Evaluated the lead levels at boiling water in clay pots and impact of the lead contaminated diet on nutritional, biochemical status of male rats

Mona S. El Kutry* and El-Sayed A. El-Sahar

Abstract

Background: For centuries, people have been using clay or earthen pots for cooking. Also, many studies indicated a contrariety from safety or danger of it. Our study aims to assess the lead concentration in boiling water in clay pots. Contaminated the diet with lead acetate and evaluated the nutritional, biochemical statuses, and histological studies for male albino rats.

Step A: Ten samples of the distilled water and/or tap water were boiled in the clay pots (glaze and/or unglazed). Then, it was left to cool for either 2 or 4 h.

Step B: Thirty male albino rats divided three groups as follows: group 1: fed on basal diet (negative control), group 2: fed on basal diet + 466.5 mg/kg body wt of lead acetate (L1), group 3: fed on the basal diet + 933 mg/kg body wt of lead acetate (L2).

Results: Lead concentrations in distilled water samples boiled in glazed clay pots were significantly higher than the negative control. Tap water samples boiled in glazed clay pots showed lead concentrations that were significantly higher than that of the positive control. Whereas, lead concentrations in distilled water boiled in the unglazed clay pots and left to cool for either 2 h or 4 h showed no significant differences compared to the negative control. Besides, rats fed L1 and L2 of lead acetate had a significant decrease in BWG and food intake compared with the negative control group. Also, rats were given lead acetate at the two levels (L1 and L2) had significantly lower levels of hemoglobin, RBCs, and WBCs compared with rats fed basic diet only (negative control). Data illustrated that the rats of groups 2 and 3 have increased significantly in GOT concentration of serum, a significant increase in cholesterol and triglycerides levels, and increased significantly in creatinine, urea, and lead concentration in serum compared with the (negative control).

Conclusion: Bring the clay pots for cooking would be unglazed and natural forming, even if glazed may be having certified a lead free.

Keywords: Lead acetate, Clay glazed, Clay unglazed, Water, Liver, Kidney function
1 Background
For centuries, we have been using clay or earthen pots (glazed and unglazed) for cooking. Food cooked in clay pots will remain moist and hot, enabling cooking with lesser amounts of liquid and fat than usual. In addition, it has been reported that food cooked in clay pots has a significantly lower load of pathogenic microorganisms than that cooked in aluminum pots [1]. One concern that people have about cooking in ceramic pots is that a glaze may contain lead and/or cadmium, which could leach into foods if the glaze is improperly formulated, applied, or fired [1]. All pottery sold in the USA for cooking must be tested and certified as safe by the US Food and Drug Administration [2].

Lead (Pb) is one of the widespread environmental pollutants that induce a broad range of physiological and biochemical dysfunctions in animals [3]. Lead exposure, even at low concentrations, is toxic to animals and humans [4]. Lead concentration of 5 μg/L in drinking water may lead to a total intake of lead ranging from 3.8 μg/day in an infant up to 10 μg/day in an adult [5]. Lead toxicity can be associated with gastrointestinal disturbances such as nausea, vomiting, diarrhea, and in severe cases coma and death [5]. Chronic lead exposure can adversely affect hemoglobin formation and cause anemia. In addition, it may interfere with calcium and vitamin D metabolism, and delay nervous system development causing mental retardation [6]. Lead is absorbed through the gastrointestinal tract. Around 70 to 90% of lead goes into the bones, kidneys, and liver, with subsequent organ dysfunction [7, 8].

Therefore, the aim of the current study was to assess lead concentration in clay pots’ boiling water and to evaluate the nutritional, biochemical, and histopathological effects of lead acetate exposure in male albino rats.

2 Methods
2.1 Step A
Both clay pots (natural clay, non-pigmented) and ceramic pots (clay covered with a glaze layer) were purchased from the local Egyptian market (Figs. 1 and 2). Water was boiled in both glazed and unglazed clay pots. Then, it was left to cool for either 2 or 4 h. Water samples (Sp) were classified as follows:
Sp 1: distilled water (negative control); Sp 2: tap water (positive control); Sp 3: distilled water boiled in an unglazed clay pot and left to cool for 2 h; Sp 4: distilled water boiled in an unglazed clay pot and left to cool for 4 h; Sp 5: distilled water boiled in glazed clay pot and left to cool for 2 h; Sp 6: distilled water boiled in glazed clay pot and left to cool for 4 h; Sp 7: tap water boiled in an unglazed clay pot and left to cool for 2 h; Sp 8: tap water boiled in an unglazed clay pot and left to cool for 4 h; Sp 9: tap water boiled in glazed clay pot and left to cool for 2 h; and Sp 10: tap water boiled in glazed clay pot and left to cool for 4 h.

