

## ORIGINAL ARTICLE

## ROLE OF ANTIOXIDANT IN CHEMOTHERAPY RELATED INFERTILITY

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**Background:** Chemotherapeutic agent Doxorubicin (DOX), an anthracycline induced gonadotoxic damage has concerned physicians for decades. DOX induces damage on healthy tissues through the formation of free radicals. Vitamin C, a water-soluble non-enzymatic antioxidant was used to study its protective role on male gonads damaged by DOX. **Methods:** In this experimental study 30 male mice were divided into 3 groups of 10 mice each, A, B, and C. Group A was given normal saline 1 ml intraperitoneal (IP). Group B mice were given DOX alone (0.003 mg in 0.03 ml/gm body weight IP for 3 doses on 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> day of study). In Group C, DOX (0.003 mg in 0.03 ml/gm body weight IP for 3 doses on 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> day of study) was given and Vitamin C (0.5 mg in 0.01 ml/gm body weight per orum daily was added to medication. On completion of experiment, epididymides dissected along with testes from sacrificed animals and were placed in Petri dish containing 5 ml saline and left for a few minutes for spermatozoa to float. Neubauer chamber was used for sperm count. **Results:** The DOX administration significantly decreased ( $p<0.001$ ) number and motility of sperms as compared to controls. However co-administration of Vitamin C with DOX significantly reduced ( $p<0.001$ ) the seminiferous epithelial damage. Sperm parameters seemed to be improved as compared to DOX only and control group respectively. **Conclusion:** Vitamin C has protective role against DOX induced testicular damage.

**Keywords:** Doxorubicin, Vitamin C, Antioxidant, Oxidative damage, Sperm, Epididymis, Infertility

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## INTRODUCTION

Chemotherapy for cancer is given either alone or with radiotherapy and surgery. These chemotherapeutic drugs act mainly on proliferating cells in cancerous mass, hence it is no wonder that they cause gonadotoxic effect as testis is among the viscera which has rapidly proliferating spermatogenic cells.<sup>1</sup> Differentiating spermatogonia are more sensitive to toxic effect of these drugs.<sup>2</sup> Because of the slow rate of mitosis in stem cell spermatogonia, they are more resistant to cytotoxic drugs.<sup>3</sup> Chemotherapy benefits cancer patients by inhibiting tumour growth, but simultaneously put them at risk of side effects by damaging the non-targeted elements of the body.

Formation of reactive oxygen specie by Doxorubicin (DOX) has been a medical concern for years, as its formation results damage to sperms and testicular function. DOX leads to male infertility, however, it is a treatment of choice for solid tumours.<sup>4</sup>

In human body the level of reactive oxygen specie (ROS) is kept in minimal amount. Seminal fluid has antioxidant for the protection of spermatozoa from oxidative ROS. But in cancer patients, its level gets reduced leading to damage of spermatozoal plasma membrane. This decline in levels is observed even before the administration of

chemotherapeutic drug. Administration of anticancer further exacerbates this condition.<sup>5</sup>

Based on the fact that various unwanted effects of DOX occur through formation of free radicals, anti-oxidant therapy has been considered and has proved to be effective in mitigating the effects of DOX on testis. Action of antioxidants like tocopherol and Vitamin C reduces the activity of free radical.<sup>6</sup> The survival of animals receiving antioxidants with chemotherapy, was increased in comparison to those receiving chemotherapy alone.<sup>7</sup> It has got effects stronger than other antioxidants and also stimulate in the formation of collagen. This helps in walling off the cancer.<sup>8</sup> As an antioxidant, Vitamin C seems to interfere in free radical formation induced by DOX.<sup>9</sup> Decreased levels of Vitamin C were seen in seminal fluid of infertile patients.<sup>10</sup>

Morphological disturbance observed in testicular tissue by mercury was noticed to be less affected by co-supplementation of Vitamin C.<sup>11</sup> Potential of Vitamin C in clinical use is evident by many studies. Its benefit to health may be attributed not only to its antioxidant property but also to its anti-atherogenic and immunomodulator activity.<sup>12</sup>

A nourished and healthy cancer patient has the capacity to bear the severity of cytotoxic therapy. Addition of Vitamin C as an adjuvant therapy has helped to reduce the undesired testicular effect of

DOX in our clinical application. This work was designed to study the changes in number of sperms in DOX affected testes with co-administration of antioxidant Vitamin C.

## MATERIAL AND METHODS

Thirty male NMRI mice aged 5 weeks were acquired from animal house of Ojha Campus, Dow University of Health Sciences, Karachi. Five mice were kept in each tagged cage under normal circadian rhythm, food, and water *ad libitum*. They were housed under observation for a week before beginning of the study.

