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Some Studies on the Toxic Effects of Prolonged Lead Exposure in Male Rabbits: Chromosomal and Testicular Alterations

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Abstract: The aim of the present study was to investigate the toxic effect of prolonged lead exposure on the chromosomal and testicular tissue of male rabbits. Twenty mature male New Zealand rabbits were used in four groups, the first group kept as a control. The second, third and fourth groups received low (15mg/kg b.wt), medium (20mg/kg b.wt) and high (30mg/kg b.wt) doses of lead acetate, respectively. Lead acetate was given by oral route for 12 weeks (five days a week). By the end of the experiment, animals were injected with colchicine 3 h before slaughtering.The epididymis were excised and sperm was collected for morphological abnormalities of the sperm shape. Bone marrow metaphases were prepared and scored for chromosomal aberrations. Also pathological examination of the testes and epidedemis were examined. Result revealed a statistically significant ($p < 0.01$) increase in the number of abnormal sperm in treated animals at the three tested doses. Lead acetate at the three doses increased the percentage of chromosomal abnormalities.Pathological examination of testicular tissues showed degenerative changes of spermatogonia and spermatocytes to advanced degeneration and vacuolation with pyknosis and necrosis of spermatogonia and setoli cells. The hyaline degeneration and edema in the center of semniferous tubules were detected and in high dose, the lumen of semineferous tubules showed atrophied and free from spermatocytes. In conclusion, lead acetate has genotoxic and cytotoxic effect in male rabbit and may contribute in reduction of fertility.

Key words: Rabbits · Lead Acetate · Chromosomal Abnormalities · Sperm Abnormalities · Pathological Changes

INTRODUCTION

Lead (Pb) is a soft, grey-blue heavy metal found ubiquitously and is a common cause of poisoning in domestic animals throughout the world. Health hazards from increased Pb exposure as a result of industrial and environmental pollution are recognized. It has been found to produce wide range of biochemical and physiological dysfunctions [1]. Pb can be present in the air in the vicinity of factories, because of its persistence in the environment; exposure to lead has become a major public health concern [2]. In eukaryotic cells, this metal is usually genotoxic through a mechanism that until now has not been well characterized and possibly involves indirect damage to DNA, either by affecting the stabilization of chromatin or by interacting with repair processes [3,4].

Several studies in animals showed significant increases in the chromosome aberrations rate in bone marrow cells after intraperitoneal administration of lead acetate in rats [5] and mice [6]. Other authors did not find any increase in the frequencies of chromosomal aberrations in mice fed with lead acetate [7]. In human, most of studies using chromosomal aberrations test were performed in occupationally exposed workers, there is a considerable controversy regarding the ability of lead to cause chromosomal damage on exposed individual [8]. Several studies reported increases in the frequency of chromosomal aberrations in human populations exposed to lead [9-13]. However, other workers found no effects of lead exposure on chromosomal aberrations frequency [14-16].

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Many metal ions (lead, mercury, arsenic, cadmium, chromium, nickel, vanadium, copper, lithium) exert a wide variety of adverse effects on reproduction and development, including influence on male and female subfertility or fertility, abortions, malformations and birth defects [17,18]. Several studies have reported declines in semen quality associated with exposure to Pb [19-20]. Reproductive dysfunction by lead has distinct morphological and biochemical features such as, decrease sperm quality and alters sperm morphology, as well as low androgen levels [21,22]. Serum testosterone level decreased after lead exposure in male rabbit [23]. In the animal model, lead has a primary toxic effect on the hypothalamic pituitary unit, a primary effect on the testes and acts at all levels of the reproductive axis [24]. Although evidence of a genetic risk associated with lead exposure actually exists, there are still confiicting data on the conditions under which its genotoxicity becomes apparent. In addition, little is known about cytogenetic in rabbit treated with lead acetate [25]. So, the present study has been undertaken on rabbits to investigate the chronic effects of lead acetate on chromosomal aberrations, sperm abnormalities and pathology of testes.

MATERIALS AND METHODS

Animals: Twenty mature New Zealand male rabbits were used and maintained in individual wire cages, given limited food and water. Rabbits were reared in the animal house, National Research Center, Egypt. The animals had 2700-3500 g body weight and were divided into four groups, each of five animals. The first group served as a control. The second group (low dose group) was given lead acetate at a dose of 15 mg/kg daily. The third group (medium dose group) was given lead acetate orally at a dose of 20mg /kg daily. The fourth group (high dose group) was given lead acetate of 30mg/kg. All dosed were orally administered over an experimental period of 12 weeks (5 day per week).

