ORIGINAL ARTICLE

Effect of Lead Acetate on Basement Membrane of Seminiferous Tubules of Adult Rat Testis and Protective Effects of *Ficus Carica*: A Histological Study

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Abstract

Objective: To determine the effects of lead acetate and *Ficus carica* on disruption of basement membrane in seminiferous tubules of adult rat testis.

Study Design: An experimental study.

Place and Duration of Study: Department of Anatomy, Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad, from March to November 2017.

Methodology: Thirty male adult Sprague Dawley rats were selected and divided into three groups, each with ten animals. All treatments were given once daily for a period of eight weeks. Control was labelled as group A. Group B was administered lead acetate at a dose of 30 mg/kg body weight. Group C was treated with lead acetate at a dose of 30 mg/kg body weight and *Ficus carica* at a dose of 80 mg/kg body weight. Animals were dissected 24 hours after the last dose. Testis were treated, fixed and stained for histological study. Disruption of basement membrane in seminiferous tubules was scored morphometrically on a scale of 0 (normal) to 3 (>70% tubules showing disruption) and statistically analysed.

Results: Significant number of seminiferous tubules showed disruption of basement membrane in group B (20%) as compared to group A (0%). Less severe disruption of membrane was seen in group C as compared to group B, which was statistically not significant (p=0.082).

Conclusion: Lead acetate causes significant disruption of basement membrane in seminiferous tubules of testis of adult rats but subsequent administration of *Ficus carica* reduces the effects on short term.

Key Words: *Basement membrane disruption, Ficus carica, Lead acetate, Seminiferous tubules, Testis.*

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.[1]

As a result of human activities, global lead contamination remains compelling and is inferable to the markedly expanded circulation of lead in environment.[2] Lead equally affects women and men.[3] 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.[4] 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.[5]

Lead is the leading cause of reproductive toxicity among metals, causing alterations in fertility.[6] Decreased fertility is due to alterations in the action of sex steroid hormones, specifically estrogen in the uterus.[7] Lead effects testicular growth by decreasing the seminiferous tubular diameter.[8] 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.[9]

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*.[10]

Components like amino acids, phenolic components, organic acids, fatty acids, aliphatic alcohols, hydro-

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carbons, and volatile compounds have also been isolated. Fig has numerous medicinal properties and is used as an anti-inflammatory, hypoglycemic, hypo-lipidemic, anticancer, and heamostatic 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.[11] 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,[12] 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.[13]

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 I: Frequencies and percentages of disruption of basement membrane (N=10) of control group A and experimental groups B and C.

Parameter	Findings	Group A	Group B	Group C
Disruption of basement membrane	No effect 0	10 (100%)	0 (0.0%)	2 (20%)
	Slight effect 1	0 (0.0%)	1 (10%)	3 (30%)
	Moderate effect	20 (0.0%)	3 (30%)	4 (40%)
	Severe effect	30 (0.0%)	6 (60%)	1 (10%)

N = Number of rats

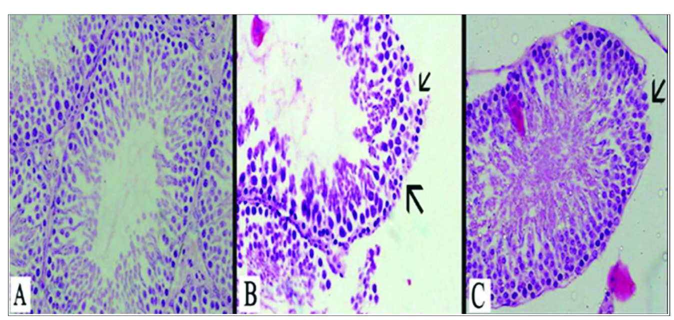

Figure 1: Photomicrograph showing comparison of disruption of basement membrane. **(A)** Intact basement membrane in control group A, **(B & C)** showing disruption of basement membrane (arrow): 40X, H&E.

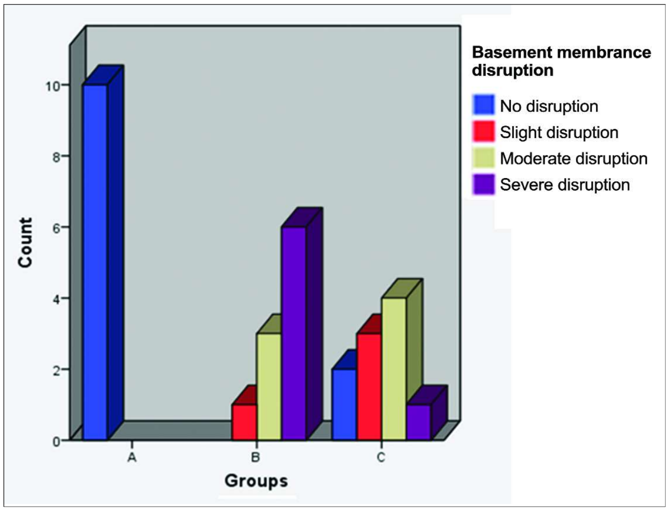

Figure 2: Cluster bar chart showing frequency of disruption of basement membrane (N=10) among the control group A and experimental groups B and C.

