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Lead Toxicity and the Hypothalamic-Pituitary-Testicular Axis

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Abstract

Environmental exposure to toxic levels of lead (Pb) occurs in a number of industries with potential adverse effects on the reproductive capacity of exposed men. Clinical and animal studies indicate that abnormalities of spermatogenesis result from toxic lead exposure, but eventual histopathologic alterations involved have not been identified. To explore putative abnormalities in the reproductive gonadotropic axis following lead intoxication, experimental animals when exposed to low levels of lead, 65 days old animals were treated with distilled water containing 0,0 mg (control), 10 mg lead (Pb)/Kg/day and 15 mg lead (Pb)/Kg/day intraperitoneally for 20 days. At the end of treatment, the animals were sacrificed and the blood collected for luteinizing hormone (LH) and testosterone assays. The testis was processed for histological analysis. The results showed a high serum concentration of LH and testosterone in lead-treated animals compared to controls. Histological examination of testis showed deformities in testicular morphology of lead intoxicated animals with gross damage within the somniferous tubules. A strong correlation was established between LH and testosterone suggesting an alteration in the endocrine components of the gonadotropic axis. Histological examination of pituitary gland showed some degenerative changes in endocrine cells of lead group. Changes in -LH and testosterone levels suggest that Pb exposure during the critical time of sexual differentiation induces reproductive axis abnormalities in adulthood. In conclusion, lead has a gonadotoxic effect by decreasing LH and testosterone levels and damaging the testis seminiferous tubules. Catalase activity was significantly reduced in the lead group following 65 days of exposure which possibly indicates that lead might had other mechanisms of action, such as increasing oxidative damage.

Keywords: hypothalamic-pituitary axis, lead, sex hormone

Introduction

Lead is considered as one of the major environmental pollutants. It may have different origins such as contaminated food, unsanitary preservation of food, lead hydrous piping, industrial pollution, road traffic and drinking water (López-Carrillo *et al*., 1996). Exposure to lead could damage nervous systems by provoking neuropsychological disturbances (Hogstedt *et al*., 1983), motor and sensory conduction velocities (Seppäläinen *et al*., 1983), and alter the heme synthesis (Piomelli, 1981). It can also induce acute nephrotoxicity (Goyer, 1989), cause disturbances of the reproductive system (Apostoli *et al*., 1998; Goyer, 1990) and increase blood pressure (Hertz-Picciotto and Croft, 1993; Schwartz, 1995). Some other studies have shown that it can increase the risks of lung cancer (Anttila *et al*., 1995). In animals, several experimental studies have reported impairment of the spermatogenesis (Der *et al*., 1976; Gorbel *et al*., 2002) but the mechanisms implied in the pathogenesis are not yet completely understood. Indeed, some works suggested a direct toxic action on the hypothalamic-pituitary axis, such as the study of (Stumpf *et al*., 1980) which mentioned a fall in the number of spermatozoa in the testis; In addition, other studies showed that the toxicity of lead appears primarily on the interstitial cells by the inhibition of enzymes synthesis implied in the steroidogenesis and of the hormonal receivers (Thoreux-Manlay *et al*., 1995; Wiebe *et al*., 1983). It has been demonstrated that chronic lead exposure, for 8 weeks, resulted in an appreciable accumulation of lead in different rat regions (Sandhir *et al*., 1994). This increase was accompanied by an increase in lipid peroxidation and a concomitant decrease in the activities of catalase and other antioxidant enzymes (Sandhir *et al*., 1994). In other studies, the activity of catalase in erythrocytes from workers occupationally exposed to lead was also significantly lower than that of control subjects (Cardona and Lessler, 1974; Sugawara *et al*., 1991).Therefore; it seems that chronic lead administration could reduce catalase activity in different organisms and tissues. The present work was aimed to investigate eventual action of lead on the pituitary and testicular components of the gonadotropic axis underlying alterations in reproductive function.

Materials and methods

Animals

Sixty-five days old animals were used for this study. Rats were housed in temperature controlled rooms (25°C) with constant humidity (40-70%) and 12/12 h light/ dark cycle prior to use in experimental protocols. All the procedure performed on animals were approved and conducted

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in accordance with the National Institute of health Guide (Reg. No. 488/160/1999/CPCSEA).

Experimental design

Three groups of rats were constituted (groups A, B, and C, n = 10). Control group (A) were drinking distilled water. The experimental groups received daily 10 mg/kg, group (B) and 15 mg/kg for group (C) to the end of the experimental design by intraperitoneal injections of lead acetate for 20 days.

At the end of the experimental design, the animals were sacrificed; the brains were removed immediately by opening the cranial cavity. During the course of the treatment, body weight of animals was recorded every 2 days.

