Egyptian Journal of Anatomy, Jan. - July 2009; 32(1-2):59-72

Original Article

Protective Effects of Erdosteine on the Testes of Cisplatin- Treated Adult Male Albino Rats: A Light and Scanning Electron Microscopic Study

Seham H. Refaat, Ghada A. Wassif, Rania A. Salah El Din, and Atef I. Shabana

Anatomy Department, Faculty of Medicine, Ain Shams University

ABSTRACT

Background: Cisplatin is considered a potent chemotherapeutic drug used in clinical oncology but causes testicular damage as a side effect. Erdosteine is a mucolytic drug possessing an antioxidant capacity.

Aim of the Work: The present study was planned to evaluate the possible protective effect of erdosteine against cisplatin-induced testicular toxicity in rats.

Material and Methods: Forty adult male albino rats were divided into four groups, ten rats each: control, erdosteine-treated (50 mg/kg body weight/day, orally for 7 days), cisplatin-treated (a single dose of 7mg/kg intraperitoneally) and erdosteine/cisplatin-treated. Erdosteine was administered 24h before cisplatin injection and was continued until animal sacrifice. Six days after cisplatin administration, all rats were anaesthetized with ether. The scrotum of each animal was incised and the testes were removed and processed to be examined by both the light and scanning electron microscopy.

Results: Examination of the testicular specimens of the cisplatin-treated group revealed severe testicular damage with significant reduction in the tubular diameter and germinal epithelium thickness compared to the control group. Shrunken tubules, germ cell loss and apoptotic changes in most of the cells, especially the primary spermatocytes and round spermatids were noticed. The mature spermatids appeared markedly decreased in number and the existing ones showed abnormal forms. Increased interstitial edema and apparent decrease in the Leydig cells were also observed. Erdosteine administration before cisplatin showed amelioration of the testicular architecture. Despite the mild degenerative changes in some spermatogenic cells, cell loss was markedly decreased and most of the tubular lumina were full of mature spermatids. Apparent increase in the Leydig cells and decreased interstitial edema was also noticed.

Conclusions: Erdosteine partially protected the rat testes against the cisplatin-induced toxicity most probably via its potent antioxidant and radical scavenging activities.

Corresponding Author: Dr. Ghada Wassif, Anatomy Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt, Mobile: 0164941926.

Key Words: Erdosteine, protection, cisplatin, toxicity, testes, rats, adults, light microscopy, scanning electron microscopy.

INTRODUCTION

Cisplatin (Cis-diamminedichloroplatinum- II, CP), a chemotherapeutic agent has been successfully used in clinical oncology against diverse types of malignancies, including cancers of lung, bladder, ovary and testis *(Rosenberg, 1999)*. Testicular germ cell tumors are the most common malignancy in adult men between 15 and 45 years of age *(Fossa, et al. 1988)*. The cytotoxic effects of cisplatin and others chemotherapeutic drugs result, in part, from their interaction with DNA inhibiting fundamental cellular processes including replication, transcription and DNA repair with subsequent apoptosis *(Wozniak and Blasiak, 2002)*. Moreover, there is much evidence that the binding of cisplatin to non- DNA targets (especially proteins) may contribute to its cytotoxic mechanism of action. On the other hand, the dose of the drug and the metabolic condition of the cell subjected to cisplatin, may determine that the cancer cells die through apoptosis or cell necrosis *(Gonzalez, et al. 2001; Fuertes, et al. 2003)*.

Despite the potent antineoplastic action of cisplatin, it induced severe side effects including ototoxicity, gonadal toxicity, peripheral neuropathy and nephrotoxicity *(Stadnicki, et al. 1975;*

Drasga, et al. 1983; Thompson, et al. 1984; Appenroth, et al. 1988). Cisplatin chemotherapy results in azoospermia in most men, with a recovery of spermatogenesis in about 50% after 2 years and 80% after 5 years and the rest are rendered permanently infertile. These toxic effects led researchers to additional methods that could preserve fertility in men undergoing traditional anticancer therapy. Hormonal manipulation to enhance recovery of spermatogenesis and cryopreservation of testicular tissue were suggested *(Howell and Shalet, 2005)*.

Cisplatin cytotoxicity is also believed to be a result of generation of free radicals and induction of oxidative injury *(Baliga, et al. 1999)*. The overproduction of reactive oxygen species (ROS) results in defects in the integrity of the cell membrane of spermatozoa, as a result of lipid peroxidation, error in spermiogenesis and detrimental effects on sperm functions *(Sanocka and Kurpisz, 2004)*. Many antioxidant agents have been studied in experimental and clinical trials to reduce or prevent cisplatin- induced testicular toxicity *(Choudhury and Jagdale, 2002; Ateşşahin, et al. 2006; Türk, et al. 2008)*.

Erdosteine is a mucolytic drug that has been used in the treatment of chronic obstructive pulmonary diseases. The antioxidant and free radical scavenging capacity of erdosteine has been studied in the recent years. These protective activities were via its active metabolites *(Braga, et al. 2000; Fadillioglu and Erdogan, 2003)*. Erdosteine proved to ameliorate the cisplatin- induced renal failure in a dose- dependent manner in rats *(Özyurt, et al. 2004)*. So, the aim of the present study was to evaluate the possible protective effect of erdosteine on the testes of cisplatin-treated albino rats using light and scanning electron microscopy.

MATERIAL AND METHODS

Animals: Forty adult male albino rats weighing 200- 250 gm were used in the present study. The animals were kept in the animal house in the Medical Research Center and Bilharizial Researches, Faculty of Medicine, Ain Shams University hospitals. Animals were housed in separate cages under conventional and controlled conditions (12/12 light / dark cycle; 23-25c). The rats were maintained on standard laboratory diet with free access to water.

Drugs: Cisplatin (cisplatin/ Merck, Merck generiques) was obtained as a solution (50 mg / 50 ml isotonic solution) and administrated as a single intraperitoneal injection (i.p.) in a dose of 7 mg/ kg body wt. Erdosteine (Mucotec/ Global Napi pharmaceuticals, Egypt) was obtained as capsules (150 mg). Capsules were dissolved in distilled water and given orally by gastric intubation as a single daily dose of 50 mg/kg body wt for a week. The first dose was given 24h prior to cisplatin injection and was continued for 6 days *(Özyurt, et al. 2004)*.

Experimental design: Animals were randomly divided into four equal groups, 10 rats each.

- **Group I (Control):** Rats only received distilled water in a dose of 0.5ml/ rat/ day.
- **Group II (Erdosteine- treated):** Rats received a single oral daily dose of erdosteine dissolved in distilled water) 0.5ml/ rat/ day) for 7 days.
- **Group III (Cisplatin- treated):** Rats were injected i.p. with a single dose of cisplatin
- **Group IV (Erdosteine/ Cisplatin- treated):** Rats were given a single dose of erdosteine 24h before cisplatin injection and continued until the sacrifice of the animals.

After six days of cisplatin administration, animals of all groups were anaesthetized with ether. The scrotum of each animal was incised. The testes were exposed and dissected. The right testis of each rat of each group was fixed in Bouin's liquid for 48 hours, sliced and processed for paraffin embedding. Sections of 5-7um were cut and stained with Hematoxyline and Eosin *(Bancroft and Gamble, 2002)* for their histopathological examination. The left testis of each rat of each group was immersed in 2.5 % buffered gluteraldehyde for 24 hours. Some specimens were processed to prepare to semithin sections. Other specimens were processed to be examined by scanning electron microscope. For preparation of semithin sections, tissue samples (1mm) were washed 3 times in phosphate buffer at PH 7.4, post fixed in 1% osmium tetraoxide for 1-2 hours. After dehydration in ascending grades of alcohol, the specimens were cleared in three changes of propylene oxide and finally embedded in gelatin

capsules filled with fresh spurr-premix. The capsules were kept in the oven at 60oc for 48 hours for polymerization. Semithin sections of 0.5-1µm were cut using a glass knife and stained with toluidine blue to be examined by light microscopy. For scanning electron microscopic study, the buffered gluteraldehyde fixed tissue samples were dehydrated in ascending grades of ethyl alcohol (50%- 70%- 80%- 90%- 100%). The tissues were then further dehydrated in a mixture of 1:1 absolute alcohol and acetone 100% and then in 100% acetone twice; fifteen minutes for each. The specimens were then dried at critical point using liquid carbon dioxide in BALTEK CPD030 critical point dryer. The specimens were fixed on aluminum stubs and then sputter coated with gold using BALTEK-SCD005. Some specimens were cut with freeze fracture after immersion in liquid nitrogen. The testes of all groups were examined using scanning electron microscope Philips XL30 *(Robinson, et al. 1987)*.

