Full Length Research Paper

Maternal lead exposure and pregnancy outcome in Wistar albino rats

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Lead (Pb) is a heavy metal environmental pollutant and toxicant. The present study investigated dose-related effects of maternal Pb exposure on pregnancy outcome. Wistar albino rats were gavaged with Pb nitrate (4 or 8 mg/kg) or vehicle daily from gestation day 0 until delivery. Length of pregnancy was noted. Number, birth weight and physical characteristics of pups were registered. Neonatal mortality was also recorded between days 1 to 15 post-delivery. Lead treatment significantly ($p < 0.05$) inhibited maternal body weight gain and caused abortion of pregnancy dose-dependently (4 mg/kg: 67.7%, 8 mg/kg: 100%). Besides, all occurring births in 4 mg/kg Pb-exposed rats were preterm ($p = 0.0023$) with morphological abnormalities in the head and limbs, and about 33% were still births. Also, average number and birth weight of Pb-exposed rats offspring were significantly ($p < 0.0001$) lower compared to controls. Furthermore, while body weights of offspring of control rats increased significantly ($p < 0.0001$) over time, those of Pb-exposed rats decreased significantly ($p = 0.0077$). Neonatal survival was 0% in Pb treated rats and 100% in control. The results demonstrate that maternal Pb exposure adversely and dose-dependently affects pregnancy outcome.

Key words: Developmental toxicity, gestation, heavy metals, lead, reproductive toxicity, resorption.

INTRODUCTION

Lead is a heavy metal environmental pollutant which occurs naturally as lead oxide or lead salts. With its characteristic (dense, ductile, malleable, and corrosion resistant) properties, the metal has wide industrial applications in modern society. Lead is being used in production of paints, batteries, water pipes, gun bullets, x-ray and atomic radiation protection, eye cosmetics, base metal utensils, and also as additive in gasoline (Florea and Busselberg, 2006). Thus, the metal is a major environmental pollutant and it has been detected in every facet of environmental and biological systems (Payne, 2008; Bilandzˇic et al., 2009; Clark et al., 2009), particularly in industrialized cities. Lead is a known

impairments in both human and experimental animals toxicant which causes functional and structural (Goswami and Bhattacharya, 2000; Loumbourdis et al., 2003; Reza et al., 2008). It is one of the oldest harmful agents known to mankind as its toxicity has been observed and documented since historic times of the Greeks, Romans and Arabs and even the Egyptians (Ahmad et al., 2003). The metal is readily absorbed through the gastrointestinal tract and distributes into soft body tissues such as kidney, bone marrow, liver and brain, but bioaccumulates in the blood and bone. Human exposure to lead occurs through a combination of inhalation and oral exposure; the former being more significant for occupationally exposed groups,

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while the latter contributes a greater proportion of those for the general population (CDC, 2005). Acute toxicity is related to occupational exposure, and is not very common because of the measures employed by regulatory bodies like the Environmental Protection Agency (EPA), Consumer Product Safety Commission (CPSC), and CDC to reduce lead use: stoppage of lead in gasoline, reduction of the amount of lead in paints. Chronic toxicity on the other hand, is much more common and occurs at blood lead levels (BLL) of 40 to 60 µg/dl, which in severe cases, is characterized by persistent vomiting, encephalopathy, lethargy, delirium, convulsions, and coma (Flora et al., 2006; Pearce, 2007).

