Effect of dietary calcium on maternal and fetal lead toxicity and teratogenicity in rats

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SUMMARY

Lead is being a deleterious environmental pollutant that induce a broad range of physiological and biochemical dysfunctions. Repeated exposure to lead or lead salts during organogenesis exerts toxic actions including teratogenicity, embryotoxicity and abortion. Studies of metabolism and toxicity of lead have revealed important interactions between lead and calcium. Diets low in calcium may increase the gastrointestinal absorption and toxicity of lead.

The purpose of this study was to investigate the effect of orally administered lead (26.8 mg/kg B.wt. or 13.4 mg/kg B.wt.) alone or in combination with dietary calcium (1.0%) on pregnant rats and their feti during organogenesis (day 6-15 of gestation). Some maternal biochemical values were estimated and evaluated on the serum of pregnant rats. Lead (at the high dose) intoxication during organogenesis exhibited a significant decrease in the body weights of dams and their feti, the number of live feti, the fetal crown-rump length and induced a high percentage of visceral and skeletal malformations. Maternal values of serum ALT, cholesterol, triglycerides, urea, creatinine and uric acid were elevated while, levels of AST and ALP did not differ from the control group.

Lead in-combination with calcium decreased significantly the incidence of visceral and skeletal abnormalities per fetus (dose dependant) and improved significantly the levels of the aforementioned hepatorenal values. In contrast, there were no significant differences between data of calcium supplemented group and lead intoxicated rats on mother body weight and fetal values.

It is inferred from our study that, lead has a potentially serious effects on the pregnant rats and their feti and these deleterious effects could be limited by dietary calcium supplementation.

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INTRODUCTION

Human civilization and concomitant increase in industrial activity has gradually redistributed many toxic metals from the earth crust to the environment and increased the possibility of human and animal exposure. Among the various toxic elements, heavy metal lead is especially prevalent in nature due to their high industrial use. Lead is a cumulative poison and is toxic even at low dose (Chowdhury and Chandra, 1987). Lead toxicity is more clear in the newly born animals and human (Galhome et al., 2000) and it accumulates to a greater extent in females than in males (Klevay, 1972).

Lead exposure has many undesired effects, including neurological (Moreira et al., 2001 and Soltaninejad et al., 2003), behavioural (De-Marcio et al., 2005), immunological (Bunn et al., 2001), renal (Vargas et al., 2003), hepatic (Randa, 2006), and especially haematological dysfunctions (Sivaprasad et al., 2003).

Lead exposure also increases the incidence of infertility, abortion (Abd El-Hamed et al., 2008), stillbirth, fetal death and macrocephally (Needleman et al., 1984). Little attention was given to the possible embryo toxic effects of lead. Information is scanty for animals, as for human regarding the amount of lead reaching the embryo before implantation and during organogenesis causing malformation (Gerber et al., 1980).

Studies of metabolism and toxicity of lead have revealed important interactions between lead and some essential dietary elements like calcium (Chowdhury and Chandra, 1987). Diets low in calcium may increase the gastrointestinal absorption and toxicity of lead (Mahaffey, 1981; Miller et al., 1990 and Bogden et al., 1995). Despite the well–documented effects of dietary calcium on lead absorption, metabolism and toxicity during pregnancy have not been adequately studied. Pregnancy has profound effects on calcium homeostasis (Pitikin, 1992). Increasing dietary calcium during pregnancy could reduce lead toxicity (Hallberg et al., 1991).

The objective of this work was to determine the effect of interaction between dietary calcium and lead on the mothers and their feti during organogenesis.

MATERIALS AND METHODS

Chemicals:

- Lead (Pb): Lead acetate trihydrate (C₄H₆O₄Pb₃H₂O) of M.W. 379.33, was provided by Riedel Dehaen, Hanover, Germany. Each one gram of Pb is found in 1.8307 g of finally powders of lead acetate.
b- Calcium (Ca): Calcium carbonate (CaCO$_3$) of M.W. 100 was purchased from BDH Chemicals Ltd Poole, England.

c- Glacial acetic acid: It was added to the drinking water at a concentration of 12.5 µl/L to prevent formation and precipitation of lead carbonate (Bogden et al., 1995).

