Original Article

Effect of Ficus carica on lead acetate induced changes in diameter of seminiferous tubules of adult rat testis

Ayesha Asad, Shabnam Hamid, Saima Qureshi

Department of Anatomy, Army Medical College, National University of Medical Sciences, Rawalpindi, Pakistan

Objective: To assess the effect of Ficus carica on lead acetate induced changes in diameter of seminiferous tubules in testis of adult rats.

Methodology: Thirty healthy adult male Sprague-dawley rats were selected and divided into three groups; each consisting of 10 animals. Group A served as control. Group B was given lead acetate 30 mg/kg bodyweight/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 hematoxylin and eosin. Diameter of seminiferous tubules was measured. The data were analyzed using SPSS version 22. Results were considered significant at $p \leq 0.05$.

Results: Diameter of seminiferous tubules remained within normal limits in control group A. In experimental group B, significant increase in diameter of seminiferous tubules was observed. In experimental group C, the diameter decreased as compared to experimental group B.

Conclusion: Lead acetate induced increase in diameter of seminiferous tubules and administration of Ficus carica significantly prevented the increase in diameter. (Rawal Med J 202;46:745-748).

Keywords: Diameter, Ficus carica, seminiferous tubules.

INTRODUCTION

The testes are paired structure for production of germ cells. There are several factors in the environment which adversely affect the sperms. The use of metals is an integral part of our daily lives. Lead is the most widely studied occupational and environmental pollutant and general population is exposed to it through water, air and food.[1] Toxicity by lead can affect the function and structure of gonads, and can cause alterations in fertility.[3] Reduced libido, chromosomal damage, anomalous spermatogenesis (decreased mobility and count), abnormal prostatic function, infertility and variations in serum testosterone are common outcomes of lead poisoning in males.[2] Changes in epididymal epithelium may be an essential contributory factor in infertility caused by lead.[4]

Toxic effects of lead acetate on adult male rats revealed decrease in weight of testis, degeneration and necrosis of spermatogenic and interstitial cells of leydig and absence of spermatogenesis at high doses.[5] Phenolic compounds are the major components of *Ficus carica* and the antioxidant properties were attributed to them. 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] Beneficial effects of Ficus carica include hepatoprotective, hypoglycemic, hypolipidemic, anticancer, antipyretic, scavenging activity and immune response, in addition to antioxidant activity.[6] The aim of this study was to assess the effect of Ficus carica on lead acetate induced changes in diameter of seminiferous tubules in testis of adult rats.

METHODOLOGY

This study was approved by Ethical Review Committee of Army Medical College Rawalpindi and was carried out in the Departments 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. 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 NIH standardized lab diet for two months. Water was provided *ad libitum.*

Rats were divided into three groups (n=10 in each group). Group A rats served as controls were untreated. Group B was given Lead acetate 30mg/kg/day.[8] Group C was given Lead acetate 30mg/kg/day and *Ficus carica* 80mg/kg/day.[9] All doses were administered using oral gavage once daily for a period of eight weeks.

By the end of eight weeks, the animals were sacrificed; dissected and fresh testis specimens were taken out. The sections were stained with hematoxylin and eosin (H&E) for routine histological study of testis under light microscope. The diameters of seminiferous tubules with profiles that were round or nearly round were estimated for each animal and a mean + SD of the seminiferous tubule diameter was determined. Three sections per animal were observed with the help of ocular micrometer at 40X magnification.

One tubule was selected from each section and diameter was calculated from the center of each tubule by taking the average of two measurements, D1 and D2 (perpendicular to one another).[10] 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 diameter in micrometer. Measurement tool 'straight' was selected and the diameter to be measured was calculated by drawing a straight line. The measurements were then analyzed and recorded.

Statistical Analysis: SPSS 23 was used for statistical analysis. Significant difference was determined using one way analysis of variance (ANOVA) followed by post Hoc Tukey test. Results were considered significant at $p \leq 0.05$.

RESULTS

The mean diameter of seminiferous tubule for control group A was 378.02±3.85 µm (Table 1). On intergroup comparison, control group A was statistically significant as compared to experimental group B (p=0.000*) and experimental group C (p=0.000*) (Table 2).

