DOI: https://doi.org/10.53350/pjmhs2023173573

ORIGINAL ARTICLE

Impact of Antioxidant on Altered Quantity of Testicular Leydig Cell During Anti Cancerous Therapy an Experimental Study

AISHA ABDUL HAQ[1], DURESHAWAR REHMAN[2], SADIA IQBAL[3], UZMA HAMEED[4], AISHA HASSAN BROHI[5], AMNA MUGHAL[6]
[1]*Associate Professor Anatomy Department Dow Medical College DUHS, Karachi.*
[2]*Lecturer Anatomy Department King Saud bin Abdul-Aziz University for Health Sciences, Riyadh,KSA.*
[3]*Assistant Professor Anatomy Department Dow Medical College DUHS, Karachi.*
[4]*Assistant Professor Anatomy Department Dow International Dental college DUHS, Karachi.*
[5,6]*Assistant Professor Anatomy Department Dow Medical College DUHS, Karachi.*
Corresponding author: Aisha Abdul Haq, Email: aisha.haq@duhs.edu.pk, Cell: +92 335-2333326

ABSTRACT

Statement of Problem: Patients undergoing chemotherapy has been struggling long with side effects on tissues which were not targeted for therapy. Chemotherapeutic agents Doxorubicin (DOX) used in this study induced testicular damage, had been a major concern of oncologists for decades. Studies so far has more focus on the adverse impact of drug on germ cells and very few observed its effect on Leydig cell secreting testosterone. To date few studies are available that have used antioxidant therapy like vitamin C to assuage the oxidative damage induced by DOX on supporting cell like Leydig.

Objectives: To demonstrate the altered numbers of Leydig cells produced by DOX in testes of mice and designed to study the changes in quantity of Leydig cells in DOX affected interstitial cells with co-administration of antioxidant ascorbic acid.

Research Design: An experimental study to determine the effect of dox on testicular leydig cells and to assess the ameliorating effects of vitamin c on it.

Duration of Study: This study was conducted from June 2022 to February 2023

Place of Study: Research was conducted at Dow university of health sciences (DUHS) Karachi at institute of basic medical sciences with collaboration of its animal house and Dow Diagnostic Research and Reference Lab DUHS.

Sampling technique: The samples were chosen randomly. In this experiment drugs used include doxorubicin hydrochloride, vitamin c and normal saline on 6th, 8th and 10th day of the study.

Methodology: Male mice of five weeks old were segregated into 3 groups. Group- A mice were treated with normal saline, DOX alone was given to group- B animals and DOX + Vitamin C to group- C. At the end of experimental study, testes of sacrificed animals were preserved in Bouin's fluid and later processing, staining etc. were performed.

Results and Statistical Analysis: By using SPSSS version 16, statistical analysis was done, using ANOVA test to evaluate the significance of parameter among different groups studied. Widening of interstitium with significant Leydig hyperplasia ($P < 0.00$) as compared to controls were seen in group B However co-administration of Vitamin C with DOX significantly decrease was observed in comparison to Group A.

Practical Implications: The practical implications of this study for the community are that cancer patients undergoing chemotherapy can potentially benefit from co-administration of vitamin C to reduce the adverse effects on testicular tissue, which may help preserve fertility and hormone production. Improving the quality of life for cancer patients by providing an accessible and potentially effective way to manage chemotherapy-induced side effects.

Conclusion: This study suggested that the antioxidant Vitamin C has a role in ameliorating the effects produced by DOX on testis by showing change in altered quantity of leydig cell.

