Histological and Morphometric Study on the Possible Protective Effect of Vitamin E on the Cadmium Intoxication of Adult Male Albino Rat Testis

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Abstract

Background: Male infertility is one of the major health problems in life and approximately about 30% of this problem is due to male factors. Recent studies have illustrated that the testis is exceedingly sensitive to cadmium (Cd) toxicity. More important, Cd and other toxicants, such as heavy metals may account for the recent declining fertility in men among developed countries by reducing sperm count and testis function. Cd-exposed mammals, many target organs are affected including the testes, brain, liver and kidneys. Vitamin E (α-tocopherol) is a fat soluble vitamin which regulates oxidation processes in the body as it acts as a powerful antioxidant. Vitamin E supplements enhance the reproductive performance and is called an anti-sterility vitamin.

Aim of the Study: The present study was designed to investigate the protective effect of vitamin E as a model of powerful antioxidant against cadmium-induced testicular injury in male rats.

Material and Methods: 18 healthy male Sprague-Dawley rats, age 3-4 months and about 200-250g body weight were purchased from Animal House, College of Medicine, Assiut University. All animals were housed in stainless-steel cages at room temperature at a natural photoperiod. The animals were randomly divided into three groups (6 rats each) as the following: Group I (Control group). Group II (Cd-received group) received cadmium chloride at a dose of 0.5mg/100g body weight/day orally for 8 weeks. Group III (combined Cd and vit E-received group) received cadmium chloride (same dose in group II) and vitamin E at a dose of 100mg/kg/day orally for 8 weeks. By the end of the experiment the animals were firstly anaesthetized by ether then whole body perfusion fixation was done. Testicular weight was estimated and then fixed in the gluteraldehyde solution till processed for histological examination. Semithin sections were stained using Toluidine blue and were examined with light microscope. Scanning electron microscopic slices were done. Quantitative analysis including testicular weight, seminiferous tubular diameter, height of the seminiferous tubule epithelium and estimation volume density (Vv). Data were presented as means±SEM. Statistical analysis of data were calculated for significance using the student t-test.

Results: Quantitative results: Cd decreased significantly both the height of the seminiferous tubule epithelium and volume density of the seminiferous epithelium and interstitium. These changes were minimized by co administration of vit E with Cd. Histological results: Cd-received animals: The seminiferous tubule epithelium shows areas of destruction both by light microscopy and scanning-EM. Abnormal pyknotic small oval cells with condensed chromatin appears separated from the basal lamina. The interstitial spaces are widened with areas of tissue destruction and edema both by light microscopy and scanning-EM. These changes were ameliorated by co administration of vit E with Cd.

Conclusion: Cd exerts a harmful effect on male reproduction. Vitamin E could correct all the adverse effects of Cd administration on the testis structure. Thus, it is recommended to use vitamin E wherever the toxicity with Cd is the matter of concern.

Key Words: Vitamin E – Cadmium (Cd) – Albino testis.

Introduction

MALE infertility is one of the major health problems in life and approximately about 30% of this problem is due to male factors [1-3].

Cadmium (Cd) is a heavy metal and a major environmental toxicant. The general population is exposed to Cd via contaminants found in drinking water and food, while occupational exposure to Cd usually takes place during mining or manufacturing of batteries, dyes, plastics, electrochemistry and paint pigments [4]. It can be found in soils following insecticides, fungicides, sludge and commercial fertilizers containing Cd that are used in agriculture [5]. Sunflower seeds, peanuts, flaxseed, and linseed absorb and accumulate Cd from the soil in a manner similar to that of tobacco [6]. Bivalve mollusks and crustaceans are filter feeders that accumulate metals from the aquatic environment indepen-dent of environmental pollution, and contami-nated waters could further increase the content of metals [7]. Because of its high rates of soil-to-plant transfer, Cd is a contaminant found in most human food-
stuffs, which renders diet a primary source of exposure among non-smoking, non occupationally exposed populations [8,9,10]. Tobacco smoke is another important source of Cd exposure [11].

Cd has been found to produce wide range of biochemical and physiological dysfunctions in humans and laboratory animals [12]. Chronic Cd poisoning can result in nephrotoxicity, osteoporosis, cardiovascular diseases, testicular necrosis, prostatic and testicular cancers, renal failure and neurodegenerative conditions [13-15]. In Cd-exposed mammals, many target organs are affected including the testes, brain, liver and kidneys. It also involved in carcinogenesis in multiple organs including kidney, prostate, liver and pancreas [16-18]. Cancer mortality was found to be associated with environmental exposure to Cd [19-21]. The testis is extremely sensitive to Cd toxicity [22,23]. Cd impairs reproductive capacity by causing severe testicular degeneration, seminiferous tubule damage and necrosis in rats [24]. Cd It may also cause severe damage to embryos [18,25].

