# EFFECT OF TRANSPLACENTAL-FETAL BARRIER AND LACTATIONAL TRANSFER OF LEAD ON PROLACTIN GENE EXPRESSION, DNA FRAGMENTATION AND CHANGES IN THE BRAIN OF EXPOSED MOTHERS AND THEIR OFFSPRING IN ALBINO RATS

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## ABSTRACT

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The present study was conducted to report the effect of prenatal and postnatal treatment of lead (Pb) on some antioxidant defense system in cerebrum and cerebellum of dams and of their foeti, histopathological lesions and DNA fragmentation in brain of intoxicated dams. Experiments were carried out on pregnant Wister rats, they were divided into four groups as follows, Group I (pregnant group, n=10) received lead acetate in their drinking water with concentration of 0.1mg / ml from the 0 day of pregnancy till parturition, Group II (pregnant and lactating group, n=10) were treated as that of group I, but lead exposure extended to the lactation period (21 days after parturition). The suckling pups were allowed to suckle their mothers during the exposure period. Group III (lactating group, n=10) were allowed to deliver without any treatment then they were exposed to lead acetate in their drinking water in the same concentration during the lactation period and their pups suckled them till weanling (21 days), Group IV (control group, n=30) were left without any treatment from the 0 day of pregnancy till the end of lactation (21 days). At the end of each exposure period, dams as well as their pups were weighted and sacrificed by decapitation. The brains were obtained and weighed and dissected into two regions, cerebrum and cerebellum. In pups tissue of three littermates was pooled, weighed and homogenized. The obtained results showed that there was a significant ($p < 0.05$) decrease in the activity of superoxide dismutase, catalase and glutathione in both cerebrum and cerebellum of lead treated rats at the three experimental periods. In addition, a significant elevation of lipid peroxidation was recorded in cerebrum and cerebellum of treated rats and their pups at the three different stages of the experiment was observed. The present data revealed that cerebrum of the treated dams and their pups was more affected than cerebellum. The histopathological examination of cerebrum and cerebellum of treated dams showed marked alterations. DNA fragmentation percent increased while prolactin gene expression showed significant declination especially in pregnancy and lactation group. This study concluded that prenatal and postnatal exposure to Pb induced marked alteration on some antioxidant defense system in cerebrum and cerebellum of dams and of their foeti, marked increase in DNA fragmentation percent in brain of intoxicated dams. Moreover, there was significant declination in prolactin gene expression especially in pregnancy and lactation group.

***Keywords:*** *Prenatal, postnatal, lead exposure, placental barrier, DNA, prolactin expression*

## INTRODUCTION

The most important source for lead environmental pollution is the use of petroleum that contains high lead levels (Grobler *et al.*, 1992). The study of accumulation and effects of heavy metals on living organisms is a matter of topical interest in connection of global environmental pollution (Teodorova *et al.*, 2003). Concern has been expressed that changes in physiology during pregnancy increase the turnover of bone, which could raise maternal blood lead concentrations to levels

that would harm fetus (Manton *et al.,* 2003). Exposure of rats to Pb after weaning failed to show behavioural alterations (Kuhlmann *et al.,* 1997). In contrast, animals exposed during gestation (Yang *et al.,* 2003), or during both gestation and lactation led to long-term cognitive deficits in offspring. Lorenzo *et al.* (1977) found considerably higher Pb concentration in the milk than the blood of Pb exposed lactating rabbits and a study by Keller and Doberty (1980) reported that Pb exposed mouse dams transmitted a significantly greater amount of Pb to their offspring through their milk than by in utero exposure (transplacental). In contrast Miller *et al.* (1998) suggested that the lead concentration in the milk of exposed rats was insufficient to causes significant exposure to the nursing pups. In both human and experimental animals, Pb readily crosses the placental- fetal barrier (Donald *et al.,* 1986), causing a direct relation between the Pb –exposed mother and the possibility for irreversible developmental damage to her offspring (Rom, 1976). Pb accumulate in the fetus from the second trimester and onward (Bhattacharayya, 1983), but during lactation, it is excreted into the milk, which continues the risk to nursing offspring (Namihira *et al.,* 1993 and Hallen *et al.,* 1995). Prenatal exposure to levels of Pb has been involved in behavioral and neurochemical alterations detected in both suckling and adult rats (Moreira *et al.,* 2001 and Widzowski *et al.,* 1994). It is also found that lower level in utero Pb exposure may be related to defects in post-fetal grown and post-natal behavioural development (Bellinger *et al.,* 1986). Prolactin is another major hormonal mechanism for modifying calcium metabolism during pregnancy and lactation, increasing calcium absorption and placental transfer of calcium. Prolactin was described by De Burbure *et al.* (2006), as a sensitive indicator of early effects in toxicological research. The present study elucidates DNA fragmentation, prolactin expression due to exposure to lead.