Histomorphometric study: In each group, the transverse sections of the testes stained with Hematoxyline and eosin were examined by an image analyzer computer system using the soft ware Leica Quin 500 MC program. Ten random non-overlapping fields/ testis specimen/ group were examined at 100x magnification and the tubular diameter and the height of the germinal epithelium of the examined seminiferous tubules were measured. The readings were obtained for each group and the mean values were calculated.

Statistical analysis: All data were collected and analyzed using the Statistical Package for the Social Sciences (SPSS) software program (version 13; SPSS Inc., Chicago, IL, USA). All values were expressed as mean ± SEM (standard error of mean). Student's t- test was used to compare the mean of the four groups. Probability (P) value was considered statistically significant if < 0.05 and highly significant if <0.01.

RESULTS

I-Histological Results Light microscopic examination of the stained sections of the control group (Group I) revealed that the parenchyma of the testis was formed of numerous oval or rounded seminiferous tubules (STs) with narrow interstitial tissue spaces in between. Each tubule was surrounded by a regular thin basement (limiting) membrane and lined by five or six layers of spermatogenic cells in addition to the supporting Sertoli cells (Fig. 1-A). The germinal epithelium consisted of spermatogonia, primary spermatocytes, hardly detected secondary spermatocytes, young (round) and mature (elongated) spermatids (Fig. 1-B). The spermatogonia lay in contact with the basement membrane beside the Sertoli cells. Mitotic figures could be detected in some spermatogonia. The primary spermatocytes represented the next one or two rows of cells. They were large round cells having nuclei with coarse chromatin (pachytene stage) (Figs. 2- A, B). Next to the primary spermatocytes, the round spermatids were staked in three to four layers. They possessed lightly stained cytoplasm and round nuclei with pale granular chromatin. Mature (late or elongated) spermatids appeared in most of tubules with flattened, elongated nuclei and their tails were directed towards the lumen. The Sertoli cells rested on the basement membrane and almost extended to the lumen. They were pyramidal in shape with large pale oval nuclei with prominent nucleoli (Figs. 1-B, 2-A). In the interstitial spaces, the Leydig cells were observed singly or in groups embedded in loose connective tissue that was richly supplied by blood capillaries. They showed either round or oval nuclei with prominent nucleoli (Figs. 1-B, 2- B). Scanning electron microscopic examination of the control group showed several round seminiferous tubules with regular outlines and separated by narrow interstitial spaces (Fig. 3-A). They were full of all types of germ cells, lying close to each other. Mature spermatids occupied completely the lumina of the tubules (Figs. 3-B, 4-A, B). Each elongated spermatid exhibited an oval head and a single straight tail, of uniform thickness directed towards the lumen (Figs. 4-A, B).

Light and scanning microscopic examination of erdosteine- treated group (Group II) did not reveal any apparent histological differences between this group and the control one. All of the spermatogenic cell types constituting the successive stages of spermatogenesis were observed. The lumina of most of the seminiferous tubules were occupied by mature elongated spermatids (Figs. 5, 6-A). The latter showed oval heads and elongated straight tails of apparently similar thickness to the control group (Fig. 6-B).

Under Light microscopy, sections of the cisplatin- treated group (Group III) showed severe testicular damage and extensively wide interstitial

spaces. Seminiferous tubules exhibited various degrees of affection; focal depletion of germ cells ,detachment of the germinal epithelium towards the lumina, or showed shrinkage with irregular basement membrane (Figs. 7-A, B). Moreover, some of them were lined with few degenerated cells and their lumina were filled with acidophilic hyaline material (Fig. 7-C), while others appeared distorted with loss of a part of their basement membrane (Figs.7-A, D). Severely affected tubules showed remnants of degenerated cells with cytoplasmic dense bodies resting on a thickened and wrinkled basement membrane. The primary spermatocytes appeared small in size with irregular outlines. Most of the round spermatids showed highly vacuolated cytoplasm and ruptured cell membrane. Wide intercellular spaces among the spermatogenic cells were encountered as well (Fig. 8-A). Some of these tubules also showed areas completely depleted of germ cells and the Sertoli cells were the only cell type observed lining the tubules. Few degenerated cells were seen exfoliated towards the lumen (Fig. 8-B). The spermatogonia were hardly detected in the severely affected tubules (Figs. 8-A, B).

However, some of the seminiferous tubules showed normal height of the germinal epithelium but most of spermatogenic cell types exhibited morphological abnormalities. Primary spermatocytes appeared swollen with either densely stained cytoplasm and large- sized hyperchromatic nuclei or lightly stained cytoplasm and nuclei with ill- defined nuclear membranes (Figs. 9, 10). The round spermatids showed ring- shaped, sharp delineated and condensed marginal chromatin. Remnants of degenerated elongated spermatids were also observed (Fig. 9) Moreover, Adluminal apoptotic bodies were detected (Fig. 10). The dilated interstitial spaces showed scanty number of Leydig cells, accumulation of intercellular vacuolated material in addition to dilated and congested interstitial blood vessels (Fig. 11). Scanning electron microscopic examination of the sections of group III revealed that the majority of seminiferous tubules appeared compressed with wrinkled basement membrane. Most of them were occupied with spermatogenic cells while few appeared empty and completely depleted of cells (Figs. 12-A, B). Intercellular spaces were observed in between the germ cells (Fig. 13-A). The young spermatids appeared polymorphic in shape. The mature ones showed apparent decrease in number as compared to the control group. Most of the elongated spermatids exhibited abnormal forms, thin short or forked tails (Figs. 13- B).

Light microscopic examination of the stained sections of erdosteine / cisplatin – treated group (Group IV) revealed maintenance of the general architecture of the tubules with preservation of the normal height of germinal epithelium in addition to the diminution of the intertubular spaces as compared to group III (Fig. 14). Moreover, the interstitial tissue showed decreased vacuolation and abundant Leydig cells (Fig. 15). However, the improvement was not complete, as some tubules showed mild intercellular spaces as well as degenerative changes in some germ cells. Few primary spermatocytes showed lightly stained nuclei and ill- defined nuclear membrane. Some round spermatids showed small, dark and pyknotic nuclei. (Figs. 16- A, B). Scanning electron microscopic examination of group IV showed that most of the seminiferous tubules had regular outlines and the germinal epithelium showed full height. The lumina of some of the tubules were occupied with mature spermatids while others appeared free (Figs. 17-A, B).

II-Morphometric Results: Statistical analysis of the mean values of the seminiferous tubular diameter and the germinal epithelial height in the erdosteine- treated group (Group II) revealed insignificant change ($P < 0.5$) in comparison to the control one. Cisplatin- treated group (Group III) showed a highly significant decrease ($P < 0.001$) in the mean diameter of the seminiferous tubules and the germinal cell height compared to the control group. Erdosteine treatment before cisplatin (Group IV) revealed insignificant increase in the tubular diameter ($P < 0.2$) as well as the germinal epithelial height ($P < 0.1$) compared to cisplatin-treated group (Tables 1, 2).

Table 1: mean diameters of the seminiferous tubules in different groups.

Groups	Mean (µm)	SDM	P Value	Significance
Control (I)	230.71	± 40.43	-	-
Erdosteine (II)	211.98	± 26.8	<0.5*	NS
Cisplatin (III)	174.64	± 22.1	<0.001*	HS
Erdosteine+ Cisplatin (IV)	203.38	± 82.93	<0.2**	NS

- P* value in each group (II, III, IV) was in comparison with the control value, P** value in group IV was compared with group III.
- N.S. = non significant; P < 0.001 was regarded highly significant (H.S).

Table 2: mean height of the tubular germinal epithelium in different groups.

Groups	Mean (µm)	SDM	P Value	Significance
Control (I)	70.16	± 14.57	-	-
Erdosteine (II)	72.52	± 11.41	<0.5*	N.S.
Cisplatin (III)	41.16	± 3.55	<0.001*	H.S
Erdosteine+ Cisplatin (IV)	44.78	± 6.06	<0.1**	N.S.