Determination of testosterone and LH serum levels

Blood was collected from abdominal vein of all anesthetized rats after scarification. Serum was separated for assessment using (ABOTT) kits.

Histological procedure

Specimens from testicular tissues and pituitary were fixed in 10% neutral buffer formalin, dehydrated in ascending grades of ethanol alcohols, cleared in xylol, casted, blocked, cut at 2-5 µm thickness and stained with hematoxylin-eosin for microscopic examination (Bancroft *et al*., 1996).

Lead acetate concentration

Brain lead levels were measured in rats treated with lead acetate (10 mg/kg and 15 mg/kg). The brain collected at 20 days following this treatment. Digestion of dry brain samples was performed in a medium with nitric acid and 30% hydrogen peroxide (Krachler *et al*., 1996). The lead acetate content in brain homogenates was measured by atomic absorption spectrophotometry (Perkin Elmer 1100 B) (Chakraborty *et al*., 1996).

Catalase activity determination

The whole brain was homogenized in a phosphate buffer (50 mmol/I; pH 7.0) with digitonin (0.01%). Brain homogenates were centrifugated at 10,000 rpm for 10 min in an Eppendorf microcentrifuge. Supernatant aliquots were used to determined brain catalase levels. Catalase activity was assayed spectrophotometrically in the supernatants by measuring the decrease in absorbance of H_2O_2 at 240 nm (Aebi, 1984). Protein levels were determinated from supernatants (Bradford, 1976).

Statistical analysis

The results obtained from control and lead-poisoned animals are expressed as means ± SEM. Statistical significance was determined by using Student's unpaired t-test. A p value <0.05 was considered significant.

Results

Body and brain weights

Administration of lead acetate showed a significant decrease ($p<0.05$) in body weight of treated rats. The decrease is 28.57% and 38.09% in rats treated at 10 mg/kg and 15 mg/kg respectively when compared with control. Brain weight also decrease significantly ($p<0.05$) in treated groups; the decrease was 14.20% and 26.70% in rats treated at 10 mg/kg and 15 mg/kg respectively (Tab. 1).

Tab. 1. Effect of lead acetate on body, brain weights

Parameters/ group	Control (g)	10 mg/kg (g)	15 mg/kg (g)
Body weight	210±2.88	145±1.60*(-28.57)	130±1.98**(-38.09)
Brain weight	1.76	1.51* (-14.20)	1.29** (-26.70)

Values in brackets are % decrease (-). $^*p<0.05$; $^{**}p<0.01$

Elemental analysis

The results of Student's t-test on lead residues in rat brain treated 20 days with 10 mg/kg and 15 mg/kg of lead acetate revealed that the group treated with 15 mg/kg of lead acetate had significantly higher concentration of lead in their brains than the group treated with 10 mg/kg ($p<0.01$). The mean ± SEM for the lead-treated group at 15 mg/kg was 2.95±0.39 µg of lead/g of brain, and for lead at 10 mg/kg group, it was 0.94±0.21 µg of lead/g of brain compared with the control (Fig. 1).

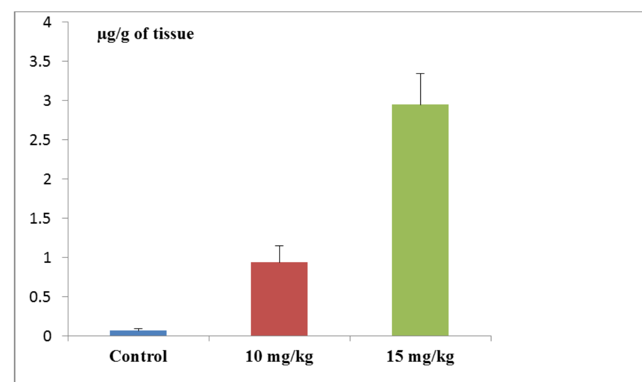

Fig. 1. Effect of lead acetate on lead concentration in rat brain. Values are mean ± SD. $^*p<0.05$; $p<0.01$

Catalase activity

The catalase activity comparisons revealed a significant decrease in brain catalase activity in animals treated with lead acetate during 20 days. The decrease is 39.04% and 45.9 % in rat treated at 10 mg/kg and 15 mg/kg respectively when compared with control ($p<0.01$) (Fig. 2).