## MATERIALS and METHODS

### 2.1. Animals, tissue preparation and sampling

Experiments were carried out on Wister rats obtained from the breeding unit of the veterinary hygiene and management department, Faculty of Veterinary Medicine, Cairo University. Animals were housed with free access to water and food in animal room with 12 / 12hours light/ dark cycle, at 25 ± 2 $^{o}$C. They were matted one week after their arrival (three females and one male per cage). On 0 day of pregnancy (presence of sperm in vaginal smears) the dams were divided into four groups as follows, Group I (pregnant group;G1): in this group pregnant rats (n=10) received lead acetate in their drinking water with concentration of 0.1mg / ml (Keller and Doherty, 1980) from the 0 day of pregnancy till parturition, Group II (pregnant and lactating group;G2): pregnant rats(n=10) were treated as that of group I, but lead exposure extended to the lactation period (21 days after parturition). The suckling pups were allowed to suckle their mothers during the exposure period. Group III (lactating group;G3): in this group pregnant rats (n=10) were allowed to deliver without any treatment then they were exposed to lead acetate in their drinking water in the same concentration during the lactation period and their pups suckled them till weanling (21 days), Group IV (control group;C1,C2,C3): in this group pregnant rats (n=30) were left without any treatment from the 0 day of pregnancy till the end of lactation (21 days). At the end of each exposure period, dams as well as their puppies were weighted and sacrificed by decapitation. The brains were obtained immediately by opening the cranial cavity, weighed and dissected (Glowinski and Iverson, 1966), into two regions, cerebrum and cerebellum. Due to the small amount of tissue in case of puppies especially in group one, tissue of three littermates was pooled. The tissue was weighed and homogenized (1 mg tissue / 4 ml phosphate buffer saline (PBS) in ice /cooled 140mMPBS using 10stocks in a Teflon/ glass homogenizer. The tissue was maintained at 0-4 $^{o}$C throughout the dissection and homogenization procedure. The homogenate was centrifuged for 15 minutes at 1000 g at 4 $^{o}$C and the supernatant was centrifuged again at 18000 g for 15 minutes at 4 $^{o}$C. Blood samples were collected immediately after decapitation of animals in tubes pre- treated with 10 µ l of heparin while in pups in the first group (pregnant group) blood samples were taken by heart puncture.

### 2.2. Chemicals

Lead acetate (99.8%) and other chemicals were obtained from Riedel Dehan AG- Seelze – Hannover – Germany. Lead acetate in the form of pure crystals and made soluble in water by addition of 1-2 drops of acetic acid.

### 2.3. Enzymes assay

The final supernatant was used for the evaluation of the activities of Super Oxide Dismutase (SOD) (Mc Cord and Fridovich, 1969), Catalayse (Luck, 1963), Reduced Glutathione (GSH) (Moron *et al.,* 1979), Lipid Peroxidation (LPO) (through the accumulation of thiobarbituric acid reactive substance, TBARS) by the assessment of Malondehyde level (MDA) (using method of Chanarin, 1989) and Protein content determined according to Lowry *et al.* (1951).

### 2.4. Histopathological studies

Small pieces of cerebrum and cerebellum of dam's brain were histopathologically examined (Carleton *et al.,* 1987).

**2.5. DNA fragmentation assay**

The DNA fragmentation assay was conducted according to Sellins and Cohen (1987). Tissues were lysed in 1 mL buffer (10 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.5% Triton X-100). The pellets containing total intact DNA (designated P) and the supernatants containing smaller fragments of DNA (designated S) were treated separately with 0.5 mL of 25% TCA. Both sets were left over night at 4 °C, and precipitated DNA was collected by centrifugation. Each pellet was treated with 80 µL of 5% TCA followed by heat treatment at 90 °C for 15 min. One mL freshly prepared diphenylamine (DPA) reagent was added to each sample, tubes were allowed to stand overnight at room temperature, and the optical density was recorded at 600 nm. Percentage DNA fragmentation was calculated as follows: DNA fragmentation% = [S/(S + P)] × 100, where S is the optical density of the supernatant and P the optical density of the pellet.

**2.6. Determination of prolactin expression level**

Total RNA was isolated from blood using RNA easy blood Mini Kit; Qiagen. RNA concentration was measured spectrophotometrically (according to Kit manufacture). The isolated RNA was subjected to reverse transcription polymerase chain reaction. The reaction was performed at 44°C for 60 min. A 170-base pair (bp) fragment of the prolactin gene was amplified by polymerase chain reaction (PCR) using forward (5'- AGTCTGTTCTGGTGGCGACT- 3') and reverse (5'- GAAGTGGGGCAGTCATTGAT-3') primers. Cycles applied were: denaturation 95°C, 5 min; followed by 35 cycles. Each cycle consisted of 30 s at 95°C, 30 s at 60°C, 30 s at 72°C and final extension 72°C, 10 min. A 452-base pair (bp) fragment of the glyceraldehyde 3 phosphate dehydrogenase gene was amplified, as internal standard, by polymerase chain reaction (PCR) using forward (5'- ACCACAGTCCATGCCATCAC- 3') and reverse (5'- TCCACCACCCTGTTGCTGTA-3') primers. Cycles applied were: denaturation 95°C, 5 min; followed by 35 cycles. Each cycle consisted of 30 s at 95°C, 30 s at 60°C, 30 s at 72°C and final extension 72°C, 10 min. The PCR products were electrophoresed on 2%agarose.

