Original Article

Protective Role of Vitamins E in Doxorubicin Induced Toxicity in Rat Testes: A Histomorphometric Study

Farooq Khan, Nighat Ara, Shamila Hafizi, Nomanullah Wazir, Fahadullah and Ambareen Hamayun

ABSTRACT

Objective: To observe the antioxidant effect of vitamin E on the toxicity of doxorubicin of rat testes.
Study Design: Analytical experimental randomized control study
Place and Duration of Study: This study was conducted at the Department of Anatomy, Peshawar Medical College Peshawar from February 2013 to July 2013.
Materials and Methods: Thirty two rats were randomly divided into two main groups; normal and the experimental groups. Experimental group was further divided into the toxic and vitamin E groups. The toxic group (B-I) was given doxorubicin at the dose of 2 mg/kg body weight, i.e. weekly for four weeks. While the vitamin E group (B-II) was introduced with the oral administration of vitamin E at the dose of 150 mg/kg daily for four weeks along with the doxorubicin i.e. weekly for four weeks. After scarifying the animal according to the protocol the testis were sectioned and then preparatory slides were used to apply the basic stains i.e. H and E stain, PAS stain and Massan Trichrome stains.
Results: Group B-I treated with Doxorubicin only showed marked decreased in body weight, testicular weight, decreased height of epithelium and germ cells count as compared to B-II.
Conclusion: The simultaneous use of vitamin E as an antioxidant, can protect the toxic effects of Doxorubicin and thus damage to the testes.
Key Words: Doxorubicin, Testicular toxicity, vitamin E

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INTRODUCTION

Cancer continues to represent the largest cause of mortality in the world. It takes almost six million lives every year.[1] Chemotherapy involves the use of chemical agents to stop the growth and eliminate cancer cells even at distant sites from the origin of primary tumor.[2]

Among these chemotherapeutic agents, Doxorubicin has long been widely used for its potent efficiency.[3] Doxorubicin belongs to a class of medications called anthracyclines. It works by slowing or stopping the growth of cancer cells in the body. It is derived from the algae, Streptomycespeucetius Sp. Caesius.[4]

Doxorubicin can impair the motility of sperms[5], induce germ cell apoptosis[6], and result in testicular damage ultimately.[7] The exact mechanism of doxorubicin testicular toxicity is still completely not known, but doxorubicin induced cardiomyopathy implicates the breakage of DNA continuity, overload of oxidative stress, and apoptosis of cells.[8]

An antioxidant is a molecule that inhibits the oxidation of other molecules. The most popular and abundant antioxidant vitamin is vitamin E, which is used worldwide nowadays. Supplementation of vitamins E has protected the testicular tissues and sperms.[09,10]

Vitamin E is an essential fat-soluble nutrient that serves as an antioxidant and is also used in cell signaling, regulation of gene expression and immune functions. It was discovered by Evans and Bishop in 1922, as a necessary dietary factor for reproduction of rats[11].

As a fat soluble antioxidant, it stops the production of reactive oxygen species formed when fat undergoes oxidation[12,13,14]. Vitamin E has many biological functions. As an antioxidant, vitamin E acts as a peroxyl radical scavenger, preventing the propagation of free radicals in tissue, by reacting with them to form a tocopheryl radical by the help of a hydrogen donor i.e.: vitamin C , and thus return to reduced state[15].

Vitamin E also plays a role in neurological functions[16], and inhibition of platelet aggregation.[17,18] Vitamin E also protects lipids and prevents the oxidation of polyunsaturated fatty acids.[19]

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MATERIALS AND METHODS

Thirty two male albino rats of Sprague Dawley strain, 8 weeks of age, weighing 200-220 gm were procured from the animal house of Peshawar Medical College animal house. These animals were kept in solid bottom polypropylene cages. Group 1 (control group): animals of this group received intraperitoneal normal saline injection once a week for a period of four weeks. Group 2 (experimental group): Sub-group I. Animals of this group received intraperitoneal doxorubicin at 2mg/kg body weight, on weekly basis for a period of four weeks. Sub-group II. Animals of this group received intraperitoneal doxorubicin at 2mg/kg body weight, on weekly basis for a period of four weeks and Vitamin E, at a dose of 150mg/ kg body weight daily for four weeks. On 28th day, animals were euthanized and organs were collected in 10 % neutral buffered formalin to be processed for paraffin embedding, 0.5 µm thick sections were cut on rotary microtome and were stained with Haematoxalin and Eosin for routine microscopy. Sections were also stained with Masson's Trichrome to see the changes in the connective tissue elements of the stroma. PAS stain was also applied to see the integrity of the basement membrane and the carbohydrates contents of the cellular and non-cellular elements of the testis. Following observations were made under the microscope; thickness of seminiferous epithelium, number of spermatogenic cells/cross section of seminiferous tubule, integrity of Basement membrane by PAS stain, demonstration of the connective tissue by Masson's Trichrome and demonstration of Muscular tissue By H and E stain. Data was analyzed by using the SPSS version 15, and the P value was considerably significant statistically.