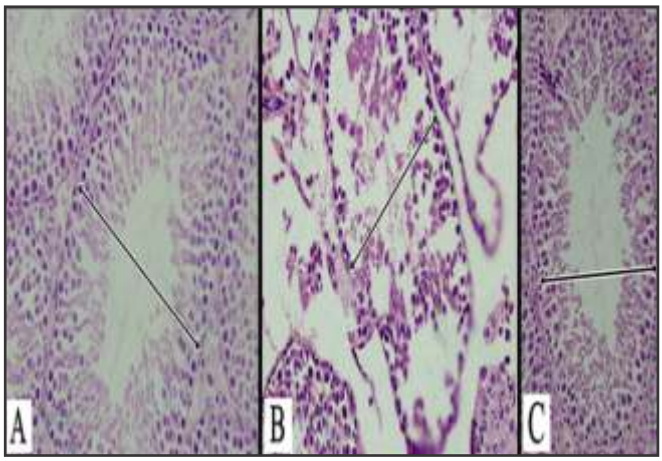
Table 1. Mean diameter of seminiferous tubule all groups.

Parameter	Control group A	Experimental group B	Experimental group C
Diameter of seminiferous tubule (µm)	378.02 ± 3.85	425.68 ± 5.42	392.43 ± 3.93

Table 2. Comparison of p values of diameter in all groups.

Parameter	Control group A vs. B	Experimental group A vs. C	Experimental group B vs. C
Diameter of seminiferous tubule (µm)	0.000*	0.000*	0.000*

Figure 2: Photomicrograph showing comparison of seminiferous tubular diameter in control group A. experimental group B and C: 40X, H&E.



The diameters were significantly increased in experimental Group B, which showed detrimental effects of lead acetate. The experimental group B and C had mean diameter of 425.68±5.42 µm and 392.43±3.93 µm, respectively and when experimental group B and C were compared, the values were statistically significant (p-value=0.000*). The increase in diameters in experimental group C depicted that *Ficus carica* had propitious effects.

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.[12] Oxidative stress caused by lead acetate may lead to the production of free radicals and changes in oxygen free radical scavenging enzyme system and blemish membrane framework.[13]

The nutriments 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.[14] *Ficus carica* has been conventionally passed down in the medication of distinct diseases such as digestive disorders including decreased appetite, colic, dyspepsia, loose motions and other disorders.[15]

In the present study, lead acetate treated rats showed significant changes in seminiferous tubules with apparent blood vessel congestion These results were in accordance with the previous studies conducted by Elgawish and Abdelrazek who documented dose dependant testicular damage in lead acetate treated rats.[8] In another study, performed by Dorostghoal et al showed pronounced degeneracy of most seminiferous tubules. [16] A study by Sudjarwo et al postulated that lead arouses the production of reactive oxygen species, thus generating oxidative contamination to different organs causing changes in membrane physiology, which is in accordance to our study.[13]

Hassan et al.[17] also strengthens our conclusion that toxicity caused by lead causes testicular impairment, this may be the due to its potential to elicit peroxidation in lipid bilayer evoking cell death and oxidation stress. One of the reasons for oxidative stress is high amount of polyunsaturated fatty acids in cells of testis, this was proposed by El-Khadragy.[18] However, Kata et al postulated a decrease in diameter of seminiferous tubules, which is not in accordance with the current study.[19]

Our results are similar to results of Haredy et al which documented that antioxidant enzyme activity within the seminiferous tubules significantly improved by *Ficus carica* treatment, the reason being that flavanoids is one of its main components and are antioxidant boosters.[20] Abu Bakar et al initiated that quercetin defers oxidant abuse and apoptosis which is caused by free radical oxidation, this is in accordance with our study.[21] Arvaniti et al described that *Ficus carica*'s antioxidant capability is directly proportional to the extent of phenolic compounds.[22]

Phenolic compounds performs its functions by hydrogen donators, free radical scavengers, singlet oxygen quenchers , this was proposed by Fahmy et al and is in agreement with our study.[15] Our results are similar to study by Samsulrizal et al which reinforces that *Ficus carica* vitiates testicular damage in rats by causing a significant abate in oxidative stress markers, free radicals counterbalancing, expanding cell efficiency.[23]

CONCLUSION

Our study proposes that lead acetate causes significant increase in diameter of seminiferous tubules of testis of adult rats which is caused by inhibition of antioxidant enzymes, this inhibition leads to significant surge in free radicals in testicular tubules. This in turn affects reproductive prolificity in rats. *Ficus carica* concurrently administered will have beneficial effects on seminiferous tubules.

Author Contributions:
Conception and design: Shabnam Hamid
Collection and assembly of data: Ayesha Asad
Analysis and interpretation of the data: Ayesha Asad
Drafting of the article: Saima Sohail
Critical revision of the article for important intellectual content: Shabnam Hamid
Statistical expertise: Ayesha Asad
Final approval and guarantor of the article: Shabnam Hamid
Corresponding author email: Ayesha Asad: ashi_asad@live.com
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