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Histological Changes of Testis in Mice After Administration of Doxorubicin HCL (Adriblastina) Cytotoxic Drug

EIMAN AL-HAZMI[1], SOAD SHAKER[1], SAMMAR AL-SAGAFF[2] and SALEH KORAIUM[3]

[1]*Department of Medical Biology,* [2] *Clinical Anatomy, Faculty of Medicine and* [3]*Department of Biology, Faculty of Science, King Abdulaziz University Jeddah- Saudi Arabia*

ABSTRACT. Ninety adult male mice MF1 (in breading) strain aged 35-45 days, with weight of 18-20 grams were used in this study. Doxorubicin (adriamycin (Adriblastina)) the cytotoxic drug used for treatment of many cancer types was injected intravenously via tail vein in a single dose (0.02mg/mice) or as repeated doses (0.01mg/2-3 times/ week/ mice) three times per week. Testicular tissue and epididymis were taken after intracardiac perfusion from both control and treated mice, processed for 5μ paraffin section, stained by haematoxalin and eosin (H&E), Periodic acids schief (PAS) for polysaccharides. The tissues from single dosed group were taken after 3, 5, 7, 10, 15 and 20 days after injection. Those treated with repeated doses were taken after 1, 2 and 3 weeks from starting injection. The results showed that doxorubicin could alter histological structure of testicular tissue in early periods of single dose injection. Vascular congestion of interstitial tissue vessels and focal separation of germ cells were observed, and the animal exhibited slight increase in body and testicular weight. Drastic changes increased in intensity after 7, 15 and 21 days were marked degeneration of apoptotic (programmed cell death pattern) was observed in spermatogenic cells. It seems to be stage specific degeneration. Spermatogonia and mature sperms seem to resist toxicity although alteration in head and tail parts were observed in sever cases (3 weeks of repeated doses). The evidences of apoptotic changes involved in testicular toxicity are the absence of any inflammatory reaction or any features of necrotic degeneration. Sertoli; the supporting cell seems to be affected functionally in early stages, possibly loosing their junction with germ cells leading to their observed disorganization and separation. Later in single dosed group and after repeated doses they showed vacuolated or ballooned cytoplasm and irregular small size nuclei. Interstitial or Leydig cells were apparently not changed in early period of doxorubicin administration, however after 21 days of single dose and 2-3 weeks of repeated doses proliferation of Leydig cells were observed among severely degenerated tubules. The cells showed cytoplasmic vacuoles and strained deeply by osmic acid. Doxorubicin was suggested to exert its action through interference with DNA synthesis, altering microtubule assembly or interference with cellular metabolism. An extensive ultra-structural, histochemical and hormonal assays are needed to shed more light upon doxorubicin degenerative effects observed in the present work.

Introduction

A nnmber of research studies had indicated decreasing sperm counts and increasing reprodnctive problems in animals and humans upon nsing cytotoxic drngs (Wyrobek, 1983). Whether these drugs act directly on spermatogenic cells (Soranio, *et al.,* 2000) or throngh intervention with Sertoli and Leydig interstitial cells (Kotovskii & Shamanov, 1985) it is the interest field of recent researches.

Sertoli eells play a key role in spermatogenesis (Sapori *et al,* 1986 & Sharpe, 1993), whereas Leydig eells are the main sonrce of androgen production. Both types of cells can be readily affected by toxieants and chemical drugs (Papadakis, *et al.,* 1999). Alterations in the funetions of these eells may lead to a change in the hormonal balance, distnrbed the process of spermatozoa developinent and iinpaired male fertility (Monses *et al.,* 2000 & 2001).

Doxornbicin was nsed as both antibioties (Suwalsky *et al.,* 1999) and anti caneer drug (Baquiran & Gallagher, 1998). Its testicular toxicity was proved in both animals and human (Adachi *et al.,* 2000; Tsnnenari *et al.,* 2000). Body weight, spermatogenic activities and serum levels of enzymes responsible for testosterone prodnction were significantly decreased by doxorubicin treatment (Kang *et al.,* 2002 & Adachi *et al.,* 2000).

The present study was designed to investigate the effect of both single and repeated intravenous doxorubicin administration on morphology of testicular tissues, spermatogenic cells in particular Sertoli and Leydig interstitial cells of adult male mice.

Material and Methods

Animals

Ninety MF1 male mice, weighing 18-20 gram, were used in this study. The animals age ranges from 35-45 days (maximum fertility age). The animals were randomly divided into three groups: Group I (n=10) served as controls, Group II (n=40) for single dose injection and Group III (n=40) for repeated doses injection with Doxorubicin.

Doxorubicin drug administration

Doxorubicin hydrochloride (Adriblastina) was purchased in vial contains 10mg powder dissolved in 5 ml solution. Therapeutic doses were transformed for mice using Paget and Barnes schedule (1964). Doxorubicin hydrochloride was injected intravenously via tail vein as a single dose (0.02 mg/m^2) to the animals of group II and every 3 days/week for three weeks in a dose of (0.01 mg/m^2) for group III animals. Control animals group I were given 0.9% saline solution by the same dose and route as tbose treated animal. The animal weights were recorded before and after drug administration before dissection.

Specimens collection

The animals of group II (single dose) were killed after 3, 5, 7, 10, 15 and 21 days by cervical decapitation and immediate pericardial perfusion with 10% neutral buffered formalin done after dissection to ensure good fixation, then testes were removed and weighted. In group III (repeated doses) animals were killed after 1, 2 and 3 weeks. Cross sectional slices were taken from mid testicular tissue, re-fixed in the same fixative for 24

hours till processing for 5μ paraffin sections. The slides were stained by Haematoxylin and Eosin and periodic acid Schiff (PAS) (Drury & Wallington, 1980). Other slices were fixed in 2.5% glutaldehyde in phosphate buffer (pH 7.4) for 3-4 hours, washed and post fixed in 1% osmic acid (2 hours), washed and processed for paraffin sections. Using light microscope, histological changes were photographed and compared to control.

Results

MF1 mice (inbreeding strain) were known to simulate cancerous patients in possessing low immunity compared to others strains. The results of the present study showed that Doxorubicin administration to MF1 mice led to an increase in aggressive behavior, loss of hair and loose stools. In early days (5-7 days) of treatment, body weight was increased in all treated animals (group II and group III) (Table 1), whilst they showed continuous decrease after 7 days of treatments till the end of experiment. For the testicular weight, slight increase was observed after 3, 5, 7 days (Table 2), then continuously decreased.

Table (1). Average of body weight (gm) after single and repeated dose administration

Period		MF1	
		Control group (gm)	Experimental group (gm)
Single dose	3 days	21.38	21.73
	5 days	23.25	24.89
	7 days	24.35	29.92
	10 days	25.94	25.63
	15 days	27.02	25.04
	21 days	29.24	24.39
Repeated dose	One week	24.76	26.89
	Tow week	26.83	24.51
	Three week	29.25	22.16

Table (2). Average of testies weight (gm) after single and repeated dose administration.

Period		MF1	
		Control group (gm)	Experimental group (gm)
Single dose	3 days	0.05	0.06
	5 days	0.06	0.08
	7 days	0.07	0.09
	10 days	0.08	0.07
	15 days	0.09	0.05
	21 days	0.1	0.04
Repeated dose	One week	0.07	0.03
	Tow week	0.07	0.02
	Three week	0.09	0.02

Histological studies

Control animals

Histological studies of testicular tissue were similar to that observed in other mammals. The parenchyma is made up of seminiferous tubules. Among the tubules located interstitial cells of Leydig in contact with few thin walled capillaries. The tubules are lined by few Sertoli cells that lie among germinal or spermatogenic epithelium. Most of the seminiferous tubules of control animals showed all stages of spermatogenic cells namely

from the basement membrane to the lumen, spermatogonia; primary spermatocytes; secondary spermatocytes, spermatids and spermatozoa (Fig.1).