The main targets of lead poisoning have been identified to include the hematopoietic (Jacob et al., 2000; Anetor, 2002), nervous (White et al., 2007; Sansar et al., 2012), cardiovascular (Navas-Acien et al., 2007), and renal tissues (Ekong et al., 2006). Furthermore, like cadmium and other heavy metals, lead has been demonstrated to be a testicular toxicant, causing various forms of reproductive dysfunctions in males (Foster et al., 1993; Benoff et al., 2003; Makhlouf et al., 2008). Additionally, lead exposure has been linked to increasing prevalence of various abnormalities of the female reproductive system. Tolerable BLL in pregnant women have been suggested to be < 10 µg/dl (Kaul et al., 2002; ATSDR, 2005) whereas BLL ≥ 10 µg/dl may adversely affect pregnancy outcome, resulting in increased risk of gestational hypertension, reduced length of gestation, miscarriage, spontaneous abortion, and preterm delivery (Torres-Sanchez et al., 1999; Sowers et al., 2002; Vigeh et al., 2004). Earlier studies had equally reported that increase maternal BLL strongly correlate to low birth weights in humans (Odland et al., 1999), and sponta-neous abortion (Borja-Aburto et al., 1999). Furthermore, it has been observed that other complications during pregnancy such as anemia, proteinuria and essential hypertension, toxemia, and hyperemesis occur at BLL > 10 µg/dl (Kaul et al., 2002; ATSDR, 2005). However, a clear threshold of BLL for these sensitive effects has not been identified as even BLL of < 5 µg/dl have been reported to cause adverse pregnancy outcomes (ATSDR, 2007; Zhu et al., 2010), while other investigators have reported lack of association between maternal BLL and adverse pregnancy outcomes like spontaneous abortion, low birth weight, intrauterine growth retardation, prema-ture rupture of membranes and congenital anomalies (McMichael et al., 1986; Rahman and Hakeem, 2003).

The major mechanism of lead induced toxicity in most biological systems has been reported to be via oxidative stress (Flora et al., 2012). On a molecular level, proposed mechanisms of action for lead toxicity involve funda-mental biochemical processes. These include alteration of the actions of bivalent cations (for example, Ca^{2+}, Mg^{2+} and Fe^{2+}), and interaction with proteins, especially those with sulfhydryl, amine, phosphate and carboxyl groups (Lidsky and Schneider, 2003; ATSDR, 2005). Lead also inactivates antioxidant enzymes by displacing zinc ions which serve as important co-factors for such enzymes (Flora et al., 2007). Although, a number of studies have reported on the toxic effects of Pb and other metals in animals and humans, there is need for a continuous evaluation of Pb toxicity as human exposure to low Pb levels continues because of the widespread use of lead and its ubiquitous nature (CDC, 2005). Furthermore, the persistence of lead and other metals in pregnant and breast feeding mothers, and their toxic effects on various developing organs have increased the concerns of their safeties in public health. Moreso, metal-induced toxicity in pregnant animals is related to the age of pregnancy, animal species, as well as the amount of exposure (Parzyck et al., 1978; Andrews et al., 1994; Bellinger, 2005; Karri et al., 2008; Thompson and Bannigan, 2008). Thus, data on the impact of maternal Pb toxicity in relation to dose, age of pregnancy, and duration of exposure are of special concerns.

In a number of previous studies, lead exposure to pregnant animals at a daily dose range of 4 to 32 mg/kg and different stages of pregnancy has revealed various toxicological effects on fetal development including low birth weight (Chandra et al., 1983; Lewis and Pitts, 2004); severe hematological abnormalities (Sharma et al., 2012), impairment of implantations, resorptions and malformations (Mogra et al., 2009). However, maternal and fetal effects of similar levels of prenatal lead exposure from conception to term have not been studied in the rat, which this work aims to address.

MATERIALS AND METHODS

Chemical

Lead nitrate (Burgoyne Burgoynes & Co, India) was obtained from the Department of Pharmacology, University of Port Harcourt.

Animals

Non pregnant female Wistar albino rats weighing between 170 to 190 g and male albino rats weighing between 200 to 220 g were obtained from the animal house of the University of Port Harcourt, Port Harcourt, Nigeria. The animals were housed four per cage, with a 12 h light/dark cycle. They were supplied with standard laboratory chow and tap water *ad libitum*. The experimental protocol followed the Guide for Care and Use of Laboratory Animals (CCAC, 2009) and was approved by the Committee for Ethics in Animal Experimentation of the University of Port Harcourt.

Experimental design

Animals were divided into different groups and allowed to mate

Table 1. Effect of Pb exposure on body weight of pregnant Wistar albino rats

Dose (mg/kg)	Body weight (g)			
	Day 0	Day 7	Day 14	Day 21
Control	175.00±4.18	196.60±4.80*	207.00±4.36**	221.60±7.81***[b]
4	187.00±4.06	192.00±6.44	190.00±2.74	184.00±5.79
8	175.40±4.08	176.00±10.17	179.00±9.92	169.40±5.78

Data expressed as mean ± SEM, n = 6. Data analyzed by ANOVA, followed by Bonferroni's multiple comparison test. * Significant at P < 0.05 (Day 0 vs Day 7). ** Significant at P < 0.01 (Day 0 vs Day 14). *** Significant at P < 0.001 (Day 0 vs Day 21). [b]Significant at P < 0.05 (Day 7 vs Day 21).