Chromosome Aberrations: Animals were injected with colchicine (0.6 mg/kg b. wt) for 3h prior to collecting samples. Bone marrow metaphases were prepared according to Yosida and Amano [26]. Slides were stained with 7% Giemsa stain in phosphate buffer (pH 6.8). 500 well spread metaphases (5 animals/group) were scored for chromosomal aberrations. The types of aberrations in bone-marrow cells including gaps, breaks, deletions, fragments, centric fusions and polyploidy were scored.

Sperm-shape Abnormalities: The epididymis were excised and sperm was collected in isotonic sodium citrate solution (2.2%). Smears were prepared and sperms were stained with Eosin Y. At least 1000 sperm per animal (5 animals/ group) were assessed for morphological abnormalities of the sperm shape.

Histopathological Examination: After the complete necropsy, testicular and epididymal specimens were collected from all experiment groups and fixed in 10% neutral buffered formalin for the histopathological examination. After proper fixation, the tissue was rinsed with water and dehydrated in ascending grades of alcohols, cleared in xylene and embedded in paraffin. Tissue blocks were cut into thin sections (2-5 micron) and routinely prepared and stained with haematoxylin and eosin (HandE).

Statistical Analysis: The significance of the results from the control and treated data was calculated using (t- test) for chromosome aberrations in bone marrow cells and sperm- shape abnormalities.

RESULTS

Chromosomal Abnormalities: The lead acetate doses of 15, 20 and 30 mg/kg b.wt induced significant increase in the percentages of chromosomal aberrations in bone marrow of male rabbit even after excluding gaps (Table 1). The intensity of the effect is a function of lead concentration. High incidence of chromosomal aberrations observed after treatment with the highest tested dose 30 mg/kg b.wt where the mean percentage of aberrant cells reached 13.20±0.68 compared with 2.20±0.56 for control.

Concerning the different types of chromosomal aberrations, it was clearly indicated that gaps are the most sensitive type of aberrations to be induced after lead administration. Fragments, breaks, deletions and centric fusions were also recorded. Numerical chromosomal aberrations in the form of polyploidy were observed with the three doses (Table 1).

Sperm Abnormalities: Table (2) represents the number and mean percentage of sperm shape abnormalities. The mean percentage of sperm shape abnormalities for animals treated with three doses of lead acetate significantly ($p<0.01$) increased than control animals. The effect was dose dependent. The mean percentage of sperm abnormalities reached 17.96±0.48 after treatment with the highest dose 30 mg/kg b.wt. which significantly ($p<0.01$) exceed that of the control.

Table 1: Percentage of chromosomal aberrations induced in bone marrow of male rabbit after treatment with different doses of lead acetate for 8 weeks.

Treatments	Abnormal Metaphases			No. of different types of metaphases					
		Mean (%)±SE							
mg/kg b.wt	No.	Including Gaps	Excluding Gaps	G.	Frag. and/or Br.	Del.	C.F.	M.A.	Polyp.
I. Control	25	5.00±0.48	2.20±0.56	14	7	3	0	0	1
II. Lead acetate									
15mg	55	11.00±0.54**	7.80±0.68 **	16	19	7	3	8	2
20 mg	68	13.60±0.58**	9.60±0.65**	20	24	6	5	11	2
30 mg	85	17.00±0.78**	13.20±0.68**	19	33	9	4	15	5

Total number of examined metaphases 500 (5 animals/group)

** Highly significant $p < 0.01$ level (t-test)

G.: Gap; Frag.: Fragments; Br. Breaks; Del.: Deletions; C. F.: Centric Fusions; M.A.: Multiple Aberrations; Polyp:Polyploidy.

Table 2: Percentage of sperm abnormalities induced in male rabbit after treatment with different doses of lead acetate for 8 weeks.

