Original Article

Protective Effects of Curcumin and Selenium on Testicular Toxicity Induced by Lead Acetate in Adult Male Albino Rats [Structural and Biochemical Study]


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ABSTRACT

Background: Several industrial products [such as paints, cosmetics, and petrol] contained metals like lead [Pb], which are harmful to people. The reproductive organs are the primary organs affected by heavy metal toxicity through the production of oxidative stress. Selenium and curcumin could help to counteract this toxicity.

Aim of the work: We investigated the possible role of Selenium or curcumin in improving the testicular toxicity caused by the intake of Lead orally in albino rats.

Materials and Methods: 40 adult male albino rats were selected and unsystematically separated into 4 groups [10 rats/each] included: Group 1: control group; Group 2: Pb-exposed group; Group 3: Pb plus Selenium and Group 4: Pb plus Curcumin. Blood samples were collected for examination of serum biochemical parameters, serum Testosterone, LH, FSH levels, Total Anti-oxidant Capacity [TAC], testicular tissue oxidative stress parameters and testicular histology were evaluated.

Results: Oral administration of Pb to rats for 4 weeks disturbs the testicular structure and function with a significant increase in the oxidative stress enzymes, deposition of collagen fibers and anticaspase-3 in the testis. While, administration of either Selenium or Curcumin with Pb-exposure induced significant improvement of all the testicular changes.

Conclusion: Chronic lead exposure causes deteriorating changes in the testicular structures and functions which could be improved by oral intake of either Selenium or Curcumin by their antioxidant, antifibrotic, and antiapoptotic properties.

Keywords: Lead; Testis; Oxidative stress; Selenium; Curcumin.
INTRODUCTION

Humans are continuously exposed to pollution with several heavy metals and metalloids, through air, water, and food [1]. There are many industries that use the heavy metal lead [Pb]. Overexposure to Pb is a serious problem in many countries as a result. Pb can highly accumulate in the body and is excreted in a gradual, steady manner. Also, Lead is one of the most hazardous heavy metals that may eventually accumulate in different organs because of its lengthy biologic half-life [2].

Overexposure to metals can be harmful and have numerous acute and long-term toxic effects on a variety of organs, particularly the reproductive organs and their ability to function normally. It can also result in splenomegaly, nephrotoxicity, damage to the haemopoietic system, and damage to the central and peripheral nervous system [3, 4]. Lead causes obvious testicular damage and interferes with the amounts of gonad/pituitary hormones such follicle-stimulating hormone [FSH], testosterone, and luteinizing hormone in the blood [ LH] [5]. As the testis is so susceptible to lead exposure, its harmful effects could seriously impair the germ cells; indicating poor-quality sperm, and ultimately lead to infertility. Hence, lead has emerged as a major cause of concern for human reproduction and general health [6].

Although the precise mechanism causing the combined low dosage chronic lead exposure is still unknown, oxidative stress generated by each of these metals may be connected to their combined toxicity [7]. The primary targets of lead toxicity are antioxidant enzymes including glutathione peroxidase [GPx], catalase [CAT], and Superoxide dismutase [SOD]. Lead toxicity also increases the generation of free radicals [hydroperoxides [HO2], and hydrogen peroxide [H2O2]] or reactive oxygen species [ROS], which induce cellular injury [8].

As a result of their antioxidant, anti-inflammatory, and anti-apoptotic properties, several natural and synthetic substances have been explored for their probable action to alleviate the harmful effects of lead on the reproductive system [9, 10]. Recently, multiple studies demonstrated that selenium is one of the most significant bio elements that may have antioxidant benefits against heavy metal-induced oxidative damage [11, 12]. Selenium [Se] is integrated into selenoproteins in the form of selenocysteine to provide a variety of biological features. The male reproductive function was tightly related to Se, and Se deficit decreased the amount of testicular selenoproteins and diminish the quality of mouse sperm [13].

Moreover, traditional medicine such as herbal plants [medicinal plants] and other medications have been utilized extensively to treat various reproductive diseases worldwide during the past ten years [14]. Curcumin is a naturally occurring herbal substance that has high antioxidant, anti-inflammatory, and anti-angiogenic effects. It has the potential to be used as a natural remedy for curing and preventing many disorders [15]. Several studies have explored the potential beneficial effects of curcumin on male hormone levels, testicular cells, and sperm parameters [16, 17].

Therefore, we aimed to investigate the effect of lead on the structure and functions of the testis, and compare the influence of Selenium and Curcumin in the case of oral combination with Lead to alleviate the possible testicular-toxicity caused by Lead exposure in male albino rats through histological, immunohistochemical and biochemical parameters.

