Dept. of Anatomy
Fac. of Medicine, Assiut University.
Head of Dept. Prof. Dr. R.S. Mohammed.

QUANTITATIVE AND QUALITATIVE STUDIES ON THE SEMINIFEROUS EPITHELIUM OF ALBINO RATS UNDER THE EFFECT OF CYTOTOXIC DRUGS

(With 2 Tables & 26 Fig. and 3 Diagrams)

By

A.T. GALAL; A.M.M. HASSANEIN*
NAGWA M.EL-SAWI**; MADIHA M. MOHAMMED***
and SOAD S. ALI***

(Received at 2/2/1994)

دراسات نوعيه وكميه لتأثير بعض أدوية السرطان على النسيج الطلائى للانيبيبات المنويه فى الفأر الأبيض

أحمد جلال ، عبد الفتاح حسنين ، نجوى الصاوى
مديحه محمد ، سعاد على

أجرى هذا البحث لدراسة تأثير السيسبلاتينيم ، الميثوتركسات والاندوكسان على النسيج الطلائى للانيبيبات المنويه فى الفأر الأبيض .

أظهرت النتائج أن عقار السيسبلاتينيم ليس له تأثير واضح على كل من تركيب الانيبيبات المنويه ومستوى هرمون التيستستيرون فى الدم .

أدى حقن عقاري الميثوتركسات والاندوكسان الى ضمور فى حجم الانيبيبات المنويه ونقص عدد خلاياها الجرثوميه التى ما فتئت أن اعتراها تغيرات تراجعيه .

كما أدى حقن هذين العقارين إلى إنخفاض واضح فى مستوى هرمون التيستستيرون فى الدم .

*: Zoology Dept., Faculty of Science, Assiut University.
**: Chemistry Dept., Faculty of Science, (Sohag), Assiut University.
***: Histology Dept., Faculty of Medicine, Assiut University.

SUMMARY

The present work aims to study the qualitative and quantitative changes of the rat's seminiferous epithelium after administration of three commonly used cytotoxic drugs; Cisplatinum, methotrexate and endoxan. Treatment with cisplatinum revealed no remarkable changes in both the seminiferous epithelial elements and the serum testosterone level. Administration of endoxan and methotrexate led to severe lesions. The seminiferous tubules were markedly decreased in size and perimeter. The germ cells underwent degeneration, necrosis and dysfunction represented by a pronounced serum testosterone withdrawal.

INTRODUCTION

The cytotoxic drugs are group of drugs used in the treatment of malignant diseases. All of these drugs are toxic to both normal and tumour cells *(CLIFFORD, 1979)*. These drugs may cause severe toxicity to different body organs and tissues *(CALABRESI and GHABNER, 1991)*. The most affected tissues are bone marrow, liver, kidney and testis *(CARTER, 1975)*. Ovarian as well as testicular damage and dysfunction by cytotoxic drugs were observed by *CHAPMAN (1982) and OOMMEN* et al *(1991)*.

In various studies on the seminiferous tubules of mammals, two distinct trends have emerged in the choice of criteria for the identification of the stages. The first method used the development of the acrosomic system of spermatids. This method provided classifications with larger number of stages *(CLERMONT, 1960, L962 & 1972 and CLERMONT and LEBLOND, 1953 & 1955)*. The other method was based on the nuclear morphology of spermatids simultaneously with the position of the more mature spermatids within the seminiferous epithelium *(ROOSEN-RUNGE, 1962 and ORTAVANT* et al., *1977)*. In such classifications eight stages in rat *(ROOSEN-RUNGE, 1962; AND MORSI* et al., *1985)* and mice *(HASSANEIN and MOHAMMED, 1991)* were recognized.

The present work aimed to study the quantitative and qualitative changes that occur in the rat testes under the effect of three commonly used cytotoxic drugs; Cisplatinum, Methotrexate and Cyclophosphamide; Endoxan. Biochemical evaluation of the serum testosterone levels were estimated at different treatments with these cytotoxic drugs.

