Indian Journal of Science and Technology Vol. 4 No. 12 (Dec 2011) ISSN: 0974- 6846

Effect of doxorubicin on the biochemical activities of the male reproductive system of white mice, *Mus musculus*

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Abstract

Doxorubicin is one of the chemotherapeutic agents in treating cancer. The present study was undertaken to investigate whether doxorubicin had any adverse effect when administered at the recommended dose. For this purpose, the effect of Doxorubicin on LDH, glucokinase, alkaline phosphatase, acid phosphatase and sialic acid at the recommended therapeutic dosage were studied. Doxorubicin treatment resulted in decreased protein, glycogen and alkaline phosphatase level and increase in glucokinase, LDH, ADP and sialic acid. When sufficient withdrawal time was given, most of these changes reverted to normal. From the outcome of this study it may be assumed that it is safe to administer doxorubicin at the recommended dosage (0.5-1 mg / kg body weight) as the effects are temporary and reversible.

Keywords: Doxorubicin, glycogen, lactate dehydrogenase, acid phosphatase, alkaline phosphatase

Introduction

Of the various chemotherapeutic agents, anthracyclines play an important role (Goodman & Gilman, 1975).The anthracyclines are derived from *Streptomyces* species and are tetracyclic chromophore antibiotics. Doxorubicin is one such anthracyclines which has been used effectively in treating acute leukemias and malignant lymphoma and solid tumours (Shinde *et al.,* 2010; Kaiserová *et al.,* 2007). Doxorubicin has a broad spectrum of potent activity against many different types of cancers, including a variety of solid tumours (Martindale, 1996). The drug is particularly beneficial in a wide range of sarcomas. It is the single most agent for treating metastatic adenocarcinoma of the breast.

Chemotherapy for cancer is often associated with adverse effects (Hoekman *et al.,* 1999). The toxic manifestations of doxorubicin are many such as myelosupression, thrombocytopenia, anaemia, stomatitis, gastrointestinal disturbances and alopecia. Many of these manifestations are reversible (Goodman & Gilman, 1975). Cardiomyopathy (Martindale, 1996) and gonadal injury are other toxic manifestation. Gonadal injury by antineoplastic drugs, though commonly observed, has been relatively less investigated than 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; Morris, 1988; Ward *et al*., 1988). Although the above data indicate that doxorubicin is a testicular toxicant and is dose dependent, not much work has been carried out to study its effects at the recommended therapeutic doses. Hence, an attempt has been made to study the effect of doxorubicin on the reproductive system especially carbohydrate metabolism using white mice as study organism.

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 Toxiology, (FIPPAT), Padapai. The animals were housed in polypropylene cages and provided with standard pelleted feed. Food and water were provided *ad libitum.*

Experimental design

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

Doxorubicin has been given in doses of 0.5- 1 mg/kg daily for 2-6 days (Goodman & 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 blood was collected and left undisturbed .The serum thus obtained was used for the assay of protein. The tissues like the testes, seminal vesicle and epididymis were immediately removed, cleaned from the adhering tissue and weighed individually. The tissues were used for biochemical analysis. Serum protein was estimated by the method of Lowry *et al*. (1951). Glycogen was estimated using the method of Hassid and Abraham (1957). Lactate dehydrogenase was studied using the method of King (1965), Glucokinase was estimated based on the method of McLean and Brown (1966). Alkaline phosphatase was

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Table 1. Effect of Doxorubicin on the total organ/tissue protein (in mg/gm wet weight) and serum protein (in mg/dl) of the male reproductive system of Mus musculus