**2.7. Statistical Analysis**

The obtained data was analyzed statistically by using SAS user guide (2004) to determine one way analysis of variance (ANOVA).

## RESULTS

**3.1. Effect of Lead acetate on enzymes activity**

**3.1.1. Effect of Lead acetate on enzymes activity of treated dams**

The obtained results showed that there was a highly significant ($p < 0.01$) decrease in superoxide dismutase activity in both cerebrum and cerebellum of lead treated rats at the three experimental periods (table 1). Moreover, values of catalase activity revealed higher significant decrease ($p < 0.01$) in cerebrum at the 1st and 2nd treated groups and significant decrease only ($p < 0.05$) in the same region of brain in 3rd group (lactation only). While in cerebellum of lead treated rats, the catalase values showed higher significant decrease only in the 2nd group (pregnancy and lactation) and significant decrease ($p < 0.05$) in the 1st and 3rd groups (table 2).

Regards to glutathione values the current study showed that there was higher decrease in their concentration in cerebrum and cerebellum of both lead treated groups (2nd and 3rd groups) while in the 1st group (pregnancy only) a significant decrease in glutathione concentration in the brain region was observed. In addition, a higher concentration of lipid peroxidation was recorded in cerebrum and cerebellum of treated rats at the three different stages of the experiment was observed except a significant decrease ($p < 0.05$) in lipid peroxidation value in cerebellum of the 3rd group (lactation group) (table 2).

**3.1.2. Effect of Lead acetate on enzymes activity of pups**

The obtained results showed that there was a significant ($p < 0.05$) decrease in superoxide dismutase activity of both cerebrum and cerebellum in pups resulted from lead treated dams at the three experimental periods (table 1). Moreover, values of catalase activity revealed a significant decrease **(**$p < 0.05$) of both cerebrum and cerebellum in pups resulted from lead treated dams at the three experimental periods.

Regards to glutathione values the current study showed that there was a significant decrease in their concentration in cerebrum of pups in the three experimental groups. While its value showed significant decrease ($p < 0.05$) only in cerebellum of pregnant group and higher significant decrease ($p < 0.01$) in the same region in pregnant and lactating and lactating groups respectively (table 2). In addition, a significant increase ($p < 0.05$) in concentration of lipid peroxidation was recorded in cerebrum and cerebellum of pups resulted from lead treated dams in pregnant and lactating groups (1st and 3rd groups). While in pregnant and lactating group (2nd group) the results denote a highly significant ($p < 0.01$) increase in both brain regions of pups resulted from lead treated dams in 2nd group (pregnancy and lactation group) (Table 1).

**Table 1:** Effect of Lead acetate on the activity of Superoxide dismutase (SOD), Catalase (CAT), reduced glutathione (GSH) and Lipid peroxidation (LPO) in cerebrum and cerebellum of pups from lead treated dams

| Groups | SOD (unit / g protein) | | CAT(unit / mg protein) | | GSH (µ mole/ g tissue) | | LPO (µ mole/ g tissue) | |
|---|---|---|---|---|---|---|---|---|
| | Cerebrum | Cerebellum | Cerebrum | Cerebellum | Cerebrum | Cerebellum | Cerebrum | Cerebellum |
| G1 | 98.3± 3.15* | 104± 4.12* | 90.11± 2.53* | 90.2± 2.54* | 0.118± 0.03* | 0.142± 0.03* | 1.44± 0.06* | 1.72± 0.05* |
| C1 | 110.5±3.72 | 113.6±3.15 | 97.2±2.82 | 101.2±2.95 | 0.125±0.02 | 0.132±0.03 | 1.25±0.05 | 1.52±0.04 |
| G2 | 90.2± 4.36* | 101.6± 3.15* | 88.3± 2.17* | 90.3± 2.65* | 0.113± 0.03* | 0.112± 0.04** | 1.92± 0.04** | 1.95± 0.05** |
| C2 | 110.4±2.85 | 119.3±2.65 | 98.7±3.15 | 105.2±3.62 | 0.128±0.03 | 0.135±0.05 | 1.42±0.02 | 1.43±0.07 |
| G3 | 102.1± 3.72* | 116.4± 3.24* | 95.5± 2.43* | 98.3± 3.14* | 0.122± 0.02* | 0.128± 0.02** | 1..52± *0.02 | 2.25± 0.06* |
| C3 | 115.2±4.35 | 125.2±3.75 | 103.7±2.76 | 108.1±3.42 | 0.135±0.01 | 0.142±0.03 | 1.33±0.03 | 1.96±0.07 |
| LSD at P<0.05 | 10.2 | 7.6 | 6.5 | 8.2 | 0.002 | 0.003 | 0.14 | 0.15 |
| LSD at P< 0.01 | 22.3 | 18.5 | 15.8 | 19.6 | 0.018 | 0.011 | 0.28 | 0.32 |

