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Chemotherapy: Affected Morphological and Morphometry of Testes and Improvement by Antioxidants

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ABSTRACT

Background: Taking into account, the adverse effects of chemotherapy on male fertility, Chemotherapy has been a major area of concern for physicians. Chemotherapeutic agent Doxorubicin (DOX) profoundly affect the morphometric of testes that is indicative of damage to this important reproductive organ. Antioxidants being free radical scavenger could be used in order to ameliorate the Undesirable effect of anti-cancer agents

Objective: To demonstrate change in weight of testes and thickness of germinal epithelium after consumption of Doxorubicin and to observe the effect of ascorbic acid on these changes

Material and Methods: Experimental study was conducted on mice which were divided in 3 groups A, B and C. They received saline intraperitoneally (IP), DOX IP and DOX + Ascorbic acid per oral respectively. On completion sacrificed animals were dissected and testes were weighed and later sections were selected for morphometric measurement of germinal epithelium and were observed in H&E stained slides under 40x objective and 10x ocular lenses of light microscope

Result: Ascorbic acid seems to be significantly affecting the declined weight of the organ and on disturbed morphometry of germinal epithelium in mice induced with chemotherapeutic agent DOX

Conclusion: Ascorbic acid seems to be significantly affecting the declined weight of the organ and on disturbed morphometry of germinal epithelium in mice induced with chemotherapeutic agent DOX

Keyword: Chemotherapy, antioxidant, weight, germinal epithelium, mice.

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INTRODUCTION

Cancer is considered leading cause of death universally. Among the conventional treatments for cancer, chemotherapy is frequently used to treat malignancies. However, anticancer medicines are associated with a significant side effects[1]. Each one of drug with its own set of unwanted effects cause disturbance in quality of one's life. One of the major etiology of harmful reactions, for instance target of DNA by drugs, is the increase amount of reactive oxygen species (ROS) and hence in turn excessive oxidative stress.[1]

For years Production of ROS by Doxorubicin (DOX) has been major concern, as its production leads to damage to both the sperms and function of testes [1]. Though it is the treatment of choice for solid tumors and many others, DOX leads to the path of male infertility.[2]

Infertility is one of the major problems following chemotherapy in cancer treatment.[1]

Chemotherapy affect fertility of young males by influencing on function of testes. Cancer being controlled by chemotherapy, however seems to bear a child after treatment becomes a significant issue. Dysfunction of testes is the commonly seen side effects for a longer period of time in men receiving chemotherapy.[3,4]

Infertility observed after chemotherapy could be directly due to damage of the germ cells or indirectly because of effect on endocrine control of somatic cells. Proliferation of sertoli cell during their infancy period in fact makes them a target of toxicity of cancer treatment in childhood.[5,6]

To ameliorate these undesirable effects, several dietary supplements have been tested, out of which increase popularity is gained by antioxidants as adjuvant in therapy.[7]

prevention of damage to cell by destroying oxidized free radicals by antioxidant is gaining its relevance in chemotherapy treatment as adjuvant.[8]ascorbic acid also is observed in improving Dox induced dysfunctional systolic and diastolic with damage to the structure [9]. All these indicate a cardioprotective action of ascorbic acid on Dox mediated cardiomyopathy by declining the oxidative stress, apoptosis, and inflammation, also improving ascorbic acid transporter proteins.[9] combination with ascorbic acid likely to be of potential interest because it can potentiate the ant proliferative action of DOX.[10] literature reveals that vitamin c is a powerful antioxidant that is water soluble and it also scavenges the ROS [11, 12].

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Deficiency of this vitamin is characterized by increase in oxidative stress and damage to tissues that includes oxidant-induced cell death.[11,12]

Prognosis of survival may be improved by antioxidants like ascorbic acid through improving the therapeutic index of co administered chemotherapeutic drugs.

Rationale of study was to demonstrate both morphological changes in terms of weight of testes and morphometry of organ by examining thickness of germinal epithelium that are produced by anthracycline DOX on the testes of mice and designed to compare these changes in DOX affected testes with combination of ascorbic acid

MATERIAL AND METHODS

Only healthy and active five weeks old NMRI strain of male mice were selected for the experiment and were chosen randomly. It was an experimental study conducted for 10 months at Institute of Basic Medical Sciences Dow University (262/DUHS-11). It was taken into account that there was least weight difference within and among groups. The samples were chosen randomly. Estimation of the sample size was done as statistically advised; at least 8 animals were required in each group for statistical analysis. They were divided in to A, B and C groups. Each had 10 animals.

Group A (Control Group):
Control group received 1 ml of normal saline IP on sixth, eighth and tenth day of the study.

