Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqi_jms_alnahrain @yahoo.com
http://www.colmed-nahrain.edu.iq/

Histopathological Changes of the Mice Placenta Exposed to Lead Acetate

Ibtisam J Sodani *MSc*
High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Lead is one of the most dangerous metals and could be incorporated in various body tissues and thus exposure to it stills a major medical problem in both environmental and occupational setting.

Objectives To detect the deleterious and toxic effects of the lead acetate on the weight and histological features of mice placenta at different dosages.

Methods A prospective study including eighty mature pregnant mice were divided into two groups (experimental and control groups). Forty pregnant mice were divided into two major experimental groups (G1and G2) according to the level of the dose. The other forty animals with same age divided at the same way as in the previous experimental groups considered as control groups (C). Injections of lead acetate 0.1, 0.4 mg/kg body weight /day intraperitoneally (G1, G2 respectively) were started at the first day of gestation and continued for (17 or 20 days). When the female in each experimental and control groups reach day 17 of gestation 10 animals of each group were scarified, whereas the other 10 animals were left to be scarified at 20 day post coitum (dpc). Weight of placentas was recorded, in addition, histological study for these placentas were done.

Results Prenatal lead acetate injection to pregnant mice intraperitoneally for 17 day cause a significant reduction ($P<0.05$) in placental weight and a highly significant reduction ($P<0.01$) in placental weight was recorded at 20 dpc experimental groups (G2) but there was no significant decrease in weight of placenta for 17 and 20 dpc in experimental groups (G1) In addition placentas belongs to (G1 and G2) mothers revealed histopathological alterations in the various component of the placenta.

Conclusion Administration of low dose of lead acetate intraperitoneally to pregnant female mice causes significant decrease in weight of their placenta. Lead acetate also causes adverse effects on the histological features of fetal placenta.

Key words Lead, Placenta, Placental transport toxicology, Toxic trace elements, Metallothionein, Reproductive toxicology

Introduction

During recent decades concerns have been raised about human infertility that might stem from exposure to environmental contamination [(1)]. Lead (Pb) is one of the most environmental pollutant [(2)], known to be a poisonous compounds for centuries [(3)], it is consistently observed to be a reproductive toxicant, causing decreased fertility and pregnancy loss [(4)]. Furthermore, life in utero as a developing embryo and fetus may be the most vulnerable period for lead toxicity to occur [(5)].

The placenta is generally described as the interface between the mother and the outside world and the developing fetus [(6)], it anchors the developing embryo to the uterine wall and connects it to the maternal blood stream, thus supplying the embryo with ions and metabolites and providing a waste-removal mechanism for the embryo [(7)]. More reliable evidence for the

permeability of the human placenta to lead began to appear in the 1930's, when chemical analytical techniques for detecting lead in blood and other tissues were developed [8]. The study of Georgieff MK et al clearly demonstrated the presence of lead in fetal tissues and left little doubt that the human placenta was permeable to this toxic trace metal [9]. Abundant early evidence of this hazard came from reports that women working in lead exhibited unusually high rates of sterility, spontaneous abortion, and stillbirths [10]. A study of Hertz-Picciotto et al. which was conducted on apparently normal humans, indicated that placental transfer of lead began as early as the 12th week of gestation and that the total lead content in fetal tissues increased throughout pregnancy [11]. Furthermore, maternal blood lead concentration was highly correlated with umbilical cord lead suggesting the transplacental movement of lead to the fetus, which crosses the placenta by passive diffusion and it has measured in the fetal brain as early as the end of first trimester (13 weeks). The high Pb^{++} levels in the umbilical cord blood propose that the essential placental barrier for the Pb^{++} passage to the fetus does not exist [6]. Moreover the mean concentration of lead in women was 2.56 µg/d and in umbilical cord blood was 2.01µg/d. A positive correlation was noted between lead concentration in maternal and umbilical cord blood (r=0.59) [12].

The toxic action of Pb^{++} on the fetus is expressed by decreased intra-uterine growth, low birth weight [13], chromosomal aberration, macro-cephaly, miscarriages, still birth and early death of offspring [14]. A study by Bressler et al showed that during early gestation inorganic lead crosses the placental membranes rapidly and in significant amounts, even at very low maternal blood levels, and the yolk sac placenta is the primary transfer site for lead [15]. In addition, the generalized distribution of radioactivity observed in the embryos indicated that all major organ systems are exposed to lead ions during this very critical period of development [6]. In this study, the histopathological changes of the developing placenta, and the weight of the placenta of mice will be studied after injecting the pregnant mice with different dose of lead acetate.