Vitamin E (α-tocopherol) is a fat soluble vitamin and thought to protect tissues by reducing or preventing oxidative damage [26,27]. Insufficient intake of vitamins has been reported to produce deleterious effects on the process of spermatogenesis and production of normal sperms [28-31]. Dietary intake of antioxidants from natural products with vitamins E and C can protect sperm DNA from oxidative stress in the rat testis [32]. Vitamin E regulates oxidation processes in the body as it acts as a powerful antioxidant [33-36] preventing membrane damage mediated by free radicals [37]. Vitamin E, can inhibit reactive oxygen species (ROS) [38-40], and thus terminate lipid peroxidation and stabilize the molecular composition of cellular membranes [41,42]; therefore, it prevents the harmful effects of ROS on cells.

This vitamin was reported to reduce oxidative stress in the testis [43]. It is called an anti-sterility vitamin [44] as it is associated with normal function of the male reproductive system [45]. Supplementation with vitamin E has also been shown to increase sperm concentration, improve sperm motility and enhances sperm and semen quality and fertility [46].

**Aim of the study:**

The present study was designed to investigate the protective effect of vitamin E as a model of powerful antioxidant against cadmium-induced testicular injury in male rats.

**Material and Methods**

I- **Scope, place and time of study:**

Scope of study included histological (light and scanning electron microscopic) and morphometric analysis and the study was conducted between June, July and August 2012 in Assiut University, Egypt. The scanning electron microscopic study done in King Khalid University, KSA.

II- **Study design:**

The study was experimental design post-test only one control group. The animals were kept for one week for adaptation before they randomly divided into one control and two experimental groups.

III- **Inclusion and exclusion criteria:**

For inclusion criteria, the rats were albino rats, males, of age 3 months for all rats at the beginning of the study and weighted 200-250gm.

For exclusion criteria, the rats died before the end of the experiment and showed violent activities were excluded.

IV- **Animals:**

A total number of 18 male albino rats (average weight 200-250gm.) were randomly used in this study. The animals were isolated in clean properly ventilated cages in the Animal House of Assiut University under normal conditions with an appropriate temperature, standard diurnal changes with free access to food and water. Each cage contained three rats. All animals of the three groups were at age of 3 months at beginning of the experiment. They were kept for one week adaptation before intervention. They were exposed for a period of 8 weeks of intervention.

V- **Animal grouping:**

The animals were divided into three groups (one control and two experimental):

Group I (Normal control group): Receives distilled water as sole drinking source.

Group II (Cd-received group): Received cadmium chloride orally by gavage tube.

Group III (Combined Cd and vit E-received group): Received cadmium chloride and vitamin E orally by gavage tube.

VI- **Drug dosage:**

1 - Cadmium chloride given at a dose of 0.5mg/100g body weight/day [47]. It dissolved in warm distilled water and given orally by gavage tube for 8 weeks.
2- Vitamin E at a dose of 100mg/kg/day [34]. It is given orally by gavage tube for 8 weeks.

The cadmium chloride was collected from Sigma Chemical Co., St Louis, MO, USA. Vitamin E (z-tocopherol) was obtained from Pharco Company for Pharmaceuticals, Alexandria, Egypt. It is dispensed in the form of soft gelatinous capsules each containing 1000mg of z-tocopherol acetate.

VII- Study variables:

Independent variables included the one doses only of Cadmium chloride and vitamin E. The dependent variables included the testicular tissues.

VIII- Experimental analysis:

Two methods of analysis were used in the present study:

A- Histological examination; in which the putative effects of the administration of cadmium alone and combined cadmium and vitamin E on the testis were observed by studying the morphological changes of the seminiferous epithelium and interstitium. This was attained by light and scanning electron microscope using:

1- Toluidine blue staining for semithin sections.
2- Ultrathin sections by scanning electron microscope.

