Effect of Doxorubicin on the morphology, histology and karyology of male reproductive system of white mice, Mus musculus

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Abstract
Doxorubicin (adriamycin) an anti tumour antibiotic is effective against solid and non-solid malignant tumour. It is known to produce reproductive toxicity. An attempt has been made to study the effect of Doxorubicin on the histological and morphometric changes in the male reproductive system and karyological changes in the bone marrow of mice, Mus musculus at the prescribed dosage of 0.5-1 mg/ kg body weight. The histological observations in the present studies showed no apparent damage to the testis and there was no difference in spermatogonial, spermatocyte or spermatid population. Epididymis and the seminal vesicle too showed no apparent damage in the dosage studied. Chromosome analysis of the bone marrow cells did not show any significant aberrations. At the dose designed in the present investigation all the changes observed were transitory and did not impair the normal functioning of the reproductive tissues.

Keywords. Doxorubicin, histology, testis, morphometry, karyology

Introduction
Cancer remains a hugely expensive public health problem both in terms of economy and the amount of human suffering it produces (Weiss, 1995). McGardy (1993) aptly describes a cancer cell as “a savage cell which somehow corrupts the forces which normally protects the body, invades the well ordered society of cells surrounding it, colonizes distant areas and as a finale to its cannibalistic orgy of flesh consuming flesh, commits suicide by destroying the host.”

Gonadal injury by antineoplastic drugs like Doxorubicin, though commonly observed, has been relatively less investigated when compared to their other adverse effect (Ward et al., 1988). Doxorubicin exhibits profound toxicity to the reproductive system, adversely affecting male fertility (Shamberger et al., 1981). Doxorubicin is very effective, but exhibits reproductive toxicity at high doses (Lu & Meistrich, 1979; Meistrich, 1982; Ward et al., 1988).

Doxorubicin causes severe degenerative changes in germinal cells, atrophy in the diameter size of seminiferous tubules and germinative cell thickness. Meistrich et al., (1990) showed that translocations could result in heritable mutations and doxorubicin elevated DNA fragmentation and toxicity. Kamendulis et al., (1994) proved that DNA damage is an early causal event in toxic cell death caused by alkylating hepatotoxicants. At doses of 6 mg/kg, doxorubicin is a weak inducer of chromosomal mutation (Meistrich et al., 1990).

Although the above data indicate that doxorubicin is a testicular toxicant which is dose dependent not much work has been carried out to study the effects of Doxorubicin at the recommended therapeutic doses. Therefore an attempt has been made to study the histological and morphometric changes in the tissues related to the male reproductive system and the karyological changes in the somatic cells of the bone marrow and the effect after the cessation of the administration of the drug.

Materials and methods
Experimental animal and laboratory maintenance
Male white mice of inbred Swiss strain, 45±5 days old and of 30±5 gm body weight were selected and used for experiment. The animals were obtained from Fredrick Institute of Plant Protection and Toxicology (FIPPAT), Padapai. The animals were housed in polypropylene cages and provided with standard pelletized feed. Food and water were provided ad libitum.

Experimental design
The animals were divided into three groups. The group I (0.9% saline, intraperitoneally, every 24 hours and sacrificed on the 6th day), group II (0.5 mg/ kg of Doxorubicin intraperitoneally, every 24 hours and sacrificed on the 6th day) and group III (0.5 mg/ kg intraperitoneally, every 24 hours for 5 days with a withdrawal period of 6 days and sacrificed on the 13th day).

Methodology
Doxorubicin has been given in doses of 0.5-1 mg/kg daily for 2-6 days (Goodman and Gilman, 1975) Based on this a dose of 0.02mg/ animal was obtained for the present study by dissolving 1.0 mg of Doxorubicin in 5 ml of 0.9% saline.0.01 ml of this solution was administered intraperitoneally with a 25 gauge needle with a disposable syringe. The animals were sacrificed by cervical dislocation. The tissues like the testis, seminal
vesicle and epididymis were immediately removed, cleaned from the adhering tissue and weighed individually. The organs and tissues were processed for histological studies as per the methods of Pearse (1980; 1985). The volume estimation of the various regions of the male reproductive system was done according to the principles of Elias & Pauly (1966). Chromosome preparation and aberration scoring was done according to the methods of Murthy (1983).

