INTRODUCTION
Exposure to heavy metal impairs reproductive capacity by causing severe testicular degeneration, seminiferous tubule damage and necrosis in rats.1-3 Zinc (Zn) is an anti-oxidant trace element that is present in all organs, tissues, and fluids of the body. It is required for cell proliferation, differentiation, normal growth, immune functions, and wound healing.4 Zn is an essential mineral for spermatogenesis and a hepatocellular Metallothionein (MT) inducer, and zinc co-treatment protects tissues against free radicals and oxidative stress.5 Metallothionein (MT), a cysteine-rich heavy metal binding protein, protects tissues from heavy metal toxicity and hydroxyl radical attack through sequestration and anti-oxidising heavy metals.3,5,6 The beneficial role of zinc against cadmium-induced testicular damage has also been reported, although its anti-oxidant mechanism is unclear.3,4,7,8 Nevertheless, several mechanisms have been proposed for zinc. One mechanism is that zinc's protection of the testes is mediated by the induction of MT against heavy-metal toxicity.3,5 Alternatively, zinc may directly antagonize the toxic effects of heavy metal and/or stabilize cell membranes and protect lipid peroxidation by free radicals.2,4,6,9 Lead (Pb) on the other hand is a toxic non-essential trace element.10

ABSTRACT
Objective: To determine the effects of lead and zinc on testes.
Study Design: Randomized control trial.
Place and Duration of Study: Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, from August 2003 to December 2005.
Methodology: Sixty adult (90 days old) albino rats were obtained from animal house JPMC for the study and divided into 3 groups. Group A received injection normal saline 1 cc intraperitoneally daily for 8 weeks. Group B received lead chloride in a dose of 10 mg/kg body weight intraperitoneally daily. Group C received zinc chloride in a dose of 1 mg/kg body weight before half an hour of injection of lead chloride in a dose of 10 mg/kg body weight intraperitoneally daily so that to provide pre-treatment. On the day of completion of treatment the animals were sacrificed testes removed and fixed in Bouin's fluid. Testes were dehydrated in the ascending strength of alcohol, 5 µm thick sections were cut and stained with PAS Iron Hematoxylin. Student's t-test was used for statistical analysis with significance at p < 0.05.

Results: The mean diameter of seminiferous tubule was 291.91±1.18, 198.53±1.67 and 288.77±1.11 µm in groups A, B and C respectively. Diameter of seminiferous tubules decreased by 31.99% in group B (p < 0.001; CI 97.736) as compared group A and while group B comparing with group C, the diameter of seminiferous tubules was decreased by 31.25% (p-value = 0.076; CI -94.264 to -86.203). Mean thickness of germinal epithelium was 96.19±1.01, 50.69±1.20 and 94.94±0.54 µm in groups A, B and C respectively. Thickness of germinal epithelium decreased by 47.30 in group B (P < 0.001; CI 42.503 to 48.496) as compared to group A and while comparing group B with group C, the thickness of germinal epithelium was decreased by 46.61% (p=-44.25; CI -46.704 to -41.787).

Conclusion: Zinc prevented toxic effects of lead on germinal epithelium in the albino rats.

intake of zinc reduces the accumulation and toxicity of lead, probably by decreasing its intestinal absorption.\textsuperscript{14} Sub-chronic oral lead intoxication may affect body organs and testes for several years even in absence of continued exposure.\textsuperscript{15,16} Many studies have shown that reproductive toxicity is an important feature of lead toxicity.\textsuperscript{17-19} During lead exposure, it accumulates in testis tissue in a dose dependent manner.\textsuperscript{17,18} Lead toxicity induces a significant increase in apoptotic cell death in the seminiferous tubules of young growing rats.\textsuperscript{17} It is also associated with disruption of spermatogenesis and histoarchitecture and lowered enzyme activities in testis.\textsuperscript{18}

This study was conducted to document the effect of zinc on lead-induced toxic effects on the testes.

**METHODOLOGY**

The experimental study was carried out at Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, from August 2003 to December 2005. Sixty adult male albino rats were obtained from Animal House, BMSI, and JPMS and maintained at food and water ad libitum. The animals were randomly divided into three groups: A, B and C each group consists of 20 animals. The animals from each group were kept in separate cages.

Control Group-A was divided into 4 subgroups (A1, A2, A3 and A4) based on period of treatment (1, 3, 5 and 8 weeks respectively), each subgroup consisting 5 animals. This group received injection normal saline 1 cc intraperitoneally daily for their respective period of treatment.

Lead Group-B was divided into 4 subgroups (B1, B2, B3 and B4) based on period of treatment (1, 3, 5 and 8 weeks respectively) each subgroup consisted of 5 animals. This group received lead chloride in dose of 10 mg/kg body weight in the distilled water intraperitoneally daily for 8 weeks.\textsuperscript{7}

Ameliorated group was divided into 4 subgroups (C1, C2, C3 and C4) based on period of treatment (1, 3, 5 and 8 weeks respectively) each subgroup consisted of 5 animals. Each subgroup was injected lead chloride in dose of 10 mg/kg body weight in the distilled water intraperitoneally daily. Zinc chloride was injected intraperitoneally daily at least 2 hours prior to lead chloride (pre-treatment) in a dose of 1 mg/kg body weight.\textsuperscript{7} On the day of completion of period of treatment, animals were weighted and sacrificed under deep ether anaesthesia.