Treatments mg/kg b.wt	Abnormal sperm		No. of different types of sperms				
	No.	Mean (%)±SE	Amorphous	Triangle	Small	Big	Coiled tail
I. Control	73	1.46±0.49	47	18	2	1	5
II. Lead acetate							
15mg	315	6. 30. 0±0.41**	120	91	27	32	45
20 mg	651	13.02±0.58 **	405	115	36	41	54
30 mg	898	17.96±0.48**	509	213	44	51	81

Total number of examined sperms 5000 (1000 /animal, 5 animals/group)

** Highly significant $p < 0.01$ level(t-test).

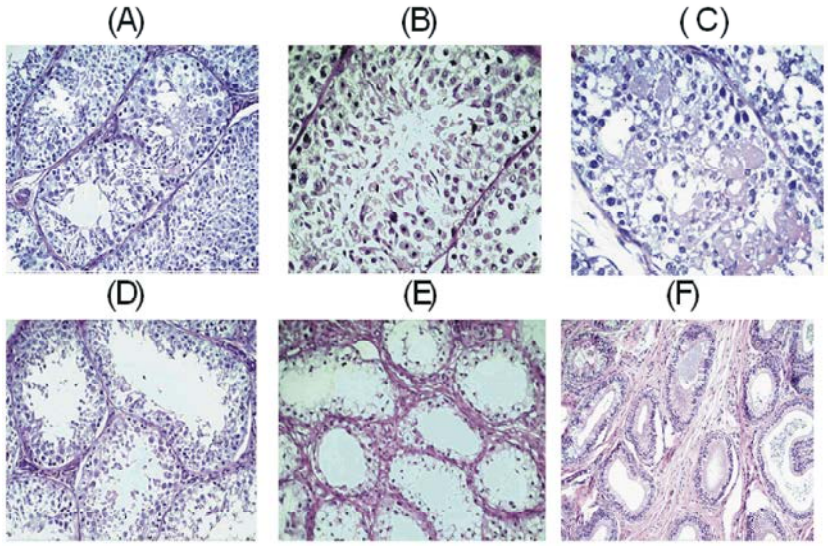

Fig. 1: Seminiferous tubules of lead toxicity rabbit, showing (A) vacuolation and degeneration of spermatogonia, lake of spermatocytes, and central edema (low dose group, H&E X 200), (B) pykness of spermatogonia and sertoli cells with very low number of spermatocytes (H&EX 400), (C) necrosis, hyaline degeneration andedema in the center of seminiferous tubule (high dose group, H&EX400), (D) pyknosis of spermatogonia and sertoli cells with absence of spermatocytes (high dose group, H&EX400), (E) pyknosis, necrosisof spermatogonia and sertoli cells with absence of spermatocytes, oedema and Proliferation of interstitial cells (H&E X 400) and Epididymis showing some lumen of ducts are free from spermatozoa, other contain homogenous fluid andsomecontain few spermatied (high dose group, H&EX200).

Histopathological Examination: Microscopical examination of the testis of the control rabbits were within the normal histological structure limit. Examination of seminiferous tubules of lead toxicity rabbit at low chronic dose group (15 mg/kg), showed vacuolation and degenerative changes of most spermatogonia (Fig. 1A) and piknotic changes of spermatocytes. The center of most semineferous tubules showed moderate number of spermatozoa and edema (Fig. 1B). While examination of seminiferous tubules of lead toxicity rabbit at medium chronic dose group (20mg /kg), showed pynkness of spermatogonia, necrosis hyaline degeneration and edema of spermatocytes (Fig. 1C), also focal areas of vacuolar degenerative changes appeared in the cytoplasm of the spermatogenic epithelium, degenerative changes of spermatogonia and abnormal distribution of spermatozoa. The Lumen of the seminiferous tubules were free from spermatozoa in most examined semineferous tubules and contain oedematous hyaline materials and debris of shedded cells, also proliferation of interstitial cells between the semineferous tubules and oedema was detected (Fig. 1D). At high chronic dose (30mg/kg) advanced degeneration and necrosis of spermatogenic and interstitial Leydig cells were shown, also the lumen of most semineferous tubules were free from any spermatozoa, the semineferous tubules were irregular in outline and shrinkage and atrophied (Fig. 1 E). With respect to epididymis of high dose group, some lumens of ducts are free from spermatozoa, others contain homogenous fluid and some contain few spermatied (Fig. 1F).