Hormonal parameters

At the end of the 20-day treatment period, neither the plasma LH nor testosterone was found to be different from those of the control animals (Tab. 2). However, after 20 days of intoxication, the two hormones under-

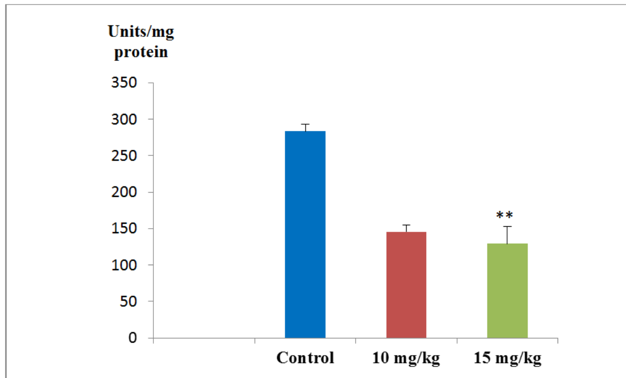

Fig. 2. Effect of lead acetate on catalase activity in brain rats. Values are mean ± SD. *$p<0.05$; ** $p<0.01$

went a significant animals treated at 15 mg/kg and 10 mg/kg (respectively 2.70±0.49; 3.58±0.45 *vs* 4.98±0.62 ng/ml, $p<0.01$; 2.18±0.58; 4.38±0.40 *vs* 6.82±0.73 ng/ml, $p<0.01$).

Tab. 2. Plasma LH and testosterone after 20 days of intraperitoneally exposure to lead acetate

Groups	LH (ng/ml)	Testosterone (ng/ml)
Control	4.98±0.62	6.82±0.73
10 mg/kg	3.58±0.45*	4.38±0.40*
15 mg/kg	2.7±0.49**	2.18±0.58**

Data are means ±SD. NS: significant difference ** $p<0.01$; * $p<0.05$

Histopathologic changes of testis

Compared with the control group (Fig. 3A), there were significant histopathological changes in Pb-treated group. The main pathological changes induced atrophication of seminiferous tubules, the number of Leydig cells appeared to be lowered in the interstitium, and parts of Sertoli cells and Leydig cells showed shrunken cells with pyknotic nucleus with evidence of destruction (Fig. 3B), these histopatological changes are less marked in rats Pb-treated at 10 mg/kg (Fig. 3C).

Histopathologic changes in pituitary gland

The fact that the testis is activated by a gonadotropic hormones principle had well been demonstrated in a number of ways. If a particular cell type in the pituitary is responsible for secretion of the gonadotropic hormone. Reference to Fig. 4A will reveal a normal pituitary gland with uniform polygonal cells with round nuclei and clear cytoplasm. However, in Pb-treated group at 10 mg/kg showed some degenerative changes in endocrine cells (Fig. 4B). The cells outlines tended to be indistinct, the cytoplasm stained less uniformly and cells had distorted, seemingly pyknotic. In Pb treated group at 15 mg/kg showed more exaggerated features, hypertrophy of endocrine cells and glial cells (Fig. 4C).

Discussion

The present data show that the toxic effects of lead occur at the level of the pituitary and gonadal components of the gonadotropic axis. Such effects of lead acetate on the hypothalamic-pituitary axis are dose-dependent. Exposure of rats to lead had a detrimental effect on growth of animals; the decrease in body weight could be due to toxic

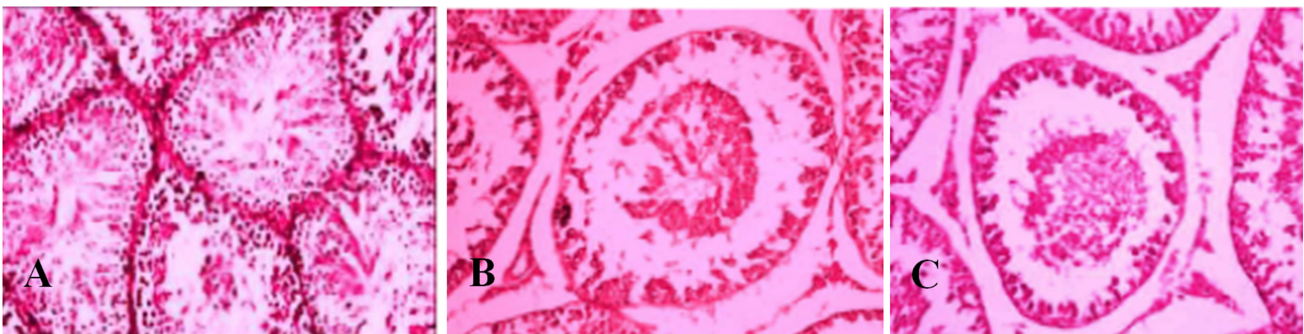

Fig. 3. Testicular histopathologic changes by HE staining (X200). Structure of seminiferous tubules was normal in control group; (B) Atrophification of seminiferous tubules, the number of leydig cells appeared to be lowered in the interstitium in Pb-treated at 15 mg/kg; (C) Histopathological changes are lesser in Pb-treated at 10 mg/kg