	Testis	Caput	Corpus	Cauda	Seminal Vesicle	Serum
Control	0.1748 ± 0.0006 (a)	0.1217 ± 0.0038 (a)	0.2828 ± 0.0034 (a)	0.2469 ± 0.0044(a)	0.1303 ± 0.0005(a)	24.0333 ± 0.1007(a)
Experimental	0.07467± 0.0129 (b)	0.0057 ± 0.0009 (b)	0.1680 ± 0.0087 (b)	0.1004 ± 0.0012(b)	0.0647 ± 0.0012(b)	23.4867 ± 0.1007(b)
Withdrawal	0.0805 ± 0.0019 (b)	0.0083± 0.0010(b)	0.1847 ± 0.0013 (c)	0.1013 ± 0.0018(b)	0.0712 ± 0.0006(b)	23.5867 ± 0.0451(b)

The given values are Mean ±SD. Means in the same column with different letters are significantly different (p<0.05)

estimated by the Andersch and Szczypinski (1947), specific activity of acid phosphatase and sialic acid was estimated using the method of Warren (1959).

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

Tissue and serum protein

Antineoplastic drugs, heavy metals and pesticides are known to affect the structure, functions and biochemical composition of reproductive organs (Sehgal & Pandey, 1984). The results of biochemical analysis of serum and tissue proteins as a result of doxorubicin treatment are shown in Table 1.

The serum protein level showed a significant decrease in the experimental group and this level was sustained in the withdrawal group. This could be due to the cytotoxic effect of the drug doxorubicin (Patil & Balaraman, 2009). The decrease in protein level observed in this study is supported by Friedrich *et al*., (1990) who stated that anthracyclines like doxorubicin acted by the depression of protein synthesis.

The testicular protein of the experimental group showed a highly significant decrease from that of the control. The withdrawal group showed a non significant increase from the experimental group. The testis contains several types of protein in addition to histones. These include specialized contractile protein (Mohri, 1968), acidic proteins (Kadohama & Turkington, 1974) and basic proteins, some of which disappear when spermatogenesis is abnormal (Kistler *et al*., 1973). The highly significant decrease in protein may be due to defective spermatogenesis. Though the protein value of the withdrawal group did not reach the control value it showed an increase over the experimental which indicates that if time is given, it would have returned to normalcy. The epididymal protein level in all three different regions, the caput, corpus and cauda showed a highly significant decrease from that of the control value. The withdrawal value for protein for all the three tissues had shown an increase from the experimental value with the protein value of the corpus region being significantly higher than the control group; however the values did not approach the control value. Reduction of Leydig cells, as was observed in the histological preparations of the testis of the experimental group could also have caused reduction in the testicular androgen. This observation is also supported by Eik-Nes and Hall (1965) who reported that almost all testicular androgen are formed and secreted by Leydig cells. Brookes (1981) reported that the synthesis and secretion of most of the epididymal proteins is under androgenic control. Hence, when the level of testicular androgens decreases, the epididymal protein level also decreases. The withdrawal group if given sufficient time could approach the control value.

The seminal vesicle showed a similar trend as the epididymal region. The proteins of the seminal vesicle of rat are androgen regulated (Fawell *et al*., 1986). The depletion of androgen could be a possible reason for the observed protein decrease in the experimental group.

Glycogen

The results of doxorubicin treatment on the glycogen content of the male reproductive system of the miceis depicted in Fig.1a. Glycogen has been demonstrated in the testis and Sertoli cells (Cavazos & Mallampy, 1954). The testis showed a highly significant decrease in glycogen levels which eventually increased in the withdrawal group. This is indicative of a tendency to return to the normalcy. In the presence of oxygen glucose

Fig.1a. Effect of Doxorubicin on Glycogen content of the male reproductive system of Mus musculus

