INTRODUCTION

The usage of metal is indispensable part of our daily lives. Due to low cost and abundance, lead is pervasively used in batteries, paints, construction, plumbing, bullets, glazes and enamels. It is the most extensively studied occupational and environmental pollutant. Water, air, and food are the main sources of lead exposure to general population.

As a result of human activities, global lead contamination remains compelling and is inferable to the markedly expanded circulation of lead in environment. Lead equally affects women and men. People most vulnerable to lead are those who come into closest contact in production processes. Upto three million workers per year are exposed to lead, according to National Institute of Occupational Safety and Health (NIOSH), in United States. Cardiovascular, reproductive, renal and central nervous systems are majorly affected by lead, depending upon the level and duration of exposure. Due to lead exposure, central nervous and renal systems are more affected in children.

Lead is the leading cause of reproductive toxicity among metals, causing alterations in fertility. Decreased fertility is due to alterations in the action of sex steroid hormones, specifically estrogen in the uterus. Lead effects testicular growth by decreasing the seminiferous tubular diameter. Toxic effects of lead acetate on adult male rats avowed that the weight of testis decreased, deterioration and annihilation of spermatogenic and Leydig cells of interstitium and lack of spermatogenesis at elevated doses.

The common fig (Ficus carica, Fc) is an extensively used fruit. Figs belong to family Moraceae, native to the Middle East and Western Asia, is rich in minerals, vitamins and antioxidants. Antioxidant in the plants was the main reason behind activity against infertility. Abundance of beneficial vitamins including vitamin A, B1 and B2 are present in figs. Ficus carica have numerous bioactive compounds such as mucilage, flavonoids, enzymes, nicotinic acid, and tyrosine. High levels of polyphenols, flavonoids, anthocyanins and antioxidant capacity are the potential health-promoting constituents of Ficus carica.

Components like amino acids, phenolic components, organic acids, fatty acids, aliphatic alcohols, hydro-
carbons, and volatile compounds have also been isolated. Fig has numerous medicinal properties and is used as an anti-inflammatory, hypoglycemic, hypolipidemic, anticancer, and hemostatic agent. Figs inhibit ROS (reactive oxygen species) production which has a positive effect on the sperm count and causes an increase in the number of sperms.

The rationale of this study was to observe the possible effects of *Ficus carica*, as effects of fig on testis have not been studied in local setting earlier.

The objective of this study was to analyse response of lead acetate and *Ficus carica* on disruption of basement membrane in seminiferous tubules in testis of adult rats.

**METHODOLOGY**

The research was authorised by the Ethical Committee of the Army Medical College, Rawalpindi. It was conducted in the Department of Anatomy, Army Medical College, in association with National Institute of Health (NIH), Islamabad and Armed Force Institute of Pathology (AFIP), Rawalpindi. Lead acetate was purchased from local pharmaceutical supplier. *Ficus carica* was acquired from local market. Thirty adult healthy male Sprague Dawley rats, nine to eleven weeks of age, weighing of 200 - 250 gm, were used for this experiment. Rats were maintained in a well-airy room, with 20 - 26°C temperature range and dark-light sleep cycle of 12 hours throughout the duration of experiment. Rats were given standard laboratory diet, provided by NIH. Water was provided *ad libitum*. All doses were administered through oral gavage needle once daily for eight weeks.

Group A rats, which served as controls (untreated). Group B was given lead acetate 30 mg/kg/day via oral gavage; whereas, Group C was given lead acetate 30 mg/kg/day and *Ficus carica* 80 mg/kg/day via oral gavage, separately. By the end of eight weeks, the animals were sacrificed, dissected, and fresh testis specimens were taken out. Testis were placed in 10 percent formalin and processed into 5-micron thick sections using rotary microtome. The sections were stained with haematoxylin and eosin (H&E) for routine histological study of testis under light microscope. Image J v1.48 was used to open each image, and disruption of basement membrane was measured in ten consecutive high power fields (HPF), by considering equally spaced consecutive fields selected from right to left for each H & E stained specimen. Seminiferous tubules in each field were then observed for basement membrane disruption and given a score from 0 to 3. The score core was assigned as 0 = normal seminiferous histology, no disruption of seminiferous epithelium, 1 = slight effect, ≤50% of the tubule cross-section shows disruption of seminiferous epithelium, 2 = moderate effect, ≥50% tubule cross-sections shows disruption of seminiferous epithelium, and 3 = severe effect, >70% of tubule cross-section shows disruption of seminiferous epithelium.

Statistical Package for Social Sciences version 22 was used for analysing the data. The parameter was expressed as mean + standard deviation. Significant difference was determined using cross tabs, followed by chi-square test. The p-value of <0.05 was considered significant.

