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Original Research Article

The ameliorative effect of methanolic red carrot extract and vitamin E against cadmium-induced testicular toxicity in rats

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ABSTRACT

The current study aimed to investigate the effects of methanolic red carrot extract (MRCE) against cadmium intoxication on testis of adult Wister albino male rats. For that purpose, forty eight Wister albino male rats were randomly divided into four groups (12 rats per group). Group 1 (normal control), animals received corn oil. Group 2 (cadmium group), animals received cadmium chloride ($CdCl_2$) at a dose of (5 mg/ kg BW). Group 3 (cadmium &Vit. E group), animals received vitamin E at a dose of (400mg/kg BW) and $CdCl_2$at a dose of (5 mg/kg BW). Group 4 (cadmium & red carrot extract, animals received methanol: water red carrot extract (1:1) at a dose of 400 mg/kg and $CdCl_2$ at a dose of (5 mg/kg BW). All rats were received their corresponding treatment orally by gastric gavage daily for 4 weeks. Result of organ weight analysis in Cd –exposed rats showed a decrease in testes weight. On the contrary, MRCE and vitamin E prophylactic co-treatments with cadmium showed significant increase in testis weights in comparison to cadmium group ($P<0.05$). Moreover, sperm concentrations were reduced markedly with cadmium while they were upturned greatly after prophylactic co-treatment with either MRCE or vitamin E ($P<0.05$). Moreover, cadmium induced a significant increase in testicular malondialdehyde (MDA) and significant decrease of total antioxidant capacity (TAC) but both MRCE and vitamin E supplementation succeeded markedly to produce a significant reduction in testicular MDA and noticeable increase of TAC level. Thus, MRCE and vitamin E could be considered optimal prophylactic treatments to protect the testis of rats from cadmium intoxication.

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1. Introduction

Cadmium is a heavy metal, which is widely used in industry, affecting human health through occupational and environmental exposure. It exerts multiple toxic effects and has been classified as a human carcinogen by the International Agency for Research on Cancer. Cadmium affects cell proliferation, differentiation, apoptosis and other cellular activities (El-Demerdash et al., 2004). International and governmental agencies have made efforts to control and lower the cadmium exposure to the general public in recent years. It may cause severe damage to embryos and the reproductive organs in adults including the ovary and testes, which are sensitive to cadmium toxicity (Thompson and Bannigan, 2008). Cadmium may account for the recent declining fertility associated with reduced sperm count and testis function in men in developed countries (Siu et al., 2009).

Previous study reported that spermatogenesis is disturbed by free radical toxicity. Thus, the study of oxidative stress is a determinant in exploring some aspects affecting fertility(Aruldhas et al., 2005). Cadmium depletes many essential metal antioxidants including selenium in the body (Sato and Takizawa, 1982). Oxidative stress occurs as a result of an increase in Cadmium-induced peroxidation of membrane lipids in the organs where it accumulates (Liu et al., 1996).

Cadmium is a known endocrine disruptor by affecting the synthesis and/or regulation of several hormones (Henson and Chedrese, 2004, Darbre, 2006).Testosterone plays a crucial role in the regulation of Sertoli cells TJ-permeability barrier (Chung and Cheng, 2001), which is consistent with recent reports that androgen promotes the blood testis barrier "BTB" integrity and cell adhesion function in the testis (Meng et al., 2005, Wang et al., 2006). In male rodents, it is well established that Cd significantly alters the circulating levels of several hormones (e.g., testosterone, LH, FSH) (Lafuente et al., 2004).

Previous studies have shown that Cadmium impairs the testosterone production in isolated Leydig cells without affecting their viability (Laskey and Phelps, 1991), demonstrating that steroidogenic disruption in Leydig cells is likely to be an initial target of Cadmium toxicity as an endocrine modulator. Cadmium exposure results in an increase in reactive oxygen species like hydrogen peroxide, hydroxyl radicals and superoxide radical ions, leading to increase lipid peroxidation, change intercellular stability, damage to deoxyribonucleic acid (DNA), membranes and consequently inducing cell death (Stohs et al., 2001).

Cadmium can also modify hormone levels by affecting the hypothalamic pituitary-testicular axis in different aspects, not only via its effects on Leydig cells. For instance, Cd affected the circadian pattern release of noradrenaline, a regulator of hypothalamus hormone secretion, which resulted in changes in the daily pattern of plasma testosterone and LH levels (Lafuente et al., 2004). In addition, plasma levels of pituitary hormones (e.g., LH, FSH, prolactin, ACTH) were also modified after Cd exposure (Lafuente et al., 2003).

