Original Article

Effect of Vit.C on Lead Toxicity

Protective Effect of Vitamin C on Diameter of Seminiferous Tubules and Spermatogenesis of Albino Rats with Lead Toxicity

1. Mujahid Akbar Mamoun 2. Syed Tariq Ali Rizvi 3. H. Mohammad Fareedullah Alvi

1. Asstt. Prof. of Anatomy, 2. Senior Demonstrator of Anatomy, 3. Asstt. Prof. of Anatomy,
Quad-A-Azam Medical College, Bahawalpur

ABSTRACT

Objectives: The study was undertaken to investigate whether lead toxicity can reduce the diameter and spermatogenesis of testes of albino rats. If the reduction occurs, with what dose the animals can be protected by vitamin C against lead toxicity.

Study Design: Experimental study.

Place and Duration of Study: This study was carried out at the Department of Anatomy, PGMI, Lahore from March 2007 to August, 2007.

Materials and Methods: In this study, 90 animals (albino rats) were taken from National Health Institute Islamabad. These were divided into five groups. Each group has 18 animals as group A,B,C,D and E.

Results: The lead treated animals reduced 16% of diameter in 4 weeks as reported by Harvey. The loss of diameter and reduction in spermatogenesis was due to lead toxicity. In another study by Biswas and Gosh, the animals gave same results with lead toxicity in 14 days. In this experiment it has proved that lead toxicity reduced the body weight of albino rats and this toxicity can be protected with heavy dose of vitamin C.

Conclusion: Vitamin C reduces the toxic effects of lead on diameter of seminiferous tubules and spermatogenesis of rats, which is shown in this study.

Key Words: Lead toxicity, Seminiferous tubules, Vitamin C

INTRODUCTION

Lead is a heavy metal present in earth crust. It is also end product of uranium disintegration. Lead is a common environmental toxic metal used by human beings for thousands of years. Lead is present as inorganic metal in lead oxide, lead chloride, lead sulfide etc and as organic metal in lead tetra ethyl chloride etc. Lead can replace the trace metals from human body such as calcium, copper, chromium, manganese and magnesium. Its absorption enhances from gastrointestinal tract if these trace elements are deficient in human beings.[1] No organ of the body is immune for lead poisoning if it is exposed to it chronically. Lead is continuously emitted from the industries.[2] The mechanism by which blood lead concentration increases depends upon both ingestion and inhalation. It can be mobilized from bone where lead is deposited.[3] It can catalyze the oxidative reaction and produce excessive reactive oxygen species.[4] Beverages and acidic foods can dissolve the lead from improperly glazed containers.[5] Heavy metals exert their toxic effects by combining with more reactive groups reducing the appetite and body weight.

MATERIALS AND METHODS

For this study, 90 animals (albino rats) were taken from National Health Institute Islamabad. These were divided into five groups. Each group has 18 animals as group A,B,C,D and E . The animals of group A were given 1 cc normal saline daily intraperitoneally. Group B animals were given lead acetate 10 mg/kg body weight daily intraperitoneally. Group C animals were given lead acetate 10 mg/kg body weight and vitamin C 250 mg/kg body weight daily intraperitoneally. Group D animals were given lead acetate 10 mg/kg body weight and vitamin C 500 mg/kg body weight daily intraperitoneally. Group E animals were given lead acetate 10 mg/kg body weight and vitamin C 1000 mg/kg body weight daily intraperitoneally.

In the beginning of experiment, Group A was divided into subgroup A1, A2 and A3. Group B into subgroup B1, B2 and B3. Group C, D and E were divided into C1, C2 and C3,D1,D2 and D3 and E1, E2 and E3 respectively. Subgroup 1 was sacrificed after 5th week, subgroup 2 was sacrificed after 6th week and subgroup 3 was sacrificed after 7th week. Lead acetate was purchased from Anarkali near King Edward Medical University, Lahore.

Statistical Analysis: All values were presented by SPSS version 17. The diameter of seminiferous tubules of all the same subgroups were taken and compared as A1, A2 and A3 etc, and with each other as A1, B1 and C1 etc. The significant and insignificant P values were calculated by ANOVA. The diameter was measured under low power microscope with electrometer. ANOVA was applied and P value was calculated. All these values are presented in the form of tables and graphs.

