THE EFFECT OF FICUS CARICA ON LEAD ACETATE INDUCED CHANGES IN THE HEIGHT OF GERMINAL EPITHELIUM OF SEMINIFEROUS TUBULES OF ADULT RAT TESTES

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ABSTRACT

Objective: To evaluate the effect of Ficus carica on lead acetate induced changes in the height of germinal epithelium of seminiferous tubules in testes of adult rats.

Study Design: Randomized controlled trial.

Place and Duration of Study: The study was conducted for a period of 8 weeks at Departments of Anatomy and Pathology, Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad Pakistan.

Material and Methods: Thirty healthy adult male Sprague-dawley rats were selected by simple random sampling and divided into three groups. Each group consisted of 10 animals. Group A served as control. Group B was given lead acetate 30 mg/kg body weight/day. Group C received Ficus carica 80 mg/kg bodyweight/day in addition to lead acetate 30 mg/kg bodyweight/ day. The dose was administered once a day for a period of 8 weeks. Twenty-four hours after administration of last dose, the animals were sacrificed and testes were removed. Testicular tissue was processed and stained with haematoxylin and eosin. Height of germinal epithelium of the seminiferous tubules was measured. The data was analyzed using SPSS version 22. Results were considered significant at p-value<0.05.

Results: In experimental group B, significant decrease in height of germinal epithelium was observed as compared to the control group of the germinal epithelium. In experimental group C, the height increased as compared to experimental group B.

Conclusion: Our study proposes that the laboratory induced deleterious effects of lead acetate in the testes of adult rats may be limited by administering concomitant supplement of Ficus carica in adult rats.

Keywords: Ficus carica, Germinal epithelium, Lead acetate.

INTRODUCTION

The testes are the principal organs of reproduction, consisting of germ cells. The organ maintains a continuous supply of spermatids by nurturing germ cells. Within the environment, there are many hazardous factors which negatively affect the germ cell - spermatid balance, hence interfering with reproduction.

Appearance wise metals are hard, shiny and malleable. Manufacturing of construction products, varnishes, wires, stainless steel and cooking utensils etc. involves metals as an integral part. Within the periodic table, lead belongs to post-transitional metals. They have high density. Lead is soft and moldable. Due to abundance and low cost, lead is extensively used in construction, plumbing, batteries, bullets, paints, enamels and glazes.

Amongst the occupational and environmental pollutants, lead is the most widely studied metal. For past 6000 years, the hazardous nature of lead is known to man. Through air, food and water, general population can be exposed to lead[1]. Human beings, be it male or female, are equally affected by lead[2]. Lead tops the list of reproductive toxicity caused by metals. Toxicity caused by lead can affect the gonadal anatomy and physiology, and can also cause infertility[3]. The common results of lead poisoning in males are decreased libido, chromosomal

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damage, anomalous spermatogenesis, decreased mobility and count of sperm, abnormal prostatic function[2]. Lead toxicity causes changes in epithelium of epididymis, which may be an integral contributory factor in infertility[4]. In adult male rats, decrease in weight of testes, degeneration and necrosis of spermatogenic and interstitial cells of leydig and absence of spermatogenesis at high doses are all manifestations of toxic effect of lead[5].

For centuries vegetables, fruits and nuts have been used for their beneficial health effects on humans. Ficus carica (common fig) is widely used for its beneficial effects on reproductive health. The presence of antioxidants in the plants was the main reason behind their activity against infertility. The antioxidant properties of fig were attributed to phenolic compounds. Figs contain a wealth of beneficial vitamins including vitamin A, vitamin B1 and vitamin B2. Diverse compounds such as mucilage, enzymes, flavonoids, nicotinic acid and tyrosine are plentiful in Ficus carica. High levels of polyphenols, flavonoids, anthocyanin's and antioxidant capacity are the potential health-boosting components of Ficus carica[6].

Although many studies have asserted that exposure to lead acetate have critical effects on testicular structure and function. But the beneficial effects of Ficus carica have not been studied on testicular tissue damaged by lead acetate. Thus, the aim of the study was to evaluate the protective effects of fig on germinal epithelium.