The testes were fixed in Bouin's fluid for 24 hours, before they were cut longitudinally into 2 equal halves and again post-fixed in fresh Bouin's fluid for next 24 hours. The tissues were dehydrated in the ascending strengths of alcohol, cleared in xylene. Infiltrated and embedded in paraffin wax, the tissue blocks were made, cut into 5 \( \mu \)m thick sections with the help of rotatory microtome. The sections were mounted on albumenized glass slides and stained with PAS-Iron Hematoxylin. Morphological study of testes was done and morphometric study of germinal epithelium was measured with the help of ocular micrometer scale under light microscope.

The level of significance (p) was calculated by the help of student's t-distribution table. The significance level was considered as p \( \leq \) 0.05. All the calculations were done utilizing, SPSS 15.0.

**RESULTS**

On external examination of testes in control group, they appeared grayish white in colour, soft in consistency and blood vessels were present on the surface (Figure 1). While on opening the scrotal sacs of lead treated group, a large quantity of blood was observed in the vicinity of testes, they were firm in consistency. Large number of blood vessels was seen on the surface with bleeding and necrotic areas (Figure 2). On examination of testes of lead + zinc group finding were similar to control group except slight increased in vascularity on surfaces and small quantity of haemorrhage seen in vicinity (Figure 3).

In the control group, there was regular and compact arrangement of tubules with intact interstitium (Figure 4). Section showed the large number of seminiferous tubules with regular and intact basement membrane. The germ cells were arranged regularly and all line of cells were present from spermatogonia to spermatid which attached to the sertoli cells. The lumen contained the spermatooza without sough. The interstitial spaces between the seminiferous tubules showed the interstitial cell of Leydig.

Sections of testes of group B showed shrinkage of seminiferous tubules with degeneration and marked widening of interstitial space with area of necrosis (Figure 5). Basement membrane of most of tubules were distorted and ruptured. Arrangement of germinal epithelium was disturbed most of cells degenerated and the vacuoles appeared between the germinal epithelium. The lumen contained slough with a few numbers of spermatooza. Most of interstitial cells of Leydig degenerated.

Sections of testes of group were showing seminiferous tubules distorted but intact basement membrane (Figure 6). There was slight widening of interstitial spaces seen, with slight degeneration. Germinal cells arranged regularly with very few vacuoles in between. Lumens were narrow and contained spermatooza without slough and interstitial space contained cells of Leydig.
The diameter of seminiferous tubules were recorded at different time intervals in different groups (Table I). Mean diameter of seminiferous tubules decreased by 31.99% from 291.91±1.179 µm (group A) to 198.54±1.672 µm (group B). Comparing group B with group A, there was statistically significant decreased in diameter of seminiferous tubules (p < 0.001; CI 89.023 to 97.736).

Mean diameter of seminiferous tubules decreased from 291.91±1.179 µm (group A) to 288.77±1.112 µm (1.07%, group C). There was insignificant decreased in diameter of seminiferous tubules while comparing group A with group C and (p=0.076; CI -0.339 to 6.631). Mean diameter of seminiferous tubules deceased by 1.25% from 288.77±1.112 µm in group C to 198.536±1.672 µm (group B). Comparing group C with group B, there was statistically significant decrease in diameter of seminiferous tubules (p < 0.001; CI-94.203 to -86.203).

The thicknesses of germinal epithelium were recorded at different time intervals in different groups (Table II). Mean values of thickness of germinal epithelium (Figure 4) decreased by 47.30% from 96.19±1.01215 µm (group A) to 50.69±1.20064 µm (group B). Comparing group B with group A there was statistically significant decreased in thickness of germinal epithelium (p < 0.001; CI 42.50378 to 48.49622). Mean thickness of germinal epithelium decreased by 1.30% from 96.19±1.01215 µm (group A) to 94.94±0.54202 µm (group C). There was insignificant decrease in thickness of germinal epithelium while comparing group A with group C (p=0.274; CI -1.024 to 3.532). Mean thickness of germinal epithelium deceased from 94.94±0.542 µm in group C to 50.69±1.200 µm 46.61% decreased. Comparing group B with group A there was a statistically significant decrease in the thickness of germinal epithelium (p < 0.001; CI -41.704 to -41.787).

### Table I: Mean ±SEM diameter of seminiferous tubules of testes in different groups of albino rats at different time intervals.