Values indicate Mean ± SE (Standard Error), Number of animals= 6, LSD = Least Significant Difference between groups, G1 = Pregnancy group, G2= Pregnancy and lactating group, G3= Lactating group, C1, 2, 3 = Control group

**Table 2:** Effect of Lead acetate on the activity of Superoxide dismutase (SOD), Catalase (CAT), reduced glutathione (GSH) and Lipid peroxidation (LPO) in cerebrum and cerebellum of treated and control dams.

| Groups | SOD (unit / g protein) | | CAT(unit / mg protein) | | GSH (µ mole/ g tissue) | | LPO (µ mole/ g tissue) | |
|---|---|---|---|---|---|---|---|---|
| | Cerebrum | Cerebellum | Cerebrum | Cerebellum | Cerebrum | Cerebellum | Cerebrum | Cerebellum |
| G1 | 162.5± 1.32** | 168.15± 3.86** | 131.3± 2.25** | 143.5± 5.92* | 0.161± 0.03* | 0.182± 0.06* | 3.72± 0.15** | 2.84± 0.12** |
| C1 | 230.2±5.63 | 235.6±4.71 | 158.6±4.92 | 158.5±6.24 | 0.175±0.06 | 0.201±0.04 | 1.95±0.16 | 1.98±0.14 |
| G2 | 140.6± 1.73** | 143.35± 5.72** | 118.3± 3.45** | 128.15± 3.72** | 0.142± 0.04** | 0.163± 0.03** | 4.82± 0.18** | 4.16± 0.17** |
| C2 | 235.2±6.54 | 248.2±6.85 | 162.5±3.85 | 162.5±4.61 | 0.185±0.05 | 0.225±0.06 | 2.25±0.15 | 2.35±0.15 |
| G3 | 186.5± 1.54** | 193.14± 4.51** | 142.5± 3.82* | 156.2± 5.83* | 0.173± 0.04** | 0.196± 0.02** | 2.99± 0.11** | 2.21± 0.18* |
| C3 | 240 ±6.32 | 252.46±3.92 | 168.6±4.72 | 171.28±6.2 | 0.196±0.03 | 0.233±0.05 | 2.35±0.11 | 1.92±0.16 |
| LSD at P<0.05 | 14.85 | 18.92 | 12.53 | 13.72 | 0.007 | 0.009 | 0.18 | 0.23 |
| LSD at P< 0.01 | 30.3 | 37.5 | 25.7 | 27.5 | 0.018 | 0.021 | 0.41 | 0.47 |

Values indicate Mean ± SE (Standard Error), Number of animals= 5, LSD = Least Significant Difference between groups, G1 = Pregnancy group, G2= Pregnancy and lactating group, G3= Lactating group, C1, 2, 3 = Control group

### 3.2. Histopathological examination of cerebrum and cerebellum of lead treated dams and their pups

Hemotoxylin / eosin sections of cerebrum and cerebellum of lead treated dams were evaluated under light microscopy. After lead treatment, transverse sections of treated cerebrum in pregnant group showed hyalinization in the wall of the blood vessels in the cerebral tissue (Fig. 1), focal gliosis was observed in cerebral tissue (Fig. 2). In group II (pregnancy and lactation) microscopic evaluation showed focal extravasations of red blood cells in the cerebral tissue (Fig. 3) and also hemorrhages in the gray matter was observed (Fig. 4). In cerebellum of

treated dams in group II, degeneration of the perkinje cells was noted (Fig. 5). In group III (lactation group) microscopic examination of cerebrum showed neuronal degeneration and chromatolysis (Fig. 6). Light microscopic examination of cerebrum and cerebellum of pups resulted from treated dams showed no remarkable histopathological alterations in the three experimental groups. Both cerebrum and cerebellum of rat in group IV (control group) showing normal histological structure (Fig.7& Fig. 8).

**3.3. DNA fragmentation assay**

The DNA fragmentation percentage showed a marked increase in the pregnancy and lactation group in the female cerebrum whereas, there was no significant effect among the other treated groups. The DNA fragmentation percentage showed significant increase among the treated groups in the female cerebellum (Fig. 9) such that the highest percentage was seen in the pregnancy and lactation group. The results showed that the cerebrum was more affected than the cerebellum (Fig.10). The DNA fragmentation percentage showed marked increase in the lactation and the pregnancy and lactation groups in the fetal cerebrum. The DNA fragmentation percentage showed significant increase among the treated groups in the fetal cerebellum and the highest percentage was reported among the pregnancy and lactation group (Fig. 11& Fig.12). The results showed that the cerebrum was more affected than the cerebellum. By comparing the results of the female and feti, the female brain tissue were found to be more affected that the fetal ones.

