areas of tubulonecrosis with mononuclear cells infiltration (Fig. 2D) were noticed in renal cortex and corticomedullary junction. Some renal tubules were dilated with thinning of renal epithelium. OXT+CoQ10 rats showed mild vacuolation of some renal tubular epithelial cells, mild perivascular lymphocytic infiltration and detachment of renal epithelium in some renal tubules (Fig. 2E). Besides, most of renal histoarchitecture were nearly normal (Fig. 2F).

Testis and Epididymis

Seminiferous tubules of control (Fig. 3A) and CoQ10 (Fig. 3B) testis were histologically normal with multiple layers of spermatogenesis. Testis of OXT group showed some degenerated buckled hyalinized seminiferous tubules (Fig. 3C) with selective destruction to primary spermatocytes. Mild interstitial congestion and edema were also noticed in OXT group. OXT+CoQ10 group (Fig. 3D) had few degenerated seminiferous tubules; however, other tubules appeared without significant histopathologic alterations (Fig. 3E). Regarding to epididymis (Fig. 4), all groups had epididymal tubules packed with sperms without significant histopathologic findings except in OXT group; there was desquamated germinal epithelium inside the lumina of caput epididymis tubules.
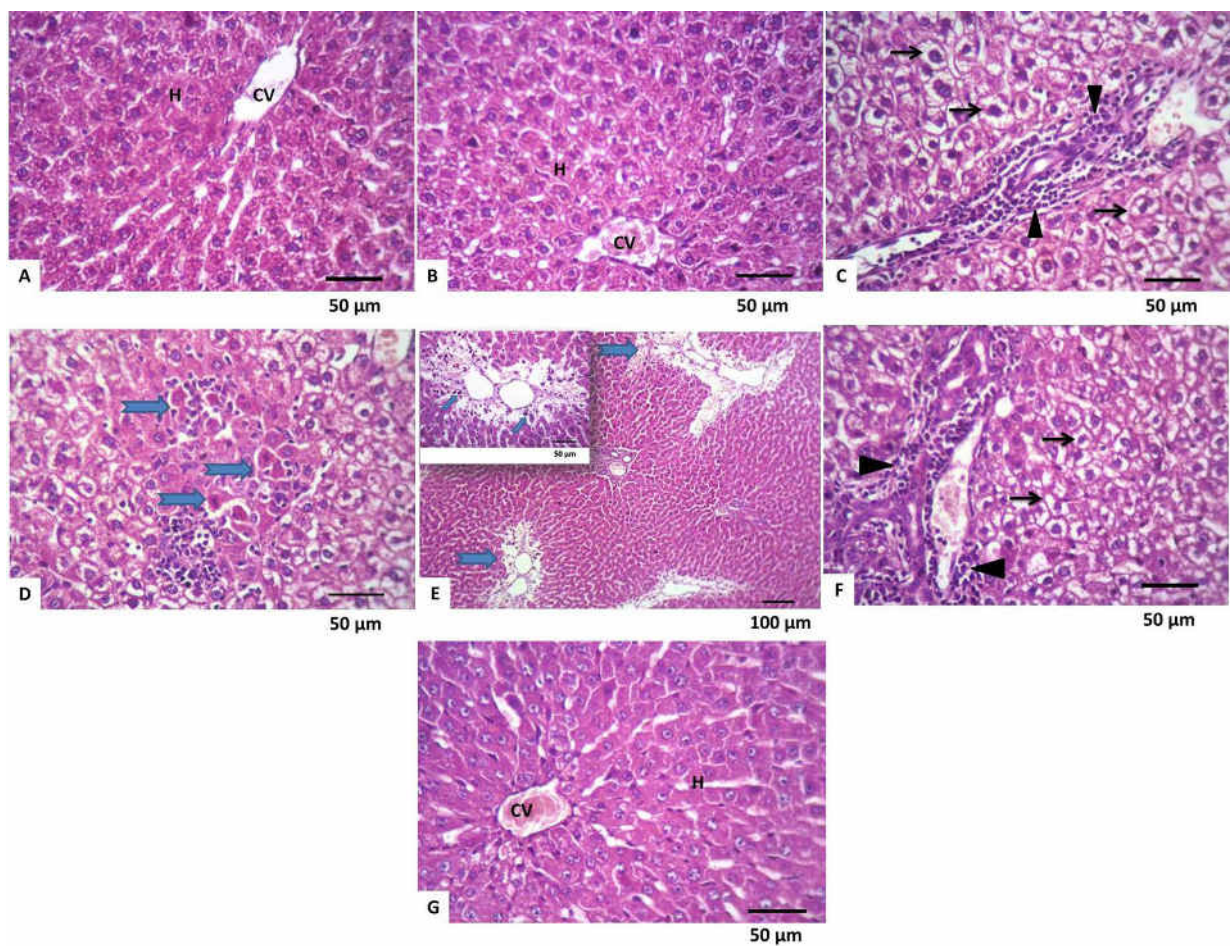