Data analyses were carried out using SPSS statistical package. Analysis of variance (ANOVA) was used to determine differences between various data sets. Tukey's multiple range test was used to resolve difference among treatment mean. A value of p<0.05 was used to indicate significant difference.

Results and discussion

Body weight and tissues

The effect of Doxorubicin on the body weight and the weight of various tissues are shown in the Table 1 & 2. Doxorubicin administration produced a significant decrease in the body weight. The overall trend showed a decrease in the individual tissue weights too. The reduction in weight was significant in all the tissue studied except cauda and corpus. The weight of the body and the tissues of group III increased when compared to the group II except in cauda and corpus. The group III animals may reach the weight of the group I animals if sufficient time is given. Pristas et al., (1992) have shown Doxorubicin induced decrease in body weight in mice. Ward et al., (1998) also reported a dose related reduction in the weight of testis epididymis and seminal vesicle at the dose of 1 mg/kg body weight.

Histology

The results of the histological preparation of the testis from Doxorubicin are shown in Plate 1 & Fig 1. The testis shows that there is no observable difference between the groups with regard to spermatogonial, spermatocyte or spermatid population. Leydig cells show a marginal degeneration in the group II which show recovery in the group III. Ward et al., (1998) illustrated the presence of large vacuoles in the cytoplasm of Sertoli cells is indicative of spermatogenic epithelial degeneration and is dose related. Patil and Balaraman (2009) have reported vacuolization and fibrinoid debris in the seminiferous tubule when male rats were treated with 15 mg/kg of Doxorubicin. Sonmez et al. (2005) and Saalu et al. (2006) have reported that germinal epithelium is more sensitive to cytotoxic drugs and oxidative stress. At low doses there are no large vacuole formations but on increasing the dose from 3 mg/kg to 12 mg/kg, the number of these large vacuoles increases extensively. No such vacuoles could be observed in the present study and no apparent damage to the testicular tissue could be detected in the experimental group. In accordance to the literature cited, the low dose of doxorubicin employed in the present study could be a possible explanation for the absence of any testicular damage.

Table 1. Effect of Doxorubicin on the body weight (in gms) of white mice, Mus musculus

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>33.69±1.03(a)</td>
</tr>
<tr>
<td>II</td>
<td>30.67±0.21(b)</td>
</tr>
<tr>
<td>III</td>
<td>30.64±0.42(b)</td>
</tr>
</tbody>
</table>

The given values are Mean ±SD. Means within a column with different letters are significant(p<0.05)

Table 2. Effect of Doxorubicin on the total organ / tissue weight (in mg) / of male reproductive system of white mice, Mus musculus

<table>
<thead>
<tr>
<th>Testis</th>
<th>Caput</th>
<th>Corpus</th>
<th>Cauda</th>
<th>Seminal Vesicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group - I</td>
<td>109.72 ± 0.74(a)</td>
<td>31.67 ± 1.05(a)</td>
<td>4.10 ± 0.36(a)</td>
<td>33.66 ± 0.13(a)</td>
</tr>
<tr>
<td>Group - II</td>
<td>97.03 ± 2.35(b)</td>
<td>28.40 ± 0.50(a)</td>
<td>4.00 ± 0.10(a)</td>
<td>29.90 ± 0.21(b)</td>
</tr>
<tr>
<td>Group - III</td>
<td>109.70 ± 1.8(a)</td>
<td>31.45 ± 2.31(a)</td>
<td>4.07 ± 0.6(a)</td>
<td>30.91 ± 1.54(b)</td>
</tr>
</tbody>
</table>

The given values are Mean ±SD. Means within a column with different letters are significant(p<0.05)
Effect of Doxorubicin on the histology of the various regions of the male reproductive system of the white mice Mus musculus