2.1.1 Collection of water samples
Tap water was collected in clean, 6-L capacity plastic containers from different three locations of Cairo, Egypt. The taps were pliable to run for at least 5 min before filling. Each sample was collected three times during a period of about 5 h for each location.

2.1.2 Samples preparation and analysis
All pots were properly washed several times using distilled water before starting the experiment. Either distilled water or tap water was boiled in the pots. Water was left to cool for 2 h or 4 h. Then, it was collected carefully in polythene bottles (250 mL), properly labeled, and sent for analysis. The ten samples were taken to the Desert Research Center, Cairo, Egypt for chemical analysis according to the Association of Official Analytical Chemists (AOAC) [9].
2.2 Step B
Lead acetate Pb(C₂H₃O₂)₃, was purchased from Sigma Company, Cairo, Egypt. Levels of exposure were set at 466.5 mg/kg body weight (wt) (L₁), while high level (L₂) of lead acetate (Pb) equals 933.0 mg/kg body wt according to Subranamoorthy [10] and Nicholas and cheremisinoff [11].

Basal diet was formulated from the natural ingredients according to the National Research Council [12] with some modification according to Reeves et al. [13].

2.2.1 Animals
Thirty male albino rats weighing 118 ± 5 g were obtained from the National Research Center, Cairo, Egypt. All animals were offered food and water ad libitum throughout the experimental period. The rats were divided into three groups (10 rats each) as follows:

Group 1: fed on basal diet (negative control)
Group 2: fed on basal diet+466.5 mg/kg body wt of lead acetate (L₁)
Group 3: fed on basal diet+933.0 mg/kg body wt of lead acetate (L₂)

During the experimental period (6 weeks) animals were inspected daily, and food intake and body wt were recorded twice/week. At the end of the experiment, the overnight-fasted rats were anesthetized, and blood samples were taken for biochemical analysis. Then, animals were sacrificed by cervical dislocation, with their livers and kidneys removed and weighed for determination of relative organ weight (organ/100 g live animal BW) and were prepared for histopathological examination. Rats in group 3 were found to have urinary bladder stones, and they were analyzed in a private medical laboratory; morphological characteristics were represented in results section. Food efficiency ratio (FER) was determined according to Chapman et al. [14].

2.2.2 Biochemical analysis
Estimation of serum total protein and albumin were carried out as described by Henry et al. [15] and Webster et al. [16], respectively. Globulin was calculated from the difference between total soluble protein and albumin concentrations. In addition, we assessed serum levels of glucose, glutamic oxaloacetic transaminase (GOT) and serum glutamic pyruvic transaminase (GPT), creatinine, urea, triglycerides, and cholesterol according to Tietz [17], Stanley and Sam [18], Henry and Canon [19], Patton and Crouch [20], Fossati and Principe [21], and Thomas [22], respectively. We determined hemoglobin (HB) concentration as described by Schalm [23] and erythrocyte count according to Wintrobe [24]. Serum lead concentration was estimated according to AOAC [9]. Livers and kidneys were dissected and prepared for histopathological examinations according to Drury and Wallington [25].

2.2.3 Statistical analysis
Water sample was analyzed using T test according Excel 2010. However, blood sample was analyzed using ANOVA according to computer SPSS, 2015.

3 Results
3.1 Step one result
Lead concentrations in distilled water boiled in the unglazed clay pots and left to cool for either 2 h (Sp 3) or 4 h (Sp 4) showed no significant differences compared to the negative control (Sp 1). Whereas lead concentrations in distilled water samples boiled in glazed clay pots (Sp 5 and Sp 6) were significantly (p < 0.05) higher (26.00 μg/L and 36.00 μg/L, respectively) than that of the negative control (Sp 1) as shown in Table 1.

Table 2 shows that lead concentrations in tap water (Sp 2) was 13.00 μg/L. Its concentrations in boiling tap

| Parameter | Distilled water (Sp 1) (negative control) (Pb μg/L) | Clay pot natural (unglazed) (Pb μg/L) | Clay pot glazed (Pb μg/L) |
|-----------|---------------------------------------------------|--------------------------------------|---------------------------|
| Groups    | < DL                                              | After 2 h (Sp 3)                      | After 4 h (Sp 4)           |
|           | < DL                                              | After 2 h (Sp 5)                      | After 4 h (Sp 6)           |
|           | 13.00 ± 0.5*                                     | 16.00 ± 0.3*                         | 36.00 ± 0.8*               |

All data represented as mean ± SD
*p < 0.01
doses (L1 and L2) had significantly lower levels of hemoglobin, RBCs, and WBCs compared with negative control. The report of the urinary calculus analysis shows that the stone was one large, oval-shaped, appearing in light color, with dimensions of 2 × 1.5 cm, and a weight of 1.5 g. Its chemical composition was calcium phosphate.

