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Adverse Effects of Lead Acetate on Female Reproductive System of Mice

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Abstract:

Lead exposure is considered as a serious environmental issue. Lead is known to exert its toxic effects on various vital organs of the body. The present study was performed to analyse the toxic effects of lead acetate on female reproductive system of mice at two different doses namely 2 and 10 mg/ kg body weight for 30 days. The result of the study indicated that lead acetate significantly and dose dependently decreased to body weight, uterine and ovarian tissue weights of female mice. It also altered the cyclicity of estrous cycle, reduced protein and glycogen levels and increased the cholesterol levels in mice. Various researchers have studies the effect of very high dose of lead on the female reproductive system. However the results of the present study indicate that lead exerts toxic effect on female reproductive system even at very low dose. Lead is found to exert its adverse effects on female reproductive system at low concentration also. This emphasizes the need to minimize the exposure of subjects to this toxicant which is indeed very difficult to achieve in this era of rapid industrialization.

Keywords: Estrous cycle, Female reproduction, Lead acetate, Swiss mice.

1.0 Introduction:

Metals such as copper, iron, tin etc have been used by humans since time immemorial.A great variety of metals have found application in industries, agriculture and medical sector.This has led to an increase in their levels in the environment. Lead, which is one such metal,has found extensive application in storage batteries and cable industries. In addition to this, various lead compounds are used as insecticides and as fuel additives. The widespread use of lead has resulted in major environment pollution. Lead toxicity was recognized as early as300 BC during which a case of lead poisoning has been recorded by Hippocrates.Lead exists in environment both in inorganic and organic forms. It is found as lead salt, as tetraethyl lead, as tetramethyl lead and as metallic lead etc.Occupational exposure to lead is also common as research reports have indicated high lead levels in workers working in smelting, battery manufacturing industry, painting industry, typesetting industries and even in stained glass artists.

Lead is ubiquitous than most other toxic metals. Humans are exposed to this metal through air, water and food. Although it is well known that the main target for lead toxicity is red blood cells, it is also known to exert its toxicity on reproductive organs in males and females. Lead has been recognised as a reproductive hazard since the days of ancient Rome (Gilfillan, 1965). It has been used by some people as a spermicide and as an abortifacient in females. Female exposure to lead has been associated with amenorrhea and other menstrual disorders, infertility, spontaneous abortions, still births and neonatal deaths (Gerber,*et al.,* 1980).Experimentally induced lead toxicity in animals has found to decrease fertility, caused delay in vaginal opening, resulted in ovarian atrophy and altered ovarian cycle (Mattison,*et al.,* 1983). The sites of action include the hypothalamus, pituitary, ovaries and uterus. Prenatal exposure to lead can result in spontaneous abortions. Exposure to lead is reported to be detrimental to implantation and embryonic survival (Mattison, 1983). Lead acetate is found to induce tetarogenic effect and maternal toxicity in animals (Mogra,*et al.,* 2009). Previous studies have determined the toxicity of lead on female reproductive organs especially ovaries. Taupeau,*et al.,* (2001) have reported lead-induced damage in the process of ovarian folliculogenesis at two different doses 10 mg/kg/day for 15 days or 10 mg/kg/week for 15 weeks. Qureshi,*et al.,* (2010) have studied the protective role of vitamins C and E on lead acetate-induced chronic reproductive toxicity in females at a dose of 160mg/kg and 320mg/kg for 3 months and have reported decrease in fertility of these mice. Sharma,*et al.,* (2013) have determined the

detrimental effect of lead acetate-induced toxicity on postnatal development of ovary at a dose of 266 mg/kg/body weight (8 mg/animal/day) and 1066 mg/kg/body weight (32 mg/animal/day). Similarly Sharma and Mogra (2013) have also studied the toxic effects of gestational and lactational exposure of lead acetate on implantation and on some neonatal parameters in mice at three different doses of 266 mg/kg body weight, 532 mg/kg body weight and 1064 mg/kg body weight. A recent study has also reported the adverse effect of lead on both male and female reproductive system at a dose of 640mg/ kg body weight (Sharma *et al.,* 2012) and reported that exposure of lead acetate in female mice induced miscarriages in females and resulted in infant mortality.