MATERIAL AND METHODS

Histological Study:

Twenty-four adult male albino rats of nearly the same age, weighing from 120 to 180 g. were used. The animals were divided into four equal (six animals each) groups. The first group received Methotrexate in a dose of 0.5 mg/rat. The second group received Cisplatinum in a dose of 0.69 mg/rat. The third group received Endoxan in a dose of 1.0 mg/rat. The fourth group received normal saline and was kept as control. The dose for each drug was calculated according to the table designed by *PAGET and BARNES (1964).* Each group received only one drug daily for six successive days. The drugs were administered via the intraperitoneal route by a tuberculin syringe. At the end of the course of treatment the animals were scarificed by cervical dislocation. Control and experimental animals were dissected and specimens of testes were removed and fixed in formol-alcohol-glacial acetic acid solution, dehydrated in ascending series of ethyl alcohol, cleared in xylol and embedded in paraffin wax. Paraffin sections were cut at 7 μ thickness and stained with haematoxylin & eosin and PAS-haematoxylin according to *DRURY and WALLINGTON (1980).*

The identification of the various stages of the seminiferous epithelial cycle, in the present investigation, was based on the morphological changes of the germ cell nuclei and the local arrangement of the spermatids. The perimeter of the seminiferous tubules was measured in PAS-haematoxylin stained sections with digitizer of IBM-computer.

Fourty-eight rounded cross sections of the seminiferous tubules from each testicle representing all the stages of the cycle were examined. The number of all various types of cells (spermatogonia, primary and secondary spermatocytes, spermatids and Sertoli cells) occupied the seminiferous tubules was counted.

The spermatogonia were classified into type-A and type-B. The intermediate type was counted together with type A. The preleptotene and leptotene phases were also counted together. The diakinesis was counted together with the diplotene.

The percentage of cross sections of the tubules at a given stage of the seminiferous epithelial cycle was taken as the relative frequency of this stage.

The data were statistically analysed. Analysis of variance was used at 0.05 level of significance for the number of cells of the seminiferous epithelial cycle according to *SIMPSON* et al. *(1960).*

The graphics of the data were computerized.

Biochemical Study:

Blood samples from all groups were collected directly from the heart for determination of serum testosterone using I^{125} radioimmuno assay kit (Coat-A-Count) supplied by DPC'S diagnostic products corporation, Los Angeles, California.

RESULTS

Histological Findings:

The seminiferous tubules of control rats are lined with spermatogenic cells which is highly modified stratified cuboidal epithelium. The seminiferous epithelium contains two distinct categories of cells; Sertoli cells and the germ or spermatogenic cells.

Eight stages of the seminiferous epithelial cycle were recognized (Fig. 1-8). These stages are characterized by:

Stage (1): Predominant types A & B spermatogonia, leptotene and pachytene phases of primary spermatocytes and one generation of rounded spermatids.

Stage (2): Abundant type A-spermatogonia, zygotene and pachytene spermatocytes and ovoid spermatids.

Stage (3): Appearance of diplotene and few diakinesis primary spermatocytes. The zygotene phase is still present. The elongated spermatids are arranged in bundles.

Stage (4): Presence of zygotene spermatocytes, secondary spermatocytes with rounded nuclei; each nucleus contains about six chromatin particles, and bundles of elongated spermatids.

Stage (5): Presence of type A spermatogonia, zygotene sperm- atocytes and two generations of spermatids; young rounded spermatids and bundles of elongated spermatids.

Stage (6): Type B-spermatogonia appeared in this stage and are predominating beside type A spermatogonia. Pachytene, rounded spermatids and bundles of elongated spermatids are present.

Stage (7): Presence of types A & B spermatogonia and pachytene spermatocytes. The rounded and the elongated spermatids are arranged centripetally around the circumference of the seminiferous tubules.

Stage (8): Presence of types A & B spermatogonia, pachytene, rounded spermatids and mature spermatozoa.

The perimeter of the seminiferous tubules and the average number of the cells of seminiferous epithelial cycle are shown in Table 1 and Fig. 27 and 29. The frequency of the eight stages of the seminiferous epithelial cycle is shown in Fig. 28.

In treated animals with methotrexate, the size of the tubules was smaller than that of control (Fig.23 and 24). Few degenerated cells and necrotic areas were noticed in the tubules; and the nuclei of most of the germ cells appeared pyknotic . A significant decrease in the perimeter of the seminiferous tubules was observed (Table 1 and Fig. 29). A significant decrease in the number of Sertoli cells and a significant increase in the number of type B-spermatogonia, Zygotene, oval and elongated spermatids was noticed (Table 1 and Fig. 9-11 and Fig. 27). A remarkable increase in the frequency of stage I was observed as compared with control (Fig. 28).

Treatment with cisplatinum induced no remarkable changes compared to that of the control except that the lumen of some seminiferous tubules contained degenerated cells (Fig. 25). All the cellular stages of the seminiferous epithelial cycle were nearly the same as in the control rats (Fig. 12-15). The perimeter of the seminiferous tubules and the average number of cells of the seminiferous epithelial cycle are illustrated in Table (1) and Fig. 27,29. The number of type B spermatogonia was significantly decreased. The frequency of all stages is shown in Fig. 28. An increase in the frequency of stage I was noticed comparing with control.

