Lead-induced adverse effects on the reproductive system of rats with particular reference to histopathological changes in uterus

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ABSTRACT

Objectives: This study was undertaken to elucidate the adverse effect of lead on female reproductive system following in vivo exposure in rats.

Materials and Methods: Animals of Group II, III and IV received lead acetate in drinking water (30, 100 and 300 ppm, respectively) for 28 days whereas Group I served as control. Lead levels in digested blood and bone samples were measured using atomic absorption spectrophotometer.

Results: Marked and a significant decrease in per cent body weight gain was observed in rats of Group IV and III, respectively, compared to that in the control group. Relative uterine weights were found to decrease by 27% in Group III and IV compared to control and low dose lead treated (30 ppm) rats. Lead levels were found to increase in a linear manner in blood along with a marked increase in bone levels in 100 ppm exposure group while there was a decrease in both the blood and bones levels at 300 ppm exposure. Compared to plasma progesterone levels in rats of the control group, a nonsignificant (12.46–21.13%) reduction in plasma progesterone were observed in different lead-treated groups. No apparent gross pathological lesions were observed in any of the vital organs, including uterus. However, histopathological examination of uteri of different groups revealed lead-induced dose-dependent inflammatory changes, which were characterized by thickening of the endometrium, narrowing of uterine lumen, damage to endometrial glands and vacuolar degeneration in endometrial epithelial cells.

Conclusion: Findings of this study suggest lead-induced pathophysiological alterations in myometrium, which in turn may affect the reproductive efficiency of animals.

KEY WORDS: Blood, bones, histopathology, inflammation, lead, uterus

Introduction

Among the several factors responsible for infertility and consequential productivity losses in dairy animals, malnutrition, ovulatory or hormonal imbalances and infectious agents have been recognized as the major ones. However, information on impact of environmental toxicants on female reproduction and how these affect reproduction is almost obscure. Adverse effects like altered spermatogenesis, increased sperm pathologies and testicular degeneration due to lead have been studied well in males,[1] however, studies on female reproductive toxicology are very less and differ from males due to differences in gametogenesis, access of the germinal cells and also because of the revolving nature of female breeding function.[2,3] Severe cases of lead poisoning have been reported to be associated with sterility, miscarriage, abortion, premature delivery, and infant mortality.[4] Lead crosses the placenta during pregnancy and has been associated with intrauterine deaths, prematurity, and low birth weight.[5] In experimental animals, chronic exposure to lead may cause inhibition of menstruation, ovulation and follicular growth in monkeys,[6] delay in vaginal opening in pubertal rats[7] and decrease in frequency of implanted ova and of
pregnancies in mice. In primates, prolonged exposure to lead blocks ovarian and luteal functions by reducing progesterone, luteinizing hormone and follicle-stimulating hormone levels. Lead-induced reduction in number of primordial follicle and increase in number of atretic follicles have been reported in ovaries of mice while in uterus, it damages endometrium, myometrium and perimetrium, along with reduction in uterine gland and decrease in height of columnar cells in mice. In view of the limited laboratory studies on impact of lead on female reproductive system in rats, present study was undertaken in female rats by exposing them to three different lead levels selected based on biomonitoring studies in cows and buffaloes in and around Mathura.

**Materials and Methods**

**Experimental Animals**

Healthy adult female Wistar rats were procured from the Laboratory Animal Resource Section, Indian Veterinary Research Institute, Izatnagar; Bareilly and acclimatized in departmental animal house under standard managemental and feeding conditions before starting the experiment. Animals (100–120 g) were divided into four groups: Group I contained six animals while Groups II, III and IV contained eight animals each. Group I was kept as control and rats received triple distilled water only while rats of Group II, III and IV received lead acetate at 30 ppm, 100 ppm and 300 ppm in triple distilled water (w/v), respectively continuously for 28 days. Animals of all the groups had free access to respective drinking water (s). These three dose levels of lead (30, 100 and 300 pm) for the present study were selected based on an earlier study undertaken in our laboratory, where rats were exposed to lead in drinking water at 100 ppm level for 28 days and lead-treated rats were found to have the plasma levels of 2.20 ppm (unpublished observation). Further, cows and buffaloes around Mathura have been observed by us to have the circulating blood lead levels of more than 1.00 ppm (1.37 ± 0.24 ppm in cows and 1.10 ± 0.10 ppm in buffaloes). In view of the above and also to simulate the circulating blood levels of more than 1.0 ppm in experimental rats, we had selected one higher dose and one lower dose of lead for the present study to investigate the toxic effects of lead on rat myometrium and hormonal status.

