Effect of lead toxicity on mineral metabolism and immunological factors in rats

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**Key words:** lead acetate, mineral, immunity, turmeric

**ABSTRACT:**

This study was carried on 40 male albino rats to evaluate the toxic effects of Lead on minerals and immunological factors. The rats were divided into four equal groups. Group I kept as control group, group II was fed 1.0% Turmeric powder for 11-week, group III was injected (i.p) with 8 mg/kg bwt lead acetate trihydrate, (from 3rd to 11th week), given 4 days a week, rats in group IV were fed Turmeric 1% before and along with Lead at the same previous dose. Blood samples were collected for biochemical analysis at the end of experiment. The results revealed that a significant increase in serum Ca, Ph, Cu, Fe, uric acid and creatinine levels following Lead toxicity. Meanwhile, serum Zn and IgG levels and AST enzymatic activities were significantly decreased. On the other hand, serum IgM and ALT levels were nonsignificantly decreased in lead group. Combined treatment of lead exposed animals with Turmeric powder had improved immunity without any protective effect against lead toxicity on mineral and kidney functions. The results indicate that administration of lead acetate caused alterations in minerals, immunological factors, liver and kidney functions and Turmeric powder fails to normalize these factors.

1. **INTRODUCTION**

Lead is one of the major environmental pollutants (toxin) in the modern world whose higher concentrations particularly in industrial zone; adversely affect the vitality and production performance of domestic animals (Kwatra et al., 1986). Lead usually induce adverse effects on the central nervous system as irritating, immunosuppressive, genotoxic, teratogenic, nephrotoxic and other toxic effects on the haematopoietic system. Lead is also known to modify the metabolism of trace elements and nutrients (Levander, 1979). Also, lead administration decreased liver copper level whereas additional dietary copper increased the liver lead level (Bafundo et al., 1984). It was postulated that lead interferes with copper and iron metabolism (Klauder and petering., 1977).

Turmeric is the rhizome of Curcuma longa had been widely used as a spice and coloring agent in many foods, it had been used as medicinal plant for
treatment of atherosclerosis, anemia, hemorrhoids, hepatitis, hysteria, indigestion, inflammation, skin diseases, urinary diseases, wound, bruise healing, psoriasis and anorexia (Ishita et al., 2004). Curcumin from turmeric ameliorate oxidative stress and it is considered a potent antioxidant inhibitor of lipid peroxidation than other flavonoids, which have a single phenolic hydroxyl group (Eybl et al., 2006; Phan et al., 2001). The effective antioxidant property of curcumin by inhibition of the utilization of vitamins C and E in the liver, thus maintaining their levels (Rukkumani et al., 2003). Also It has been used as an antioxidant in toxicity studies of several metals including cadmium (Daniel et al., 2004), copper (Nair et al., 2005), iron (Manjunatha and Srinivasan, 2006), lead (Dairam et al., 2007) and selenium (Padmaja and Raju, 2004).

The aim of the present study is to show the effect of lead on liver and kidney functions, mineral status and immunological factors in rats and investigate the protective role of turmeric against the adverse effects of lead toxicity.

2. Materials and METHODS

Plants: Turmeric Rhizomes were obtained from local market at Alexandria, Egypt and dried then minced to be fine powder. Chemicals: lead acetate trihydrate extra pure from Merck, Darmstadt Germany.

Animals and experimental design: Forty males albino rats aged three months, weighting (160-240 g), were purchased from the breeding unit of Faros University. The animals were housed in steel mesh cages and maintained for two weeks acclimatization period on basal diet consisted of (bread and corn) and drinking water ad libitum. The housing cycle was 12:12 hr light –dark cycle. Then rats were divided into four groups (10 rats each) as the following: group I- control group were fed basal diet for 11 week, group II-turmeric administrated group received 1% turmeric powder mixed with basal diet for 11week, group III-lead intoxicated group were i.p injected with with 8 mg lead acetate trihydrate/kg bwt, (from 3rd to11th week) for 4 days a week for 9 weeks, group IV- turmeric protected group were fed turmeric powder before lead intoxication with the same previously mentioned doses.

