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HISTOMORPHOMETRIC COMPARISON ON DOXO-TOXICITY WITH SAFETY EVALUATION OF ANTIOXIDANTS WITH VITAMIN C IN ALBINO RAT TESTES

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ABSTRACT

Objective: To observe the histological effect of conjoint antioxidant in comparison to vitamin C on the doxo-toxicity induced on rat testes. **Methodology:** The following experimental randomized control analysis research was conducted on male albino rats. This study was conducted in the PCSIR laboratory complex and in Peshawar medical college during February- July 2019. The Rats were arbitrary divided into two major groups. The normal and the experimental groups. Experimental group was further subdivided into toxic (B-I), conjoint group (B-II) and Vitamin C (B-III) groups. The normal group animals were given i.p. injection of placebo on weekly basis, while in the experimental group B-I group was introduced to doxorubicin at a dose of 02 mg/kg i.p. body weight weekly for four weeks. While the conjoint group (B-II) were given i.p. of same dose with combine therapy of Vit. C and E orally on daily basis. While the vitamin C (B-III) groups were given orally on daily basis. After oblations of the animal were used according to the protocol, the testes were taken out and sectioned. The prepared slides were used for the basic stains i.e: H and E, Periodic Acid Schiff stain and Massan Trichrome

stains. Data of the experimental groups was analyised by the SPSS version 21, and the P value was considerably significant statistically. **Results:** The current research was conducted to observe the doxo-toxicity in Albino rats, and to find out the possible protective therapies in combination in contrast to vitamin C. B-I group treated with Doxorubicin only manifest notable decreased body weight, testicular weight, reduced height of seminiferous epithelium and count of germ cells as compared to B-II and B-III. Also there were positive and good results in conjoint therapy comparable to single antioxidants. **Conclusion:** It is concluded that simultaneous use of antioxidants comparable to single, can shows better results to prevent damage to testes.

KEYWORDS: Doxorubicin, Testicular toxicity, Histomorphometery, vitamin C, Vitamin E.

INTRODUCTION

Cancer represents to be the largest cause of transience in the world, taking about six million lives each year.[1] Chemo-therapy involved in the treatment chemical agents use to eliminate and stop the growth of the cancer cells even at remote sites from its origin of primary tumor.[2]

It is an abnormal cell growth is a group of diseases involving the capability to invade and spread to other parts of the body.[3]

Amid these therapeutic anticancerous agents, Doxorubicin is among the widely used for its influential efficacy.[4] It belongs to a group of anthracyclines. It MOA shows slowing by blocking the cancer cell growth in the body. The algae, Streptomycespeucetius Sp. Caesius[5] is a specious from which it is derived.

Doxorubicin can harm the locomotion and effect the epithelial height of testes,[6] and can induce germ cell autophagy,[7] which results in the testicular destruction ultimately.[8]

The potential side effect is Damage to the testicular germinal epithelium is affected due to the cancer therapy, and in men it is of particular concern having tumors with high cleanse rates in the middle age people.[9]

The antioxidant molecule reduces the oxidation of other molecules which affect the testicular epithelium. The most importantly used antioxidants are vitamins currently i.e: ascorbic acid

(vit. C) and vitamin E, which are used worldwide. Supplementation of antioxidants has prevented the testicular tissues and sperms.[10]

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 of weight, patent testicular degeneration of epididymis, vas deference and the halting of spermatogenesis.[11] Antioxidants individually or in combination pattern can protect normal cells against some of the toxicities produced by these therapeutic agents.

High concentration of Vitamin c is seen in the immune cells, and is used quickly in infections. It is not yet known that how vitamin C interacts with the immunity. Hypothesis been made that it to modulate the phagocytic activities, cytokines production and lymphocytic reaction with the number of cell adhesion molecules in monocytes.[12]

- Ascorbic acid i.e Vit.C is a water soluble agent, which enables it to scavenge free radicals in aqueous environments, such as the inside of cells as well as extracellular body fluids.[13]

Due to its fat solubility activity of vitamin E, stops the production of reactive oxygen species experiences fat oxidation.

It acts as a peroxyl radical scrounger due to antioxidant activity, which preventing the spread of free radicals in organism, by acting with hydrogen donor, forming a tocopheryl radical. It also shields the lipids and arrests the oxidation of poly unsaturated fatty acids.[14]

Different researchers use different patterns to see the effect of different regime and their valuable results. However in this study we have seen the single verses conjoint effect of antioxidents to prevent the toxic effect of chemotherapy.