Although many previous researchers have reported the ill effect of lead toxicity on female reproductive system of mice, the dose of lead acetate used to induce toxicity was found to be much higher. Thus we attempted to study thesubacute toxicity of lead acetate on female reproductive system at much less dose as compared to many studies. The present study was undertaken to evaluate physical and biochemical changes in female reproductive organs of mice at two different doses of lead treatment 2 and 10 mg/ kg body weight/day for a period of 30 days.

2.0 Materials and Methods:

2.1 Chemical:

Chemicals used in the present study were purchased from Sisco Research Laboratories Pvt. Ltd, Mumbai India and were of analytical grade.

2.2 Animals:

Healthy adult, Swiss strain female albino mice (*Musmusculus*) weighing approximately 30-35 gm were used in the present experiment. All animals were fed with standard chow and water adlibitum and maintained under standard laboratory conditions of temperature (25 ± 2ºC) and relative humidity (50 – 55%) at 12 h light/ dark cycle in the animal house of Department of Zoology, Gujarat University, Ahmedabad. The experimental protocol were assessed and approved by "The committee for the purpose of control and supervision of Experiment on Animals" (Reg – 167/ 1999/ CPCSEA), New Delhi, India and animal ethical guidelines laid down by Prevention of Cruelty to Animals Act, 1960 (59 of 1960) Government of India were rigorously followed.

2.3 Experimental Protocol:

Thirty female mice were randomly segregated in three groups: control, low dose and high dose lead acetate-treated animals (the dose was chosen based on our preliminary study). Each group of animals were caged separately and administered the test substance as follows:

1. Group I control: Animals of group I were injected intraperitoneally with 0.2ml normal saline/ mouse/ day for 30 days.2. Group II Low dose: This group of mice were administrated with 0.2 ml oflead acetate ip at a dose of 2mg/Kg body weight per day for 30 days. 3. Group III High dose: Animals of this group received 0.2 ml lead acetate ip at dose of 10mg/ Kg body weight/ day for 30 days.

2.4 Gravimetric Analysis:

The body weight of all animals was recorded daily. On the 31st dayanimals were sacrificed by cervical dislocation and the female reproductive organs namely uterusand ovary were dissected out, blotted free from blood and weighed and used for further analysis. The relative uterine and ovarian organ weights were also calculated by dividing the organ weight by body weight and multiplying by 100.

2. 5 Estrous Cycle:

The estrous cycle is the female reproductive cycle in mice which is of 4-5 days duration with stages namely proestrous, estrous, metaestrous and diestrous. A medical dropper containing 1-2 drops of saline was gently inserted into the vagina of mice and vaginal fluid was aspirated and smeared on a clean slide and stained for 4-5 min with Ehrlich's haematoxylin and observed under light microscope.

2. 6 Protein Estimation:

Protein content was estimated in ovary and uterus by the method of Lowry,*et al.,*(1951) using bovine serum albumin as a standard. When protein reacts with phenol reagent of FolinCiocalteu, a deep blue colour develops. The colour development is due to two reactions occurring simultaneously i.e., the reaction of alkaline copper sulphate solution with peptide bonds and reduction of phosphomolybdic and phosphotungstic acids present in the protein. The blue colour that develops is quantitatively proportional to the total protein, which was measured at 540 nm. The protein content was expressed as mg/100 mg tissue weight.

2. 7 Phosphorylase Activity (E.C. 2.4.1.1):

The activity of phosphorylase in the uterus of control and lead acetate treated mice was estimated by the method of Cori,*et al.,* (1943). The

enzyme phosphorylase hydrolyses the substrate glucose-l-phosphate.A known amount of tissue was homogenised in distilled water. 0.1 ml of tissue homogenate was added to 0.2 ml sodium citrate buffer (0.1 M; pH 5.9) and 0.3 ml of 0.15 M potassium fluoride and 0.05 ml of 0.2 M glucose-1-phosphate and mixed well, incubated for 15 minute at 37°C after which 1 ml of 10% TCA was added to stop the reaction.The inorganic phosphorus formed at the end of the reaction was measured by using the method of Fiske and Subbarow (1925).