- P* value in each group (II, III, IV) was in comparison with the control value, P** value in group IV was in comparison with group III.
- N.S. = non significant; P < 0.001 was regarded highly significant (H.S).

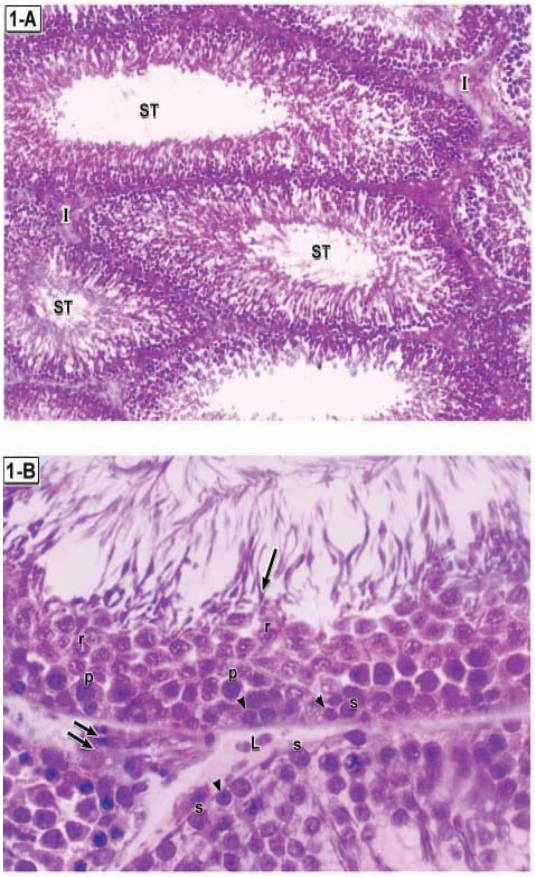


Fig. 1- A, B: Photomicrographs of testis sections of rats of the control group:
A- Section showing seminiferous tubules (ST) with narrow interstitial tissue spaces (I) in between. H & E; X100
B- section showing the different cells lining the tubule including spermatogonia (arrowhead), primary spermatocytes (p), round spermatids (r) and mature spermatids (arrow) in addition to Sertoli cells (S). Note the presence of Leydig cells (L) near the interstitial blood vessel (double arrows). H & E; X400

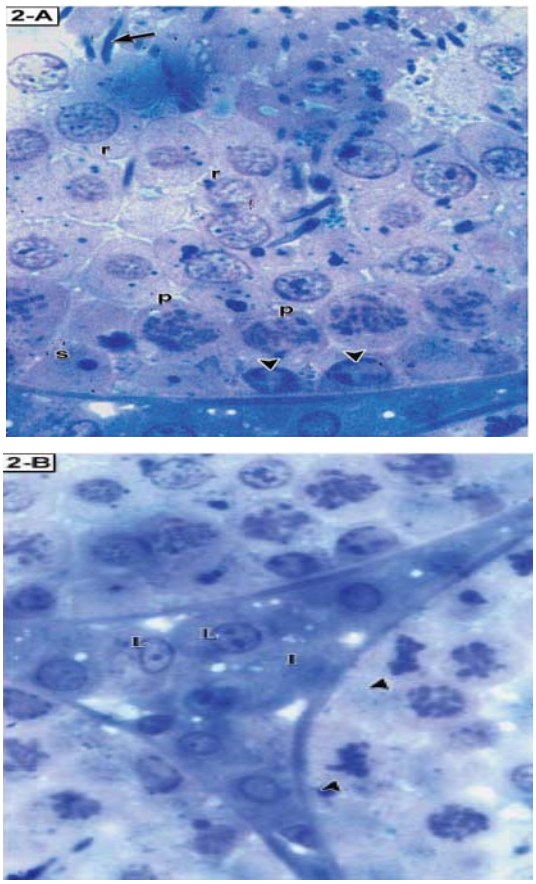


Fig. 2- A, B: Photomicrographs of semithin sections of rat testis of the control group:
A- Section showing the cells lining the seminiferous tubule, spermatogonia (arrowhead) and Sertoli cells (S) resting on a regular basement membrane. Note the presence of pachytene primary spermatocytes (p), round spermatids (r), and mature spermatids (arrow). Toluidine blue; X1000
B- Section showing the interstitial space (I). Notice the Leydig cells (L) with round or oval nuclei and apparent nucleoli. Note the mitotic figures (arrowhead) in some spermatogonia. Toluidine blue; X1000

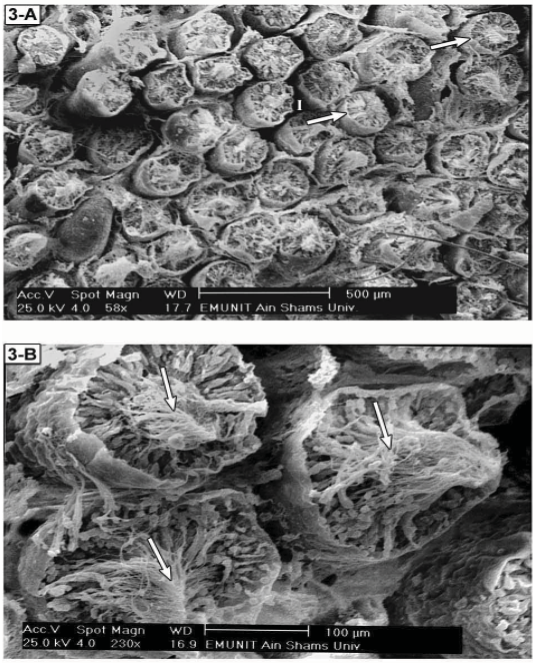


Fig. 3 -A, B: A Scanning electron micrographs of testis sections of rats of the control group showing:
A- Regular seminiferous tubules (arrow) with minimal interstitial spaces (I) in between. SEM X58
B- The seminiferous tubules lined with full height germinal epithelium. Notice that their lumina are completely occupied by mature elongated spermatids (arrow). SEM X230

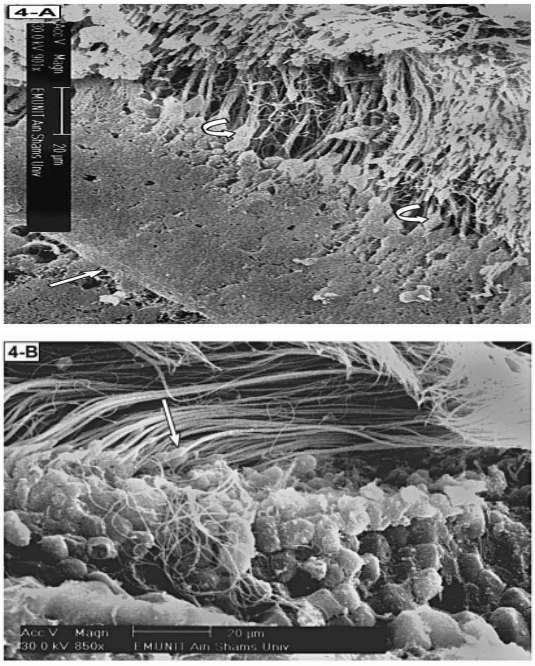


Fig. 4- A, B: Scanning electron micrographs of testis sections of rats of the control group:
A- A freeze fracture section showing the full thickness of the spermatogenic epithelium resting on the limiting basement membrane (arrow). Note the numerous mature spermatids (curved arrow). SEM X901
B- Different types of cells constituting the spermatogenic epithelium. Note each mature spermatid with an oval head (arrow) and a single straight tail of uniform thickness directed towards the lumen. SEM X850