Methods

This experimental study was carried out at high Institute for infertility diagnosis and assisted reproductive technologies, Al-Nahrain University, A total of 80 mature female Swiss-Webster mice, weighing 25-30 g and age of about 8-10 weeks were divided equally into two groups (experimental and control groups). Forty animals were divided into two major experimental groups (G1and G2) according to the level of the dose (20 animal/group). Each major group subdivided into two minor experimental groups (10 animals/group) according to different periods for killing during gestation period (day17, and day20). The other 40 animals with same age divided at the same way as in the previous experimental groups considered as control groups (C) injected intraperitoneally by normal saline only. Vaginal smear were performed to all the adult female mice to detect heat stage for mating. Females in the estrous phase were left with mature healthy males for mating (1 male/1 female). In mice, day 1 of pregnancy was designated by the presence of a copulatory plug in the vagina [16].

The pregnant female was transferred into separate cage. Injection of lead acetate (0.1, 0.4 mg/kg body weight/day intraperitoneally for the experimental groups G1, G2 respectively were started at the first day of gestation and continued for (17 or 20 days) while the two parallel control groups were injected normal saline with the same rout and dose as that used in the experimental groups. At 17 and 20 days of gestation, 10 animals of each group were scarified at each of these intervals. The embryos were dissected into placental corn and embryonic portions using a scissor. Placentas were removed and placed in a dish containing worm normal saline. The numbers of placentas were recorded washed and weighed using sensitive electrical balance, then fixed in Bouin's solution, paraffin sections with 5 micron thickness were prepared and stained with hematoxylin eosin stain for histological study [17]. Data from treated and

control groups are expressed as mean ± standard error (SEM) and analyzed using student t-test to compare values from experimental and control groups at individual time points. Differences between groups were considered significant at (P<0.05) and highly significant at (P<0.01) [18].

Results

A significant decrease (P<0.05) in weights of placenta belongs to mothers injected with 0.4 mg/kg body weight (b.w.) of lead acetate (G2) at 17 dpc while highly significant reduction (P<0.01) was recorded at 20 dpc, in comparison with that of control group (C) as shown in figure 2 and table 1, while the result showed no significant decrease in weights of placenta belongs to mothers injected with 0.1 mg/kg b wt. of lead acetate at 17 and 20dpc,in comparison with that of control group as shown in figure 1 and table 1.

Table 1. Changes in weight of placenta associated with administration of (0.1, 0.4 mg/kg b.w.) (G1) and (G2) of lead acetate to pregnant female mice for 17 and 20 dpc

Placenta weight	Control		Treatment			
	17 day	20 day	0.4mg/k.b.w. 17 day	0.4mg/k.b.w. 20 day	0.1mg/k.b.w. 17 day	0.1mg/k.b.w. 20 day
Mean	0.10284	0.11145	0.05831*	0.00988**	0.11736	0.09928
SD	0.02874	0.03050	0.04404	0.02786	0.06121	0.01545
SE	0.0079	0.00847	0.01223	0.00774	0.01700	0.00429

k.b.w.: Kilogram body weight, * = p<0.05, ** = P<0.01

Histological observations

1. Non-treated experimental group (Control group) [C].
   The histological sections of placenta belong to mothers from control group showed huge numbers of villi. Each villous contain a mesenchymal core, containing fetal capillaries. The villous surface area exposed to the lacuna filled with maternal blood. Between the villous capillaries and maternal blood is a continuous layer of syncytiotrophoblast supported by a layer of proliferating cytotrophoblast cells (Figures 3-5).
2. Experimental group female mice treated with 0.1 mg/kg b.w. of lead acetate at 17 and 20 dpc (G1); The histology of placenta belong to these mothers showed a reduction in the thickness and distruption of trophoplastic septa, necrotic area in the labrinth zone and deciduas, cystic degeneration of glycogen cells, appoptosis of trophoblast and stromal cells of placental tissue, irregular dilatation of maternal sinusoid, fibrin depostion around villi and calcification and hyalinized villous spots (Figures 6-9).
3. Experimental group female mice treated with 0.4 mg/kg b.w. of lead acetate at 17 and 20dpc (G2); the histology of placenta in this experimental group showed the same histological observations as in G1 but more extensive and promanent (Figures 10-12).

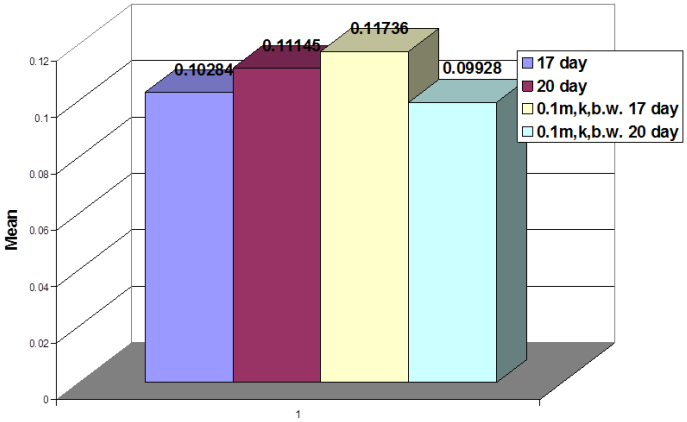

Figure 1. Changes in weight of placenta associated with administration of (0.1 mg/kg b wt.) (G1) of lead acetate to pregnant female mice for 17 and 20 dpc