Table II: Showing comparison of p-values of disruption of basement membrane (N=10) among control group A and experimental groups B and C.

	Group A vs. B	Group A vs. C	Group B vs. C
Disruption of basement membrane	0.000*	0.006	0.082

≤0.05 P-value is statistically significant.
** = highly significant; N = Number of rats*

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 voluminuous spectrum of anatomical, physiological and biochemical effects in humans.[14] *Ficus carica* is widely used dried fruit having potent antioxidant activity.[10] The nutritional combination of dried *Ficus carica* verified and found that it has the premium nutriment aggregate, consisting of minerals and vitamins.[15] 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.[16] 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.[17] According to the study by Khaki,[16] 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.[5] 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.[18]

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,[19] which are responsible for its antioxidant activity and making it free radical scavenger.[20] Alkaline phosphatase is normally present in basement membrane, it helps in maintaining integrity and permeability,[21] 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.[22] On comparing experimental group B with experimental group C, the p-value was 0.082, and found to be statistically insignificant.

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

1. Sajitha G, Jose R, Andrews A, Ajantha K, Augustine P, Augusti K. Garlic oil and vitamin E prevent the adverse effects of lead acetate and ethanol separately as well as in combination in the drinking water of rats. *Indian J Clin Biochem* 2010; **25**:280-8.
2. Tong S, Schirnding YEV, Prapamontol T. Environmental lead exposure: a public health problem of global dimensions. *Bull World Health Organ* 2000; **78**:1068-77.
3. Winder C. Lead, reproduction and development. *Neurotoxicology* 1993; **14**:303-17.
4. Staudinger KC, Roth VS. Occupational lead poisoning. *Am Fam Physician* 1998; **57**:719-26.

5. Tomer V, Sangha JK, Ramya H. Pesticide: An appraisal on human health implications. *Proc Natl Acad Sci India Sect B Biol Sci* 2015; **85**:451-63.
6. Qureshi N, Sharma R. Lead toxicity and infertility in female Swiss mice: A review. *J Chem Biol Phys Sci* 2012; **2**:1849.
7. Tchernitchin AN, Gaete L, Bustamante R, Báez A. Effect of prenatal exposure to lead on estrogen action in the prepubertal rat uterus. *ISRN Obstet Gynecol* 2011; **2011**:329692.
8. Ahmad I, Sabir M, Yasin KF. Study of the effects of lead poisoning on the testes in albino rats. *Pak J Med Res* 2003; **42**:97-101.
9. Hamadouche NA. Reproductive toxicity of lead acetate in adult male rats. *Am J Sci Res* 2009; **3**:38-50.
10. Joseph B, Raj SJ. Pharmacognostic and phytochemical properties of *Ficus carica* Linn-An overview. *Int J Pharmt Res* 2011; **3**:8-12.
11. Hessler J, Lehner N. Planning and designing research animal facilities: Academic Press; 2011.
12. Schneider CA, Rasband WS, Eliceiri KW. NIH image to image J: 25 years of image analysis. *Nat Methods* 2012; **9**: 671-5.
13. Lysiak JJ, Turner SD, Nguyen QAT, Singbartl K, Ley K, Turner TT. Essential role of neutrophils in germ cell-specific apoptosis following ischemia/reperfusion injury of the mouse testis. *Biol Reprod* 2001; **65**:718-25.
14. El-Tantawy WH. Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats. *J Tradit Complement Med* 2016; **6**:327-31.
15. Zubair R, Baig A, Aliyu I. Non-toxic antiproliferative effect of *Ficus carica* fruit extracts on estrogen receptor positive breast cancer cell (MCF-7). *J Chem Pharm Res* 2015; **7**:815-21.
16. Khaki A, Tubbs R, Shoja M, Rad J, Khaki A, Farahani R, *et al*. The effects of an electromagnetic field on the boundary tissue of the seminiferous tubules of the rat: A light and transmission electron microscope study. *Folia Morphol* 2006; **65**:188-94.
17. Dixit A, Goyal R. Evaluation of reproductive toxicity caused by indigo carmine on male Swiss albino mice. *Pharmacology* 2013; **1**:218-24.
18. Wang J, Zhu H, Yang Z, Liu Z. Antioxidative effects of hesperetin against lead acetate-induced oxidative stress in rats. *Indian J Pharmacol* 2013; **45**:395.
19. Badgujar SB, Patel VV, Bandivdekar AH, Mahajan RT. Traditional uses, phytochemistry and pharmacology of ficus carica: A review. *Pharm Biol* 2014; **52**:1487-503.
20. Patil V, Patil V. *Ficus carica* Linn. An overview. *Res J Med Plant* 2011; **5**:246-53.
21. Liu Z, Sun Y, Su L, Sun Y, Kong S, Chang X, *et al*. Effects of cisplatin on testicular enzymes and Sertoli cell function in rats. *Fundam Toxicol Sci* 2015; **2**:137-45.
22. Aitken RJ, Roman SD. Antioxidant systems and oxidative stress in the testes. Molecular mechanisms in spermatogenesis: *Springer;* 2009; p. 154-71.