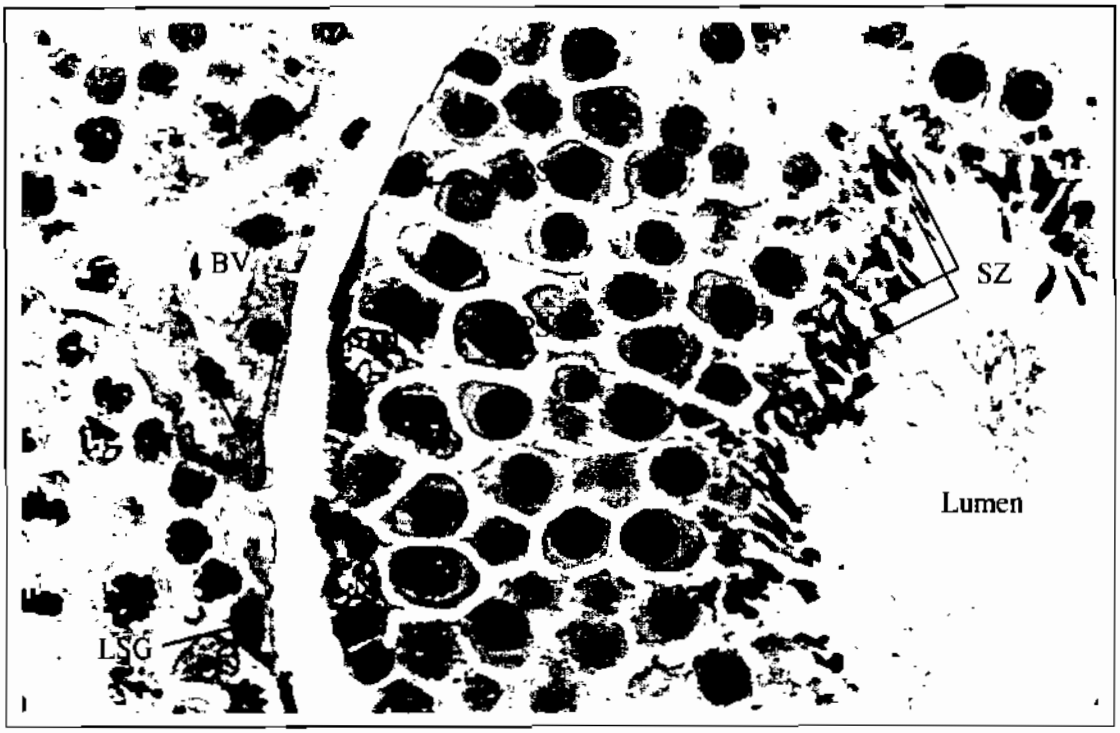

Fig. (1). Photomicrograph of control mouse testis showing the parts of seminiferous tubules. Notice the normal appearance of germinal epithelium; dark spermatogonia (DSG), primary spermatoeyte (PS), secondary spermatocyte (SS), early spermatid and mature sperms (SZ) with curved shaped heads. Groups of interstitial cells (IC) surrounding blood vessels (BV) were observed among the tubules.

(40 x 3.3 x 1.25 Haematoxyline & Eosin (H&E))

Treated animals

Doxorubicin administration led to histological changes in testicular tissues, which is dose and time dependant. In single dose treated animals (group II), slight changes in the form of mild separation of spermatogenic cells and vascular congestion of interstitial blood vessels were observed in early days (3, 5, 7 days) of drug administration (Fig. 2). From 10 to 21 days, the changes became more severe; where spermatogenic cells underwent apoptotic changes (programmed cell death). The changes are in the form of deeply stained nuclei (Fig. 3), complete absence of one or two stages of germ cell leaving only basal spermatogonia and sperms, multinucleated cells were observed in some tubules (Fig.4). Vacuolation and loss of all stages were observed after 15 and 21 days of single dose administration (Figs. 5 & 6). Spermatogonia and Sertoli cells were the last to be affected; the latter undergo vacuolation, their nuclei became irregular and lose their euchromatic appearance.

In group III group (repeated doses administration), histological changes were more evident and severe after two and three weeks of doxorubicin injection. There is loss of most germ cells after 15 days leaviug only ghosts of their cell membranes (Fig.7). Specimens from animals with longer period of injection (3 weeks), testicular parenchyma showed progressive changes, massive vacuolation of spermatogenic and Sertoli cells were observed and only their degenerated nuclei were left (Fig. 8).

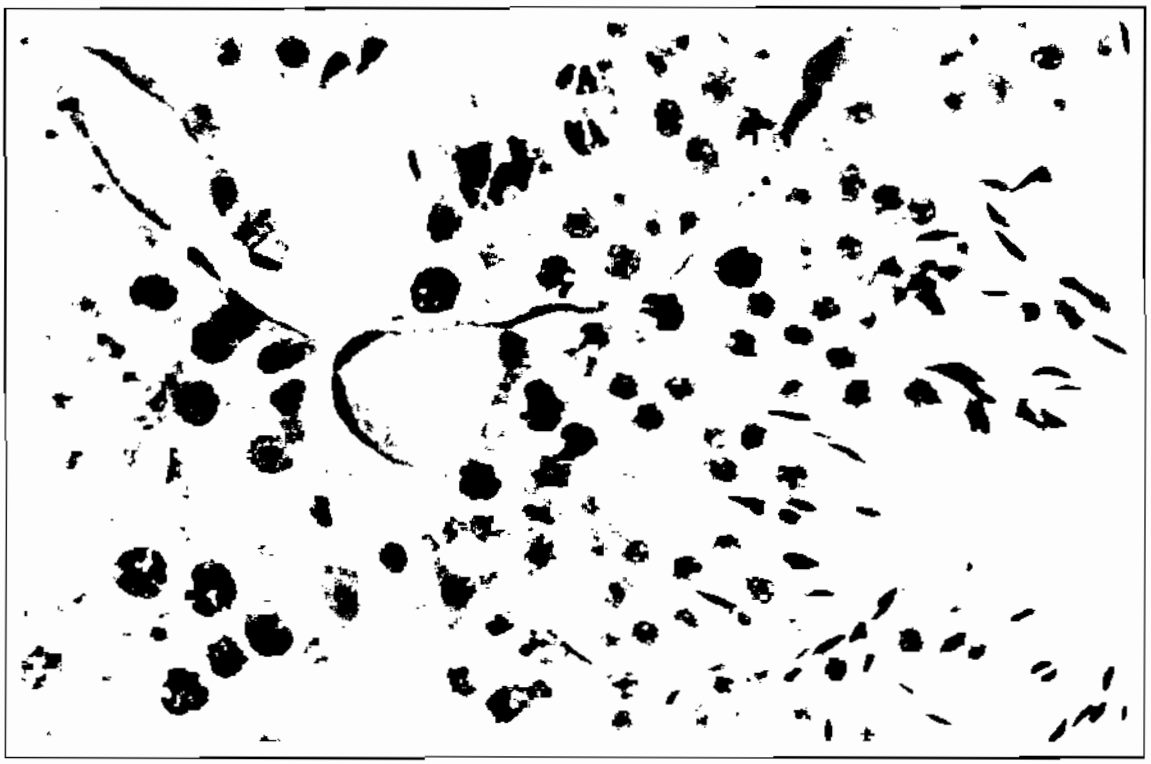

Fig. (2). Cross section of 3 seminiferous tubules after 5 days of doxorubicin administration showing separation of spermatogenic cells, and congestion of interstitial blood vessels (BV).
(40 x 3.3 x 1.25 H&E)

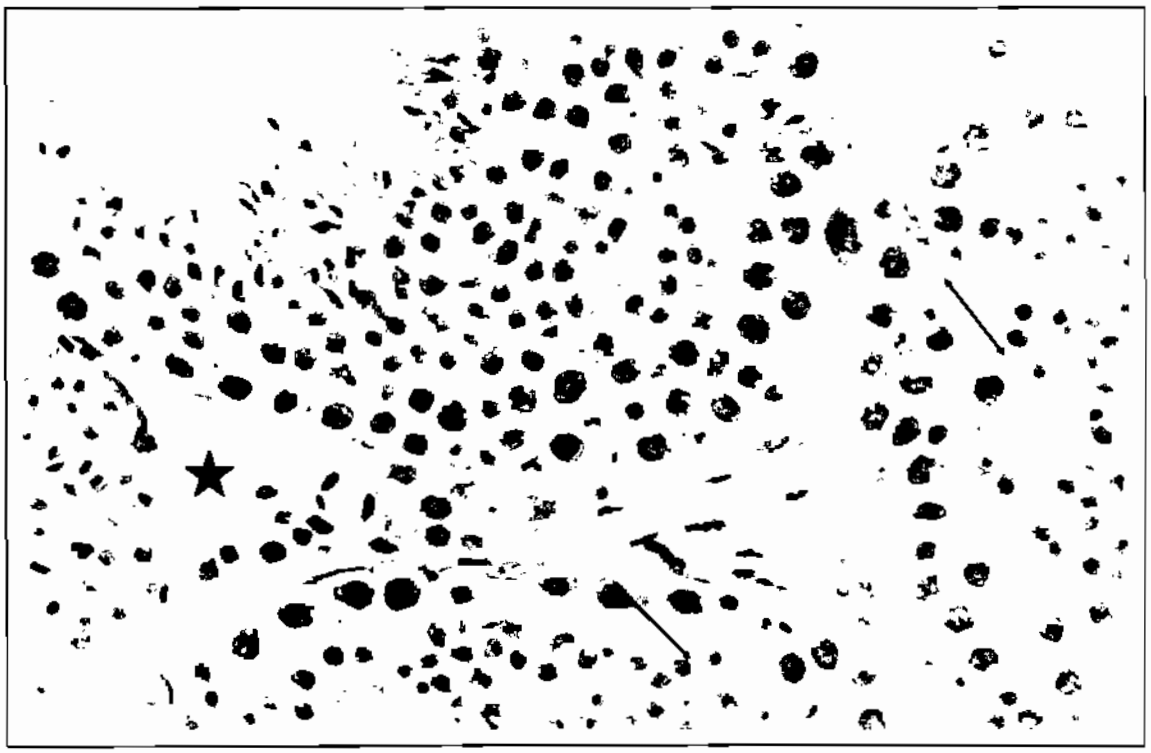

Fig. (3). Seminiferous tubule of mice after 7 days of doxorubicin showing separation of spermatogenic cells (arrow). Notice the congested blood vessels in the interstitial tissue (star).
(40 x 3.3 x 1.25 H&E)