B- Quantitative analysis; in which the putative effects of the administration of cadmium alone and combined cadmium and vitamin E on the testis were noticed by studying the differences in testis between the control and experimental groups. This was attained by measuring the following parameters:

1- Testicular weight (TW).
2- Seminiferous tubular diameter (DTD).
3- Height of the seminiferous tubule epithelium (HSTE).
4- Estimation volume density of the seminiferous tubule epithelium (VvEp).
5- Estimation volume density of the interstitium (VvIS).

IX- Experimental methodology:

At the end of the experiment the animals were firstly anaesthetized by ether then whole body perfusion fixation was done. Each animal’s four limbs were fixed in a dissecting dish through its four limbs. The chest was opened to expose the heat for intracardiac perfusion. Sacrification was done by intracardiac perfusion with 10ml of heparinized isotonic saline. Heparinization was achieved to facilitate testicular perfusion in using a whole body perfusion technique via the cardiac route. Heparin was used in a dose of 130 IU/100gm body weight [48]. This was followed by 2.5% cacodylate buffered glutraldehyde. The perfusion was done through the left ventricle while the right atrium was perforated to prevent rupture of the circulation by over distension. The wall of the scrotum was dissected and both testes were extracted. The epididymic and other extra parts of the spermat cord were removed. Testicular weight was estimated and then fixed in the glutaraldehyde solution till processed for histological examination. The right testis subjected for scanning electron microscopic study while the left testis for toluidine blue and quantitative study.

For semithin sections (0.5-lµm) the specimens were fixed in 4% gluteraldehyde, were stained with Toluidine blue [49] and were examined with light microscope. These sections also were subjected to morphometric study.

For scanning electron microscopy testicular tissue were fixed with glutaraldehyde in phosphate buffer for 4 hours. After the samples were washed and dehydrated, then dried by the critical point in acetone method. Testis samples were mounted on an aluminium stub and then coated with gold. The prepared samples of testis were examined under the scanning electron microscope. The photographs of testis tissue were recorded using SEM 6390 LV JEOL in King Khalid University, KSA.

Quantitative analysis:

1- Testicular weight: Using a sartorial balance capable of measuring with a precision of 0.01mg.
2- Seminiferous tubular diameter: Tubular diameter was measured using the scale slide. Five slides from each animal were drown using a camera Lucida (Leitz Wetzlar, Germany) and object piece X40. The slides were chosen from the mid area of the testis. The equation used was: Magnification= Length in image/true length.
3- Height of the seminiferous tubule epithelium: Tubular epithelial height was measured by the same technique described in measuring the tubular diameter.
4- Calculation volume density (Vv): This was done using the point counting technique. A measuring area (about 9cm x 9cm) on a graph paper was divided into 100 point (10 x 10 point). This diagram was placed against Lucida lens, and the slide was placed against object piece X40. The number of points fallen on the seminiferous epithelium was calculated (P_Ep) and the number of points fallen on the interstitial tissue was
calculated \((P_{IS})\), while the total number \((P_{IT})\) was constant and equal to 100 points Abdel Hakem [50]. The volume density of the seminiferous tubules to the testis was calculated as follow: \(Vv_{Ep} = P_{Ep}/P_{IT}\) and \(Vv_{IS} = P_{IT}/P_{IT}\).

**X- Statistics:**

Data were presented as means±SEM. Statistical analysis of data were tested for significance using the student \(t\)-test through the computerized statistical package “Prism”. Values were considered significant at \(p<0.05\).

**Results**

**Quantitative results: (Table 1 and Charts 1-5).**

The mean±SD of the testicular weight, seminiferous tubule diameter, seminiferous tubular epithelium height, volume density of seminiferous tubular epithelium \((Vv_{Ep})\) and volume density of the interstitum \((Vv_{IS})\) of the control animals as compared to that of cadmium received and combined cadmium and vitamin E received animals (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>C (n=6)</th>
<th>Cd (n=6)</th>
<th>Cd+Vit E (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicular weight (gm)</td>
<td>1.97±0.2</td>
<td>1.8±0.5</td>
<td>1.89±0.3</td>
</tr>
<tr>
<td>Seminiferous tubule diameter (µm)</td>
<td>271±12.4</td>
<td>257±11.8</td>
<td>267.3±9.4</td>
</tr>
<tr>
<td>Seminiferous tubular epithelium height (µm)</td>
<td>74.8±5.7</td>
<td>68.7±2.3</td>
<td>73.2±3.1</td>
</tr>
<tr>
<td>(Vv_{Ep}) (%)</td>
<td>75.2±4.6</td>
<td>70.3±1.5</td>
<td>73.2±3.1</td>
</tr>
<tr>
<td>(Vv_{IS}) (%)</td>
<td>14.8±1.7</td>
<td>12.8±1.2</td>
<td>13.2±1.2</td>
</tr>
</tbody>
</table>

\(*\): Significant \((p<0.05)\).

