Original Article

Lithium & Vit. E

# Lithium Induced Histological Alteration in Testes of Albino Rats and Their Amelioration with Vitamin E

**1. Muhammad Rashid Ahmed 2. Muhammad Mazhar Hijazi 3. Bushra Sherwani 4. Hafiz Syed Imran ul Haque 5. Masood Ahmed 6. Muhammad Arif**

1. Asstt. Prof. of Anatomy, BMU, Karachi 2. Prof. of Anatomy and Cell Biology, Ummul Qura University, KSA 3. Asstt. Prof. of Ophthalmology, AJK MC, Muzaffarabad, AJ&K 4. Assoc. Prof. of Pharmacology, DUHS, Karachi 5. Prof. of Anatomy and Histology, Qasim University, KSA 6. Prof. of Pharmacology, AJK MC, Muzaffarabad, AJ&K

## ABSTRACT

**Objective:** To evaluate the lithium induced histological alteration in testes of albino rats and their amelioration by Vitamin E.

**Study Design:** Experimental study

**Place and Duration of Study:** This study was conducted at department of Anatomy, Baqai Medical University, Karachi from July 2010 to August 2010.

**Materials and Methods**: The rats were assigned into three experimental groups (eight rats/group): control group, lithium group and lithium plus vitamin E treated group. Lithium (50 mg/kg/day) and vitamin E (50mg/kg/day) were given intraperitoneally for 21 days. At the end of experiment, rats were sacrificed and testes removed and processed for routine H&E. Slides were studied for histological examination under light microscope.

**Results**: Lithium treated rats showed decreased body and testicular weights, spermatogenic cells such as primary and secondary spermatocytes and spermatids were decreased, very little spermatozoa were seen in lumen of seminiferous tubules, significant increase in tubular count observed while tubular diameter, germinal epithelial thickness, number and size of nuclei of leydig cells were highly significantly reduced. In lithium plus vitamin E treated group, body and testicular weight, primary and secondary spermatocytes, spermatids were restored near to control. Tubular lumen also showed many spermatozoa. Tubular diameter, germinal epithelial thickness, number and size of nuclei of leydig cells were also returned to control.

**Conclusion:** Our study conclude that lithium causes detrimental effect on testicular morphology through oxidative stress and vitamin E provided protection through its antioxidative property.

**Key Words:** Lithium, Vitamin E, Testes

**Citation of article: Ahmed MR, Hijazi MM, Sherwani B, Haque HSI, Ahmed M, Arif M. Lithium Induced Histological Alteration in Testes of Albino Rats and Their Amelioration with Vitamin E. Med Forum 2015;26(9): 21-25.**



## INTRODUCTION

Lithium carbonate is the most prescribed drug in psychiatric illness especially in bipolar disorder. In spite of its extensive use, lithium has wide ranges of side effects at therapeutic dose, including thyroid disorders andnephrogenic diabetes insipidus[1].

Lithium also causes substantial adverse effects on male reproductive system[2]. Many studies indicated that lithium reduced FSH, LH, prolactin and testosterone level[3, 4]). At therapeutic dose, lithium inhibited the testicular hydroxysteroid dehydrogenase activity, steroidogenesis and spermatogenesis [3-6]. At higher dose, lithium decreased the weight ofr eproductive organs as well as secretions of prostate and seminal vesicle. Furthermore, spermatozoa were absent in lumen of epididymis and vas deferens. Histological examination showed the degeneration of germinal epithelium and leydig cells and vacuolization of cytoplasm of sertoli cells [6, 7].

Vitamin E is a fat soluble vitamin that considered as main antioxidant of body. Many studies indicated that vitamin E ameliorates the reproductive toxicities causes by various toxic stimulants[8, 9]. Vitamin E had a protective role on biochemical and morphological changes on testes induced by pre and post natal administration of ethanol[10]). In another study, pretreatment of vitamin E ameliorated the histopathological alteration of testes following cadmium chloride administration [11]. These results support the concept that vitamin E has a protective role on reproductive system, so we designed to observe the effects of vitamin E on lithium induced morphological alteration in testes of albino rats.