Table 2. Effect of maternal Pb exposure on pregnancy outcome in Wistar albino rats.

Dose (mg/kg)	No. of rats that gave birth	Total no. of pups delivered	Total no. of still births	No. of abnormal pups	Physical characteristics of pups
Control	6	61	0	0	Normal and active
4	2	9	3	9	Abnormally shaped heads and limbs. Also, weak and less active

freely. Female rats were closely observed and mating was confirmed through vaginal smear examination by the presence of vaginal copulation plug (day 0 of pregnancy). Pregnant rats were randomly distributed into 3 experimental groups of six rats each. Each group was exposed to lead as nitrate at 0, 4, or 8 mg/kg/day (equivalent to ≈ 0, 20 or 40 ppm lead) by oral gavage from day 0 of pregnancy until delivery. Length of pregnancy and number and birth weights of pups delivered were recorded. Physical characteristics of the pups were also registered by close and careful physical examination of their physical appearances (structures) for any abnormality. In addition, survival of pups from day of delivery to 15 days post-delivery was also registered.

Statistical analysis

Statistical analyses of data were performed using GraphPad Prism 5 software. Data were expressed as means ± standard error of means (SEMs). Statistical differences between the groups were evaluated by paired Student's t test or analysis of variance (ANOVA). Comparisons between groups were made by the Newman-Keuls or Bonferroni's multiple comparison test to compare parameters across time. Differences yielding P-values < 0.05 were considered statistically significant.

RESULTS

Body weights of control pregnant rats significantly (p < 0.05) increased over time, but there was no significant (p > 0.05) difference in body weight of rats treated with lead (Pb) throughout pregnancy (Table 1). When compared to average body weight at conception (day zero of pregnancy), maternal weight increased time-dependently during pregnancy in the control group, p < 0.05 (Table 1).

Their body weights on days 14 and 21 were higher than weight obtained on day 7, although only that of day 21 was significant, p < 0.05. There was also weight gain between days 14 and 21, but this was not significant, p > 0.05 (Table 1). Furthermore, all six control pregnant rats gave birth; 2 out of 6 gave birth in the 4 mg/kg Pb-exposed; while none gave birth in the 8 mg/kg-exposed rats (Table 2). This corresponds to 66.7 and 100 % abortion, respectively. Furthermore, average length of pregnancy of the occurring births in 4 mg/kg Pb-exposed rats (16.50 ± 1.50 days) was significantly (p = 0.0023) lower compared to control: 22.17 ± 0.48 days (Figure 1). The average number (4.50 ± 0.50) and birth weight (3.82 ± 0.12 g) of the delivered pups were also significantly (p < 0.0001) lower compared to the corresponding values (10.17 ± 0.31 and 5.76 ± 0.08 g, respectively) obtained in control animals (Figures 2 and 3).