\(\text{ns}\): Non-significant \((p>0.05)\).

\(n\): Number of animals in each group.
Histological results:

Control animals:

Normal histological architecture of the testes of control animals noticed by toluidine blue stain (Fig. 1). The seminiferous tubules surrounded by basal membrane formed of myoid cells. Each tubule composed of Sertoli cells and germ cells of various stages of development.

Scanning electron microscopy:

The normal architecture of testicular seminiferous tubules and interstitial spaces shown in the control rats (Figs. 5,8). The seminiferous tubules are contact with each other and with interstitial tissues in-between them. The outer surface of the seminiferous tubules covered with single layer of flat myoid cells, whose nuclei appeared as small bulges. The myoid cells appeared to be arranged in a continuous single cell layer. The seminiferous tubules present different stages of development and flagellae of spermatids at its centre.

Cadmium received animals:

The seminiferous tubules are surrounded by thick basal lamina (Figs. 2,3). The seminiferous tubule epithelium shows areas of destruction and edema in between cells. Abnormal small oval cells with darkly stained condensed chromatin appeared separated from the basal lamina. The interstitial spaces are widened with areas of tissue destruction and edema. The interstitial blood vessels were congested and lined by thick fenestrated endothelium.
**Scanning electron microscopy:**

The seminiferous tubules appeared surrounded by myoid cells (Figs. 6, 9). The seminiferous tubular epithelium is damaged leaving many cavities. The interstitial tissues were interrupted with extensively widened spaces.

**Cadmium and vitamin E received animals:**

The degenerative picture of the cadmium received rats is ameliorated. The seminiferous tubules were surrounded by myoid cells. The seminiferous epithelium was more or less similar to control with scattered focal loss but less than that of cadmium received rats alone.

The interstitial tissues were more or less similar to the control (Fig. 4).

**Scanning electron microscopy:**

The seminiferous tubules appeared surrounded by myoid cells (Figs. 7, 10). The seminiferous tubular epithelium showed areas of loss, but less than that of the cadmium received rats alone. The interstitial tissues showed wide spaces but less than that of the cadmium received rats alone.

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**Fig. (5):** Scanning electron micrograph of testicular tissue of control rat showing seminiferous tubule (ST) lined with flat myoid cells (arrow head) and interstitial spaces (IS) in between. The lumen of the seminiferous tubule shows the presence of mature elongated spermatids (Sr) with long flagellae. (X200, bar = 1.00µm).

**Fig. (6):** Scanning electron micrograph of testicular tissue of cadmium received rat showing seminiferous tubule (ST) lined with flat myoid cells (arrow head) and wide interstitial spaces (IS) in between. Some of the seminiferous tubules are collapsed and lined by irregular basal lamina. Tissue destruction (*) within the seminiferous tubules and interstitium are noticed. (X200, bar = 1.00µm).

**Fig. (7):** Scanning electron micrograph of testicular tissue of cadmium and vitamin E received rat showing seminiferous tubule (ST) lined with flat myoid cells and wide interstitial spaces (IS) in between. Tissue destruction (*) within the seminiferous tubules and interstitium are noticed. (X200, bar = 1.00µm).

**Fig. (8):** Scanning electron micrograph of testicular tissue of control rat showing seminiferous tubule (ST) with flat myoid cells (arrow head). The seminiferous tubule shows different stages of spermatocytes with the presence of mature elongated spermatids (Sr) and its long flagellae towards the lumen. (X600, bar = 20µm).

**Fig. (9):** Scanning electron micrograph of testicular tissue of cadmium received rat showing seminiferous tubule (ST) with flat myoid cells (arrow head) and irregular basal lamina. Part of the seminiferous tubules is collapsed. Tissue destruction (*) within the seminiferous tubule is noticed with the presence of mature elongated spermatids (Sr) and its long flagellae towards the lumen. (X600, bar = 1.00µm).

**Fig. (10):** Scanning electron micrograph of testicular tissue of cadmium and vitamin E received rat showing seminiferous tubule (ST). Tissue destruction (*) within the seminiferous tubule is noticed. (X600, bar = 1.00µm).
Discussion

The present study was preferred on rats as rodent tests are especially sensitive to the toxic effects of Cd exposure [51]. Also we preferred studying cadmium as it a major environmental toxicant. Exposure to Cd via contaminants found in drinking water and food [52], occupational exposure [5] also high.