Epidemiologic studies show that abundant intake of vegetables, fruits, and beans reduces Cd-induced damage to the human body, due to their content of vitamins, dietary fiber, and flavonoids. Additional studies have shown. that flavonoids are specifically effective against Cd induced damage (D'Andrea, 2015). Carotenes in the carrot extract include β-carotene,α-carotene, γ–carotene, lycopene, cryptoxanthin, leutin and many partly degraded carotenoids such as abscisic acid, trisporic acid,-apocarotenoids, e.g. violaxanthin (Olson, 1989). Some of the previous active principles have the potential to minimize the deleterious effects of free radicals including the peroxy radicals (Burton, 1989).This confirms that carrot extract

could effectively protect tissues against the free radical mediated oxidative stress as evidenced by significantly decreased TBARS levels in serum. Red carrot extract was used to protect against cadmium intoxication by means of reducing oxidative stress in testis of Wistar rats.

Thus, the present study aimed to investigate the effect of cadmium chloride on testis of male rat and to investigate the possible protective effect of methanolic red carrot extract as a model of powerful antioxidant against cadmium chloride-induced testicular toxicity in rats.

2. Material and methods

2.1 Chemicals

Cadmium chloride was purchased from Sigma Aldrich Company, USA while vitamin E was purchased from local pharmacy (safe pharma, Egypt).

2.2 Preparation of Methanolicred carrot extract

Red carrot roots were purchased from El-Minia vegetable market (El-Minia city, Egypt). The red carrot roots were authenticated by Dr. Ahmed Ali Mahmoud, professor of natural products, Faculty of Science, Minia University, Minia, Egypt. Methanolic red carrot extract (MRCE) was prepared in the natural products lab, Faculty of Science, Minia University according to the method described by Zykevičiūtė-Laugks et al. (2013).

2.3 Animals

Forty eight adult Wistar albino male rats (weighing 180 - 220 gm) were purchased from lab animal center of Physiology Department, Faculty of Veterinary medicine, Beni-Suef University. The rats were allowed to acclimatize two weeks before the start of experiment. Animals were housed in plastic cages at room temperature (22±3°C) and photoperiod (12h L:12h D) with free access to water and rat ration (21% protein).

2.4 Experimental design

Forty eight adult Wister albino male rats were randomly divided into four groups (twelve rats each)as follows: 1) First Group (normal control) where animals received corn oil, 2) second group (cadmium group) where rats received 5 mg cadmium chloride /kg BW (Hassanin and Safwat, 2014), 3) Third group (cadmium chloride +Vit. E group) where rats received 5 mg cadmium chloride /kg BW and 400 mg Vit. E /kg BW (Layachi and Kechrid, 2012) and 4) Forth group (cadmium chloride + red carrot extract) where animals received methanolic red carrot extract at a dose of 400 mg/kg and cadmium chloride at a dose of 5 mg/kg BW. All rats were received their corresponding treatment orally by gastric gavage daily for (30 days) thirty days.

2.5 Sampling and tissue preparation

2.5.1. Body weight and testes weight

At the end of the experiment, the rats were weighed using digital balance and the weight was recorded to be subjected for statistical analysis and the rats' testes weight for all groups was also recorded.

2.5.2. Serum samples

At the end of the experimental period (4 weeks), blood samples were collected from the medial canthus of the eye by heparinized capillary tubes in a clean centrifuge tubes. They were left for 20 mins. at room temperature to clot and then centrifuged for separation of blood serum which was separated in Eppendorf tubes and stored at – 20^{o}c until its use for biochemical assay.

2.5.3 Tissue preparation

Rats were sacrified by decapitation and abdominal incision done immediately and testes were collected and divided into 2 parts, one part was suspended in physiological saline (0.9 %) and then was kept at – 20^{o}C for measurement of some biochemical parameters and the other part

was placed in 10% neutral buffer formalin, for histopathological examination.

2.5.4. Epididymal semen samples

Epididymis was excised and put on glass slide on which physiological saline was present and then was dissected for small pieces for gaining of semen which was diluted with saline. Then one drop of semen was pipetted on Neubauer chamber of hemocytometer slide for counting of sperms.

2.7. Biochemical examination

2.7.1. Determination of oxidative and antioxidant markers

The levels of malondialdehyde (MDA) were measured in testicular tissue homogenates using colorimetric kit depends on thiobarbituric acid (TBA) reaction. TBA reacts with MDA in acidic medium at 95°C for 30 min to form thiobarbituric acid reactive product. The absorbance of the resultant pink product can be measured at 534 nm as outlined by (Ohkawa et al., 1979, Kei, 1978). Moreover, total antioxidant capacities (TAC) in testicular homogenates were determined using colorimetric kit as outlined by (Koracevic et al., 2001).

