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RAPID COMMUNICATION

Coenzyme Q10 Ameliorates Cadmium Induced Reproductive Toxicity in Male Rats

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Summary

This study aimed at investigating the protective role of CoQ10 against cadmium (Cd)-induced reproductive toxicity in male rats. Adult male Wistar rats were exposed to an acute dose of Cd (25 mg/kg bwt; Cd group), Cd+CoQ10 (25 mg/kg bwt Cd+10 mg CoQ10; Cd-Q10 group) and distilled water (control) *in vivo* for 15 consecutive days and semen quality was assessed. A significant reduction was noted in sperm concentration, progressive motility, morphology and DNA integrity in both Cd- and Cd-Q10 groups in comparison to control indicating Cd-induced testicular lipid per oxidation (LPO) and decline in indigenous antioxidant defense system as measured by total antioxidant capacity (TAC) ($p<0.05$). However, simultaneous co-administration of CoQ10 along with Cd (Cd-Q10 group) was able to improve sperm concentration, motility, progressive motility, morphology, DNA integrity, and testicular TAC as well as lower LPO compared to Cd group ($p<0.05$). Results indicate that used dose of CoQ10 is capable of moderately ameliorating reproductive toxicity of Cd by improving semen quality and reducing testicular oxidative stress.

Key words

Rat • Sperm • Oxidative stress • DNA damage • Cadmium

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Cadmium (Cd) is a persistent heavy metal with a wide range of industrial consequences (Adamkovicova *et al*. 2016). Its routes of entry to the organism include inhalation of contaminated air, fumes and dusts, tobacco smoke, contaminated of food and water, and occasionally through ingestion at work place (Oyinloye *et al*. 2016). Basic mechanism of Cd toxicity involves excessive production of reactive oxygen species (ROS) leading to lipid per oxidation (LPO) and eventually oxidative stress. Co-enzyme Q10 (2, 3-dimethyl-6-ten-isoprene paraben-zoquinone) is a naturally occurring lipophilic antioxidant molecule located primarily in the hydrophobic domain of mitochondrial inner membrane of phospholipid bilayer and other plasma membranes. It stimulates cell growth and inhibits cell death (Tawfik 2015) by scavenging free radicals and preventing the instigation and transmission of LPO in cellular bio-membranes (Cervellati and Grecoa 2016). The aim of this *in vivo* study was to investigate the efficacy of CoQ10 in combating the reproductive toxicity of Cd in male rats. After obtaining approval from the

institutional animal ethics committee, male Wistar rats of reproductive age were administered cadmium chloride ($CdCl_2$) orally every morning for 15 consecutive days, and divided into 3 groups each consisting of 5 animals. Control group received distilled water, whereas Cd group received Cd at 25 mg/kg bwt which is 1/5th of LD_{50}, and Cd-Q10 group received Cd + CoQ10 (25 mg/kg bwt + 10 mg/kg bwt), respectively. Initial and final body weights were recorded prior to sacrifice by cervical dislocation on 16th day. Pair of testes and cauda epididymis was dissected out from each animal by laprotomy, gently rinsed in phosphate buffered saline (PBS, pH 7.4), cleaned off the adhering tissues and weighed. Cauda epididymis of each animal was gently minced in 2 ml PBS and incubated at 37 °C for semen analysis including DNA integrity (Sarathchandiran *et al.* 2014). Sperm concentration, motility and progressive motility were assessed by mounting the sperm suspension in Makler Counting Chamber (Sefi Medical Instruments, Germany) and observed under 20x magnification using phase contrast microscope (Labomed-LX 300) (Roychoudhury *et al.* 2010a). Sperm morphology was examined using Giemsa staining (Roychoudhury *et al.* 2010b). Sperm DNA integrity was studied by measuring the DNA fragmentation index under fluorescent microscope (Olympus-CX 31-TR) using acridine orange dye (Varghese *et al.* 2009). Testicular oxidative stress was determined by LPO measured as the amount of malondialdehyde produced (Paunovic *et al.* 2017) and TAC was measured in testis by commercially available kit (Roychoudhury *et al.* 2016). Each experiment was performed thrice. Significant differences between the experiments were evaluated using one way ANOVA with Scheffe's *post hoc* comparison of SPSS version 21 software. Differences from control at $p<0.05$ were considered significant. In comparison to control, there was a significant decline in testicular weight in both Cd- and Cd-Q10 groups ($p=0.035$). Sperm concentration, motility, progressive motility, morphology, DNA integrity and testicular TAC were significantly lower in both Cd- and Cd-Q10 groups compared to control ($p<0.05$). Marked elevation was noted in the level of LPO in both Cd- and Cd-Q10 groups in comparison to control ($p<0.05$). Co-administration of CoQ10 was capable of moderately recovering the toxic effect of Cd as sperm concentration, motility, progressive motility, morphology, DNA integrity and testicular TAC increased significantly in Cd-Q10 group, while a significant reduction in LPO was noted in Cd-Q10 group in comparison to Cd group, respectively ($p<0.05$) (Figs 1A-C).

