

INFLAMMATION OF INTERSTITIAL CONNECTIVE TISSUE SPACE INDUCED BY LEAD ACETATE AND AMELIORATIVE AFTERMATH OF FICUSCARICA ON RAT TESTIS

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ABSTRACT

Objective: To observe inflammation of interstitial connective tissue space caused by Lead acetate in rat testis and ameliorative after math caused by Ficuscarica.

Study Design: Laboratory based experimental study.

Place and Duration of Study: Anatomy Department, Army Medical College Rawalpindi together with NIH (National Institute of Health) Islamabad, from Mar to Nov 2017.

Methodology: Sprague Dawley male rats, 30 in quantity were chosen and 10 animals each partited into 3 groups. Treatments were given for 8 weeks, once daily. Group A was control group. Group B was treated with dosage of 30 mg/kg of Lead acetate. Group C was given dosage of 30 mg/kg of Lead acetate as well as 80 mg/kg of Ficuscarica. Twenty four hours after the concluding dose, animals were vivisected. For histological study, testis were fixed and stained with Haematoxylin and eosin. Interstitial connective tissue space thickness was morphometrically and assessed by SPSS version 22. A *p*-value <0.05 was considered significant in results.

Results: Interstitial space thickness was significantly increased due to inflammation (>3 times normal) in group B in comparison to groups A and C. Thickness of space was slightly increased (<2 times normal) in group C in comparison to groups A due to reduction in inflammation.

Conclusion: There was increased thickness of interstitium due to inflammation, cellular congestion and lymphocytic infiltration in rat's testis because of lead acetate but concomitant dose of Ficuscarica protects against inflammation, venous congestion of interstitial space.

Keywords: Ficuscarica, Inflammation, Interstitial connective tissue space, Lead acetate.

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INTRODUCTION

In modern world, metals are part and parcel of day to day routine. One of the post transitional metal is lead. Lead is flexible, with elevated density and decreased melting point¹. Due to easy availability and being cheap, lead is widely used in daily use products such as; toy batteries, paints, construction, and varnishes. To make the lipstick holding on lips for longer durations; lipsticks have higher lead content which is six times greater than authorized by Food and Drug Administration². Lead is the much largely prepensed vocational and natural pollutant. Lead has been precarious, for last 6000 years. Lead exposure to humans occurs via water, air and food³.

Greatly increased circulation of lead is due to worldwide lead spoliation due to human activities⁴. Lead exposure is dangerous for health of humans as it interferes with biochemical processes. Abundant workplace exposure of lead in workers have been found to have higher level of lead concentration in the blood⁵. Females and males are uniformly affected by lead⁶.

According to NIOSH-National Institute of Occupational Safety and Health, 3 million employees are divulged to lead poisoning⁷. The amount and time of lead exposure afflicts cardiovascular system, reproductive system, kidney and central nervous system⁵.

Within metals, lead is noted cause of reproductive toxicity. Structure and function of reproductive organs can be changed by lead poisoning, leading to changes in fertility⁸. Adverse after math caused by Lead acetate in adult male rats caused reduction in testicular weight, spermatogenic and Leydig cells showed degeneration and noxiousness and no signs of spermatogenesis at large doses along with inflammation of interstitium.

Vegetables, fruits and nuts have been known for a long time to improve health of human beings. Oxidative chain reactions that are constantly occurring in human body can be broken down by antioxidants. Ficuscarica, Fc (common fig) is widely used. Fig is a part offamily Moraceae. Fig constitutes the biggest genera of herbal medicines⁹. Fig is abundant in copper, magnesium, fiberand otherminerals. Fig is one of the largest sources of antioxidants. Infertility in humans can be cured by fig, as it has antioxidant properties.

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High levels of polyphenols, flavonoids, bioactive compounds like arabinose, carotenes, xanthoxolane the plausible strength-bolstering components of *Ficus carica*.