The animals were divided into A, B, and C groups, each group of 10 animals. Control group had 10 male mice and received normal saline 1 ml IP on 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> day of the study. Group B had 10 animals and received DOX in dose of 0.003 mg/g or 0.003 mg in 0.03 ml/gm body weight IP<sup>13</sup> up to 3 doses on 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> day of study (total cumulated dose 0.009 mg/gm). Group C comprised of 10 animals and received DOX in a dose of 0.003 mg/gm or 0.003 mg in 0.03 ml/gm body weight IP and Vitamin C in dose of 0.5 mg/gm or 0.5 mg in 0.01 ml/gm body weight PO<sup>14</sup>. DOX was given on 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> day of experiment, and Vitamin C was given daily.

At the end of the experiment, epididymides were dissected free from the testes, pieced in a Petri dish, and was kept in 5 ml of normal saline for 5 minutes to let the spermatozoa float in saline. Sperms were counted using a Neubauer chamber.

Data was analyses on SPSS-16. ANOVA was applied to find the statistical difference between the groups. Tukey-multiple Comparisons post Hoc test was applied to check the pair-wise comparison at 95% confidence interval.

## RESULTS

The mean sperm count was  $3.02 \pm 0.41$  ( $\times 10^6$ ) in controls,  $0.90 \pm 0.31$  ( $\times 10^6$ ) in DOX group, and  $1.75 \pm 0.37$  ( $\times 10^6$ ) in DOX+Vitamin C group. The sperm count of DOX group was significantly less than control group. The sperm count of DOX+Vitamin C group was significantly less than control group, but it was significantly more than DOX group. (Table-1, 2).

**Table-1: Sperm count in the groups ( $\times 10^6$ )**

Groups	Mean $\pm$ SD	95% CI
Control (A)	3.02 $\pm$ 0.41	2.72–3.32
Doxorubicin (B)	0.90 $\pm$ 0.31	0.67–1.12
Doxorubicin+Vit C (C)	1.75 $\pm$ 0.37	1.48–2.01

**Table-2: Comparison of sperm count among groups ( $\times 10^6$ )**

Groups	Mean $\pm$ SD	p	95% CI	
A vs B	3.02 $\pm$ 0.41	0.90 $\pm$ 0.31	<0.001*	50.96–73.90
B vs C	0.90 $\pm$ 0.31	1.75 $\pm$ 0.37	<0.001*	-1.23– -0.463
C vs A	1.75 $\pm$ 0.37	3.02 $\pm$ 0.41	<0.001*	0.88–1.66

\*Tukey HSD Post Hoc Multiple comparisons test

## DISCUSSION

As a strong antioxidant, Vitamin C seems to interfere with DOX induced free radical formation. Marked decrease in levels of various antioxidants including Vitamin C was seen with the treatment of DOX. Scanty numbers of studies are available on the role of Vitamin C as an antioxidant on the effects of DOX and the interpretable data is sparse. Few reports have been published on the ameliorating effect of Vitamin C on testicular toxicity of DOX in mice observed under light microscope.<sup>11,12</sup>

In our study, there was a significant decrease in the sperm count in the DOX group, when compared with controls, whereas significant increase ( $p < 0.001$ ) in count was seen in DOX+Vitamin C treated group in comparison to DOX only group. A significant improvement in count was seen in DOX+Vitamin C treated group but was still less than control group.

Reduced sperm count in our study after exposure to DOX was in agreement to observations of Kato *et al*<sup>16</sup>, Zanetti *et al*<sup>17</sup>, Vendramini *et al*<sup>18</sup>, Patil *et al*<sup>19</sup>, and Sahafi *et al*<sup>20</sup> who reflected degeneration occurring in seminiferous tubules. However none of these studies addressed the ameliorating effect of Vitamin C on sperm count.

Sperm analysis from the epididymis also suggested that Vitamin C could provide partial protection to the survival of spermatogenic cells depicted by improvement in sperm count in DOX+Vitamin C group. This indicates that spermatogonia resist the toxic effects of DOX with co-administration of Vitamin C.<sup>18</sup> This in turn supports the evidence that in male reproductive system, Vitamin C has a vital role as far as spermatogenesis is considered.<sup>21</sup> Fazeli *et al*<sup>22</sup> demonstrated remarkable improvement in motility and percentage of live sperms after Vitamin C supplementation. They found it effective in decreasing the percentage of abnormal sperms.

None of the studies so far discussed both the drugs together affecting sperm count. Some studies show harmful effects of DOX and other metabolites having same mode of action on testes. There are other studies showing improvement in testicular and epididymal sperm parameters affected by other drugs having same mode of action as doxorubicin, but none has observed improvement in sperm parameters by combining both drugs. Our study revealed mitigating effects of vitamin C on the drastic changes in testicular parameters produced by DOX chemotherapy.

## CONCLUSION

Vitamin C plays a significant role in ameliorating the damage of testes induced by DOX, showing improvement in the epididymal sperm parameters.

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