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Original article

Cadmium chloride–induced testicular toxicity in male wistar rats; prophylactic effect of quercetin, and assessment of testicular recovery following cadmium chloride withdrawal

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ABSTRACT

This study assessed the effect of quercetin (QE) on cadmium chloride ($CdCl_2$) – induced testicular toxicity, as well as the effect of withdrawal of $CdCl_2$ treatment on same. Thirty male Wistar rats aged 10 weeks old and weighing 270–300 g were assigned into 5 groups and used for this study. Rats in groups 1–4 were administered vehicle, $CdCl_2$ (5 mg/kg bwt), $CdCl_2$ + QE (5 mg/kg bwt and 20 mg/kg bwt, respectively) or QE (20 mg/kg bwt) orally for 4 weeks. Group 5 rats received $CdCl_2$, with 4 weeks recovery period. Results showed that cadmium accumulated in serum, testis and epididymis, decreased body weight, testicular and epididymal weights, sperm count, motility and viability. Cadmium decreased serum concentrations of reproductive hormones, but increased testicular glucose, lactate and lactate dehydrogenase activity. Cadmium decreased testicular enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic (glutathione, vitamins C and E) antioxidants, and increased malondialdehyde and hydrogen peroxide. Cadmium down-regulated Bcl-2 protein, up-regulated Bax protein, increased Bax/Bcl-2 ratio and cleaved caspase-3 activity. Histopathology of the testis showed decreased Johnsen's score and Leydig cell count. These negative effects were attenuated by QE administration, while withdrawal of $CdCl_2$ did not appreciably reverse toxicity. We conclude that QE better protected the testis from $CdCl_2$ toxicity than withdrawal of $CdCl_2$ administration.

1. Introduction

Cadmium (Cd) is a hazardous heavy metal largely available in the environment, especially in industrialized settlements [1]. Along with other heavy metals, cadmium has been listed in the International Register of Potentially Toxic Chemicals of the United Nations Environment Program [2], and the World Health Organisation (WHO) estimated 500 μg/week cadmium as the safe level for human ingestion [1]. Mclellan *et al.* [3] reported that cadmium absorption in humans was slow following oral exposure, with availability of cadmium in the gut 3–5 weeks after ingestion, thus, a possibility of a cumulative toxic effect. Studies have demonstrated that cadmium accumulates in the liver and other organs of the body following oral exposure [4], thus, further confirming its poor elimination.

Several studies have shown that acute or chronic cadmium exposure results in generation of large amount of free radicals and consequently, oxidative stress in virtually all the organs of the body [5,6]. It has been shown that cadmium toxicity has pre–testicular, testicular and post–testicular effects. The pre–testicular effects include suppression of the hypothalamo-pituitary-gonadal axis and decreased serum levels of follicle stimulating hormone (FSH) [7,8]. The reported testicular effects of cadmium toxicity have been named to include; decreased steroidogenic marker enzymes [3β hydroxyl steroid dehydrogenase (3β-HSD) and 17β hydroxyl steroid dehydrogenase (17β-HSD)] activity, decreased sperm count, daily sperm production, motility, viability and antioxidant enzymes, and marked histological changes in the testis [9–13]. Cadmium has also been shown to up-regulate p53 and Bax, and down-regulate Bcl-2 gene expressions in the testis, thus increasing

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germ cell apoptosis [14]. Gennart *et al.* [15] reported that workers exposed to cadmium for 24 years had no fertility deficits. The authors measured the urinary concentration of cadmium in 83 workers and reported a mean value of 6.94 μg of cadmium/g of creatinine. On the contrary, Akinloye et al. [8] reported that cadmium was found in high concentrations in the serum and seminal plasma of infertile male Nigerians in a fertility clinic study, thus pointing out the likely negative effect of the metal on male fertility.

Quercetin (QE), a flavonoid, is an antioxidant of plant origin. It is contained in large amounts in fruits and vegetables [16]. Studies have shown that quercetin is a powerful antioxidant owing to its ability to scavenge free radicals [16,17]. This property of quercetin may have informed its use today as an antioxidant supplement. Studies have shown that quercetin significantly reversed reproductive toxicity in male rats exposed to cadmium by lowering oxidative stress [16,17]. Although there are reports about the ameliorative effects of QE on cadmium – induced reproductive toxicity in male rats, so much is still desirable, especially as it concerns its mechanism of action and possible reversal of negative effects after withdrawal from exposure. In this study, we examined the effect of exposure to cadmium chloride on the testis, the ameliorating effect of QE (a strong antioxidant) on cadmium – induced testicular toxicity, as well as the effect of administration and subsequent withdrawal of cadmium on testicular toxicity in adult male Wistar rats.