RESULTS

The rats which were treated with lead acetate showed a decrease in diameter and the rats which were treated with both lead acetate and vitamin C showed improvement as compared to those rats which were only treated with lead acetate as evident from table No. 1. The rats which were treated with maximum dose of vitamin C (1000 mg/kg body weight daily) showed the most improved diameter and spermatogenesis. Similarly in the subgroup 2 the effect of lead toxicity and its reversal by vitamin C is more pronounced as given in Table No. 2. The mean diameter of seminiferous tubules of subgroup B2 was 234.16 ± 28.53 µm and the mean diameter of seminiferous tubules of A2 was 266.41 ± 18.1 µm (given in Table No. 2). This showed a significant weight loss in these rats, only treated with lead acetate. The effect of lead toxicity was reversed by vitamin C. The higher the dose of vitamin C, the greater reversal was seen as evident from Table No. 1 and 2. In subgroup 3, the effect of reversal of lead toxicity with vitamin C was the most significant as given in Table No. 3.

Table No. 1 Diameter of Seminiferous Tubules in µm of Subgroup 1

Sub group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group A 1	6	242.9667	35.13122	14.34226	206.0987	279.8346	194.70	284.20
Group B 1	6	227.6500	23.91792	9.76445	202.5497	252.7503	193.10	260.90
Group C 1	6	230.6500	17.78637	7.26126	211.9843	249.3157	209.80	256.20
Group D 1	6	235.9667	19.66842	8.02960	215.3259	256.6074	208.60	260.50
Group E 1	6	238.5833	45.98419	18.77297	190.3259	286.8408	170.20	280.60
Total	30	235.1633	28.78003	5.25449	224.4167	245.9100	170.20	284.20

ANOVA					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	900.325	4	225.081	.243	.911
Within Groups	23120.085	25	924.803		
Total	24020.410	29			

Table No. 2: Diameter of Seminiferous Tubules of subgroup 2

Sub group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group A 2	6	266.416	18.196	7.428	247.320	285.512	241.70	291.60
Group B 2	6	234.166	28.532	11.648	204.223	264.110	206.40	280.30
Group C 2	6	241.466	26.047	10.634	214.131	268.802	208.20	278.70
Group D 2	6	246.866	17.675	7.215	228.317	265.415	217.40	261.70
Group E 2	6	260.066	19.690	8.038	239.402	280.730	231.30	282.50
Total	30	249.796	24.099	4.399	240.797	258.795	206.40	291.60

ANOVA					
	Sum of Squares	Df	Mean Square	F	P value
Between Groups	4223.808	4	1055.952	2.092	0.112
Within Groups	12619.342	25	504.774		
Total	16843.150	29			

Table No. 3: Diameter of Seminiferous Tubules of subgroup 3

Sub group	N	Mean	Std. Deviation	STD. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group A 3	6	263.316	17.445	7.122	245.008	281.624	236.40	283.80
Group B 3	6	221.166	36.561	14.926	182.797	259.536	200.70	293.80
Group C 3	6	237.383	43.104	17.597	192.148	282.618	190.20	308.30
Group D 3	6	244.166	31.501	12.860	211.108	277.225	197.80	280.90
Group E 3	6	260.850	28.273	11.542	231.178	290.521	206.80	290.20
Total	30	245.376	34.104	6.226	232.641	258.111	190.20	308.30

ANOVA					
	Sum of Squares	Df	Mean Square	F	P value
Between Groups	7276.495	4	1819.124	1.719	0.177
Within Groups	26454.178	25	1058.167		
Total	33730.674	29			

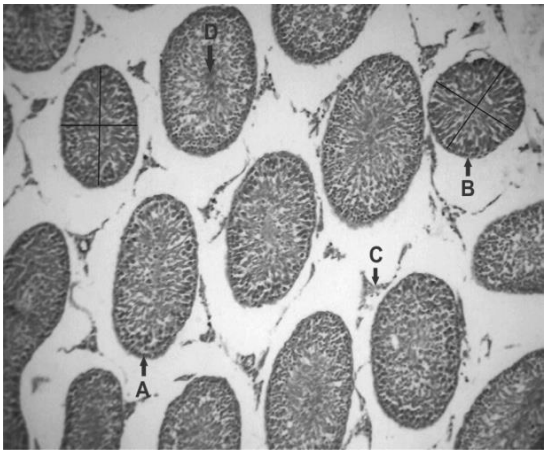

Figure No. 1: Photomicrograph of testicular tubules of group A1.