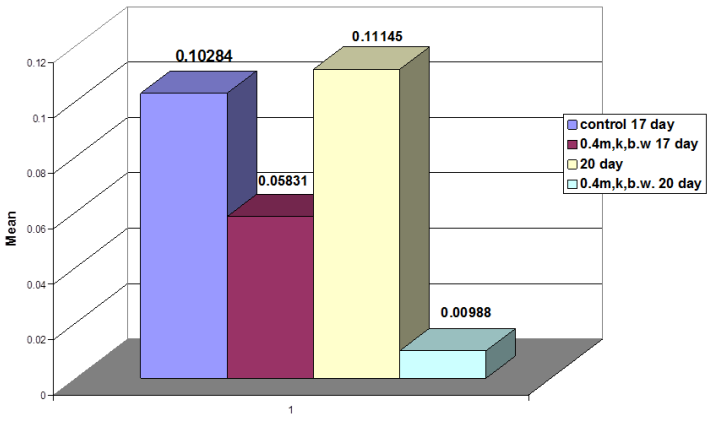

Figure 2. Changes in weight of placenta associated with administration of (0.4 mg/kg b.w.) (G2) of lead acetate to pregnant female mice for 17 and 20 dpc

Discussion

In the current study the results revealed that injection of lead acetate to the mothers at dose (0.1 mg/kg b.w.) for 17 and 20 dpc (day post coitum) did not affect significantly the weight of placenta but the results demonstrated that the placental weight at dose (0.4 mg/kg b.w.) at 17 dpc was significantly decreased ($P<0.05$) and highly significant decreased ($P<0.01$) in 20 dpc compared with control group, these finding is agree with the studies of Wang Yun-Ying et al [19] who cited that fetal body weight, body length and placental weight were significantly lower ($P<0.05$) in the lead exposed rats.

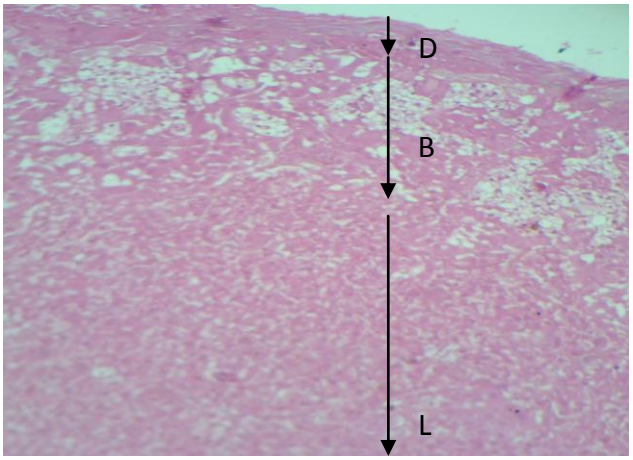

Figure 3. Micrograph illustrate normal structure of placenta from full term mice fetus (control group, 20dpc), showed the labyrinth zone (L) wich contain the maternal sinusoid and the trophoblastic septa. The basal zone (Junctional zone) (B) wich comprised of spongiotrophoblast cells, trophoblastic gaint cells and glycogen cells. Decidual zone (D) (Maternal part of placenta), wich is a vascular densely packed with decidual cells..(10X, H&E)

The decrease in placental weight may be attributed to the fact that trophpblastic cells are specialized for nutrient transfer, energy storage, hormone production and invasion [20]. Moreover tissue oxygen levels regulate the proliferation and differentiation of human trophpblast cells [21]. Lead readily crosses the placental-fetal barrier [22], during different gestational period and has a traumatic effect on the trophpblast, leading to interference of nutrition and oxygen exchange between mother and fetuses. The blood supply to the placenta was also interfered, leading to reduction of placental weight and retardation of development of fetuses [23]. In lead-treated animals, placental blood flow per embryo weight is reduced [24].