2. 8 Glycogen Content:

The glycogen content of the uterus was analyzed by the method of Seifter, et al., (1950). The glycogen present in tissue is converted to glucose, which reacts with anthrone reagent to give a green coloured product which was read at 620 nm. The glycogen concentration was expressed as mg/100 mg tissue weight.

2. 9 Cholesterol Content:

The cholesterol content of the ovary was estimated by the method of Zlatkis,*et al.*, (1953). Cholesterol forms a coloured complex with ferric chloride ($FeCl_3$) in the presence of concentrated sulphuric acid and glacial acetic acid which can be measured at 540 nm. The cholesterol content was expressed as mg/100 mg of tissue weight.

2. 10 Statistical Analysis:

The data was statistically analyzed using students t test. The level of significance was accepted with *p < 0.05.

3.0 Results and Discussion:

Repeated intraperitoneal administration of lead acetate to mice resulted in whitish scar on skin and abdominal tissues and they were observed to be more lethargic. However control mice were normal in appearance.

3. 1 Body Weight and Organ Weight:

The changes in body weight and absolute and relative weight of ovary and uterine tissues is depicted in Table 1. Results indicated a dose-dependent significant reduction in body weight of female mice treated with lead acetate for 30 days as compared to control mice. Similarly intraperitoneal administration of lead acetate (2 and 10 mg/kg body weight) in mice for 30 days caused reduction in ovary and uterine weights in a significant manner. Repeated intraperitoneal administration of lead acetate (in saline) to mice for 30 days caused appearance of whitish scar on the skin at the sight of injection and also in the abdominal soft tissues. Such appearances were not observed in the control mice. Lead acetate intoxication is reported to cause calcification of soft tissue (Vandeputte,*et al.,* 1990). Our results also suggested significant reduction in body weight of mice after treatment with lead acetate (Table 1). Taupeau,*et al.,* (2001) who have worked on lead toxicity have reported no change in body weight gain and ovarian weight of mice intoxicated with lead acetate. However our results corroborated with the findings of Qureshi,*et al.,*(2010) who have also reported that body weight of female mice treated with lead decreases significantly.The results of the present study suggested a significant reduction in absolute and relative ovarian weights and uterine weighs in both low and high dose lead acetate treated animals. Rao (1989) reported that male rats administered with 4 mg and 6 mg/kg body weight of lead acetate caused a general reduction in growth rate and tissue weight.

3. 2 Estrous Cycle:

Estrous cycle in control mice was of 4-5 days. However lead acetate treated mice showed a drastic change in their estrous cycle. In low dose treated mice the cyclicity stopped after 17 days of treatment at diestrous stage (Table 2). The change in estrous cycle of high dose lead acetate treated mice was more severe in which the cyclicity stopped after 14 days of treatment (Table 2).

In our study we observed highly altered cylicity of estrous cycle in lead acetate treated mice (Table 2). It was found to be stopped at diestrous stage. The effect was more pronounced in high dose treated animals. However the control mice showed regular estrous cycle and normal duration of each phases of estrous cycle. An estrous cycle is a rhythmic reproductive cycle occurring in sexually mature female mammals which depend upon the periodic release of gonadotropic releasing hormones, gonadotropins and sex hormones and gives a fair index of ovarian and uterine function. The stages of estrous cycle are regulated by female reproductive hormones namely estrogen and progesterone synthesized in the ovary. It is known that proestrous stage predominantly consists of nucleated epithelial cells, estrous consists of anucleatedcornified cells, metaestrous consisting of leukocytes and epithelial and cornified cells and diestrous mainly consisting of leucocytes. Alterations in menstrual cycle and premature menopause after prolonged exposure to lead have also been observed by Laughlin,*et al.,* (1987) in rhesus monkeys. Lead acetate was found to influence the estrous cyclicity in Swiss mice

administered at dose of 160 mg/kg body weight and 320 mg/kg body weight for 30 days (Qureshi*et al.,* 2010). In the present study, the reason for interruption of estrous cycle may be due to hormonal imbalance.