Fig has numerous medicinal properties. Fig raised the plasma antioxidant levels and is used as a haemostatic, anti-inflammatory and anti-helminthic agent. Fig causes reduction in blood glucose and blood cholesterol levels. Fig also fights against cancer. Fig aggrandized in sperm count & sperm numbers by suppressing ROS production.

The testes are paired ovoid organs that reside within scrotum. Average testicular dimensions are 3-4 cm in length, 2-2.5 cm in breadth and 2-3 cm in anteroposterior diameter. A single lobule of testes has 1-4 seminiferous tubules, where production of sperms takes place. The tissue between the seminiferous tubules is the interstitial connective tissue space¹⁰.

The objective of the research was to evaluate the inflammation of interstitium by Lead acetate in testis of adult rats and probable fruitful aftermath of *Ficus carica*.

The rationale of my study was to evaluate the fruitful effects of fig on organs, whose anatomy is disrupted by noxious metals in the environment.

METHODOLOGY

The research was a laboratory based experimental study, IERB approval number ERC/MS-17/Anatomy, of the AMC Rawalpindi. This research was conducted in the Department of Anatomy, Army Medical College, jointly with Armed Force Institute of Pathology (AFIP) Rawalpindi and NIH - National Institute of Health (NIH), Islamabad. Male adult Sprague Dawley healthy rats, 30 in number were taken. Age of the rats was between 9-11 weeks; rats weighing 200-250gms were used for this practical, 20-26°C temperature was maintained for rats and they were put in a well-ventilated room. Twelve hour night-day sleeping cycle was maintained during the whole time of practical¹¹. Standard laboratory diet was given to rats arranged by NIH. Rats were given water ad libitum. The drug and fig were given via oral gavage needle day to day for 8 weeks.

Rats in group A served as untreated controls. Group B rats were treated with Lead acetate 30mg/kg/day¹² whereas group C rats were treated with 30mg/kg/day Lead acetate and 80mg/kg/day *Ficus carica*¹³. On reaching term of experiment i.e. 8 weeks, the animals were vivisected and right specimens of testis were removed. Testes were put in formalin with a

concentration of 10%, in duly labeled separate plastic containers. The coronal section of each right testis was taken. Before embedding, it was ensured that the sections were oriented perpendicular to their long axes. Specimens were put in duly labeled tissue tek cassettes and processed in Leica TP 1020 automatic tissue processor. Sections were processed through the increasing concentration of alcohol from 70-100%, cleared in xylene. Paraffin wax with melting point 58°C was used for infiltration and embedding. For this purpose, paraffin embedding center LEICA EG 1160 was used. The blocks were allowed to solidify on a cold plate and then in a rotary microtome 5-micron thick sections were made. H&E - Haematoxylin and eosin stain was brought to use for staining the sections, this stain is used to view histological slides of testis in a light microscope. Right testes of 30 animals were observed. One section per animal was observed. Total 30 interstitial spaces were observed in each group. From each section one tubule was chosen and interstitial connective tissue space thickness was estimated by calculating 3 distances of one interstitial space from one seminiferous tubule's basement membrane to the surrounding seminiferous tubule basement membrane¹⁴. An objective micrometer with 40X magnification was used to measure the interstitial space in each testes section. Digital camera by Olympus (12-mega pixel) was used to take pictures of section from each slide. The images were then transferred to laptop. Image J v1.4815 was used to open each image. To calculate the thickness in micrometer, a scale was set at 40X. 'Straight' tool was chosen for calculation and the thickness to be calculated was measured by making a straight line. The mean of the 3 distances was taken and average mean was calculated to attain the thickness in whole of the interstitial space for every Haematoxylin & Eosin stained section¹⁴. Statistics were evaluated with the help of statistical package for social sciences version 22. The thickness of the space was articulated as mean ± standard deviation. Difference was found out with the help of one way analysis of variance (ANOVA) and after that post Hoc Tukey test was applied. Significant *p*-value was taken as <0.05.