Animals:
Fifty female albino rats of Wister strain weighing 130 – 140 g and twenty five male albino rats of the same strain weighing 140 – 150 g were purchased from the National Research Centre. The rats were allowed to acclimate to the laboratory environment for one week and food and distilled water were provided.

Teratologic study:
After the acclimation period, two female rats were caged with a proven fertile male. In the morning vaginal smears were examined from each rat for the presence of sperms. Pregnant rats were removed to a separate cage and it was considered as day zero of pregnancy. After being identified as pregnant, the fifty rats were assigned to five treatment groups as follows (10 rats / group):

Group (1): Control rats were fed on normal diet and distilled water during the gestation period.

Group (2): Pregnant rats were fed on normal diet and were intubated orally by stomach tube with lead 1/400 LD$_{50}$ (26.8 mg/ kg B.wt.) (Fatma, 1992) from day 6-15 of gestation.

Group (3): Pregnant rats were fed on normal diet containing Ca (1.0%) as calcium carbonate (Shackelford et al., 1994) and were intubated orally by stomach tube with lead 1/400 LD$_{50}$ from day 6-15 of gestation.

Group (4): Pregnant rats were fed on normal diet and were intubated orally by stomach tube with lead 1/800 LD$_{50}$ (13.4 mg/ kg B.wt.) from day 6-15 of gestation.

Group (5): Pregnant rats were fed on normal diet containing Ca (1.0 %) as calcium carbonate and were intubated orally with lead 1/800 LD$_{50}$ from day 6-15 of gestation.

Fetal gross, visceral and skeletal examinations:
The pregnant rats were kept under daily observation until the 20th day of gestation, afterwards they were weighed and anaesthetized with ether for performance of caesarian sections.

The number of live, dead and resorbed feti were counted in each uterine horn and examined for gross abnormalities. The fetal body weight and fetal crown-rump length were recorded. Half of the
live feti were fixed in Bouin’s solution and the other half in 95% ethanol. The former feti were hand-sectioned and examined by a hand lens for internal visceral examination, (Wilson, 1972). The latter feti were cleared with KOH solution (2%), stained with alizerin red stain and examined by a hand lens for skeletal examination (Hayes, 1988).

**Maternal biochemical analysis:**

Blood samples were collected in the teratologic study from each group of pregnant dams from the retro-orbital venous plexus on day 20 of gestation. Blood samples were left to form clot then serum samples were obtained after centrifugation at 3000 rpm for 15 minutes. Serum samples were separated for measuring the concentration of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Reitman and Frankel, 1957), alkaline phosphatase, ALP, (Tietz et al., 1986), cholesterol (Watson, 1960), triglycerides (Foster and Dunn, 1973), urea (Coulombe and Favreau, 1963), creatinine (Husdan and Rapaport, 1968) and uric acid (Barham and Trinder, 1972).

**Statistical analysis:**

The obtained data were statistically analysed using ANOVA test and least significant difference (LSD) as comparative of means as well as Fischer Exact Probability test according to the method described by (SPSS 14, 2006).

**RESULTS**

Exposure to lead (1/400 LD50) during organogenesis exhibited a significant decrease in the body weights of dams and feti, the number of live feti and the fetal crown-rump length while, the low dose of lead (1/800 LD50) decreased only the fetal body weight and length (Fig., 1) comparing with control group. Calcium – lead (1/400 LD50 and 1/800 LD50) in-combination did not improve mother body weights or fetal values comparing with lead intoxicated groups. Both tested concentrations of lead did not cause death or resorption of the feti (Table, I).

**Teratologic study: Fetal gross and visceral examination:**

In fetal examination, lead at concentration levels of 1/400 LD50 and 1/800 LD50 induced gross abnormalities limited to haematoma (Fig., 2) at different parts of the body 64.6 – 47.9%, respectively while, Ca-lead in-combination decreased these percentages to 33.3 – 19.1%, respectively (Table II). In the same table the two tested concentrations of lead evoked a high % of visceral malformations involving cerebral haemorrhage, dilatation of central and lateral ventricles (Fig. 3) and/or hydrocephaly of the brain, anophthalmia and/or microphthalmia (unilateral
or bilateral), thickening of the ventricular septum of the heart (Fig. 4), dilatation in the renal pelvis (Fig. 5) and intra-abdominal intra-thoracic haemorrhage (IATH). The incidence of fetal gross abnormalities and visceral malformations per fetus (incidence/fetus) of both lead concentrations (1/400 and 1/800 LD$_{50}$) showed significant differences comparing with control group; 7.044, 2.995 and 0.1056, respectively (dose-dependent).

