CADMIUM-INDUCED TESTICULAR TOXICITY: COMPARATIVE EFFECTS OF PRE- AND POST-TREATMENTS WITH VITAMINS C AND E

[1]ADAIKPOH, M. A. [*] and ORHUE, N. E. J.

[1]Department of Biochemistry, Faculty of Sciences, University of Benin, P. M. B 1154, Benin City, Nigeria

** Author for correspondence (e-mail: adaikpohtina@yahoo.com)*

ABSTRACT

Cadmium, a toxic metal, is known to exert a number of deleterious effects on health. However, it is less harmful when bound to rocks. Climate change, resulting from such phenomena as acid rain, promotes the breakdown and consequent release of bound cadmium. Flushing of inactive and active mines by flood lead to changes in cadmium levels in soil and underground water bodies. The effect of vitamins C and E on rat testis pre- and post-treatment with cadmium, using urinary creatine as marker of testicular toxicity was investigated in this study. Male Wistar rats (n=24) were divided into 4 groups; control (n=6) and test group (n=18); divided into groups that received cadmium only (CT), those treated with the vitamins before cadmium exposure (Cd24V) and those treated with the vitamins post cadmium exposure (Cd1V). Results indicate a significant ($p<0.05$) increase in urinary creatine that was temporarily relieved by the Cd1V treatment. Relative to both control and the CT-treatment groups, the Cd24V treatment significantly ($p<0.05$) reduced urinary creatine 24hrs before sacrifice,. Histological sections of the control testis showed numerous seminiferous tubules undergoing sequential spermatogenesis while sections of the CT- and Cd1V-treated rat testis showed extensive coagulative necrosis affecting all the tubules. There was also hyperplasia of interstitial cells of the leydig. Relative to the control and CT-treated rat testis, sections of the Cd24V rat testis showed numerous seminiferous tubules filled with germ cells undergoing sequential spermatogenesis. There were eosinophilic exudates in the interstitium with mild acute inflammatory cell infilterate. In this study, biochemical and histological investigations indicate cadmium toxicity of the testis. It also shows that post-treatment of cadmium-exposed rats with the vitamins were ineffective in protecting the testis while pre-treatment ameliorated the toxic effect of the metal.

Keywords: Climate change, cadmium-toxicity, urinary creatine, testis, Wistar rats

INTRODUCTION

Cadmium is a toxic environmental and industrial pollutant that is widely used as an anti-corrosive in plating metals and other alloys that are valuable in industry. With the wide application of cadmium-related products, hazardous exposure to the metal is increasing. It is particularly toxic because it bioaccumulates and is neither biodegraded nor biotransformed as a means of detoxication. Although cadmium compounds are highly toxic they are less harmful when bound to rocks. They are present in coal, soil, active and inactive mines and are usually leached from soil through reaction with acids. The dissolving action of hydrogen ions cause rocks and small-bound soil particles to break down. Flushing of inactive or active mine sites and mineralized but unmined sites will cause larger sudden increases in concentrations that will be an ever increasing danger to aquatic life with climate change (Nordstrom 2009). It has been demonstrated that cadmium induces several alterations in the tissues of laboratory animals and humans (Foulkes, 1986; Adaikpoh and Obi, 2009; Eriyamremu *et al.,* 2006; Massanyi *et al,,* 2000).

Heavy metals like cadmium induce a prooxidant state (Casalino *et al.*, 2002), thus suggesting the involvement of reactive oxygen species in the mechanism of cadmium induced testicular damage. Vitamin E, the most potent natural antioxidant readily donates its ring hydrogen from the hydroxyl group to free radicals, which then become unreactive. On donating the hydrogen atom, vitamin E itself, be-

comes a relatively unreactive free radical (Scott, 1997). After its reaction with free radicals, the reactive form of vitamin E is rapidly regenerated by vitamin C. Therefore, the consumption of foods rich in antioxidants, which are potentially able to quench or neutralize free radicals, may play an important role in the prevention of toxic effects due to cadmium.