2.8. Sperm concentration

Aliquots of sperm suspension were diluted 100 times with fresh saline, and sperm numbers were counted using a Neubauer chamber according to the method described by Aydogan and Barlas (2006).

2.9. Histopathological examination:

Testicular specimens were fixed in 10% neutral buffer formalin, dehydrated in ascending grades of ethanol alcohols , cleaned in xylol casted, blocked, cut at 2-5 µm thickness and stained with hematoxline - eosin for microscopic examination (Bancroft and Gamble, 2008).

2.10. Statistical analysis

All data were as means±SEM. Differences between the groups were determined by one way ANOVA followed by least square differences (LSD) post hoc, using SPPSS software version 16. The results were considered significant when $P< 0.05$ (Xing et al., 2009).

3. Results

3.1. Effect of methanolic red carrot extract "MRCE" against cadmium chloride intoxication on body weight, testis weight and sperm concentration

As shown in Table 1, there were no significant differences among all treatments concerning their effects on the body weights ($P>0.05$). However, $CdCl_2$ treatment decreased significantly the testicular weights when compared with control group ($P<0.05$). Interestingly, co-administration of $CdCl_2$ with either Vit. E or MRCE significantly succeeded to amend the weights toward control values ($P<0.05$).

Furthermore, Table 1 illustrated that sperm concentration was reduced significantly with $CdCl_2$treatment while it was elevated obviously with both Vit. E and MRCE prophylactic treatments in relation to $CdCl_2$ group ($P< 0.05$).

Table 1. Changes of body weight, testis weight and sperm count in rats of control, cadmium, cadmium + vitamin E and cadmium + methanolic red carrot extract groups (Mean ±SE)

	Body weight (gm)	Testes weight (gm)	Sperm count (X 10^6 / ml)
Control group	245.5±49.4[a]	1.4± 0.079[a]	220.7± 40.8[a]
Cadmium chloride group	214.5±39.5[a]	1.1± 0.057[b]	100.9 ± 17.6[b]
Cadmium chloride+ vitamin E group	232.6±35.1 [a]	1.3± 0.075[a]	223.8.± 18[a]
Cadmium chloride+methanolic red carrot extract group	241.2±42.3 [a]	1.4± 0.049[a]	235.1±27[a]

Values are represented as mean ± SEM (standard error of mean).

The different superscript letters mean a significant difference at (P< 0.05) between groups in the same column.

3.2. Effect of methanolic red carrot extract against cadmium chloride intoxication on testicular TAC and MDA activities

The results presented in Figs. 1& 2 stated that $CdCl_2$ treatment reduced significantly the levels of TAC but elevated markedly those of MDA in testicular tissue homogenates when compared with control group (P< 0.05).On the contrary, prophylactic treatment of rats with either Vit. E or MRCE recuperated greatly the TAC and MDA toward control levels (P< 0.05).

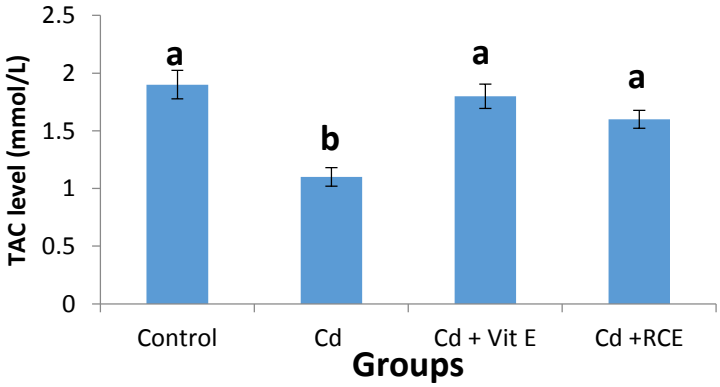

Fig. 1 Changes of testicular TAC activity in rats of control, cadmium, cadmium + vitamin E and cadmium + methanolic red carrot extract groups

Values are represented as mean ± standard error of mean (SEM).

The different superscript letters mean a significant difference at (P< 0.05) between groups.