Maternal dietary Ca (1.0%) plus lead induced remarkable improvement in the previous aforementioned visceral lead malformations, Calcium – lead (1/800 LD$_{50}$) in-combination prevented completely the appearance of anophthalmia and/or microphthalmia comparing with lead intoxicated group. The incidence of fetal gross abnormalities and visceral malformations per fetus at both concentrations of Ca-lead (1/400 and 1/800 LD$_{50}$) in-combination differed significantly from lead intoxicated groups; 3.2942, 0.8289, 7.0441 and 2.995, respectively (dose-dependant), (Table II).

**Skeletal examination:**
In skeletal examination (Table, III) concurrent administration of Ca with lead (1/400 LD$_{50}$ and 1/800 LD$_{50}$) decreased the percentage of skeletal anomalies of skull (incomplete ossification with open fontanellae), sternabrae (missing) and limbs (incomplete ossification and missing of phalanges).

Feti exposed to lead (1/400 LD$_{50}$) plus Ca showed a decrease in the incidence of rib (missing, fused and/or incomplete ribs), (Fig. 6) and vertebral column (incomplete ossification and/or missing vertebrae) malformations (Fig. 6) while, Ca – lead (1/800 LD$_{50}$) in-combination gave complete protection from the above-mentioned skeletal anomalies. Ca-lead (1/400 and 1/800 LD$_{50}$) in-combination improved significantly the incidence of skeletal anomalies per fetus (dose-dependant) comparing with lead intoxicated groups 5.6101; 1.6751; 13.104 and 4.9293, respectively.

**Maternal biochemical analysis:**
Effect of lead (1/400 LD$_{50}$ and 1/800 LD$_{50}$) on some maternal biochemical values in serum of rats during organogenesis revealed that lead had a significant elevating effect on the levels of ALT, cholesterol, triglycerides, urea, creatinine and uric acid. Meanwhile, AST and ALP activities showed no differences comparing with control rats. Calcium supplementation to both tested lead concentrations improved significantly the levels of serum ALT, triglycerides and creatinine together with cholesterol, urea and uric acid comparing with non Ca – treated group (Table IV).
Fig. (1): Feti from a dam rat intoxicated with lead (13.4 mg/kg B.wt.) (A) during organogenesis showing decreased fetal size more than control (B).

Fig. (2): A fetus from a dam rat intoxicated with lead (26.8 mg/kg B.wt.) during organogenesis showing haematoma at different parts of the body.

Fig. (3): Cross section in brain of rat feti obtained from a dam intoxicated with lead (26.8 mg/kg B.wt.) (A) during organogenesis showing dilatation of brain ventricles (central and lateral) compared with control (B).

Fig. (4): Cross section in heart ventricles of rat feti obtained from a dam intoxicated with lead (26.8 mg/kg B.wt.) (A) during organogenesis showing thickening of the ventricular wall compared with control (B).

Fig. (5): Cross section in kidney of rat feti obtained from a dam intoxicated with lead (26.8 mg/kg B.wt.) (A) during organogenesis showing dilatation of the renal pelvis compared with control (B).