Nicholson *et al.*, (1989) reported that acute and sub-chronic administration of some testicular toxicants caused a significant rise in urinary creatine in rats. This report has since been collaborated by others (Gray *et al.,* 1990; Timbrel, 2000). The purpose of this study is to use urinary creatine as a marker of testicular toxicity in rats exposed to cadmium and to compare the effectiveness of vitamins C and E treatment on rat testis pre- and post- cadmium exposure.

MATERIALS AND METHODS

Experimental Design

Twenty-four male rats (Wistar strain) weighing between 180-200 g were used for this study. They were allowed a 2-week acclimatization period and consequently divided into four groups of six rats each, such that the average weight between groups did not exceed ± 20 g. The control group (CT) received neither cadmium nor vitamins C and E while the second group was treated with cadmium only (Cd). The third group (Cd1V) was treated with cadmium on day 1 of study and vitamins C and E for 28 days. The fourth group (Cd28V) was pre-treated with vitamins C and E for 28 days before cadmium exposure on day 28. Cadmium in the form of $CdCl_2$ (5 $mgKg^{-1}$ body wt) or its vehicle (normal saline) was administered subcutaneously while vitamins C (100 $mgKg^{-1}$ body wt) and vitamin E (150 $mgKg^{-1}$ body wt.) were administered daily by gavage. All animals were allowed free access to commercial rat chow (Ewu Feeds and Flour meals Ltd Edo State, Nigeria) and water throughout the period of study. Rats from each group were kept in metabolic cages so that faecal output, feed consumption and urine can be monitored. The urine samples were collected every 24 hours for 3days as well as 24hr before animal sacrifice. The animals were weighed once every week and accordingly, the dose of $CdCl_2$ and vitamins were adjusted on weekly basis.

At the end of the study period, animals were necropsized under chloroform (BDH, Poole, England) anesthesia. The abdominal regions were opened, the testes were excised trimmed free of connective tissue and fixed in buoin solution for histological examination.

Biochemical Analysis

Determination of Creatine and Creatinine in Urine

The determination of creatine in urine is based on *in vitro* conversion of creatine to creatinine under either acid or alkaline conditions, followed by measurement of creatinine with the Jaffe reaction method as described by Bonsnes and Taussky (1945). Urine is treated with picric acid and heat to force conversion of creatine to creatinine; sodium hydroxide is then added to create the alkaline conditions needed for the jaffe reaction. The increase in absorbance at 520nm resulting from the formation of creatinine – picrate adduct allows an estimate of the total creatinine concentration. The creatinine concentration in the original sample is also measured and a correction is made for this endogenous creatinine. The observed increase in creatinine after acid and heat treatment is reported as creatine.

Calculation

Absorbance of unknown urine sample / Absorbance of standard x Concentration of standard (177mmol / L)

The creatine standard was used to calculate the creatine concentration while the creatinine standard was used to calculate the preformed creatinine concentration. The creatine concentration for each tube was calculated by subtraction of the initial creatinine concentration (attributable to endogenous creatinine only) from the final concentration (attributable to endogenous creatinine plus creatinine formed by creatine condensation).

Histological Examination of Testis

The testes of one rat from each group was serially sectioned and fixed in Bouin solution for 48hr. The specimen was then dehydrated

through graded series of alcohol and cleared in three changes of xylene before they were embedded in paraffin. Serial sections, each of 4 μm thickness, were made and stained with hematoxylin and eosin according to standard method. Histological assessment was performed under light microscopy.

Statistical Analysis

Results were expressed as means ± (STD). Analysis of variance (ANOVA) was used to test for differences between treatment effects while Turkey multiple comparison tests was used to test for significant differences between the treatment means. Values are considered significant at $p < 0.05$.