Fig. 1: Liver of a rat stained with HE. (A) Control, (B) Coenzyme Q10, (C-D-E) Oxytetracycline and (F-G) Oxytetracycline+Coenzyme Q10 group. Central vein (CV) and hepatocytes (H). Hepatocytic vacuolation (black arrows), periportal mononuclear infiltrates (arrowheads) and hepatocytic necrosis associated with mononuclear infiltrates (blue notched arrows).
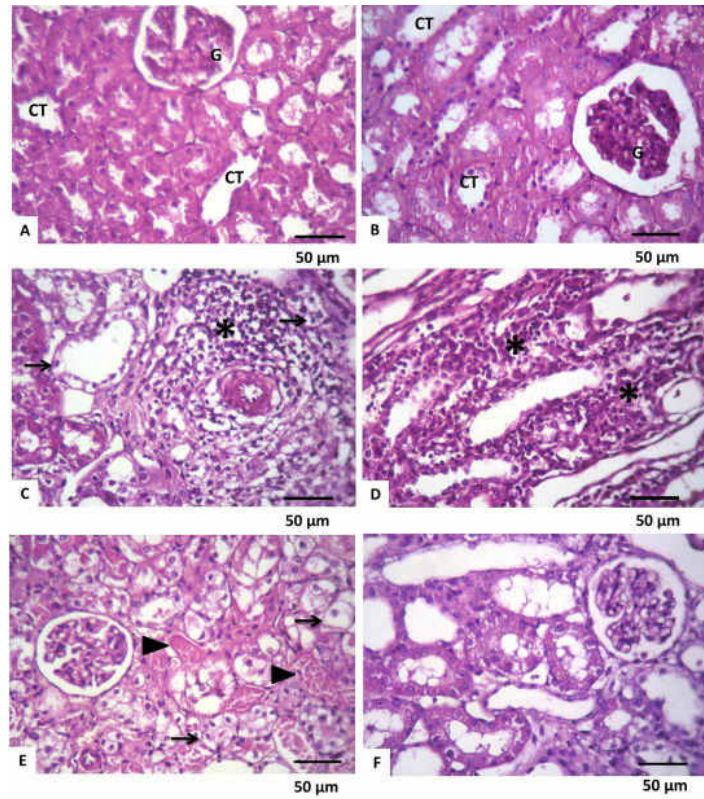
Fig. 2: Kidney of a rat stained with HE. (A) Control, (B) Coenzyme Q10, (C-D) Oxytetracycline and (E-F) Oxytetracycline+Coenzyme Q10 group. Glomerulus (G) and renal convoluted tubules (CT). Mononuclear cells infiltrate (asterisk), vacuolated renal tubular epithelium (black arrows), and detached renal epithelium (arrowheads).
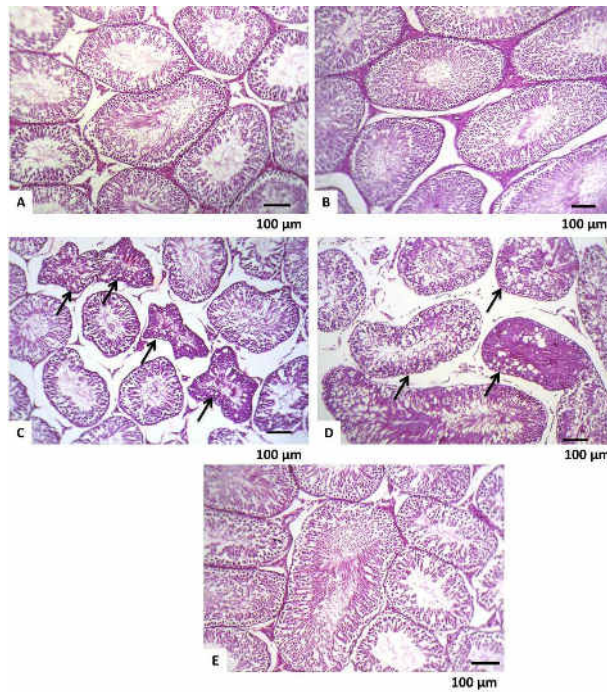