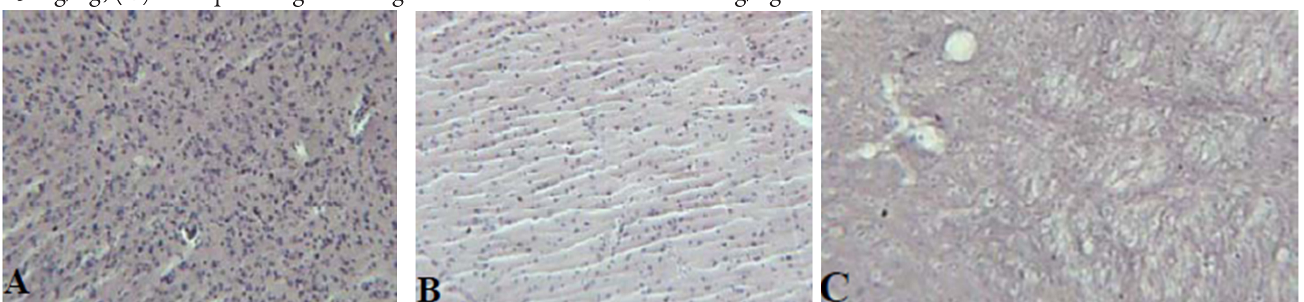

Fig. 4. Pituitary histopathologic changes by HE staining (X100). Pituitary of control; (B) pituitary of Pb-treated at 10 mg/kg; (C) Pituitary Pb-treated at 15 mg/kg

effects of lead on cellular metabolism (Wapnir *et al*., 1977) and not due to underfed or undernourished conditions. It has been suggested that loss in body weight in general on heavy metal exposure can be attributed to the enhanced synthesis of glucose from noncarbohydrate sources, that is, metabolization of fat deposits (Tandon and Tewari, 1987). Alcoholism is associated with increase in metabolic rate and increased brown fat thermogenesis in rats and humans, which in turn, may be responsible for the decrease in growth of the animals (Ahmed, 1995). Further, lead is possibly acting together, resulting, in poor growth of animals. Although significant decrease in the weight of brain has been observed, the percentage decrease in weight of rats treated with lead was greater than that of the brain control, which could be the result of high vulnerability of brain cortex towards various toxic exposures of lead. Reduced weights of various brain areas, including cerebral cortex, cerebellum, and hippocampus, also have been reported by Lampert *et al*. (1967) during lead encephalopathy in suckling rats. Lorton and Anderson (1984) reported a number of alterations in lead-exposed cerebellum, including a decrease in molecular layer width, granular cell density and dendritic arborisation, after a decrease in total weight. The distribution of lead in the various regions of brain does not appear to be uniform. It has been reported that in rats there is a selective accumulation of lead in the hippocampus (Fjerdingstad *et al*., 1974). In our study it has been observed that lead levels in the brain increased significantly in treated rats as compared with the control. Kuhlmann *et al*. (1997), Flora and Seth (2000) have also reported differential accumulation lead levels in brain. Klein and Koch (1981) postulated that in lead-exposed animals, lead concentrates in cerebellum and cerebral cortex. An almost similar observation was given by Collins *et al*. (1982), rats increased significantly after lead treatment for 6 weeks. However, in cerebellum, lead has tendency to accumulate in white matter rather than cortical grey matter, either by transport to edema fluid formed in the cortex as result of vascular damage or by direct passage over the damaged in the white matter (Lindh *et al*., 1989). This, however, is possibly due to an effective blood-brain barrier, known to restrict the entry of heavy metals in brain, and many such examples in literature confirm our findings (Flora and Tandom, 1987; Pal *et al*., 1993). This is also supported by the observation that immature animals accumulated more lead in the brain because of the underdeveloped blood-brain barrier (Willes *et al*., 1977). Further, our results are in agreement with those of Flora and Tandom (1987), who reported significantly higher accumulation of lead in the whole brain of rats coexposed to lead and ethanol. Metal toxicity or lead toxicity affects the normal histological structure of the brain and causes disturbances in the normal functions performed by them. Clasen *et al*. (1974) reported cerebral edema, muscular changes, and focal cerebellar atrophy involving Purkinje's and granular cells and neural degeneration as a result of lead encephalopathy in rat brain. Lead damages the nerve cells and ganglia and alters the cell structure (Fantin *et al*., 1985). Histologically, in the present study, disorganization of cells in the successive layers of cerebral cortex was seen in the lead-treated group. Lead and its ions induced oxidative stress in cells by several distinct mechanisms. Because lead has a high affinity for sulfhydryl residues in proteins, it has been proposed that the toxicity of lead is the result of its ability to act as a nonspecific enzyme inhibitor. It also exerts its toxic effects by combining with oxygen and sulfur-containing bioligands. It has been advocated as a calcium mimic as well as it causes oxidative stress in cells, which is detrimental to a cell's survival (Lawton and Donaldson, 1991; Zelikoff *et al*., 1988). The oxygen radicals that are normally produced within the body are usually kept in check by complex multifactorial protective enzymes, which include catalase, superoxide dismutase and glutathione peroxidase, which can check the free radicals originating either in the mitochondria or in the cytoplasm. However, the brain is one organ that is, at first instance, susceptible to peroxidase damage (Julka *et al*., 1992). Lead can alter the cellular redox state by inhibiting the enzymes involved in antioxidant defense, that is catalase, glutathione peroxide, glutathione and superoxide dismutase, which function as blockers of free radical process (Dormandy, 1978). Most of these enzymes are metalloenzymes, w sulfhydryl groups which are essential for their activities, and the oxidation of these groups that are essential for their activities, and the oxidation of these groups by lead may result in partial or complete inhibition of antioxidant defense system. The reduction of antioxidant enzymes may result in alteration in membrane integrity, thereby increasing the susceptibility of the membrane to metal exposure (Floche and Zimmermann, 1970). The decrease in catalase could be the result of in a decrease in substrate levels, H_2O_2 (Aebi, 1974), or a reduced synthesis of enzyme itself as result of higher intracellular concentration of lead/trace metal. Concerning the hormonal levels, our results showed a decrease in both plasma LH and testosterone in the animals exposed to lead for 20 days. Reproductive hormones play an important role in the regulation of spermatogenesis and sperm development. Our results well correlate with previous studies showing that in rat the effects of lead involve multiple action sites on male reproductive hormones although the most important part of these disorders probably occurs in the hypothalamic-pituitary axis (Ronis *et al*., 1996; Sokol *et al*., 1985). For example, depending on lead exposure levels and duration, signals within and between the rat's hypothalamus and pituitary appears to be disrupted by lead (Sokol *et al*., 2002). In a study of lead-exposed rats hyper responsiveness to stimulation with gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) was demonstrated (Sokol, 1987). In addition, McGregor (1990) reported a positive correlation between serum LH levels and duration of occupational lead exposure. Testosterone, the main male sex hormone, is