RESULTS

Throughout the monitoring period, cadmium significantly ($p<0.05$) increased urinary creatine levels, with the highest level recorded on the third day of study (Tables 1). Daily treatment with vitamin E and C post cadmium -exposure resulted in significant ($p< 0.05$) reduction in urinary creatine levels within the 72hr monitoring period. However, the protection provided by the vitamin treatment was overwhelmed 24hr before the animals were sacrificed, resulting in significant ($p< 0.05$) elevation by 42.8% when compared with the control. The temporary significant ($p<0.05$) decrease in urinary creatine of the Cd24V treatment group on day 2 of the vitamin treatment was not evident on the 3rd day (Table 2). However, 24hrs before the animals were sacrificed, a significant ($p< 0.05$) reduction in urinary creatine was observed relative to both the control and the CT treatment groups.

Histological sections of the control testis showed numerous seminiferous tubules undergoing sequential spermatogenesis while sections of the CT and Cd1V-treated rat testis showed extensive coagulative necrosis affecting all the tubules (Plate 1). There was also hyperplasia of interstitial cells of the leydig. Relative to the control and CT-treated rat testis, histological sections of the Cd24V rat testis showed numerous seminiferous tubules filled with germ cells undergoing normal sequential spermatogenesis. There were eosino-

Table 1: The Effect of Post-treatment with Vitamins C and E on Cadmium-induced Changes in Urinary Creatine Levels in Rat.

	URINARY CREATINE (mg /dl)			
Groups	DAY 1	DAY 2	DAY 3	24hrs Before Sacrifice
Control	25.98 ± 2.01	45.37 ± 4.10	65.13 ± 10.56	30.54 ± 0.26
Cadmium Only	33.38 ± 2.99*	82.68 ± 3.69*	85.93 ± 4.42*	45.46 ± 2.44*
Cd1V	27.92 ± 1.36**	35.85 ± 2.75***	67.77 ± 3.46**	43.61 ± 1.27*

Values are mean ± SD (n = 6)
**Values on the same column differ significantly from control (p< 0.05).*
*** Values on the same column are significantly (p<0.05) different from the cadmium only group.*
**** Values on the same column are significantly (p<0.05) different from both control and cadmium only groups*

Table 2: The Effect of Pretreatment with Vitamins C and E on Cadmium-induced Changes in Urinary Creatine Levels in Rat.

Groups	URINARY CREATINE (mg / dl)			
	DAY 1	DAY 2	DAY 3	24hrs Before Sacrifice
Control	20.14± 1.88	42.28 ± 3.89	55.30 ± 9.24	34.02 ± 0.30
Cadmium Only	26.08 ±2.01*	77.21 ± 4.01*	72.94 ± 3.36*	50.44± 3.22*
Cd24V	29.00 ±1.29*	22.02 ± 3.81***	81.84 ± 2.70*	19.55 ± 1.52***

Values are mean ± SD (n = 6)

*Values on the same column differ significantly from control ($p < 0.05$).