Fig. 3. Section of Corpus

Fig. 4. Section of Cauda

Fig. 5. Section of Seminal vesicle

Sections of caput epididymis (Fig. 2) show no observable difference in the histological preparations, between all the three groups. Neither the columnar principal cells with microvilli nor the sperms in the lumen show any change. The same is true of the corpus and the cauda (Fig. 3 & 4). Sections of the corpus however presented an interesting observation. The overall size of the corpus showed remarkable difference between the three groups. The corpus of group III showed a decrease in size with deterioration of most of the tubules when compared to the group I, while group III showed an increase in the corpus size as well as the number of tubules with respect to the experimental. However, it never reached the size of the group I. Considering only the histological details no apparent drug effect could be accounted for in the epididymis. However, to decide if the changes in the size of the corpus epididymis of the treated animals can induce strain on its functional capacity needs further study.

Histological preparations of the seminal vesicle (Fig. 5) clearly demonstrate the difference in the villous mucosa. The villous mucosa gets obliterated and the primary, secondary and tertiary folds are not clearly visible in group II. The luminal volume increases and the lumen is vacuolated in group II. The smooth muscle layer

Table 3. Effect of Doxorubicin on the spermatogonial, spermatocyte and spermatid population (per 100 of total cells) of male white mice Mus musculus

<table>
<thead>
<tr>
<th>Group</th>
<th>Spermatogonia ±SD</th>
<th>Spermatocyte ±SD</th>
<th>Spermatid ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>18.69 ± 0.25(a)</td>
<td>35.52 ± 0.30(a)</td>
<td>45.42 ± 0.34(a)</td>
</tr>
<tr>
<td>II</td>
<td>17.26 ± 0.13(b)</td>
<td>33.78 ± 0.26(b)</td>
<td>46.94 ± 0.18(b)</td>
</tr>
<tr>
<td>III</td>
<td>16.60 ± 0.49(c)</td>
<td>32.65 ± 0.43(c)</td>
<td>43.30 ± 0.72(c)</td>
</tr>
</tbody>
</table>

The given values are Mean ±SD; Means within a column with different letters are significant (p<0.05)
found in between the connective layer and the villous mucosa is thinner in the in the experimental. However these changes recuperate in group III. Hence it can be reasoned that even if the histological changes witnessed in group II probably impaired the function, the changes are transitory and could be reverted back to normal if sufficient withdrawal time is given.

**Cell count in testis sections**

The percentage cell count of spermatogonial, spermatocyte and spermatid population of the testis was done and the results are tabulated in Table 3. All the three cell types showed a significant decrease in group II. The spermatogonial, spermatocyte and the spermatid values further decreased in group III. The decrease in the cell count further proves the reproductive toxicity of the drug Doxorubicin. Reduction in spermatogonial population is supported by Meistrich (1982) who has demonstrated that drugs like Doxorubicin causes killing of the spermatogonial stem cells. This finding is further supported by Russell and Russell (1991). Mouse testicular stem cells were killed at high doses (Lu & Meistrich, 1979). Ward et al., (1988) demonstrated dose dependent damage to the seminiferous tubules, which was reflected in the testicular and epididymal sperm content.

Though the testicular damage or injury due to doxorubicin is reversible in most cases (Shamberger et al., 1981), the values of the cell count further decreased in group III, and this may be because the time needed for the recuperation after the cessation of the administration of the drug and the values may return to the control value if sufficient time is given. Similar trend was obtained where the various enzymes like LDH, Glucokinase, acid phosphatase and alkaline phosphatase changed on the administration of Doxorubicin but reverted back to the control value (Sridevi, 2011).

**Volume of the testis tubules**

Volume and densities of tubular lumen and seminiferous epithelium can also give information about the degree of testicular damage as a consequence of germ cell death (Vendramini et al., 2010). Observation made on the volume of the seminiferous tubule and interstitial stroma of the testis were tabulated and shown in Fig 6. The seminiferous tubule showed an insignificant increase in volume in the group II, which further decreased significantly in the group III when compared to group II.