Methodology

The Male Albino rats Strain of Sprague Dawley, aging of 08 weeks, weight 250-300gm, (n= 24), were pick up from the animal house of National Institute of Health Islamabad. These animals were kept in special prepared cages for experimental purposes. Inclusion and exclusion criteria were applied according to the protocol. Care of the rat's i.e: food and water were regularly given with well established with well equipped and trained staff in the animal house. Trails were conducted as per ethical committee approved.

1. Control group

Rats in this group receive i.p., saline injection, for a month duration on weekly basis.

2. Experimental groups

This group was further subdivided into the following sub groups.

Sub group B-I.

These animals received i.p., doxorubicin at 2mg/kg/BW, per week for a month.

Sub group B-II.

This group contains animals which received i.p., doxorubicin at 2mg/kg/BW, per week for a month, and Vitamin C, at a dose of 500mg/ kg/BW, per oral daily for a month.

Sub group B-III.

This group contains animals which receiving i.p., doxorubicin at 2mg/kg/BW, per week for a month, and combination of Vitamin E and vitamin C, at a dose of 500mg/ kg/BW daily for a month orally.

After the completion of experimental duration the animals were euthanized by using the overdose of ether in a jar and then organs (testicals) collected and placed in 10 % buffered formalin neutral solution for processing in paraffin embedded, the organs were sectioned 0.5 µm of thickness by microtome and were then stained for histological purpose with H & E for routine microscopic analysis. The slides were also used to stain by Masson's Trichrome to observe the histology in the stroma of connective tissue.

Application of PAS stain was use to see the Basement membrane integrity.

Following observations were made under the microscope.

i. Testicular Seminiferous epithelium.
ii. Spermatogenic cells/mm.[3]
iii. Integrity of the Basement membrane.
iv. Connective tissue stroma by using the Masson's Trichrome.

RESULTS

The current study was conducted to observe the doxorubicin toxicity in male Albino rats, and to find out any possible preventive effects of antioxidants such as vitamin C and combine C and E (Conjoint group).

General physical Appearance and Behavior of the animals

All the rats in control group and the experimental groups (groups B-I, B-II and B-III) remained active and healthy with normal feeding behavior.

After five weeks, the animals in groups B-II and B-III were more active than group B-I animals. Their MBW was 230 and 240 gm respectively which was significantly higher than group B-I animals which was 225 gms.

Body weight (gm)

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

At the beginning of the study

At the start of the research, the mean weight of rats in control group was 194.5 gms + 4.7, In experimental group i.e: group B-I was 195.50 gms + 4.6, group B-II was 196.27 gms + 4.27, and in group B-III was 197.2 gm + 5.1 respectively. The mean difference in weights of control group and antioxidants treated groups observed insignificant (P>0.009).

At the end of the study

At the end of the research, the mean weights of rats in control group was 245 gm+ 4.6, in group B-I was, 227.5 + 6.2, in group B-II, was 237 gms + 4.7, in group B-III was 245.2 gms + 4.8 respectively.

The body weight was increased in control group and became notably higher in contrast to the B-I. The weight of the experimental groups i.e. groups B-II and B-III was significantly increased to the group B-I which was treated with Doxorubicin only.

Graph and Table 01

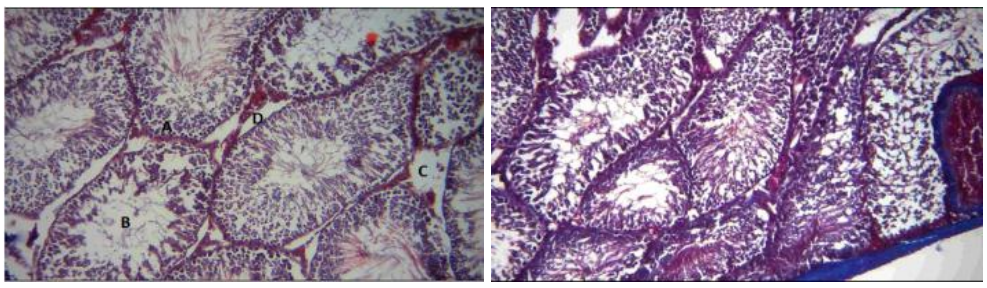

Figure 1: Photomicrograph showing normal verses B-I group with loss of connective tissue stroma with Massan Trichrome stain, X180.