Fig. (6): Feti from a dam rat intoxicated with lead (26.8 mg/kg B.wt.) during organogenesis (A) showing missing of coccygeal vertebrae (B) missing and fused ribs (B) compared with control (C).
Table (I): Effect of lead 1/400 LD$_{50}$ (26.8 mg/Kg B.wt) or 1/800 LD$_{50}$ (13.4 mg/Kg B.wt) orally administered at days 6-15 of gestation period with and without Ca (1.0%) to rats during organogenesis on fetal values and mother body weight.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mother body weight (g)</th>
<th>Fetal body weight (g)</th>
<th>No. of live feti</th>
<th>Fetal crown-rump length (cm)</th>
<th>Resorbed feti</th>
<th>Dead feti</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>205.0 ± 1.79 A</td>
<td>3.81 ± 0.049 A</td>
<td>9.6 ± 0.42 A</td>
<td>4.14 ± 0.09 A</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lead (1/400 LD$_{50}$)</td>
<td>183.60 ± 1.51 a</td>
<td>2.81 ± 0.044 aB</td>
<td>5.8 ± 0.66 aB</td>
<td>2.49 ± 0.19 a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lead (1/400 LD$_{50}$) + Ca</td>
<td>189.20 ± 1.24</td>
<td>2.97 ± 0.049 a</td>
<td>6.6 ± 0.56 aC</td>
<td>2.89 ± 0.27 a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lead (1/800 LD$_{50}$)</td>
<td>193.80 ± 3.96</td>
<td>3.01 ± 0.047 a</td>
<td>9.4 ± 0.32bc</td>
<td>2.90 ± 0.27 a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lead (1/800 LD$_{50}$) + Ca</td>
<td>199.60 ± 1.53</td>
<td>3.18 ± 0.076 b</td>
<td>9.6 ± 0.55bc</td>
<td>3.20 ± 0.38 a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F-calculated</td>
<td>3.477#</td>
<td>51.314#</td>
<td>11.125#</td>
<td>3.769#</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

# Significant at P < 0.05 Using ANOVA test

Aa, Bb, Cc, Dd significantly different between two comparison groups against capital letter using LSD at P < 0.05
Table (II): Effect of lead 1/400 LD$_{50}$ (26.8 mg/Kg B.wt) or 1/800 LD$_{50}$ (13.4 mg/Kg B.wt) orally administered at days 6-15 of gestation period with and without Ca (1.0 %) to rats during organogenesis on fetal gross abnormalities and visceral malformations.

<table>
<thead>
<tr>
<th>Malformation %</th>
<th>Haematoma</th>
<th>Cerebral haemorrhage</th>
<th>Brain</th>
<th>Eye</th>
<th>Heart</th>
<th>Kidney</th>
<th>IATH</th>
<th>Incidence/ fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>0.7</td>
<td>0.3</td>
<td>0.0156 a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead (1/400 LD$_{50}$)</td>
<td>64.6</td>
<td>12.5</td>
<td>37.5</td>
<td>31.3</td>
<td>56.3</td>
<td>31.3</td>
<td>38.6</td>
<td>7.0441 b</td>
</tr>
<tr>
<td>Lead (1/400 LD$_{50}$) + Ca</td>
<td>33.3</td>
<td>9.0</td>
<td>32.6</td>
<td>4.7</td>
<td>30.4</td>
<td>26.1</td>
<td>8.7</td>
<td>3.2942 c</td>
</tr>
<tr>
<td>Lead (1/800 LD$_{50}$)</td>
<td>47.9</td>
<td>8.7</td>
<td>31.8</td>
<td>22.7</td>
<td>36.4</td>
<td>29.5</td>
<td>10.5</td>
<td>2.9950 d</td>
</tr>
<tr>
<td>Lead (1/800 LD$_{50}$) + Ca</td>
<td>19.1</td>
<td>4.5</td>
<td>13.0</td>
<td>-</td>
<td>2.4</td>
<td>9.3</td>
<td>4.7</td>
<td>0.8289 e</td>
</tr>
</tbody>
</table>

Haematoma: at different parts of the body
Brain: Dilatation of central and lateral ventricles and/or hydrocephalus
Eye : Anophthalmia and/or microphthalmia (unilateral or bilateral)       Heart : Thickening of the ventricular septum
Kidney : Dilatation of renal pelvis                        IATH : Intra-abdominal/ intra-thoracic haemorrhage

a, b, c, d, e significant difference between different letter using Fischer Exact Probability test.
Table (III): Effect of lead 1/400 LD\textsubscript{50} (26.8 mg/Kg B.wt) or 1/800 LD\textsubscript{50} (13.4 mg/Kg B.wt) orally administered at days 6-15 of gestation period with and without Ca (1.0 %) to rats during organogenesis on fetal skeletal malformations.