RESULTS

At the beginning of the study, average weight of animals in group A was 193.5±4.7 in group B-I was 192.0±4.60, group B-II was 195.5±4.27. The difference in the weights of control group and antioxidant group showed insignificant (P>0.009). At the end of the study, average weights of animals in control group was 246±4.6, in group B-I was 228.5±6.2, in group B-II was 238±4.8 respectively. The body weight was increased in group A and became significantly higher as compared to experimental group B-I as shown table 1.

The weights of testes of all the groups were measured and compared. The average weight in control group was 1.54±0.044, in group B-I the weight was 1.46±0.030 in group B-II was 1.52±0.038 grams. There was a significant difference in body weight of control group and group B-I with p value of (<0.0052). So far the weights of testes is concerned, the result of present study indicate that the most affected group was B-I which was only given doxorubicin as compared to the groups B-II, which was treated with antioxidants in addition to doxorubicin during the experiment. This confirms the protective role of antioxidants when given in combination with doxorubicin as shown in table 2.

In control group the testes were pink, firm in consistency and ovoid in shape. The H and E stain showed tunica albuginea with dark pink fibers running in bundles showing compact arrangement. These fibers are identified as collagen fibers. There were marked changes in the experimental group B-I. There is decreased in the germ cells count in group B-I, also there is decreased is epithelial height of the seminiferous epithelium as compared to the experimental group B-II as seen in Figs. 1-6.

The height of the seminiferous epithelium in all the groups was measured and compared. The average height in control group was 9.8±0.044 µm in group B-I was 9.3±0.035 µm in group B-II was 9.7±0.038 µm. There was significant difference in epithelial height among the control group and the group B-I with a p value of (<0.0051). So far the height of epithelia is concerned; the results of present study indicate that the most affected group was the B-I which was given doxorubicin only as compared to the groups B-II, which received both doxorubicin and antioxidant as shown in table 3.

The average germ cells count of all the groups were measured and compared. The average germ cells count in control group was 293.35±0.044 cells/HPF, in group B-I was 217.625±0.030 cells/HPF, in group B-II was 253.87±0.038 cells/HPF. The difference in amount of germ cells count among the control group and the antioxidant treated groups was insignificant (P<0.001) as shown in Table 4.

Table No.1: Body weight of animals in control group

Animal number	Initial weights of animals (gm)	Final weights of animals (gm)	Difference in weights (gm)
A1-A4	193.5	246	52.5
BI-1— BI-8	192	213.13	21.13
BII-1--- BII-8	195.5	233	37.5

Table No.2: Weight of testis in all groups

Animal number	Initial weights of right testis (gm)	Mean weights of left testis (gm)	Mean average weight of testis (gm)
A1-A4	1.54	1.53	1.54
BI-1— BI-8	1.46	1.47	1.46
BII-1--- BII-8	1.51	1.53	1.52

Table No.3: Epithelial height (thickness) of all groups

Animal number	**Mean Thickness (µm)**
A1-A4	9.9
BI-1---BI-8	8.52
BII-1---BII-8	9.3

Table No.4: Mean germ cells count in all group

Animal number	**Mean germ cell count (cell/HPF)**
A1-A4	293.5
BI-1---BI-8	217.63
BII-1---BII-8	253.87

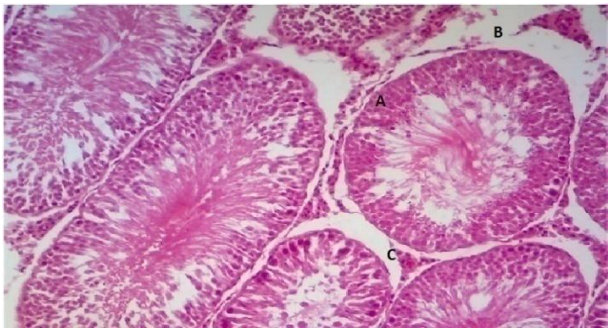

Figure No. 1: Photomicrograph of 5µm thick section of testis of a control group showing normal histoloarchitecture of the seminiferous tubules A, interstial space B and cells of Leydig C. H and E; X 180