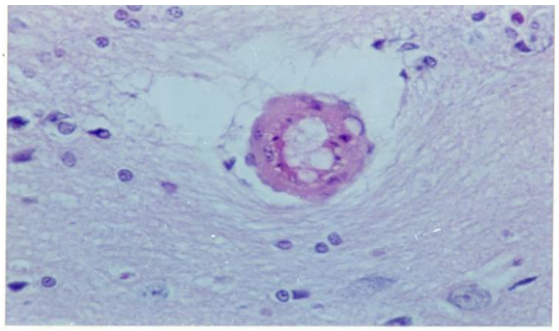

**Fig. 1:** Brain of rat in group I (pregnant group) showing hyalinization in the wall of the blood vessels in the tissue of cerebellum (H&E X:160)

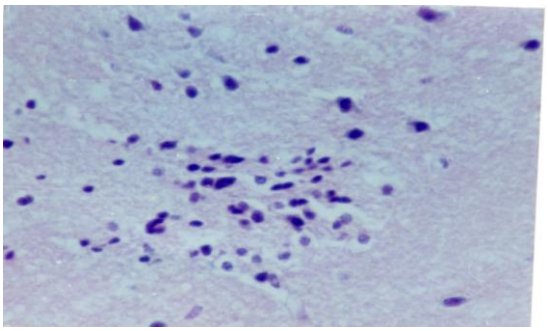

**Fig. 2:** Brain (cerebrum) of rat in group I (pregnant group) showing focal gliosis in the cerebral tissue (H&E X:160)

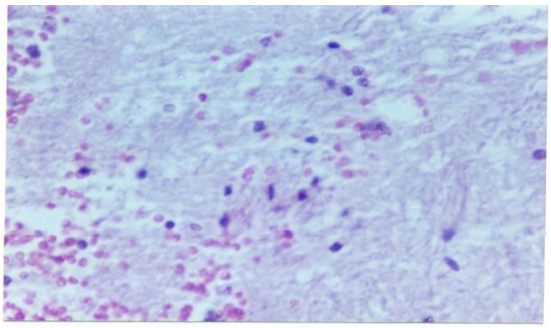

**Fig. 3:** Brain (cerebrum) of rat in group II (pregnant and lactating group) showing focal extravasation of red blood cells in the cerebral tissue (haemorrhage) (H&E X:160)

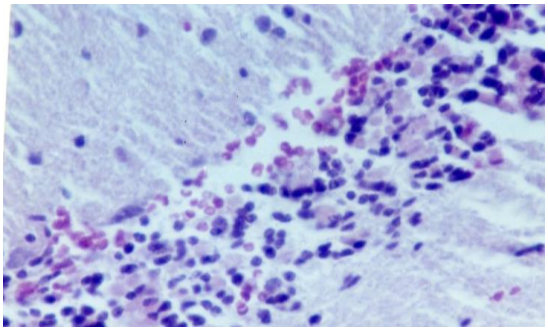

**Fig. 4:** Brain (cerebrum) of rat in group II (pregnant and lactating group) showing focal extravasation of red blood cells in the gray matter (haemorrhage) (H&E X:160)

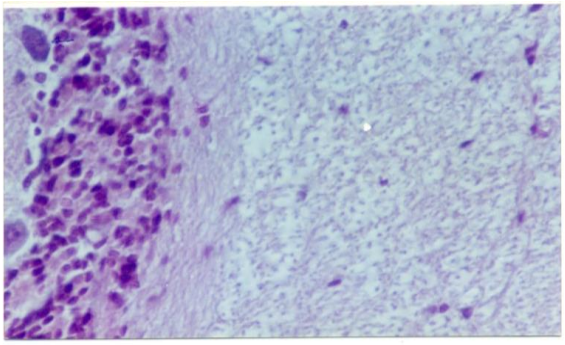

**Fig. 5:** Brain (cerebellum) of rat in group II (pregnant and lactating group) showing degeneration of pyrking cells (H&E X:160)

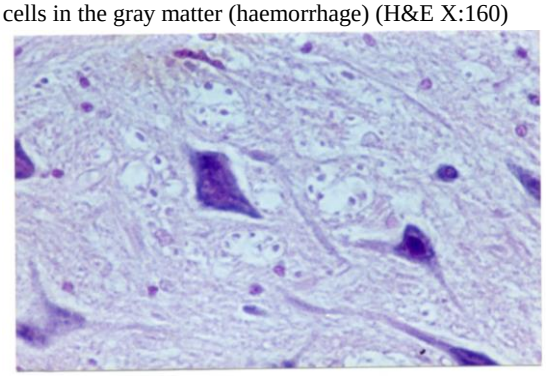

**Fig. 6:** Brain (cerebrum) of rat in group III (lactating group) showing neuronal degeneration and chromatolysis in the cerebral tissue (H&E X:160)

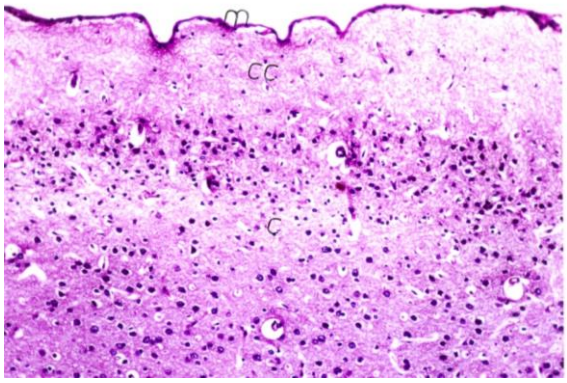


**Fig. 7:** Brain (cerebrum) of rat in group IV (control group) showing normal histological structure of cerebral cortex and cerebrum (H&E X:160)