Group B (DOX group):
DOX group had DOX in dosage of 0.003 mg/g or 0.003 mg in 0.03 ml /gm body weight IP [13] up to 3 doses on sixth, eighth and tenth day of study (total cumulated dose 0.009 mg/gm).

Group C: (DOX + ascorbic acid group)
Group C received DOX in a dose of 0.003 mg/gm or 0.003 mg in 0.03 ml /gm body weight IP [13] and Ascorbic acid in dose of 0.5 mg/gm or 0.5 mg in 0.01ml/gm body weight P.O.[14] DOX on sixth, eighth and tenth day of experiment and ascorbic acid was given daily basis.

Animals were anaesthetized by deep ether anesthesia and were dissected through midline abdominal incision. After identification, testes were freed from surrounding tissues and were weighed on digital scale. After fixation in Bouin's fluid for 24 hours, longitudinal sections of testes were taken and were placed in properly marked and labelled cassettes for further processing. Five µm thick sections were obtained from rotary microtome and were mounted on marked glass slides. For thickness and in each animal five sections were selected. In each section five fields and in each field three circular or semicircular tubules were selected. In total, fifteen tubules were selected in each section and were observed in H&E stained slides under 40x objective and 10x ocular lenses of light microscope. To observe the statistical difference among the groups, One Way Analysis Of Variance was used and then Tukey post Hoc test was applied to check the pair wise comparison at 5% level of significance

RESULTS

Absolute weight (mg) of testes of mice:

Comparison of absolute weight of testes between the control group (A) and DOX group (B)

The mean weight ± SD of testes of control mice was 0.10 ± 0.02 mg. The mean weight ± SD of testes of DOX group was 0.04 ± 0.02 mg. When the comparison of these two groups was done, P value of < 0.001 was seen for the testes at 95% C.I as shown in table-1

The highly significant reduction of absolute testicular weight was observed in the DOX group.

Comparison of absolute weight of testes between DOX group (B) and DOX + Vitamin C group (C)

The mean weight ± SD of DOX treated group was 0.04 ± 0.02 mg, whereas the mean weight ± SD of testes of DOX + Vitamin C treated mice was 0.08 ± 0.03 mg. When the comparison of these two groups was done, P value = 0.006 was seen at 95% C.I as shown in table-1

The significant increase of absolute testicular weight was observed in the DOX + Vitamin C group.

Comparison of absolute weight of testes between the control group (A) and DOX + Vitamin Ctreated group (C)

The mean weight ± SD of testes of control mice was 0.10 ± 0.02 mg, whereas the mean weight ± SD of testis of DOX + Vitamin C treated group was 0.08 ± 0.03 mg. With the comparison of two groups, P value of 0.507 was obtained at 95% C.I as shown in table-1

The non-significant decrease of absolute testicular weight was observed in the DOX + Vitamin C group.

Table 1. Comparison of Mean ± SD of Absolute Weight (mg) of Testes in Different Groups

Groups	Mean ± SD	P value Among Groups		95% Confidence Interval	
		Groups	P value	Lower Bound	Upper Bound
Control (A)	0.10 ± 0.02	A vs B	< 0.001*	0.023	0.08
DOX (B)	0.04 ± 0.02	B vs C	0.006*	-0.068	-0.013
DOX + Vitamin C (C)	0.08 ± 0.03	A vs C	0.507*	-0.015	0.04

* Tukey HSD Post Hoc Multiple comparisons test

Thickness of germinal epithelium (µm):
Comparison of thickness between the control group (A) and DOX group (B)
The mean ± S.D of thickness of germinal epithelium in control was 58.42 ± 5.663 µm and in the DOX group was 3.925 ± 2.438 µm. When the mean thickness of germinal epithelium was compared between control and DOX groups, the P value turned out to be < 0.001 at 95% C.I as shown in table-2, figure 1.

A significant decrease was seen in the mean thickness of germinal epithelium of DOX group.

Comparison of thickness between the DOX group (B) and DOX + Vitamin C group (C)
The mean ± S.D of thickness of germinal epithelium in DOX group was 3.925 ± 2.438 µm and in the DOX + Vitamin C was 38.70 ± 16.65 µm. When the mean thickness of germinal epithelium was compared between DOX and DOX + Vitamin C groups, the P value turned out to be < 0.001 at 95% C.I as shown in table-2 figure 1 and 2.

A significant increase was seen in the mean thickness of germinal epithelium of DOX + Vitamin C group.

Comparison of thickness between the control group (A) and DOX+ Vitamin C group (C)
The mean ± S.D of thickness of germinal epithelium in control was 58.42 ± 5.663 µm and in the DOX + Vitamin C group was 38.70 ± 16.65 µm. When the mean thickness of germinal epithelium was compared between control and DOX + Vitamin C groups, the P value turned out to be < 0.001 at 95% C.I as shown in table-2

A significant decrease was seen in the mean thickness of germinal epithelium of DOX + Vitamin C group.