MATERIAL AND METHODS

This randomized controlled trial study was approved by Ethical Review Committee of Army Medical College Rawalpindi and was carried out in the Department of Anatomy and Pathology, Army Medical College Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad. Thirty healthy adult male Sprague-dawley rats, 9-11 weeks of age, with weights ranging from 250-350 gm. were housed in separate cages in a well-ventilated and spacious room. Animals were selected via simple random sampling. Cycles of 12 hours light and 12 hours dark were maintained under a temperature range of 20-26°C with the help of central temperature regulating system[7]. Rats were fed

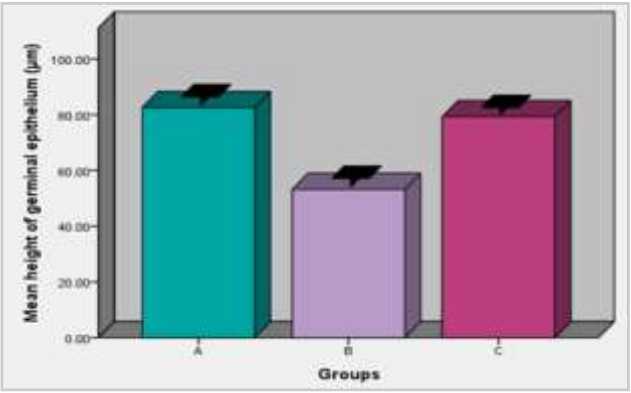

Figure-1: Bar chart showing comparison of mean values of height of germinal epithelium (µm) among the control group A and experimental group B and C.

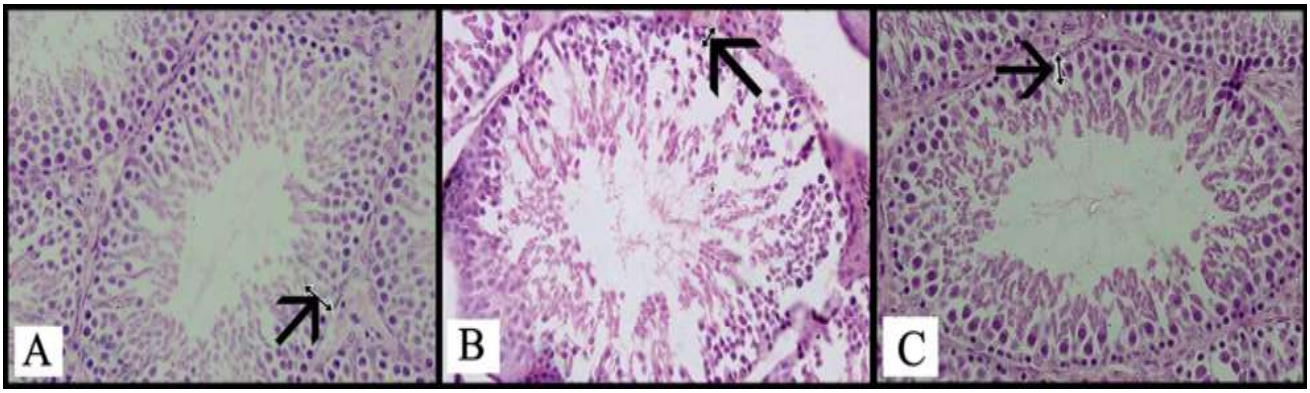

Figure-2: Photomicrograph showing comparison of height of germinal epithelium in control group A and experimental group B and C. Arrows showing the height of germinal epithelium: 40X, H&E.

NIH standardized lab diet for two months. Water was provided ad libitum. All doses were administered using oral gavage once daily for a period of 8 weeks.

Rats were divided into three groups (n=10 in each group) by lottery method. Group A rats served as controls remained untreated. Group B was given Lead acetate 30mg/kg/day[8]. Whereas group C was given Lead acetate 30mg/kg/ day and Ficus carica 80mg/kg/day[9]. By the end of eight weeks, the animals were sacrificed; dissected and fresh right testes specimens were taken out. Testes were placed in 10 per cent 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. Right testes of thirty animals was observed. Three sections per animal were observed. Total thirty seminiferous tubules were observed in each group. One tubule was selected from each section and from each tubule height of germinal epithelium was taken from basement membrane towards the center till spermatids at three different points[10]. Then mean height was taken for each animal. In each tubular cross-section, the height of germinal epithelium was observed with the help of ocular micrometer at 40X magnification. Images were taken from each section with the help of Olympus digital camera (12-mega pixel). The images were then transferred to laptop. Each image was opened in Image J v1.48[11]. A scale was set at 40X to measure the height in micrometer. Measurement tool 'straight' was selected and the height to be measured was calculated by drawing a straight line. The measurements were then analyzed and recorded. Results were expressed as means for each specimen in micrometers and taken as final reading for that specimen.