<table>
<thead>
<tr>
<th>Malformation %</th>
<th>Malformation %</th>
<th>Incidence/ fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Skull</td>
<td>Sternebrae</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead (1/400 LD\textsubscript{50})</td>
<td>80</td>
<td>71.4</td>
</tr>
<tr>
<td>Lead (1/400 LD\textsubscript{50}) + Ca</td>
<td>20</td>
<td>66.7</td>
</tr>
<tr>
<td>Lead (1/800 LD\textsubscript{50})</td>
<td>52.9</td>
<td>47.6</td>
</tr>
<tr>
<td>Lead (1/800 LD\textsubscript{50}) + Ca</td>
<td>8.3</td>
<td>40</td>
</tr>
</tbody>
</table>

Skull: Incomplete ossification of skull with open fontanellae
Sternebrae: Missing
Limbs: Incomplete ossification and/or missing phalanges
Ribs: Missing, fused and/or incomplete
Vertebral column: Incomplete ossification and/or missing vertebrae.

a, b, c, d significant difference between different letter using Fischer Exact Probability test.
Table (IV): Effect of lead 1/400 LD<sub>50</sub> (26.8 mg/Kg B.wt) or 1/800 LD<sub>50</sub> (13.4 mg/Kg B.wt) orally administered at days 6-15 of gestation period with and without Ca (1.0%) on some maternal biochemical values in serum.

<table>
<thead>
<tr>
<th></th>
<th>ALT (Iu/L)</th>
<th>AST (Iu/L)</th>
<th>ALP (Iu/L)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td>14.0 ± 0.24&lt;sup&gt;A&lt;/sup&gt;</td>
<td>14.0 ± 0.27</td>
<td>92.0 ± 1.68</td>
<td>60.0 ± 1.19&lt;sup&gt;A&lt;/sup&gt;</td>
<td>177.0 ± 5.61&lt;sup&gt;A&lt;/sup&gt;</td>
<td>29.0 ± 1.76&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.64 ± 0.011&lt;sup&gt;A&lt;/sup&gt;</td>
<td>3.00 ± 0.068&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lead (1/400 LD&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>25.8 ± 0.29&lt;sup&gt;ab&lt;/sup&gt;B</td>
<td>14.9 ± 0.26&lt;sup&gt;A&lt;/sup&gt;</td>
<td>94.6 ± 1.73</td>
<td>140.0 ± 2.78&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>363.0 ± 11.52&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>40.0 ± 1.50&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>1.00 ± 0.020&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>4.30 ± 0.097&lt;sup&gt;aB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lead (1/400 LD&lt;sub&gt;50&lt;/sub&gt;( + Ca)</td>
<td>18.0 ± 0.30&lt;sup&gt;bc&lt;/sup&gt;C</td>
<td>13.9 ± 0.17</td>
<td>94.0 ± 1.72</td>
<td>70.2 ± 1.18&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>227.0 ± 7.20&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>34.0 ± 1.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.76 ± 0.015&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.93 ± 0.089&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lead (1/800 LD&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>20.0± 0.24&lt;sup&gt;abD&lt;/sup&gt;B</td>
<td>13.8± 0.25</td>
<td>92.2 ± 1.68</td>
<td>67.3 ± 1.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>231.0 ± 7.33&lt;sup&gt;abc&lt;/sup&gt;C</td>
<td>37.0 ± 1.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.90 ± 0.014&lt;sup&gt;abC&lt;/sup&gt;</td>
<td>3.80 ± 0.086&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lead (1/800 LD&lt;sub&gt;50&lt;/sub&gt;( + Ca)</td>
<td>14.0 ± 0.23&lt;sup&gt;bcd&lt;/sup&gt;B</td>
<td>13.7 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.2 ± 1.68</td>
<td>65.0 ± 0.66&lt;sup&gt;bC&lt;/sup&gt;</td>
<td>189.0 ± 5.99&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>35.0 ± 2.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.70 ± 0.011&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>3.60 ± 0.081&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>F-calculated</td>
<td>31.254#</td>
<td>4.511#</td>
<td>0.504</td>
<td>88.19#</td>
<td>89.521#</td>
<td>6.354#</td>
<td>51.124#</td>
<td>31.57#</td>
</tr>
</tbody>
</table>

# Significant at P < 0.05 Using ANOVA test
Aa, Bb, Cc, Dd significantly different between two comparison groups against capital letter using LSD at P < 0.05
DISCUSSION

In this study orally administered lead in a dose level of 1/400 LD$_{50}$ to dams from day 6-15 of gestation, decreased the body weight of mothers and feti, the number of live feti and the fetal crown-rump lengths, while the low dose of lead (1/800 LD$_{50}$) decreased only the fetal body weight and length. These results coincided with those reported by Fatma (1992) and Bogden et al. (1995). Hammond et al. (1993) who suggested that lead may reduce linear growth and weight gain during development. The observed retardation of fetal growth may result from an inhibition of fetal haemoglobin synthesis as lead intoxication causes anaemia (Jacquet et al., 1977; Gerber and Maes, 1978; Fatma, 1993 and Randa, 2006) and/or an action on placental blood flow (Gerber et al., 1978).