PATIENTS AND METHODS

Chemicals

Lead acetate [Pb] was acquired from Sigma-Aldrich company [USA] and diluted in distilled water before oral intake. Sodium selenite [selenium 200 MCG 90 VEG. CAPS] was purchased from SOIFICOPHARM.

Curcumin

Curcumin was bought from the National Research Center in Dokki, Giza, Egypt.

Animals

Forty adult male albino rats [120-160 g] were used in this study. Rats were housed in polypropylene cages with 12-hour light/dark cycles, at a constant room temperature of 22°C, with 10% humidity, and were given free access to water and a normal pellet meal, animal doses of lead, Selenium, and Curcumin were guided by previous studies [4, 11, 16].
Ethical considerations

Animal handling was approved by the medical ethical committee, Damietta Faculty of Medicine, Al-Azhar University, Egypt [IRB 00012367-22-11-015].

Experimental design and drug administration

After acclimation for one week, rats were separated into four groups of 10 rats each [five per cage]. For 4 weeks, all groups received the following oral treatment: Group 1 [control group] were fed normally and given access to distilled water; rats in Group 2, [Pb-group], were given 50 mg/kg of lead acetate in drinking water for 4 weeks and still receiving a normal diet; rats in Group 3, [Pb+ Selenium group] received 50 mg/kg of lead acetate in drinking water plus selenium [10 g/kg b.w.] by oral gavage daily for 4 weeks; and rats in Group 4, [Pb+ Curcumin group] received 50 mg/kg of lead acetate in drinking water plus curcumin [100 mg/kg bw.] by oral gavage daily for 4 weeks.

Blood collection, serum, and tissue preparations

Blood samples were taken from the retro-orbital plexus at the end of the study [at 28th day], and serum was then separated by centrifugation at 1200 g for 15 min, collected, and maintained at 20 °C for additional biochemical tests.

Assay of serum biochemical parameters

The serum levels of testosterone in pg/ml, luteinizing hormone [LH], and follicle-stimulating hormone [FSH] in IU/ml, and serum TAC were measured using enzyme-linked immunosorbed assay [ELISA] kits in accordance with the manufacturer's instructions.

Determination of oxidative/Antioxidative status

Both testes were quickly excised, the right testis was used for histological examination and the left testis was used for determination of the oxidative/antioxidative stress parameters, rinsed, minced in ice-cold saline buffer [20 mM Tris–HCl, 0.14 M NaCl buffer, pH 7.4], and homogenized by a homogenizer [10 percent, w/v]. The homogenized tissue was used for an initial lipid peroxidation experiment, and aliquots of the homogenate were preserved for further biochemical investigation. Following the manufacturer's directions, the activities of glutathione peroxidase [GPx], catalase [CAT], Superoxide dismutase [SOD], and tissue levels of hydrogen peroxide [H2O2], and malondialdehyde [MDA] were measured.

Evaluation of histological and immunohistochemical changes

A solution of neutral buffered formalin [10%] was used for fixation of the testicular tissue, embedded in paraffin and processed for light microscopic investigation after staining with hematoxylin and eosin [H&E] stain for structural changes, Masson trichrome stain for fibrotic changes, and anti-caspase 3 for apoptotic changes [18].

Light microscopy was used to inspect the produced slides. Photographs of the images and assessments of the immunological expression of caspase 3 and the % area density of collagen fibres were made using a Raywild E5 microscope with a Raywild M-300 digital camera with the image-analyzing system [Mvi-image program v12].

Statistical analysis

The mean±SE is used to represent data. The statistical programme SPSS for Windows [Version 21.0; SPSS Inc., Chicago, IL, USA] was used to analyze the data. The one-way ANOVA was used, and Duncan's post hoc test for multiple group comparison came next with considered statistical significance at P < 0.05.

RESULTS

Effects on hormonal levels

Lead exposure considerably reduced the levels of serum testosterone, FSH, and LH in rats compared to the control group, whereas combination oral treatment of either selenium or curcumin with lead significantly increased those levels compared to the lead-exposed group [p < 0.05] [Table 1].

Parameters of Oxidative/antioxidative stress tissue levels in the various study groups

Animals exposed to Pb demonstrated a significantly lower levels of SOD, GPX, and
CAT in testicular tissue \( [p < 0.05] \) in comparison to the control group. SOD, GPx, and CAT levels significantly increased when either selenium or curcumin was combined with Pb administration \( [p < 0.05] \), in contrast to the Pb-exposed group. Nevertheless, MDA and H2O2 levels were higher in the Pb-exposed group compared to the control group \( [p < 0.05] \). In comparison to the Pb-exposed group, those levels were significantly lower after receiving Pb in combination with either selenium or curcumin \( [p < 0.05] \) [Table 2].