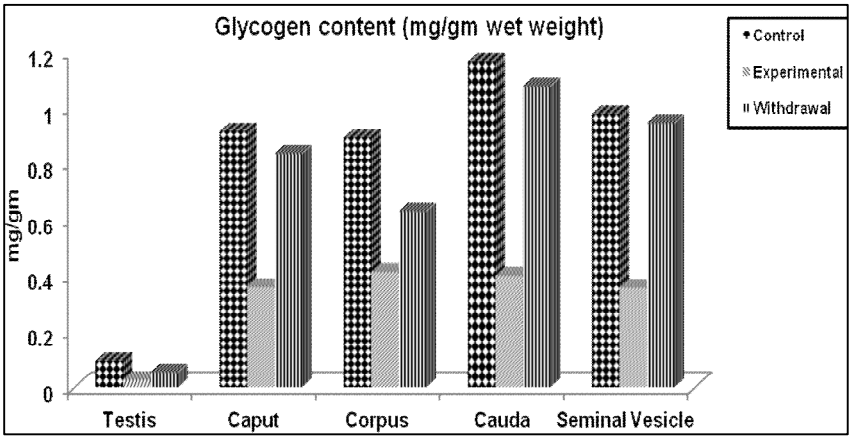

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Fig.1b. Effect of Doxorubicin on the Glucokinase activity of the male reproductive system of Mus musculus

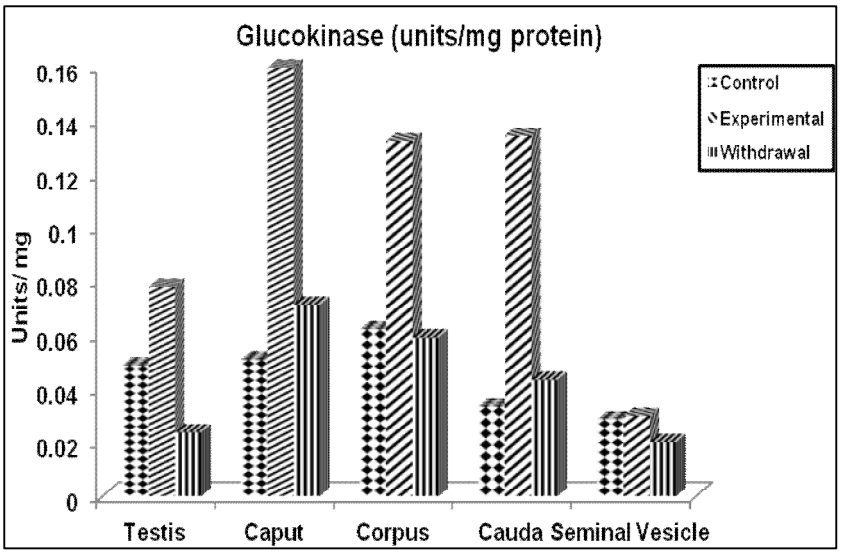
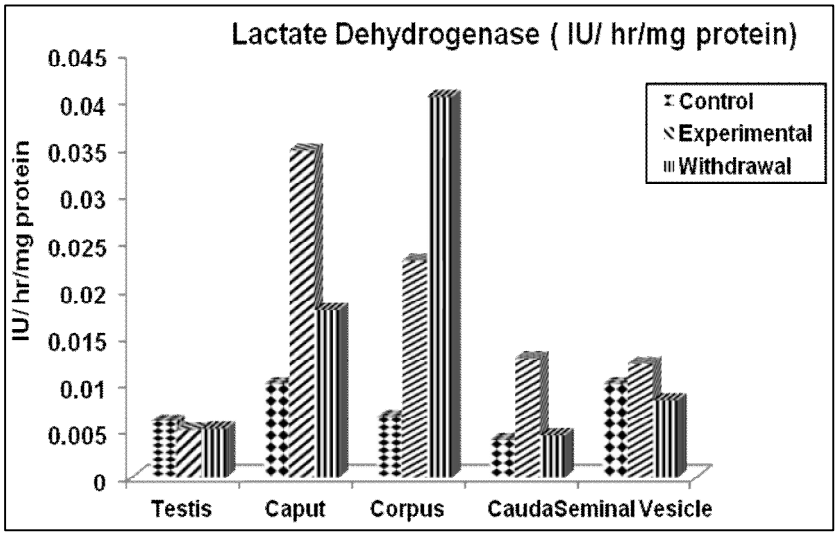


Fig.1c. Effect of Doxorubicin on the Lactate ehydrogenase activity of the male reproductive system of Mus musculus.