**RESULTS**

Thirty male adult Sprague Dawley rats, nine to eleven weeks of age, with an average weight of 300 ±2.7 gm were used in the experiment. Histologically, in all 10 (100%) specimens of Control Group A, there was no evidence of disruption of basement membrane (Table I, Figure 1-A). In experimental Group B, 6 (60%) specimens had severe disruption, 3 (30%) specimens had moderate disruption, and only 1 (10%) specimen had slight disruption. The tubules were characterised by irregular shape. The boundary of the tissue was thinner and had breaks in its continuity at multiple sites (Table I, Figure 1-B). In experimental Group C, there was no disruption of basement membrane in 2 (20%) specimens, 3 (30%) specimens showed slight disruption, 4 (40%) specimens showed moderate disruption, and only 1 (10%) specimen had severe disruption of basement membrane (Table I, Figure 1-C).

On intergroup comparison, when experimental group B was compared to control group A, the p-value was found to be highly significant statistically (p<0.001, Table II, Figure 2). On comparison of experimental group C with control group A, the difference was significant statistically (p=0.006, Table II, Figure 2). When experimental group B and C were compared, the p-value was found to be statistically insignificant (p=0.082, Table II, Figure 2).

<table>
<thead>
<tr>
<th>Parameter Findings</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disruption of basement membrane</td>
<td>No effect 0</td>
<td>10 (100%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Slight effect 1</td>
<td>0 (0.0%)</td>
<td>1 (10%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Moderate effect 20 (0.0%)</td>
<td>3 (30%)</td>
<td>4 (40%)</td>
<td></td>
</tr>
<tr>
<td>Severe effect 30 (0.0%)</td>
<td>6 (60%)</td>
<td>1 (10%)</td>
<td></td>
</tr>
</tbody>
</table>

N = Number of rats

![Figure 1: Photomicrograph showing comparison of disruption of basement membrane. (A) Intact basement membrane in control group A, (B & C) showing disruption of basement membrane (arrow): 40X, H&E.](image-url)
According to the disruption of basement membrane is due to biological stress and production of free radicals, effecting myoid cell and making them dormant, causing them to loose cell organelles and loosing tight interaction with adjacent myoid cells. Free radicals attack nearly all components of cell including proteins and DNA. It also impairs natural antioxidant defence mechanisms. Lead acetate resulting in the formation of free radicals, causing the lipid peroxidation of lysosomal membrane. This in turn leads to increased levels of lysosomol enzyme acid phosphatase.

In this study, in the experimental group C, 20% rats showed no disruption of basement membrane, 30% rats showed mild disruption, 40% showed moderate disruption and only 10% of the rat showed severe disruption, of basement membrane. On comparison of experimental group C with control group A, p-value was found statistically significant with 0.006. As previously mentioned, Ficus carica is rich in flavonoids, polyphenols, anthocyanins, and alkaline phosphatase, which are responsible for its antioxidant activity and making it free radical scavenger. Alkaline phosphatase is normally present in basement membrane, it helps in maintaining integrity and permeability, so Ficus carica helps in nourishing the depleted stores of alkaline phosphatase. Ficus carica is soluble in both lipid and aqueous environments, crossing the blood-testis barrier with ease, safeguarding the germinal epithelium and basement membrane. On comparing experimental group B with experimental group C, the p-value was 0.082, and found to be statistically insignificant.

DISCUSSION

Susceptibility of humans to lead acetate via multifarious ways mutates the function of numerous organs and tissues, leading to saturninity. Lead (Pb) is a heavy noxious metal that actuates a voluminous spectrum of anatomical, physiological and biochemical effects in humans. Ficus carica is widely used dried fruit having potent antioxidant activity. The nutritional combination of dried Ficus carica verified and found that it has the premium nutrient aggregate, consisting of minerals and vitamins. Accordingly, this study was designed to investigate the response of lead acetate on the testis of adult rats and whether co-administration of Ficus carica can modify the effects.

Scoring for basement membrane disruption was done which included score from 0 - 3. In the experimental group B, 10% of the cases had slight disruption, 30% had moderate disruption, and 60% of the rats showed severe disruption in their basement membrane. Comparison of p-value of basement membrane disruption of experimental group B as compared to control group A was less than 0.001*, which was highly statistically significant. The present study was in accordance with the study done by Khaki. He saw the effects of noxious agent on the boundary wall of seminiferous tubules, under light microscopy. Lead causes a decrease in vitamin E, which plays important role in abolishing lipid peroxidation; deficiency of this vitamin produces oxidative stress. According to the study by Khaki, disruption of basement membrane is due to biological stress and production of free radicals, effecting myoid cell and making them dormant, causing them to lose cell organelles and loosing tight interaction with adjacent myoid cells. Free radicals attack nearly all components of cell including proteins and DNA. It also impairs natural antioxidant defence mechanisms. Lead acetate resulting in the formation of free radicals, causing the lipid peroxidation of lysosomal membrane. This in turn leads to increased levels of lysosomal enzyme acid phosphatase.

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CONCLUSION

This study identified that lead acetate causes significant disruption of basement membrane in seminiferous tubules of testis of adult rats and Ficus carica, concurrently administered, will have beneficial effects on disruption of basement membrane.

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REFERENCES