Keywords: Doxorubicin, Vitamin C, Mice, Antioxidant, Leydig cells

INTRODUCTION

Traditional chemotherapy attacks both cancerous growth and healthy cells in the body. Hence targeted goal is not met. Even after combined chemotherapy approach, the situation persists. As the drug is often administered at maximally tolerated dose, visceral side effects are seen frequently. Researchers have revealed chromosomal irregularities and mutation in normal cells along with broad range of side effects on many viscera.[1] Testes and bone marrow in particular having quickly dividing cells, are more susceptible to toxic effects of the drug.[2]

Distortion in the microscopic anatomy of testis was seen with administration of Doxorubicin DOX, an anthracycline.[3] Degree of testicular damage was observed by many parameters indeed indicating oxidative stress to vascular wall.[4] Zanetti in 2010 also mentioned deterioration in histology. Drastic reduction in diameter of tubules with decrease sperms count, widening of interstitium were evident by other studies.[5] Leydig cells secreting testosterone, are considered more resistant to chemotherapy than germinal and Sertoli cells. However, Leydig cell function can be unaffected in long-term treatment of cancer survivors [6] Amplified number and size of interstitial Leydig cells were detected as a compensatory response to deteriorated epithelium.[7]

Antioxidants prevent cells in the body from damages produced by free radicals.[8] Antioxidants avoid toxic effects of anti-cancer treatment by stabilizing free radicals and preventing them to take electrons from other molecules. Antioxidants are abundant in fruits and vegetables and elevate body's own immune mechanism.[9] Both synthetic and natural antioxidants are in therapeutic use including enzymatic, scavenging and pharmacological antioxidants.[10,11] As a potentially strong antioxidant, Vitamin C seems to hinder with DOX induced free radical formation.[12,13] Distinct decrease in levels of various antioxidants including Vitamin C was seen with the management of DOX in breast cancer thus signifying the necessity to use antioxidants alongside chemotherapy.[14]

Despite their critical role in the spermatogenesis support and the conservation of spermatogonial stem cell niche, not sufficient interest has been paid to myoid Leydig cells and no investigations have focused on macrophages.[15] The upgrading the quality of lives of cancer fighters has thus become a significant public healthcare problem. Malignancy treatment-related side effects can produce both late and long-term side effects in healed patients and barrenness could be one of them.[16]

Rationale of Study: This study highlights the importance of considering potential side effects on non-targeted tissues during chemotherapy and the potential benefits of using antioxidant therapy like vitamin C. By incorporating vitamin C supplementation as part of chemotherapy management, healthcare providers may be able to reduce the risk of long-term complications related to testicular tissue damage.

Research Gap: No detailed morphological studies have been done, regarding the possible protection carried by Vitamin C to the integrity of Leydig cells affected by DOX; opening new grounds for further research to let oncologists aware about the definite morphological changes in reproductive organs of the males who take DOX for various cancers.

MATERIAL AND METHOD

Research Design: An experimental study to determine the effect of dox on testicular leydig cells and to assess the ameliorating effects of vitamin c on it.

Place and Duration of Study: This study was conducted from June 2022 to February 2023 at Dow university of health sciences (DUHS) Karachi at institute of basic medical sciences with collaboration of its animal house and Dow Diagnostic Research and Reference Lab DUHS.

Inclusion and Exclusion Criteria: Only healthy and active 5 weeks old thirty male mice were included for the study.

Sampling Technique: The samples were chosen randomly. In this experiment drugs used include doxorubicin hydrochloride, vitamin c and normal saline on 6th, 8th and 10th day of the study.

Grouping: Group a (control): Control group enclosed 10 male mice and normal saline 1 ml ip was given.

Group b (dox): 10 animals in this received dox in dose of 0.003 mg/g or 0.003 mg in 0.03 ml /gm body weight ip, [17]

Group c: (dox + vitamin c): 10 animals in this group received dox in a dose of 0.003 mg/gm or 0.003 mg in 0.03 ml /gm body weight ip [17] and vitamin c in dose of 0.5 mg/gm or 0.5 mg in 0.01ml/gm body weight p.o.[18]

Animal sacrifice and sample: each animal was weighed at the commencement and on the completion of study (33 days for one spermatogenic period of animal). Animals were anaesthetized by deep ether anaesthesia and were dissected through midline abdominal incision. After identification, testes were freed from surrounding tissues were preserved in bouin's fluid for 24hrs.

Histological processing of testicular tissue: After fixation in bouin's fluid for 24 hours, longitudinal sections of testes were taken after dehydration and infiltration, embedding with paraffin tissue blocks were made. five µm thick sections were obtained h&e-stained slides were observed for number of leydig cells under 40x objective and 10x ocular lenses. the sections were viewed and photographed by using nikon light.