utilization is curtailed and mainly stored as glycogen (Axelrod, 1967). Decrease in glycogen level in the Doxorubicin treated animals show that glucose is used *via* the anaerobic pathway. The glycogen level for the caput, corpus and the cauda epididymis showed a highly significant ($p>0.001$) decrease from that of the control value. The value for the withdrawal group in all the three region of the epididymis showed a significant increase. However, the value for the caput and corpus still were significantly lower than the control value. The value for the cauda almost reached the control value. Brookes (1981), had established that in the caput and cauda epididymis of rats, the rate limiting step in glycolysis is not the activity of the enzymes but the availability of glucose. He also reported that in the case of androgen depletion, the epididymal intermediary metabolism is entirely dependent on carbohydrate for metabolic fuel. The androgen depletion was inferred from the histological observation. Consequently a shift to intermediary metabolism which is dependent on the availability of glucose becomes imperative. Decrease in glycogen level as obtained in this study, probably indicates that most of the available glucose enters the glycolytic pathway.

The seminal vesicle also showed a significant decrease in the glycogen content of the experimental group. The value again increased in the withdrawal groups. The dependence on carbohydrate as metabolic fuel (Brookes, 1981) may be the reason for reduced glycogen content.

Glukokinase *(EC 2.7.1.2)*

The result of doxorubicin treatment on the Glucokinase activity of the male reproductive system of the mice is shown in Fig.1b. The testis of the experimental animals showed a highly significant increase in the enzyme glucokinase level when compared to the control. The enzyme level of the withdrawal group continued to remain at a higher level. The same trend is exhibited by all the epididymal region as well as by the seminal vesicle. This increase in the glucokinase activity was also reported by Geetha *et al*., (1989) in Doxorubicin treated rats. A decrease in glycogen as seen in the present study indicates that there is an active metabolism of glucose which required the production of more glucokinase. This is supported by the trend in the increase of glucokinase that was observed in this study.

Lactate dehydrogenase *(EC 1.1.1.27)*

The result of Doxorubicin treatment on the Lactate dehydrogenase (LDH) activity of the male reproductive system of the mice is shown in Fig.1c . Lactate dehydrogenase is involved in the catalysis of the reduction of pyruvate to lactate in the presence of NADH. This allows the glycolysis to proceed in the absence of oxygen, regenerating sufficient amount of NAD^+ for sustaining the glycolytic pathway (Geetha *et al.,* 1989) and it is released during cell lysis (Decker & Lohmann-Matthes, 1988).

The LDH levels in the testis of the experimental animals showed a decrease which further decreased in the withdrawal group. Reduction in the glycogen level indicates that the available glucose is actively metabolized. Since the testis depends on the glucose for carbon-di-oxide production (Middleton, 1973), it is probable that the available glucose in the testis is metabolized *via* the Kreb's cycle.

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Fig.2a. Effect of Doxorubicin on the Acid Phosphatase activity of the male reproductive system of Mus musculus.

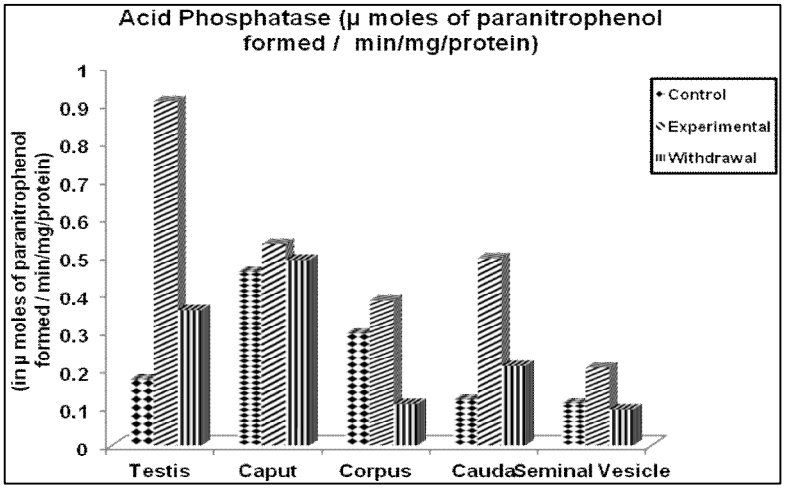


Fig.2b. Effect of Doxorubicin on the Alkaline Phosphatase activity of the male reproductive system of Mus musculus