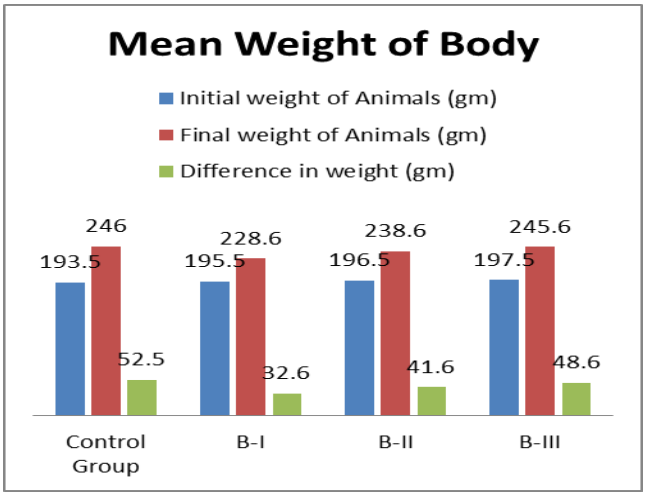

Fig. 1: Graph Showing Mean average body weight at the beginning, end and the difference in weights of all the groups.

Weight of the Testes (gm)

After the euthanization of animals, testes were taken out for gross analysis, so weights of all groups was conducted and then collate. The mean weight in control group was 1.53 gms+ 0.04, group B-I shows weight was 1.45 gms + 0.030, group B-II was 1.53 gms + 0.038 and B-III was 1.53 gms + 0.039 respectively.

There was a remarkable differences in all body weight of control group in contrast to group B-I with a p value of (<0.005).

After the analysis the testicular weight shows that current study indicate the most affected group was B-I, compared to the groups B-II and B-III, which were given antioxidants during the study, which affirms the safe role of antioxidants showing less decreased in testicular weight.

Graph and Table 02

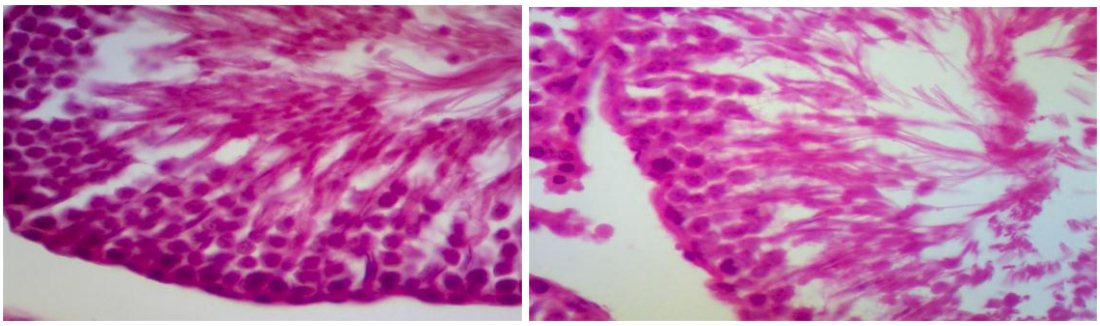

Figure 2: Photomicrograph showing B-III verses B-I group with diminished height of seminiferous epithelium with H & E stain, X360.

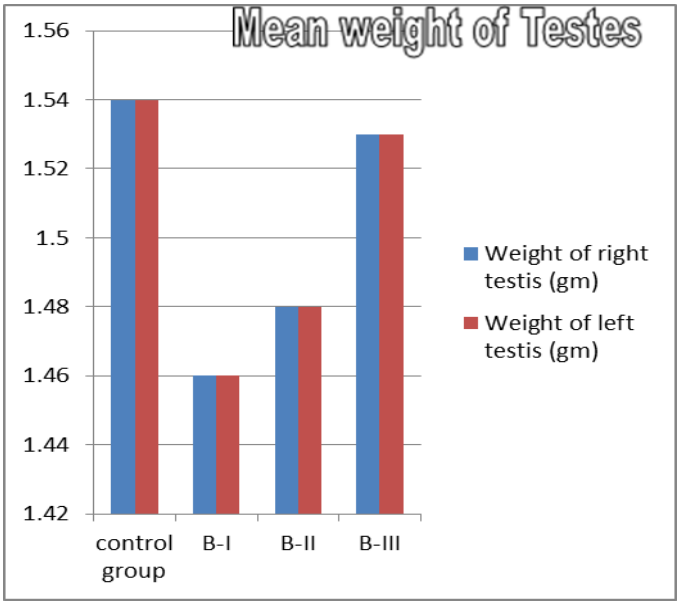

Fig. 2: Graph Showing Mean average weight of Right and Left testes in of all the groups.

