HISTOMORPHOMETRIC STUDY REVEALING THE PROTECTIVE ROLE OF VITAMINS C IN DOXORUBICIN INDUCED TOXICITY IN RAT TESTES

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ABSTRACT

Objective: To observe the antioxidant effect of vitamin C on the toxicity of doxorubicin of rat testes.

Methodology: The following Analytical experimental randomized control study was conducted on male albino rats. This study was conducted in the PCSIR laboratory complex and in Peshawar medical college during February- July 2013. Rats were randomly divided into two main groups. The normal and the experimental group. Experimental group was further divided into the toxic and vitamin C groups.

The toxic group (B-I) was given doxorubicin at the dose of 02 mg/kg body weight. While the vitamin C group (B-II) was also introduced with the oral administration of vitamin C along with the doxorubicin.

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.

Data was analyised by using the SPSS version 15, and the P value was considerably significant statistically.

Results: The current study was conducted to observe the doxorubicin toxicity in male Albino rats, and to find out any possible protective effects of antioxidant vitamin C.

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: It is concluded that simultaneous use of vitamin C as an antioxidant , can protect the toxic effects of Doxorubicin and thus damage to the testes.

Keywords: Doxorubicin, Testicular toxicity, Antioxidant, Vitamin C.

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 ascorbic acid (vitamin C) which is used worldwide nowadays. Supplementation of vitamins C has protected the testicular tissues and sperms.[09,10]

The core of the antioxidant network includes powerful antioxidants such as CoQ10 and vitamin C. In addition to their free radical fighting properties, this antioxidant has other functions that allow them to make critical contributions to our health and longevity. Vitamin C, have antioxidant role in protection of the organs against the toxicity caused by the cancer drugs. Deprivation of vitamin C make experimental animal rapidly lose weight, show marked degeneration in testes, epididymis, vas deference and the cessation of spermatogenesis.[11]

Vitamin C is found in high concentrations in the

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immune cells, and is consumed quickly in infections. It is not certain how vitamin C interacts with the immune system: It has been hypothesized to modulate the activities of phagocytes, the production of cytokines and lymphocytes, and the number of cell adhesion molecules in monocytes.[12]

Vitamin C (ascorbic acid) is water soluble, enabling it to scavenge free radicals in aqueous (watery) environments, such as the inside of our cells and extracellular body fluids.[13]

METHODOLOGY

Male Albino rats of Sprague Dawley strain,08 weeks of age, weighing 200-220gm, (n= 32), were procured from the animal house of Peshawar Medical College animal house. These animals were kept in solid bottom polypropylene cages. Experiments were conducted as per the protocol approved by the institutional animal ethics committee.

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 groups.

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 C, at a dose of 500mg/ 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.

i. Number of spermatogenic cells/cross section of seminiferous tubule.

ii. Integrity of Basement membrane by PAS stain.

iii. Demonstration of the connective tissue by Masson's Trichrome.

iv. Demonstration of Muscular tissue By H and E stain.

RESULTS

The current study was conducted to observe the doxorubicin toxicity in male Albino rats, and to find out any possible preventive effects of antioxidant such as vitamin C.

Table 01: I- Body Weight (bw) of animals in Control group.

Animal No	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 02: Weight of testis in all groups.

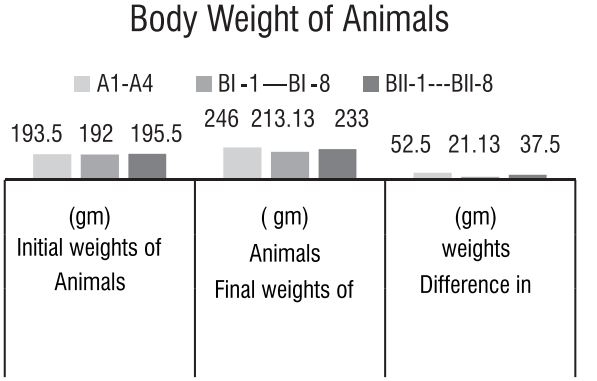
Animal number	Mean weights of Rt. Testis (gm)	Mean weights of Lf. Testis (gm)	Mean average Wt. of Testis (gm)
A1-A4	1.54	1.53	1.54
BI-1---BI-8	1.46	1.47	1.46
BII-1---BII-8	1.51	1.53	1.52