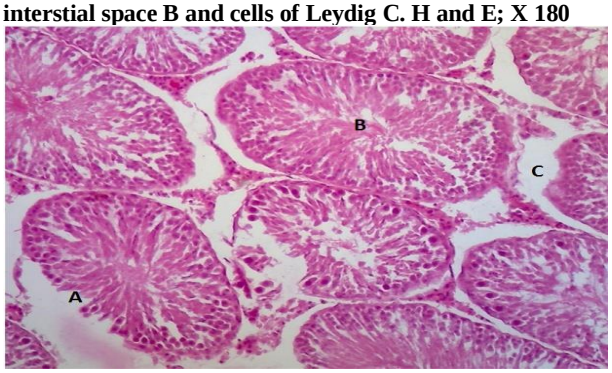

Figure No.2: Photomicrograph of 5µm thick section of a group B-I showing histopathological changes in the seminiferous tubules i.e. Disrupted Basement membrane (A), congested Lumen (B) and wide interstitial space (C). H and E; X180

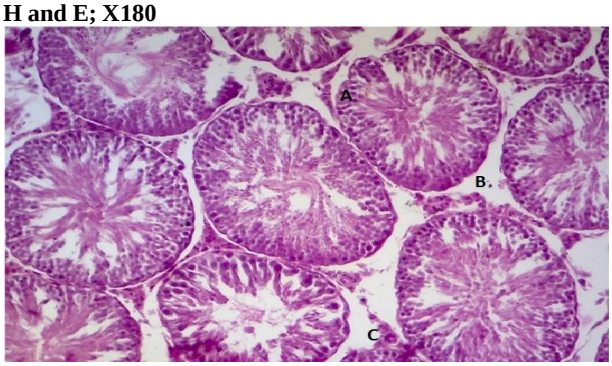

Figure No.3: Photomicrograph of 5µm thick section of control group showing normal seminiferous tubules (A), interstitial space (C), cells of Leydig (D).PAS; X180

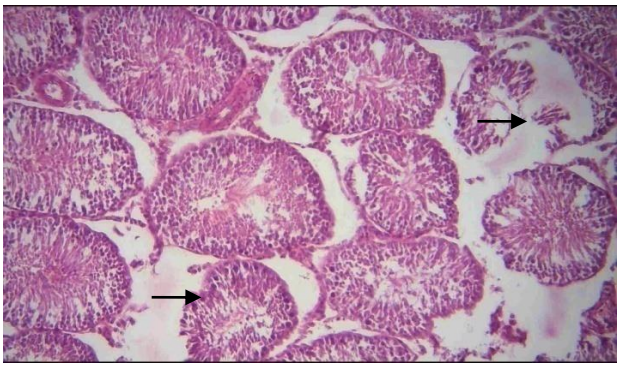

Figure No.4: Photomicrograph of 5µm thick section of a group B-I Showing sloughing of the germ cells due to disruption of basal membrane PAS; X180.

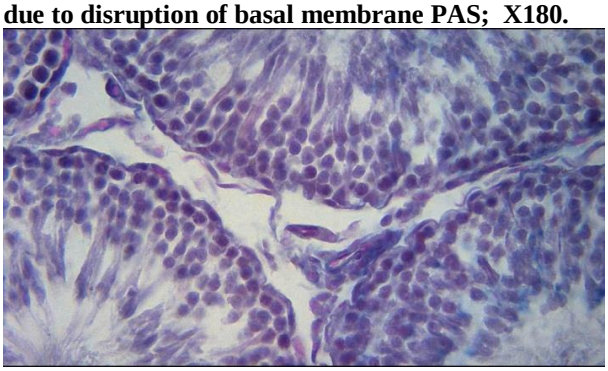

Figure No.5: Photomicrograph of 5µm thick section of control group showing normal histoarchitecture of the seminiferous tubules (A), Lumen (B), interstitial space (C), Intact basal Lamina (D).Masson's Trichome; X400