3.3 Histopathology results
Figure 4 a represents a liver section from a control rat showing the normal liver structure, with a central vein and negative control. Concerning the percentage of neutrophils did not significantly differ between the three groups.

Table 6 shows that rats given lead (L1 and L2) had significant (p < 0.05) reduction in the levels of total protein, albumin, globulin, and glucose compared with negative control rats.

Data in Table 6 illustrate the blood cholesterol and triglycerides levels. Results indicated that the animals of the group fed on Pb at L1 had a significant increase in cholesterol and triglycerides levels (p < 0.01) with a mean value of 93.0 ± 0.1 and 74.4 ± 0.7 mg/dL, compared to the negative control 85.4 ± 0.3 and 42.2 ± 0.6 mg/dL, respectively. And vice versa, results of blood cholesterol and triglycerides levels of the group fed on Pb at L2 had shown decreased significantly (p < 0.01) compared with other groups.

Table 7 report that rats fed the basal diet plus lead acetate at both low and high levels (L1 and L2) had significantly (p < 0.01) increased creatinine and uric acid concentrations compared to the negative control group. Similarly, lead-fed rats (L1 and L2) had significantly (p < 0.001) increased GOT serum concentrations with mean values of 14.6 ± 1.2 and 11.9 ± 1.2 U/L, respectively, compared to the negative control (5.80 ± 0.7 U/L). GPT serum concentrations were significantly (p < 0.05) lower in lead-fed animals (L1 and L2) with mean values of 11.7 ± 0.9 and 10.7 ± 1.7 U/L, respectively, compared to the negative control animals (12.8 ± 0.5 U/L).

Animals given lead acetate that was added to the basal diet (L1 and L2) had significantly (p < 0.001) increased serum lead concentrations at level one and two with mean values of 21.25 ± 0.8 μg/100 cm³ and 25.64 ± 1.3 μg/100 cm³, respectively, compared with rats given only basal diet (Fig. 3).

The report of the urinary calculus analysis shows that the stone was one large, oval-shaped, appearing in light color, with dimensions of 2 × 1.5 cm, and a weight of 1.5 g. Its chemical composition was calcium phosphate.

### Table 3
The impact contaminated diet with lead to body weight gain (BWG), food intake (FI), and food efficiency ratio (FER) of rats for 6 weeks

| Groups/parameters | BWG (g) | FI (g) | FER (%) |
|-------------------|---------|--------|---------|
| Group 1 negative control | 103.0 ± 3.0 | 123 ± 0.9 | 7 ± 1.8 |
| Group 2 L1Pb | 76.0 ± 3.0** | 89.3 ± 0.5* | 8 ± 1.3 |
| Group 3 L2Pb | 55.0 ± 7.0** | 7.75 ± 0.5* | 8 ± 2.0 |

*p value | p < 0.01 | p < 0.05 | No sign.

All data represented as mean ± SD

### Table 4
Effect contaminated diet with lead acetate on organs (liver, kidney, brain, spleen, testes, and heart) relative weight of rats

| Parameters | Liver (g) | Kidney (g) | Brain (g) | Spleen (g) | Testis (g) | Heart (g) |
|------------|-----------|------------|-----------|------------|------------|-----------|
| Group 1 negative control | 3.30 ± 0.24 | 0.74 ± 0.04 | 0.87 ± 0.07 | 0.41 ± 0.04 | 1.46 ± 0.27 | 0.46 ± 0.05 |
| Group 2 L1Pb | 3.53 ± 0.25* | 0.88 ± 0.08 | 0.74 ± 0.11 | 0.45 ± 0.06* | 1.84 ± 0.17* | 0.38 ± 0.03* |
| Group 2 L2Pb | 3.90 ± 0.28* | 0.84 ± 0.12 | 0.87 ± 0.05 | 0.64 ± 0.16* | 2.03 ± 0.33* | 0.36 ± 0.03* |

*p value | p < 0.05 | No sign. | No sign. | p < 0.05 | p < 0.05 | p < 0.05 |

All data represented as mean ± SD

L1Pb = 466.5 mg/kg wt while L2Pb = 933 mg/kg wt

* p < 0.05
Liver sections from rats given lead at a low dose show portal tracts with dilated, congested veins (↗), periportal necrosis of hepatocytes surrounding the portal area (►) as well as inflammatory infiltration (⇑) (Fig. 4b) (H & E stain, X 150).