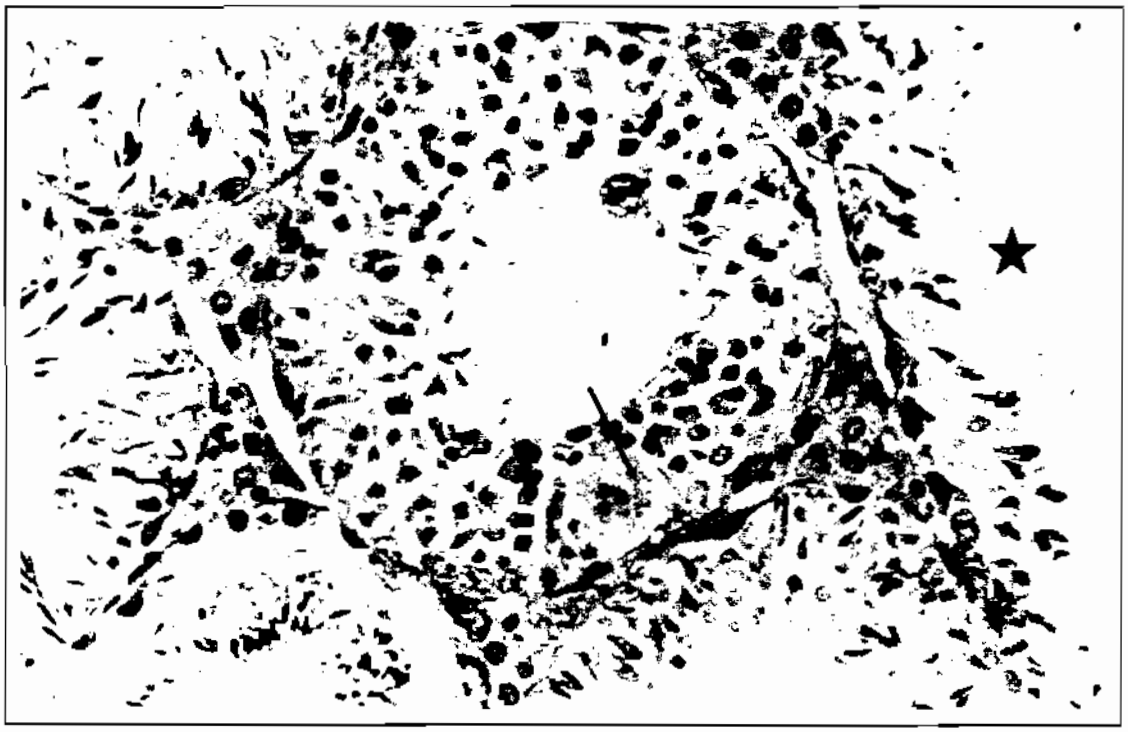

Fig. (4). Cross section of seminiferous tubules after 10 days of single dose injection showing loss of most germ cells in many tubules leaving only spermatogenie and sperm cells (star). Some multinucleated giant cells were observed (arrow).

(40 x 3.3 x 1.25 H&E)

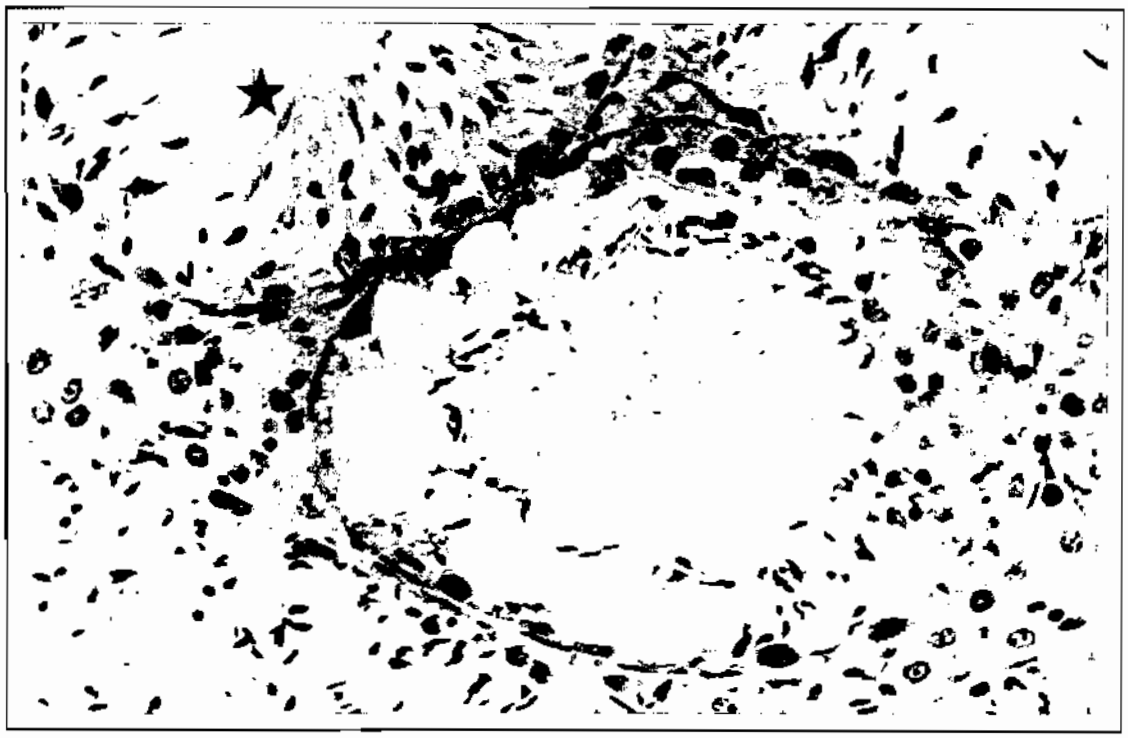

Fig. (5). Cross section of seminiferous tubules after 15 days of single dose showing disappearance of dividing germ cells; only spermatogonia and sperm heads were left (star). Some tubules showed massive vaculations. Notice the proliferating Leydig cells.

(40 x 3.3 x 1.25 H&E)

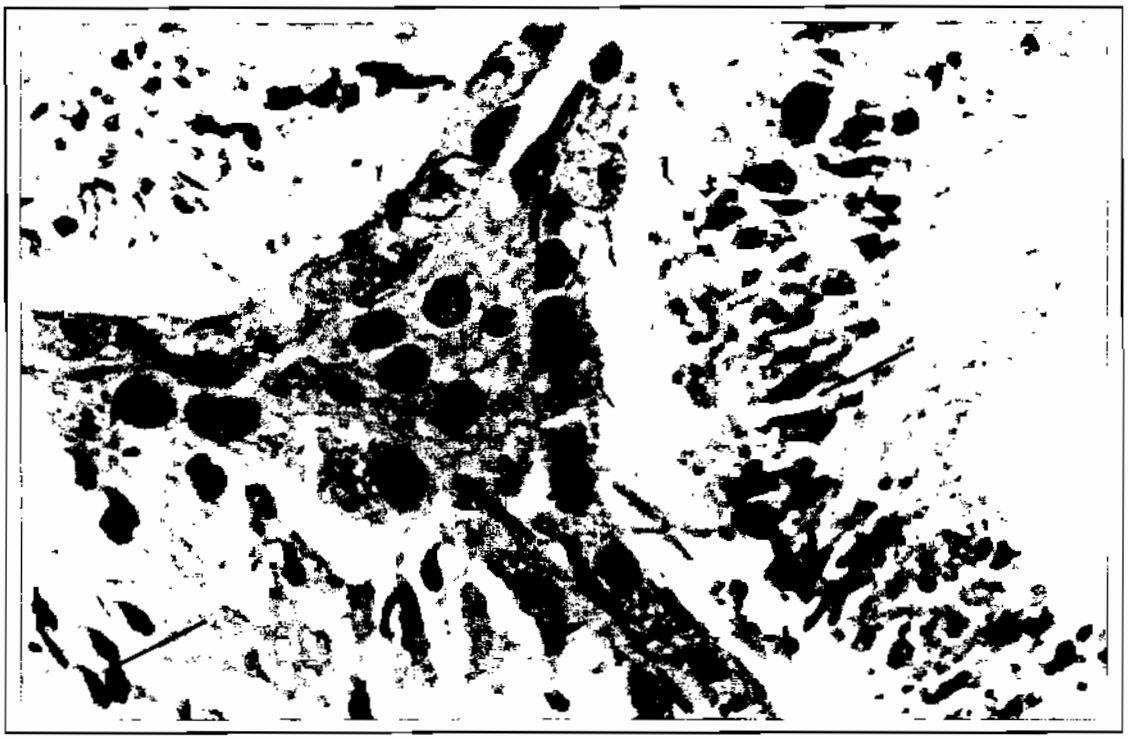

Fig. (6). Cross section of seminiferous tubules after 21 days of single dose injection showing only degenerated basal spermatognia and deformed sperm heads (arrows). The rest of germ cell layers were disappeared.