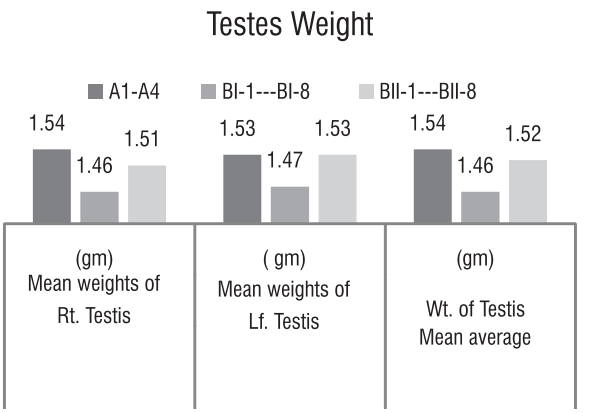
Table 03: I-Epithelial Height (thickness) of all groups.

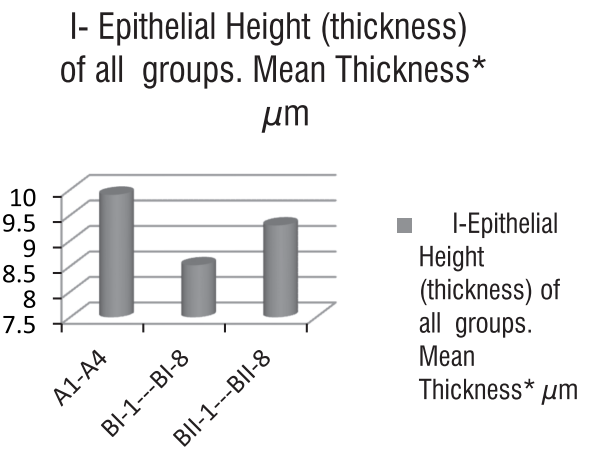
Animal No.	Mean Thickness* μm
A1-A4	9.9
BI-1---BI-8	8.52
BII-1---BII-8	9.3

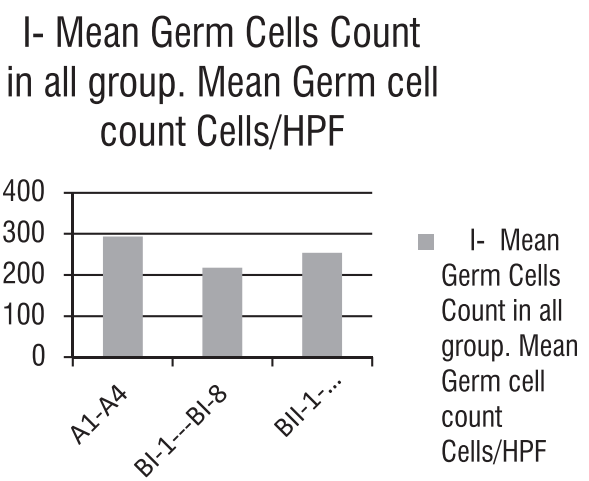
Table 04: I - Mean Germ Cells Count in all group.

Animal No.	Mean Germ cell count Cells/HPF
A1-A4	293.5
BI-1---BI-8	217.63
BII-1---BII-8	253.87









General Physical Appearance and Behavior of the animals

All the rats in control group and the experimental group (groups B-II) remained active and healthy with normal feeding behavior. After five weeks, the animals in groups B-II were more active than group B-I animals. Their MBW was 240 gm which was significantly higher than group B-I animals which was 228 gms.

Body weight (gm)

To confirm the effect of doxorubicin toxicity and

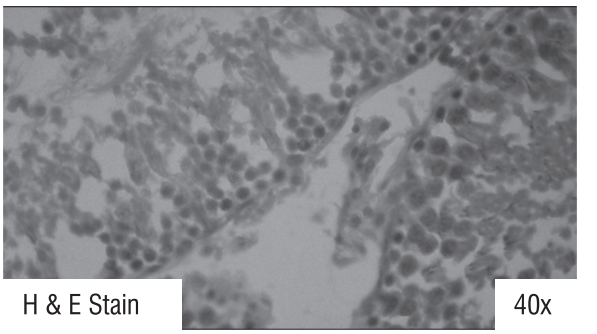


Fig. Cross section of testis of control group at 400x magnification.