**Correspondence: Muhammad Rashid Ahmed**
Asstt. Prof. of Anatomy, BMU, Karachi
**Cell No.: 03452929169**
**E-mail: dr_rashidahmed@hotmail.com**

## MATERIALS AND METHODS

Thirty six male rats of 150-200 gm weight were selected randomly for this study. They were kept in plastic cages (4 rats per cage) in the animal house of Baqai Medical University and maintained under standard temperature, 28 ±2 °C, illumination, 12 hours dark and 12 hours light cycle and humidity, 55± 5%. All animals were kept in close observation for seven days. They were fed at laboratory chow and water ad libitium. Animals were divided into three groups with eight animals in each group. Groups were as follows: Group 1 served as control, group 2 served as lithium treated, group 3 was lithium plus vitamin E treated. Control group received daily 1cc intraperitoneal injection (i.p) of normal saline for 21 days. Group 2 animals received lithium (50mg/kg/day i.p)daily for 21 days[12]. Group 3 animals received both lithium (50mg/kg/day i.p) [12] and vitamin E (50mg/kg/day i.p) for 21 days ([13]). After 21 days, weight of each animal were recorded and sacrificed by overdosing of ether anesthesia. Testes were removed from surrounding tissue and weighted by Sartorius electro balance and put into separate bottle containing 10% normal buffered formalin. Afterwards 24 hours, it was rinsed with water, dehydrated in ascending grades of alcohol, cleared in xylene-I and II and embedded in paraffin at 58 ° C. Finally, five micron thick sections were cut by rotary microtome and stained with haemotoxylin and eosin and studied under light microscope. Data were expressed as mean±SEM. One way ANOVA followed by Post Hoc tukey test by using SPSS -18. Values of $p<0.05$ was considered as significant and $p < 0.01$ was considered highly significant.

## RESULTS

**Body Weight Changes:** Mean final body weight was significantly increased in all groups when compared with their mean initial body weight. However, the weight gain inlithium group was higher than the weight gain by other group animals (Table-1).

**Table No. 1: Body, Testicular And Relative Testicular**

| Parameter | Control | Lith-treated | Lith+Vit E treated |
|---|---|---|---|
| Initial body weight (gm) | 179.63+ 5.78 | 184.37+ 4.13 | 184.88+ 5.11 |
| Final body weight (gm) | 194.87+ 5.89[a] | 213.00+ 2.62[a] | 206.25+ 2.19[a] |
| Weight gain (gm) | 15.25+ 1.53 | 28.87+ 3.7 | 21.37+ 6.09 |
| Testicular weight (gm) | 1.23+ 0.07 | 0.97+ 0.60[b] | 1.17+ 0.52[c,d] |
| Relative testicular weight | 0.62+ 0.02 | 0.45+ 0.03[b] | 0.56+ 0.02[c,d] |

Results are given as means +SEM. Lith=Lithium, Vit E= Vitamin E. [a] P <0.05 when compared with initial weight, [b] P <0.05 when compared with control group, [c] P <0.05 when compared with lithium treated group, [d] P >0.05 when compared with control group.

**General Histological Observation:** In lithium plus vitamin E treated group, the tubular lumen was more than that in lithium group. More primary and secondary spermatocytes were recognized in seminiferous tubules in lithium plus vitamin E treated and control groups than the lithium treated group. In most of the tubules spermatozoa were 2 layers in lithium plus vitamin E treated and control groups in contrast to lithium treated group in which it was one layer thick. Similarly, spermatid's layer restored to 4-5 layers in lithium plus vitamin E treated and control groups while it was 2-3 layers in lithium treated group (Figure-1& 2**)**.

**Count of the Seminiferous Tubules:** The mean tubular count of lithium plus vitamin E treated group were restored near to normal and highly significantly lower ($P< 0.01$) as compared to that of lithium group but statistically insignificant ($P>0.05$) when compared to that of control rats (Table-2, Figure -1& 2).