Feti from dams intoxicated with lead (1/400 and 1/800 LD$_{50}$) and supplemented with Ca (1.0%) did not show improvement in the fetal body weights and lengths. Shackelford et al. (1993) demonstrated that consumption of a 1.25% Ca diet during gestation period had no effect on fetal rat body weight and length, meanwhile this Ca concentration (1.25%) decreased kidney and iron concentration of the mothers (Shackelford et al., 1994). Bogden et al. (1995) found that a high dietary Ca (2.5%) plus lead during gestation period did not interfere with maintaining pregnancy and delivering normal pups but decreased the fetal body weights and lengths, as well as reduced dam and pups haemoglobin and organ iron concentration. It has been reported by Hallberg et al. (1991) that increasing dietary Ca during pregnancy could reduce lead toxicity but might simultaneously reduce iron absorption. Anaemia during pregnancy has been associated with low birth weight (Scholl and Hediger, 1994). Thus, the unimprovement of body weights and lengths of feti obtained from dams exposed to Ca and lead in our study are likely due, at least in part, to decrease iron absorption induced by Ca-lead in-combination.

Teratologic study:

In the present study dams exposed to lead (both tested concentrations) during organogenesis, their pups exhibited numerous visceral and skeletal malformations. Most of the severe visceral teratogenic abnormalities were found in heart, brain, kidney and eye. The skeletal anomalies were high in skull, sternum and limbs followed by ribs and vertebrae. The incidence of fetal gross abnormalities, visceral and skeletal malformations per fetus was significantly high (dose-dependant).
Valid comparisons of the rates of anomalies reported by several investigations in different species revealed that lead has been considered a potential teratogen depending on the dose level administered, the critical times of gestation and route of administration. In accordance to our results, different degrees of abnormalities were reported by Gerber et al. (1980) in mammals; Needleman et al. (1984); Shukla et al. (1989); Bentur and Koren (1991) in human and Fatma (1992) in rats.

Lead deposits were observed in placenta and chorionic membranes (Mestek et al., 1998). Lead is capable of crossing the placenta causing failure of implantation and/or affecting embryonic and fetal development causing death of the embryo due to its toxic action (Fatma, 1992 and Butkauskas and Srouga, 2004). Lead interferes with embryonic nutrition and energy supply at a number of sites, in addition to compete with other cations such as zinc (Uzbekov et al., 2007), iron and Ca and thereby limits their availability at critical sites (Mahaffey and Michaelson, 1980; Needleman et al., 1984 and Goyer, 1996).

About 99% of orally absorbed lead is bound to erythrocyte which can spread lead to different organs of the body (Sivaprasad et al., 2003), causing damage to them (Moussa and Bashandy, 2008). Nolan and Shaikh (1992) and Han et al. (1996) suggested that this element is strongly bound to macromolecules in the intracellular component as lead binding proteins have been isolated from brain, kidney, liver and blood (Raghavan and Gonick, 1977; Mistry et al., 1986; Guilarte et al., 1994 and Han et al., 1996). Lead has been shown to affect the function of mitochondria in every organ that reflects lead toxicity clinically: the brain, the kidney and the haemopoietic system in particular (Goyer, 1968).

Holtzman et al. (1984) have noted that the sensitivity of human and rat brain to lead toxicity may be greater in younger organisms because lead may be increasingly sequestered away from mitochondria as organism ages. Exposure of the developing brain to lead, known to interact with Ca disturbs neural tube closure and subsequent maturation of the nervous system (Lagunowich et al., 1994 and Goyer, 1996). Prenatal lead exposure had a damaging effect on Cu/Zn superoxide dismutase activity in the brain of treated fetuses reflects activation of free radical processes and impairment of the antioxidant defense system during prenatal lead exposure (Uzbekov et al., 2007). Lead is a central nervous system toxicant which can result in cerebral edema, brain
dilatation and congestion (Durgut et al., 2008).