After treatment with endoxan, the tubules were markedly decreased in size (Fig. 26). Many germ cells underwent various degenerative changes (Fig. 16-22). A highly significant decrease in the perimeter of the semini- ferous tubules was observed (Table 1, Fig. 29). The average number of cells of the seminiferous epithelial cycle is shown in (Table 1 and Fig. 27). A significant decrease in the number of Sertoli cells was observed. The frequency of all stages of the cycle was illustrated in Fig. 28.

Biochemical Analysis:

The total testosterone level was significantly decreased in the treated rats $P < 0.001$ (Table, 2) and (Fig. 29). This reduction in hormonal level was markedly pronounced for Endoxan, and minimal for Cisplatinum.

DISCUSSION

The eight stages of the seminiferous epithelial cycle observed in the testes of control albino rats are similar to those mentioned by *ROOSEN - RUNGE (1962); SWIERSTRA & FOOTE (1963); ORTAVANT (1977); MORSI ET AL. (1985) and HASSANEIN & MOHAMMED (1991).*

The cellular elements of all stages of the semini- ferous epithelial cycle of rats treated with cisplatinum is nearly the same as in control. Also, this drug produced minimal serum testosterone withdrawal. In line with these findings were the observations of *HANDELSMAN et al. (1988).* On the other hand, treatment with methotrexate and endoxan showed prominent lesions in the seminiferous tubules with degeneration of most germ cells. Sertoli cell number showed a significant decrease, while type B spermatogonia showed a significant increase in number. Although there is no significant difference between the number of Sd spermatids of control and treated animals with the different cytotoxic drugs, the sperms in treated animals appear morphologically abnormal and unhealthy.

In agreement with the present testicular damaging effect of methotrexate and endoxan were the previous observations of *CHAPMAN (1982) and MEISTRICH et al. (1982).* The latter added that these drugs did not show significant stem cell killing.

The present results could be supported by the fact that methotrexate and endoxan disrupted the function of the hypothalamic-hypophseal-gonadal axis; they decreased FSH level by suppressing the release of FSH-releasing hormone from the hypothalamus resulting in the regression of spermatogenesis and reduction in testosterone secretion as were reported by *KERR et al. (1986) and RISBRIDGER et al. (1989).*

The drastic effect of methotrexate and endoxan could be also attributed to their action as an alkylating agents on DNA cross-link *(RICHTER et al., 1970)* and hence interfere with the integrity of the germ cell elements leading to their degeneration *(FARMER, 1987).* Testicular degeneration could be secondary to the toxic effect of drugs upon the epithelial cells of the blood vessels of the testes *(CALABRESI and GHABNER, 1991). EL-KADY et al. (1993)* added that the main action of methotrexate is upon cell metabolism and cell enzymes.

Moreover, *RICHTER et al. (1970)* decided that the damaging effect of endoxan and methotrexate to mature and immature germ cell elements rendering to irreversible azoospermia and infertility. *LENTZ et al. (1977)* emphasized that a "Safe" regimen to endoxan treatment has not been established for either immature or mature gonad.

The toxic effect of methotrexate and endoxan on the testicular function is similar, to some extent, to those produced by opiates such as heroin and morphine *(MENDELSON et al., 1975; EL-HOSSINY et al., 1988 and HASSANEIN and MOHAMMED, 1991).* It may be stated that these cytotoxic drugs destroy some

Leydig cells leading to the suppression of testosterone secretion.

From the aforementioned effects of the cytotoxic drugs on testes, it would be recommended to restrict their use as can as possible.

REFERENCES

Calabresi, P. and Ghabner, A. (1991): Chemotherapy of Neoplastic diseases, In: (Godman and Gilman ed.) "The pharmacological basis of therapeutics" . Pergman press 8th edition.

Carter, C. (1975): Cited from Clif D.P. (1979): The role of cytotoxic drugs in the surgical management of head and neck malignancies. J. Laryng. Otology 93: 1151-1180.

Chapman, R.M. (1982): Effect of cytotoxic therapy on sexuality and gonadal function. Semin-oneol., 9(1): 84-94.

Clermont, Y. (1960): Cycle of the seminiferous epithelium of the guinea pig. A method for identification of the stages. Fertility and Sterility. 11: 563-573.

Clermont, Y. (1962): Quantitative analysis of spermatogenesis of the rat: a revised model for the renewal of spermatogonia. Am. J. Anat., 111: 111-129.

Clermont, Y. (1972): Kinetics of spermatogenesis in mammals: Seminiferous epithelium cycle and spermatogonia renewal. Physiol. Rev., 52 (1):198-236.