Table 1: Changes in body weight and organ weight of control and lead acetate treated mice

S.No	Groups	Parameters				
		Body weight (g)	Absolute uterine weight (g)	Relative uterine weight (g)	Absolute ovarian weight (g)	Relative ovarian weight (g)
1	Control (I)	32.16 ± 0.60	125.75 ± 12.95	391.37 ± 36.69	17.36 ± 0.82	54.03 ± 2.62
2	Lead acetate – Low dose (II)	29.02 ± 0.87*	99.18 ± 8.26*	308.10 ± 26.93*	13.82 ± 0.71*	42.76 ± 2.56*
3	Lead acetate – high dose (III)	26.07 ± 0.70*	83.63 ± 9.68*	278.11 ± 31.25*	11.15 ± 0.62*	37.08 ± 1.85*

Results are expressed as mean ± S.E.M; n = 10

* Significant at level $p < 0.05$ as compared to control (group I)

Table 2: Changes in Estrous cycle of female control and lead-acetate treated mice

Days of Treatment	Groups		
	Control (I)	Lead acetate – Low dose (II)	Lead acetate – high dose (III)
1	D	E	D
2	P	D	EP
3	M	EP	P
4	ED	P	E
5	EP	E	M
6	E	E	D
7	M	M	EP
8	P	D	EP
9	E	EP	P
10	EP	EP	E
11	EP	P	M
12	P	P	D
13	E	E	P
14	E	E	ED
15	M	E	D
16	D	M	D
17	LD	D	D
18	LD	D	D
19	P	D	D
20	E	D	D
21	E	D	D
22	M	D	D
23	EP	D	D
24	EP	D	D
25	P	D	D
26	M	D	D
27	LD	D	D
28	LD	D	D
29	P	D	D
30	M	D	D

P – Proestrous, E – Estrous, M – Metaestrous, D – Diestrous, ED – Early diestrous, LD – Late diestrous, EP – Early proestrous, n = 3

Table 3: Lead acetate-induced changes in protein content of mice

S.No	Groups	Uterine protein content (mg/ 100mg tissue weight)	Ovarian protein content (mg/100 mg tissue weight)
1	Control (I)	13.82 ± 1.76	10.22 ± 0.55
2	Lead acetate – Low dose (II)	10.02 ± 0.78*	8.33 ± 0.41*
3	Lead acetate – high dose (III)	8.07 ± 0.975*	7.92 ± 0.47*

Results are expressed as mean ± S.E.M; n = 10

* Significant at level $p < 0.05$ as compared to control (group I)