Blood samples were collected from the retro-orbital plexus on 0, 15th and 29th day in heparinized tubes for the detection of lead levels whereas, at the end of the experiment, all the animals were sacrificed under xylazine-ketamine anesthesia. Uteri were collected for determining its absolute and relative weights and the volume was adjusted to 5 ml by adding double acid mixture (nitric acid and 70% perchloric acid, 3:1) and digested slowly till white fumes emanated from it. The final volume was adjusted to 10 ml by adding triple distilled water.

For digestion of bone samples, whole tibia was cleaned and dried in hot air oven before weighing and grinding it in a crucible, which was kept on a heater for 15–30 min to make it smoke free. The bone ash (1 g), prepared in muffle furnace (550°C for 2 h), was mixed with 1 ml concentrated nitric acid and heated on a hot plate to dry. After cooling, 2 ml concentrated hydrochloric acid was added and kept for 15 min. The resultant mixture was then filtered through Whatman filter paper (No. 1) and the final volume was adjusted to 50 ml by adding triple distilled water.

Lead levels in digested blood and bone samples were measured using atomic absorption spectrophotometer (Model AAS 400; Perkin Elmer, USA).

**Plasma Progesterone Levels**

Progesterone (P₄) levels in plasma samples of different groups of rats were determined using commercially available ELISA kit (Labserv, Fisher Scientific, India).

**Histopathological Examination**

Uteri of different groups of rats were processed for routine histopathological examination. Approximately, 5–6 μm thick sections were prepared and stained with hemotoxyline and eosin following standard staining technique and examined under light microscope.

**Statistical Analysis**

Results are expressed as mean ± standard error of the mean and analyzed by one-way ANOVA, followed by Tukey’s post-hoc test using SPSS version 19 (SPSS Inc., IBM).

**Results**

**Effect of Lead on Body and Uterine Weights**

Apparently, no untoward clinical signs were observed in rats of any of the three lead-treatment groups. Per cent change in body weight gain in animals of different experimental groups on days 0, 7, 14, 21 and 28 are presented in Table 1. Absolute and relative weights of uterus as well as per cent change in uterine weights of rats of different groups are summarized in Table 2. Compared with the control group, there was 27% decrease in relative uterine weights in rats of Groups III and IV, but it was not statistically significant.

**Effect on Blood and Bones Lead Levels**

Blood lead levels after 14 and 28 days exposure in rats of different treatment groups (30, 100 and 300 ppm) are illustrated in Figure 1 while lead levels in bone are illustrated in Figure 2.

**Effect of Lead on Plasma Progesterone Levels**

Compared to the control group (10.03 ± 3.93 ng/ml; n = 4), plasma progesterone levels did not differ significantly between different lead-treatment groups, however, these values were markedly lower in 30 and 100 ppm lead-treated groups as the respective values in these groups were found to be 8.70 ± 2.52 ng/ml and 7.91 ± 1.40 ng/ml. However in 300 ppm group, plasma progesterone level was surprisingly almost comparable to that in control group.

**Histopathological Studies**

Alterations in cellular architecture of uterus of different lead-treatment groups are shown in Figures 3-5. Compared

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to the uteri of control group, uterus of rats treated with 30 ppm lead acetate (Group II) revealed uniform thickening of endometrium with projections leading to narrowing of lumen, besides chronic inflammation and damage to endometrial glands [Figure 3a and b]. There was extensive thickening of endometrium with projections and marked narrowing of the lumen in rats of Group III (100 ppm). In addition, vacuolar degeneration in endometrial epithelial cells, cystic degeneration of goblet cells, damage to endometrial glands and chronic inflammation were also observed as shown in Figure 4a and b. Uterine inflammatory changes in rats of Group IV (300 ppm) were more severe and characterized by massive thickening of the endometrium with projections and conspicuous narrowing of lumen. In addition, there was sloughing off of the lining endometrial epithelial cells, damage to endometrial glands and marked chronic inflammation as shown in Figure 5.