Statistical analysis: To find the statistical difference between the groups, One Way Analysis of Variance (ANOVA) or Kruskal Wallis test was used. In case of significant result, Tukey – multiple Comparisons post Hoc test was applied to check the pair wise comparison at 5% level of significance (95% confidence interval C.I).

RESULTS

Number of Leydig cells in interstitial space: **Comparison of number of Leydig cells between the control group (A) and DOX group (B):** The mean ± S.D of cell count in interstitial spaces in control was 18.67 ± 2.54 and in the DOX group was 44.64 ± 11.17. When the comparison was done between the two groups, the observed P value was < 0.001 at 95% C.I as shown in table-1, graph-1, figures 1 and 2 (a, b)

A significant increase was observed in the mean Leydig cells count of DOX group.

Table-1: Comparison of Mean ± SD of number of Leydig cells in interstitial space in different groups

Groups	Mean ± SD	P value among groups		95% Confidence Interval	
		Groups	P value	Lower Bound	Upper Bound
Control (A)	18.67 ± 2.54	A vs B	< 0.001*	-34.27	-17.65
DOX (B)	44.64 ± 11.17	B vs C	< 0.001*	16.755	32.39
DOX + Vitamin C (C)	20.06 ± 6.09	A vs C	0.883*	-9.21	6.42
* Tukey HSD Post Hoc Multiple comparisons test					

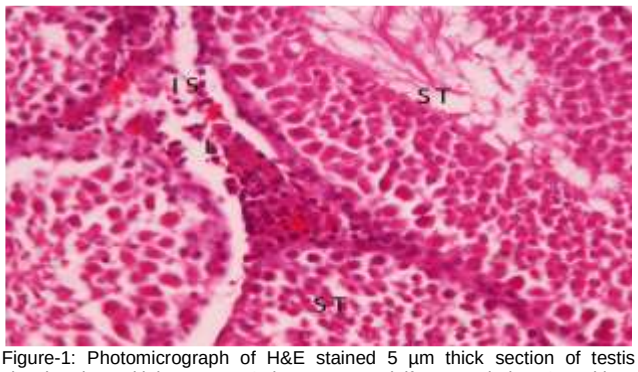


Figure-1: Photomicrograph of H&E stained 5 µm thick section of testis showing interstitial spaces IS between seminiferous tubules ST with L Leydig cells L in control mouse at x400.

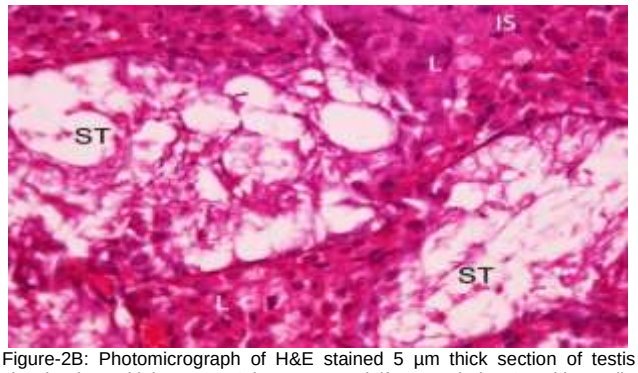


Figure-2B: Photomicrograph of H&E stained 5 µm thick section of testis showing interstitial spaces IS between seminiferous tubules ST with Leydig cells L in control mouse at x400.

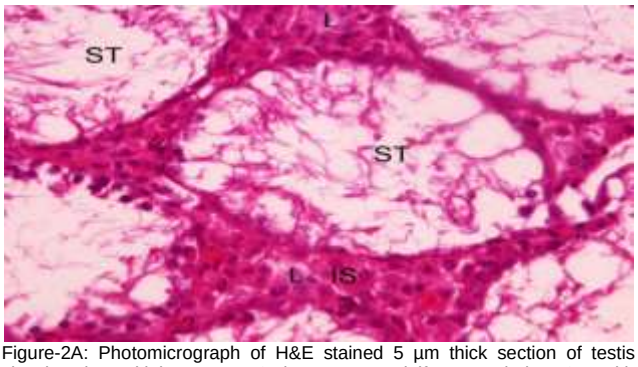


Figure-2A: Photomicrograph of H&E stained 5 µm thick section of testis showing interstitial spaces IS between seminiferous tubules ST with increase number of Leydig cells L in DOX mouse at x400.