Biochemical methods: at the end of experiment, rat were sacrificed, blood sample was collected and centrifugated at 3000 rpm for 10 minutes in room temperature; the serum was separated and kept in clean stopper glass vial at -20 °C unit assay. Serum was subjected to the following parameters; serum Calcium (Schmidt et al.,1997) phosphorous (Goldenberg and Fernandez, 1966), zinc (Johnsen and R.Eliasson, (1987), copper (Abe et al.,1989), iron (Ramsay, 1957), IgG and IgM (Narayanan, 1982; Price et al., 1983), urea and uric acid (Rock et al., 1987), creatinine (Henry, 1984), ALT and AST(Young, 1990).

Statistical analysis: by one way, ANOVA according to SAS, (1996).

3. Results

Table (1) illustrated that serum calcium was significantly decreased at (P<0.01) in turmeric group as compared to control group. On the other hand, serum calcium, phosphorous, iron, copper were significantly increased at (P<0.01) in lead group and protected group as compared to control group.

However, serum zinc was significantly decreased at (P<0.01) in lead group and protected group as compared to control group.
Lead is one of the major environmental pollutants (toxin) in the modern world whose higher concentrations particularly in industrial zone; adversely affect the vitality and production performance of domestic animals (Kwatra et al., 1986). Lead usually induce adverse effects on the central nervous system as irritating, immunosuppressive, genotoxic, teratogenic, nephrotoxic and other toxic effects on the haematopoietic system. Lead is also known to modify the metabolism of trace elements and nutrients (Levander, 1979). Also, lead administration decreased liver copper level whereas additional dietary copper increased the liver lead level (Bafundo et al., 1984). It was postulated that lead interferes with copper and iron metabolism (Klauder and Petering, 1977).

Turmeric is the rhizome of Curcuma longa had been widely used as a spice and coloring agent in many foods, it had been used as medicinal plant for treatment of athersclerosis, anemia, hemorrhoids, hepatitis, hysteria, indigestion, inflammation, skin diseases, urinary diseases, wound, bruise healing, psoriasis and anorexia (Ishita et al., 2004). Curcumin from turmeric ameliorate oxidative stress and it is considered a potent antioxidant inhibitor of lipid peroxidation than other flavonoids, which have a single phenolic hydroxyl group (Eybl et al., 2006; Phan et al., 2001). The effective antioxidant property of curcumin by inhibition of the utilization of vitamins C and E in the liver, thus maintaining their levels (Rukkumani et al., 2003). Also it has been used as an antioxidant in toxicity studies of several metals including cadmium (Daniel et al., 2004), copper (Nair et al., 2005), iron (Manjunatha and Srinivasan, 2006), lead (Dairam et al., 2007) and selenium (Padmaja and Raju, 2004).

The aim of the present study is to show the effect of lead on liver and kidney functions, mineral status and immunological factors in rats and investigate the protective role of turmeric against the adverse effects of lead toxicity. Table (2) illustrated that serum AST activity was significantly decreased at (p<0.01) in turmeric group, lead group and protected group respectively as compared to control group. Meanwhile, serum ALT activity was not significantly changed in lead group and turmeric group as compared to control at p<0.01. Serum urea was not changed in the four groups. Serum uric acid and creatinine were significantly increased at (p<0.01) in lead group and protected group respectively as compared to control group.

### Table (1): The mean values of serum Calcium, phosphorus, iron, copper and zinc in Lead and Turmeric administrated groups

<table>
<thead>
<tr>
<th>Item</th>
<th>Control group</th>
<th>Turmeric Group</th>
<th>Lead group</th>
<th>Lead+ Turmeric</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean± S.E</td>
<td>Mean± S.E</td>
<td>Mean± S.E</td>
<td>Mean± S.E</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>6.77±0.05b</td>
<td>6.34±0.15c</td>
<td>7.57±0.12a</td>
<td>7.41±0.10a</td>
</tr>
<tr>
<td>Ph(mg/dl)</td>
<td>4.34±0.12b</td>
<td>4.32±0.09a</td>
<td>5.15±0.08a</td>
<td>5.27±0.14a</td>
</tr>
<tr>
<td>Fe(µg/dl)</td>
<td>1.33±0.04b</td>
<td>1.34±0.03a</td>
<td>3.09±0.15a</td>
<td>3.23±0.36a</td>
</tr>
<tr>
<td>Zn(µg/dl)</td>
<td>150.90±4.43a</td>
<td>147.2±2.7a</td>
<td>120.60±4.15b</td>
<td>112.60±3.16c</td>
</tr>
<tr>
<td>Cu(µg/dl)</td>
<td>120.10±1.12c</td>
<td>126.68±0.93c</td>
<td>140.40±1.12b</td>
<td>165.20±4.14c</td>
</tr>
</tbody>
</table>

