Ultrastructural effects of lead acetate on the spleen of rats

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Abstract: In this experiment, the ultrastructural effects of orally administered lead acetate on rat (Rattus rattus) spleen were studied. Rats weighing approximately 200–250 g were used in this experiment. They were divided into five groups, each one including five rats. Group I served as the normal control group while the others served as the experimental groups. The rats were exposed to oral administrations of lead acetate of 2.5 mg dissolved in 1 mL of drinking water per day. The administration lasted 96, 144, 336, and 432 h. The animals were anesthetized using chloroform inhalation and the peritoneum was stripped open and the spleen was removed and prepared for histological observation using a staining technique. Hypocellular white pulp, enlargement of venous sinusoids, clustering of heterochromatin in nucleus, vacuolation in the cytoplasm, swelling of mitochondria, and the distortion of rough endoplasmic reticulum cisterns were observed upon electron microscopic examination of the spleen tissue cells. These cytopathologic alterations indicate that lead acetate has some drastic toxicological effects on cellular structures.

Key words: Rats, spleen cells, lead acetate, ultrastructure, electron microscope

1. Introduction
Lead compounds are among the most common causes of animal and human intoxication, due to their vast natural availability and widespread industrial and daily usage (Sharma et al., 1980; Deveci, 2006; Suradkar et al., 2010). Organic and inorganic lead compound consumption is common, especially in paints and coatings, storage batteries, water ducts, ceramic glazes, plastic or rubber products and compounding, as well as in the printing, pesticide, and petroleum industries. Extensive use of lead causes not only environmental pollution, but also intoxication of various organisms as it becomes involved in the food chain (Şanlı and Kaya, 1992; Dewrée et al., 2007; Suradkar et al., 2010).

Exposure to various lead compounds via nasal, oral, or dermal routes causes intoxication (Nordberg et al., 2007). Although values below a certain blood level may be tolerated, it is well known that even levels beneath the safety margin may also alter some biologic functions (Pounds et al., 1991; Berny et al., 1994). Chemical compounds that are unknown and not necessary for biologic functions of the organisms are classified as xenobiotics. These compounds are not the part of the metabolism and they do not have nutritional value. Food and cosmetic additives, drugs, herbicides, pesticides, solvents, and combustion products may be included in this category (Romert, 1991).

While many reports are available concerning light microscopic assessment of the mammalian spleen structure and functions (Cesta, 2006; Suttie, 2006; Ibe et al., 2010), electron microscopic studies are few or scarce. In recent reports, the pathologic effects of xenobiotics, especially heavy metals, on organisms have been evaluated thoroughly (Gram et al., 1986). Histopathologic studies carried out to recognize these toxic changes are mainly focused on the liver, kidneys, brain, thymus, small intestines, and blood. Various studies have shown the histochemical and histopathological effects of lead on hematologic, neurologic, gastrointestinal and immunological systems, correlated with dosage and exposure time applied by light microscope. Therefore, the effect of lead on the rat spleen’s ultrastructure was examined by electron microscope in this study.

2. Materials and methods
Twenty-five male and female rats of 200–250 g were used in this experiment. Group I was used as a control group and they did not receive any treatment. The remaining groups were given a daily oral administration of 0.5 mg of lead
acetate [lead (II) acetate trihydrate = \((\text{CH}_3\text{CO}_2\text{)}_3\text{Pb.H}_2\text{O}\)] (2.5 mg/kg) dissolved in water. These groups, dosage, and exposure times are summarized in the Table.

All animals were sedated with intramuscular injections of 0.01 mg keta and the specimens were obtained from the spleen. Spleen tissues of 1 mm³ were fixed in glutaraldehyde (3%) and phosphate-buffered saline (pH 7.2) at 4 °C for 3 h and postfixed with 1% osmium tetroxide for 1 h. Osmium tetroxide was washed away with the same buffer. Ethyl alcohol was used for dehydration and the specimens were embedded in Araldite CY-212.

Thin cross sections were double-stained with saturated uranyl acetate (20 min) and lead citrate (10 min) (Sato, 1967). A Jeol JEM 100 CX-II electron microscope was used for the examination of the specimens.

The experiment was carried out in accordance with the Ankara University guidelines for the care of the experimental animals. Additionally, the guiding principles for experimental procedures presented in the World Medical Association's Declaration of Helsinki regarding animal experimentation were followed in this study.

3. Results

3.1. Group I (control group)
Specimens obtained from the control rat spleen showed normal nucleus, organelles, and mitochondria with typical inner membrane and cristae. Normal tiny structural anatomy was preserved. Lymphocytes, both in the white and red pulp of the spleen, exhibited normal structures (Figure 1).

3.2. Group II: specimens obtained at 96 h after daily administration of 2.5 mg/kg dose of lead acetate
Lower magnification examinations revealed erythrocyte stasis in the venous sinusoids of the red pulp, with macrophage and lymphocyte infiltration at neighboring sites. Vacuolation was observed in the cytoplasm of some of these cells. Multiple microvacuolations were also detected in some erythrocytes (Figure 2).