DISCUSSION

The increased percentages of chromosomal aberrations in our study supported previous data from human and animal studies including rats and mice. Muro and Goyer [27] detected chromosome gaps and breaks by 1% lead acetate in cultured mouse leucocytes. An increase in the frequency of chromosomal aberrations was reported in human lymphocytes treated *in vitro* with lead acetate [28-29]. Similarly, an increase in the frequency of sister chromatid exchange has been reported in human lymphocytes after lead sulphate treatment [30]. Lorencz *et al*. [31], who found increases in numerical aberrations in Wistar rats treated with different doses of lead acetate. Aboul-Ela [32] found only structural aberrations like chromatid gaps, deletions and fragments in bone marrow cells of male Swiss mice after oral administration of lead acetate. Celik *et al*. [33] had observed that lead acetate significantly increased the micronucleus frequency in peripheral blood of rats. In other studies, however, no significant increase was found in the frequency of chromosomal aberration and micronuclei in lead-exposed cultured peripheral blood lymphocytes [34]. Bauchinger and Schmid [3] failed to observe any significant change in the chromosomal aberrations in CHO cells exposed to different concentrations of lead acetate. The variability found in the different studies could be due to the infiuence of different experimental variables that may act as confounding factors, such as duration and route of lead exposure, cell culturing time following the exposure, smoking habits and simultaneous exposure to other toxic agents that could act by modifying the genotoxic response of the cells to lead exposure [8]. In addition, different types of cells have different susceptibility to the genotoxic action of lead. This difference could be due to the presence of proteins, such as metallothionein in erythrocytes that sequestered lead into a nonbioavailable form, protecting the individual from the toxicity of metal [36].

The mechanisms for these genotoxic responses may involve indirect damage to DNA affecting the stabilization of chromatin [37] or interacting with repair processes [4]. Lead is believed to covalently interact with tertiary phosphate ions in nucleic acids and proteins [38]. Lead is reported to affect the fidelity of DNA synthesis in vitro[3]. In a study performed by Jagetia and Aruna [40], it was reported that lead nitrate treatment increased erythropoiesis in mouse bone marrow as evidenced by an increase in the %PCEs. Similarly, in another study, lead acetate administration has been reported to increase the cell proliferation in rat kidney by 40-fold [41]. The increase in cell population in some organs/tissues of rats indicates that lead led the tumor formation in some tissues, particularly kidney tissue. In contrast, Sata *et al*. [42] reported a decrease in cells of the immune system in workers exposed to high lead levels.

These results showed that exposure of male rabbits to the three doses of lead acetate had adverse effect on the sperm. It increased the percentage of sperm shape abnormalities. These finding coordinate with results reported by Oliveira *et al*. [43] who found a decrease in the percentage of sperm motility and intact acrosomes in mice treated with lead acetate. Also, Mendiola *et al*. [44] reported a significant positive association between the percentage of immotile sperms and seminal plasma levels of lead and cadmium in men. Moreover, Leiva *et al*. [45] reported a reduction in epididymal sperm number and daily sperm production in male rats treated with lead

acetate and explained this reduction in sperm number that Lead acetate administration inhibited spermatogenesis by reducing the length of the stages related to spermiation and onset of mitosis [45].

The present study clearly demonstrated that chronic exposure of an adult male rabbits to lead acetate can seriously alter the testicular tissues which started the changes with vacuolar degeneration till necrosis and atrophy of semineferous tubules, the changes were dose dependants according to the experimental groups. Chronic low and moderate groups showed vacuolation and degenerative changes of most spermatogonia arrest of spermatogenesis and pyknotic changes of spermatocytes. The center of most seminiferous tubules showed moderate number of spermatozoa and edema. While high dose group showed pyknosis of spermatogonia, necrosis hyaline degeneration and edema of spermatocytes, advanced degeneration and necrosis of spermatogonia and interstitial (Leydig) cells and abnormal distribution of spermatozoa. These results indicated that in adult male rabbits, lead targets testicular spermatogenesis and sperm within the epididymis to produce reproductive toxicity. These findings support the results from other reports that lead acetate can seriously alter the testes and reproductive tract in male rats treated with lead acetate for 90 days [37,46-48]. In this respect, Vaziri and Sica [49] reported that lead exposure enhances intracellular reactive oxygen species (ROS) production and lipid peroxidation, which may lead to tissue damage. Epididymal change in our results showed free of sperm at high dose which considered an important contributory factor in infertility caused by lead, this result are coincident with the study conducted by Marchlewicz *et al.* [50]. These results indicated that lead acts as spermicidal agent in case of high exposure for a long time, also our experiments acted that toxic effects of lead on reproductive system in male rabbits were dose-dependent, these findings correspond with observations of Sokol [51].

In conclusion, lead has a genotoxic and cytotoxic effects in male rabbits and its effect is based on the dose-effect relationship.

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