(40 x 3.3 x 1.25 H&E)

Fig. (7). Cross section of seminiferons tubules after 2 weeks of repeated doxorubicin injection showing complete loss of spermatogenic cells. Notice the proliferation and dark staining of interstitial cells.

(40 x 3.3 x 1.25 H&E)

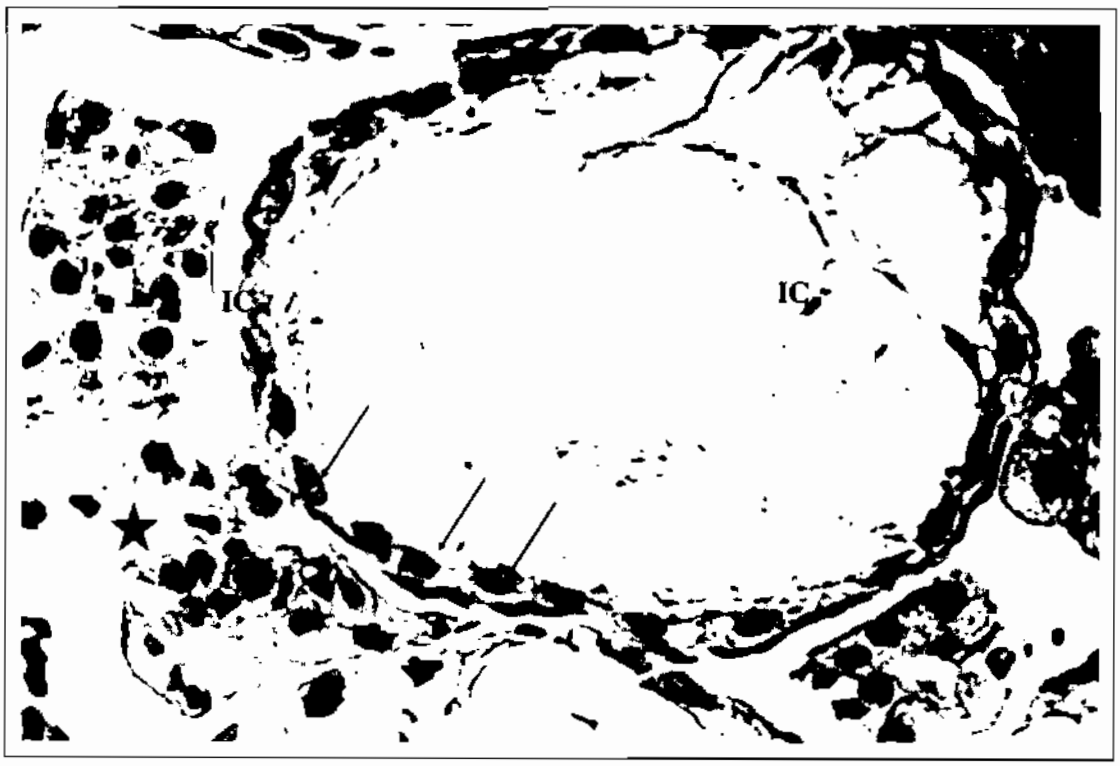

Fig. (8). Cross section of atrophied seminiferous tubules after 3 weeks of repeated doxorubicin injection only the degenerated Sertoli cell nuclei were seen (arrows), the spermatogenic cells are degenerated leaving ghosts of their cell membranes. Interstitial cells are hypertrophied and showed tiny vacuoles (star).

(40 x 3.3 x 1.25 H&E)

Sertoli cells

Plate (1a) showed Sertoli cells in control animals, they can be identified by their oval or pear shaped vesicular nuclei lying perpendicular on basement membrane of seminiferous tubules. The nuelei showed acidophilic nucleolus surrounded by 2 rounded basophilic bodies.

Starting from 10 days up to 21 days of single dose experiment, the nuelei of Sertoli cells appeared to lose their regular pyriform or oval shape, looked irregular and smaller. The cytoplasm in some cells showed vacuolation (Plate1b) and it lost their junction with germ cells leading to their observed disorganization and separation in latter stages. The changes in Sertoli cells seemed to be more sever in repeated doses. After one week of doxorubicin injection their nuclei get darker, and they appeared dark and irregular in shape after 2-3 weeks of treatment with vaculation of cytoplasm (Plate1c & d).

Interstitial cells (Leydig cells)

Plate (2a) showed the normal Leydig interstitial cells lying between seminiferous tubules. They are polyhedral in shape with large nuclei. The cytoplasm contains few tiny vacuoles. After doxorubicin single dose injection, no significant changes were observed in interstitial cells after 3, 5 or 7 days. However, with appearance of degenerative changes in seminiferous tubules after 10-21 days, the cells start to increase in size and amount with appearance of more cytoplasmic vacuoles (Plate 2b), and they stained faintly by PAS (Plate 3b).

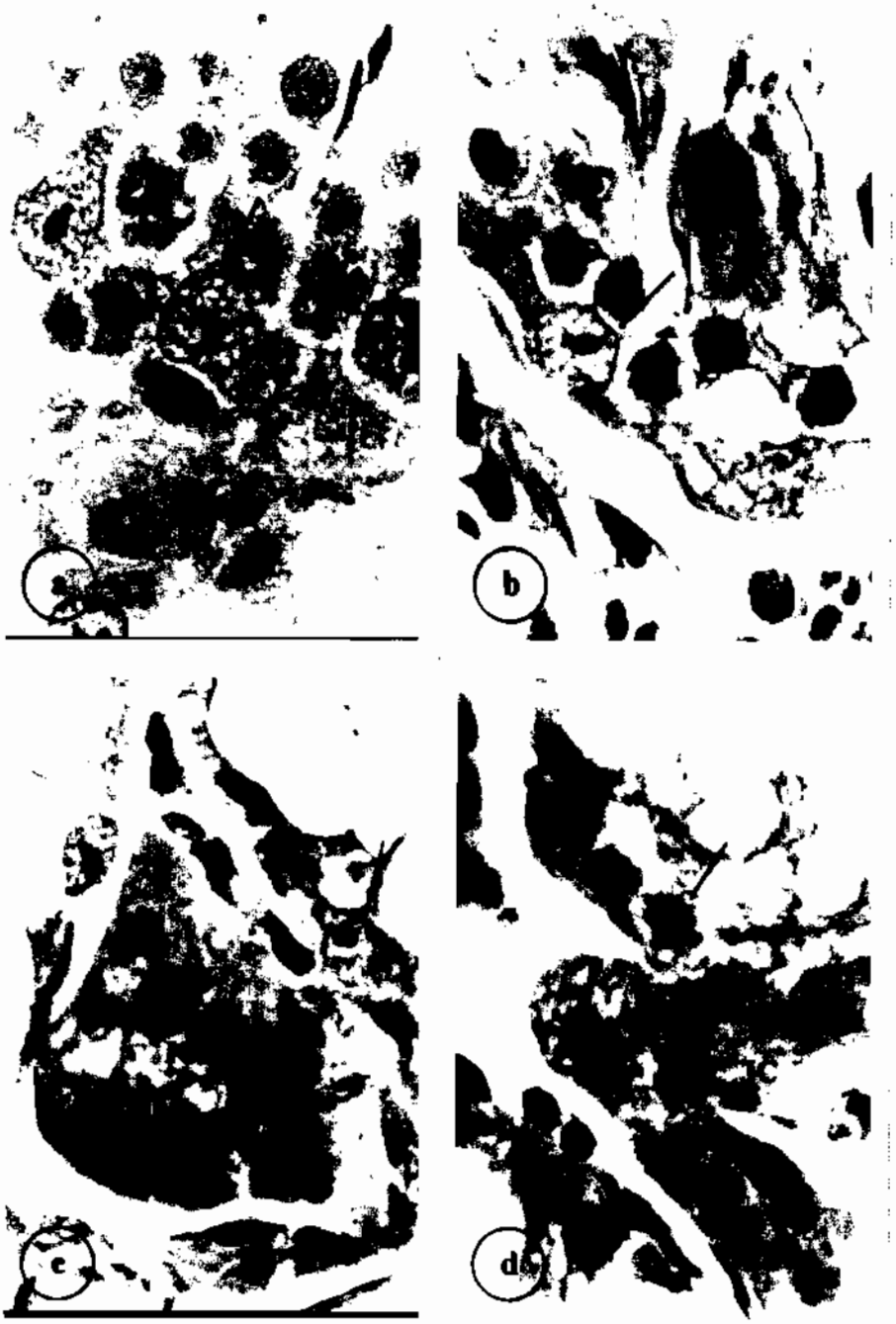

Plate (1).