Scarce lymphocyte and plasma cells were observed to have early stages of nucleoplasm vacuolation and

### Table.
Experiment groups, daily dosage, and administration time.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Numbers of rats in each group</th>
<th>Daily dosage (mg)</th>
<th>Administration time (h)</th>
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<tbody>
<tr>
<td>Group I (Control)</td>
<td>5</td>
<td>------</td>
<td>00.0</td>
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<tr>
<td>Group II</td>
<td>5</td>
<td>2.5</td>
<td>96 h</td>
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<tr>
<td>Group III</td>
<td>5</td>
<td>2.5</td>
<td>144 h</td>
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<tr>
<td>Group IV</td>
<td>5</td>
<td>2.5</td>
<td>336 h</td>
</tr>
<tr>
<td>Group V</td>
<td>5</td>
<td>2.5</td>
<td>432 h</td>
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nucleolemma loosening. Plasma cells were defined by their partially-dilated, homogenously filled, and well-organized rough endoplasmic reticulum cisterns. Swelling of mitochondria and cristae loss was clearly identified in these cells (Figure 3).

At a higher magnification, plasma cells located in the red pulp of the spleen were shown to be dilated rough endoplasmic reticulum cisterns. Cristae loss and vacuolation was documented in swollen mitochondria in this area (Figure 4).

3.3. Group III: specimens obtained at 144 h after daily administration of 2.5 mg/kg dose of lead acetate

A tiny cross section obtained from the marginal region revealed normal organelle structure, with significant swelling of mitochondria and cristae loss in some cells (Figure 5). In another specimen, venous sinusoids were found to be filled with erythrocytes and eosinophils. Cytoplasm of some cells was filled with highly electron-dense materials. Swelling of mitochondria and cristae loss were evident (Figure 6).

Figure 3. A lymphocyte (Lc) and a plasma cell (Pc) in the red pulp of a rat spleen in the 96 h exposure group (Group II). Early stages of nucleoplasm vacuolation and nucleolemma distortion (thick arrow) were seen in the lymphocyte. The plasma cell (Pc) had dense homogeneous material and rough endoplasmic reticulum (rer). Swelling of mitochondria and cristae loss (m) in this cell were clear.

Figure 4. A plasma cell in the red pulp section of a rat spleen from the 96 h exposure group (Group II). Dilated rough endoplasmic reticulum cisterns (rer) were specific. Swelling of mitochondria and distortion of cristae (m) were seen (N). Nucleus (N), heterochromatin (ht).

Figure 5. Cross section from a rat spleen in the 144 h exposure group (Group III). Cristae loss in mitochondria (m), erythrocytes (e), and an eosinophil (el) were significant in this cell. Granule (G).

Figure 6. The red pulp compartment of a rat spleen in the 144 h exposure group (Group III). Erythrocytes (e) with an eosinophil (el) could be seen in the sinusoidal lumen. Swellings and cristae loss in mitochondria (m) were noted.
3.4. Group IV: specimens obtained at 336 h after daily administration of 2.5 mg/kg dose of lead acetate

The marginal zone and the neighboring red pulp were examined thoroughly in this group. It was noted that most of the mitochondria had transformed into vacuoles (Figure 7). Erythrocytes and eosinophils were also observed (Figure 8).

Swelling of mitochondria and cristae loss in lymphoid cells of the periarterial lymphatic sheath were significant. Swelling of mitochondria was even more pronounced in this group (Figure 9).

3.5. Group V: specimens obtained at 432 h after daily administration of 2.5 mg/kg dose of lead acetate

Degeneration of spleen cells was noteworthy at this stage. Cytoplasmic organelles could not be identified in most of the cells; instead, cytoplasm was filled with a homogeneous material. Mitochondria lost their normal structure and extreme swelling occurred, transforming them to vacuoles. Unrecognizable electron-dense structures were encountered in the midmarginal zone (Figure 10).

Figure 7. The marginal zone of a rat spleen in the 336 h exposure group (Group IV). Vacuolation of mitochondria (m) and enlargement in perinuclear area (V) were distinct.

Figure 8. The marginal zone of a rat spleen in the 336 h exposure group (Group IV). Vacuolation of mitochondria (m) and also erythrocytes (e) with an eosinophil (el) were seen in the sinusoidal area (S). Macrophage (M).

Figure 9. The red pulp of a rat spleen in the 336 h exposure group (Group IV). Vacuolation and swelling of mitochondria were observed (m). An erythrocyte (e) also was seen.