The data were entered in the database using statistical package for social sciences (SPSS version 22). Diameter of seminiferous tubule was expressed as mean ± standard deviation and the significant difference was determined using one way analysis of variance (ANOVA) followed by post Hoc Tukey test. Results were considered significant at $p \leq 0.05$.

Table-I: Comparison of mean values of height of germinal epithelium in control group A and experimental groups B and C.

Parameter	Control group A Mean ± SD	Experimental group B Mean ± SD	Experimental group C Mean ± SD	*p*-value
Height of germinal epithelium (µm)	82.56 ± 4.52	53.18 ± 4.53	79.24 ± 3.18	<0.001

Table-II: Comparisons of mean values of height of germinal epithelium in control group A and experimental groups B and C.

(I) Group	(J) Group	Mean Difference(I–J)	Std. Error	*p*-value
A	B	29.377*	1.925	<0.001*
	C	3.324	1.925	0.214
B	A	-29.377*	1.925	<0.001*
	C	-26.052*	1.925	<0.001*
C	A	-3.324	1.925	0.214
	B	26.052*	1.925	<0.001*

p-value<0.05 is considered statistically significant.

RESULTS

This study were conducted to evaluate the effect of Ficus carica on lead acetate induced histomorphological changes in testis of adult rats. For this purpose thirty Sprague-Dawley male rats were equally divided into three groups.

Mean height ± SD of germinal epithelium in group A was 82.56 ± 4.52µm (normal limit values of germinal epithelium[12,13]), while it was 53.18 ± 4.53µm and 79.24 ± 3.18µm in experimental groups B and C respectively (table-I). The mean ± SD of height of germinal epithelium of control group B was statistically significant as compared

to experimental group A. When experimental group C was compared to control group A, the *p*-value was statistically insignificant (*p*-value=0.214) (table-II; fig-1 & 2). On intergroup comparison, experimental group B showed high statistical significance (*p*-value <0.001*) when compared with experimental group C (table-II; fig-1 & 2).

DISCUSSION

Lead (Pb) is a heavy noxious metal that actuates a voluminous spectrum of anatomical, physiological and biochemical effects in humans[14]. The nutriments (nutrition) combination of dried Ficus carica testified that it has the premium nutriment aggregate amid the dried fruits, constituting a substantial wellspring of minerals and vitamins[15].

In the current study, slides were observed for histomorphological examinations of testicular specimens. Slides were observed for height of germinal epithelium. The experimental group B which was exposed to lead acetate had significant decrease in height of germinal epithelium in comparison with group A which served as control. This decrease was highly statistically significant with *p*-value of <0.001*. The result of this study are in accordance with the conclusion of Kata study[16], showing a decrease in the height of germinal epithelium. Mittal *et al*[17] explained that deleterious effects are produced by free radicals in living systems. Reactive oxygen species causes inflammation and tissue injury. Free radicals attack nearly all components of cell including proteins and DNA. It also impairs natural antioxidant defence mechanisms[18]. Lead acetate causes the formation of free radicals, causing the lipid peroxidation of lysosomal membrane. This in turn leads to increased levels of lysosomal enzyme, acid phosphatase[19]. Alkaline phosphatase (ALP) is found in interstitial cells, seminiferous tubule and basement membrane, playing a key role in transfer of materials from sertoli cells to germinal cells and growth of the germinal epithelium[20]. In this study, the height of germinal epithelium in experimental group C was close to control group A with *p*-value of 0.214, which was statistically insignificant. The free radical scavenging activity is mainly because of phenols. Ficus carica is rich in phenols and phytosterols[21]. Phenols restore the germ cells number and sertoli cells morphology causing an increase in height of germinal epithelium. While comparing experimental group B with experimental group C, *p*-value was 0.000* which is highly statistically meaningful, denoting a significant increase in height of germinal epithelium in experimental group C in comparison to experimental group B. Ficus carica improves the status of oxidation by causing a decrease in lipid peroxidation and increasing the antioxidants and isoflavones like biochanin A, beta-sisterol and alpha-amyrin[21].

CONCLUSION

Our study proposes that the laboratory induced deleterious effects of lead acetate in the testes of adult rats may be limited by administering concomitant supplement of Ficus carica in adult rats.

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Author's Contribution

Ayesha Asad conceived the idea, created the manuscript, Afnan Gulanalyzed the data. Maria Yousafdid the critical analysis and Brig. Khadija Qamar revised the manuscript.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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