LEAD INDUCED NEPHROTOXICITY WITH SPECIAL REFERENCE TO PROXIMAL TUBULE IN ALBINO RATS

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ABSTRACT
Lead Pollution is one of the most important problems of environmental and occupational origin and is widely regarded as risk to health. Kidneys are particularly exposed to the untoward toxic effects of lead as they form its major route of excretion. The effects of lead on renal systems are mediated by the generation of Reactive Oxygen Species, which are thought to be the initiators of per oxidative damage to the cell membranes. The present study was designed to observe the nephrotoxic effects of lead on proximal tubules with increasing time period. Based on the study, it can be stated that lead induced nephrotoxicity particularly damages the structure of proximal tubules and the damage is more pronounced with increasing time period.

Keywords: Lead Pollution, Proximal tubules, Nephrotoxicity, Reactive Oxygen Species, Per oxidative Damage.

INTRODUCTION
Lead is one of the mankind’s oldest environmental and occupational toxins (Chatterjee and Rana, 2002). Exposure to Lead can occur from a multitude of sources. Worldwide six categories of products account for most cases of lead exposure i.e., gasoline additives, food can soldering, lead based paints, ceramic glazes, drinking water systems and folk remedies (Markowitz, 2000).

A high content of lead in petrol is a serious issue, as the end product of it is release of lead into the environment. Unfortunately lead isotopes are very stable and do not decay for millions of years.

Lead enters the body through multiple routes and gets distributed and stored in almost every organ resulting in its defective function (Pocock, 1980; Hodgkins et al., 1991).

Since kidneys are the major route of excretion of lead, this organ is particularly exposed to its toxic effects (Noorafshan, 1998). The primary effects of lead on renal functions are thought to be mediated via damage to cell membranes (Oberley et al., 1995). Reactive oxygen species are the initiators of per oxidative damage to membranes (Sandhir et al., 1994) which involves oxidative degradation of polyunsaturated fatty acids, resulting in impaired membrane function and structural integrity, decrease in fluidity, inactivation of a number of membrane bound enzymes (Sidhu et al., 2004) and finally cell death (Othman and El-Missiry, 1988).

The proximal tubular cells are particularly vulnerable owing to their high energy demand such as reabsorptive and secretory functions (WHO, 1991). Lead accumulates in mitochondria and causes both structural and functional alterations. The effects include mitochondrial swelling, inhibition of respiratory functions and energy (ATP) production. Consequently energy dependent processes including tubular transport are impaired (Kathuria et al., 2004).
MATERIALS AND METHODS

The study was done on a total of 40 albino rats in the department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Post-graduate Medical Centre (JPMC), Karachi.

The animals were divided into two main groups of 20 animals each.

- Group A served as control
- Group B rats received Inj. Lead Acetate (Merck, Germany) 8 mg/kg body weight intraperitoneally. The two groups were further subdivided into four subgroups of five animals each according to the period of treatment i.e., one, two, four and six weeks.

The animals were sacrificed under ether
anesthesia at the end of experimental period. The kidneys were dissected out and were fixed in alcoholic formalin for 24 hours. The kidneys were processed, 5µ thick longitudinal sections were made on a rotatory microtome and were mounted on albuminized glass slides. The slides were stained with Periodic Schiff haematoxylin technique. The light microscopic study of proximal tubules was

Figure 3: Mean nuclear diameter of proximal tubular cells of kidneys of albino rats in different groups at variable time interval.

Table-1
Mean* number of proximal tubules in cortical region of kidneys of albino rats in different groups at variable time interval.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment received</th>
<th>No. of proximal tubules per unit area under high magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st Week</td>
</tr>
<tr>
<td>A (n=20)</td>
<td>Control</td>
<td>17.796 ± 0.204</td>
</tr>
<tr>
<td></td>
<td>A1 (n=5)</td>
<td>15.942 ± 0.693</td>
</tr>
<tr>
<td>B (n=20)</td>
<td>Lead acetate treated</td>
<td>18.864 ± 0.319</td>
</tr>
<tr>
<td></td>
<td>B1 (n=5)</td>
<td>17.854 ± 0.352</td>
</tr>
</tbody>
</table>

* Mean ± SEM; *P value ≤ 0.05 means statistically significant.

Table-2
Mean* number of nuclei of proximal tubules cells of kidneys of albino rats in different groups at variable time interval.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment received</th>
<th>No. of nuclei per unit area under high magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st Week</td>
</tr>
<tr>
<td>A (n=20)</td>
<td>Control</td>
<td>18.864 ± 0.319</td>
</tr>
<tr>
<td></td>
<td>A1 (n=5)</td>
<td>17.854 ± 0.352</td>
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<td>Lead acetate treated</td>
<td>18.864 ± 0.319</td>
</tr>
<tr>
<td></td>
<td>B1 (n=5)</td>
<td>17.854 ± 0.352</td>
</tr>
</tbody>
</table>

* Mean ± SEM; *P value ≤ 0.05 means statistically significant.
done. The micrometric observations included proximal tubular count under 8x ocular and 40x objective; proximal tubular nuclear count and nuclear diameter under 8x ocular and 100x oil immersion objective using ocular micrometer scale (for nuclear diameter).