RESULTS

Thirty Sprague Dawley, male adult rats; whose age was between 9-11 weeks, were taken for this research. The average weight of rats was 300 ± 2.7gm.

The histology of the slides showed that Experimental group C and control group A displayed regular architectonic with closely arranged, oval or

semi-round seminiferous tubules (fig-1A & C). Group B showed pronounced changes with small diffuse seminiferous tubules and wide connective tissue space of interstitium due to inflammation, because of the hypoplasia of seminiferous tubules (fig-1B). Mean thickness \pm SD of connective tissue space of interstitium in Control group A was 111.43 ± 5.03 (μm), it was eminently statistically significant in comparison to experimental group B and C (p -value ≤ 0.01) Mean thickness \pm SD of interstitial space of experimental groups B and C were 140.92 ± 3.42 (μm) and 125.53 ± 3.59 (μm)

respectively (table-I, fig-2) (given at end of document, after the references). While doing comparison within the groups, increase statistical significance was showed by group B (p -value ≤ 0.01) with groups C (table-II) (given at end of document, after the references).

DISCUSSION

Lead acetate exposure is the leading health risk with universal magnitude¹⁶. Lead altersthe functioning of various vital body organs. There are cellular, structural and biological effects in humans caused by lead

Table-I: Comparison of mean value of thickness of interstitial connective tissue space within control group A and experimental groups B and C.

	Group A Mean \pm SD	Group B Mean \pm SD	Group C Mean \pm SD
Thickness of interstitial connective tissue space (μm)	111.43 ± 5.03	140.92 ± 3.42	125.53 ± 3.59

Table-II: Comparison of p-values of thickness of interstitial connective tissue space among control group A and experimental groups B and C.

	Group A vs. B	Group A vs. C	Group B vs. C
Thickness of interstitial connective tissue space	0.000*	0.000*	0.000*

p-value ≤ 0.05 is statistically significant, *Highly significant

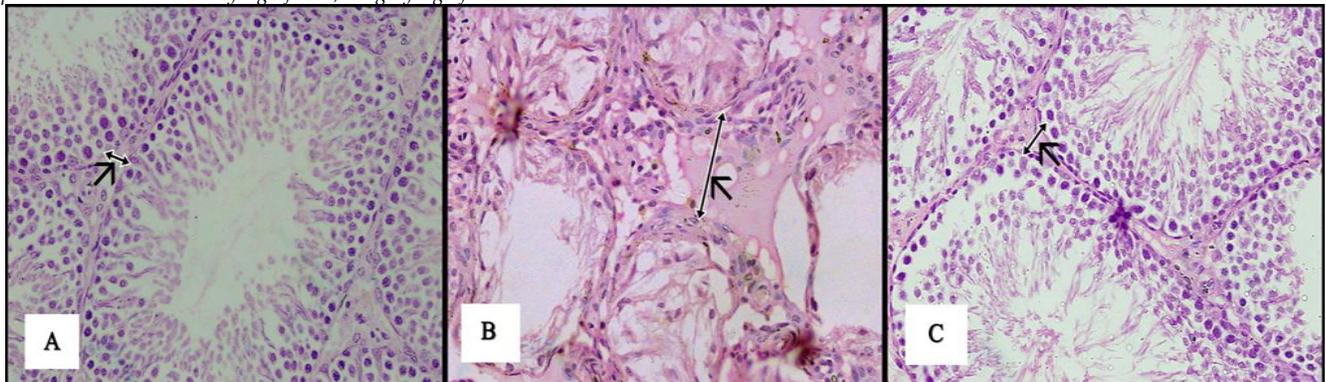


Figure-1: Photomicrograph depicting comparison of interstitial connective tissue space. (A) Measurement of interstitial connective tissue space thickness (Arrow) in control group A, (B) Increased interstitial connective tissue space due to inflammation (Arrow) in experimental group B, (C) Decreased interstitial connective tissue space (Arrow) in experimental group C as compared to group B: 40X, H&E.