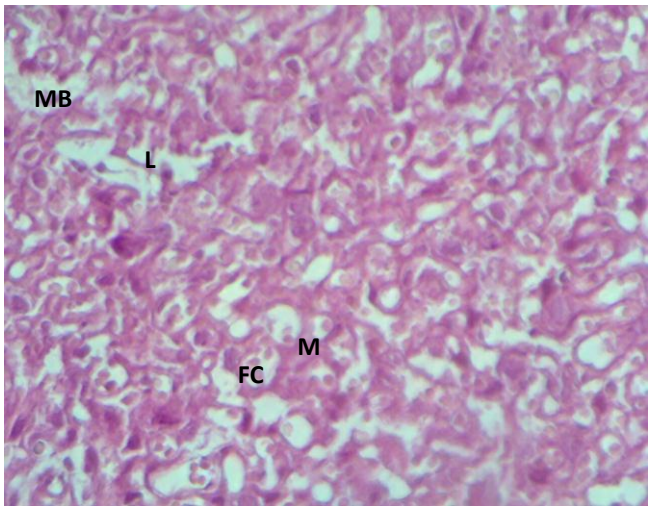

Figure 4. Micrograph illustrate placenta from full term mice fetus,(control group, 17dpc), the villi are seen to have a highly cellular core of mesenchyme (M). Maternal blood (MB) in the surrounding lacuna (L). The trophoblas is reduced to a thin layer of syncytiotrophoblast only and the fetal capillaries (FC) tend to be located in the periphery of the core (40X, H&E)

In addition placental growth, development and aging are crucial to the overall being well of the fetus and are controlled by many of endocrine signals, including steroids and growth factors [25]. Trophopblast giant cells are situated at the maternal-placental interface and are one of the major endocrine cells of the placenta, they synthesize and secret steroid and peptide hormones [16]. Estrogen is known as an inhibitor of placental growth [26]. The regulation of

estrogen biosynthesis in the placenta is very important for human embryos because altering placental function can cause permanent effects on embryos [27]. Lead exposures in utero significantly reduce steroid production [28], and have antiestrogenic activity [29].

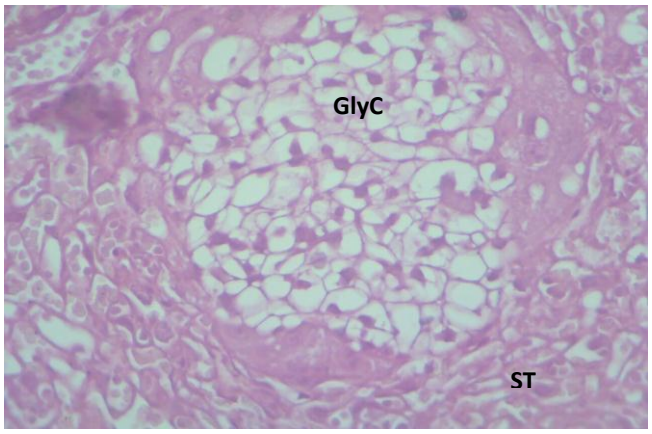

Figure 5. Micrograph revealed the basal zone of placenta belong to mother from control group (20 dpc). The histological section showed a well developed spongiotrophoblasts (ST) around clusters of glycogen cells (GlyC) (40X, H&E)

The decrease in placental weight may also be attributed to the mitotic inhibition, apoptosis, degeneration and/or necrosis of trophoblast, which are induced by direct placental injury or nonspecific effects associated with conditions of an excessively unfavorable maternal environment [30], leads to a lack of cells populations required for later normal histogenesis, resulting in small placenta [23]. Furthermore injury to cytoblasmic organelles may interfere with the nutrition and oxygen exchanges between mother and fetus, and may contribute to abnormal pregnancy outcome [19], and low birth weight [31].

The histological sections of placenta of the fetuses belong to mothers from experimental groups showed alterations in the various components.

In the lead-exposed placenta, expression of lead toxicity was detected in the necrotic area in the labyrinth zone at 17 and 20 dpc in G1 and G2 compared to control group (Figure 8, Figure 10, Figure 12) this observation agree with the studies of Satoshi et al [32], who cited that histological examination of the placenta revealed that there was apparent damage and patchy necrosis of the villous syncytiotrophoblastic cells in the study group in comparison to the control group.

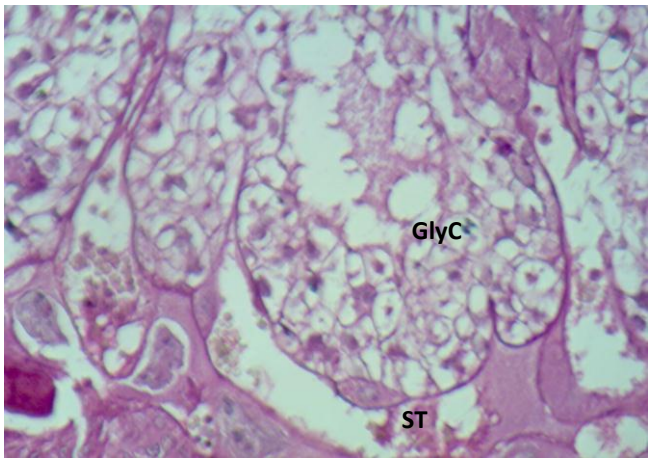

Figure 6. A histological section of placenta belong to mother treated with 0.1 mg/kg b.w. of lead acetate at 17 dpc, showed cystic degeneration of glycogen cells (GlyC) and apoptosis of spongiotrophoblast cells (ST) in basal zone. (40X, H&E)