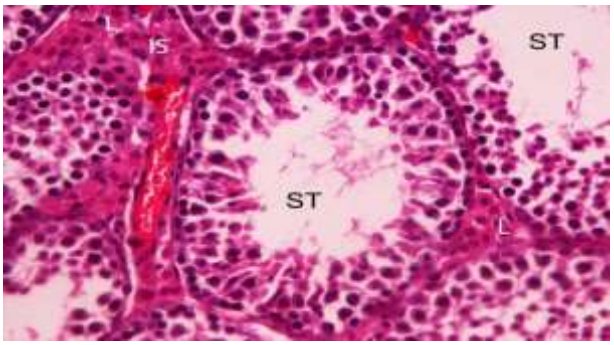


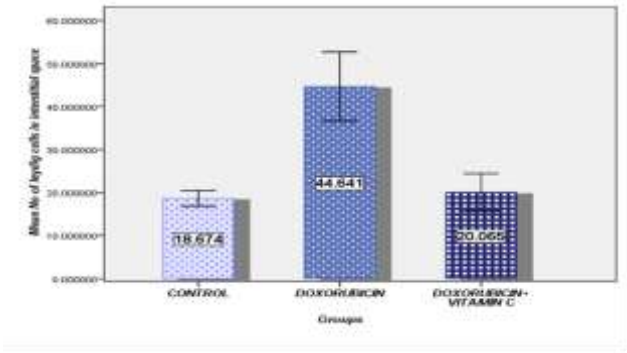
Figure-3: Photomicrograph of H&E stained 5 µm thick section of testis showing diminished widening of interstitial spaces IS between seminiferous tubules ST with number of Leydig cells L in DOX + Vitamin C administered mouse at x400.

Comparison of number of Leydig cells between the DOX group (B) and DOX + Vitamin C group (C): The mean ± S.D of cell count in interstitial spaces in DOX group was 44.64 ± 11.17 and in the DOX + Vitamin C was 20.06 ± 6.09. When the comparison was done between the two groups, the observed P value was < 0.001 at 95% C.I as shown in table-1, graph-1, figures 2 (a, b) and 3.

A significant decrease was observed in the mean Leydig cells count of DOX + Vitamin C group.

Comparison of number of Leydig cells between the control group (A) and DOX+ Vitamin C group (C): The mean ± S.D of cell count in interstitial spaces in control was 18.67 ± 2.54 and in the DOX + Vitamin C group was 20.06 ± 6.09. When the comparison was done between the two groups, the observed P value was = 0.883 at 95% C.I as shown in table-1, graph-1, figures 1 and 3.

A non-significant increase was observed in the mean Leydig cells count of DOX + Vitamin C group.



Graph-1: Comparison of mean number of Leydig cells in interstitial spaces between three groups. Values are expressed in mean ± 95% C.I of error bar

DISCUSSION

In the present study number of Leydig cells in testis of mice were observed in control and after the treatment with DOX and co-administration of Vitamin C with DOX. The P value of number of Leydig cells in interstitial space turned out to be < 0.001 when comparison was made between control and DOX treated. This P value indicated the definite increase in number of Leydig cells in DOX treated testes and decrease in number after providing them with the treatment of DOX + Vitamin C. A significant P value observed when controls were compared to the DOX group and a nonsignificant change of P value (0.883) was seen between control and DOX + Vitamin C groups.