Fig. 3: Testis of a rat stained with HE. (A) Control, (B) Coenzyme Q10, (C) Oxytetracycline and (D, E) Oxytetracycline+Coenzyme Q10 group. Degenerated hyalinized seminiferous tubules (black arrows).
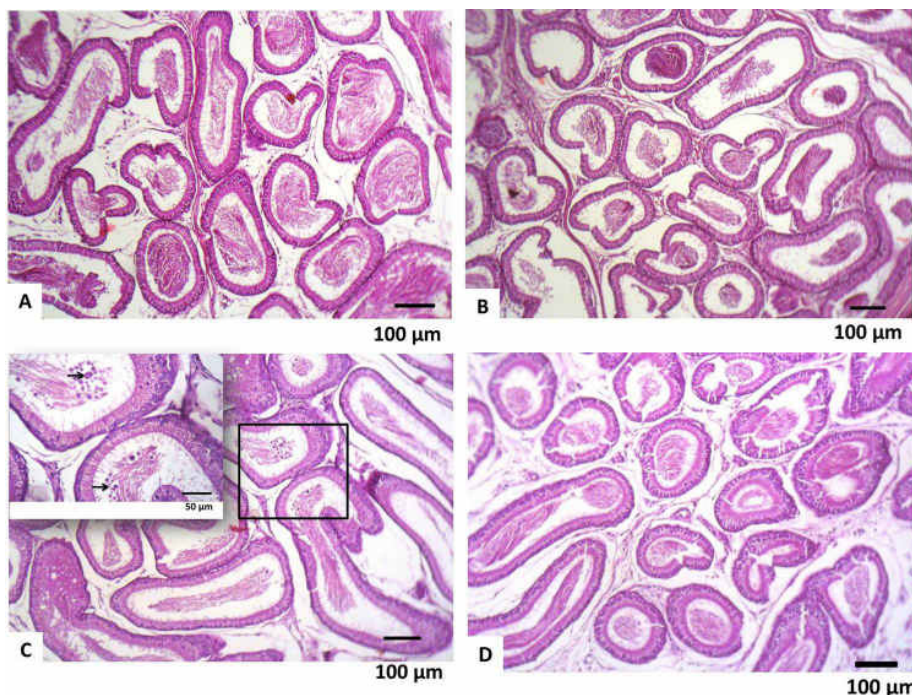
Fig. 4: Epididymis of a rat stained with HE. (A) Control, (B) Coenzyme Q10, (C) Oxytetracycline and (D) Oxytetracycline+Coenzyme Q10 group. Desquamated germinal epithelium (black arrows).

DISCUSSION

Improper use of OXT at high doses induces hepato-renal toxicity (Naseer and Alam, 1987). To our knowledge, this study is the first to document the plausible protective efficacy of CoQ10 against hepatorenal and reproductive toxicity induced by OXT in male rats. Our results indicate that OXT treatment significantly decreased body weight of rats and increased the relative liver and kidney weights. Hematological results revealed reduction in HB concentration and RBCs count in OXT treated groups. These results were parallel to those reported by Hue *et al.* (2015). Our study revealed that OXT induced significant leukopenia which may be due to its immunosuppressive effect. OXT immunosuppressive effect was reported in rainbow trout when administered for 14 days (Yonar, 2012) and in pigeon for 50 days (Al-Ankari, 2005). However, single dose of long acting OXT did not affect immune status of rats (Ravikumar *et al.*, 2016). In the current study, OXT treatment increased serum GGT, ALP, AST, total bilirubin and decreased serum total protein and albumin. These results reflect the hepatic damage caused by OXT treatment. The increase in serum AST levels mirrors the injury of cell membrane and leakage of cytoplasmic enzymes to the blood (Janbaz *et al.*, 2004). The increase in serum levels of ALP, GGT and bilirubin is due to biliary lesions (Moss and Butterworth 1974). Moreover, the reduction in serum albumin and total protein concentrations indicates failure of protein synthesis by hepatocytes (Zimmerman and Seeff *et al.*, 1970). These functional alterations were long-established histopathologically. The hepatic structural changes prompted by OXT were diffuse hepatocytic vacuolation, mononuclear cell infiltrates in portal areas and hepatic necrosis. OXT induced hepatic damage may be due to inhibition of β oxidation of free fatty acids and lipoprotein secretion in liver (Letteron *et al.*,

2003) and increased cholesterol and triglyceride biosynthesis. In our experiment, OXT did not elevate serum total cholesterol and triglycerides and no steatosis was observed in OXT treated rats. The study of Pari and Gnanasoundari (2006) is in agreement with our findings as they described the hepatic histopathological alterations induced by OXT (200mg/kg/day for 15 days) without steatosis. Our results revealed increase in hepatic MDA and decrease in GSH and catalase enzyme in OXT treated groups. These findings were consistent with the studies of Jayanthi and Subash (2010) and Abdel-Daim and Ghazy (2015). Moreover, serum urea and creatinine values were elevated by OXT treatment indicating renal dysfunction (Gnanasoundari and Pari, 2006). Renal lesions caused by OXT were in line with previous reports of Tarara *et al.* (1976), Gnanasoundari and Pari (2006) and Shabana *et al.* (2012). CoQ10, a component of electron transport chain, has antioxidant, anti-inflammatory, and anti-apoptotic properties against acetaminophen hepatotoxicity and metabolic-stress-induced liver damage (Vasiliev *et al.*, 2011, Fouad and Jresat, 2012). CoQ10 adjusts oxidative phosphorylation and inhibits lipid peroxidation (Bargossi *et al.*, 1994). In our study, CoQ10 treatment in part countered lipid peroxidation (MDA) induced by OXT and enhanced GSH and catalase levels in liver and kidneys. On the basis of histopathological description and lesion scoring, CoQ10 treatment ameliorated the degenerative and necrotic lesions prompted by OXT in liver and kidneys. Our study has shown that OXT administration has deleterious effect on sperm motility/viability. Furthermore, the current work reported for the first time that OXT causes moderate damaging effect in testis via oxidative stress action as it provoked lipid peroxidation and a reduction in levels of testicular GSH and catalase. These effects were associated with significant decrease in serum testosterone level. The adverse effect of OXT on sperm motility noticed in the current work is consistent with previous