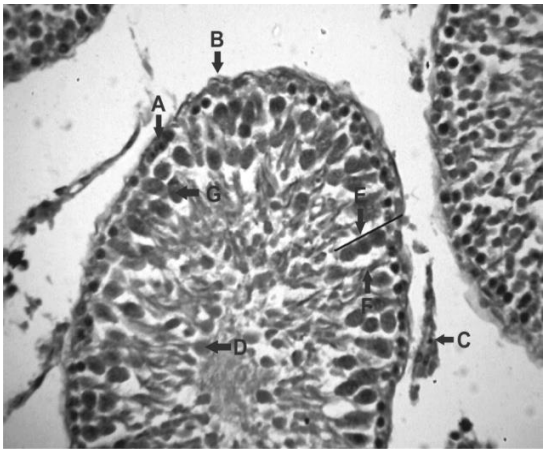

Fig No.2: Photomicrograph of testicular tubules of group A1.

A. A continuous single basal layer of spermatogonia. A. A single continuous basal layer of spermatogonia.
B. An intact basement membrane. B. An intact basement membrane without any disruptionn. C. Leydig cells C. Leydig cells visible.
D. Spermatids with the debris of spermatocytes in the lumen.D.Spermatids in the lumen and debris of spermatocytes. H & E stain, X 100.
E. Number of Epithelial cell layer reduced (4-5). F. Sertoli cell
G. Normal Size of Primary spermatocyte, number reduced. H & E stain, X 400.

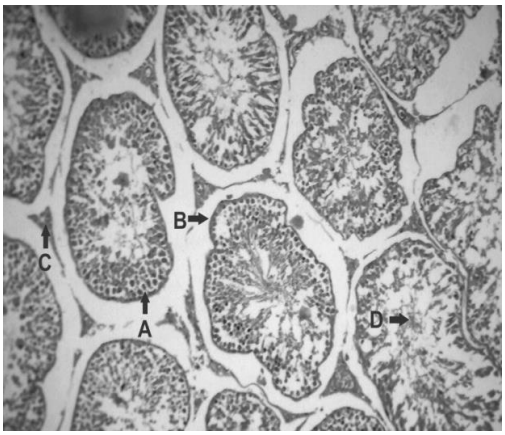

Figure No.3. Photomicrograph of testicular Tubules of group B1.

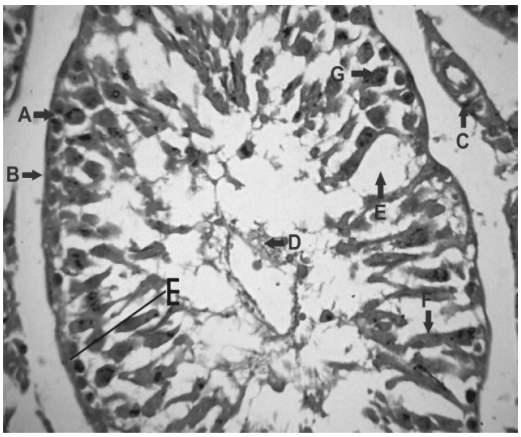

Figure No. 4. Photomicrograph of testicular tubules of group B1.

A. A continuous single basal layer of spermatogonia. A. A single continuous basal layer of spermatogonia.
B. An intact basement membrane. B. An intact basement membrane without any disruptionn. C. Leydig cells C. Leydig cells visible.
D. Spermatids with the debris of spermatocytes in the lumen. D. Spermatids in the lumen and debris of spermatocytes.
H & E stain, X 100. E. Number of Epithelial cell layer reduced (4-5). F. Sertoli cell
G. Normal Size of Primary spermatocyte, number reduced. H & E stain, X 400.