(a): A part of seminiferous tubule of control group showing the pear shaped nuclei of Sertoli eells (➞). Notice the well defined nueleoli in it.

(100× 3.3 × 1.25 H&E)

(b): A pieture shows the changes and irregularity in Sertoli cells nuclei (➞) and the atrophy of spermatogenic cells in 15 day single dose treated mice.

(100× 3.3 × 1.25 H&E)

(c): A magnified picture showing loss of nonnal appearance of Sertoli cell nuclei (➞) and its vacuolated cytoplasm. (IC) group of hypertrophoied interstitial cells after 2 weeks of repeated dose injection.

(100× 3.3 × 1.25 H&E)

(d): A picture showing the more sever changes in Sertoli cells. the nuclei (➞) are irregular in shape and darkly stained . proliferating interstitial cells (IC) contain tiny cytoplasmic vacnoles.

(100× 3.3 × 1.25 H&E)

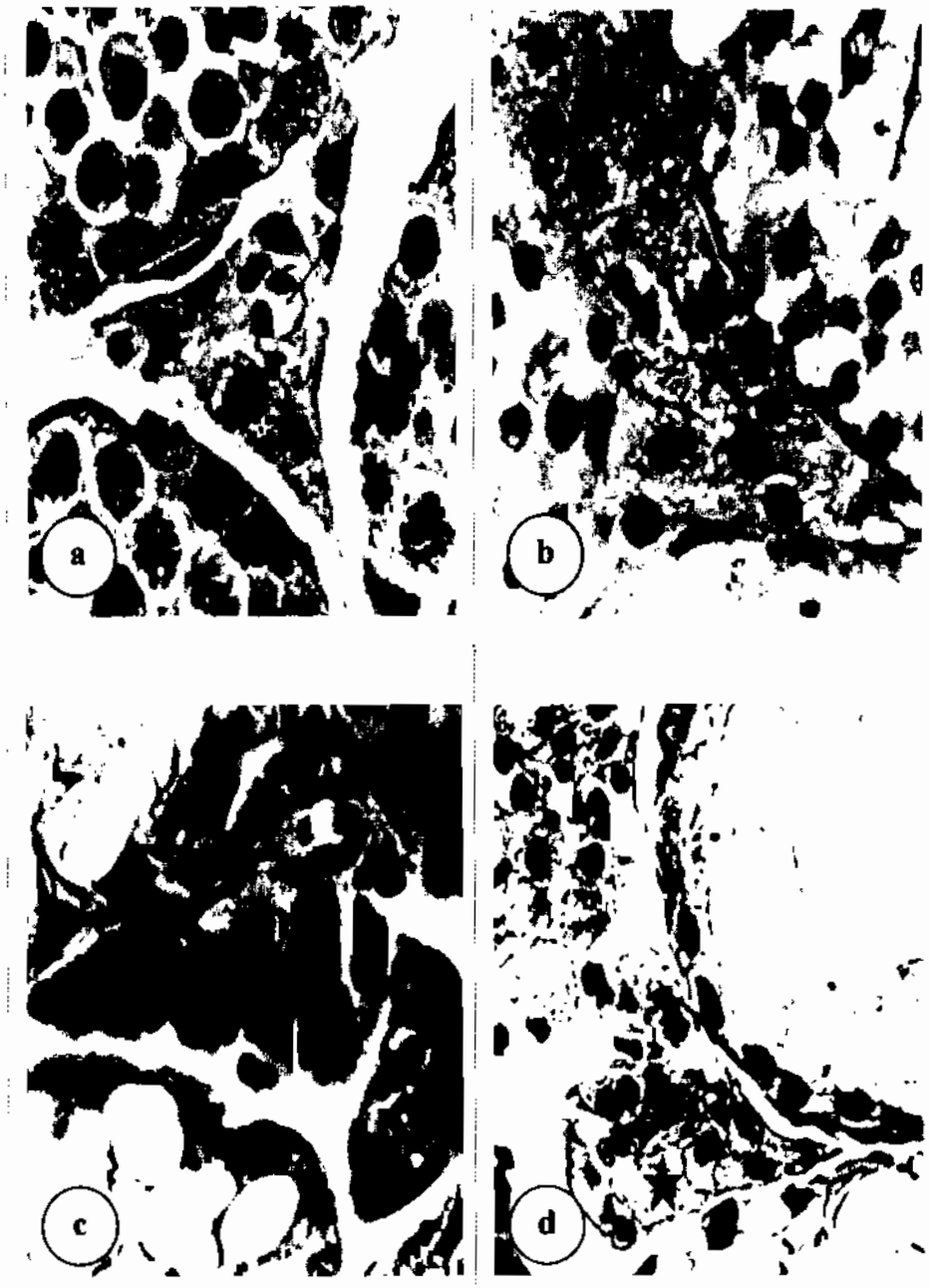

Plate (2).

(a): A part of control mice testes showing the Leydig cells in the interstitial tissue near fine blood capillaries (BV) ➡ sec the different shape and size of nuclei (star).

(40× 3.3 × 1.25 H&E)

(b): A part of testis after 10 days single dose treated mice showing atrophy of spermatogenic cells inside the somniferous tubules and the appearance of somc cytoplasmic vacuoles and proliferation of interstitial cells (star). See the vacuoles inside the cells.

(40× 3.3 × 1.25 H&E)

(c): A picture of proliferating Leydig cells (star) after 2 weeks of repeated dose injection showing an increase in cytoplasimic staining (star).

(40× 3.3 × 1.25 H&E)

(d): A picture of Leydig cells (star) after 3 weeks of repeated dose injection showing increasing in cytoplasmic vacuoles size. Notice the loss of most germ cells nearby seminiferous tubule.

(40× 3.3 × 1.25 H&E)