studies reported similar observation in bucks (Onakpa *et al.*, 2010), and rats (Oyedeji *et al.*, 2013). In contrast to our observation, sperm viability in these studies showed no change, probably because lower doses of OXT were used (20-25mg/kg). In an in-vitro assay, minocycline at dose as low as 50µg/ml was found to be toxic to bull sperm (Ahmad and Foote, 1986), likewise tetracycline was reported to cause dose-dependent irreversible decline in human sperm motility besides a decreased viability (Hargreaves *et al.*, 1998). Impairment of sperm motility by tetracyclines family might be due to its ability to chelate calcium (Caswell and Hutchison, 1971), which is of paramount importance for sperm movement and hyperactivation necessary for fertilization (Darszon *et al.*, 2011). The elevated levels of lipid peroxidation recorded hereby in testis of OXT-treated rats implicates that OXT might has prompted the same process in epididymal spermatozoa resulting in a decrease in their motility. Spermatozoa are highly susceptible to oxidative stress, particularly lipid peroxidation because of their great content of polyunsaturated fatty acids in plasma membrane. Oxidative stress is well recognized as a cause of male infertility due to sperm damage, deformity, and induced DNA fragmentation (Agarwal *et al.*, 2008). Reports studied the effect of tetracyclines on histological structure of testis and epididymis are few. Histopathological examination of OXT testicular sections showed moderate degenerative changes in some seminiferous tubules, which did not affect spermatogenesis. This result explains why sperm count did not change in response to OXT treatment. These observations agree with an early study described that OXT administration for eight days in rats has negligible effects on spermatogenesis (Timmermans, 1974). The subtle changes in testis may be attributed to OXT- induced reduction in testicular GSH and catalase decreasing tissue ability to resist lipid peroxidation. An increase in relative testis weight was noted, that may due to circulatory disturbances such as congestion and edema in testis. Also, epididymal tubules of OXT group had desquamated germinal epithelium. These structural alterations may be attributed to oxidative stress and decrease in serum testosterone levels induced by OXT. Testosterone is necessary for development and function of male reproductive tissues (Mooradian *et al.*, 1987). The observed reduction in serum testosterone concentration associated with OXT treatment is in line with a previous study that described same observation after tetracycline administration in men (Pulkkinen and Maenpaa, 1983). Contrary to this result, Hamalainen and co-workers (1987) reported that OXT has no effect on circulating testosterone level, possibly due to differences in dosage of the drug. There is no substantial evidence on mechanism of action of OXT-induced decrease in testosterone; it is speculated that disturbance in enterohepatic circulation of steroids is partially responsible for this phenomenon (Pulkkinen and Maenpaa, 1983). Whether or not OXT can directly inhibit testosterone synthesis is still questionable. In our work, supplementation of CoQ10 to OXT-treated rats resulted in relatively improved sperm motility/viability, showed androgenic action evidenced by elevated serum level of testosterone, restored relative testis weight and improved testicular and epididymal histoarcitecture. These findings are consistent with studies demonstrated that oral supplementation of CoQ10 improved sperm motility in idiopathic infertile men (Balercia *et al.*, 2004; Balercia *et al.*, 2009; Safarinejad 2009; 2012). Furthermore, CoQ10 as antioxidant suppressed lipid peroxidation in testis and boosted levels of testicular GSH and

catalase, two naturally occurring testicular antioxidant enzymes. Similarly, oral CoQ10 induced an increase in seminal catalase and super oxide dismutase activity in infertile men (Safarinejad *et al.*, 2012).

In conclusion, among other possible mechanisms, OXT administration induced hepatorenal and male reproductive toxicity via oxidative stress. Moreover, CoQ10 could provide partial protection against OXT-induced hepatorenal as well as reproductive dysfunction in male rats through limitation of oxidative stress triggered by OXT.

CONFLICT OF INTEREST

Authors state that there are no conflicts of interest.

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