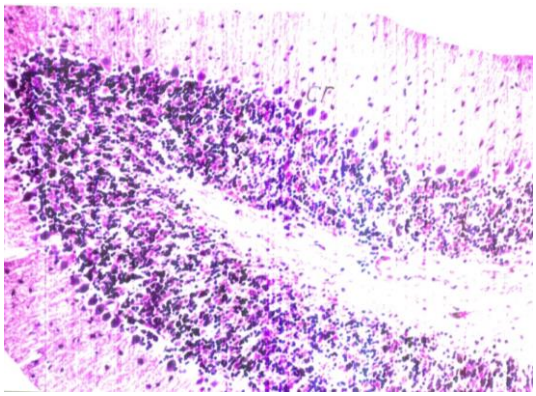


**Fig. 8:** Brain (cerebellum) of rat in group IV (control group) showing normal histological structure of cerebellum (H&E X:160)

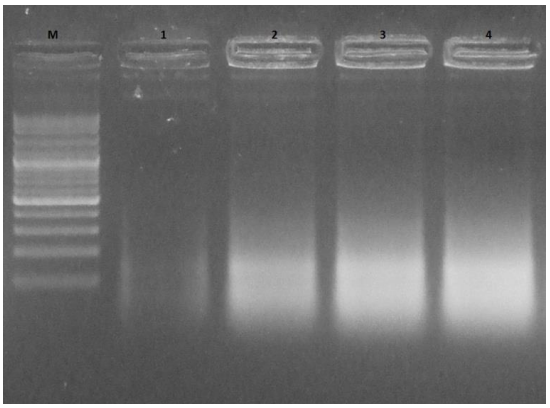


**Fig. 9:** Analysis of DNA fragmentation. Agarose gel electrophoresis of low molecular weight DNA extracted from fetal cerebellum analyzed by electrophoresis through a 2% agarose ge. M refers to 100 bp DNA ladder, Lane 1: negative control group; lane 2: pregnant group; lane 3: pregnancy and lactation group; lane 4: lactation group

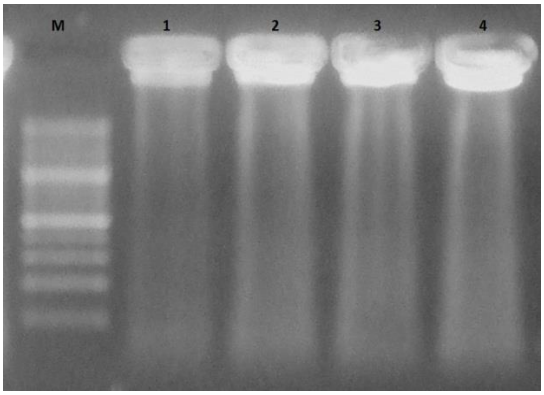


**Fig. 10:** Analysis of DNA fragmentation. Agarose gel electrophoresis of low molecular weight DNA extracted From fetal cerebrum analyzed by electrophoresis through a 2% agarose ge. M refers to 100 bp DNA ladder, Lane 1: negative control group; lane 2: pregnant group; lane 3: pregnancy and lactation group; lane 4: lactation group

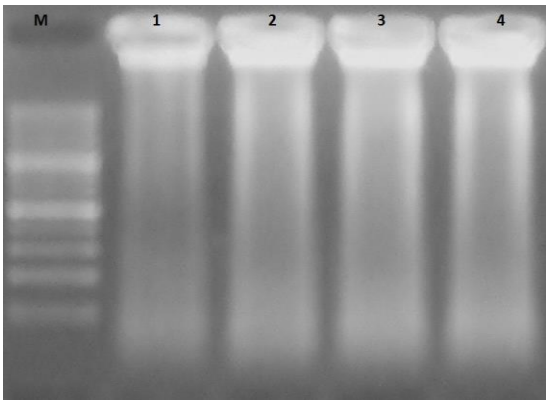


**Fig.11:** Analysis of DNA fragmentation. Agarose gel electrophoresis of low molecular weight DNA extracted from female cerebellum analyzed by electrophoresis through a 2% agarose ge. M refers to 100 bp DNA ladder, Lane 1: negative control group; lane 2: pregnant group; lane 3: pregnancy and lactation group; lane 4: lactation group