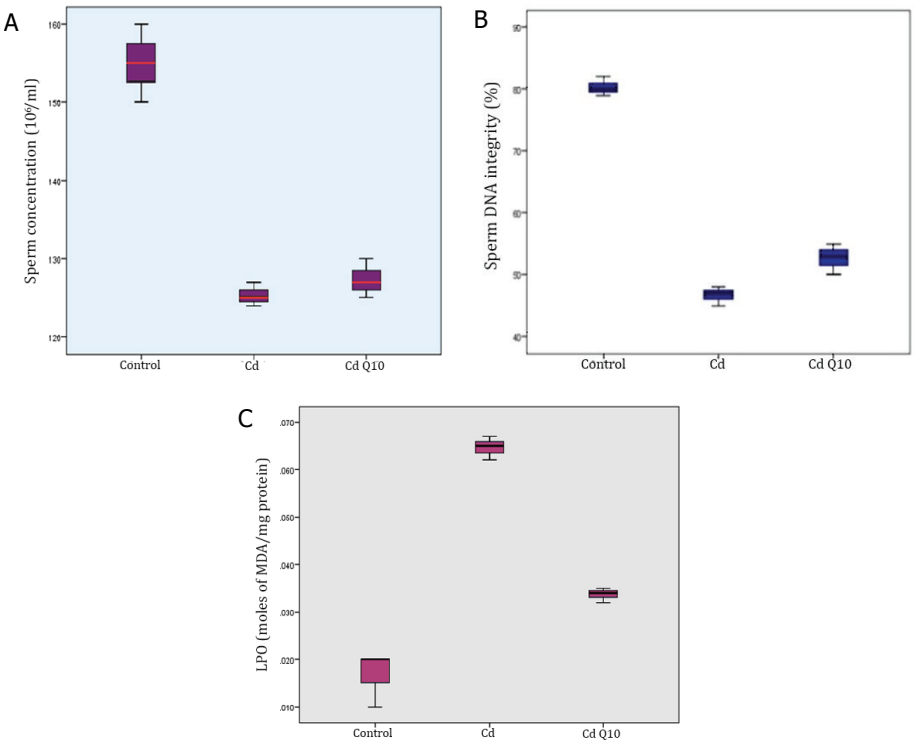

Fig. 1. Moderate amelioration of cadmium (Cd) induced reproductive toxicity by coenzyme Q10 (CoQ10) in male rats: (**A**) sperm concentration, (**B**) sperm DNA integrity, and (**C**) testicular lipid peroxidation (LPO) in control, Cd and Cd-Q10 groups

Spermatogenesis is a complex process that involves a phase of proliferative expansion, meiosis, and cytodifferentiation (Jan *et al.* 2012). In the rat, the coordinated spatial associations of developmental germ cell types occurs in a time regulated fashion, and accordingly the seminiferous epithelium can be subdivided

into fourteen stages (Leblond and Clermont 1952, Oakberg 1956). The time necessary for a cell to ascend one stage above the original cell in a spermatogenic cycle map is 13.3 days and the duration of spermatogenesis is 53.2 days in Wistar rats as used in the present study (Huckins 1965). A number of characteristics including the timing of differentiation process and spermatozoa morphology are also unique to rats although the general process of spermatogenesis is highly conserved across mammals (de Krester and Kerr 1988, Russell *et al.* 1993). In earlier studies, Cd was found to induce necrosis in testicular tissue that adds to weight loss in testes (Wang *et al*. 2007, Toman *et al*. 2011). Chronic administration of a lower dose of Cd (5 mg/kg bwt) for a period of 6-8 weeks caused a decline in sperm concentration, motility, progressive motility and morphology in rats (Akunna *et al*. 2017, El-Demerdash *et al*. 2004). Cadmium was also found to impair rabbit sperm motility, morphology and membrane integrity even at a much lower dose (0.62 µg $CdCl_2$/ml) *in vitro* (Roychoudhury *et al*. 2010). In the present study, Cd-induced toxicity was evident from reduction in sperm concentration, motility, progressive motility, morphology, DNA integrity and TAC together with an increase in testicular LPO in both Cd- and Cd-Q10 groups, which is directly related to male fertility and recognized to have putative roles in diminishing sperm quality through testicular damage (Akunna *et al*. 2017). Cadmium is known to alter testicular function by inducing oxidative stress (Amara *et al*. 2006) which is a common pathology in approximately half of all infertile men, and is further associated with pathogenesis of sperm DNA damage (Aitken *et al*. 2010). A lower dose of 1 mg/kg bwt administered to adult male rats for a period of 5-8 weeks showed elevated LPO and relative depletion of testicular antioxidant levels (Acharya *et al*. 2008). Coenzyme Q10 is a potent antioxidant with the capacity to neutralize tocopheroxyl radicals in its reduced form ubiquinol (Nagaoka *et al*. 2000). Protective effect of CoQ10 has been shown on sperm motility, LPO and DNA fragmentation *in vitro* (Talevi *et al*. 2010). Coenzyme Q10 when administered to adult male rats at 10 mg/kg bwt was able to ameliorate testicular toxicity induced by Cd at the dose 5 mg/kg bwt (Abdel-Hady and Abdel Rahman 2011). In the present study, moderate improvement in sperm concentration, motility, progressive motility, morphology, DNA integrity and testicular TAC was noted in Cd-Q10 group with 25 mg/kg bwt Cd co-administered along with 10 mg CoQ10 for a period of 15 days in comparison to Cd group with 25 mg/kg bwt Cd-exposure.

In conclusion, our results indicate that CoQ10 at a low dose of 10 mg/kg bwt used in the present study was possibly capable of nullifying the ability of a high dose of 25 mg/kg bwt Cd to replace redox-active metals and hence did not allow Cd to hinder redox scavenging enzymes. These findings support the use of CoQ10 as a potential antidote in the management of Cd induced reproductive toxicity and male infertility through mechanisms involving reduction of testicular oxidative stress and improvement of semen quality.

Conflict of Interest

There is no conflict of interest.

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