A careful macroscopic analysis of the pups showed abnormality in Pb-exposed rats, with abnormally shaped skull bone, short forelimbs and with complete absence of hindlimb in some, compared to controls. In addition, 3 out of 9 delivered pups of the Pb-exposed rats were stillbirths, while the live pups (6) were weak and less active compared to controls (Table 2). Furthermore, average body weight of Pb-exposed offspring significantly (p < 0.05) decreased over time post-delivery, while that of control significantly (p < 0.001) increased (Figure 4). Average body weights of offspring of the control rats on days 5, 10 and 15 postpartum were all significantly (p < 0.0001) higher compared to their birth weight (day 1). In addition, their weights on days 14 and 21 were higher, p
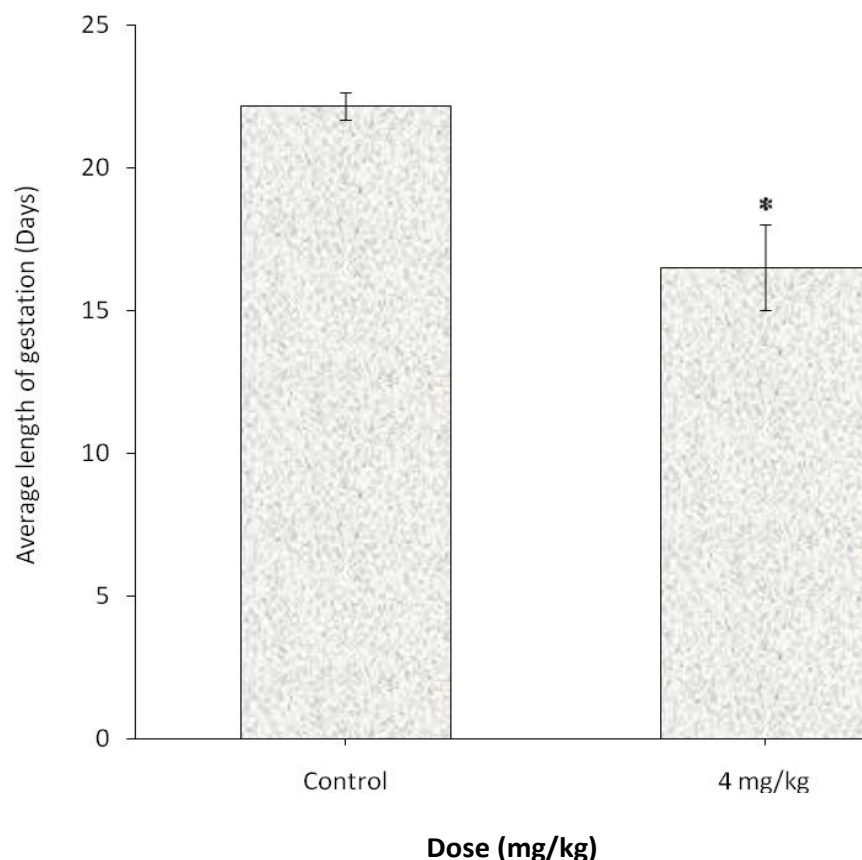
Figure 1. Effect of maternal Pb exposure on pregnancy in Wistar albino rats. Values expressed as mean ± S.E.M. Data analyzed by unpaired student t test. *Represents value significantly different from control at $P < 0.01$. Pregnancies of 8 mg/kg Pb exposed animals were aborted.

< 0.0001 (that is, significant weight gain), compared to that of day 7. There was significant weight gain, $p < 0.0001$ between days 14 and 21 well as in the control group (Figure 4). On the other hand, average body weights of Pb-exposed offspring on days 5 and 10 postpartum were significantly ($p > 0.05$) lower compared to their birth weight (day 1). However, their weight on day 10 was not significantly ($p < 0.05$) different from that of day 5 (Figure 4). Furthermore, all offspring of Pb-exposed rats died before 15 days post-delivery, while offspring of controls survived (Figure 4).

DISCUSSION

Lead is a widespread environmental toxicant essentially derived from industrial activities. High blood lead levels (BLL) ≥ 10 μg/dl in pregnant women have been asso-ciated with adverse pregnancy outcomes, including increased risks of pregnancy hypertension, spontaneous abortion, low birth weight, reduced offspring, and neurobehavioral development (Borja-Aburto et al., 1999; Odland et al., 1999; Torres-Sanchez et al., 1999; Sowers et al., 2002). However, considerable uncertainty remains regarding the specific malformations and dose-response relationships of Pb. Also, maternal or fetal Pb toxicity depends on a number of factors, including dose (Bellinger, 2005).