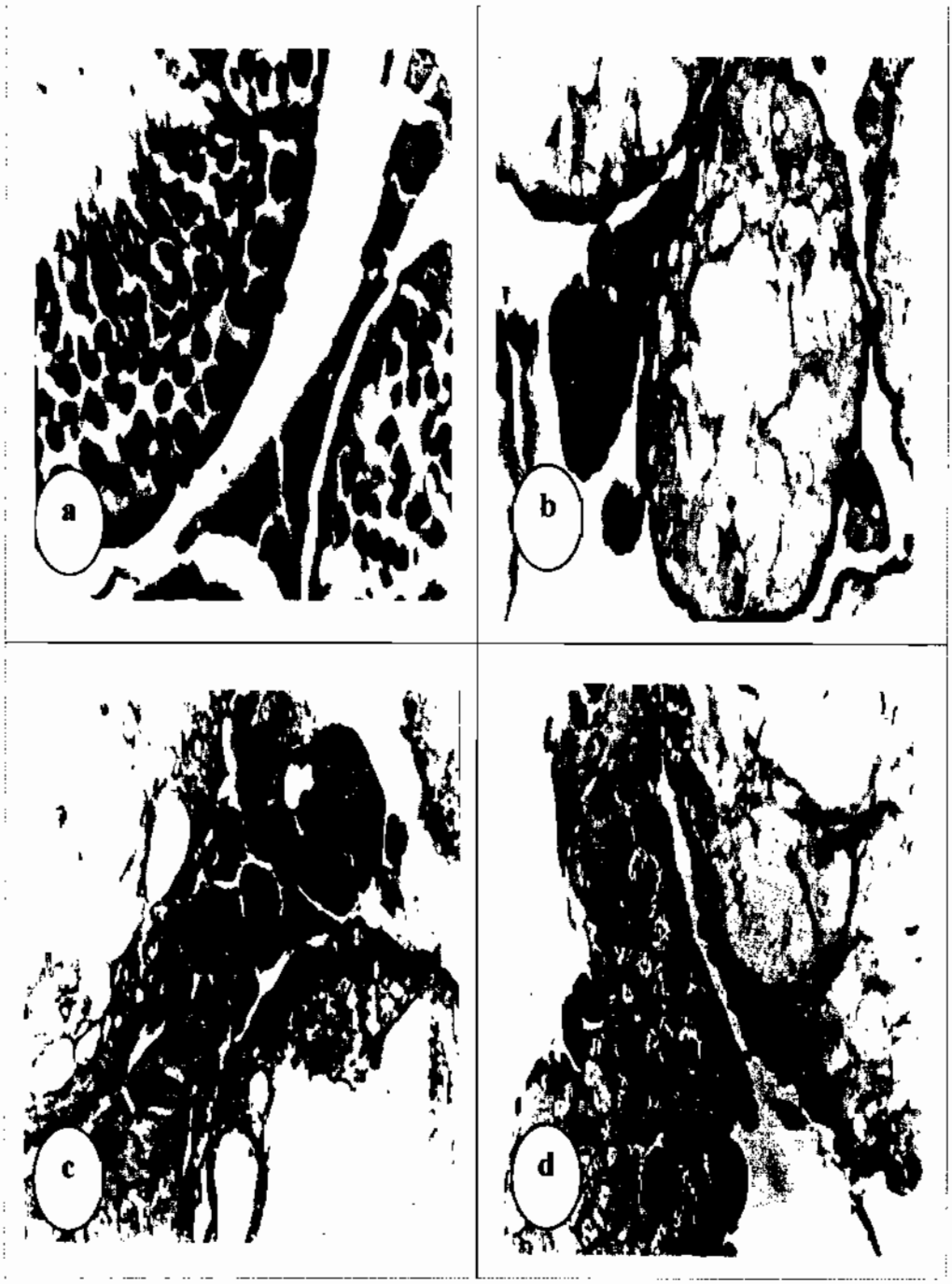

Plate (3):

(a): A picture of a part of control mice testis showing Leydig cells and parts of seminiferous tubules stained for carbohydrates.

(20× 3.3 × 1.25 PAS)

(b): A picture of 15 days single dose treated mice showing little inercase in PAS stained substance in some Leydig eells and the basement membrane of seminiferous tubules.

(20× 3.3 × 1.25 PAS)

(c): Two weeks after repeated dose injection PAS stained substance was increased in the basement membrane of seminiferous tubules. Diffused staining for carbohydrates was observed in Leydig cells.

(20× 3.3 × 1.25 PAS)

(d): A 3 weeks repeated dose testis showing diffused staining of proliferating Leydig cells

(20× 3.3 × 1.25 PAS)

After 2 weeks of repeated doses of doxorubicin injection, there was a significant increase in leydig cells relative to degenerated seminiferous tubules (Plate 2c). The cytoplasm of these cells stained intensely with eosin and its affinity to PAS staining increase (Plate 3b), few granules stained by osmic acid (Plate 4c). After 3 weeks, the cytoplasmic vacuoles increase (Plate 2d), accompanied by marked affinity to osmic acid stain (Plate 4d). Slight increase in PAS positive material was observed in the basement membrane of Sertoli cells and in proliferating Leydig cells in late stage of treatment in 15 day single dose and 2-3 of group III of repeated dose (Plate 3c & d).

Histological studies of epididymal content in control and experimental mice

Plate (5.a) showed the normal appearance of epididymal lining cells and contents in mice. The lining is either of columnar or pseudostratified columnar ciliated type. The sperms could be identified by their curved (sickled- shaped) heads. (Plate 5b) showed the decease in sperm content after 10-15 days of doxorubicin single dose injection. There is frequent alteration of sperm heads. Some tubules contain degenerated sperms and desquamated spermatogenic cells. After 2 weeks of repeated doses the tubules contain degenerated sperms without apparent heads or just showed reticulated fluids (Plate 5c). The lining epididymal cells looked layered, with clear Golgi region and ciliated borders.

In case of 3 weeks of repeated doses where there is marked destruction of seminiferous tubules in the affected animals, the epididymal tubules were empty from any sperms or just contain reticulated materials (Plate 5d).

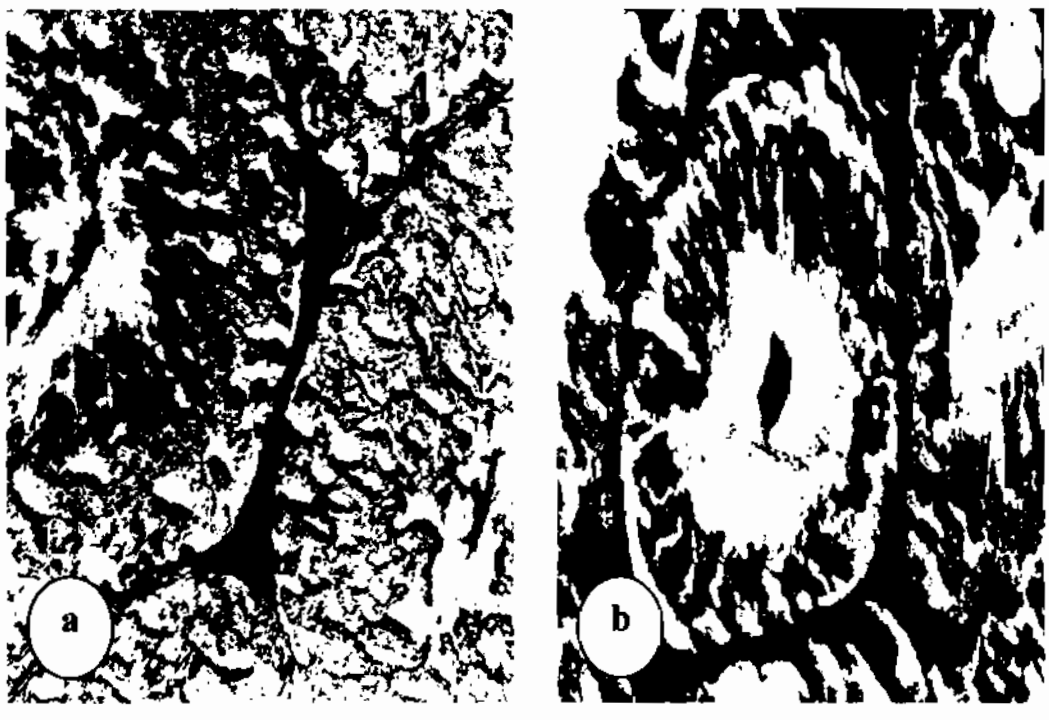

Plate (4):

(a): A part of mice testis of control group showing the stained osmium tetra-oxide interstitial cells and late stages of germ cells.

(20× 3.3 × 1.25 Osmic acid)

(b): A part of testes of 10 days single dose treated mice showing an increase of osmophilic substance in degenerated germ and interstitial cells.

(20× 3.3 × 1.25 Osmic acid)

Plate (4):

(c): A part of testes of 21 days single dose treated mice showing an increasing of osmophilic staining of interstitial cells.

(20× 3.3 × 1.25 Osmic acid)

(d): A part of testes after 2 weeks of repeated dose showing an increase in staining degree of interstitial cells.

(20× 3.3 × 1.25 Osmic acid)

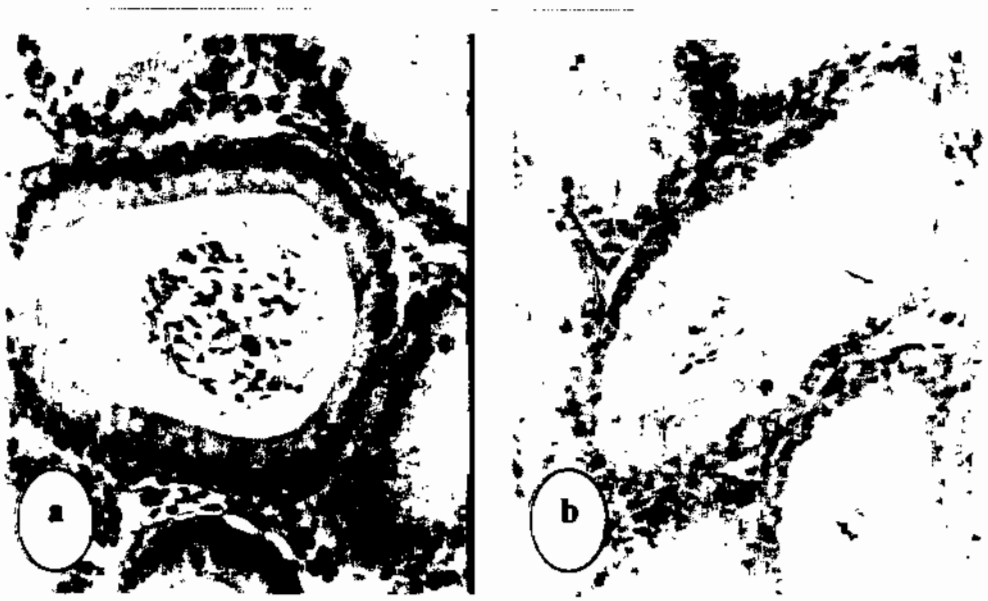

Plate (5):

(a): Parts of epididymal toboles of control group showing a mass of sperms with sickle shaped heads sperm.