Histological appearance of testes

Histological analysis was seen that control group shows pinkish color, its firm in consistency and of ovoid shape. The tunica albuginea shows dark pinkish fibers arranged in bundles showing compact aggregation of collagen fibres in H and E stain. Increased changes were seen in the B-I group. Marked decreased in the germ cells count and diminish seminiferous epithelial height in contrast to the i.e. B-II and B-III, respectively.

Height of seminiferous epithelium (µm)

The germinal epithelial height in all groups was analyzed and compared with each other. The average height in placebo group was 9.9 µm + 0.04 in group B-I was 8.5 µm + 0.04 in group B-II was 8.64.7 µm + 0.038 and in group B-III was 8.64.7 µm + 0.039. There was high difference in seminiferous epithelium among the placebo group with the group B-I with a p value of (<0.0051).

So far the germinal epithelium is concerned; the current study indicates the most affected group was B-I, in contrast to B-II and B-III, which received both doxorubicin and antioxidants.

Graph and Table 03

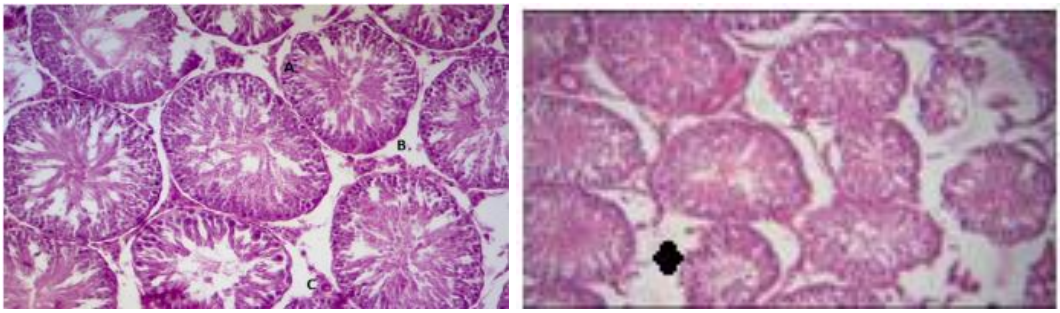

Figure 3: Photomicrograph showing normal verses B-I group with sloughing of the germ cells (✹) due to disruption of basal membrane PAS stain, X180.

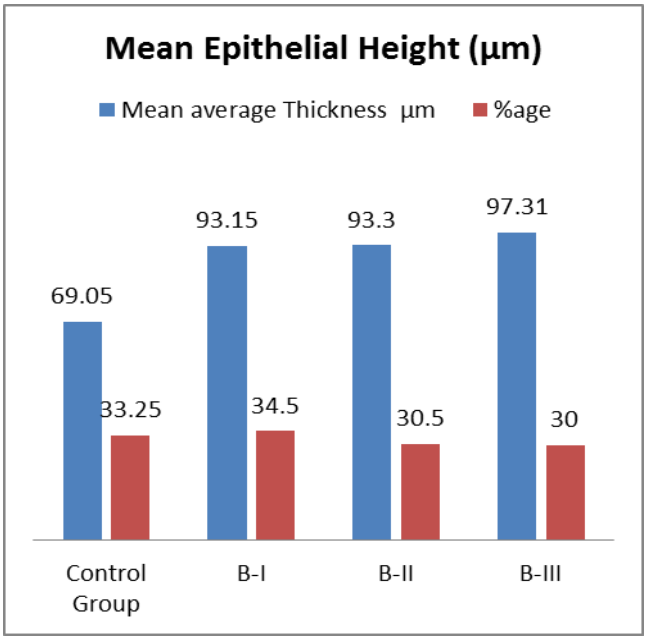

Fig. 3: *The thickness of the seminiferous epithelium was measured at 10 random Locations and An average mean was worked out as the average thickness of the seminiferous epithelium under 10x.

Germ cells count

The mean germ cells count was compared and analyzed in all groups. The mean germ cells count in placebo group was 290.0 cells/HPF + 0.04, in group B-I was 217.5 cells/HPF + 0.03, in group B-II was 253.5 cells/HPF + 0.04, and in group B-III was 283.66 cells/HPF + 0.03. The statistics showing the count of germ cells among the placebo group and the antioxidants treated groups was insignificant (P<0.001).