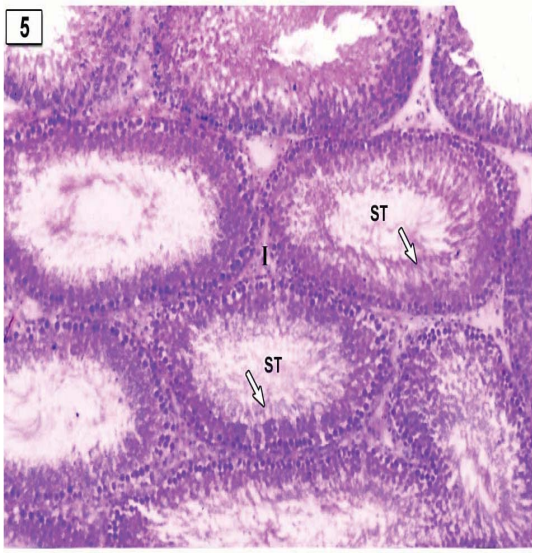


Fig. 5: A Photomicrograph of a section of rat testis of erdosteine- treated group showing apparently normal seminiferous tubules (ST) with narrow interstitial spaces (I) in between. Notice the full thickness of the germinal epithelium Note also the mature spermatids (arrow) in most of the tubules. H & E; X100

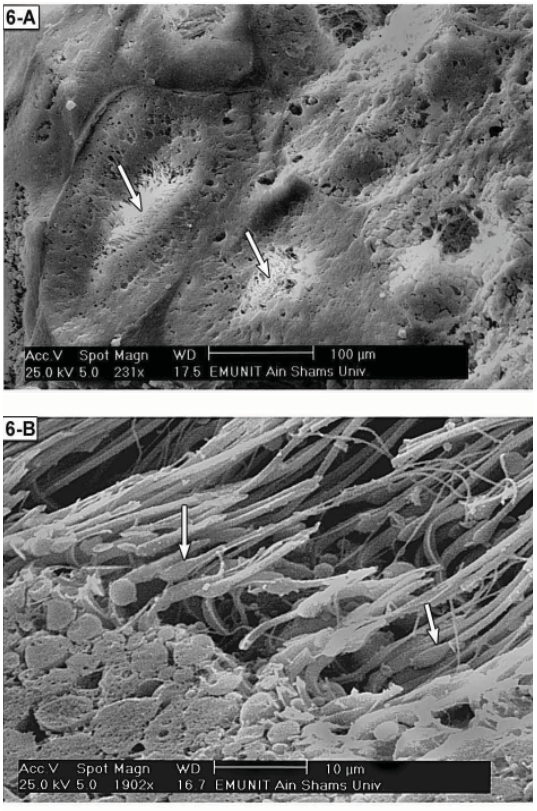
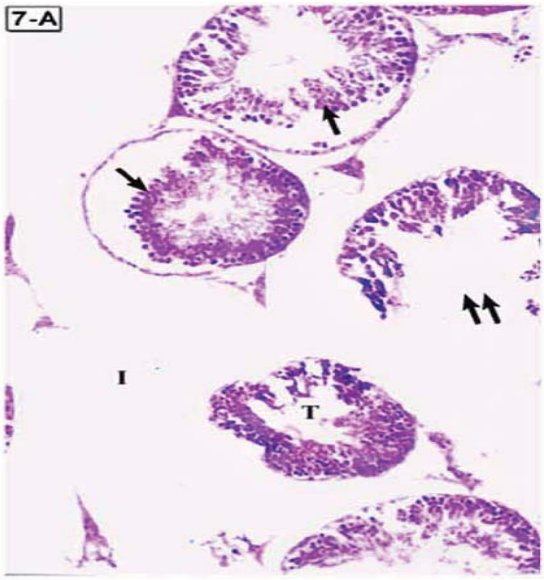
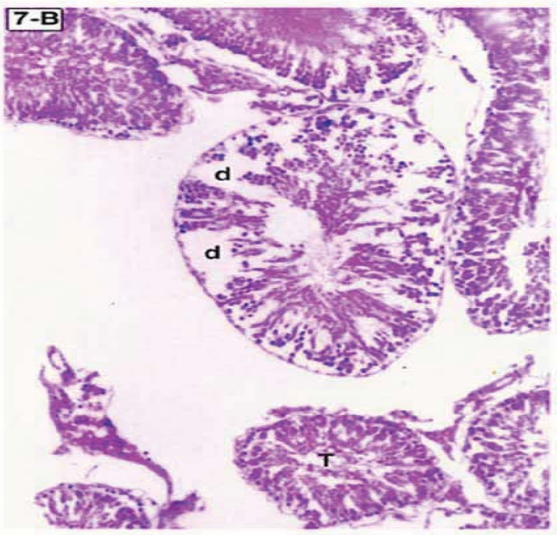
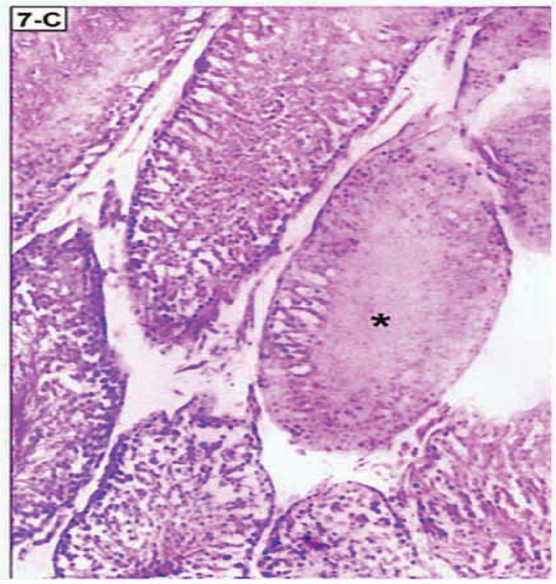


Fig. 6- A, B: A freeze fracture scanning electron micrographs of testis sections of rats of the erdosteine- treated group showing:
A- Oval seminiferous tubules with regular outlines and the lumina of most of them are occupied by mature spermatids (arrow) SEM X231
B- Apparently normal- shaped mature (elongated) spermatids (arrow). SEM X1902







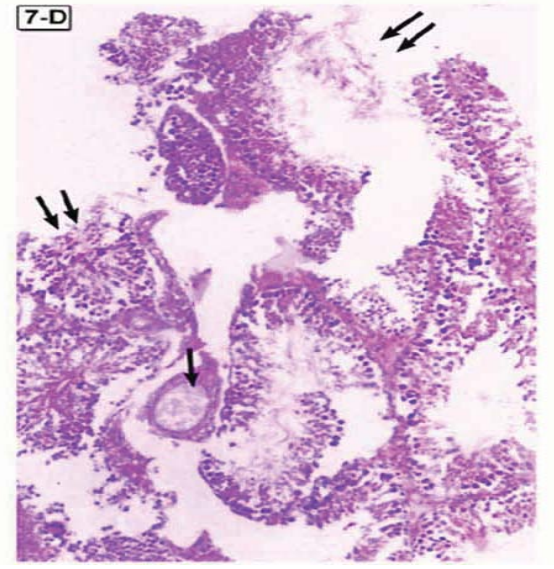
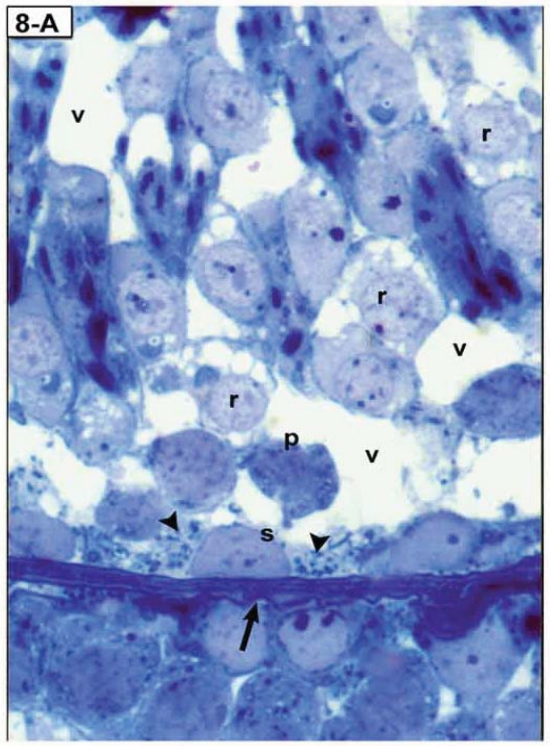