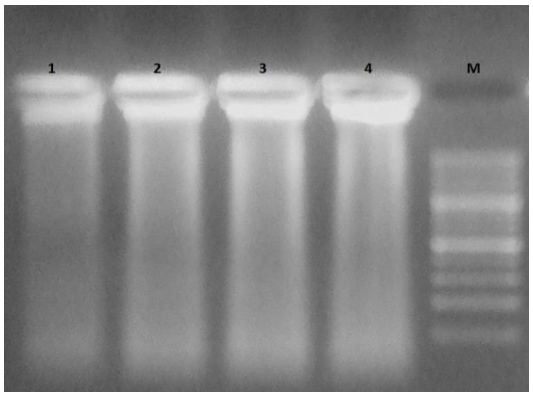


**Fig.12:** Analysis of DNA fragmentation. Agarose gel electrophoresis of low molecular weight DNA extracted from female cerebrum analyzed by electrophoresis through a 2% agarose ge. M refers to 100 bp DNA ladder, Lane 1: negative control group; lane 2: pregnant group; lane 3: pregnancy and lactation group; lane 4: lactation group

**3.4. Prolactin expression level**

The prolactin expression relative to GAPDH showed significant declination among the exposed groups if compared to the negative control. The most affected group was the pregnant and lactation, as it showed the lowest prolactin expression level.

## DISCUSSION

The effect of Pb on fetal growth, intrauterine development and postnatal status has long been of concern in occupational and environmental medicine. More recently, several large epidemiological studies have reported deficits in early infant development observed in children born to mothers whose blood lead levels during pregnancy were only slightly elevated as compared to a control group (Dietrich *et al.,* 1990). Lead (Pb) pass readily to the fetus through the placenta (Karpela *et al.,* 1986) and is also found in milk during the lactation period. The effect of these divalent metal on the developing CNS may be due to the fact that immature organism absorbs them to greater extent than does the adult (Mc Michel *et al.,* 1988). Hallen *et al.* (1995) recorded that continuous Pb exposure during gestation and lactation in rats resulted in milk Pb levels approximately 2.5 times higher than the blood Pb levels. When Pb exposure was terminated at parturition the milk Pb bevels were at a level similar to those of blood Pb at day 15 of lactation. This indicates that the lactational transfer after recent exposure of Pb in dams is considerably higher than placental transfer.

Antonio *et al.* (2003) mentioned that cadmium and Pb intoxication during pregnancy and lactation have important effects on both body and brain weight. Zhang *et al.* (2009) mentioned that heavy metal ions are toxic to the CNS because blood brain barrier is immature and protein complexes sequestering metals in mature tissues are not present. Both metal (Cd and Pb) are likely transferred from dams to pups in the first three weeks after birth (Bhattacharayya, 1983). Cd and Pb intoxication during pregnancy and lactation has critical effects on the body and brain weights of pups. The same results were also recorded by Kahloula *et al.* (2009) they revealed that Pb exposure during pregnancy and lactation period in rats causes loss in both body and brain weight. They attributed this reduction in body and brain weight to the endocrine and biochemical mechanisms underlaying the growth suppression produced mainly by gestational and lactational lead exposure are related with decreases in growth hormones associated factors (Ronis *et al.,* 1998).

The direct neurotoxic actions of lead include apoptosis (programmed cell death). Correlations between maternal and umbilical cord blood lead levels confirmed the transfer of lead from the mother to the fetus (Gardella, 2001), a new born infants blood pb was shown to reflect that of the mother (Schell *et al.,* 2003). Moreover, the increase in lead level in breast milk with increasing maternal blood pb levels represents an additional risk to the newborn infant (Li *et al.,* 2000) in rats. These results came in the same line with ours such that the highest DNA fragmentation was apparent in the pregnancy and lactation groups in the fetal cerebrum and cerebellum. Exposure to low to moderate concentrations (10 nM to μM) of lead ions induced apoptosis in cell culture (Gilley *et al.,* 2003) and in developing and adult rats (He *et al.,* 2000). Exposure to low to moderate levels of lead during development (0–21days), resulting in signs of apoptosis in the cell. It was suggested that lead bind to the internal metal-binding site of the mitochondrial transition pore, subsequently open the transition pore, and initiate the cytochrome C-caspase activation cascade leading to apoptosis. Low-level lead exposure inhibits NOS activity, which is involved in memory, in the rat hippocampus, the cerebral cortex, and the cerebellum (Emerit *et al.,* 2004).

In dams and their corresponding pups, effects of Pb exposure induced statistically significant changes in all enzymes evaluated in all regions. The observed enzyme activities (SOD, Catalyse and Glutathione) showed significant decrease and this indicate that free radical generation is progressively increased with the increase in exposure spams in all the regions during treatment period. Also the present study revealed that dams and their pups exposed to Pb during the three experimental periods showed significant increase ($p < 0.05$, $p < 0.01$), in the concentration of lipid peroxide in the two tested regions of brain. This elevation was marked in dams and their pups during gestation and lactation treatment more than gestation or lactation only. Also significant increase in lipid peroxidation was higher in cerebrum than in cerebellum in all treatment period. It was known that lead and its ions induce oxidative stress in cells by several distinct mechanisms. Because lead has a high affinity for sulfhydryl residues in protein, it has been proposed that the toxicity of lead is the result of its ability to act as a non specific enzyme inhibitor. It also exerts its toxic effects by combining with oxygen and sulfur- containing bioligands (Sidhu and Nehru, 2004). The oxygen radicals that are normally produced within the body are usually kept in check by complex multifactorial protective enzymes, which include SOD, CAT and GSH, which can check the free radicals originating either in the mitochondria or in the cytoplasm. However, the brain is one organ that is at first instance, susceptible to peroxidase damage because of several factors, such as high oxygen tension, low mitotic rate, high lipid content,

as well as low antioxidant concentration (Julka *et al.*, 1992).