produced and secreted by Leydig cells of testis in response to LH stimulation. Lead concentrations in the Semen at a mean of 2 µg/dl have been reported to be inversely correlated to serum testosterone among occupationally-exposed men (Alexander *et al*., 1997). The findings suggest the involvement of other hormonal and/or hormonal feedback pathways (s) than disruption of testosterone secretion in the reproductive hormonal axis by lead, such as a lack of reflex in response to plasma testosterone, direct inhibitory androgen in Leydig cells (Wiebe *et al*., 1983), or defects in LH regulation at the pituitary level (Sokol *et al*., 1985). Molecular mechanisms underlying histopathological examination have revealed disturbance degeneration in Leydig cells among rats, thereby suggesting Leydig cells as a target for lead intoxication (Mohsen Vigeh *et al*., 2011). Our histopathological examination of rats intraperitoneally administered lead acetate showed alteration in the testis with a marked degeneration and necrosis of germ cells lining seminiferous tubules, as well as interstitial odema and complete absence of germ cells. The histological changes in testis of lead administered rats are in agreement with those reported by Hachi *et al*. (2008). Furthermore, Garu *et al*. (2011) monitored the developmental effects on testes of male offspring of lead exposed Swiss mice during gestation and lactation. The results revealed that lead induced apparent damage and reduction in the number, changes in shape and size of developing seminiferous tubules. Oral exposure of lead acetate changed the arrangement and shape of spermatogonial cells and reduced the number of Sertoli cells (Garu *et al*., 2011). It also diminished the development of Leydig cells.

In conclusion, the results of our study demonstrate that daily intraperitoneal administration of lead acetate in rats during 20 days induced a significant fall in the secretion of LH and testosterone. Although we cannot exclude a direct toxicity of lead on the interstitial cells and the Sertoli cells, it seems that the major target of lead toxicity in the reproductive hypothalamic-pituitary axis via a reduction, in the LH secretion. Further studies are still required to explain the mechanism of lead toxicity and especially to clarify the role of hypothalamic-pituitary axis in testicular dysfunction.

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