Liver sections from rats administered high-dose lead show portal tracts and periportal spaces (↑), periportal necrosis of hepatocytes that surround the portal area (►), and some nuclei are pyknotic (►) and inflammatory infiltration (⇑) (Fig. 4c) (H & E stain, X 300).

Figure 5a shows a section in a control rat kidney with normal structure (H & E stain, X 300).

Kidney sections from rats fed lower doses of lead show renal corpuscle congestion and hypercellular (↑). Observed the highly ale generated tubules (►). Also, notice the shrinkage of an anther one associated with wide urinary space (↓) (H & E stain, X 300).

Figure 5c represents a kidney section from rats fed higher doses of lead. It shows hemorrhagic areas in the interstitium (↑) and inflammatory infiltration beside the congested renal corpuscle (►). The notice shows the denigration of the renal tubules (#) (H & E stain, X 300).

4 Discussion

From ancient to recent century, people were used to cooking in clay pots. Our study investigated the lead concentration in the water was boiled in two kinds of clay pots (glazed and unglazed). The best kind of clay for cooking a clay pot were unglazed as natural form (Fig. 1). Whether the glazed layer in the local market is contaminated with lead is reported by the FDA [2].

Lead may be present in the glazes or decorations covering the surface of some traditional pottery. If the pottery is not manufactured properly, this lead can leach into food and drink that is prepared, stored, or served in the dishes. Loading food of Pb differs according to the cooking method and the acidity of food and time.

Our results show that lead concentrations in distilled water boiled in glazed clay pots were 26.00 and 36.00 μg/L at 2 h and 4 h, respectively, which are significantly higher than its concentrations in the negative control water sample. These results explain the distilled water contaminated by lead carried out from the glazed layer of a clay pot. A previous case was a report of a woman presenting to the hospital with nonspecific chronic abdominal pain and unexplained anemia. She was suspected of having lead toxicity, and hence blood lead level was ordered. The lead concentration in her blood was markedly elevated. Her son had also an elevated blood lead level. Both the patient and her son had substantial lead exposure as they were using ceramic pots and mugs, whose glaze covering was found to have 17%, the lead by weight [26].

Diaz-Ruiz et al. [27] used clay containers to prepare and store lemonade, which was supplied as drinking water to pregnant rats throughout the gestational period. They found that these containers leached about 200 μg/
Lead. Moreover, pregnant rats had elevated lead levels in their blood (2.5 μg/dL). Examination of the neonates’ brain revealed increased lead content in the hippocampus and cerebellum.

Our results indicated that distilled water boiled and stored for 2 h and 4 h in unglazed clay pots had lead concentrations less than the detectable limits. Tap water that was prepared in unglazed clay pots had the same level of lead found in tap water with no significant difference between 2 and 4 h. The advert results of tap water were prepared in a clay pot (glazed), resulting to a significant increase in lead concentration of about 36.00 and 52.00 μg/dL through 2 h and 4 h, respectively. Anyway, the tolerable admission level of lead in a review of the latest scientific evidence was 25 μg/kg BW per week [5].

Lead is a far-reaching and non-biodegradable poison that is critically dangerous to human beings, with several sources for exposure. Therefore, our study results in Step B aimed to assess its toxicity. In the present study, rats fed L1 and L2 of lead acetate had a significant decrease in BWG and food intake compared with the negative control. This could be due to the toxicity induced on the gastro-intestinal tract as manifested by symptoms such as colic, diarrhea, nausea, and loss of appetite, which agreed with the findings of the WHO [28].

Ebrahimi et al. [29] reported that lead-induced oxidative stress adversely suppresses feed efficiency and growth performance in chicken. On the other hand, our data illustrated that the weights of the liver, spleen, and testes of rats given lead acetate at two levels were significantly (p < 0.05) higher compared with the negative control. This result is compatible with that of the liver enzymes. Where lead-fed rats (L1 and L2) had significantly (p < 0.001) increased GOT serum concentrations compared to the negative control, but GPT serum concentrations were significantly (p < 0.05) lower in lead-fed animals (L1 and L2) compared to the negative control animals. Furthermore, histopathological examination of liver sections from lead-fed rats (L1 and L2) showed portal tracts with dilated, congested veins, pericellular necrosis of hepatocytes, and cellular inflammatory infiltration.

On the other hand, the effect of toxicity induced by lead at the two levels of exposure on total organs relative weight (liver, kidney, brain, spleen, testes, and heart) varied depending upon the type of organ affected, adverse effects of lead toxicity, type of administration, and doses administered.