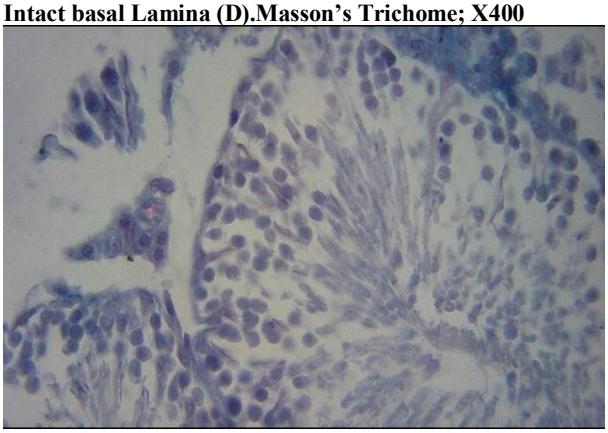

Figure No.6: Photomicrograph of 5µm thick Masson Trichome stained section of a group B-I showing normal histoarchitecture of the seminiferous tubules. Masson's Trichome; X 400.

DISCUSSION

Cancer is considered as a major health problem that has become the most common leading cause of death throughout the world. To fight against this *deleterious* disease a number of anti cancer medicines have been introduced in the field of medicine. Doxorubicin is one of the commonest medicines used for this purpose. It is worth mentioning that doxorubicin like any other drug is also having adverse effects on the body of the individuals being treated. To reduce the adverse effects

of doxorubicin during chemotherapy co-administration of antioxidants have been proved to be beneficial.

After administration of the doxorubicin for a period of 4 weeks, we observed 20% weight loss in the animals that were treated with doxorubicin only (group B-I), however none of the remaining experimental groups showed any statistically significant weight loss. Loss of appetite due to adverse effects of doxorubicin on GIT is an obvious reason for the weight loss in this particular group. Therefore, our finding is in accord with the result of a study conducted by Harvey (1987), who found that there was a significant weight loss in those albino rats that were treated with doxorubicin. Harvey attributed this weight loss to appetite loss and gastrointestinal disturbances.[20] The current study is also in close agreement with the study of Der R, Fahim et al. (1974), where they found that the animals of the experimental groups had 16% loss of the body weight as an adverse effect of doxorubicin which had led to ulcerative lesions at the sites of the injections.[21]

We found that there was a significant loss in the weight of testes of group B-I animals as compared to animals of the control group. Groups B-II animals protected with vitamin E did not show remarkable loss of testicular weight during the experiment. Our study is in agreement with a study conducted by Evenson and Jost, (1993) who recorded loss in weights of testes of animals treated with doxorubicin.[22] Our study is also in conformity with the study done by Patil and Balaraman (2009) who reported testicular weight loss of animals treated with doxorubicin for a period of 5 weeks.[23]

This finding of the present study correlates with the results of a similar study conducted by Lu and Meistrich (1979), where it was found that even a low dose of doxorubicin (1 mg/kg.b.w.) could target the germ cells and spermatogonia, leading to a decrease in the height of seminiferous epithelium.[24]

So far the germ cells count is concerned, we found that group B-I showed marked decrease in germ cells count as compared to the groups B-II which were given antioxidant along with doxorubicin during experiments. In this aspect our study stands in complete harmony with the study conducted by Ward et al. (1988), who reported doxorubicin induced reductions in germ cells count.[25] The present study also strongly supports the findings of a study conducted by Biswas NM (1996) and Ghosh (2002), in which vitamin C was given to rats being treated with doxorubicin[26]. Their results showed a significant elevation in the activities of the testes, and an increase in germ cells count, which may be due to the direct stimulatory effect of the vitamin on the enzyme i.e. 3β-HSD (hydroxysteroid dehydrogenase deficiency) and 17 β-HSD.[27,28] It may also be due to antioxidant effect of vitamin C against oxidative stress induced by doxorubicin.[29-31]

CONCLUSION

Simultaneous use of antioxidant vitamin C can prevent the testicular damage which can be caused by doxorubicin toxicity.

Author's Contribution:

Concept & Design of Study:	Farooq Khan
Drafting:	Nighat Ara, Shamila Hafizi
Data Analysis:	Nomanullah Wazir, Fahadullah, Ambareen Hamayun.
Revisiting Critically:	Farooq Khan, Nighat Ara
Final Approval of version:	Farooq Khan

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

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