Clermont, Y. and Leblond, C.P. (1953): Renewal of spermatogonia in the rat. Am. J. Anat., 93:475-502.

Clermont, Y. and Leblond, C.P. (1955): Spermiogenesis of man, monkey, ram and other mammals as shown by the "Periodic acid-Schiff" technique. Am. J. Anat., 96: 229-253.

Clifford, P. (1979): The role of cytotoxic drugs in the surgical management of head and neck malignancies. J. Laryng. and Otology, 93, 1151-1180.

Drury, R.A.B. & Wallington, E.A. (1980): Carleton's histological technique, Oxford, New York, Toronto, Oxford University Press.

El-Hossiny, K.M.; Seliem, S.M. and Ali, S.S. (1988): The effect of chronic toxicity of morphine and propoxyphene on testicular functions. Zagazig first annual conference on medical Physiology, pp. 83-96.

El-Kady, M.M.; Al-Hussaini, A.A.; Mohammed, M.M. and Saad, M. (1993): Ototoxicity of three commonly used cytotoxic drugs. Assiut. Med. J. 17(2): 11-23.

Farmer, P.B. (1987): In (Tlaskell C.M. ed.). "Cancer treatment". Saudnders W.B. Company 3rd ed. 1990.

Handelsman, D.J.; Peng, S. and Sikka, S. (1988): Effect of gonadotropin-releasing hormone analogs on Cisplatinum induced spermatogenic damage. Int. J. Androl., 11(5), 425-435.

Hassanein, A.M.M. and Mohammed, M.M. (1991): Quantitative studies on the effect of morphine on the testicular structure of the albino mice. Bull. Fac. Sci.; Assiut Univ., 20(1-E); 183-198.

Kerr, J.B.; Bartlett, J.M. and Donachie, K. (1986): Acute response of testicular interstitial tissue in rats to the cytotoxic drug ethan dimethanesulphonate. An ultrastructural and hormonal assay study. Cell Tissue Res.,243 (2): 405-414.

Lentz, R.D.; Steffes, M.W. and Michael, A.F. (1977): Postpubertal evaluation of gonadal function following cyclophosphamide therapy before and after puberty. J. Ped., 91(3), 385-394.

Meistrich, M.L.; Finch, M. and Da-lunha, M.F. (1982): Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. Cancer Res. 42(1), 122-131.

Mendelson, J.H.; Mendelson, J.E. and Patch, V.D. (1975): Plasma testosterone levels in heroin addiction and during methadone maintenance. J. Pharmacol. Exp. Ther., 192:211-217.

Morsi, G.A.; Selim, A.A. and Hassanein, A.M. (1985): The effect of acute cold on the testis of the albino rat. Assiut Med. J., 9(3): 494-516.

Oommen, R.; Jose, C.C. and Vishwanathan, F. (1991): Ovarian and testicular damage by cytotoxic drugs. Indian J. Cancer 28(3): 119-123.

Ortavant, R.; Court, M. and Hochereau de Reviers, M.T. *(1977):* Spermatogenesis in domestic animals. In: "Reproduction in domestic animals" (eds. H.H. Cole and P.T. Cupps). Academic Press, New York.

Paget and Barnes (1984): Evaluation of drug activities pharmacemetrics, laurence and Bacharach Nol. 1, Academic Press, New York.

Richter, P.; Calamera, J.C. and Morgenfeld, M.C. (1970): Effects of chlorambucil on spermatogenesis in the human with malignant lymphoma. Cancer, 25: *1026-1303.*

Risbridger, G.; Kerr, J. and De-Kretser, D. (1989): Differential effects of the destruction of Lydig cells by administration of ethanedime thane sulphonate to postnatal rats. Biol. Reprod., 40(4), 801-809.

Roosen-Runge, E.C. (1962): The process of spermatogenesis in mammals. Biol. Rev., 37:343-377.

Simpson, G.G.; Roe, A. and Lewontin, R.C. (1960): Quantitative Zoology. New York, Harcourt, Brace & World. Inc.

Swierstra, E.E. and Foote, R.H. (1963): Cytology and kinetics of spermatogenesis in the rabbit. J. Reprod. Fertility, 5:309-322.

Legends

Figs. (1-8): T.S. of testis of control rat showing the seminiferous tubules in different stages of spermatogenesis. (H & E. X 400).

Figs. (9-11): T.S. of testis treated with methotrexate showing an increased number of type B-spermatogonia (B) and a decreased number of Sertoli cells (S). (H & E. X 400).

Figs. (12-15): T.S. of testis treated with cisplatinum showing that the cellular stages of the seminiferous epithelial cycle is nearly the same as in the control. (H & E. X 400).