Fig. 7- A, B, C, D: A Photomicrographs of testis sections of rats of the cisplatin- treated group showing:
A- Seminiferous tubules with extensive large interstitial spaces (I) in between. Note the detached germ cells from the tubular basement membrane towards the lumen (arrow). Notice also a shrunken tubule (T) with wrinkled basement membrane as well as the loss of part of the basement membrane of another one (double arrows). H & E; X100
B- A tubule with focal cell depletion (d). Note a shrunken tubule (T) with irregular festooned basement membrane. H & E; X100
C- A tubule lined with few degenerated germ cells. Note the presence acidophilic hyaline material (asterisk) in its lumen. H & E; X100
D- Distorted tubules with loss of part of the basement membrane (double arrows). Notice the thickened, dilated and congested interstitial blood vessel (arrow). H & E; X100



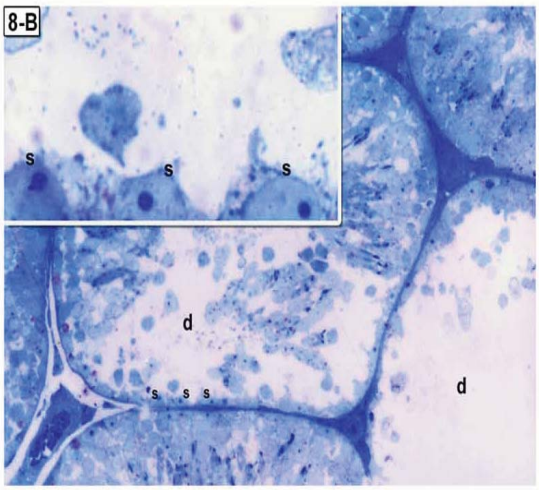


Fig. 8- A, B: Photomicrographs of testis semithin sections of rats of the cisplatin- treated group showing:
A- Cell remnants with cytoplasmic dense bodies (arrowhead) beside the Sertoli cells (S). Note the primary spermatocytes (p) with irregular outlines, the round spermatids (r) with vacuolated cytoplasm, disrupted cell membrane and the large intercellular spaces (v). Notice also the thickened and wrinkled basement membrane (arrow). Toluidine blue; X1000
B-Massive depletion (d) of most of the spermatogenic cells with luminal exfoliation of few degenerated germ cells. Note the inset showing part of a tubule lined with Sertoli cells (S) only. Toluidine blue; X200; Inset X1000

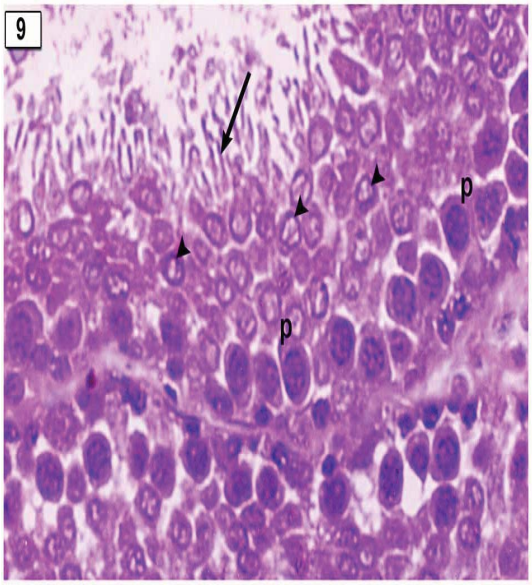


Fig. 9: Photomicrograph of section of rat testis of the cisplatin- treated group showing a tubule lined with all types of spermatogenic cells. Note the swollen primary spermatocytes (p) with highly acidophilic cytoplasm and hyperchromatic nuclei. Notice also the ring– shaped nuclei of the round spermatids (arrowhead) and the luminal remnants of the degenerated elongated spermatids (arrow) H & E; X600

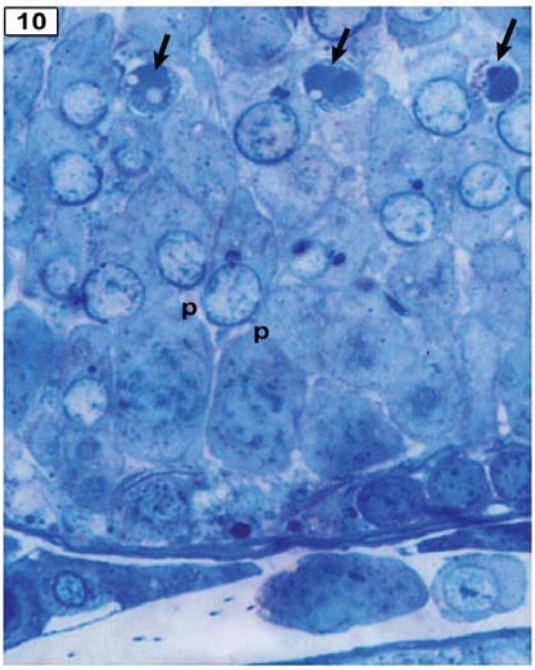


Fig. 10: Photomicrograph of semithin sections of rat testes of the cisplatin- treated group showing swollen primary spermatocytes (p) with lightly stained nuclei and ill defined nuclear membrane. Note the adluminal apoptotic bodies (arrow). Toluidine blue; X1000

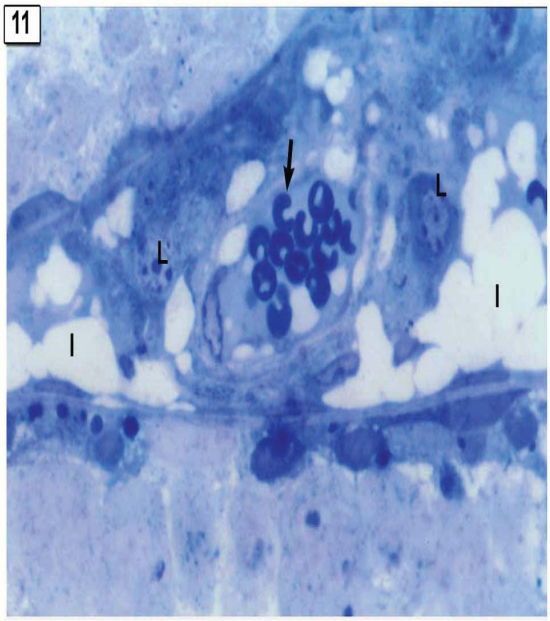
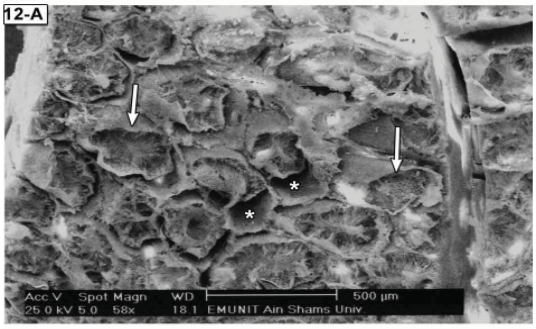


Fig. 11: A Photomicrograph of a semithin section of rat testis of the cisplatin- treated group showing scanty Leydig cells (L) compared to the control group (Fig.2-B). Notice the wide and vacuolated interstitial tissue (I) and the dilated congested interstitial blood vessel (arrow). Toluidine blue; X1000



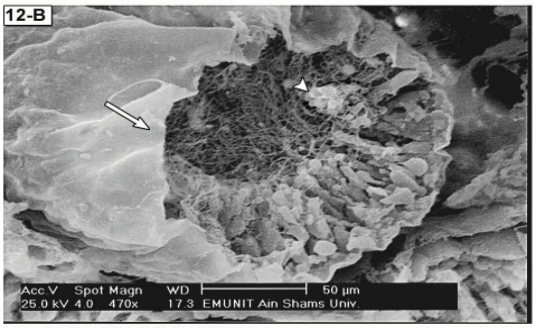
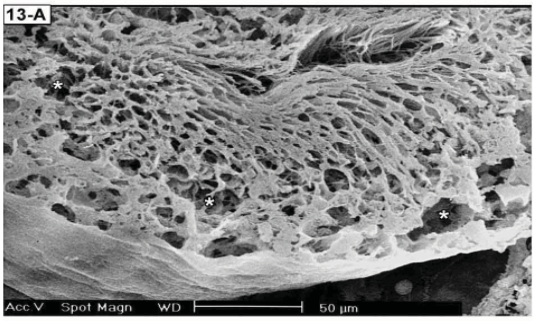