Normal levels of lead exposure to humans from the soil range between 50 parts per million (ppm) and 400 ppm, however, this could be much higher especially in industrialized environments (NRC, 1980). Other sources of lead exposure include drinking water, food, dust, air, and paint. Previous works have demonstrated Pb-induced toxicity on neurobehavioral activities in the brain using dose levels of 2 to 8 mg/kg/day (Chandra et al., 1981; 1983). Similar dose range has been used to study the effect of Pb on reproductive functions: ovarian function
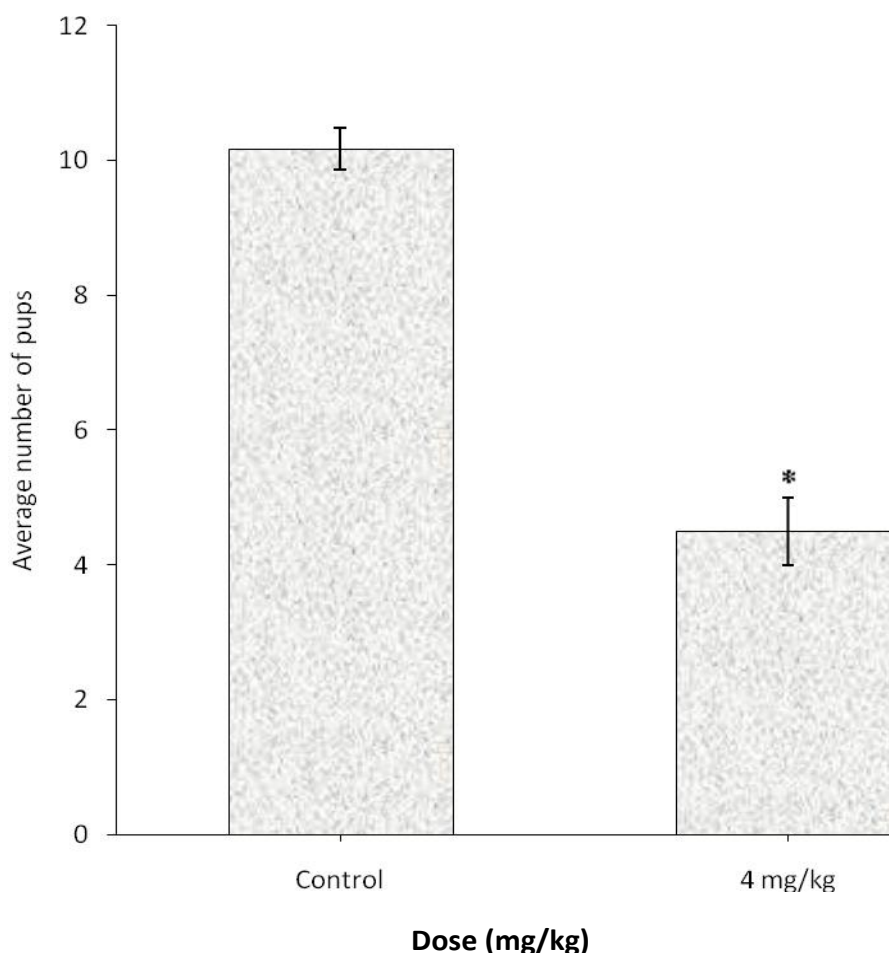
Figure 2. Effect of maternal Pb exposure on number of pups delivered in Wistar albino rats. Values expressed as mean ± SEM. Data analyzed by unpaired student t test. *Represents value significantly different from control at P < 0.0001. Pregnancies of 8 mg/kg Pb exposed animals were aborted.

in female rhesus monkeys (Franks et al., 1989), prenatal development of mice (Mogra et al., 2009). In the latter, pregnant mice were exposed to 8 to 32 mg/kg/day on different days between days 10 and 20 of pregnancy. But there are limited reports on the toxicological impact of lead exposure during pregnancy from conception to delivery at these dose levels.

Though, it has been reported that perinatal exposure to lead nitrate (3 mg lead/kg/day) results in no overt signs of toxicity in maternal monkeys (Hopper et al., 1986), inhibition of maternal body weight gain by Pb in the present study suggests that Pb may affect maternal health and normal development and growth of the rat embryo at higher dose levels (4 and 8 mg/kg in this study). Healthy body weight and adequate weight gain during pregnancy is vital for the wellbeing of mother and

normal development of baby (Derbyshire, 2007). Additionally, exposures to high levels of Pb (≥ 8 mg/kg) from onset of pregnancy to delivery may result in loss of all pregnancies as females exposed to the highest daily dose of lead (8 mg/kg) in our study were not able to sustain pregnancy.

This finding is different from the observation of Sharma et al. (2012) who reported serious hematopathology in surviving neonates, following oral maternal exposure of 8, 16, and 32 mg/kg Pb from 10th day of gestation to 21st day of lactation in rats. Our result, which is novel, may be due to the earlier period of Pb exposure.