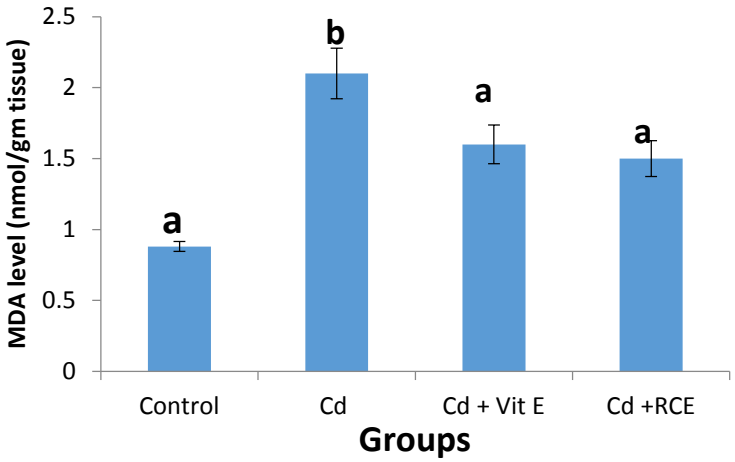

Fig. 2 Changes of testicular MDA level in rats of control, cadmium, cadmium & vitamin E and cadmium & methanolic red carrot extract groups

Values are represented as mean ± standard error of mean (SEM).

The different superscript letters mean a significant difference at ($P< 0.05$) between groups .

3.3. Effect of methanolic red carrot extract against cadmium chloride intoxication on histopathological findings

The microscopical findings indicated that $CdCl_2$ treatment induced degeneration of spermatogenic cells in addition to degeneration and reduction of the number of spermatids. Also, the blood capillaries of interstitial tissue were congested (Fig.3B). On the other side, both Vit. E and MRCE treatments improved greatly the deteriorative changes observed with $CdCl_2$. The seminiferous tubules appeared normal and contained normal spermatogenic cells and huge amount of spermatids. Also, interstitial tissues appeared with normal Leydig cells and uncongested blood capillaries (Fig. 3C, D).