Discussion

Oral exposure is one of the important routes of lead entry in humans and animals. Gastrointestinal absorption of lead is around 40% in children and 10% in adults while it is around 10% in nonruminants and <3% in ruminants.[14] After absorption, almost 99% lead binds to erythrocytes and the remaining diffuses into soft tissues and bones, where it equilibrates with blood lead and the lead accumulated in erythrocytes, soft tissues and rapidly growing bones is mostly responsible for its toxic effects.[15]

In the present investigation, compared to control group, 28 days lead exposure produced significant ($P < 0.05$) reduction

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**Table 1:**

| Time interval (in days) | Group I (control, n=6) | Group II (Pb 30 ppm, n=8) | Group III (Pb 100 ppm, n=8) | Group IV (Pb 300 ppm, n=8) |
|-------------------------|------------------------|---------------------------|-----------------------------|---------------------------|
| 0                       | 0                      | 0                         | 0                           | 0                         |
| 7                       | 22.31±2.2              | 13.95±1.88                | 14.22±3.3                   | 16.9±2.5                  |
| 14                      | 31.44±3.7              | 22.40±2.82                | 25.20±3.8                   | 22.57±4.22                |
| 21                      | 36.49±4.2              | 28.92±2.63                | 26.69±5.2                   | 29.33±4.63                |
| 28                      | 40.48±3.2              | 31.40±1.92                | 24.17±4.27*                 | 29.36±5.23                |

Data are presented as mean±SEM. *$P < 0.05$ versus control. SEM = Standard error of mean.
A reduction in plasma progesterone levels was observed in rats of Group II (8.78 ± 2.52 ng/ml) and III (7.91 ± 1.40 ng/ml) following 28 days exposure to lead at 30 and 100 ppm, respectively. Decrease in serum progesterone level following lead-treatment has been attributed to increasing in activities of five β-reductase, a progesterone-metabolizing enzyme, in liver and uterine homogenates.\[10]\]

Relative uterine weight was reduced by about 27% in rats of Group III (100 ppm) and Group IV (300 ppm), but decline was not statistically significant. Our observations are somewhat in conformity with the observations of Wiebe et al.\[20]\] who too failed to observe any significant changes in the ovary, uterus and fallopian tubes weights in rats exposed to lead during pregnancy and lactation. But, Dumitrescu et al.\[21]\] observed progressive decline in uterine weight along with fallopian tube following 6 months exposure to lead at 100 and 150 ppb. Differences between our study and those reported by Dumitrescu et al.\[23]\] may be attributed to the difference in dose and duration of lead exposure.

Histopathological studies on uteri of different treatment groups in the present study revealed its dose-dependent deleterious effects on myometrium. Cystic degeneration of goblet cells and sloughing off of endometrial lining epithelial cells was observed in uteri following exposure at 100 and 300 ppm levels. Degenerative and inflammatory changes in uterus including decrease in height of columnar cells and undistinguished areas of blood vessels, lymphatics and connective tissues have been documented by Qureshi and Sharma.\[10]\] In utero or gestational exposure to lead has been reported to cause necrosis of uterine glands and destruction of uterine lining cells.\[22]\] Importance of endometrial glands and their secretions in maintaining estrous cycles, conceptus survival and growth at the peri-implantation stage has been reported by Gray et al.\[23]\] Therefore, lead may produce deleterious effect on in-utero physiological functions by altering uterine glandular secretions like enzymes, growth factors, cytokines, lymphokines, hormones and transport proteins essential for development of conceptus\[24]\] or may even alter dynamics of uterine membrane receptors and ion channels;\[25]\] studies in these aspects are in progress.

Based on the results of present study, it is inferred that accumulation of lead in blood and long bones occurs maximally at 100 ppm level it produces subtle utero-toxic effects which are likely to affect reproduction even without any apparent observable toxic effects.

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