2. Materials and methods

2.1. Chemicals

Cadmium chloride, quercetin (≥95% HPLC) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St Louis, MO, USA). All other chemicals were of the best possible analytical grade.

2.2. Laboratory animals

Thirty (30) male albino Wistar rats aged 10 weeks old, weighing 270–300 g were used for this study. The animals were purchased from the Department of Agriculture, University of Calabar, Nigeria. The rats were acclimatized for 7 days in the animal house of the Department of Physiology, and exposed to 12/12 h light/dark cycle. This study protocol was approved by the institutional animal ethics committee, and experimental animals had access to feed and water *ad libitum*, and were handled in accordance with the provisions outlined in the animal ethics handbook of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria.

2.3. Experimental design and study protocol

The rats were randomly divided into 5 groups (n = 6) namely; control group (vehicle: 0.5 ml of 2% DMSO in distilled water), $CdCl_2$ treated group (5 mg kg^{-1} bwt in 2% DMSO), QE treated group (20 mg kg^{-1} bwt in 2% DMSO), $CdCl_2$ + QE treated group (same dose as in $CdCl_2$ and QE treated groups, respectively), and $CdCl_2$ recovery group (same dose as in $CdCl_2$ treated group, with 4 weeks recovery period). The doses for quercetin and $CdCl_2$ were selected from previous studies; Farombi *et al.* [17] for quercetin, and El-Demerdash *et al.* [18] and Alkhedaide *et al.* [19] for $CdCl_2$ respectively. Cadmium chloride was shown to cause significant oxidative stress at this dose [18,19], as observed in our previous investigation on female Wistar rats [20]. Our choice of solvent (2% DMSO in distilled water) for $CdCl_2$ and QE was well tolerated in our previous study [20]. Cadmium chloride and quercetin were administered *per os* once daily (between 8.00 and 9.00 h), for 4 weeks. At the end of 4 weeks treatment period, rats in the control, $CdCl_2$, $CdCl_2$ + QE and QE groups were euthanized, while rats in the $CdCl_2$ recovery group were allowed another 4 weeks without treatment, but with *ad libitum* access to feed and water. The rats were thereafter euthanized and parameters of male reproductive function assessed.

2.4. Assessment of body weight, weights and relative weights of organs

The body weight of each rat was measured weekly. At the end of the treatment period, the animals were euthanized and the testis, epididymis, kidney and liver were harvested, cleared of adjourning tissues and weighed. The relative weight of the organs were computed from the absolute weight with relation to the final body weight of the animal and expressed as g/100 g body weight. The testis and epididymis were separately fixed in freshly prepared phosphate buffered saline (PBS).

2.5. Preparation of the testicular and epididymal tissue homogenates

The left testis and epididymis were each homogenized separately in 0.1 M phosphate buffer (pH 7.4) using Heidolph homogenizer with a Teflon pestle. Thereafter, the homogenates were centrifuged at 5,000*g* for 15 min in a cold centrifuge (4 °C). The supernatants were collected and used for biochemical analysis.

2.6. Measurement of cadmium concentration in serum, testis and epididymis

At the end of the 4 weeks treatment period and recovery (in the case of $CdCl_2$ recovery group), the concentration of Cd was assessed in serum, testis and epididymis by Atomic Absorption Spectrophotometry (AAS) as previously described [4].

2.7. Assessment of serum reproductive hormone concentrations

After euthanasia, blood was collected through cardiac puncture into plan sample bottles and allowed for 30 min without agitation. Thereafter, the blood was centrifuged at 4,000*g* for 10 min to collect serum for assessment of the concentrations of GnRH, FSH, LH and testosterone. The serum concentrations of these hormones were assessed using commercially available ELISA kits with reference to the manufacturer's instructions.

2.8. Assessment of testicular glucose, lactate and LDH activity

Glucose concentration in the supernatant was assessed using an ACCU-CHEK glucometer (Roche Diagnostics, St Louis, MO, USA) and expressed as mg/dL. Testicular lactate concentration was determined using the method of Lowry *et al.* [21] Testicular LDH activity was assessed using the method of Wahlefeld [22], with sodium lactate and nicotinamide adenine dinucleotide (NAD) as substrates. The increase in absorbance at 340 nm resulting from the formation of reduced NAD (NADH) was used for the calculation for LDH activity.

2.9. Assessment of enzymatic antioxidants in the testis

The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were assessed in the testis homogenates. SOD activity was assessed using the method of Marklund and Marklund [23]. This method is based on inhibition of pyrogallol auto-oxidation. The amount of enzyme that gives 50% inhibition of pyrogallol auto-oxidation is considered as 1 unit of enzyme activity. The absorbance was read at 425 nm and results expressed as Units/mg protein. CAT activity was assessed using the