Fig. (16-22): T.S. of testis of rat treated with endoxan showing degeneration in the different stages of the cycle. (H & E. X 400).

Fig. (23): Seminiferous tubules of a control rat. (H & E.X 100).

Fig. (24): Seminiferous tubules of a rat treated with methotrexate. (H & E. X 100).

Fig. (25): Seminiferous tubules of a rat treated with cisplatinum (H & E. X 100).

Fig. (26): Seminiferous tubules of a rat treated with endoxan (H & E. X 100).

Table (1):Average number of cells of seminiferous epithelial cycle and the mean perimeter of the seminiferous tubules of albino rats at different treatments with cytotoxic drugs

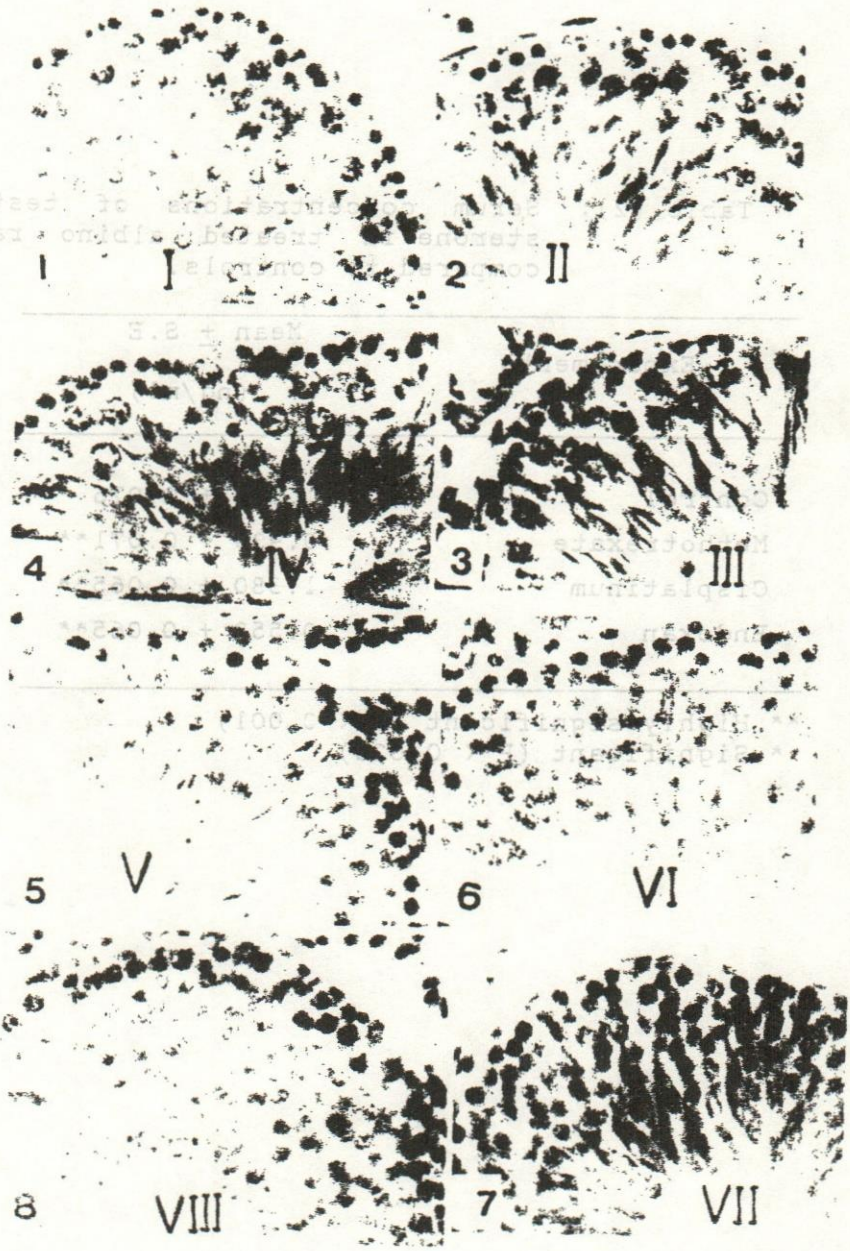
Number	Experiments	Sertoli cells	Spermatogonia			Primary Spermatocytes				Secondary Spermatocytes	Spermatids				Perimeter of Seminiferous tubules	t
			Type "A"	Type "B"	Total	Preleptotene	Zygotene	Pachytene	Diplotene & Diakinase		Sa	Sb	Sc	Sd		
(1)	Control	83.0±1.63	117.17 ± 10.02	184.66 ± 8.16	301.83 ± 15.58	9.5±0.67	266.83 ± 14.79	336.00 ± 33.59	66.0 ± 5.69	52.83 ± 5.5	883.67 ± 46.97	125.83 ± 6.31	387.33 ± 33.15	534.0 ± 24.71	1.351 ± 0.051	
(2)	Methotrexate	44.0±1.56*	142.17 ± 3.73	313.33* ± 9.45	455.5* ± 12.21	7.67±0.42	443.5* ± 11.86	401.83 ± 10.58	87.33 ± 2.33	38.00 ± 1.69	1427.00* ± 27.19	215.0* ± 6.55	664.17* ± 17.86	505.83 ± 27.09	0.927 ± 0.034	3.89*
(3)	Cisplatinum	82.0±5.39	151.83 ± 3.56	58.0* ± 2.62	209.83* ± 4.62	9.67±1.76	266.67 ± 4.05	362.17 ± 20.80	70.83 ± 2.52	46.33 ± 4.08	995.00 ± 77.50	105.83 ± 7.89	576.0* ± 32.53	468.5 ± 19.01	1.241 ± 0.033	1.02
(4)	Endoxan	45.0±1.29*	105.33 ± 5.14	236.16* ± 9.40	342.00 ± 11.53	6.67±0.33	328.33 ± 10.66	482.0* ± 12.89	93.0* ± 2.18	25.33* ± 1.47	1081.00 ± 51.71	227.16* ± 8.94	493.83 ± 20.48	662.5 ± 38.29	0.830 ± 0.011	5.66**
Significant difference between		No. 1 & 2 No. 1 & 4	—	No.1&2 No.1&3 No.1&4	No.1&2 No.1&3	—	No.1&1	No.1&4	No.1&4		No.1&4	No.1&2	No.1&2 No.1&4	No.1&2 No.1&3	—	No.1&2 No.1&4	