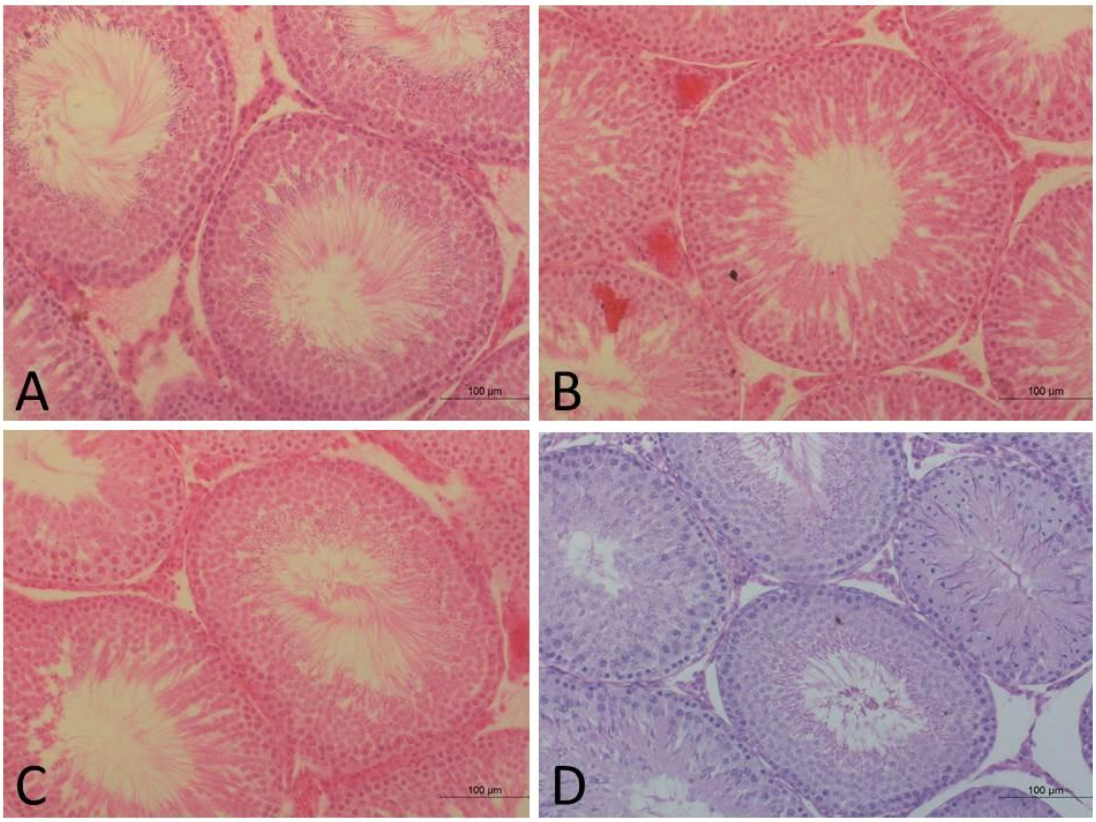

Fig. 3 A photomicrograph of testis in adult male albino rat (A) control group showing normal seminiferous tubules containing normal spermatogenic cells and huge amount of spermatid. Note, interstitial tissue containing blood capillaries and Leydig cells. (B) Cadmium chloride group showing seminiferous tubules containing degenerated spermatogenic cells and moderate amount of spermatid. Note, interstitial tissue containing congested blood capillaries. (C) Methanolic red carrot extract +cadmium chloride group showing normal seminiferous tubules containing normal spermatogenic cells and huge amount of spermatid. Note, interstitial tissue containing blood capillaries and Leydig cells. (D) Vit E + cadmium chloride group showing normal seminiferous tubules containing normal spermatogenic cells and few degenerated cells as well as moderate amount of spermatid. (H&E) stain X200.

5. Discussion

Cadmium is a known heavy metal and has gonadotoxic potentials (El-Demerdash et al., 2004). In this study cadmium treatment induced lipid peroxidation in testicular lipid membranes and this resulted in oxidative stress and increased tissue levels of lipid peroxide which was proven by an increased level of testicular malondialdehyde "MDA" which is considered one of the most common indicators of oxidative stress. This agrees with the reports of Tandon et al. (2003) and Alvarez et al. (2004). Malondialdehyde is commonly known as a marker of oxidative stress as it is one of the final products of polyunsaturated fatty acids peroxidation in the cells and this was supported by the evidence stated that an increase in free radicals is usually accompanied by overproduction of MDA. On the other side, rats that were coadminstered with cadmium and either Vitamin E or MRCE showed a significant decrease of testicular MDA level compared to the cadmium treated ones and this result agrees with the result of Burton (1989).

The result of the present study also showed a decreased testicular antioxidant status which was manifested by a reduction in testicular total antioxidant capacity "TAC" in cadmium-treated rats and this agrees with the findings of El-Demerdash et al. (2004) and Shen and Sangiah (1995). On contrary, the prophylactic administration of either Vit. E or MRCE succeeded in the restoration of the testicular TAC levels to approximately its levels in control group. This protection by a reduction in the level of LPO and the enhancement of the antioxidant status in the testis could be due to the antioxidant power of the components of the MRCE and vitamin E.

This study also found that administration of cadmium caused a significant weight loss of the testis and a reduction in epididymal sperm concentration. Cadmium has been reported to induce necrotic degeneration of testicular tissues (Wang et al., 2007) which may lead to weight loss of testis (El-Demerdash et al., 2004) and this was supported by the result of histopathological examination of rats' testes of the cadmium treated group .

Interestingly the current study found that the prophylactic administration of either vitamin E or MRCE with cadmium achieved a noticeable ameloriation of rats' testes weight and epididymal sperm concentration.

Zhang and Hamauzu (2004) reported that red carrots are good sources of natural antioxidants containing many different antioxidant components. These antioxidants include carotenoids, vitamins, phenolic compounds, flavonoids, dietary glutathione and endogenous metabolites which have been shown to function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors, and synergists. Flavonoids and phenolic derivatives, present in carrot roots play also an important role as antioxidants. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions. Flavonoid compounds with low redox potential had the ability to release electrons or hydrogen atoms, which could generate stable semiquinone-type radicals via the phenolic hydroxyl group reacting with free radicals, resulting in the termination of the free radical chain reaction and reducing biological membrane LPO (Renugadevi and Prabu, 2009, Abdel-Aziem et al., 2011, Prabu et al., 2011, Wen et al., 2013, Arafa et al., 2014, ZHANG et al., 2015).

Experimental studies have shown that flavonoids restored the depletion of antioxidants (Abdel-Aziem et al., 2011, Arafa et al., 2014) as they antagonized the effect of cadmiumon antioxidant enzyme activity (Vicente-Sánchez et al., 2008, Prabu et al., 2011, Gong et al., 2014, Arafa et al., 2014) or increased GSH content(Ghosh et al., 2010) improving the antioxidant capacity of the body.

Carrots provide, not only the major dietary fiber component of food, but also a range of micronutrients, including minerals, vitamins and antioxidant compounds, such as carotenoids and polyphenols (Augspole et al., 2014). Increased consumption of fruits and vegetables containing high levels of phytochemicals has been recommended to prevent chronic diseases related to oxidative stress in the human body (Liu, 2003, Rao and Rao, 2007, Pandey and Rizvi, 2009).

Finally, it can be concluded that cadmium induced a testicular damage through creating oxidative stress and reducing antioxidant status and the prophylactic administration of either vitamin E or MRCE markedly succeeded in amelioration of these changes through their antioxidant power.

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