<table>
<thead>
<tr>
<th>Group</th>
<th>First week (1)</th>
<th>95% CI (1)</th>
<th>Third week (2)</th>
<th>95% CI (2)</th>
<th>Fifth week (3)</th>
<th>95% CI (3)</th>
<th>Eighth week (4)</th>
<th>95% CI (4)</th>
<th>p-value</th>
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<tr>
<td>A</td>
<td>271.48±</td>
<td>(η = 20)</td>
<td>279.10±</td>
<td>(η = 5)</td>
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<td>1.64112</td>
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<td>B</td>
<td>234.25±</td>
<td>(η = 20)</td>
<td>229.19±</td>
<td>(η = 5)</td>
<td>211.47±</td>
<td>(η = 5)</td>
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<td>C</td>
<td>263.48±</td>
<td>(η = 20)</td>
<td>-275.29±</td>
<td>(η = 5)</td>
<td>279.36±</td>
<td>(η = 5)</td>
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**Table II:** Mean ±SEM diameter of seminiferous tubules of testes in different groups of albino rats at different time intervals.

<table>
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<tr>
<th>Group</th>
<th>First week (1)</th>
<th>95% CI</th>
<th>Third week (2)</th>
<th>95% CI</th>
<th>Fifth week (3)</th>
<th>95% CI</th>
<th>Eighth week (4)</th>
<th>95% CI</th>
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<tr>
<td>A</td>
<td>82.56 ± 3.82</td>
<td>8.89149 to 75.67</td>
<td>88.21 ± 3.82</td>
<td>18.84362 to 70.37</td>
<td>90.41 ± 3.82</td>
<td>12.29359 to 72.19</td>
<td>31.31756 to 69.31</td>
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<tr>
<td>B</td>
<td>70.10 ± 3.82</td>
<td>-0.46124 to 73.36</td>
<td>66.90 ± 3.82</td>
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<td>(n = 5)</td>
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<tr>
<td>C</td>
<td>82.56 ± 3.82</td>
<td>-11.99566 to 70.30</td>
<td>-86.25 ± 3.82</td>
<td>-21.17141 to 65.33</td>
<td>89.16 ± 3.82</td>
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<td>(n = 5)</td>
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</table>

**DISCUSSION**

This study was designed to examine the protective effect of zinc against lead-induced changes in testes. It suggests that Pb decreased the concentration of blood Zn when both Pb and Zn were administered intraperitoneally. It has been recognized that the Zn cannot cross biological membranes by simple diffusion and need a trafficking system for its cellular uptake and release Pb influences absorption or distribution of Zn in blood or other tissues through interfering the trafficking system of Zn.20

The toxic effects of Pb on male reproductive system could be ameliorated by Zn supplementation. As evidence has shown that Zn exists in spermatozoa within the seminiferous tubules and helps spermatogenesis, Pb may result in disruption of the metabolic functions of enzymes containing Zn, inducing testicular damage.18,20 Batra et al. reported that there was a 30% reduction in Pb deposition in the testes when Zn was co-administered.18 The protective effect of Zn on reproductive toxicity of Pb may be attributed to competition between Pb and Zn, or reduction of available Pb-binding sites in the testicular tissue.

The lead intoxication induced significant reduction in the width of germinal epithelium and number sertoli cells in lead exposed animals. The number of primary spermatocytes and spermatogonia were decreased after treatment. These findings showed that lead induced morphometric changes were irreversible. This study showed that the morphometric changes could be ameliorated by zinc.21

Reduced width of germinal epithelium that was seen in this study seems to be due to damage of germinal cells as it was reported previously by other researcher.16,22 Lead intoxication mainly affected spermatids.22 Lead-induced apoptosis of the germinal cells which was reported by Adhikhari et al. is possible mechanism for loss of germinal epithelium.17 Batra et al. observed significant reduction in type A spermatogonia after lead toxicity associated with decrease of other germ cell populations. In another study complete arrest of spermatogonia was seen in lead treated rats.19,22

There is not much evidence about the reversibility of effects of lead on germinial epithelium. The previous study showed that lead induced reproductive toxicity is reversible in pre-pubertal rats but not in adult animals.24 According to findings of study there was no reversibility in reduction of germinal epithelium width and number of spermatogonia in primary spermatocytes in lead treated animals as compare to zinc treated animals. The animals with lead + zinc showed very little disarrangement of germinal epithelium.16

Zinc is well known anti-oxidant. It is not only an essential trace element that is present in all organs and tissues but also plays important role in many body functions including testosterone production and spermatogenesis.5,4 Zinc reduces the toxic effects of lead with an anti-oxidant mechanism.7,8 Several mechanisms have been proposed for the protection provided by zinc. One mechanism is that zinc may stabilized lipid membrane and protects lipid peroxidation by free radicals, there by protecting tissues.3,4,7

These results in an animal model need corroboration and validation for applicability in other species.

**CONCLUSION**

In the present study, zinc played a protective role against lead toxicity in rat testes. However, further studied are needed to elucidate the protective role of zinc in human.

**REFERENCES**


Protective effect of zinc over lead toxicity on testes