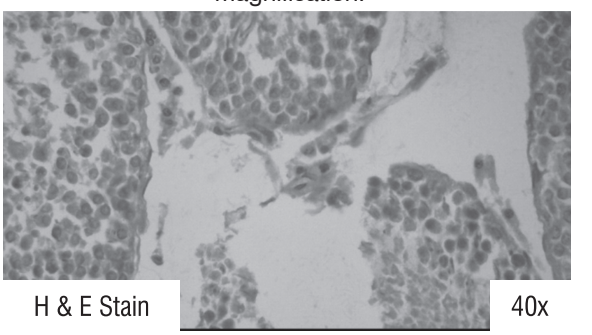


Fig. Cross section of testis of Group II at 400x magnification.

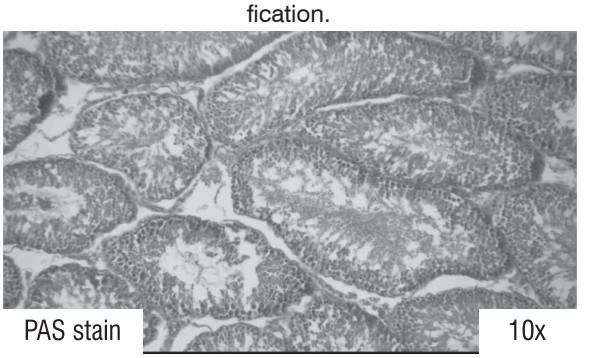


Fig. Cross section of testis of control group at 100x magnification.

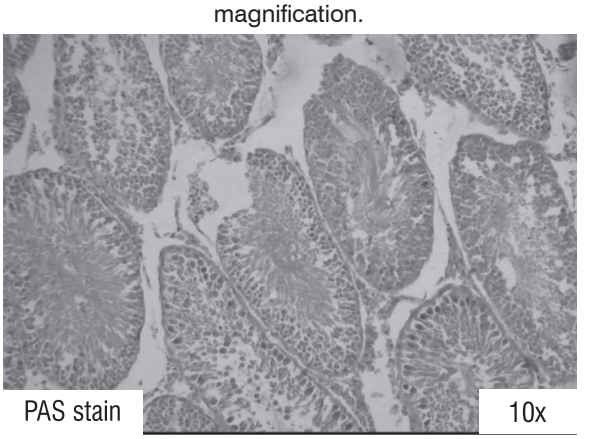


Fig. Cross section of testis of Group I at 100x magnification.

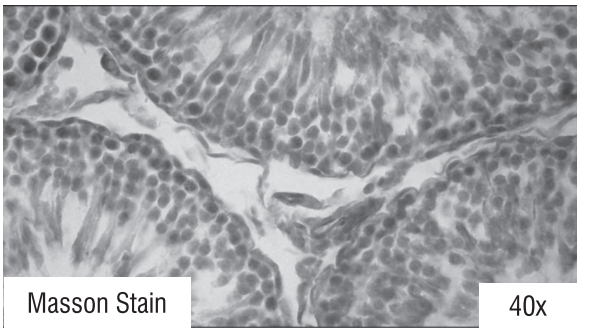


Fig. Cross section of testis of control group at 400x magnification.

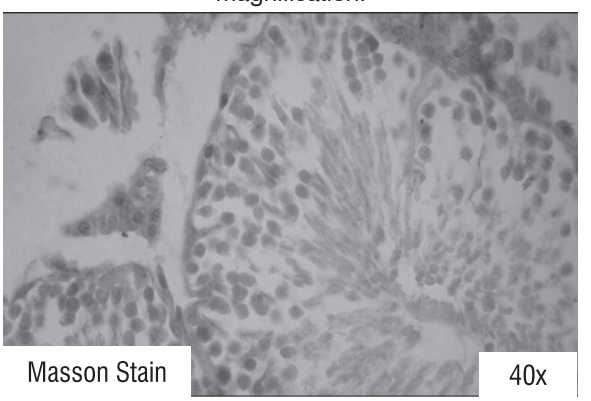


Fig: Photomicrograph section of testis group B-I at 400x

the protective effect of anti-oxidant i.e. vitamin C, pre and post experimental weighing was done.