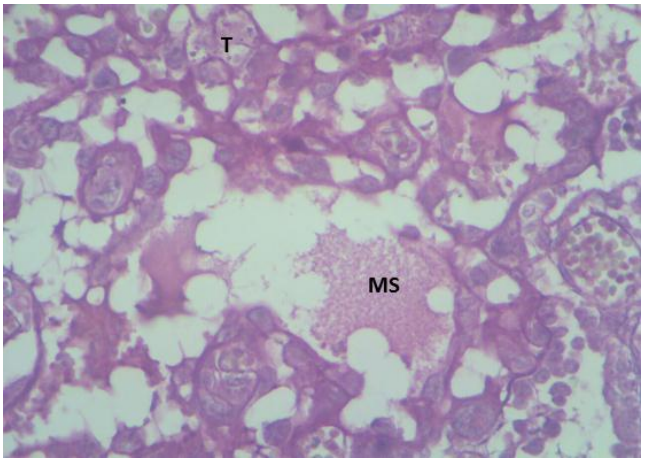

Figure 7. A histological section of placenta belong to mother from prenatal exposed to lead acetate at dose 0.1 mg/kg b.w. at 17 dpc, showed vacuolar degeneration and apoptosis of trophoblasts (T) with fibrin deposition around the villi and irregular dilatation of maternal sinusoid (MS) in the labyrinth zone. (40X, H&E).

This is also agreement with the previous studies conducted by Zhonghua et al who cited that in the experimental group of lead poisoning rats, the placenta showed focus necrosis in the deciduas and increased the trophoblast giant cells [23].

Moreover, trophoblast in the fetal part of the placenta are a common toxicological target tissue for some drugs and chemicals, because they have high proliferative activity and constitute a major structural component of the fetal part of the placenta [32].

Histological observations of basal zone in this study revealed a cystic degeneration of glycogen cells in both experimental groups (G1 and G2) (Figure 6), which is a condition describing abnormal retention of extensive cytoblasmic vacuolation within glycogen cells.

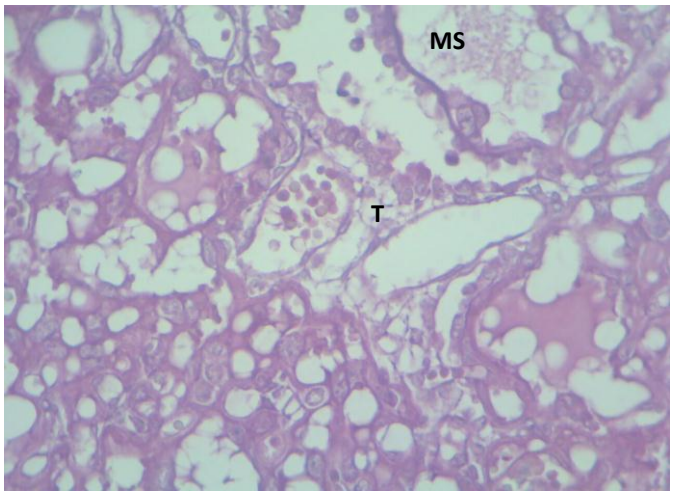

Figure 8. The micrograph illustrate placenta belong to mother treated with 0.1 mg/kg b.w. lead acetate at (20dpc), revealed villus necrosis (arrow), vacuolar degeneration of trophoblasts (T), fibrin deposition and irregular dilaation of maternal sinusoid (MS) in the labyrinth zone. (40X, H&E)

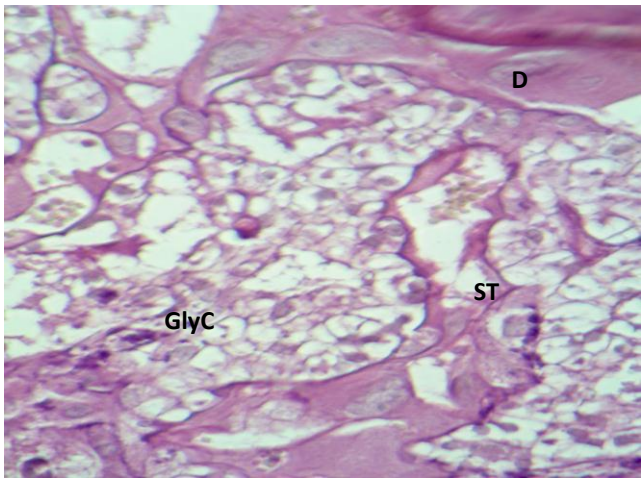

Figure 9. The micrograph revealed the basal zone of placenta belong to mother treated with 0.1 mg/kg b.w. lead acetate at (20dpc), The histological section showed apoptosis of spongiotrophoblasts (ST) around clusters of degenerative glycogen cells (GlyC). Note fibrin deposition in ducidual zone (D). (40X, H&E)