Figure 10. The marginal zone of a rat spleen in the 432 h exposure group (Group V). Cell organelles could not be differentiated in most of the cytoplasm of the cellular elements. Cell vacuoles were full of homogeneous material (v). Vacuolation of mitochondria (m) and electron-dense materials (white star) were also seen.
4. Discussion
A toxic material for living organisms, lead has no beneficial role in the metabolism. It accumulates over time, depending on the habitat of the organisms. Lead exposure is widespread in urban areas, whereas exposure is minimal in rural regions. This is mainly due to the use of leaded fuel. Fuel additives release lead compounds into the environment upon combustion, which has irreversible harmful effects on humans and other organisms (Eisler, 1988).

Ninety percent of the particles taken in by inhalation are held by the lungs. The particles reaching to the alveolus are then transferred to circulation. Lead is ingested orally at a rate of about 0.300–0.500 mg per day. As the excretion rate of lead is very slow, it accumulates in the body. Lead accumulates in the soft tissues of children and adults, whereas bone is the main site of accumulation for the elderly. It is distributed to cartilages, nerves, thymus, thyroid, pancreas, kidneys, lungs, spleen, and muscle tissue via blood circulation. Kidneys excrete 75% of the daily lead intake without altering it. The rest of the lead accumulates in nonviable tissues, such as nail and hair, and a small percentage is excreted by sweating (Ellenhorn and Barceloux, 1990; Klaassen et al., 1996).

Toxicological studies reported contradictory results in terms of lead dosage. Yagminas et al. (1990) administered doses of 0.05, 0.10, 0.20, 0.5, and 1.00 mg/kg to young rats. However, Grzybek et al. (1990) administered a single dose of 150 mg/kg to adult rats. Hashmi et al. (1989) tried 2% lead and Martinez et al. (1993) applied a daily dosage of 0.006 mg to mice. The acute lethal dosage for rats was determined to be 150 mg/kg. In this study, chronic lead acetate dosage was 2.5 mg/kg per day.

Histopathologic assessment revealed that the most significant alterations occurred in the mitochondria. Swelling of mitochondria and cristae loss were observed in the 96 h group and thereafter. Evaluation of daily 2.5 mg lead acetate administered to the group after 432 h (Group V) showed a total loss of normal mitochondrial structure with extreme swelling and vacuole formation and total cristae loss. Alteration of mitochondrial structure will inevitably impair cell functions. Our results comply with earlier studies conducted on rats, frogs, and carps (Goyer and Krall, 1969; Hoffman et al., 1972; Fewler et al., 1980; Kendall and Scanlon, 1985; Hashmi et al., 1989; Tomczok et al., 1991b; Martinez et al., 1993; Kazanci and Ayvalı, 1995; Deveci, 2006).

Vacuole formation of various sizes was also noted in our study. The number of vacuoles increased with lead exposure time. This finding also complies with the earlier studies on rats and carps (Grzybek et al., 1990; Tomczok et al., 1991a, 1991b; Kazanci and Ayvalı, 1995; Deveci, 2006).

Partially distorted heterochromatin nuclei were encountered, which has been previously observed in some studies (Grzybek et al., 1990; Tomczok and Tomczok, 1990; Fowler and DuVal, 1991; Tomczok et al., 1991b; Kazanci and Ayvalı, 1995; Deveci, 2006). Nuclear changes clearly indicated cellular death. On the other hand, we could not clarify whether cellular death was due to lead exposure or if it was a result of normal cellular activity. It was probable that lead had played a role in cellular death.

Electron-dense particles in the cytoplasm, indicating lead accumulation and deep nuclear invaginations, were documented. Liver, spleen, blood, and renal tissues were also shown to entrap lead in rat, frog, carp, and camel studies (Hoffman et al., 1972; Fowler et al., 1980; Kendall and Scanlon, 1985; Grzybek et al., 1990; Fowler and DuVal, 1991; Tomczok et al., 1991a, 1991b; Kazanci and Ayvalı, 1995; Deveci, 2006).

After 96 h of lead exposure, dilated endoplasmic reticulum with electron-dense material was noted in Group II. This finding is consistent with other studies (Hoffman et al., 1972; Colle et al., 1980; Tomczok et al., 1991a, 1991b; Kazanci and Ayvalı, 1995; Deveci, 2006). Spleen necrosis after 432 h of lead administration (Group V) might accelerate degeneration mechanisms. Some of the structural changes showed early regeneration signs in Group V. An organ's resistance to a toxic material is based on its resistance potential to that toxic material.

The increase of electron-dense material in macrophages and lysosomes is a significant finding exhibited in our study, which was also observed by Hofman et al. (1972) in rat liver and kidney tissue, and by Fowler et al. (1980) in rat kidney tissue.

Cytopathologic alterations and mitochondrial distortions observed during the study would inevitably impair cellular functions. Similar degenerative findings in mitochondria have been described by earlier studies, but, to our knowledge, changes in spleen fine structure due to lead intoxication have not been described. Consequences of damaged oxidative phosphorylation on spleen functions and the immune system need further histochemical and immunological studies.

These cytopathologic alterations indicate that lead acetate has some drastic toxicological effects on the cell structure of living beings.

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References