Moreover, Bennet et al. (1986); Pollock and Ibels (1986) and Oberley et al. (1995) proved that rats exposed to lead since conception till 3 week old revealed a significant renal damage. In addition, lead is expected to cause consistent increase in the vagal activity and other cardiac disorders such as atrioventricular conduction defects (Myerson and Elsheshawi, 1963 and Al-Dhaheri et al., 1995) and degenerative changes in the heart (Durgut et al., 2008). Lead accumulation in skeleton where it appears to compete with Ca for the binding sites (Grandjean and Olsen, 1984):

The aforementioned literatures might be the reason of appearance of numerous visceral and skeletal lead malformations encountered in this study.

In our study Ca-lead in combination of both tested concentrations improved the incidence of visceral and skeletal abnormalities per fetus (dose-dependant) comparing with non Ca-treated groups. Calcium is an essential mineral that plays an important role in the development and maintenance of bones, nerve conduction, muscle contraction and blood clotting (Ginty et al., 1998 and Kimberly and Brien, 1998). The relationship which exists between Ca and lead is complex. Six and Goyer (1970); Koroleve and Sukhanov (1996) and Moussa et al. (2001) demonstrated that body retention of lead and the severity of clinical lead-toxicity are inversely related to the level of dietary Ca. Increased dietary Ca levels appeared to decrease intestinal absorption of lead (Quarterman et al., 1978 and Fullmer, 1991). It is evident that the effect of dietary Ca on lead toxicity could be mediated by changes in intestinal lead absorption, tissue distribution, elimination or any combination of these factors (Meredith et al., 1977).

Braton et al. (1978) proposed that Ca may inhibit lead absorption via physical competition between Ca and lead for common binding sites on intestinal binding proteins for absorption. At the molecular level, lead may be competing with Ca to alter critical cell functions such as ion transport, function of heme-containing enzymes and energy productions. Impaired energy metabolism results in reduction of the normal function of the cell (Goyer, 1990). Fullmer and Rosen (1990) reported that lead in the presence of a low Ca 0.05% diet inhibits intestinal Ca absorption, while consumption of 1.2% Ca diet improves Ca absorption. Lead decreases Ca levels in blood, kidney, liver, brain and femur muscles (Moussa et al., 2001). Lead inhibi-
its mitochondria uptake of Ca in brain (Goldstein, 1977). Lead concentrations in maternal and fetal whole blood, organ (brain, kidney and liver) and femur were substantially reduced in rats fed increasing amounts of Ca (2.5%) in diet (Bogden et al., 1995). Moussa et al. (2001) mentioned that increasing dietary Ca (0.3-2.7%) can alleviate many of the toxic effects of lead as indicated by decreasing lead levels in rat organs (kidney, liver and brain), femur and blood. The effects of dietary Ca on organ lead accumulation seem to be specific. High dietary Ca is known to reduce tissue uptake of lead and protect pig tissues from pathological changes associated with lead accumulation (Hsu et al., 1975).

The aforementioned results of the previous authors may explain the improvement in the teratological effects which have been induced by Ca in lead intoxicated rats in this study.

Maternal biochemical analysis:
In the current study mothers exposed to lead from day 6-15 of gestation exhibited an elevation in serum ALT, cholesterol, triglycerides, urea, creatinine and uric acid. Lead is known to exert deleterious effects upon a wide variety of biological systems (Hoffman et al., 1972; Trejo et al., 1997 and Randa, 2006). The rise in serum ALT activity recorded in this work may be attributed to drastic changes caused by lead in hepatic tissues. The enzyme ALT is more specific for liver diseases reflecting membrane permeability changes and necrosis (Reynolds, 1986). The accumulated lead in liver can act directly by damaging the hepatocytes, primarily by destroying the permeability of cell membrane with resultant release of cellular enzymes leading to increase their values (Todorovic et al., 2005). It has been reported that serum ALT was elevated significantly more than AST on lead exposure (Hassanin, 1994) which indicates liver damage (Shalan et al., 2005) and development of fibrosis (Lanter, 1975).