Fig. 12- A, B: Scanning electron micrographs of sections of rat testes of the cisplatin- treated group showing:
A- Compressed seminiferous tubules lined with germ cells (arrow), few of them appeared empty (white asterisk). SEM X58
B- A tubule with wrinkled basement membrane. Note the depletion of the spermatogenic cells on one side of the tubule (arrow). Notice also the luminal exfoliated germ cells on its opposite side (arrowhead). SEM X470



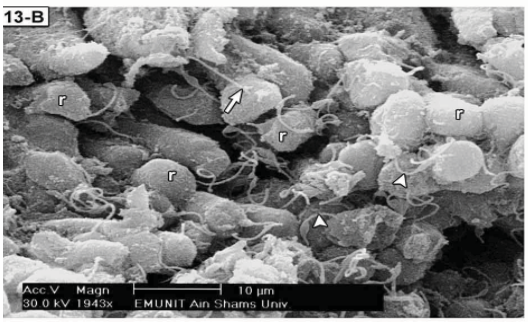


Fig. 13- A, B: Scanning electron micrographs of sections of rat testes of the cisplatin- treated group showing:
A- Intercellular vacuoles (asterisk) in between the different germ cells. SEM X462
B- Polymorphic round spermatids (r) and few scattered abnormally- shaped mature spermatids. Notice some of the later with thin short (arrowhead) or forked tail (arrow). SEM X1943

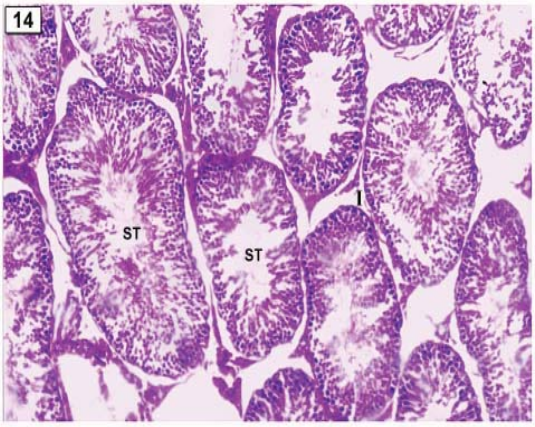


Fig. 14: Photomicrograph of a section of rat testis of the erdosteine /cisplatin- treated group showing apparently normal seminiferous tubules (ST) with diminution of the interstitial spaces (I) in between compared to group III (Fig.7-A). H & E; X100

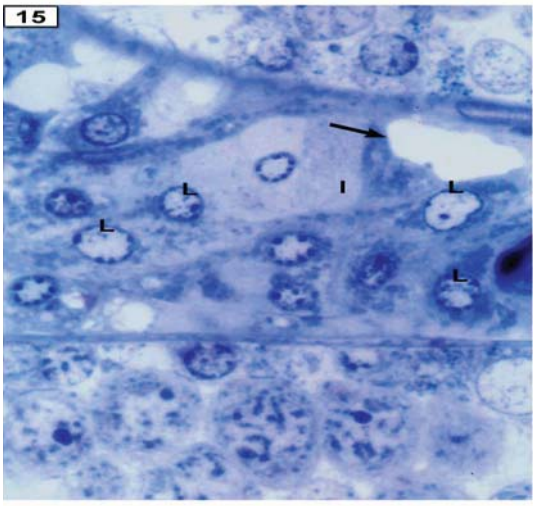
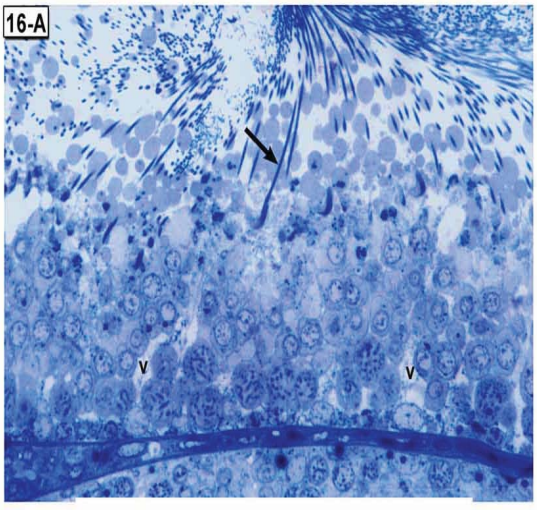


Fig. 15: A Photomicrograph of a semithin section of rat testis of the erdosteine/ cisplatin- treated group showing apparently abundant Leydig cells (L). Notice the decreased vacuolation (arrow) of interstitial tissue (I) compared to group III (Fig.10). Toluidine blue; x1000



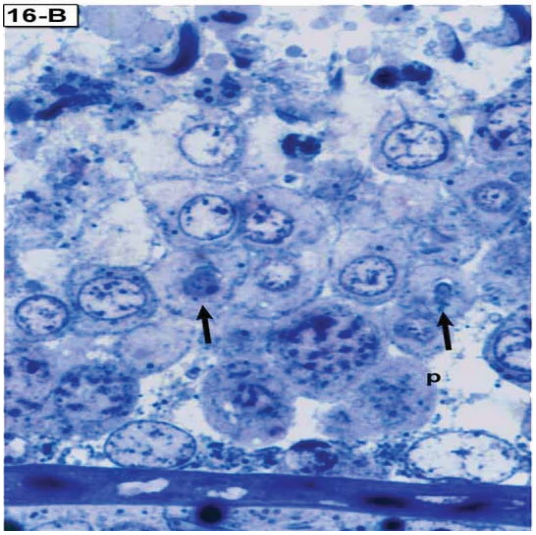


Fig. 16- A, B: Photomicrographs of semithin sections of rat testis of the erdosteine /cisplatin- treated group showing:
A- A tubule lined with all types of germ cells up to mature spermatids (arrow). Note the mild intercellular spaces (v).
Toluidine blue; X400
B- A higher magnification of the previous figure showing degenerative changes in some spermatogenic cells. Note the lightly stained nuclei and the ill defined nuclear membrane of primary spermatocytes (p). Notice also the round spermatids with small pyknotic nuclei (arrow).
Toluidine blue; X1000

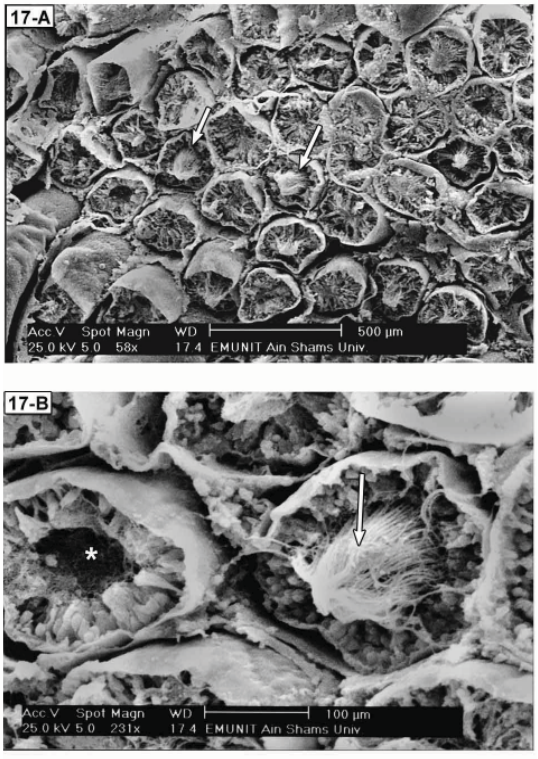


Fig. 17- A, B: Scanning electron micrographs of sections of rat testes of the erdosteine/cisplatin - treated group showing:
A- Apparently normal seminiferous tubules (arrow) with narrow interstitial spaces. SEM X58
B- A tubule with lumen full of mature spermatids (arrow) and another one free from them (white asterisk). SEM X231

DISCUSSION

Spermatogenic cells are targeted by cytotoxic agents because of their high mitotic activity. The chance of recovery of spermatogenesis and also the extent and speed of recovery are related to the agent used and the dose received *(Ishikawa, et al. 2004)*. Exposure to cisplatin results in impaired spermatogenesis with temporary azoospermia and, sometimes permanent infertility in male patients *(Colpi, et al. 2004)*. In the present research, examination of the testes of the cisplatin- treated rats' revealed severe testicular damage with a highly significant decrease in the diameter of the seminiferous tubules and the height of the germinal epithelium as compared to the control group. The primary spermatocytes appeared swollen with hyperchromatic or pale stained nuclei and ill- defined nuclear membrane. Some of the round spermatids showed nuclei with ring- shaped condensed marginal chromatin while the others showed highly vacuolated cytoplasm and ruptured cell membrane. Adluminal apoptotic bodies were also observed. *Allen et al. (1997)* found that the striking apoptotic changes observed by light microscope included cell shrinkage up to 30%, crescent- shaped masses of condensed chromatin adjacent to the nuclear envelope, and cytoplasmic and nuclear fragmentation leading to apoptotic bodies. *Seeman et al. (2003)* mentioned that administration of cisplatin resulted in elevated germ cell apoptotic rate with subsequent elimination of germ cells. Necrosis was also recorded *(Cepeda, et al. 2007)*.