Means within the same row of different letterssers are significantly different at (p<0.01). mean± S.E=mean± standard error.
Ca:calcium, Ph:phousporous, Fe: iron,Zn:zinc,Cu: copper.
Table (2): The mean values of serum AST, ALT, urea, uric acid and creatinine in Lead and Turmeric administrated groups.

<table>
<thead>
<tr>
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<th>Turmeric Group</th>
<th>Lead group</th>
<th>Lead+ Turmeric</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>152.00 ± 2.38a</td>
<td>127.60 ± 9.74b</td>
<td>106.60 ± 5.10c</td>
<td>108.00 ± 1.94c</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>59.60 ± 3.52ab</td>
<td>62.00 ± 7.28a</td>
<td>56.00 ± 3.64b</td>
<td>57.00 ± 4.52b</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>31.60 ± 0.25a</td>
<td>32.40 ± 0.24a</td>
<td>33.20 ± 0.37a</td>
<td>33.00 ± 0.64a</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>1.34 ± 0.11c</td>
<td>2.62 ± 0.22b</td>
<td>7.36 ± 0.36a</td>
<td>8.14 ± 0.45a</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.60 ± 0.02b</td>
<td>0.63 ± 0.03b</td>
<td>0.75 ± 0.04a</td>
<td>0.76 ± 0.02a</td>
</tr>
</tbody>
</table>

Means within row of different letters are significantly different at (p<0.01). mean± S.E=mean± standard error.
AST :Aspartate transaminase, ALT: Alanine transaminase.

Table (3): The mean values of serum IgG, IgM in Lead and Turmeric administrated groups.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control group</th>
<th>Turmeric group</th>
<th>Lead</th>
<th>Lead+ Turmeric</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (mg/dl)</td>
<td>801.00 ±44.23a</td>
<td>720.80 ±49.71b</td>
<td>421.80 ±14.12a</td>
<td>536.60 ±73.14c</td>
</tr>
<tr>
<td>IgM (mg/dl)</td>
<td>37.80 ±1.20b</td>
<td>33.80 ±0.51b</td>
<td>33.20 ±1.20a</td>
<td>77.80 ±3.12a</td>
</tr>
</tbody>
</table>

Mean within the same row of different letters are significantly different at (p<0.01). mean± S.E=mean± standard error.
IgG: immunoglobulin G, IgM: immunoglobulin M.

4. DISCUSSION
Lead is cumulative poisons reduces major organ functions, increasing health hazard including nephrotoxicity, hypertension, gastrointestinal and neurological dysfunction (Lokith, 1993). Lead is more toxic to newly born animals and human (Gallhom et al., 2000). Also, lead alters metaphyseal and growth plate morphology of bone. It bind to growth plate cartilage matrix sites normally associated with calcium and phosphorous in lead exposed animals (Hamilton and Ofatherty.,1995). when Lead was injected subcutaneously in soft tissues will precipitate with calcium and phosphorous to form lead hydroxyappetite when it was injected subcutaneously in soft tissues (McClure, 1980).

In this study, serum calcium and phosphorous levels in male rats intoxicated group showed a significant increase compared with control rats, which is similar to the recorded data of Yamaguchi and Yamamoto (1974) who reported that serum calcium concentration was significantly increased by administration of lead. This was attributed to the liberation of calcium from bone. Missoun et al. (2010) recorded that of calcium and phosphorus increase in serum of rats administered with lead acetate in drinking water for 8 week. This may be due to impairment of renal function or inhibitory action of lead on cation transport in tissues of rats. In addition, lead has direct effect on osteoblast function including inhibition of active vitamin D3 stimulated synthesis of...
ostecalcin, a major noncollagen constituent of bone important mineralization (Ronis, 2001). This is confirmed due to lead had direct effects on local regulation of bone cell function via interference with calcium homeostasis and calcium regulated secondary messenger system via disruption of cAMP signals (Pouds et al., 1991).