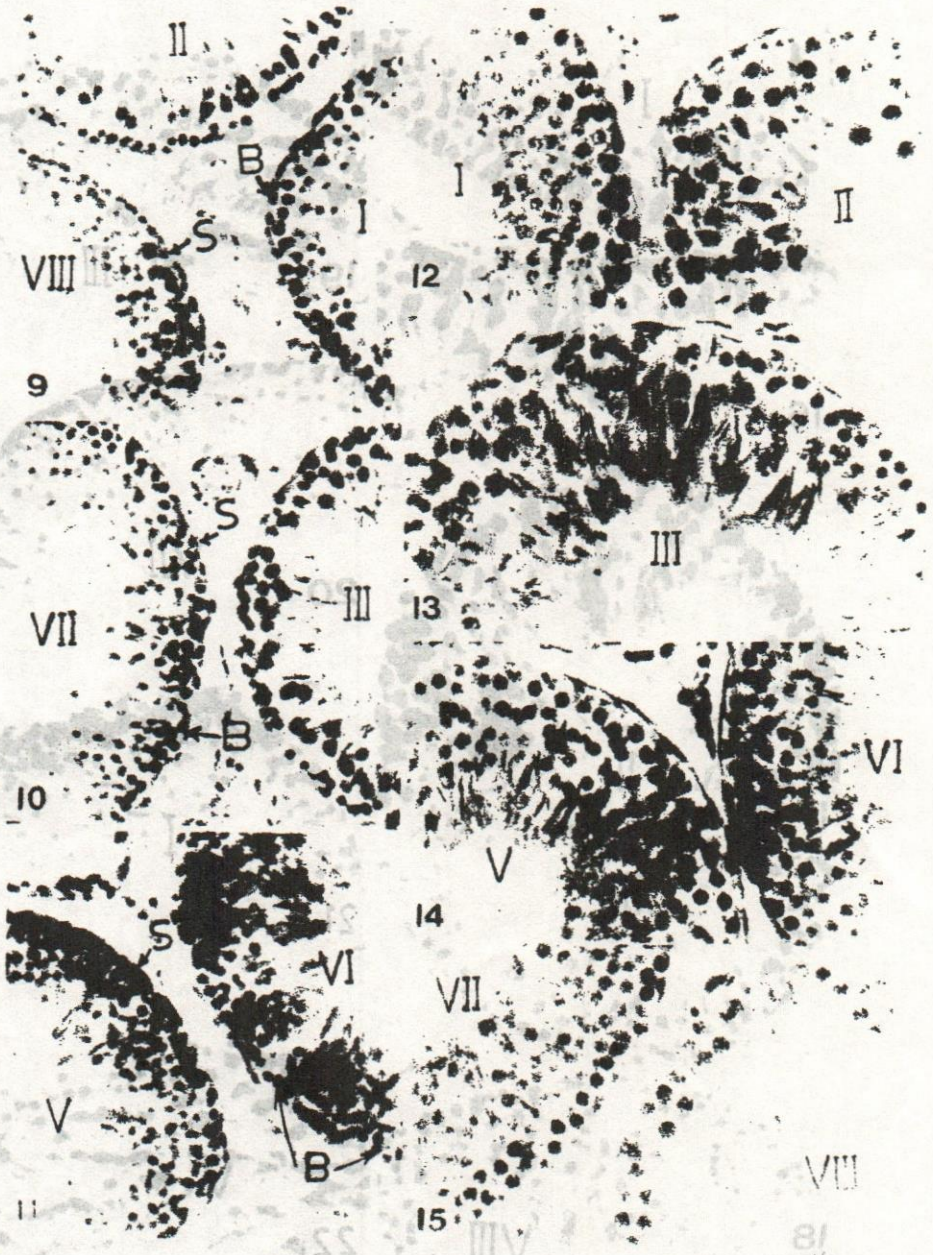
** Highly significant (P < 0.001)
* Significant (P < 0.025)