In the present study, some of the severely affected tubules showed shrinkage associated with severe degenerative changes in most of the germ cells. Other tubules showed germ cell depletion and some areas appeared lined only with Sertoli cells. Spermatogonia were hardly detected. *Schrader et al. (2002)* observed that human stem cell spermatogonia (type A) survived chemotherapy and suggested that they formed the basis for the recovery of spermatogenesis. However, high doses or prolonged duration of administration could destroy the spermatogonia leading to sustained or irreversible failure of germ cell production and consequently infertility. *Boekelheide (1993)* reported that cell vacuolation, apical sloughing, shedding of cellular material into the lumen and the massive germ cell loss were all manifestations of Sertoli cell injury. Spermatogenesis is supported and regulated by Sertoli cells. One of the

major functions of Sertoli cells is to form blood-testis barrier which provide a stable environment for cell differentiation. Sertoli cells also are the source of tubular fluid. Disruption of blood testis barrier or Sertoli cells tight junctions may alter the tubular environment and may result in germ cell loss and impaired spermatogenesis. Both the morphological and biochemical properties of Sertoli cells were affected by cisplatin administration *(Pogach, et al. 1989; Sawhney, et al. 2005)*.

In the present work, the examined sections of cisplatin- treated testes showed scanty Leydig cells, widening of interstitial spaces and accumulation of intercellular vacuolated material. Congested and dilated interstitial blood vessels were also noticed. In accordance with the present findings, *Lirdi et al. (2008)* mentioned that telangiectasia followed by endothelial disruption induced interstitial edema in cisplatin- treated rats. *Aydiner et al. (1997)* reported that cisplatin not only induced significant reduction in the Leydig cells but also resulted in alterations in their morphology. *Amin and Hamza (2006)* added that a decrease in number of Leydig cells and subsequently decreased levels of testosterone may affect directly or indirectly the interstitial tissue and lymphatic spaces provoking interstitial testicular edema. In the current study, light and scanning microscopic examination of the cisplatin- treated group revealed apparent decrease in the mature spermatids and the existing ones appeared as luminal cell remnants or exhibited abnormal forms. The present findings are in agreement with those of *Ateşşahin et al. (2006)* who explained them by the cisplatin- induced generation of reactive oxygen species (ROS). The oxidative stress can overwhelm the antioxidant defense systems and initiate changes in lipid and/ or protein layers of sperm plasma membranes. Additionally, changes in sperm DNA can be induced leading to its denaturation and fragmentation *(Sanocka and Kurpisz, 2004)*.

Recently, much attention has been focused on the protective effects of antioxidants against cisplatin-induced toxicity *(Amin and Hamza, 2006; Ateşşahin, et al. 2006 and Lirdi, et al. 2008)*. Erdosteine has been used as a mucolytic drug and as an enhancer of respiratory ventilation in the treatment of patients with chronic obstructive lung diseases. Erdosteine, which is a thiol derivative, possesses 2 blocked sulfhydryl groups which are released following first-pass metabolism. It exhibits a potent antioxidant and radical scavenging capacity. The structural feature responsible for its protective activities is the –SH group after its hepatic metabolism *(Dechant and Noble, 1996; Braga, et al. 2000; Fadillioglu and Erdogan, 2003)*. The most common side- effect of this group of drugs (mucolytics, such as N-acetylcysteine) is the gastric mucosal irritation. However, erdosteine is associated with a low incidence of adverse events because its mucolytic effect emerges after hepatic metabolism. A previous study indicated that the tolerable dose of erdosteine without side effects was 900 mg/day in humans and 500-1000 mg/kg/day in rats *(De Giovanni, et al. 1991)*. *Özyurt et al. (2004)* investigated the optimum dosage to ameliorate cisplatin –induced nephrotoxicity in rats. Three different doses of erdosteine were tested 25, 50 &75 mg/kg. The suggested optimum dose was 50 mg/kg which was used in the present work. Erdosteine was administered orally 24 hours before a single cisplatin i.p. injection and continued until the end of the present experiment (i.e.7 days). Histological examination of the sections of erdosteine/cisplatin- treated rats showed maintenance of their general architecture. Preservation of the normal height of germinal epithelium as well as diminution of the intertubular spaces was observed. The tubules appeared lined with all types of spermatogenic cells up to mature spermatids indicating complete process of spermatogenesis. Moreover, a reduction of the interstitial edema associated with an apparent increase in the Leydig cells as compared to the cisplatin- treated group was noticed. Although degenerative changes in some germ cells were detected, cell depletion was markedly decreased. Therefore, prophylactic erdosteine treatment conferred partial protection against cisplatin- induced testicular toxicity as it previously proved to ameliorate the cisplatin-induced renal failure *(Özyurt, et al. 2004)*.

In conclusion, erdosteine seems to be a promising protective drug against cisplatin- induced toxicities in rats. Further studies are recommended to investigate the protective effects of erdosteine against cisplatin in humans.

REFERENCES

Allen, R. T., Hunter, W. J. and Agrawal, D. K. 1997. Morphological and biochemical characterization and analysis of apoptosis. Journal of Pharmacological and Toxicological Methods 37(4):215-228.

Amin, A., and Hamza, A. A. 2006. Effects of Roselle and Ginger on cisplatin-induced reproductive toxicity in rats. Asian Journal of Andrology 8(5):607-612.

Appenroth, D., Winnefeld, K., and Braunlich, H. 1988. Nephrotoxicity and pharmacokinetics of cisplatinum in young and adult rats. Biomedica Biochimica Acta 47(8):791-797.

Atessahin, A., Sahna, E., Turk, G., et al. 2006. Chemoprotective effect of melatonin against cisplatin-induced testicular toxicity in rats. Journal of Pineal Research 41(1):21-27.

Aydiner, A., Aytekin, Y., and Topuz, E. 1997. Effects of cisplatin on testicular tissue and the Leydig cell-pituitary axis. Oncology 54(1):74-78.

Baliga, R., Ueda, N., Walker, P. D., and Shah, S. V. 1999. Oxidant mechanisms in toxic acute renal failure. Drug Metabolism Reviews 31(4):971-997.

Bancroft, J. D., and Gamble, M. 2002. Marsland, Glees and Erikson's technique. In Theory and practice of histological techniques, edited by J. D. Bancroft and M. Gamble, Churchill Livingstone. p. 125.

Boekelheide, K. 1993. Sertoli cell toxicants. In The Sertoli cell, edited by L. D. Russell and M. D. Griswold. Clearwater, Florida: Cache River Press. p. 551-575.

Braga, P. C., Dal Sasso, M., and Zuccotti, T. 2000. Assessment of the antioxidant activity of the SH metabolite I of erdosteine on human neutrophil oxidative bursts. Arzneimittel-Forschung 50(8):739-746.

Cepeda, V., Fuertes, M. A., Castilla, J., et al. 2007. Biochemical mechanisms of cisplatin cytotoxicity. Anticancer Agents Med.Chem. 7(1):3-18.

Choudhury, R. C., and Jagdale, M. B. 2002. Vitamin E protection from/potentiation of the cytogenetic toxicity of cisplatin in Swiss mice. Journal of Chemotherapy (Florence, Italy) 14(4):397-405.

Colpi, G. M., Contalbi, G. F., Nerva, F., et al. 2004. Testicular function following chemo-radiotherapy. European Journal of Obstetrics, Gynecology and Reproductive Biology 113 Suppl 1:S2-S6.