However, the present data is in contrary to the results of Hamilton and Ofatherine (1995) who stated that serum calcium and phosphorous were not altered by lead in drinking water suggesting that lead did not affect plaque mineralization through a reduction of serum calcium and phosphorous. In this respect Anetor et al. (2005) showed a significant hypocalcaemia in lead exposed groups. Hypocalcaemia reflects perturbation of calcium metabolism.

The finding regarding phosphate level was consistent with that of Papaionnu et al., (1978) who found significant increase of phosphate level in lead exposed workers. Lead is known to interfere with cell membrane and may also increase cell breakdown (Choice and Richter, 1972). The increase in phosphate in this study may be due to cell membrane damage as a result of exposure to lead. An increase in serum inorganic phosphorus was detected in bucks exposed to 8 mg lead acetate/kg b. wt. for 4 months (Desouky et al., 2001). In contrast to our results, unchangeable inorganic phosphorous were noticed in rabbits exposed to oral dose of lead for long periods (Walid, 1997).

In this study the recorded increase in serum copper level was agreed with (Kasperczyk et al., 2012) who found that plasma Cu level was significantly higher compared with the control group and correlated positively with lead concentrations. The results were explained by (Kasperczyk et al., 2004) who showed that lead exposure was associated with an elevated activity of superoxide dismutase isoenzyme that contains Cu and Zn (CuZn-SOD) in both serum and erythrocytes. Therefore, an increase in the Cu level, which was observed in the present study may be caused by increased CuZn-SOD activity. This enzyme is part of the antioxidant defence system and its activity may be elevated because of lead induced oxidative stress (Kasperczyk et al., 2005). The increase in plasma Cu levels may also be caused by competitive displacement of the metal from tissues by lead ions. Moreover, lead and Cu compet for binding sites on proteins, such as the ATPase complex (Qian et al., 2005), also the increased bioavailability of displaced Cu may induce ROS generation via the Fenton reaction and contribute to oxidative stress enhancement.

In the present study, there was significant decrease in serum zinc level in lead group. Similar results had been reported by Dioka et al., (2004) who observed that the blood Zn level decreased by 34% in artisans who were occupationally exposed to lead. The decrease of zinc concentration after lead exposure may be due to the imbalance of metabolism produced by impairing zinc status in zinc- dependent enzyme which is necessary for many metabolic processes (Nabil, 2012). The decrease in zinc level may be attributed to 1) Hypoalbuminemia where most of plasma zinc is protein bound (Victery et al., 1981), 2) competition of lead with essential elements such as zinc (Klassan, 1991), 3) stimulation of urinary excretion of zinc and interfering with its reabsorption in kidney (Morawiec, 1991) and 4) inhibition of G.I. absorption of zinc due to lead –zinc interaction at the molecular level in the G.I.T. (Victery et al., 1987). More over exposure to Pb can decrease the absorption rate and biologic availability of Zn in the body, mainly because of their competition for binding to the sulfhydryl (-SH) group site(s) in various enzymes, other proteins (especially metallothionein (MT)), and tissues (Telisman, 1995).

There was significant increase in serum iron level in this study, whenever anemia caused on account of lead
poisoning can be hemolytic anemia (Vij, 2009). Progressive destruction of RBCs and increasing fragility of RBCs membrane may be a cause of increasing iron level. Lead directly affects the hematopoietic system through restraining the synthesis of hemoglobin by inhibiting various key enzymes involved in the heme synthesis pathway. It also reduces the life span of circulating erythrocytes by increasing the fragility of cell membranes. The combined aftermath of these two processes leads to anemia (Guidotti et al., 2008; Cornelis, 2005).

Aminotransferases are the most frequently used and most specific indicators of hepatic injury and represent marker of hepatocellular necrosis (Rosen, 2001). Our finding showed a significant reduction in serum AST and non significant decrease in serum ALT in lead exposed group. Absolute levels of Aminotransferases correlate poorly with the severity or extent of hepatocellular damage and do not provide reliable prognostic information. Conversely, patients with a “burnt out” cirrhotic liver may have misleadingly low AST and ALT levels (Michael and Aijaz, 2009). Our results were similar to that reported by Singh et al., (1994) who mentioned that oral administration of lead acetate to rats decreased significantly the activities of ALP, AST and ALT after 4 months of treatment.