The degenerative cells undergo cytolysis and subsequently coalesce into multiple large cysts that are filled with multiple clusters of residual glycogen cells and cell debris. Placental apoptosis was also clearly obvious in both endothelial cells, trophoblast and stromal cells of placental tissue in both experimental groups (G1 and G2) (Figure 7, Figure 9, Figure 11), this correspond to work of Wang Yun-Ying et al [19], who cited that cell cycle arrest and DNA damage in trophoblast leads to apoptosis and mitotic inhibition in the labyrinth zone inducing growth arrest. Moreover, DNA fragmentation that was indicative of apoptosis was clearly present in basal and labyrinth zone of the placenta at each stage of gestation. This may be attributed to some lead-induced damages that may occur as a consequence of its propensity for disrupting the delicate pro-oxidant/antioxidant balance that exists within mammalian cells. The mechanism for lead induced oxidative stress includes the effect of lead on membrane, DNA, and antioxidant defense system of cells [33]. Lead induced oxidative stress might result from accumulation of 5-amino levulinc acid (ALA), a potential endogenous source of free radicals, induced by inhibition of lead to ALA dehydratase [34].

Additionally, direct interaction of lead with biological membranes may induce lipid peroxidation. Lead exposure might also induce decrease in activities of free radicals scavenging enzymes. This is mainly attributed to high affinity of lead to sulfhydryl-groups in these enzymes [35]. Moreover lead and other heavy metals have high affinities for glutathione (GSH), which is the primary intracellular antioxidant [36]. Furthermore lead can be concentrated in the cell nucleus thus perturbing cell proliferation and DNA synthesis [19]. Beside syncytiotrophoblast has been shown to be the site of metal-lothionein synthesis, a protein that binds lead, a

mechanism for sequestering in mature tissues [37].

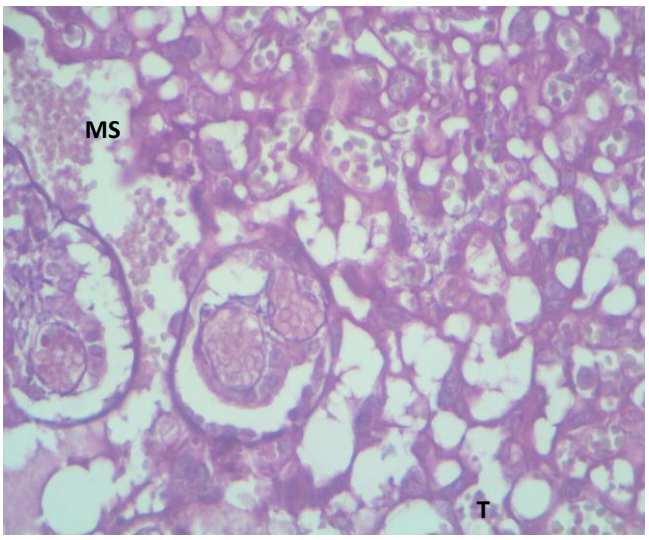

Figure 10. Micrograph illustrate placenta belong to mother treated with 0.4 mg/kg b.w. lead acetate at (17 dpc), showed extensive degeneration and necrosis of trophoblast (T), severe cavitation, irregular dilatation of maternal sinusoid (MS) and calcium deposition in the labyrinth zone. (40X, H&E)

In the labyrinth zone, a multiple cystic dilatation of maternal sinusoid was observed in some placentas on gestation day 17 and 20 in both experimental groups (G1 and G2) (Figure 6, Figure 10, and Figure 12).

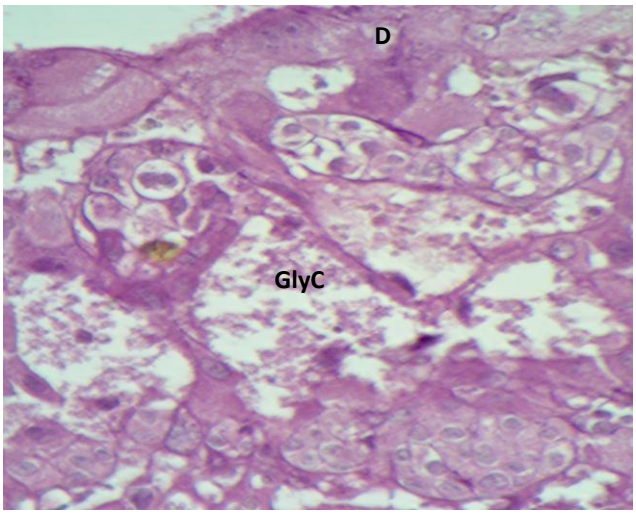

Figure 11. Micrograph illustrate placenta belong to mother treated with 0.4 mg/kg b.w. lead acetate at (20dpc), showed marked apoptosis of glycogen cells (GlyC), with extensive fibrin deposition and calcification in basal and dicidual zone (D).(40X, H&E)

There was also a deposition of fibrin in the labyrinth zone in experimental groups, (G1 and G2) (Figure 7, Figure 8, Figure 9, and Figure 11). This observation is agree with the work of Zhonghua et al [23] who found that the trophoblast in the labyrinth and trophospongium showed degeneration, fibrin deposition around the villi, and heavy deposition of fibrin in deciduas (Figure 9).