Besides, our results show that all occurring births in the lower daily lead (4 mg/kg) exposed rats were preterm with decreased birth weights and morphological abnormalities in the head and limbs. These findings were
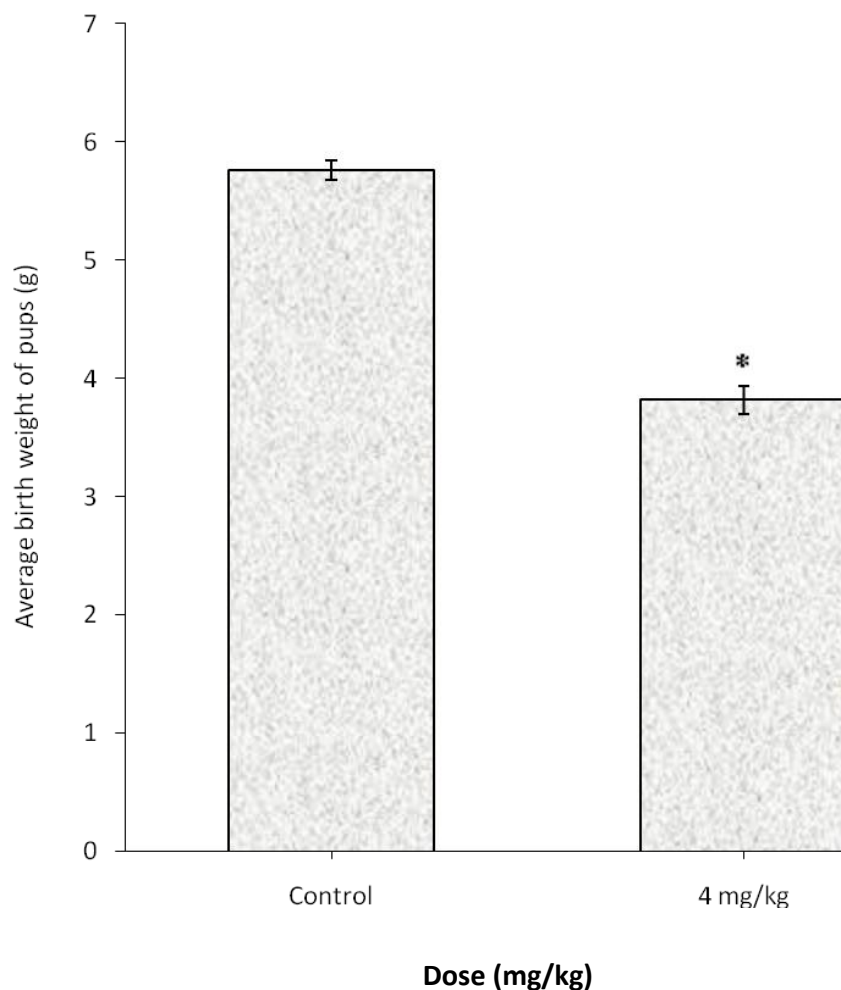
Figure 3. Effect of maternal Pb exposure on birth weight of pups in Wistar albino rats. Values expressed as mean ± SEM. Data analyzed by unpaired student t test. *Represents value significantly different from control at P < 0.0001. Pregnancies of 8 mg/kg Pb exposed animals were aborted.

similar with the results of Mogra et al. (2009), who reported impairment of implantations, resorptions and fetal malformations following prenatal exposure of lead (4, 8, 16 mg/kg) on different days between days 11 and 20 of pregnancy in the mouse. The investigators reported that the effects were most pronounced during the first trimester but declines from the 10th day of gestation. Our observation is also consistent with previous reports of spontaneous abortions, stillbirths, and miscarriages of pregnancies associated with high BLL (Torres-Sanchez et al., 1999; Borja-Abuto et al., 1999; Hertz-Picciotto, 2000). In previous studies, maternal BLL obtained in animals that received < 5 mg/kg/day of Pb was observed to be within a concentration range of 30 to 70 µg/dl,

which is associated with occupational exposure levels (Hubermont et al., 1976; Hopper et al., 1986). This indicates that the dose levels of Pb used in the present study will cause clinically relevant increase in BLL in experimental animals.