**Diameter of Seminiferous Tubules:** The mean tubular diameter of lithium plus vitamin E treated group were restored near to normal and statistically highly significant ($P<0.01$) as compared to that of lithium alone group but statistically insignificant ($P>0.05$) as compared to that of control rats (Table-2, Figure -2).
Results are given as mean±SEMLith=Lithium, Vit E= Vitamin E. [a]p<0.05 when compared with initial weight, [b] p<0.05 when compared with control group, [c] p <0.05 when compared with lithium treated group, [d]p>0.05 when compared with control group

**Count and Diameter of Interstitial Cell Nuclei:** The mean number and diameter of interstitial cell nuclei in lithium plus vitamin E treated group were restored near to normal and statistically highly significant ($p < 0.01$) as comparedto those of lithium group but statistically insignificant ($p >0.05$) as compare to those of control (Table-2, Figure 3).

**Table No.2: Morphometric analysis of seminiferous tubules and Interstitial cell nuclei**

| Parameter | Control | Lith-treated | Lith +Vit E treated |
|---|---|---|---|
| **Seminiferous Tubules** | | | |
| Count | 17.51±0.50 | 21.92±0.47 | 18.46±0.48 |
| Diameter (µm) | 269.93±4.72 [a] | 218.96±2.69 [a] | 265.36±3.80 [a] |
| Thickness of the Germinal Epithelium (µm) | 96.56±1.44 | 72.08±2.36 | 93.80±2.03 |
| **Interstitial Cell Nuclei** | | | |
| Count | 14.50±0.87 | 8.13±0.48[b] | 13.75±0.75[c,d] |
| Diameter µm) | 4.00±0.02 | 3.69±0.03 [b] | 3.98±0.01[c,d] |

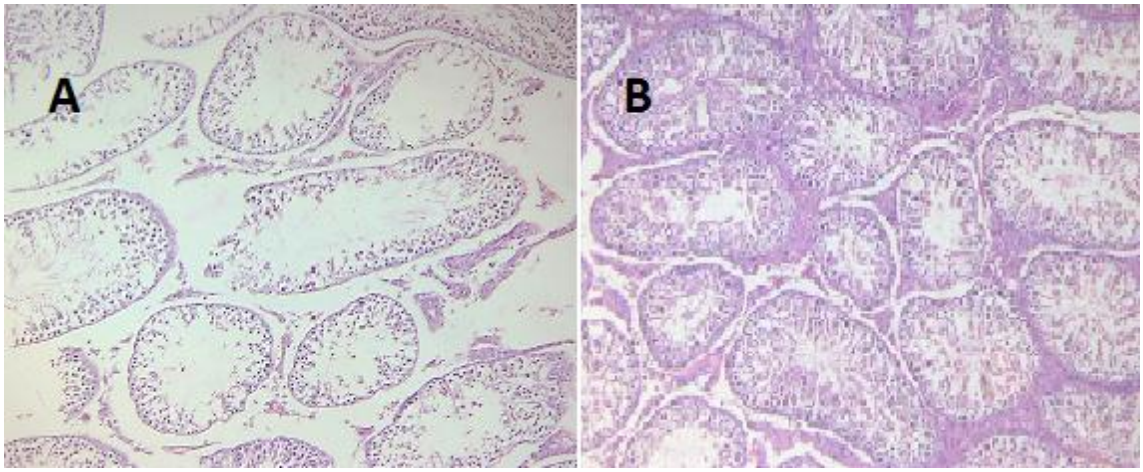


**Figure No.1: Five micron thick section of testis of Lithium (A), Lithium and Vitamin E treated (B) groups. Figure A shows more no of tubules per field, reduced germinal epithelium thickness and very little spermatozoa in lumen. Figure B shows recovery and less no of tubules per field, normal germinal epithelium thickness and more spermatozoa in lumen(10 X).**