Vitamin E preferred as preventive for cadmium destruction in the present study as traditionally, vitamin E was considered as an anti-sterility vitamin [44] and was associated with normal function of the male reproductive system [35,53].

This study confirmed the histological changes by morphometrical methods. The morphometrical methods were very sensitive parameters to evaluate threshold changes, which could not be confirmed merely by the observation of testis morphology. The sensitivity of this method permitted the detection of few differences and small modifications in relation to short period of toxicity [51].

The results of the present study revealed no significant change in testicular weight following Cd administration for 8 weeks. Blanco et al., [11] and Predes et al., [51] reported that testicular weight was diminished in relation to high dose Cd. These studies attributed this effect to the massive necrotic and degenerative Cd-induced changes as the toxic effects of Cd in the male reproductive system were dose-dependent. Brzoska et al., [8] reported significant reduction of kidney and liver weight after 12 weeks of Cd administration. The difference with present results may be attributed to the shorter period of administration in our study [54]. Another suspected explanation is that the liver and kidney were the commonest target organs for drug toxicity [55,56].

The seminiferous tubular epithelium height, volume density of seminiferous tubular epithelium and volume density of the interstitium significantly reduced in Cd-received rats. These findings were similar to the morphometric results reported by Liridi et al., [57] and Predes et al., [51]. They explained their findings by the testicular tissue damage that induced by Cd.

Our results showed germ cell loss, testicular edema and vacuolar spaces both within the germinal epithelium and the interstitium. This was in accordance with that reported by Benoff et al., [58]; Biswas et al., [59]; Yang et al., [60] and Siu et al., [23]. Noticed reduced sperm count and poor semen quality, in men exposed to Cd toxicants. Siu et al., [23] reported that in vivo acute exposure to Cd caused germ cell loss, testicular edema, hemorrhage, necrosis, and sterility in several mammalian species. They reported that the underlying mechanism(s) was not known at the time.

Also the blood vessels in the present study appeared lined by thick, fenestrated endothelium and filled with clotted blood. Primary disruption in the vascular system was considered by some investigators as one of the most important factor responsible for testicular tissue damage. Permeability changes in the capillary endothelium (which cause oedema, haemorrhage or necrosis) seem to be implicated in the histopathological mechanism of these lesions [11,15].

The interstitium of the control animals in the present study are directly continuous and all seminiferous tubules are totally surrounded by interstitial tissue. Normal blood vessels and cellularity also observed. However, in the Cd-received animals the interstitium and the tubules are completely separated without cellular connections. Fluid space and fenestrae in the interstitium were obvious. Thick walled fenestrated capillaries was observed. All of the findings were similar to that reported by Predes et al., [51]. Liridi et al., [57] claimed that the interstitial oedema observed in their study caused by a direct consequence of disruption of the endothelial layer of microvessels, allowing fluids from the blood to flow into the interstitium.

The Interstitium was the most sensitive to Cd so marked tissue destruction in the scanning electron microscopic pictures was recorded in the present study. These findings were confirmed by that reported by Blanco et al., [11,61].

Histologically we noticed that the damaging effect of Cd was ameliorated by concomitant administration of vitamin E. Still there was little testicular tissue damage but less than that of Cd-received animals alone. No abnormal spermatogenic cells observed within the seminiferous tubular epithelium. Vitamin E could normalize the damaging effect of oxidative stress induced by free radicals in rat testis and improve male fertility [43].

Moreover, vitamin E partially restored the adverse histological changes and totally restored...
the adverse morphometrical changes caused in the testis of Cd-received rats. Shalaby et al., [62]; Aruldas et al., [63] contributed this to antioxidant effect of vitamin E. In addition, investigations have also shown that vitamin E prevents toxic damages to testis and sperm after exposure to other chemicals Zhou et al., [64] and Songthaveesin et al., [68]. As vitamin E is a dietary factor, which is important for normal reproduction Azzi and Stocker [66] and Eldemerdash et al., [67] and also its requirement for testicular function is well established [40].

Conclusion:

In conclusion, cadmium has a harmful effect on male reproduction. Vitamin E could compensate for all the adverse effects of cadmium administration on the testis structure. Thus, we recommend consumption of this vitamin wherever the toxicity with cadmium is the matter of concern.

References


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