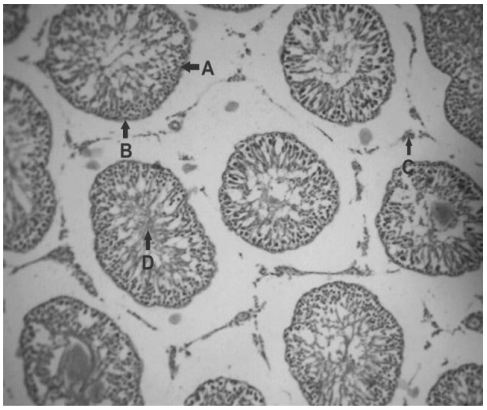

Figure No. 5: Photomicrograph of testicular tubules of group C1.

A. A single basal layer of spermatogonia.
B. An intact basement membrane.
C. Leydig cells present.
D. Spermatids in the lumen with the debris.
H & E stain, X 100.E. Epithelial cell layer reduced, 4-5 cells visible.
F. Sertoli cell present G. Primary spermatocyte reduced in size and number.

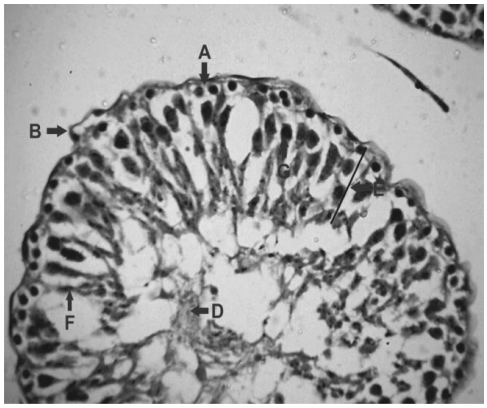

Figure No. 6: Photomicrograph of testicular tubules of group C1.

A. A single basal layer of spermatogonia.
B. An intact basement membrane.
C. Leydig cells visible.
D. Spermatids in the lumen with the debris of spermatocytes
H & E stain, X 400.

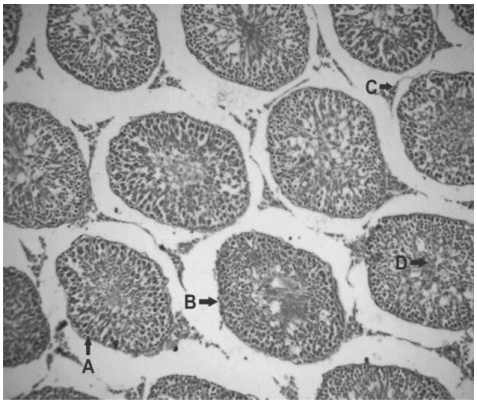

Figure No. 7: Photomicrograph of testicular tubules of group D1.

A. A single uninterrupted basal layer of spermatogonia.
B. An intact basement membrane just below stem cells.
C. Leydig cells.
D. Spermatids in the lumen
H & E Stain, X 100E. Epithelial cell layer improved (5-6 cells).
F. Sertoli cell visible normal.

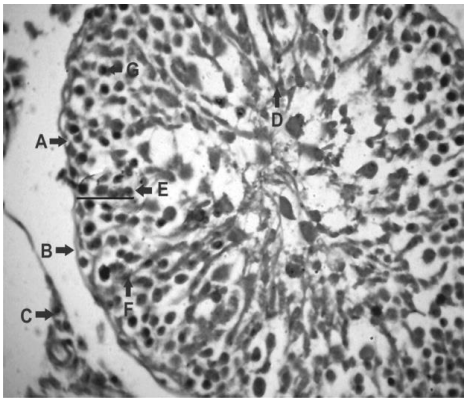

Figure No. 8: Photomicrograph of testicular tubules of group D1.

A. A single basal layer of spermatogonia.
B. An intact basement membrane.
C. Leydig cells visible
D. Spermatids in the lumen with scanty debris.
G. Normal Primary spermatocyte in size and number. H & E Stain. X 400.