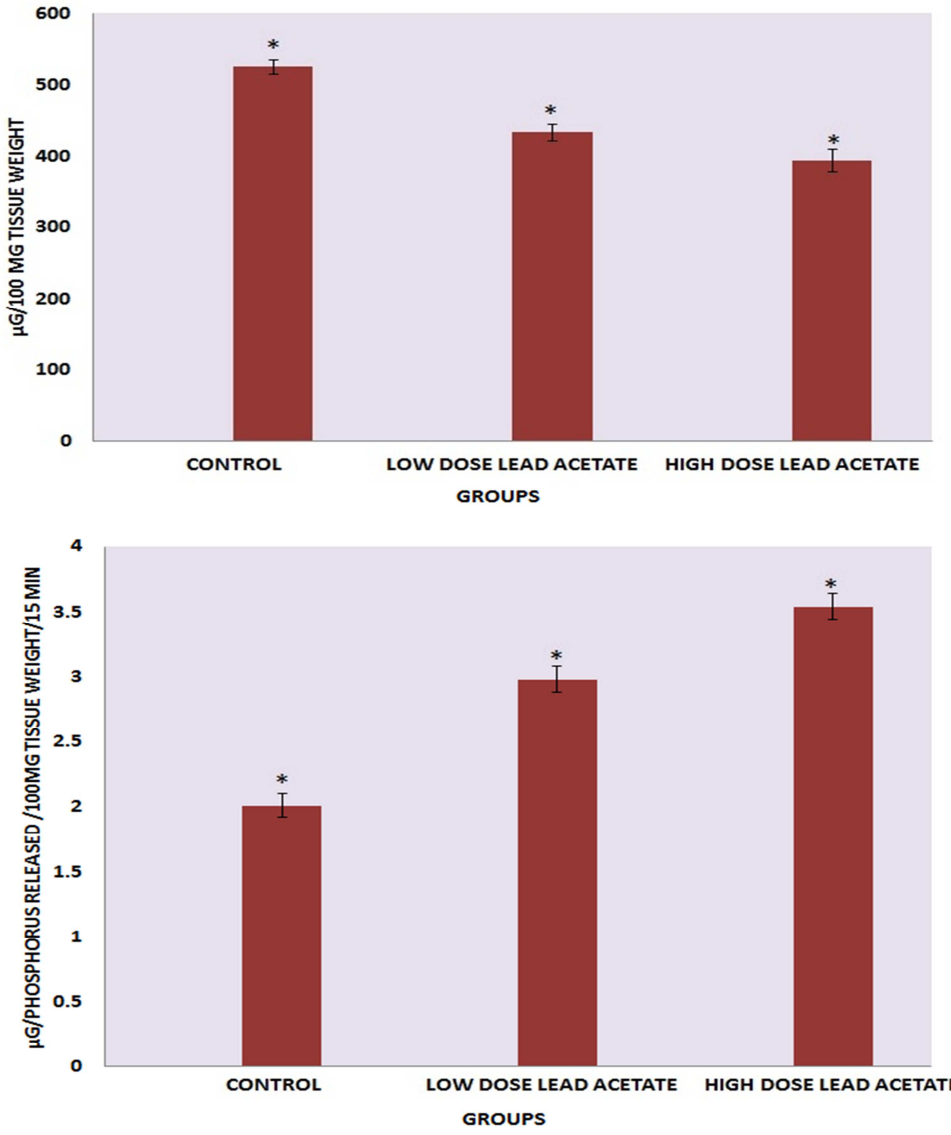

Fig 1 (A) lead acetate-induced changes in glycogen content of uterus (B) Effect of lead acetate on uterine phosphorylase activity.

Results are expressed as mean ± S.E.M; n = 10

* Significant at level $p < 0.05$ as compared to control (group I)

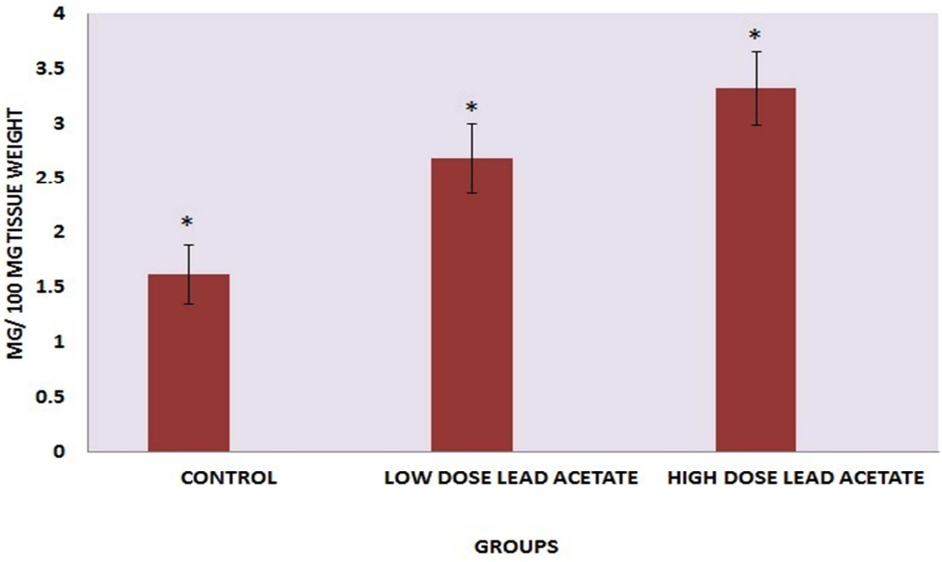

Fig. 2 Effect of lead acetate on cholesterol content of the ovary.
Results are expressed as mean ± S.E.M; n = 10
*
Significant at level $p < 0.05$ as compared to control (group I)

3. 3 Protein Levels:

The protein content in control ovary was found 10.22 mg ± 0.55 mg/100 mg tissue weight. However lead acetate treatment resulted in significant reduction in protein content of ovarian tissue in both low dose and high dose treated mice as compared to control (Table 3). Similarly significant dose-dependent reduction was observed in protein levels in uterus low dose and high dose lead acetate treated mice as compared to control animals (Table 3).Our results indicated reduction in uterine and ovarian tissue protein levels (Table 3). Triethyl lead has the ability to inhibit protein synthesis as reported by Konet,*et al.,* 1979. Also Seshadri and Khanna(1982) have reported changes in tissue concentration of proteins during lead toxicity. Lead can covalently bind to sulphydryl groups and can cause significant inhibition of protein synthesis.