Histological and immunohistochemical results

Haematoxylin and eosin-stained sections results: Seminiferous tubules containing stratified germinal epithelium were found in the control group's testes. Spermatozoa were seen in the tubules' lumina. Spermatogonia, primary spermatocytes, spermatids, and sperm make up the spermatogenic epithelium. The Leydig cells are found in the tubules that are separated from one another by loose interstitial connective tissue. The testicular structure was disturbed in the lead-exposed group, as evidenced by the presence of multiple vaculotions in many seminiferous tubules caused by the complete loss of spermatogenic epithelium, and sparse epithelial remnants with the irregularly thick basement membrane. There was also a notable broadening of the interstitial tissue which contains interstitial Leydig cells, along with congestion of interstitial blood vessels. Testicular tissue sections from the Lead & Selenium treated group almost had a normal histological structure. Only a few empty areas could still be visible due to partial epithelial desquamation, the interstitial spaces appear less widened with less congestion of blood vessels and Leydig cells show polygonal shape. In Lead & Curcumin treated group: demonstrating less dilatation of the interstitial space containing the Leydig cells and seminiferous tubules restored their shape with regular basement membrane [figure 1].

Morphometric assay of area percentage density of Collagen & caspase-3

There was a significant increase in the Percentage area density of both Collagen and Caspase-3 in the lead-exposed rats compared to the control rats, while, they were significantly lower in either Selenium-treated group or Curcumin-treated group in comparison to lead exposed group [Table 3].

Table [1]: Assay of hormones in the studied groups

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control</th>
<th>Lead-exposed</th>
<th>Selenium &amp; Lead</th>
<th>Curcumin &amp; Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone [pg/ml]</td>
<td>4.34±0.11</td>
<td>2.34±0.14ab</td>
<td>3.51±0.38ab</td>
<td>3.44±0.15ab</td>
</tr>
<tr>
<td>LH [IU/ml]</td>
<td>2.47±0.12</td>
<td>1.40±0.13ab</td>
<td>1.9±0.10ab</td>
<td>1.96±0.11ab</td>
</tr>
<tr>
<td>FSH [IU/ml]</td>
<td>2.29±0.07</td>
<td>0.89±0.05ab</td>
<td>1.85±0.10ab</td>
<td>1.80±0.12ab</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD and \([n=10]\).

a significantly different from the control group at \( p < 0.05 \).

b significantly different from the lead-exposed group at \( p < 0.05 \).

Table [2]: Determination oxidative/antioxidative stress parameters in the testicular tissue of different groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Lead-exposed</th>
<th>Selenium &amp; Lead</th>
<th>Curcumin &amp; Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum TAC [pg/ml]</td>
<td>39.81±1.5</td>
<td>20.04±1.58a</td>
<td>21.13±1.02ab</td>
<td>30.41±1.48ab</td>
</tr>
<tr>
<td>SOD [U/ mg protein]</td>
<td>8.51±0.12</td>
<td>4.25±0.07a</td>
<td>6.35±0.09ab</td>
<td>6.36±0.14ab</td>
</tr>
<tr>
<td>GPx [U/ mg protein]</td>
<td>6.79±0.08</td>
<td>3.34±0.14a</td>
<td>4.77±0.11ab</td>
<td>4.83±0.11ab</td>
</tr>
<tr>
<td>CAT [U/ mg protein]</td>
<td>74.88±2.37</td>
<td>50.89±2.56a</td>
<td>62.8±3.35ab</td>
<td>63.11±3.28ab</td>
</tr>
<tr>
<td>MDA [Umol/g protein]</td>
<td>8.82±0.11</td>
<td>12.39±0.40a</td>
<td>9.57±0.18ab</td>
<td>9.6±0.24ab</td>
</tr>
<tr>
<td>H2O2 [U/ mg protein]</td>
<td>2.45 ± 0.12</td>
<td>5.52 ± 0.19ab</td>
<td>2.41 ± 0.13ab</td>
<td>2.35 ± 0.13ab</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD and \([n=10]\).

a significantly different from the control group at \( p < 0.05 \).

