LETTERS TO THE EDITOR

Lead Induced Testicular Dysfunction in Weaned Rats

**Key words:** Lead—Testis—Biochemical changes—Weaned rats

Besides the toxic effect of lead on hematopoietic, renal and central nervous system, infertilitly, lack of libido and impotency in workers occupationally exposed to lead has also been reported. Few reports pertaining to testicular dystrophy in lead exposed animals have been reviewed by Clarkson et al. Increasing levels of lead in the biosphere and its high contents in the blood of children through pica or traffic exhaust have led us to investigate the effects of this metal on the testis of growing rats at the initiation of the pre-pubertal stage to predict its role in inducing damage to gonads in early life.

ITRC bred 40 weaned male albino rats (40–50 g) were housed in stainless steel cages and maintained in standard laboratory conditions. They were supplied with pellet diet (Pragati Feed Agency, India) and water ad libitum. The rats were randomly divided into 4 groups of 10 rats each. Rats of Group I received physiological saline daily intraperitoneally and served as controls, while rats of remaining three groups (Group II, III and IV, respectively) received lead acetate in doses of 5.0, 8.0 and 12 mg Pb+2/kg daily by the same route. The treatment schedule continued for a period of 15 days.

At the termination of the experiment, the animals were sacrificed by decapitation, testis were removed and weighed. One testes of each rat was used for biochemical and metal estimations while the other was fixed in 10% neutral buffered formaline for histopathology.

The activity of succinic dehydrogenase (SDH) (succinate: (acceptor)oxidoreductase, E.C. 1.3.99.1) was assayed according to the procedure described by Slater and Bonner. Adenosine triphosphatase (ATPase) (ATP, phosphohydrolase, E.C. 3.6.1.3) was measured by the method of Seth and Tangari. The liberated inorganic phosphorus was determined according to the method of Fiske and Subbarow. The level of cholesterol was measured by the method of Zlatkis et al. and phospholipids were determined by the method of Wagner et al. For histopathology, testis were fixed in 10% neutral buffered formalin, embedded in paraffin, sections cut at 6 μm and stained with hematoxylin eosin.

Estimation of lead in the testicular tissue was done as described by Singh et al. using Beckman's Spectrospan V (DCP-spectrophotometer). The statistical significance between control and experimental values were calculated by the Student's 't' test and p values less than 0.05 were considered to be significant.

Increasing doses of lead resulted in significant loss of body weight as well as...
Table: Effect of lead on body and testicular weights, lead content and some biochemical parameters in the testis of rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Pb 5 mg/kg</th>
<th>Pb 8 mg/kg</th>
<th>Pb 12 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Gr. I)</td>
<td>(Gr. II)</td>
<td>% change</td>
<td>(Gr. III)</td>
<td>% change</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>55 ± 4</td>
<td>45 ± 3</td>
<td>40 ± 4</td>
<td>36 ± 3</td>
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<tr>
<td>Testis weight</td>
<td>0.41 ± 0.04</td>
<td>0.30 ± 0.04</td>
<td>0.28 ± 0.01</td>
<td>0.24 ± 0.05</td>
</tr>
<tr>
<td>Cholesterol (mg/g tissue)</td>
<td>2.46 ± 0.43</td>
<td>1.56 ± 0.09</td>
<td>1.40 ± 0.05</td>
<td>1.28 ± 0.1</td>
</tr>
<tr>
<td>Phospholipids (Pi mg/g tissue)</td>
<td>1.66 ± 0.10</td>
<td>1.47 ± 0.05</td>
<td>1.34 ± 0.03</td>
<td>1.19 ± 0.09</td>
</tr>
<tr>
<td>SDH (n mol K₄Fe(CN)₆ reduced/mg/min)</td>
<td>50.16 ± 4.53</td>
<td>51.95 ± 6.73</td>
<td>55.5 ± 2.18</td>
<td>35.80 ± 2.76</td>
</tr>
<tr>
<td>ATPase (μ mol Pi liberated/mg/min)</td>
<td>1.11 ± 0.15</td>
<td>0.97 ± 0.06</td>
<td>1.02 ± 0.05</td>
<td>0.99 ± 0.06</td>
</tr>
<tr>
<td>Lead content (μg/g of wet tissue)</td>
<td>0.069 ± 0.007</td>
<td>0.071 ± 0.006</td>
<td>0.100 ± 0.010</td>
<td>0.157 ± 0.021</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E. of five rats. N.S.: Not significant.
LEAD INDUCED TESTICULAR DYSFUNCTION

in the testicular weight in group III and IV (Table). Only two animals died in group IV, a necropsy did not help to ascertain the cause of death.

Examination of sections of testis revealed no marked pathological changes in treated animals as compared to controls. The sections revealed an orderly arrangement of tubules having only sertoli cells, spermatogonial cells and spermatocytes besides normal interstitial tissue with leydig cells.

The total cholesterol contents in the testis of rats markedly decreased at all given doses of lead and was statistically significant in group III and IV. In phospholipid contents, the significant decrease was observed only at two highest doses. While at the lowest dose, the decrease in its contents was not significant compared to that of control. The activity of succinic dehydrogenase initially showed an increase at dose 8 mg/kg, while a marked inhibition was observed at the highest dose of lead as compared to that of control (Table). The activity of ATPase remained unaffected at all three doses of lead.

As shown in Table, no significant increase in lead content in the testis was noticed at lower dose level as compared to control, however, significant increase was found in group III and group IV which was dose dependent.

Lead has been reported to disrupt the spermatogenic cycle in the adult rats. These pathological changes are reported to be in the form of germinal epithelium damage, detachment of the germinal layer from basal membrane, spermatocytes and spermatids injury as well as slight oedema. But these changes have been reported only in adult rats having attained puberty. In the present study, no pathological changes could be seen in the testis of all the treated groups, however, animals exposed to different doses of lead (Group II, III and IV) exhibited dose-related response in cholesterol, phospholipid contents and SDH activity. It appears that the accumulation of lead directly exerts its effect on biochemical parameters in the testicular tissue. The dose related biochemical changes in the present study are attributed to the corresponding increase in lead contents in various treated groups. Lead deposition in testis of rats has been reported histochemically.

SDH enzyme is responsible for the energy metabolism of the cell, its decreased activity at highest dose shows the interference of lead with the cellular energetics in the testicular tissue. This enzyme is reported to be very sensitive to lead and has been found to be inhibited in the mitochondria of various organs. Decrease in cholesterol content in this study, in absence of any pathological changes, reflect another site of lead-intoxication in the testicular tissue. It assumes special significance because at weaned stage, cholesterol is regarded to be a precursor of steroidal hormone and the spermatogenesis is also reported at this age, to be preceding under the stimulus of gonadotrophic hormone. Change in cholesterol contents and glucose-6-phosphate dehydrogenase activity have been found to be related with disturbed steroidogenesis in the testicular tissue due to lead-intoxication. Such decrease in cholesterol contents may probably reflect the disturbance towards the synthesis of testosterone from Leydig cells.
The decrease in phospholipid contents may be the result of increased lipid peroxidation. Shafiqu-ur-Rehman reported increased lipid peroxides formation in the lead-exposed rats. The decomposition of phospholipids, as building blocks of bio-membranes, lead to their increased fragility and dysfunction.

Thus, in this study, lead has been found to produce marked dose-related biochemical alterations after its exposure during early life. However, further experiments are required to ascertain if the persistence of these change may be responsible for disturbed gonadal functioning late in life.

REFERENCES


(Received January 13, 1986 and in revised form March 14, 1986)