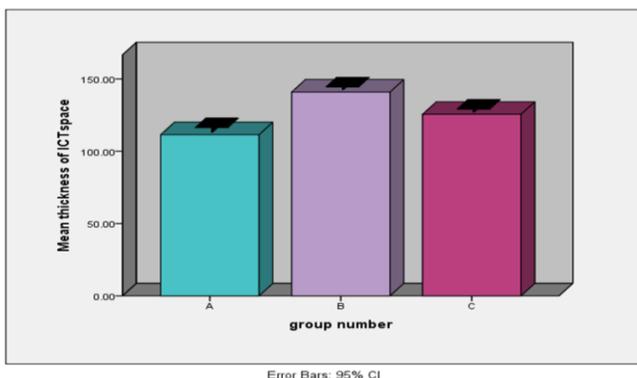


Figure-2: Comparison of mean values of thickness of interstitial connective tissue space among the control group A and experimental group B & C.

leading to extensive destruction¹⁷. Ficuscarica is extensively consumed raisin with numerous antioxidants. The antioxidants in figs stop the oxidative mechanisms causing destruction at cellular level¹⁸. The nutrients amalgam of dried Ficuscaricatells that it is the best dry frit to consume, having a variety of vitamins, antioxidants and minerals¹⁹. This research was devised to inspect Lead acetate effects on the testis of adult rats and investigate simultaneous dispensation of Ficuscarica, and its aftermath.

In this current study, histological slides of testes were studied under microscope for interstitial connective tissue space thickness. Vulnerability to clement dosage of lead acetate amends the testis architectonic with escalation in thickness of connective tissue space

of interstitium due to inflammation. In control group A the interstitial thickness remained in normal limits. We saw an escalation in the thickness of connective tissue space of interstitium because of the inflammation, there were also with distended blood capillaries in the experimental group B in comparison to control group A. This increase in thickness was highly statistically significant with p -value of <0.01 . The same conclusion was obtained by another study conducted in 2017 by Ahmed *et al*, that significant inflammation is seen in interstitial connective tissue space when noxious metal was administered¹⁴. A similar study was conducted in 2018 by Najat *et al*, who found out that interstitial connective tissue space was increased when excessive hormones were administered²⁰. One more study done in 2018 by Sudjarwo *et al*, establishes that lead acetate has deleterious effects on male reproductive system, causing distortion of seminiferous tubules and low sperm count²¹. The apprehension for escalation in interstitial connective tissue space thickness was due to the inflammation of interstitium, atrophy of seminiferous tubules, decrease in number of the Leydig cells and intertubular connective tissue space with lymphocytic infiltration and congestion of blood vessels²².

The interstitial connective tissue space in experimental group C was nigh to control group A and the p -value was found highly statistically significant (p -value ≤ 0.01). Interstitial cells, basement membrane and seminiferous tubules have alkaline phosphatase in them; its deficiency leads to hypoplasia of interstitial connective tissue space²³. Ficuscarica is gilded in xanthins, phytosterols and antioxidants. Figs by decreasing lipid peroxidation, restores alkaline phosphatase. A study conducted in 2017 by Hardey *et al*, supports the beneficial effects of Ficuscarica on rat testis stating that it helped in reversing the poisonous effects and reverted the seminiferous tubules histology to nearly normal with healthy spermatozoa²⁴. Hence exerting a shielding effect on rat testis against lead acetate poisoning.

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Disclosure

All the contributors of the article were serving at Army Medical College when the research took place.

CONCLUSION

Our research suggests that escalation in thickness of connective tissue space of interstitium was due to

inflammation, lymphocytic infiltration and cellular congestion in testis of adult rats, which was caused by lead acetate and Ficuscarica simultaneously. Ficuscarica will have defensive after math on the interstitium.

CONFLICT OF INTEREST

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

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