Graph and Table 04

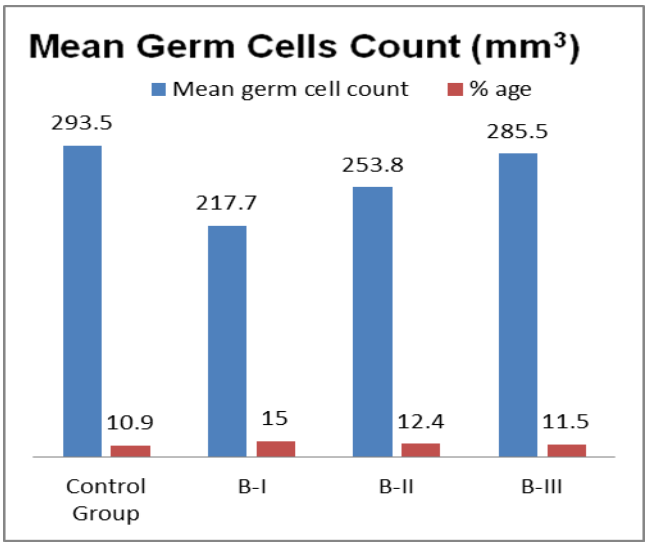

Fig. 4: Chart Showing description of Mean Germ Cells count at the end of Procedure and The difference in its weight.

DISCUSSION

Cancer is one of the major health issues that have become the leading cause of death throughout the world. Multiple trends and methods are working to fight against this *deleterious* disease multi anti cancerous medication are introduced. Doxorubicin is one of the commonest widely medicine used, despite its limiting side effects of the drug. Now multiple methods are currently used to reduce the side effects of doxorubicin administration along with the antioxidants have proved to be beneficial. Although very limited studies have been conducted in this regard.

The present study reveals to evaluate the toxic effects of doxorubicin and the preventive role of vitamin C in contrast to conjoint methods, against doxorubicin prompt histological changes in the rat's testes. For this purpose, 24 male albino rats of Sprague Dawley strain were selected.

The animals were arbitrarily are divided into a control group, and experimental groups receiving Doxorubicin (B-I), Doxorubicin and vitamin C (B-II) and Doxorubicin with vitamin C and Vitamin E (B-III).

In the current study H & E, PAS and Masson Trichrome 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.

There was a marked decreased (20%) as the dox. was given i.p.,(B-I) for a period of 4 weeks; however the remaining experimental groups showed less significance in the weight loss.

The main side effects of Dox. is the loss of appetite which shows confirm answer for the weight loss in this specific group, which was confirmed with the Harvey results conducted. Harvey accredits this weight loss was due G.I disturbance and loss of appetite.[15]

Our study is also in close relation with the study of Der R, Fahim Z et al., in which the animals of the experimental groups had 16% loss in body weight due to an adverse effect of dox. which led to injection site abscess.[16]

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.

There was a marked decreased in the testicular weight in B-I group animals in contrast to animals compared with placebo group. The antioxidant group's i.e: B-II and group B-III, didn't show comparable loss of weight of testes in the experiment.

Evenson and Jost, also saw weight loss of testes of those animals treated with doxorubicin, was in agreement with our current study.[17]

Also there was conformity of our study with the research conducted by Patil and Balaraman who proclaimed decreased in testicular weight treated with doxorubicin for a 05 weeks period.[18]

Height of seminiferous epithelium

In the present study we have seen a decline in seminiferous epithelial height in group B-I compared to the control group. This decreased was not seen in group B-II and B-III animals.

Our current research coincides with a similar study conducted by Lu and Meistrich, in which low dose of dox. (1 mg/kg.b.w.) also impair the germ cells and spermatogonia, which leads to decrease in the testicular epithelial height.[19]

Germ cells count

As far the germ cells count is agitated, there is decline in group B-I showing marked decrease in comparison to groups B-II and B-III, which were introduce to antioxidants in addition to doxorubicin.

So the study done by Ward et al., reported that doxorubicin induced decreased in germ cells count which is similar to our current research work.[20]

Also our study stands with the research by Biswas NM and Ghosh S, which showed that vitamin C and E or in combination was introduced and showed better results to rats being treated with dox.[21,22] There may be an effect of antioxidant i.e: vitamin C and E on oxidative stress by doxorubicin induced.[23]

CONCLUSION

It is concluded that that concurrent use of antioxidant i.e: vitamin C and E or in combination can protect the damage to testes caused by doxorubicin toxicity during the treatment.

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