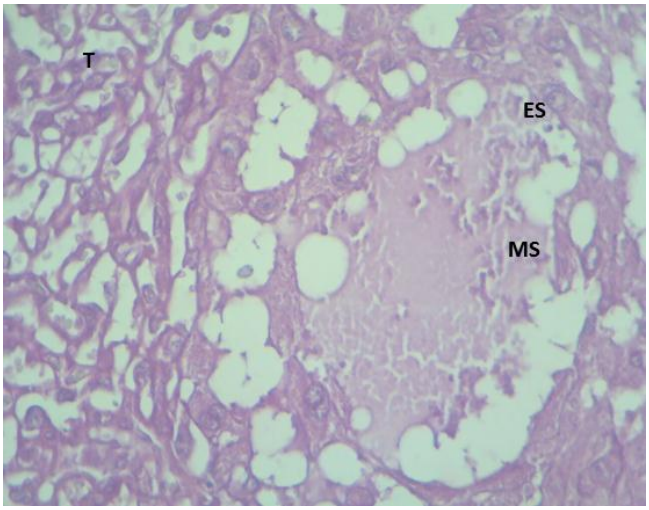

Figure 12. Micrograph illustrate placenta belong to mother treated with 0.4 mg/kg b.w. lead acetate at (20 dpc), showed severe placental changes, generalized stromal necrosis and terminal villous deficiancy, trophoblastic (T) hypoplasia, and irregular dilatation of maternal sinusoid (MS) with disrupting of endothelial cells (EC) along with cavitation in the labyrinth zone (arrow). (40X, H&E)

Calcified and hyalinized villous spots were observed in the experimental groups (G1 and G2) in comparison to the control group (Figure 10 and Figure 11) this is correspond with work of Satoshi et al [32], who proved that, since the placenta is equivalent to blood level, lead is precipitated in the term placenta along with calcium. As villi age they become necrotic, scarred with fibrous tissue, and may contain foci of calcium deposition. It is suggested, however, that fetal tissue levels may be influenced by calcium transport and intracellular calcium metabolism. On the other hand, lead may alter calcium-mediated cellular processes, producing toxicity [11]. A comparison of placental transfer of toxic metals by Tsuyoshi [27], found that lead

levels in the placenta were strongly correlated with levels of elements related to bone metabolism suggesting that placental lead may be associated with calcification and mean number of calcified and hyalinized areas per low power field were significantly higher (P> 0.01) in experimental group than in control group.