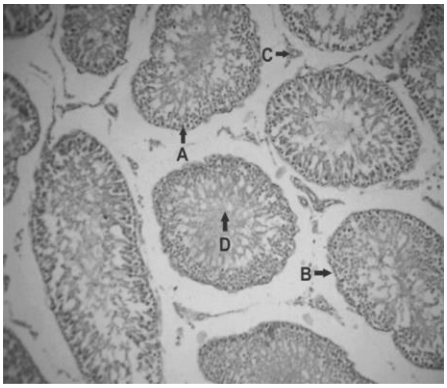

Figure No. 9. Photomicrograph of testicular tubules of group E1.

A. A regular single basal layer of spermatogonia.
B. An intact basement membrane.
C. Leydig cells.
H & E Stain, X 100.E. Epithelial cell layer.

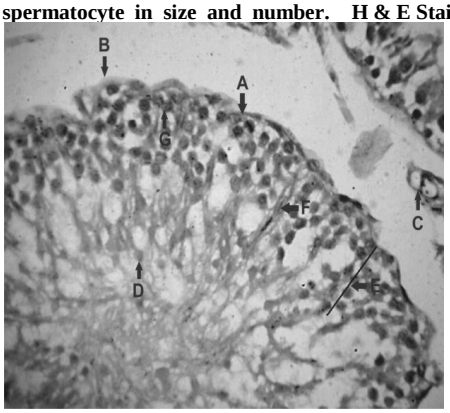

Figure No. 10. Photomicrograph of testicular tubules of group E1.

A. A single basal layer of spermatogonia.
B. An intact basement membrane.
D. Spermatids in the lumen.
F. Sertoli cell. G. Primary spermatocyte. H &E Stain, X 400.

Effect of lead toxicity and its reversal by vitamin C on diameter and spermatogenesis of testes of albino rats concluded. In table no 1 and 2 the mean diameter of semniferous tubules of rats of different subgroups showed that the rats which were treated with lead alone showed a significant decrease in mean diameter while those treated with vitamin C along with lead acetate showed better results. The subgroup C showed less decline as compared to subgroup B. The subgroup D showed increased mean diameter while subgroup E showed more increased mean diameter compared to subgroup D. This showed that the rats which were treated with higher dose of vitamin C showed better response as compared to those which were treated with lower doses of vitamin C (as evident in Table 1,2 & 3 and photograph 1 to 10).

DISCUSSION

The rats of group B which were treated only with lead showed a significant decrease in diameter over different times of scarification. This significant decrease in diameter and spermatogenesis was due to lead toxicity which were documented by Harvey.[6] According to another study by Ahmad I et al, the reason for the toxic effect of lead is accumulation of lead in testes of albino rats.[7] The group C rats which were treated with lead and vitamin C (250 mg/kg body weight daily) showed less decrease in diameter and spermatogenesis as compared to group B rats which were treated with lead only and showed a significant decrease in diameter and spermatogenesis. This shows that the toxic effect of lead is being lessen by vitamin C. The protective action of vitamin C against lead acetate can be attributed to the antioxidant action of vitamin C.[8] A study conducted by Bassem M. Raafat et al showed that administration of vitamin C with lead exposed animals exerts an obvious ameliorating as well as treatment effects.[9] The rats of group D showed more improvement in diameter and spermatogenesis as compared to group C animals, the reason behind this is that the group D rats were treated with higher dose of vitamin C as compared to rats of group C. The rats of group E were treated with the highest dose of vitamin C while all experimental groups (group B, group C, group D and group E) were given the same quantity of lead. The rats of group E showed most improved results among the experimental groups. A study by Hsu P C et al showed that vitamin C in considerable concentration showed significant reversal of lead toxicity in rats.[10] Thus greater the amount of vitamin C the more is the reversal against lead toxicity. In addition to acting as an antioxidant vitamin C also has an inhibiting effect on lead uptake on a cellular level.[11]