3. 4 Glycogen Content and Phosphorylase Activity in Uterus:

Intraperitoneal administration of lead acetate to mice for 30 days caused a significant reduction in glycogen content of uterus as compared to control mice Fig 1A. The results of glycogen estimation corroborated well with uterine phosphorylase activity in which a significant and dose dependant increase was observed after treatment with lead acetate as presented in Fig 1B. Fig 1A indicated significant decrease in uterine glycogen content with concurrent increase in uterine phosphorylase activity (Fig 1 B). Lead acetate treatment resulted in alteration of carbohydrate metabolism in uterus. The increase in phosphorylase activity indicated in glycogenolysis which resulted in low glycogen content of the uterus. Hacker, et al., 1990 have studied the changes in carbohydrate metabolism after exposure to lead and have reported a complete loss of glycogen and reduction in activity of phosphorylase in hepatic tissues. Our results corroborated well with their findings.

3. 5 Cholesterol Content of Ovary:

Lead acetate treatment to female mice for 30 days caused a significant increase in the cholesterol content of ovary as compared to that of control. The effect was more pronounced with high dose treatment (Fig 2). A significant increase in ovarian cholesterol content was seen in lead acetate treated mice (Fig 2). The result was more pronounced with high dose lead acetate treated mice than with low dose. Allouche,*et al.,* (2011) have reported similar increase in hepatic cholesterogenesis in rats treated with lead.Cholesterol is considered as an important constitute of the cell membrane acting as a precursor for steroid hormone. Thus lead induced changes in ovarian cholesterol content may be related to either a disruption of plasma membrane and/or altered steroidogenesis.Heavy metal toxicity is a grave problem as it affects the normal growth of an individual. It is known to be toxic when present in even minute concentrations and may produce reversible and irreversible damage to different body tissues depending on the route of exposure and concentration. Lead is also known to cause adverse effects to female reproductive system. Lead is also known to cause anaemia in

females, may result in frequent abortions and higher maternal concentration of lead may be responsible forlow birth weight in newborn. A study from Ludhiana, Punjab has indicated that daily mean intake of lead has increased rapidly (Sangha, *et al.,* 2001). Lead is known to induce morphological changes and may cause detrimental effects with prolonged exposure (Sandhu and Brar, 2000). It has been reported that lead decreases litter size, birth weight and the survival rate in rats (Ronis, *et al.,*1998a). A significant disturbance in estrous cycle and delay in vaginal opening has been reported in rats after lead intoxication (Ronis, et al., 1998b). Similarly lead was found to inhibit rate of pregnancy and alter the litter size and weight of pups in mice (Sharma, et al., 2012). Thus the present study evaluated the reproductive toxicity of lead acetate in female mice exposed to 2 and 10 mm/ kg body weight.

4. 0 Conclusion:

Environmental exposure of lead is a matter of great concern as evidences suggests that lead is prone to cause a manifold of adverse changes even at much lower concentration. Hence the concentration of lead is monitored vigorously by various Indian Government agencies. This is evident from one of the recent happenings in which a very popular food entity came under the regulatory scanner owing to its very high lead content. It was observed that lead acetate significantly decreased body weight, inhibited the regular estrous cycle and also lowered the uterine and ovarian weight, altered protein, glycogen metabolism and increased the ovarian cholesterol levels at much low concentration.It is concluded from the present study that intraperitoneal administration of lead acetate for 30 days in female mice drastically affects the female reproductive system. The need of the hour is to reduce the exposure of humans to heavy metals like lead which may otherwise pose a very serious environmental health hazard.

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