The interstitial stroma on the other hand showed a significant decrease in volume of group II. The value of group III showed a slight increase from group II but was however lesser than the control. According to Vendramini et al. (2010), the alteration to the volume of testis is indicative of injury to gonad. In general, germ cell death caused by anticancer drugs, including doxorubicin (Shinoda et al., 1999; Panareakis, 2002), culminates with a reduction of morphometric parameters (Stump, 2004; Lirdi, 2008). The trend observed however indicates that given sufficient recuperation time the values for the group III returned almost to the normal value and may revert back to that of the control if sufficient time is given. The above argument is supported by Shamberger et al., (1981) who stated that the testicular injury is reversible in most cases.

**Volume of the epididymal tubules**

The volume of the epithelium, lumen, and stroma of the caput, corpus and the caudal portion of the epididymis are shown in the Fig. 6.

In general the caput, corpus and the cauda of the epididymis showed a decrease in the epithelial and stromal volume, in their experimental groups which increased in group III. The lumen in the three different epididymal region showed an increase in the experimental animals which is statistically significant. The volume of the lumen of group III decreased in comparison to the group II.

Knobil & Neill (1988) observed secretion of proteins and other substances into the lumen of the epididymidal epithelium. During Doxorubicin treatment there was decrease in epididymal sperm content (Ward et al., 1988). This could be the cause for increased luminal volume and a decline in epithelial volume.

**Observations from chromosomal study of bone marrow cells**

Chromosome preparation of bone marrow revealed several aberrations. Both structural and numerical aberrations were observed and the results were tabulated in the Table 4.

The common structural aberrations observed were chromatid deletion, chromatid breaks and translocation. Another structural aberration of less frequent occurrence was the ring chromosome. Numerical aberrations include diploid and triploid cells. Another complex aberration observed was pulverized chromatid. Doxorubicin was recorded to induce translocations (Meistrich, 1982), reciprocal translocations (De Luca et al., 1990), balanced chromosomal rearrangements (Meistrich, 1982) and sister chromatid exchanges.

The numerical and structural aberrations were observed in all groups. However, their frequency of occurrence varied. The data showed that both numerical and structural aberrations increased in group II when compared to that of the control. The group III

| Table 4. Effect of Doxorubicin on the chromosomes of bone marrow preparation of white mice Mus musculus. |
|-------------------------------------------------|--------------------|--------------------|
|        | Numerical Abberation | Structural Abberation | Mitotic Index |
| Positive Control | 41.88 ± 0.15(a) | 7.47 ± 0.22(a) | 1.66 |
| Negative Control | 1.04 ± 0.01(b) | 0.99 ± 0.02(b) | 11.12 |
| Group II | 0.71 ± 0.01(c) | 1.97 ± 0.13(c) | 10.14 |
| Group III | 1.71 ± 0.006(c) | 1.68 ± 0.04(c) | 11.09 |

The given values are Mean ±SD; Means within a column with different letters are significant(p<0.05)
showed a decline in aberration from that of the group I, the decrease being statistically insignificant. Meistrich \textit{et al.}, (1990) established that even at high doses of 6 mg/kg, Doxorubicin is a weak inducer of chromosomal aberration. The aberration in the experimental groups that was observed in our present study is probably because low dose employed.

**Conclusion**

The histological observations in the present studies showed no apparent damage to the testis, epididymis and the seminal vesicle in the present dosage. The corpus region showed a decrease in size with a deterioration of most of its tubules. The morphometric investigations showed a marginal decrease in the spermatogonial, spermatocyte and spermatid population. Chromosome analysis of the bone marrow cells exhibited certain aberrations that were not significant. At the dose designed in the present investigation all the changes observed were transitory and did not impair the normal functioning of the reproductive tissues. Therefore, it may be concluded that the sub lethal dosage used in the present study is relatively safe for the reproductive tissue.

**Reference**