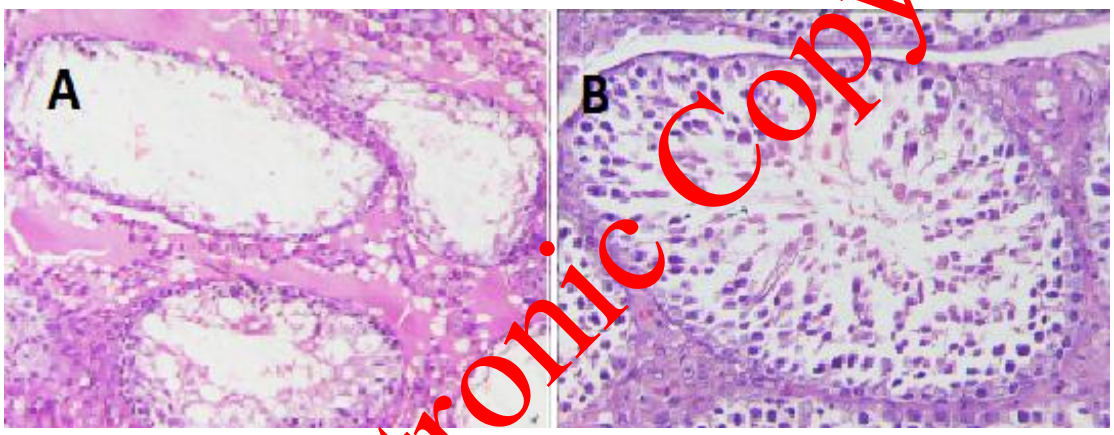




**Figure No.2: Five micron thick section of testis of Lithium (A), Lithium and Vitamin E treated (B) groups. Figure A shows reduced germinal thickness, less number of spermatogonic cells (Primary and secondary spermatocytes, spermatids). Lumen shows very little spermatozoa in it. Figure B shows recovery and more no of spermatogonic cells (primary and secondary spermatocytes, spermatids), normal germinal thickness and more spermatozo are seen (40 X).**

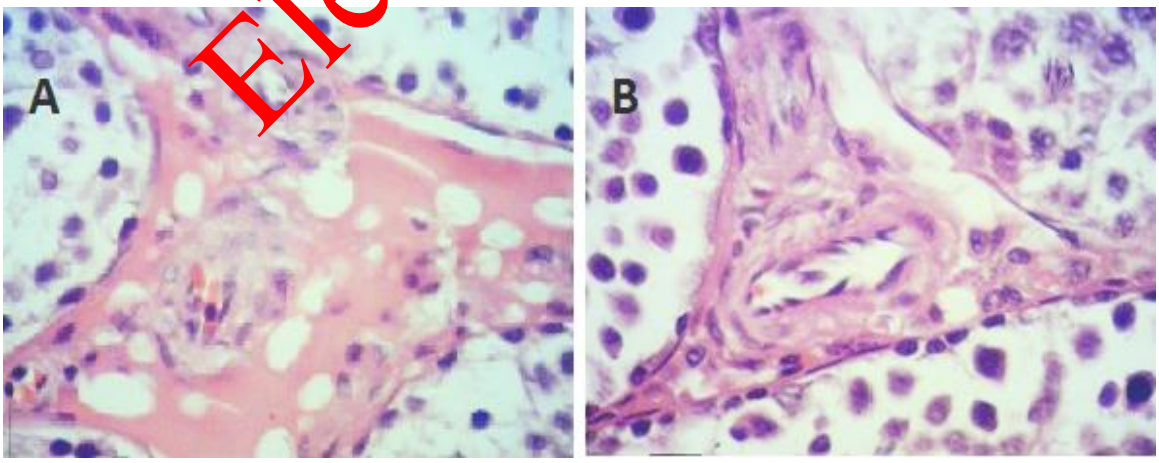


**Figure No.3: Five micron thick section of testis of Lithium (A), Lithium and Vitamin E treated (B) groups. Figure A shows widened interstitial space with reduced number and diameter of interstitial cells of leydig nuclei. Figure B shows recovery and more number and diameter of interstitial cells of leydig nuclei (100 X).**

## DISCUSSION

In this study, we demonstrated the lithium induced histological alteration in testes of albino rats and their amelioration by vitamin E.

All animals significantly increased their body weights when compared with their initial body weights. However, the weight gain by lithium alone treated animals was higher than the weight gain by other group animals. Our result closely corresponds with previous findings that lithium behaves as a nonspecific stressor to increase the body weight [14].