The significantly reduced activities of AST and ALT under the influence of lead could be explained by a possible inhibition of the synthesis of the indicated enzymes under the influence of this toxic metal (Solliqvä, 1996). A possible explanation for such differing results is the quite different in view of the experimental design and the applied doses of lead, length of exposition, the way how lead got into the organism and they also differed in that some of them were carried out on humans and others on different animal species Singh et al., (1994). Similar results was obtained by Karimi et al., (2012) who reported that AST and ALT activities and AST/ALT ratio decreased in Pb group compared to normal control group in broilers were given 400 ppm lead acetate in drinking water for 42 days. Whereas serum AST, ALT activities were normal in family manufacturing lead acid batteries (Raviraja et al., 2008). On the other hand, the present results is disagreed by the results of Khan et al., (2008) who reported that the activities of serum AST and ALT were significantly increased in lead exposed rats. Activities of ALT, AST and ALP were significantly increased in rats given daily lead acetate in diet as 500 mg/kg after 2, 4 and 6 weeks of treatment (Shalan et al., 2005; Dioka et al., (2004); Othman et al., 2004; Al-Wabel et al., 2007).

In the present study, there was a significant increase in serum uric acid and creatinine, whereas there was none significant increase in serum urea in lead exposed group. Creatinine is the breakdown product of creatine, which is an important part of the muscle. The test is performed to evaluate the kidney function. Serum/plasma creatinine is a more sensitive indicator of renal function than the blood urea nitrogen (June and Juanita, 2004). If the kidney function is abnormal, the creatinine level will increase in the blood, due to decreased excretion of creatinine in the urine (Nissi and Terra, 2004; Hecht, 2006). The presence of the increased level creatinine concentration in the blood suggests the inability of the kidney to excrete this product (Overu et al., 2004). The elevation in the serum of creatinine caused by lead suggest that renal function impairment which might result from intrinsic renal lesions, decreased perfusion of the kidney, obstruction of lower urinary tract or due to deranged metabolic process caused by this metal (Cameron and Greger, 1998).

The present results have been supported by Khalil-Manesh et al. (1992) and Abd El Rahiem et al. (2007) who mentioned that lead acetate increased serum creatinine level as compared to the control group.. Similar results have been reported by many researchers (Abdel-Razik et al., 2007; Khalil-Manesh et al., 1992).
increase in uric acid and nonsignificant increase in serum urea were in agreement with Dioka et al. (2004), who mentioned that exposure of human subjects to lead in petrol increased the concentrations of uric acid and cause nonsignificant increase in serum urea as compared to unexposed subjects.

In the present study, there was a significant decrease in serum IgG, whereas serum IgM not changed in lead exposed group. These results were in agreement with that obtained by Luster et al., (1978) who reported that chronic pre- and postnatal exposure of rats to low levels of lead resulted in a marked depression in the antibody response to sheep RBC as well as decreased serum IgG levels. Furthermore, Ayatollahi, (2002) found lower IgG levels but IgA and IgM were not affected in low-level lead-exposed individuals.

Also, a study by Undeger et al., (1996) in non-immunized lead workers and controls showed a 29% decrease in serum IgG and a 34% decrease in serum IgM.

Lead exposure resulted in oxidative stress, and oxidative stress may profoundly affect the immune function (Bajer-Bitterlich et al., 1997); it was logical to hypothesize that oxidative stress play a significant role in lead-induced immunosuppression. The mechanisms of immunotoxicity observed may be due to exposure to Pb, had been shown to be correlated to oxidative DNA damage (Schilderman et al., 1997).

5. CONCLUSION

In conclusions our results revealed that exposure to lead toxicity leads to impairment of kidney and liver functions and cause immune depression. Using of turmeric powder in diet fail to improve these functions.

6. REFERENCES


Missoun, F., Slimani, M., and Aoues, A.2010. Toxic effect of lead on kidney


Singh, B., Dhwan, D., Nehru, B., Garg, M.L., Mangal, P.C., Chand, B., Thran, P.N. 1994. Impact of lead pollution on the status of other trace metals in blood and...
Young, D.S. 1990. Effect of drugs on clinical laboratory tests.