The significantly higher number of interstitial Leydig cells in DOX group observed could be correlated with increase in testosterone levels in DOX treated group of mice. Number of Leydig cells were seemed to be reduced by DOX in other study, was in contrast to our findings[19]. Few data from animal studies are obtainable on the impact of exposure of chemotherapy on Leydig cells. In comparison to our research No alteration in function and morphology of Leydig cells has been witnessed in the pre-pubertal testis after 48 hours in vitro after exposure to doxorubicin in another study[20]. Likewise, current data have stated that density of Leydig cell was unaffected after 24 h in vitro after contact with cisplatin, doxorubicin and cyclophosphamide [21].

Increase number seen in our study was in favour with findings of Hazmi in 2004 which revealed significant increase in number and size of Leydig cells in relation to distorted seminiferous tubules.[22] Increased number of Leydig cells could be compensatory to degeneration of seminiferous tubules. It agrees with previous study in which Leydig hyperplasia was seen.[23] In another study Leydig cells were among the survival cells populating the interstitial spaces beside Sertoli cells in the tubules after DOX therapy.[5]It could be due to this fact that it is considered non proliferating cells within testis and are therefore resistant to toxic effects of chemotherapeutic drug.[24]

CONCLUSION

The study delivers a proof that DOX chemotherapy effects the testicular tissues and significantly increase the number of Leydig cells while Vitamin C co-administration does change the number observed earlier with DOX. Since DOX is in demand for treatment of various cancers, the resultant damage induced by drug is unavoidable hence more research is required as limited studies accessible do not presently permit conclusions to be drawn about the influence of chemotherapy on somatic cells of testes.

Acknowledgements: The authors are grateful for the valuable contribution of all those respectable personalities, participate and help in this research work.

Conflict of Interest: There was no any conflict for current study.

Funding: Present research was self-funded

Author's contribution: Every author contribute sincerely in the present study.

REFERENCES

1. Jung K, Reszka R. Mitochondria as subcellular targets for clinically useful anthracyclines. Adv Drug Deliv Rev. 2001; 49(1-2):87-105.
2. Erdem Guzel E, Kaya Tektemur N, Tektemur A. Alpha-lipoic acid may ameliorate testicular damage by targeting dox-induced altered antioxidant parameters, mitofusin-2 and apoptotic gene expression. Andrologia. 2021 Apr;53(3):e13990.
3. van der Zanden, S.Y., Qiao, X. and Neefjes, J. (2021), New insights into the activities and toxicities of the old anticancer drug doxorubicin. FEBS J, 288: 6095-6111. https://doi.org/10.1111/febs.15583
4. Hosseini A, Zare S, Borzouei Z, Pakdel FG. Cyclophosphamide-induced testicular toxicity ameliorate by American ginseng treatment: An experimental study. International Journal of Reproductive BioMedicine. 2018 Nov;16(11):711.
5. Majd NE, Tabandeh MR, Hosseinifar SH, Sadeghi M. 210Protective Effect of Aloe vera Gel against Cisplatin-Induced Testicular Damage, Sperm Alteration and Oxidative Stress in Rats. International Journal of Fertility & Sterility. 2021 Jul;15(3):210.
6. Noorafshan A, Karbalay-Doust S, Valizadeh A, Aliabadi E. Ameliorative effects of curcumin on the structural parameters of seminiferous tubules and Leydig cells in metronidazole-treated mice: a stereological approach. Experimental and toxicologic pathology. 2011 Nov 1;63(7-8):627-33.
7. Hazmi EA, Shaker S, Sagaff SA, Korajum S. Histological changes of testis in mice after administration of Doxorubicin HCL (Adriablastina) Cytotoxic drug. Conc. Fac. Sci. 2004; 1:99-117.
8. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010 Jul;4(8):118-26. doi: 10.4103/0973-7847.70902. PMID: 22228951; PMCID: PMC3249911.
9. Ponnampalam, E.N.; Kiani, A.; Santhiravel, S.; Holman, B.W.B.; Lauridsen, C.; Dunshea, F.R. The Importance of Dietary Antioxidants on Oxidative Stress, Meat and Milk Production, and Their Preservative Aspects in Farm Animals: Antioxidant Action, Animal Health, and ProductQuality—InvitedReview. Animals 2022, 12,3279. https://doi.org/10.3390/ani12233279
10. Chawda HS. Prospective study of antioxidants, its mechanism and potential role in cancer. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2011; 2 (3):888-94.
11. Erfani Majd N, Tabandeh MR, Hosseinifar S, Sadeghi M. Protective Effect of Aloe vera Gel against Cisplatin-Induced Testicular Damage, Sperm Alteration and Oxidative Stress in Rats. International Journal of Fertility and Sterility. 2021 Jul 1;15(3):210-8.
12. Melebary SJ. Physiological and Histological Study of the Therapeutic Effect of Silibinin on Testicular Alteration in Adult Male Albino Rats. Indian Journal of Pharmaceutical Sciences. 2023 Jan 31:1-4.
13. Pehlivan FE. Vitamin C: An Antioxidant Agent [Internet]. Vitamin C. InTech; 2017. Available from: http://dx.doi.org/10.5772/intechopen.69660
14. Rostami A, Vakili S, Koohpeyma F, Jahromi BN, Aghajari ZA, Mahmoudikohani F, Saki F, Mahmoodi M, Jaberi KR, Movahedpour A, Khorchani MJ. Ellagic acid effects on testis, sex hormones, oxidative stress, and apoptosis in the relative sterility rat model following busulfan administration. BMC Complementary Medicine and Therapies. 2022 Dec;22(1):1-4.