De Giovanni, L., Fregnan, G. B., Rabitti, C., et al. 1991. Lack of gastric adverse effects of erdosteine in rats and men. International Journal of Clinical Pharmacology, Therapy and Toxicology 29(7):269-273.

Dechant, K. L., and Noble, S. 1996. Erdosteine. Drugs 52(6):875-881; discussion 882.

Drasga, R. E., Einhorn, L. H., Williams, S. D., et al. 1983. Fertility after chemotherapy for testicular cancer. Journal of Clinical Oncology: 1(3):179-183.

Fadillioglu, E., and Erdogan, H. 2003. Effects of erdosteine treatment against doxorubicin-induced toxicity through erythrocyte and plasma oxidant/antioxidant status in rats. Pharmacological Research: 47(4):317-322.

Fossa, S. D., Aass, N., and Kaalhus, O. 1988. Testicular cancer in young Norwegians. Journal of Surgical Oncology 39(1):43-63.

Fuertesa, M. A., Castillab, J., Alonsoa, C., and Perez, J. M. 2003. Cisplatin biochemical mechanism of action: From cytotoxicity to induction of cell death through interconnections between apoptotic and necrotic pathways. Current Medicinal Chemistry 10(3):257-266.

Gonzalez, V. M., Fuertes, M. A., Alonso, C., and Perez, J. M. 2001. Is cisplatin-induced cell death always produced by apoptosis? Molecular Pharmacology 59(4):657-663.

Howell, S. J., and Shalet, S. M. 2005. Spermatogenesis after cancer treatment: Damage and recovery. Journal of the National Cancer Institute. Monographs(34):12-17.

Ishikawa, T., Kamidono, S., and Fujisawa, M. 2004. Fertility after high-dose chemotherapy for testicular cancer. Urology 63(1):137-140.

Lirdi, L. C., Stumpp, T., Sasso-Cerri, E., and Miraglia, S. M. 2008. Amifostine protective effect on cisplatin-treated rat testis. Anatomical Record 291(7):797-808.

Özyurt, H., Yildirim, Z., Kotuk, M., et al. 2004. Cisplatin-induced acute renal failure is ameliorated by erdosteine in a dose-dependent manner. Journal of Applied Toxicology 24(4):269-275.

Pogach, L. M., Lee, Y., Gould, S., et al. 1989. Characterization of cis-platinum-induced Sertoli cell dysfunction in rodents. Toxicology and Applied Pharmacology 98(2):350-361.

Robinson, D. G., et al. 1987. Methods of preparation for electron microscope.Springer Verlag.

Rosenberg, B. 1999. Platinum complexes for the treatment of cancer: Why the search goes on. In Cisplatin: Chemistry and biochemistry of a leading anticancer drug, edited by B. Lippert, Wiley-VCH. p. 3-27.

Sanocka, D., and Kurpisz, M. 2004. Reactive oxygen species and sperm cells. Reproductive Biology and Endocrinology 2:12.

Sawhney, P., Giammona, C. J., Meistrich, M. L., and Richburg, J. H. 2005. Cisplatin-induced long-term failure of spermatogenesis in adult C57/Bl/6J mice. Journal of Andrology 26(1):136-145.

Schrader, M., Muller, M., Straub, B., and Miller, K. 2002. Testicular sperm extraction in azoospermic patients with gonadal germ cell tumors prior to chemotherapy--a new therapy option. Asian Journal of Andrology 4(1):9-15.

Seaman, F., Sawhney, P., Giammona, C. J., and Richburg, J. H. 2003. Cisplatin-induced pulse of germ cell apoptosis precedes long-term elevated apoptotic rates in C57/BL/6 mouse testis. Apoptosis: 8(1):101-108.

Stadnicki, S. W., Gleischman, R. W., and Schaaeppi, U. 1975. Ototoxicity of cisdichlorodiammine platinum (II) (NSC-119875): Hearing loss and other toxic effects in Rhesus monkeys. Cancer Chemotherapy Reports 59:467-480.

Thompson, S. W., Davis, L. E., Kornfeld, M., et al. 1984. Cisplatin neuropathy. Clinical, electrophysiologic, morphologic and toxicologic studies. Cancer 54(7):1269-1275.

Türk, G., Ateşşahin, A., Sönmez, M., et al. 2008. Improvement of cisplatin-induced injuries to sperm quality, the oxidant-antioxidant system and the histologic structure of the rat testis by ellagic acid. Fertility and Sterility 89(5 Suppl):1474-1481.

Wozniak, K., and Blasiak, J. 2002. Recognition and repair of DNA-cisplatin adducts. Acta Biochimica Polonica 49(3):583-596.

التأثيرات الواقية للإردوستين على الخصى المعالجة بالسيسبلاتين فى ذكور الجرذان البيضاء البالغة (دراسة باستخدام كل من المجهر الضوئى و الإلكترونى الماسح)

سهام حسن رفعت، غادة أحمد وصيف، رانيا أحمد صلاح الدين، عاطف إبراهيم شبانة

قسم التشريح - كلية الطب – جامعة عين شمس

ملخص البحث

يعتبرالسيسبلاتين عقار قوى فى علاج الأورام إلا إن أحد آثاره الجانبيه هوتلف نسيج الخصية. أما الإردوستين فهوعلاج مذيب للمخاط له تأثير مضاد للأكسده ولذلك فقد خطط هذا العمل لتقييم التأثير الواقى المحتمل للإردوستين ضد الأثر السام الناجم عن العلاج بالسيسبلاتين على الخصى فى الجرذان البيضاء.

وقد استخدم في الدراسة الحالية أربعون من ذكور الجرذان البيضاء البالغة، والتى قسمت بالتساوى إلى أربعة مجموعات، بكل منها عشرجرذان كالتالى: مجموعة ضابطة، ومجموعة معالجة بالإردوستين ٥٠مج/كج من وزن الجسم /اليوم، لمدة ٧ أيام،يعطى بالفم) ومجموعة معالجة بالسيسبلاتين (٧مج/ كجم من وزن الجسم، جرعة واحده، تعطى فى التجويف البريتونى) ومجموعة معالجة بالإردوستين والسيسبلاتين، وقد أعطى الإردوستين ٢٤ ساعة قبل الحقن بالسيسبلاتين واستمر حتى إنتهاء التجربة. ثم تم تخدير جميع الجرذان بإستخدام الإثير بعد ستة أيام من حقن جرعة السيسبلاتين و شق الصفن و إستئصلت الخصي و تم تجهيزها للفحص بكل من المجهر الضوئى والإلكترونى الماسح.

وقد أظهر فحص عينات المجموعة المعالجة بالسيسبلاتين تلفا شديدا بالخصي مع نقص كبير ذو دلالة إحصائية فى قطر الأنيبيبات المنوية و فى سمك النسيج الطلائى الجرثومى مقارنة بنظيرتهم فى المجموعة الضابطة، كما لوحظ إنكماش بعض الإنيبيبات، و فقدان لبعض الخلايا المنوية مع تغيرات الموت المبرمج لمعظم الخلايا خاصة الخلايا المنوية الأولية و سلف النطيفة المستدير. أما الحيوانات المنوية الناضجة، فقد ظهر نقص واضح فى عددها مقارنة بالمجموعة الضابطة مع تشوه الحيوانات المنوية المتبقية. ولوحظ أيضا زيادة الإستسقاء البينى مع نقص ظاهر فى عدد خلايا ليدج. أما العلاج بالإردوستين قبل السيسبلاتين فقد أدى إلى تحسن المظهر العام للخصي. فبالرغم من التغيرات التحللية فى بعض الخلايا المنوية فقد قل فقدان الخلايا بدرجة كبيرة، كما كانت معظم تجاويف الأنيبيبات مشغولة بالحيوانات المنوية الناضجة كما لوحظت زيادة فى عدد خلايا ليدج مع نقص واضح فى الإستسقاء البينى. ومن هذا يمكن إستنتاج أن الإردوستين يستطيع أن يحمى خصي الجرزان بشكل جزئى من التأثير السمى للسيسبلاتين على الأرجح عن طريق تأثيره القوي المضاد للأكسدة والمزيل للجزيئات الشاردة.