References

1. Michal F, Grigor KM, Negro-Vilar A, et al. Impact of the environment on reproductive health: executive summary. Environ Health Perspect. 1993; 101: 159-67.
2. Lefevre B. Lead accumulation in the mouse ovary after treatment-induced follicular atresia. Reprod Toxicol. 2001; 15(4): 385-91.
3. Needleman, H. Lead Poisoning. Ann Rev Med 2004; 55; 209-22.
4. Tracey JW, Alison C, Jackie MS, et al. Proceeding of the summit on the environmental challenges to reproductive health and fertility: executive summary. Fertility Sterility. 2008; 189:281-300.
5. Wmneke G, Collet W, Lilienthal H. The effects of lead in laboratory animals and environmentally-exposed children. Toxicology. 1988; 49(2-3): 291-8.
6. Goyer RA. Transplacental transport of lead. Environ Health Perspect. 1990; 89: 101-5.
7. Cronier L, Bastide B, Defamie N, et al. Involvement of gap junctional communication and connexin expression in trophoblast differentiation of the human placenta. Histol Histopathol. 2001; 16: 285-95.
8. Kehoe K, Thammann R, Cholak J. On the normal absorption and excretion of the lead. IV. Lead absorption and excretion in infants and children. J Ind Hyg. 1933; 15: 301.
9. Georgieff MK, Wobken JK, Welle J, et al. Identification and localization of divalent metal transporter-1 in term human placenta. Placenta. 2000; 21: 799-804.
10. Gidlow DA. Lead toxicity. Occup Med (Lond). 2004; 54: 76-81.
11. Hertz-Picciotto I, Schramm M, Watt-Morse M, et al. Patterns and determinants of blood lead during pregnancy. Am J Epidemiol. 2000; 152: 829-36.
12. Grazyna D, Tadeusz K, Beata K. Evaluation of transplacental gradient for cadmium and lead. Ginekologia Polska. 2002; 73: 43-9.
13. Maryfran S. Blood lead concentration and pregnancy outcomes. Arch Environ Health2002; 57: 489-96.
14. Mahaffey KR, McKinney J, Reigart JR. Lead and compounds. In: Lippmann M. ed. Environmental toxicants: Human exposures and their health effects. 2nd ed. Philadelphia: John Wiley and Sons Inc; 2000. p. 481-521.
15. Bressler JP, Olivi L, Gheong JH, et al. Divalent metal transporter 1 in lead and cadimium transport. Ann NY Acad Sci. 2004; 1012: 142-52.
16. Ain R, Canham LN, Soares MJ. Gestation stage-dependent intrauterine trophoblast cell invasion in the rat and mouse: noval endocrine phenotype and regulation. Develop Biol. 2003; 260(1): 176-90.
17. Humason Gl. Animal tissue techniques. 5th ed. Baltimore and London: The Johns Hopkins University Press; 1997. p. 361-78.
18. Daniel WW. Multiple regression and correlation. In: Daniel WW. ed. Biostatics, a foundation for analysis in the health science. 1988. p. 13-22.
19. Yun-Ying W, Ke-Xu S, Hong LI, et al. The effect of lead exposure on placental NF-KappaB expression and the consequences for gestation. Reprod Toxicol J. 2009; 27: 190-5.
20. Georgiades P, Georgiades AC, Ferguson-smith, et al. Comparative developmental anatomy of the murine and human definitive placenta. Placenta. 2002; 23: 56-75.
21. Caniggia I, Mostachfi H, Winter J, et al. Effects of oxygen on human trophoblast differentiation through TGFbeta3. Clin Invest J. 2000; 105: 577-87.
22. Qasim M, Abu-Taweel M, Jamaan A, et al. Effect of prenatal lead in the cross-fostered mice offspring. Saudi J Biol Sci. 2006; 13: 80-90.
23. Ma HY, Li H, Wang JC, et al. Effects of lead exposure to rat placenta and pups during different gestation periods. Zhonghua Yu Fang Yi Xue Za Zhi. 2006; 40(2): 101-4.
24. Geber G, Maes J, Deroo J. Effect of dietary lead on placental blood flow and on fetal uptake of alpha-amino isobutyrate. Arch Toxicol. 2007; 41: 125-31.
25. Bauer MK, Harding JE, Bassett NS, et al. Fetal growth and placental function. Mol Cell Endocrinol. 1998; 140: 115-20.
26. Bartholomeusz RK, Bruce NW, Lynch AM. Embryo survival and fetal and placental growth following elevation of maternal estradiol blood concentration in the rat. Biol Reprod. 1999; 61: 46-50.
27. Tsuyoshi N. The problem of species comparison of developmental toxicity: can we extrapolate human developmental toxicity induced by environmental chemicals from data of rodents? Yakugaku Zasshi. 2007; 127: 491-500.
28. Dearth RK, Hiney JK, Srivastava V, et al. Effects of lead exposure during gestation and lactation on female pubertal development. Reprod Toxicol. 2002; 16: 343-352.
29. Tchernitechen NN, Villagra A, Tchernitechen AN. Antiestrogenic activity of lead. Environ Toxicol Water Qual. 1998; 13: 43-53.
30. Fuentes M, Torregrosa A, Mora R, et al. Placental effects of lead in mice placenta. Am J Epidemiol. 1996; 17: 371-9.
31. Ibtisam JS. Embryological developmental changes in the gonads of male mice associated with lead administration. MSc Thesis. Institute for infertility

diagnosis and assisted reproductive technologies. Al-Nahrain University, Baghdad. 2009.
32. Satoshi F, Seigo H, Koji U, et al. Toxicological pathology in the rat placenta. J Toxicol Pathol. 2011; 24: 95-111.
33. Ahmed M, siddiqui MK. Low level lead exposure and oxidative stress: Current opinions. Clin Chem Acta. 2007; 383: 57-64.
34. Wang J, Junhui W, Zhang Z. Oxidative stress in mouse brain exposed to lead. Ann Occup Hyg. 2006; 50: 405-9.
35. Moreira EG, Vassilieff L, Vassilieff VS. Developmental lead exposure: behavioral alteration in the short and long-term. Neurotoxicol Terato. 2001; 5: 489-95.
36. Kidd P. Glutathione, systemic protectant against oxidative free radical damage. Altern Med Rev 1997; 2: 155-176.
37. Miller RK. Placental transfer and function: the interface for drugs and chemicals in the concepts. In: Farbo S, Scialli AR. Eds. Drug and chemical action in pregnancy. Pharmacological and toxicological Principles. New York: Marcel Decker Inc; 1986. p. 123-52.

E-mail: rhmr_1988@yahoo.com
Received 8th Apr. 2012: Accepted 29th Jul. 2012