15. Allen, M.; Lopes, F.; Mitchell, R.T.; Spears, N. How does chemotherapy treatment damage the prepubertal testis? Reproduction 2018, 156, R209–R233.
16. Benedict, C.; Shuk, E.; Ford, J. Fertility Issues in Adolescent and Young Adult Cancer Survivors. J. Adolesc. Young Adult Oncol. 2016, 5, 48–57.
17. Yeh YC, Lai HC, Ting CT, Lee WL, Wang LC, Wang KY, et al. Protection by doxycycline against doxorubicin-induced oxidative stress and apoptosis in mouse testes. Biochem Pharmacol. 2007; 74(7):969-80.
18. Mazumdar M, Girl S, Singh S, Kausar A, Giri A, Dull Sharma G. Antioxidative potential of vitamin c against chemotherapeutic agent mitomycin c induced genotoxicity in somatic and germ cells in mouse test model. Assam University Journal of Science & Technology. 2011; 7:10-17
19. Abdella EM and Ahmed R. Suppression of Doxorubicin Apoptotic, Histopathologic, Mutagenic and Oxidative Stress Effects in Male Mice Bone Marrow and Testis Tissues by Aqueous Rosemary Leaves Extract. IJCP. 2009; 1:35-49.
20. Nurmio, M.; Toppari, J.; Kallio, J.; Hou, M.; Söder, O.; Jahnukainen, K. Functional in vitro model to examine cancer therapy cytotoxicity in maturing rat testis. Reprod. Toxicol. 2009, 27, 28–34.
21. Smart, E.; Lopes, F.; Rice, S.; Nagy, B.; Anderson, R.; Mitchell, R.; Spears, N. Chemotherapy drugs cyclophosphamide, cisplatin and doxorubicin induce germ cell loss in an in vitro model of the prepubertal testis. Sci. Rep. 2018, 8, 1773.
22. Kopalli SR, Cha KM, Cho JY, Kim SK, Koppula S. Cordycepin mitigates spermatogenic and redox related expression in H2O2-exposed Leydig cells and regulates testicular oxidative apoptotic signalling in aged rats. Pharmaceutical Biology. 2022 Dec 31;60(1):404-16.
23. Aldhahrani A. P aullinia cupana seed extract ameliorated methotrexate-induced testicular dysfunction through the regulation of antioxidants, inflammatory, apoptosis/anti-apoptosis, and steroidogenesis-associated genes. Environmental Science and Pollution Research. 2023 Feb;30(6):15115-27.
24. Pourheydar B, Azarm F, Farjah G, Karimipour M, Pourheydar M. Effect of silymarin and metformin on the sperm parameters and histopathological changes of testes in diabetic rats: An experimental study. International Journal of Reproductive BioMedicine. 2021 Dec;19(12):1091.