(20× 3.3 × 1.25 H&E)

(b): 15 days after single dose treatment shows severe decrease in sperms. the lining columnar cells increase in height and showing prominent –ve Golgi image regions (arrow).

(20× 3.3 × 1.25 H&E)

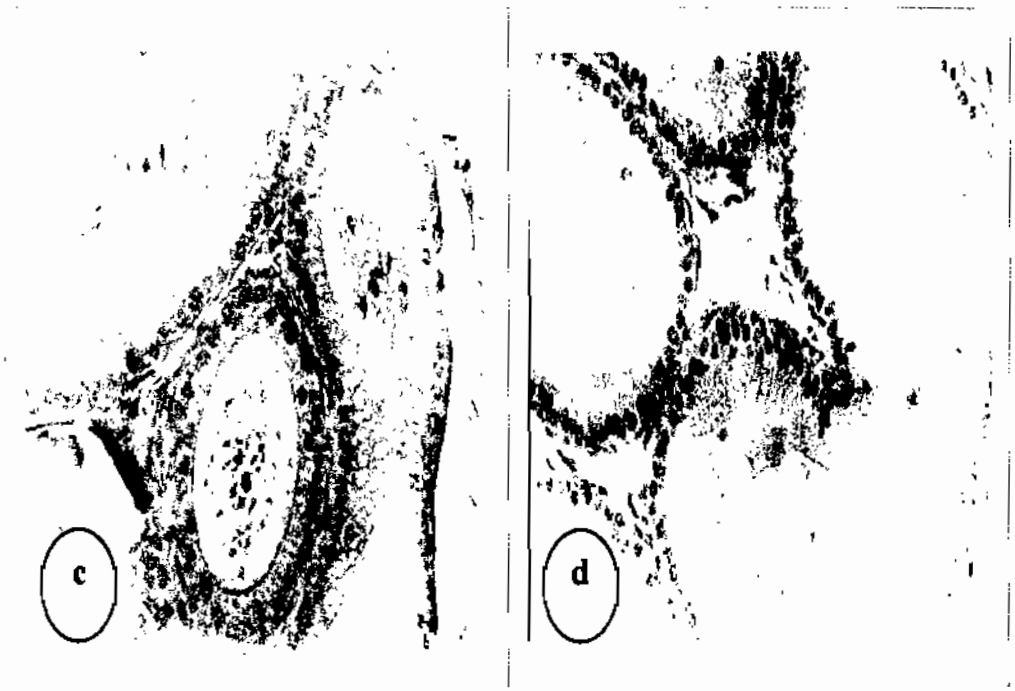

Plate (5):

(c): 2 weeks after repeated dose showing marked loss of sperm content within epididymal tubules. Only cell debris and reticulated material were seen the atrophy of most spermatogenic cells and the filling of it inside the lumen.

(20× 3.3 × 1.25 H&E)

(d): A picture of a part of epididydmis after 3 weeks after repeated dose showing complete absence of sperms within the tubules. Notice the increasing in cell height and well defined –ve Golgi image regions.

(20× 3.3 × 1.25 H&E)

Discussion

In the present study it was observed that intravenous injection of doxorubicin (as a single dose or repeated doses) leads to drastic changes in testicular parenchyma. Changes were more pronounced with repeated dose sehedule. Spermatogenic cells in dividing stages were more affected so primary spermatoeytes disappear in early stages while secondary spermatocytes and early spermatids underwent apoptotic changes in late stages. All stages completely disappeared in affected tubules at 21 days of treatment. Testicular toxicity of adriamycin was observed by (Adaehi *et al.*, 2000 & Tsunenari *et al.*, 2000) after single or repeated dose administration to rat. Multinucleated giant cells were observed within seminiferous tubules possibly may be due to fusion of macrophages to engulf deteriorated spermatogenic cells.

Similar results were reported by (Shinoda *et al.*, 1999) in meiotically dividing spermatocytes and early round spermatids. Intra tubular vacuoles observed in late stages of treatment can be attributed to shrinking and disappearance of degenerating germ cells. Similar results were reported in miee testes by (Stietiea *et al.*, 1994) after volteran administration. Another suggestion was provided by (Monsees *et al.*, 2000) that vacuoles

could be due to an increase in production of seminifrous tubular fluid by Sertoli cells. The process was known to be regulated by germ cells and androgenic hormones. The drug was proved to work through cell cycle effects. The pre-mitotic DNA synthesis was more sensitive than pre-mitotic DNA synthesis to anthracyclines group including doxorubicin (Jahnukainen *et al.*, 2000). Doxorubicin intercalate non-covalently between DNA nitrogenous bases producing helix damage (Bosser & Hortobagyi, 1994).

In the present study, degenerative changes in testicular tissue were not assoeiated with any signs of inflammatory reaction sueh as lymphocytes or neutrophil aggregation which proved that apoptosis (programmed cell death) was involved in doxorubicin induced testicular toxicity.

Most reeent researches had pointed that apoptosis is the way by which damaged spermatogenic cells were removed (Sjublom *et al.*, 1998; Shinoda *et al.*, 1999 and Jahnukainen *et al.*, 2000).

In the present study, Sertoli cells were affected morphologically in late periods of doxorubicin administration. However, in the early stagc of treatment, the Sertoli cells tight junction seems to be weakened and this lead to separation of spermatogenie ccll observed after 5-7 days of drug administration.

Sertoli cells are the nurse cells for immature sperm. Tight junction between neighboring Sertoli cells ereates a special environment that supports and protects the developing germ cells in adluminal (near lumen) compartmcnts. Its damage results in decreased sperm production, which may be permanent even if exposure to toxic agent is discontinued (Monsees *et al.*, 1996 & 2000). The resistance of Sertoli cells to doxorubicin damaging effect till late phases of treatment could be attributed to expression of a multi drug resistance (MDR) type I genes, and the plasma membrane P, glycoprotcin (P-gP) functioning as an energy depcndcnt pump for the efflux of diverse anticancer drug (Melaine *et al.*, 2002). The absence of cxpression of these genes in mitotic and meiotic germ cells probably explains their particular vulnerability to anticancer drugs observed in the present study. On the othcr hand, expression of (P-gP) in the haploid spermatozoa reflects their ability to assume thcir own anti-drug defense.

The increase in Leydig cells which was observed in the present study could be compensatory to seminiferous tubules degeneration. Similar results were observed in cryptorchid rat testes. Tubular damage might induce an increase of LH level that stimulates Leydig cell differentiation and hypereellularity (Kerr *et. al.*,1985; Barlet and Koye 1986 & El-Samamnoudy *et al.*, 1987).

Sertoli cells and Leydig cells however, can be readily affected by toxicants. Alteration in functions of these cells may lead to change in hormonal balance, disturbed development of spermatozoa and impaired male fertility (Monsees *et al.*, 2001).

The decrease or absence of epididymal contents of sperms and appearance of desquamated spermatogenic cells reflect the degcnerative ehanges in scminiferous tubules. Epididymal indcx could be used for assessment of the extent of testicular damage produced by environmental or chemical factors.

In conclusion, doxorubicin was similar to other cytotoxic drugs in having drastic effects on testicular structure, espeeially spermatogenic cell lines, Sertoli and interstitial cells suffcrcd latc during treatment. Caution should be taken during trcatment with this drug.

References

Adachi, T., Nishimura, T., Imahre, H. and **Yamamura, T.** (2000): Collaborative work to evaluate toxicity on male reproductive organs by repeated dose studies in rats. *J. Toxicol. Sci. Zoo.*, **Oct.,** 95-101

Baquiran, D. and **Gallagher, J.** (1998): *Lippincott's cancer chemotherapy handbook.* Ch1 pp. 3-5. 191-192. Lippincott. Philadelphia.

Barlett, J.M.S., Kerr, J.B. and **Sharpe, R.M.** (1986). The effects of selective destruetion and regeneration of rat Leydig cells on the interstitial distribution of testosterone and morphology of the seminiferous tubules. *J. Androl.*, **7:** 240-253.

Bosser, D. and **Hortobagyi, G.** (1994): Anthracycline antibiotics in cancer therapy. Focus on drug resistance. *Drugs,* Feb. **47**(2): 223-58.

El-Samannoudy, F., Farid, N. and **Shawky, N.** (1987): Histochemical changes in rat testis induced by unilateral crypto-orchidism and testosterone propionate injection. *Egypt. journal of histology,* **10**(1): 83-89.

Jahuukainen, K., Hou, M., Parvinen, M. Eksborg, S. and **Soder, O.** (2000): Stage-specific inhibition of deoxyribonucleic acid synthesis and induction of apoptosis by anthracyclines in cultured rat spermatogenie cells. *Biol. Reprod.* Aug **63**(2): 482-487.

Kang, J., Lee, Y., No, K., Jung, E., Sung, J., Kim, Y. and **Nam, S.** (2002): Ginseng intestinal metabolite-I (GIM-I) reduces doxorubicin toxicity in the testis. *Reprod. Toxicol.,* **16**(3): 291-8.