A number of studies showed that lead has no effect on diameter and spermatogenesis. A study conducted by Ping-Chi Hsu et al showed that there were no essential differences among the diameter either taking lead or not.[12] While Beata M. Pace et al showed in their studies that there were significant changes in pup over the first 3 weeks of lead treatment, when compared with the control group.[13] In another study, a slight reduction of diameter was observed where lead acetate was given for 14 days.[14] Thus, lead has decreased the normal diameter and spermatogenesis of the rats which is also shown in this study. The effect of vitamin C on lead levels has been clarified by studies that showed that ascorbic acid (vitamin C) decreased intestinal absorption of lead.[15] Vitamin C has a significant role in reversing the lead toxicity which is proved by a number of studies. One rat pharmacokinetic study found that intravenously administered vitamin C lowered lead tissue levels in rats that were continuously administered lead.[16] Another study showed that the adults with the highest ascorbic acid (vitamin C) levels had a 60-80% decreased prevalence of elevated blood lead.[17]

CONCLUSION

Vitamin C reduces the toxic effects of lead on diameter of seminiferous tubules and spermatogenesis of rats, which is shown in this study.

REFERENCES

1. Abdallah GM, El-sayed EM, Abo-Salem OM. Effect of lead toxicity on co-enzyme Q levels in rat tissue, Afri J Pharm and Pharmacol 2009;3(11): 568-572.
2. Suradkar SG, Ghodasara DJ, Vihol P, et al. Haemato-Biochemical alterations induced by lead acetate toxicity in wistar rats. Veteriary World. 2009; 2(11): 429-431.
3. Han S, Li W, Jamil U, et al. Effects of weight loss and exercise on the distribution of lead and essential trace elements with prior lead exposure. Environmental Health Perspectives 1999;107(8): 657-661.
4. Patrick L. The role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. Alternative medicine review. 2006;11(2): 114-127.
5. Klaassen CD. Heavy metals and heavy metal antagonists. The pharmacological basis of therapeutics. 10th ed. McGraw-Hill Medical Publishing division: New York; 2001.p.1851-1872.
6. Harvey SC. Heavy metals. The pharmacological basis of therapeutics. In: Goodman LS, Gillman A, editors. London MacMillan;1970.p. 976.
7. Ahmad I, Sabir M. Yasin KF. Study of the effects of lead poisoning on the testes in albino rats. J Med Res 2003;42:1-9.
8. Dabrowski K, Cicreszko. Ascorbic acid and reproduction in fish: Endocrine regulation and gamete quality. Aquaculture Res 2001;32:623-638.
9. Rafaat BM, Shafaa MW, Rizk AR, et al. Ameliorating effect of vitamin C against acute lead

toxicity in albino rats. Aust J of Basic and Applied Sci 2009; 3(4): 3597-3608.
10. Hsu PC, Hsu CC, Liu MY, et al. Lead induced changes in spermatozoa function and metabolism. J of Toxicol and Environ Health 1998;55:45-64.
11. Lyn Patrick ND. Lead toxicity part II: The role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. Alternative Med Rev 2006; 11: 114-127.
12. Ping-Chi Hsu, Ming-Yie Liu, Chao-Chin Hsu, et al. Effect of vitamin E and / or C on reactive oxygen species-related lead toxicity in the rat sperm. J Toxicity 1998; 128: 169-170.
13. Pace BM, Lawrence DA, Behr MJ, et al. Neonatal lead exposure changes quality of sperm and number of macrophages in testes of BALB/c mice. J of Toxicol 2005;210:247-256.
14. Turner TT, Lysiak JJ. Oxidative stress: A common factor in testicular dysfunction. J of Androl 2008; 29(3): 488-498.
15. Suzuki T, Yoshida A. Effectiveness of dietary iron and ascorbic acid in the prevention and cure of moderately long term lead toxicity in rats. J of Nutri 1979;109: 1974-1978.
16. Dalley JW, Gupta PK, Hung CT. A physiological pharmacokinetic model describing the disposition of lead in the absence and presence of L-ascorbic acid in rats. J of Toxicol 1990;50:337-348.
17. Simon JA, Hudes ES. Relationship of ascorbic acid to blood lead levels. JAMA 1990; 281: 2289-2293.

Address for Corresponding Author:
Dr. Mujahid Akbar Mamoun,
Assistant Professor of Anatomy
Quaid-A-Azam Medical College,
Bahawalpur
E-mail mujahidqmc.yahoo.com
Mailing Address;
98-B Model Town B, Bahawalpur.