It was noticed in the present investigation that lead induced a significant increase in serum cholesterol and triglycerides. Bashandy (2006) found that administration of lead to rats elevates plasma LDL (low density lipoprotein) and reduces plasma HDL (high density lipoprotein). There is evidence that linking increased of serum cholesterol and LDL levels to a higher risk for developing coronary heart diseases (Glueck et al., 1986). The increase in the levels of triglycerides in lead treated mothers indicating the breakdown of fatty acids (Knowles and Donaldson, 1996).
Elevation of urea, creatinine and uric acid recorded in serum of lead intoxicated dams reflects renal damage which was relevant with the histopathological findings (Nashwa et al., 2006). Creatinine is more diagnostic value for corrupted kidney than urea and uric acid. Increased values of creatinine may occur when there is renal impairment, the glomerular filtration rate is reduced in pre-renal and post-renal uremia, impaired blood flow and adreno-cortical insufficiency (Varley et al., 1980).

Concurrent administration of Ca with lead ameliorated the hepatorenal damage of the dams induced by lead as there was a significant decrease in serum activities of ALT, cholesterol, triglycerides, urea, creatinine and uric acid concentrations. Improvement in the previous mentioned parameters may be attributed to the protective effect of high dietary calcium in decreasing intestinal lead absorption and toxicity (Mahaffey, 1981 and Miller et al., 1990) and reducing lead accumulation in blood, liver and kidney (Bogden et al., 1995 and Moussa et al., 2001).

In conclusion, the results of the current study suggested that dietary calcium supplementation can reduce lead toxicity in pregnant rats and improve its teratogenic effects on their feti. However, dietary calcium did not improve fetal body weights and lengths recommending more studies in using calcium supplements for treatment of lead toxicity during pregnancy.

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تأثير التغذية بالكالسيوم على تسمم أمهات الفنر وتشوهات أجنتها بالرصاص

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يعتبر الرصاص من ملوثات البيئة الضارة بالصحة محدثاً كبيراً واسع المدى في الوظائف الفسيولوجية والحيوية وال تعرض المستمر للرصاص أو أملاحه أثناء فترة تخلق الأجنة يؤدي إلى تأثيرات سامة من ضمنها تشوهات الأجنة و تسممها و الأجهاض. أظهرت الدراسات الخاصة بأيض الرصاص وسميته على أنه توجد علاقة تفاعلية بين الرصاص والكالسيوم. وحيث أن الوجبات التي تحتوي على كميات ضئيلة من الكالسيوم يمكن أن تزيد من أمتصاص الأمعاء للرصاص وذلك تزيد سهولة وقود تأثير هذه الدراسة بعد تأثير أطباء الرصاص بالكمبريك 72.8 مليمجرام/كجم وزن أو 31 مليمجرام/كم وزن منفرداً أو بالاضافة إلى الكالسيوم في العلبة تتركز 24% على الفنر الحامل وأเจنتها في فترة تخلق الأجنة (اليوم 15-7 من الحمل). وقد تم قياس وتقدر بعض القيم البيوكيميائية على مصل الأمهات الحامل وأظهرت نتائج التسمم بالرصاص أثناء فترة تخلق الأجنة نقصاً معنويًا في أوزان الأعضاء الداخلية (الأحشاء) والهيكل العظمي. وقد أظهرت النتائج بالرصاص (الجرعة الأعلى) زيادة في نشاط الأستاتين أمينوتريفسيريز والكولينبروزول والدهون الثلاثية والبوريا والكرياتينين ومضخ الزورنيك في حين مستويات خيارة الفوسفاتي القلى والأسبارتين أمينوتريفسيريز لم يظهر أي تغير مقارنة بالمجموعة الضابطة.

وقد أحدثت نتائج تعاطي الكالسيوم مع الرصاص نقصاً معنويًا في نسبة حدوث التشوهات في الأجنة (الإحشاء والهيكول العظمي) ونقصاً أيضاً في تركز مستويات وظائف الكبد والكلى في الأمهات و على النقيض من هذا فقد أظهرت نتائج أجنت الأجنة الأمهات الأخرى تأثير إيجابي على تقليل سمية الرصاص وتشوهات أجنتها.

ويعتبر من هذه الدراسة أن الرصاص له أثر خطرة على أمهات الفنر الحامل و على أجنتها وأن هذه الآثار الخطرة يمكن الحد منها بواسطة أضافة الكالسيوم للعالية.

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