b significantly different from the lead-exposed group at \( p < 0.05 \).
Figure 1. Photomicrographs of sections in the testis from control group [A], lead-exposed rats [B], Selenium-treated group [C], and Curcumin-treated animals [D]. The seminiferous tubules in the control group were tightly packed, and the tubular lumen was filled with spermatogonia [curved arrow], primary spermatocytes [Sp], spermatids [St], and sperms [S]. Every tubule is sheathed by a basement membrane [arrowhead], and the Leydig cells [thick arrow] present in regular interstitial space among tubules. The testis of lead exposed groups showed excessive vacuolations [V] due to separation of many tubular germinal epithelium from its irregular thick basement membrane [arrowhead] and broadening of the interstitial space enclosing the interstitial leydig cells with congestion of its blood vessels [thin arrow]. While, testicular sections in other treated groups with either Selenium or Curcumin showed restoration of the previous changes [Hx.&E. X400] Scale bars, 100 µm.

Figure 2: Photomicrographs of testicular sections stained with Masson trichrome from control [A], lead-exposed rats [B], Selenium-treated group [C], and Curcumin-treated animals [D]: showing: no or less density of collagen fibers in the control group [A]; increased density of collagen fibers in the lead-exposed group [B]; less density of collagen fibers in the lead-exposed groups treated with either Selenium [C] or Curcumin [D] [Masson trichrome stain X-400] Scale bars, 100 µm.
**Figure 3:** Photomicrographs of Immunostained testicular sections for caspase-3 from control [A], lead-exposed rats [B], Selenium-treated group [C], and Curcumin-treated animals [D]: showing: no or less immunoexpression of caspase-3 in the control group [A]; increased caspase-3 immunoexpression in the lead-exposed group [B]; weak immunoexpression of caspase-3 in the lead-exposed groups treated with either Selenium [C] or Curcumin [D] [anticaspase-3 immunostaining X400] Scale bars, 100 μm.

**Table [3]:** Assessments of Morphometric Changes in the study groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Lead-exposed</th>
<th>Selenium &amp; Lead</th>
<th>Curcumin &amp; Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage are of Collagen density %</td>
<td>16.09 ± 0.84</td>
<td>48.02 ± 1.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.42 ± 2.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.69 ± 2.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Caspase-3 density [mm&lt;sup&gt;3&lt;/sup&gt;]</td>
<td>3.5 ± 0.14</td>
<td>17.62 ± 1.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.12 ± 1.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.76 ± 1.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD and [n=10].
<sup>a</sup> Significantly different from the control group at p < 0.05
<sup>b</sup> Significantly different from the lead-exposed group at p < 0.05

**DISCUSSION**

Metals pollution has become a serious risk due to its persistence, cumulative property, and specific lethal effects. The majority of metals released into the environment, including lead, are anthropogenic, although they are also found in natural sources such as water, air, food, soil, and rocks [19]. Due to the interference with the traditional developmental function of the male reproductive system caused by an imbalance in male and female hormone production, heavy metal exposure to humans has negative effects, such as malformations and infertility [20].

The function of the reproductive system is greatly influenced by gonadotropins, testosterone, and their interactions with regulatory areas of the central nervous system. FSH is in charge of spermatogenesis and regular testis activity, while LH is necessary for normal gonadal function. LH stimulates Leydig cells to produce testosterone, which then binds to androgen receptors [AR] in Sertoli cells to start the functional reactions necessary to promote spermatogenesis [21].

In the present study, lead exposure was found to impair the testicular function in the form of decreased serum levels of testosterone, FSH, and LH in the Pb-exposed group compared to the control group. Similar results were recorded by a previous study indicating that either lead or arsenic has reduced the testosterone, FSH, and LH in the exposed groups compared to the control groups [22]. Along with similarity, the release of FSH and LH can be decreased by lead exposure in a previous study, which has been found to be an endocrine disruptor. Leydig cells become fewer and less active as a result of a drop in LH levels, secreting testosterone in populations exposed to heavy metals [23]. This functional disturbance in the testis due to lead exposure could be
explained by the discrepancy in the Hypothalamic-Pituitary [HPT] hormonal axis, pituitary cells secrete LH in unsuitable levels and change the steroid negative feedback loop [24].

The disruption in testicular function by lead exposure in this study was associated with impairment in testicular structure in the form of excessive areas of vacuolations in many seminiferous tubules because of the complete deficiency of spermatogenic epithelium [active degeneration of germinal cells], irregular organization of epithelial cells present in the seminiferous tubules with irregular thick basement membrane and marked congested blood vessels in the wide interstitial tissue containing the Leydig cells, in the interstitium. Similar results were recorded in a recent study on testicular injuries due to subacute combined exposure to either cadmium or lead in rats in the form of degeneration and depletion of germinal cells, modifications of Sertoli cells, decrease in semen quality, and modification of spermatooza shape and size as well as, inflammatory changes [25]. Also, many human observational studies revealed that exposure to low to moderate levels of lead had a significant negative impact on sperm parameters and changes in hormone levels [26]. The ability of lead to traverse the blood-testis barrier causes detachment of Sertoli and germ cells from the basement membrane in vitro, which may be the cause of the dissociation of germ cells from the basement membrane of the seminiferous tubules [27].