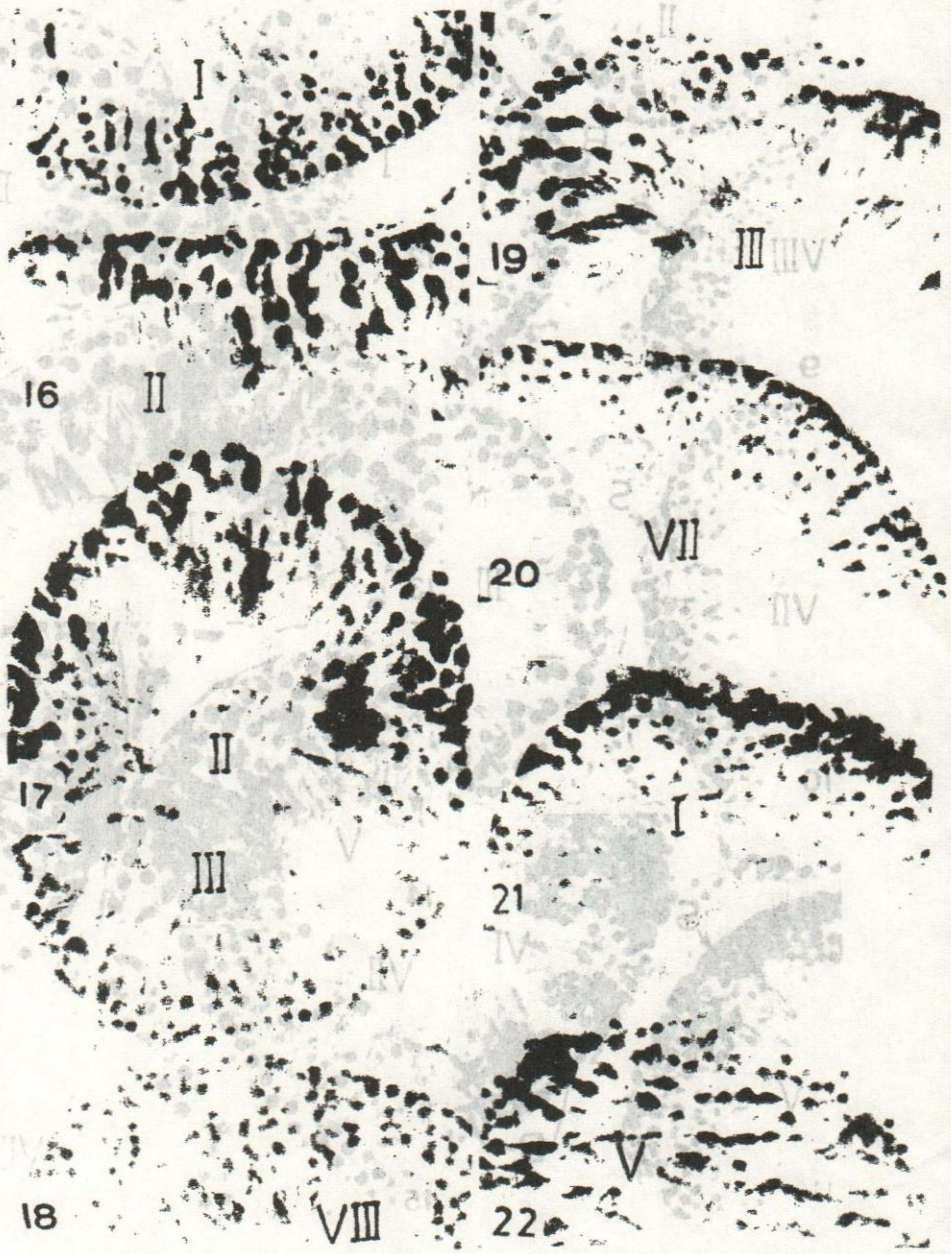
Table (2): Serum concentrations of testosterone in treated albino rats compared to controls.