*** Values a on the same column re significantly ($p<0.05$) different from both control and cadmium only groups

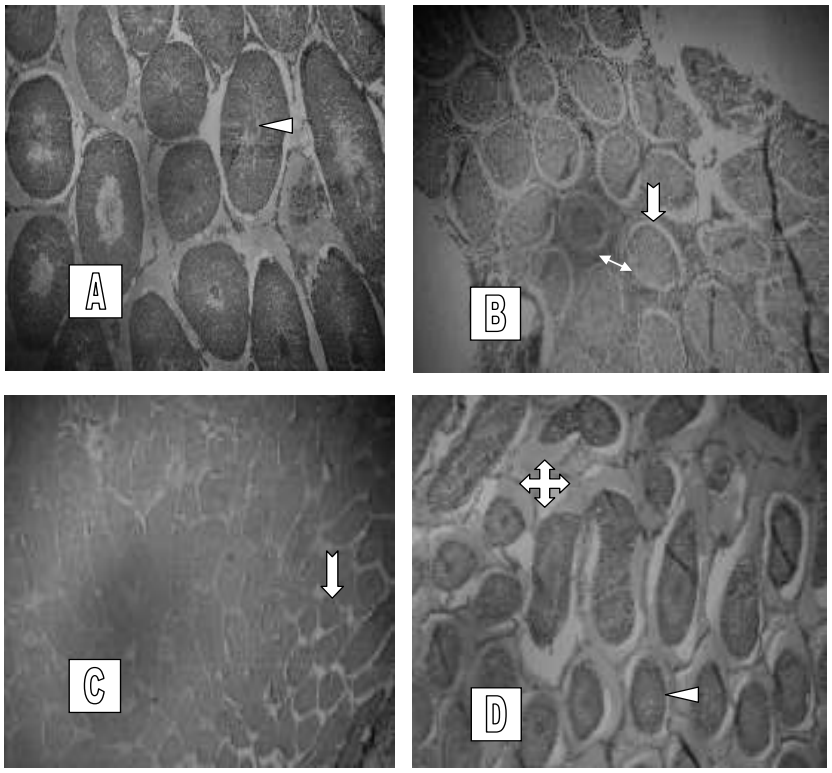

Plate 1: Histology of the testes in rat subcutaneously exposed to a single dose of cadmium-chloride (5 mg / Kg bd. wt). Most seminiferous tubules are well preserved with germ cell undergoing spermatogenesis in controls (A). In contrast histologic sections in (B) which received the CT treatment and (C) which recieved the Cd1V treatment show extensive coagulative necrosis affecting all the tubules. There is hyperplasia of the interstitial cells of the leydig. However, histologic sections of the testis in (D) which was pre-treated with the vitamins before cadmium (Cd24V) show numerous seminiferous tubules filled with germ cells undergoing normal sequential spermatogenesis. There are eosinophilic exudates in the interstitium (H&E staining x 100).

Normal spermatogenic tubule = ◅ Hyperplastic leydig cells= ↘

Necrotic tubule = ⇩ Fibrinous exudate = ✣

philic exudates in the interstitium with mild acute inflammatory cell infilterate.

DISCUSSION

The effect of vitamins C and E on rat testis pre- and post-treatment with cadmium, using urinary creatine as marker of testicular toxicity was investigated in this study. The detection of chemical damage in the male reproductive system is difficult and relies largely on sperm analysis and histopathology. However, earlier reports (Moore *et al.,* 1992; Draper and Timbrel, 1998) suggest that urinary creatine is the most sensitive and simple, non-invasive marker of acute cadmium-induced testicular damage and dysfunction, hence its use in the present study. This study shows a consistent increase in urinary creatine for 72hrs after cadmium exposure. This result is similar to earlier reports by Gray *et al*., (1990); Draper and Timbrel, (1998) and Timbrel. (2000) that acute and subchronic administration of some testicular toxicants caused a significant rise in urinary creatine in rats. Gray *et al*., (1990) attributed the elevated creatinuria to testicular necrosis and explained that doses of cadmium which did not cause testicular necrosis did not result in creatinuria and creatinaemia. Moore *et al.*, (1992) reported that creatine is associated with the cells of the seminiferous epithelium. Since similar studies with orchidectomised male rats (Gray et al., 1990) and female rats (Nicholson *et al.*, 1989) using cadmium did not result in creatinuria, it is conceivable that the elevation in urinary creatine observed in the CT treatment group in this study, is due to testicular necrosis. This result is correlated by the extensive coagulative necrosis of all the seminiferous tubules observed in the histological sections of the CT treatment group. However, it is interesting to note that the Cd24V treatment, like the CT treatment, resulted in increased creatinuria on the 3rd day of study. Thus raising the question of whether daily consumption of α-tocopherol and vittamin C at the doses used in this study is toxic to the testes. However, when this group was challenged with cadmium 24hrs before the animals were sacrificed, a 61% decrease in creatinuria was observed relative to both control and the group that was treated with only cadmium. It is not known if this significant amelioration of creatinuria would have persisted if the rats in this group were left for more than 24hrs after cadmium exposure. The protection offered by daily treatment of rats with vitamin after cadmium-exposure (Cd1V) was overwhelmed 24hrs before the animals were sacrificed (Table 1)

This study shows that pretreatment of rats with vitamins C and E is more effective at protecting against testicular toxicity than post-treatment with the vitamins in cadmium-exposed rats.

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