Kerr, J. B., Donachie, K. and **Rommerts, F.F.G.** (1985). Selective destruction and regeneration of rat Leydig cells in vivo. A new method for the study of seminiferous tubular-interstitial tissue interaction. *Cell Tissue Res.*, **242:** 145-156.

Kotovckii, E. and **Shatmanov, S.** (1985): Effect of vitamin A on the testes. *Biull. Eksp. Biol. Med.* May **99**(5): 626-8. Russian.

Melaine, N., Lienard, M.O., Dorval, I., Le Goascogne, C., lejeune, H., and **Jegou, B.** (2002): Multidrug resistance genes and p-glycoprotein in the testis of the rat, mouse, guinea pig, and human. *Biol. Reprod.,* **67**(6): 1699-1707.

Monsees, T. K., Miska, W. and **Schill, W.B.** (1996). Characterization of kininases in testicular cells. *Immune Pharmacol.*, **32:** 169-171.

Monsees, T.K., Franz, M., Gebhardt, S., Winterstein, U., Schill W.B. and **Hayatpour, J.** (2000). Sertoli cells as a target for reproductive hazards. *Andrologia,* **32:** 239-246.

Monsees, T.K., Hinkel, S., Mihm, K., Endo, F., Schill, W.B. and **Haytpour, J.** (2001). *Effects of environmental hormones on the functions of testicular cells.* Proceeding of the second status – seminar "Endocrine disruptors" Berlin, 02-04 2001, Um Welt Bundesamt, Berlin 133-136.

Paget and **Barnes,** (1964): *Evaluation of drug activities Paramacometrrics,* eds. Laurence and Bacharach, Vol.1, Academic Press., New York.

Papadakis, V., Vlachopapadopoulu, E., Van Syckle, K., Ganshaw, I., Kalmanti, M., Tan, C. and **Sklar, C.** (1999): Gonadal function in young patients successfully treated for Hodgkin disease. *Med. Pediater. Oncol.*, May **32**(5): 366-72.

Sapori, P.M.H., Chatelain, P., Vallier, P. and **Saez, J.M.** (1986). In vitro interaction between Sertoli cells and steroidogenic cells. *Biochem Biophys Res Commun,* **134**(2): 957-962.

Sharpe, R.M. (1993). *The Sertoli cell.* L.D. Russell and M.D. Griswold (eds.). Cache. River Press. Florida 391-418.

Shinoda, K., Mitsumori, K., Yasuhara, K., Uneyama, C., Onodera, H., Hirose, M. and **Uehara, M.** (1999): Doxorubicin induces male germ cell apoptosis in rats. *Arch. Toxicol.* Jun **73**(4-5): 274-281.

Sjoblom, T., West, A. and **Lahdetie, J.** (1998): Apoptotic response of spermatogenic cells to the germ cell mutagens etoposid, adriamycin, and diepoxybutane. *Environ. Mol. Mutagen.* **31**(2): 133-148.

Soranio, L., Munoz, M., Pozo, J., Contra, T., Buno, M. and **Argente, J.** (2000): Changes in gonadal function in post-pubertal male survivors of acute lymphoblastic leukemia and Hodgkin's disease. *An. Esp. Pediatr.* Oct **53**(4): 318-23.

Stietiea, F.M., Ali, S.S. and **Mohamed, S.A.** (1994). Morphological changes of the mouse testis under the influence of Diclofenac sodium: A light and electron microscopic study. *Egypt. J. Anat.*, **17**(2): 389-407.

Suwalsky, M., Hernandez, P., Villena, F., Aguilar, F. and **Sotomayor, C.** (1999): The anticancer drug adriamycin interacts with the human erythrocyte membrane. *Z. Natuforsch. C.* Mar **54**(3-4): 271-7

Tsuneuari, I., Kawachi, M., Matsumaru, T. and **Katsuki, S.** (2000): Collaborative work to evaluate toxicity on male reproductive organs by repeated dose studies in rats 10. Testicular toxicity of adriamycin observed 2 and 4 weeks after a single intravenous administration. *J. Toxicol. Sci.,* Oct. **25:** 103-15

Wyrobek A.J. (1983): Methods of evaluating the effects of environmental chemicals on human sperm production. Environ. *Health Persspect.,* Feb **48:** 53-9.

التغيرات النسيجية لخصية الفأر بعد تعاطي عقار الدكسوروبيسن هيدروكلوريد (أدريبلاستينا) المضاد للسرطان

ا.ايمان الحازمي ، ا.د. سعاد شاكر ، د. سمر السقاف ، ا.د. صالح كريم .

مستخلص. في هذه الدراسة تم استخدام عدد ٩٠ فأراً ذكراً بالغاً من سلالة MF1 داخلية التزاوج وكانت تتراوح أعمارها بين ٣٥-٤٥ يوماً وأوزانها بين ١٨-٢٠جم. استخدم في هذه الدراسة عقار الدكسوروبيسن (أدرياميسن (تحت الأسم التجاري أدريبلاستينا)) ذو السمية الخلوية والمستعمل في معالجة العديد من السرطانات حيث حقن وريدياً عن طريق الوريد الذيلي بجرعة مفردة (٠,٠٢ مجم/ فأر) أو جرعة متكررة (٠,٠١مجم /فأر) ثلاث مرات في الأسبوع. بعد التشريح حقن القلب فوراً بالفورمالين لضمان وصول المثبت لجميع الأنسجة ثم تم أخذت عينات الخصية والبربخ من كل من المجموعات الضابطة والمعاملة ومن ثم تمريرها لعمل قطاعات شمعية بقطر ٥ ميكرون وصبغت العينات بصبغة الهيماتوكسلين والأيوسين وبصبغة حامض البير أيودك شيف لفحص السكريات العديدة.

جمعت العينات من الجرعات المجموعة ذات الجرعة المفردة بعد ٣، ٥، ٧، ١٠،و ٢١ يوماً، وتلك المعاملة بجرعة متكررة تم أخذ العينات منها بعد ١ أسبوع، ٢ أسبوع، ٣ أسابيع من بداية الحقن. أوضحت النتائج أنه يمكن للدكسوروبيسن أن يغير التركيب النسيجي لنسيج الخصية في المراحل الأولية للجرعة المفردة حيث تم ملاحظة احتقان في الأوعية الدموية البين انيبيبية في النسيج البيني ، ويلاحظ تباعد وانفصال للخلايا الجرثومية كما يلاحظ زيادة طفيفة في وزن كلّ من الحيوان والخصية. أما التغيرات الشديدة في الخلايا المنوية فيتم ملاحظتها بعد ٧، ١٥، ٢١ يوماً. حيث تبدوا هذه التغيرات على شكل موت خلوي مبرمج (تحلل ذاتي)، ويظهر أن هذا التحلل يكون معتمداً على مراحل دورة الخلية، كما أظهرت خلايا أمهات المني والحيوانات المنوية الناضجة مقاومة لسمية العقار بالرغم من ظهور بعض التغيرات في رؤوس الحيوانات المنوية وذيولها في الحالات الشديدة (بعد ٣ أسابيع من الجرعات المتكررة). ومما يدل على أن التغيرات السمية الحاصلة للخصية هي تحلل ذاتي هو غياب التفاعلات الالتهابية أو أي علامات تدل على تنكرز. ويبدوا أن خلايا

سيرتولي الداعمة تتأثر وظيفياً في المراحل الأولية ومن المحتمل أنها فقدت اتصالها مع الخلايا الجرثومية مما أدى إلى عدم انتظامها وانفصالها. وفي المراحل المتأخرة من مجموعة الجرعة المفردة وبعد الجرعات المتكررة ظهرت الخلايا مليئة بالفجوات والانتفاخات السيتوبلازمية وأنوية صغيرة الحجم غير منتظمة. وظاهرياً لا تتغير خلايا ليدج في المراحل الأولية من تعاطي الدكسوروبيسين، أما بعد ٢١ يومًا من إعطاء الجرعة المفردة و٢-٣ أسبوعًا من الجرعات المتكررة تم ملاحظة تكاثر في خلايا ليدج خصوصاً بين الأنيبيبات المتأثرة بشكل كبير بالمعاملة، ويظهر سيتوبلازم هذه الخلايا فجوات تصطبغ بشدة بصبغة حمض الأوزميك.

ومن المقترح أن الدكسوروبيسين يقوم بعمله عن طريق التداخل مع بناء الحمض النووي الدنا ويقوم بالتأثير على تجمع الأنيبيبات الدقيقة أو أن يقوم بالتأثير على أيض الخلية. ونحتاج إلى دراسات موسعة للتركيب الدقيق للخلايا وتحاليل نسيجية وهرمونية وذلك لتسليط الضوء على تأثيرات الدكسوروبيسين التحليلية الملاحظة في هذه الدراسة.