Several mechanisms were studied to explain the effect of lead exposure on testicular structure and function, one of the primary causes of altered testicular structure and function and the influence of lead exposure on it is the production of free radicals and the decreased antioxidant capacity of testicular tissue [22]. In this study, on assessing the oxidative stress parameters we found that lead-exposed rats showed a significant decline in testicular tissue levels of SOD, GPX and CAT compared to the control group. Moreover, the amount of TAC in the lead-exposed group was significantly decreased as compared to those in the control group. While, the levels of MDA and H2o2 were considerably increased in the lead-exposed groups compared to the control group. Rao and colleagues previously found that oxidative damage caused by Pb-exposure, which is consistent with our findings [28]. Also, in a prior investigation on the activity of antioxidant enzymes, the effect of lead was studied in mice, the tissue levels SOD, GSH-Px, and CAT were significantly lesser in the lead exposed group compared to the control group, while, MDA levels were significantly higher in the treatment group compared to the control group [29]. This suggested that exposure to lead acetate caused oxidative stress by inhibiting the activity of this antioxidant enzyme because they form a mutually beneficial defense mechanism against free radicals and the various toxic effects of lead exposure on biological systems have been linked to an increase in MDA or lipid peroxidation as an early and sensitive effect [30].

In this study lead exposure was found to induce fibrotic changes in the testicular structure in the form of marked deposition of collagen fibers in the lead-exposed group compared to the control group. This is in agreements with the finding of several studies examining the Lead Acetate Induced - Reproductive toxicity in laboratory rats in the form of fibrous thickening of tunica albugenia, seminiferous tubular basement membrane, and interstitial tissue of epididymis [31, 32]. A previous study provided an explanation for the deposition of collagen fibers in the testis of the lead-exposed group, it hypothesized that lead stimulates macrophages, [a crucial regulator of fibrosis], which exert specific functional activities throughout the initiation, maintenance, and resolution phases of fibrosis [33]. Furthermore, Caspase-3 plays a critical part in the execution of apoptosis, which is a well-known mechanism of Pb poisoning in different organs [34, 35]. In the present study, we found that lead acetate exposure induced testicular apoptosis through activation of caspase-3 apoptotic pathway as evident by the strong immunoreactivity of Caspase-3 proteins in the testicular seminiferous tubules and interstitial cells of the lead-exposed group compared to the control group. These results are similar to the results of previous studies [36, 37] and may cause oxidation-damaged DNA in testis after lead exposure [38].

From the above findings we could hypothesize that chronic exposure to lead acetate could induce disturbance in the testicular structure and functions through oxidative stress, fibrotic and apoptotic mechanisms.

In the present study, we used either selenium or curcumin to ameliorate this testicular disturbance, and Interestingly, the use of either
Selenium or Curcumin was found to improve the biochemical, histological, immunohistochemical testicular parameters compared to lead exposed group in the form of the significant decrease in lipid and protein oxidation products [MDA & H2O2] and significant elevation in antioxidant enzymes CAT, SOD, and GPX as well as increase in serum TAC compared to lead exposed group. This suggested that their powerful antioxidant activity, which was also demonstrated in the findings of earlier studies, is the mechanism by which this effect is mediated [30, 39]. Also, the testicular damage, necrosis of seminiferous tubules and loss of germinal epithelium, increased testicular deposition of collagen fibers and increased expression of anti-apoptotic marker caspase-3 were considerably ameliorated in the groups treated with either Selenium or curcumin compared to the lead-exposed group. Similar to our results, several studies reported the protective effect of either Selenium or curcumin against the reproductive toxicity of heavy metals [25, 39-41].

Conclusion: chronic exposure to lead acetate significantly disturbs the testicular structure and functions via oxidative stress, fibrotic and apoptotic mechanisms, and may disturb the rat reproductive fertility. The intake of either Selenium or curcumin with lead acetate showed protective effects against the lead-induced testicular toxicity and this may be due to their antioxidant, antifibrotic, and antiapoptotic activity.

Conflict of Interest and Financial Disclosure: None.

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