Our study showed significant reduction in testicular weight in lithium treated group[6].Testicular weight is correlated with spermatogenic activities; therefore reduction of testicular weight could be the results of decreased number of spermatocytes, spermatids, spermatozoa and sertoli cells [15]. Co- treatment of vitamin E prevented the lithium induced reduction of testicular weight. Our result is correlated with the previous findings that vitamin E can restore the decreased body and testicular weights caused by various toxic agents [16].

The count of seminiferous tubules was highly significantly increased in lithium treated animals.Our result supports the earlier findings that Derangement of germ layer, loss of spermatids and spermatozoa cause shrinkage of tubules lead into more tubular count per field[6, 15]. Those animals who received lithium carbonate with vitamin E showed relatively decreased tubular countper field when compared with lithium alone group. These protective results could be due to that vitamin E enhances germ cells production and maintains the normal architecture of seminiferous tubules[17].

Our results showed the significant reduction in tubular diameterin lithium treated animals.Tubular diameter is correlated with the germinal epithelium and destruction of germinal epithelium lead into decreased diameter of tubules[18, 19]. These results have close correlation with findings of previous studies [2, 6]. Those animals who received lithium plus vitamin E depicted the increased tubular diameter when compared with lithium alone group that indicated the protective effects of vitamin E. Our results are also in line with the previous results when vitamin E significantly increased the diameter of atrophic tubules caused by chromium [20].

Germinal epithelial thickness was also highly significantly decreased in lithium treated animals. Two layered spermatocytes decreased into one layered and four to five layered spermatids decreased into two to three layered and merely few spermatozoa were recognized in lumen of tubules. Our results supports the previous findings that lithium treatment disarranged the germ cells, reduction in spermatogenesis, decreased primary and secondary spermatocytes, spermatid and spermatozoa[21]. Those animals who received lithium plus vitamin E showed increased thickness of germinal epithelium. These compensatory results are in accordance with previous findings when vitamin E was used with para-nonyphenol[22].

The number and diameter of nuclei of leydigcell were highly significantly reduced in lithium treated rats. These findings are in accordance with previous resultsthat lithium caused degeneration of leydig cells[6]. Co treatment with vitamin E showed increased number and diameter of interstitial cell nuclei when compared with lithium alone treated animals. These results are in line with previous reportthat supplementary effects of vitamin E increased the interstitial cells' count in cyclosporine A induced testicular toxicity[23].

## CONCLUSION

Our study showed that lithium caused detrimental effect on testicular morphology through oxidative stress and vitamin E provided protection through its antioxidative property. Further studies are needed to clarify the precise nature of lithium toxicity and protection by vitamin E.

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

## REFERENCES


1. McKnight RF, Adida M, Budge K, Stockton S, Goodwin GM, Geddes JR. Lithium toxicity profile: a systematic review and meta-analysis. The Lancet 2012;379(9817):721-8.
2. Zarnescu O, Zamfirescu G. Effects of lithium carbonate on rat seminiferous tubules: an ultrastructural study. Int J Androl 2006;29(6): 576-82.
3. Ghosh PK, Biswas NM, Ghosh D. Effect of lithium chloride on spermatogenesis and testicular steroidogenesis in mature albino rats: duration dependent response. Life Sci 1991;48(7):649-57.
4. Allagui MS, Hfaiedh N, Vincent C, Guermazi F, Murat JC, Croute F, et al. Changes in growth rate and thyroid- and sex-hormones blood levels in rats under sub-chronic lithium treatment. Hum Exp Toxicol 2006;25(5):243-50.
5. Ghosh D, Biswas NM, Chaudhuri A, Ghosh PK. Direct effects of lithium chloride on testicular delta 5-3 beta and 17 beta-hydroxysteroid dehydro-genase activities in the rat--in vitro study. Acta Physiol Hung 1990;76(4):287-90.
6. Thakur SC, Thakur SS, Chaube SK, Singh SP. Subchronic supplementation of lithium carbonate induces reproductive system toxicity in male rat. Reprod Toxicol 2003;17(6):683-90.
7. Ghosh D, Biswas NM, Chaudhuri A, Ghosh AK, Ghosh PK. Acid and alkaline phosphatase activities in lithium treated testis, prostate and seminal