Our data illustrated that rats were given lead acetate at the two levels (L1 and L2) had significantly lower levels of hemoglobin, RBCs, and WBCs compared with rats fed the basic diet only (negative control). This agrees with Gargouri et al. [30] who reported that rats treated with lead at doses of 0.344 g/kg BW for 30 days had renal damage with significant increases in hematological parameters, oxidative stress-related parameters, creatinine, urea levels in plasma, and uric acid level in urine. Also,

![Fig. 3 The effect of adding lead at two levels in the diet on lead concentration in serum of rats](image-url)
the WHO [5] and Assi et al. [31] emphasized that chronic lead exposure commonly causes hematological effects, like anemia, or neurological disturbances, including headache, irritability, lethargy, convulsions, muscle weakness, ataxia, tremors, and paralysis.

In the current study, the differential count of white blood cells (lymphocytes, reticulocytes, and monocytes) as well as total protein, albumin, globulin, and glucose levels showed a significant reduction in rats fed either doses of lead acetate (L_1 and L_2) compared with the negative control rats. This goes hand in hand with Kim et al. [32] who found reduced plasma protein level with prolonged starvation in humans and malabsorption syndrome because of some intestinal diseases such as sprue liver disease that may result in depression of protein synthesis and enhancement of albumin loss in urine.

Our results indicated that animals fed low-dose lead had a significant ($p < 0.01$) increase in cholesterol and triglycerides levels with mean values of 93.0 ± 0.1 and 74.4 ± 0.7 mg/dL, compared to the negative control which had mean values of 85.4 ± 0.3 and 42.2 ± 0.6 mg/dL, respectively. On the other hand, blood cholesterol and triglycerides levels in animals fed high-dose lead showed a significant ($p < 0.01$) reduction compared with other groups. Our interpretation of these results is that rats in group 3 had the lowest BWG and FI compared with other groups. Our finding was compatible with Kasperczyk et al. [33] who reported no significant changes in the concentration of 7-ketocholesterol and blood lipids (cholesterol, HDL, LDL, triglycerides) in blood workers exposed to lead at level one (25–40 μg/dL) and level two (40 μg/dL).
In our study, rats fed the basal diet plus lead acetate at both low and high levels (L1 and L2) had significantly increased concentrations of creatinine and urea in serum compared to the negative control group. These results coincide with the histopathology results; however, an examination of sections from kidneys of rats fed low-dose lead (L1) showed renal corpuscle congestion and shrinkage of an anther one associated with wide urinary space and highly ale generated tubules. Kidney sections from rats fed high-dose lead (L2) showed hemorrhagic areas, inflammatory infiltration, and congested renal corpuscle as well as the denigration of the renal tubules. In addition, we found urinary stones in the bladder of a few rats that were fed a high level of lead (L2). Chemical analysis of these stones revealed that they were formed of calcium phosphates. This could be interpreted in light of our finding of high blood lead concentration in rats fed high-dose lead (L2) with a mean value of 25.64 ± 0.40 μg/100 cm³. These may result from unabsorbed calcium and vitamin D in the small intestine that caused their deposition in urine and formation of the stones. Previous studies had shown a strong association between lead exposure and renal effects [34, 35]. The hidden effects of childhood lead exposure include chronic advanced renal disease or reduction in renal function in adulthood. However, continued or repetitive exposures to lead can cause decreases in the estimated glomerular filtration rate and creatinine clearance, which may develop into chronic and often irreversible lead nephropathy [36]. Dongre et al. [7] reported that lead impaired normal kidney functions. Significantly decreased total calcium, phosphorus, vitamin D, and bone mineral density and significantly increased parathyroid hormone are markers of homeostasis of calcium and phosphorus metabolism.

Finally, there are many studies that reported the side effects and the huge problem associated with exposure to lead. Abdou and Hassan [39] and Missoun et al. [40] reported that lead acetate in drinking water for 8 weeks administered by the oral route caused a renal deficiency in the affected rats.

5 Conclusion
Bringing the clay pot for cooking would be unglazed and natural forming, even if glazed may have been certified to be lead free in order to decrease the side effect caused by lead.

Try to decrease the exposed sources of lead at dusk or food contaminated with lead, so they avoid the side effects of lead on body weight, food efficiency, anemia, and weakened immunity and change the biochemical parameters in blood.

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Authors’ contributions
MS prepared the samples, clay pots, and water analysis in stage one of the experiment and made nutritional and biochemical analysis studies in stage two as well as writing. ELG prepared animals in stage two, helped in feeding, and obtained organs for the histopathology results. The authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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