The novel aspect of this study lies in elucidating the neurotoxic effect of lead and its effect on prolactin expression which is decreased in the lead treated groups. The most affected group is the pregnant and lactation group which shows a significant decrease in prolactin expression level if compared to the negative control group. The least affected group is the lactation group which show slight decrease in prolactin expression level if compared to the negative control group. In pregnancy, prolactin can be increased by exposure to lead (Lucchini *et al.*, 2000). On the other hand, negative studies have also been published on the association of prolactin with the exposure to neurotoxicants (Myers *et al.*, 2003). Meeker *et al.* (2009) reported that lead was inversely associated with prolactin level in rat. Likewise, an inverse relationship between lead and PRL was reported among pregnant women (Takser *et al.*, 2005). Our results suggest that maternal physiological statuts of pregnant females increases their susceptibility to lead toxic effects causing a significant decrease in the prolactin level of expression among all treated groups.

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## تأثير انتقال الرصاص عبر المشيمه والرضاعه على التعبير الجينى للبرولاكتين ، تفتت الحامض النووى والتغيرات الباثولوجيه فى المخ للأمهات المعرضه والمواليد فى اناث الفئران البيضاء

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أجريت هذه الدراسة لتحديد تأثير عنصر الرصاص قبل الولادة وبعد الولادة على بعض مضادات الأكسدة في المخ للأمهات والمواليد، نسبة ترسيبه فى الجسم والتغيرات التشريحية المرضية لها وتفتيت الحمض النووي في المخ المعرض للتسمم والتعبير الجينى للبرولاكتين. أجريت هذه التجربه على الفئران البيضاء الحوامل، فقد تم تقسيمها إلى أربع مجموعات على النحو التالي، المجموعة الأولى (مجموعة الحوامل، ن = ١٠) والتى تعرضت لخلات الرصاص في مياه الشرب بتركيز ٠.١ ملى جرام / مل من اليوم الأول من الحمل حتى الولادة ، المجموعة الثانية (مجموعة الحوامل والمرضعات، ن = ١٠) وعولجت كمافى المجموعة الأولى، ولكن التعرض للرصاص امتد إلى فترة الرضاعة (بعد ٢١ يوما من الولادة). سمح للمواليد بأن يرضعوا أمهاتهم خلال فترة التعرض. اما المجموعة الثالثة (مجموعة المرضعات، ن = ١٠) تركت دون أي علاج من اول يوم من الحمل وحتى الولاده ثم تعرضوا لخلات الرصاص في مياه الشرب بنفس التركيز خلال فترة الرضاعة وصغارها أرضعت منهم حتى الفطام (٢١ يوما)، المجموعة الرابعة (المجموعة الضابطة، ن = ٣٠) تركت دون أي علاج من اول يوم من الحمل وحتى نهاية الرضاعة (٢١ يوما). في نهاية كل فترة التعرض، تم وزن الأمهات والصغار ثم تم الزبيح بقطع الرأس وقد تم الحصول على المخ ووزنه وتشريحه إلى منطقتين، المخ والمخيخ. في الصغار تم تجميع الأنسجة من المخ والمخيخ. وأظهرت النتائج أن هناك انخفاض ملحوظ في نشاط بعض الإنزيمات والتى تشمل السوبر اوكسيد ديسموتاز ، الكاتلاز والجلوتاثيون في كل من المخ والمخيخ فى الفئران التي عولجت بالرصاص في المجموعات التجريبية الثلاث. وبالإضافة إلى ذلك، تم تسجيل ارتفاع كبير فى نسبة بيروكسيد الدهون في المخ والمخيخ فى الفئران المعالجة وصغارها في المراحل الثلاث المختلفة من التجربة. وكشفت النتائج الحالية أن المخ من الأمهات المعالجة كانت صغارها أكثر تأثرا فى المخيخ. أظهر فحص الأنسجة للمخ والمخيخ تغيرات ملحوظة نتيجة التعرض لخلات الرصاص. ارتفعت نسبة تجزئة الحمض النووي في حين أظهر التعبير الجيني للبرولاكتين انخفاضا ملحوظا خاصة في المجموعة التى تعرضت اثناء فترة الحمل وأثناء الرضاعة. وخلصت هذه الدراسة إلى أن التعرض قبل الولادة وبعد الولادة للرصاص يؤدى الى تغيرات ملحوظه فى بعض مضادات الأكسدة في المخ للأمهات والمواليد ، ارتفاع نسبه تفتيت الحمض النووي في المخ المعرض للتسمم وانخفاض ملحوظ فى التعبير الجينى للبرولاكتين.