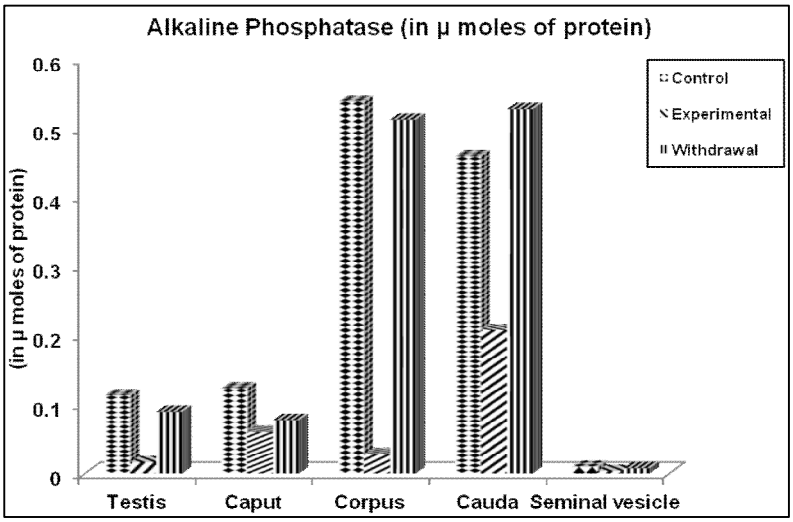
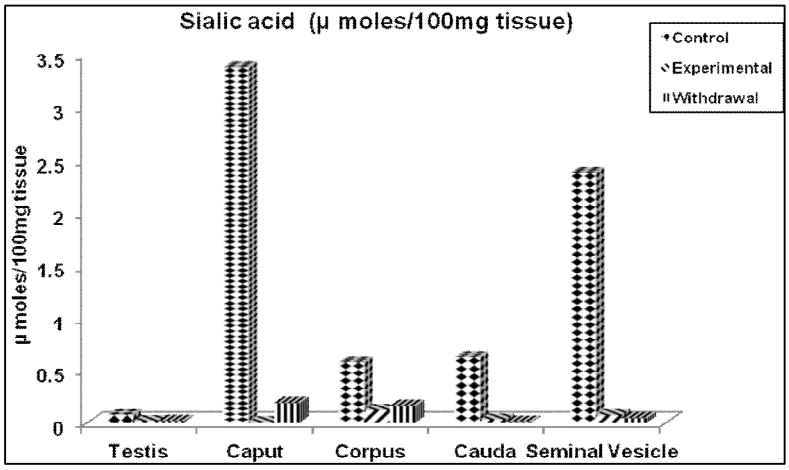


Fig.2c. Effect of Doxorubicin on the sialic acid content of the male reproductive system of Mus musculus.



The caput and the cauda epididymis of the experimental group showed an increase in the LDH value that was significant. Further, the withdrawal group showed recuperation. The corpus also exhibited a similar trend but was not statistically significant. The increase in LDH probably resulted in the increase in the regeneration of NAD^+ which is necessary for the NAD^+ utilization phase of the glycolytic pathway. Wolf and Baynes (2006) has also reported an increase in the levels of LDH .This probably indicates the sustenance of the glycolytic pathway.

The seminal vesicle showed a significant increase in the LDH value. Seminal vesicle is rich in fructose formed from glucose by the aldol reduction (Knobil & Neill, 1988). Reduction of glucose as discussed earlier in the seminal vesicle is probably because of the utilization of glucose *via* the anaerobic pathway.