Previous studies have linked exposure to metals during pregnancy to alteration of fetal environment, developmental abnormalities and phenotypic outcome of the offspring (Ronco et al., 2005). In our results, the offspring of Pb-exposed rats have poor growth with significant and time-dependent reductions in body weight and 100% mortality at postnatal day 15. This result is consistent with earlier observations of the association of fetal low birth weight with neonatal mortality and morbidity (Kramer,
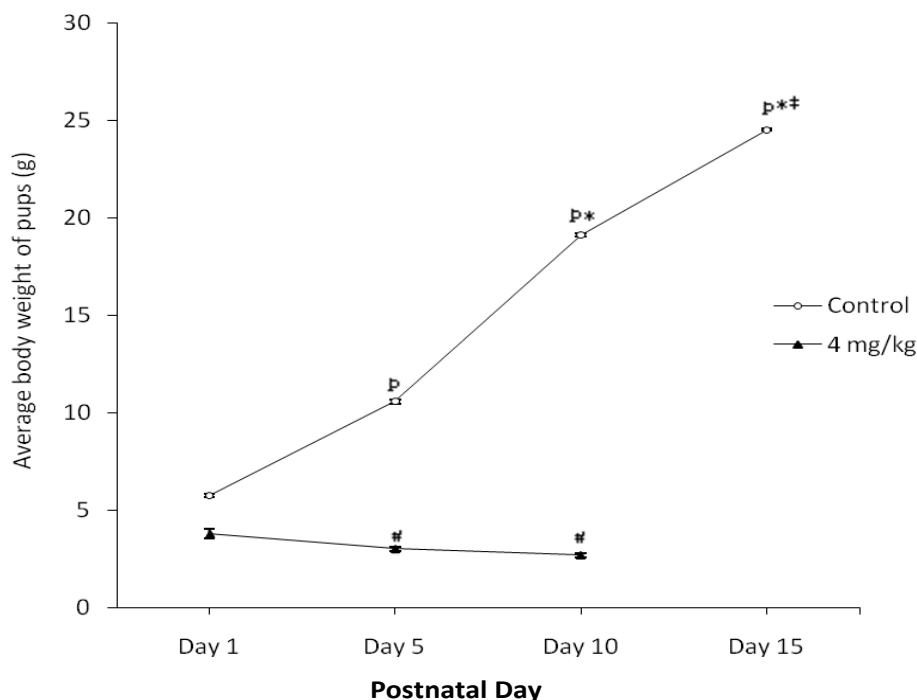
Figure 4. Daily body weight of pups of Pb-exposed and control pregnant Wistar albino rats. Values expressed as mean ± SEM. Control, n = 61; 4 mg/kg, n = 9. Data analyzed by paired Students t test, followed by Newman-Keuls or Bonferroni's Multiple Comparison test. [p]Significant at P < 0.0001 (Day 1 vs. Days 5, 10 and 15). *Significant at P < 0.0001 (Day 5 vs Days 10 and 15). [‡]Significant at P < 0.0001 (Day 10 vs. Day 15). [#]Significant at P < 0.05 (Day 1 vs. Days 5 and 10). Pregnancies of 8 mg/kg Pb exposed animals were aborted.

1987). In a related study, weight reductions have been reported in neonates of female rats that received daily injections of 5 mg/kg Pb at different stages of pregnancy by Chandra et al. (1983), but no neonatal mortality was reported. This may be due to the different exposure periods in the two studies. Also, a 3-week exposure to lead acetate (0, 50, or 250 ppm) to 21 day old rats has been reported to cause a decrease in body weight of the neonates, although, not significantly different from the control. This may be due to the differences in the dose levels and exposure times used in the studies (Lewis and Pitts, 2004).

Possible mechanisms of the abortifacient action of Pb in this study may involve oxidative stress-mediated alteration in ovarian and/or placental functions which may result in interference of fetal nutrition and oxygen utilization. Alteration in placental function may also cause inhibition of placental transport function of vital compounds like placental Zn^{2+} transport (Webb and Samarawickrama, 1981), which is critical for embryonic development and growth. This can impair normal growth and development of the embryo and also reduce fetal birth weight as observed in the significant reductions in

number and birth weight of litters of Pb-exposed rats in this study.

Conclusion

The results demonstrate that maternal Pb exposure (GD0 to term) adversely affects the pregnancy course, causes high neonatal mortality and will greatly impact serious adverse health consequences on the offspring. The results also reveal that Pb may induce complete loss of pregnancy at concentrations ≥ 8 mg/kg.

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