vesicles of adult albino rats: evidence of duration and dose dependent response. Indian J Exp Biol 1990;28(6):553-6.
8. Zubair M, Ahmad M, Ahmad N, Naveed MR, Idrees M, Sallam MA, et al. Toxic Effects of Arsenic on Reproductive Functions of Male Rabbit and Their Amelioration with Vitamin E. Global Veterinaria 2014;12(2): 213-218
9. Kalender S, Uzun FG, Demir F, Uzunhisarcıklı M, Aslanturk A. Mercuric chloride-induced testicular toxicity in rats and the protective role of sodium selenite and vitamin E. Food Chem Toxicol 2013;55:456-62.
10. Shirpoor A, Norouzi L, Khadem-Ansari M-H, Ilkhanizadeh B, Karimipour M. The Protective Effect of Vitamin E on Morphological and Biochemical Alteration Induced by Pre and Postnatal Ethanol Administration in the Testis of Male Rat Offspring: A Three Months Follow-up Study. JReprod Infertil 2014;15(3):134.
11. Kini RD, Tripathi Y, Raghuveer C, Pai SR. Effect of Vitamin E Pre-Treatment on Histopathological Changes on Rat Testis Following Cadmium Chloride Administration. J Pharm Technol 2013;6(8):874-7.
12. Nejadkey F, Nahavandi A, Dehpour AR, Mani AR. Role of nitric oxide in the gastro-protective effect of lithium. Pathophysiology. Pathophysiol 2006;13(2):85-9.
13. Kabay SC, Ozden H, Guven G, Ustuner MC, Degirmenci I, Olgun EG, et al. Protective effects of vitamin E on central nervous system in streptozotocin-induced diabetic rats. Clin Invest Med 2009;32(5):314-21.
14. Levine S, Saltzman A. Lithium increases body weight of rats: relation to thymolysis. Prog Neuropsychopharmacol Biol Psychiat 2006; 30(1):155-8.
15. Hijazi MM, Azmi MA, Hussain A, Naqvi S, Perveen R, Hijazi S. Androgenic Anabolic Steroidal-Based Effects on the Morphology of Testicular Structures of Albino Rats. Pak J Zool 2012;44(6):1529-37.
16. Banudevi S, Sharmila M, Murugesan P, Srinivasan N, Balasubramanian K, Aruldhas M, et al. Effects of Vitamin C and E on PCB (Aroclor 1254) induced oxidative stress, androgen binding protein and lactate in rat Sertoli cells. Reprod Toxicol 2004;19(2):201-8.
17. Jargar J, Yendigeri S, Dhundasi S, Das K. Protective effect of Vitamin E (α-tocopherol) on nickel-induced alteration of testicular pathophysiology in alloxan-treated diabetic rats. Int J Clin Exp Physiol 2014;1(4):290-7.
18. Sahinturk V, Guclu C, Baycu C. Protective effects of vitamin E on ethane dimethane sulfonate-induced testicular toxicity in rats. Asian J Androl 2007;9(1):117-24.
19. Damodar D, D'Souza UJ, Biswas A, Bhat S. Diazinon Affects the Cytoarchitecture of Seminiferous Epithelium in Rat Testis. Am J Biomedical Engineering 2012;2(2):13-16.
20. Chandra AK, Chatterjee A, Ghosh R, Sarkar M. Vitamin E-supplementation protect chromium (VI)-induced spermatogenic and steroidogenic disorders in testicular tissues of rats. Food Chem Toxicol 2010;48(3):972-9.
21. Ghosh PK, Biswas NM, Ghosh D. Effect of lithium chloride on testicular steroidogenesis and gametogenesis in immature male rats. Acta Endocrinologica 1991;124(1):76-82.
22. Mehranjani MS, Noorafshan A, Momeni HR, Abnosi MH, Mahmoodi M, Anvari M, et al. Stereological study of the effects of vitamin E on testis structure in rats treated with para-nonylphenol. Asian J Androl 2009;11(4):508-16.
23. Sameni H, Haghighi S, Amjad M. Protective effects of vitamin E on cyclosporine A-induced toxicity in rat testis. Koomesh J Semnan University of Med Sci 2011;12(4):419-27.