At the Beginning of the study

At the beginning of the study, average weight of animals in group A was 193.5 gms + 4.7, in group B-I was 197.50 gms + 4.60, group B-II was 196.27 gms + 4.27, in group B-III was 197.2 gm + 5.1 and in group B-IV was 191 gms + 4.7 respectively. The difference in the weights of control group and antioxidents treated groups showed insignificant (P>0.009).

At the End of the Study

At the end of the study, average weights of animals in control group was 246 gm+ 4.6, in group B-I was 228.5 + 6.2, in group B-II was 238 gms + 4.8, in group B-III was 238.2 gms + 4.8 and in group B-IV was 239 gms + 4.7 respectively.

The body weight was increased in group A and became significantly higher as compared to experimental group B-I. The weight of the experimental groups i.e. groups B-II and B-III and B-IV were significantly higher than the group B-I which was treated with Doxorubicin only.

Fig: Graph Showing Mean average body weight at the beginning, end and the difference in weights of all the groups.

Weight of the Testes (gm)

The weights of testes of all the groups were measured and compared. The average weight in control group was 1.54 gms+ 0.044, in group B-I the weight was 1.46 gms + 0.030, in group B-II was 1.52 gms + 0.038.

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.

Histological appearance of testis

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 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 groups i.e. B-II, B-III and B-IV respectively.

Height of Seminiferous Epithelium (μm)

The height of the seminiferous epithelium in all the groups was measured and compared. The average height in control group was 9.8 μm + 0.044 in group B-I was 9.3 **μm** + 0.035 in group B-II was 9.7 **μm** + 0.038. 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.

The thickness of the seminiferous epithelium was measured at 10 random locations and an average mean was worked out as the average thickness of the seminiferous epithelium under 10x.

Germ Cells Count

The average germ cells count of all the groups were measured and compared. The average germ cells count in control group was 293.35 cells/HPF + 0.044, in group B-I was 217.625 cells/HPF + 0.030, in group B-II was 253.87 cells/HPF + 0.038, in group B-III was 271.66 cells/HPF + 0.029 and in group B-IV was 279.95 cells/HPF + 0.030. The difference in amount of germ cells count among the control group and the antioxidant treated groups was insignificant (P<0.001).

PHOTOMICROGRAPHS

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. Although very limited studies have been conducted in this regard.

The present study was conducted to evaluate the toxic effects of doxorubicin and the protective role of vitamin C, against doxorubicin induced histological changes in the rat's testes. For this purpose, 24 male albino rats of Sprague Dwaley strain were selected.

The animals were randomly divided into a control group, and experimental groups receiving Doxorubicin (B-I), Doxorubicin and vitamin C (B-II).

In the current study H & E, PAS and Masson Trichome stains were used to study the histoarchitecture of the testes.

Weight of the Animals

To assess the effect of doxorubicin on the body weight of the animals, weight measurement of animals of the experimental group was measured and was compared with the body weights of the animals of the control group.

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[14]

The current study is also in close agreement with the study of Der R, Fahim Z 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.[15]

Weight of the Testes

In the current study measurement of the weight of testes was done to assess toxic effects of doxorubicin if any on the weight of testes.

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 C 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.[16]

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[17].

Height of seminiferous epithelium

In the present study we have noted a decrease in height of the seminiferous epithelium in group B-I animals as compared to the control group. This change was not observed in group B-II animals.

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.[18]

Germ Cells Count

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[19].

The present study also strongly supports the findings of a study conducted by Biswas NM (1996) and Ghosh S (2002), in which vitamin C was given to rats being treated with doxorubicin[20]. 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[21].

It may also be due to antioxidant effect of vitamin C against oxidative stress induced by doxorubicin[22,23,24].

CONCLUSION

It is concluded that that simultaneous use of an-

tioxidant vitamin C can prevent the testicular damage which can be caused by doxorubicin toxicity.

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