Acid phosphatase

The result of doxorubicin treatment on the Acid phosphatase activity of the male reproductive system of the mice is shown in Fig.2a. Acid phosphatase (ADP) is a lysosomal enzyme and is used as a marker for lysosomal activity (Trasler *et al.,*1988) and it is also as a marker for prostate cancer. The testis showed a significant increase in the ADP activity in the experimental group and reduced significantly in the withdrawal groups. All the three regions of the epididymal showed an increasing trend in experimental group with only the values for corpus and cauda being significantly different from the control group. Similarly the withdrawal group showed a decrease with changes in the corpus and cauda being significant. This is in accordance to the regional distribution of the ADP along the epididymal axis as reported by Nikkanem and Vanha-Pertula, (1977). Increase in the size of the lysosome, principal and/ or clear cells occurred during ADP activity in the epididymis (Trasler *et al.,* 1988). The increase in the value of the ADP seen in the epididymis of the present study indicates a probable increase in the lysosomal size in the cells of the epididymis of the experimental group. Further, the corpus and cauda of the experimental group shows a significant increase from its control value, unlike that of caput. This indicates a greater sensitivity of the principal and / or clear cells and the lysosomes of the caudal region of the epididymis to the activity of Doxorubicin. Hence acid phosphatase activity in the present study may be attributed to the lysosomal hypertrophy.

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The seminal vesicle also showed an increase in the ADP in the experimental group. The increase was significant. However the withdrawal almost approached the control value.

Alkaline phosphatase

The results of doxorubicin treatment on the Alkaline phosphatase activity of the male reproductive system of the mice is shown in Fig.2b. Alkaline phosphatase occurs in the plasma and helps in the breakdown of glycogen to glucose in the glycolytic pathway (Cantarow & Schepartz, 1963). There was an overall trend of decrease in the levels of alkaline phosphatase in all the tissues studied. Geetha *et al*., (1990) also observed a similar decrease in the enzyme activity.

The testis of the experimental animals showed a significant decrease in alkaline phosphatase. Though the values for the withdrawal group increased it still was significantly different from the experimental group. The epididymis of the treated group showed a significant decrease in the activity of the enzyme. Except in the caput region of the epididymis, the enzyme levels almost reached the control value. Levels of the alkaline phosphatase enzyme in the plasma arise from the routine, normal destruction of erythrocytes, leucocytes and other cells; enter the circulation during accelerated cell death (Victor, 1991). The value decreases during cell necrosis. Doxorubicin is one of the most effective cytotoxic agent (Newling, 1992; Fujihira *et al.,* 1993; Praet & Ruysschaert, 1993) and the values obtained in the present study may be due to the fact that doxorubicin is cytotoxic.

Sialic acid

The result of doxorubicin treatment on the sialic acid content of the male reproductive system of the mice is shown in Fig. 2c. The testis and the three regions of the epididymis show a significant decrease in the levels of sialic acid in the experimental group. The seminal vesicle too showed a highly significant decrease in the experimental group. The decrease in the sialic acid levels can be attributed to the role of sialic acid. Murray *et al.* (1991) stated that sialic acid occurs in all cell membranes. This indicates that Doxorubicin has probably caused some degenerative changes in the tissues, which may have caused a reduction in the levels of sialic acid in the experimental group. The values of the sialic acid in the withdrawal group did not approach the control value. This may be because the tissue needs more time to reverse the effect of doxorubicin

Conclusion

Doxorubicin induced a decrease in the protein and glycogen level. An increased level of the enzyme glucokinase, LDH, ADP and sialic acid was observed. Decrease in the level of alkaline phosphatase was observed in the present study. When sufficient withdrawal time was given most of these changes returned to the normal levels. The changes observed in the enzyme activities of LDH, glucokinase and the amount of glycogen present suggest a preference for the glycolytic pathway in the carbohydrate metabolism in the male reproductive system of the doxorubicin treated mice.

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