Table 2. Comparison of Mean ± SD Thickness of Germinal Epithelium (µm) of Seminiferous Tubules in Different Groups

Groups	Mean ± SD	P value Among Groups		95% Confidence Interval	
		Groups	P value	Lower Bound	Upper Bound
Control (A)	58.42 ± 5.663	A vs B	< 0.001*	43.13	65.86
DOX (B)	3.925 ± 2.438	B vs C	< 0.001*	-45.47	-24.07
DOX + Vitamin C (C)	38.70 ± 16.65	A vs C	< 0.001*	9.02	30.42

* Tukey HSD Post Hoc Multiple comparisons test

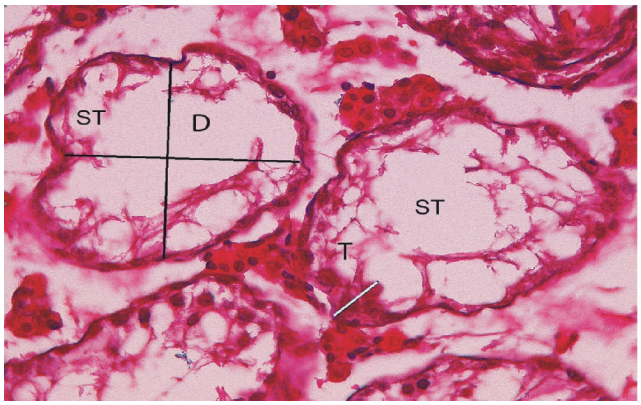


Figure 1. Photomicrograph ofH&E stained 5 µm thick section of testis showing distorted tubules with diameter D of seminiferous tubule ST and thickness of germinal epithelium T of mouseexposed to DOX at x400.



Figure-2 Photomicrograph of H&E stained 5 µm thick section of testis showing diameter D of seminiferous tubule ST and thickness of germinal epithelium T of mouse administered with DOX + Vitamin C at x400.

DISCUSSION

The DOX used in the present experiment caused reduction of absolute and relative organ weights as compared to controls in mice. This was in consistence with findings in studies by Das in 2012[15], Kato (2001)[16], Zanetti (2007)[17],Salu (2009)[18],Patil (2009)[19], and Sudha (2011).[20]They also observed decrease weight of the testes as compared to controls, after exposure to DOX. The results presented here showed that treatments with DOX caused a significant decrease in testicular weight by reducing the population of germinal cells. Decrease visceral weight in turn indicates loss of cells and presence of necrosis within organ as was observed in a study on the effect of DOX on heart in 2010.[21]A recent study in 2012 is in accordance with our study and reports by other researchers indicated that testicular weight depends on the number of germinal epithelial cells in seminiferous tubules and hence its reduction (because of decreased number of germinal cells) resulted in decreased testicular weight.[22]

Our study showed significant atrophic changes in testes of mice treated with DOX, indicated by reduction in thickness of germinal epithelium. Similar results were seen by other researches. Zanetti (2007)[17],Vendramini (2011)[23],Salu (2009)[18]and Patil in 2009[19]had mentioned germ cells death, that was followed by a decline in morphometric parameters in testicular tissue caused by anticancer drug DOX, which results in the reduction of tubular diameter and thickness of germinal epithelium. A distinctive reduction was noted in seminiferous epithelial damage in the mice treated with Vitamin C + DOX, in comparison to those solely receiving DOX. There was significant improvement in testicular weight of DOX + Vitamin C group as compared to the group administered with DOX alone. Thickness of germinal epithelium also revealed improvement. Improvement thickness of germinal epithelium observed in this study in group administered with both DOX and Vitamin C, can be matched with the findings in experiment done by Sapra and collegues.[24]This confirmed the protective role of Vitamin C against compounds that caused damage by free radical formation.[25]

CONCLUSION

Study suggested that Ascorbic Acid plays a potential role on deteriorated morphology and morphometry of testes by DOX by showing significant changes on weight of the organ along with thickness of germinal epitheliumand could be used as adjuvant therapy in cancer patients.

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AUTHOR'S CONTRIBUTION

The following authors full fill authorship criteria as per ICMJE guidelines;

Haq AA, Afzal S:	Idea conception, data acquisition, final approval, agreed to be accountable for all the work.
Rehan R, Khan M:	Design of the work, drafting the work, critical rivision, final approval, agreed to be accountable for all the work.
Ahmed H:	Data analysis, drafting of the work, final approval, agreed to be accountable for all the work.
Iqbal S:	Data interpretation, critical revision, final approval, agreed to be accountable for all the work.