**OBSERVATIONS**

The Periodic Schiff Haematoxylin stained sections of lead treated rats at 1, 2, 4 and 6 weeks were studied for micrometric observation and were compared with corresponding control (Figure 4).

The results of proximal tubular count in different groups at variable time interval are shown in Table-1. There was a significant decrease in proximal tubular count in group B1 (1 week lead treated) as compared to group A1. The decrease was highly significant in groups B2, B3 and B4 (2, 4 and 6 weeks lead treated) when compared with their corresponding control groups (Figure 1).

The result of proximal tubular Nuclear count in different groups at variable time interval are shown in Table-2. When the lead treated groups were compared with corresponding controls, a decrease in nuclear count was observed. The decrease was non-significant at first week (group B1) and moderately significant on second week (group B2). A highly significant decrease in nuclear

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Table-3
Mean* nuclei diameter of proximal tubular cells of kidneys of albino rats in different groups at variable time interval.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment received</th>
<th>No. of proximal tubules per unit area under high magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st Week</td>
</tr>
<tr>
<td>A (n=20)</td>
<td>Control</td>
<td>6.514 ± 0.049</td>
</tr>
<tr>
<td></td>
<td>A1 (n=5)</td>
<td>6.410 ± 0.056</td>
</tr>
<tr>
<td>B (n=20)</td>
<td>Lead acetate treated</td>
<td>7.288 ± 0.188</td>
</tr>
</tbody>
</table>

* Mean ± SEM; *P value ≤ 0.05 means statistically significant.

Figure 4: PAS-Haematoxylin stained, 5 µm thick, longitudinal section of kidney from group A (control) showing normal arrangement of proximal tubules.
count was observed at 4 weeks (group B3) and 6 weeks (group B4) of treatment with lead acetate (Figure 2).

The Nuclear Diameter of Proximal tubular cells in different groups at variable time intervals, are shown in Table-3. A number of enlarged nuclei were seen (Figure 5) and there was a moderately significant increase in nuclear diameter in initial periods of treatment i.e., at 1 week (B 1) and 2 weeks (B2). Later at four weeks (B3) and six weeks (B4) a highly significant decrease in nuclear diameter was observed on comparison with corresponding control groups (Figure 3) due to large number of shrunken nuclei (Figure 6).

**DISCUSSION**

Environmental chemical such as lead is capable of inducing nephrotoxicity (WHO, 1991). The kidneys are particularly exposed to
the untoward toxic effects of lead as they form its major route of excretion (Noorafshān, 1998). The proximal tubular cells are particularly vulnerable to the nephrotoxic effects owing to their high-energy demand such as reabsorptive and secretory functions (WHO, 1991).

The study of renal cortical tissue in treated group B showed a decrease in proximal tubular count progressively so that at 6 weeks the decrease is highly significant. This can be attributed initially to tubular dilatation as was indicated in the study of Khalil-Manesh et al (1992) who stated that continuous lead feeding resulted in tubular dilatation. Later with increased time period the increase in number of necrotic tubules accounts for a highly significant decrease in tubular count.

There was an initial increase in nuclear diameter and progressive clumping of chromatin in variable periods of treatment. The finding is in agreement with the study of Khalil-Manesh et al (1992) and may be attributed to the presence of lead induced nuclear inclusion bodies and pseudo-inclusions or nuclear invagination of cytoplasmic contents (Kathuria et al., 2004). In the present study there was a highly significant decrease with the presence of large number of shrunken nuclei in rats treated with lead for 4 and 6 weeks. This may be attributed to degenerative changes related to lead treatment. The progressive decrease in nuclear count in all the subgroups can be correlated with degenerative changes and with loss of cytoplasmic contents and, nuclei from distorted apical surface of the tubules.

Effects of lead on renal systems are mediated via peroxidative damage to membranes. Lead accumulates in mitochondria and causes both structural and functional alterations, including mitochondrial swelling, inhibition of respiratory functions and energy (ATP) production. Consequently energy dependent processes including tubular transport are impaired (Kathuria et al., 2004).

In the present study it was observed that administration of lead in experimental animals produced changes in the proximal tubular cells indicating its nephrotoxic effects on renal cortical tissue. The study further shows that continuous lead administration with increasing time period leads to a more pronounced and progressive increase in toxic effects on proximal tubular cells, indicated by changes seen, in micrometric observations.

REFERENCES