Experiments	Mean ± S.E (ng/ml)
Control	1.570 ± 0.075
Methotrexate	0.939 ± 0.071**
Cisplatinum	1.380 ± 0.065**
Endoxan	0.552 ± 0.065**

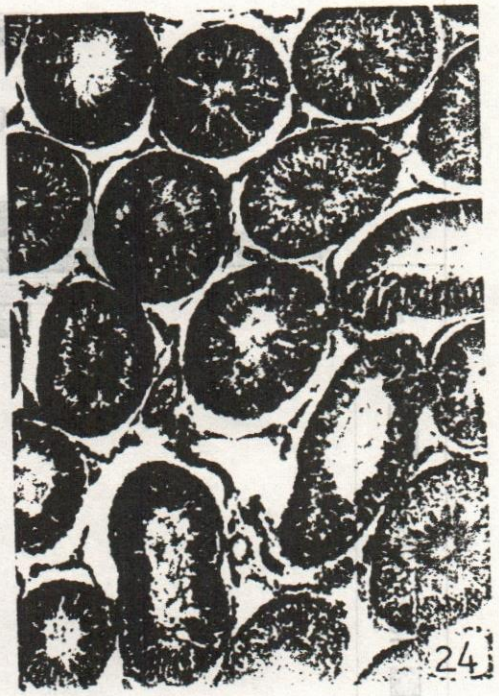
** Highly significant ($P < 0.001$)
* Significant ($P < 0.025$)

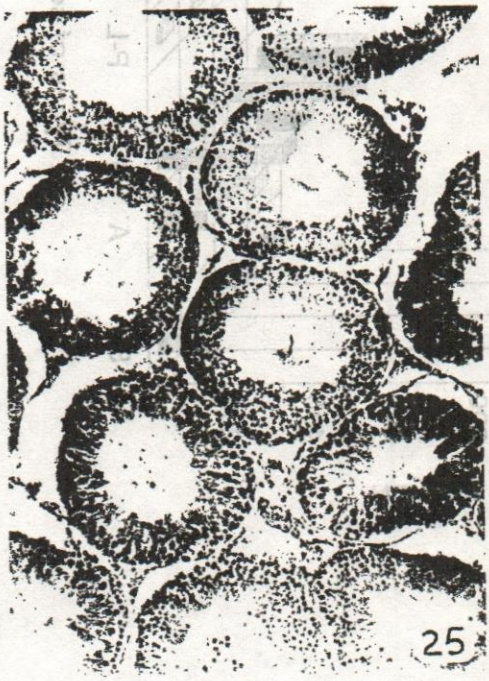

1
I
2
II
4
IV
3
III
5
V
6
VI
8
VIII
7
VII

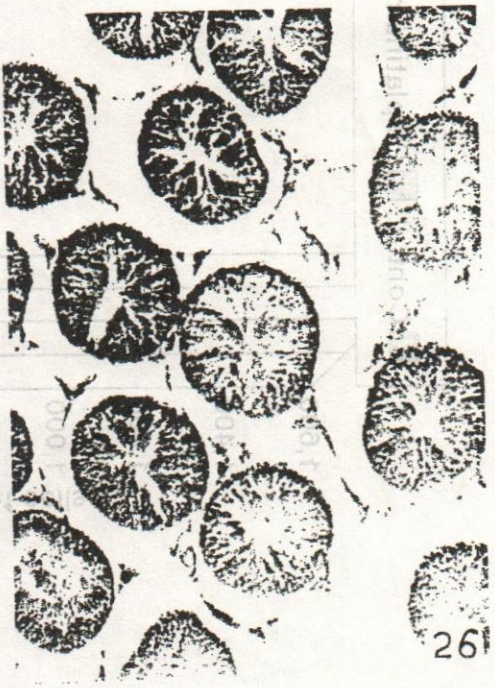

II
B
I
S
VIII
9
S
III
VII
B
10
S
VI
V
B
11
I
II
12
III
13
VI
V
14
VII
VIII
15


I
19
III
16
II
VII
20
II
17
I
III
21
18
VIII
22
V


23


24


25


26

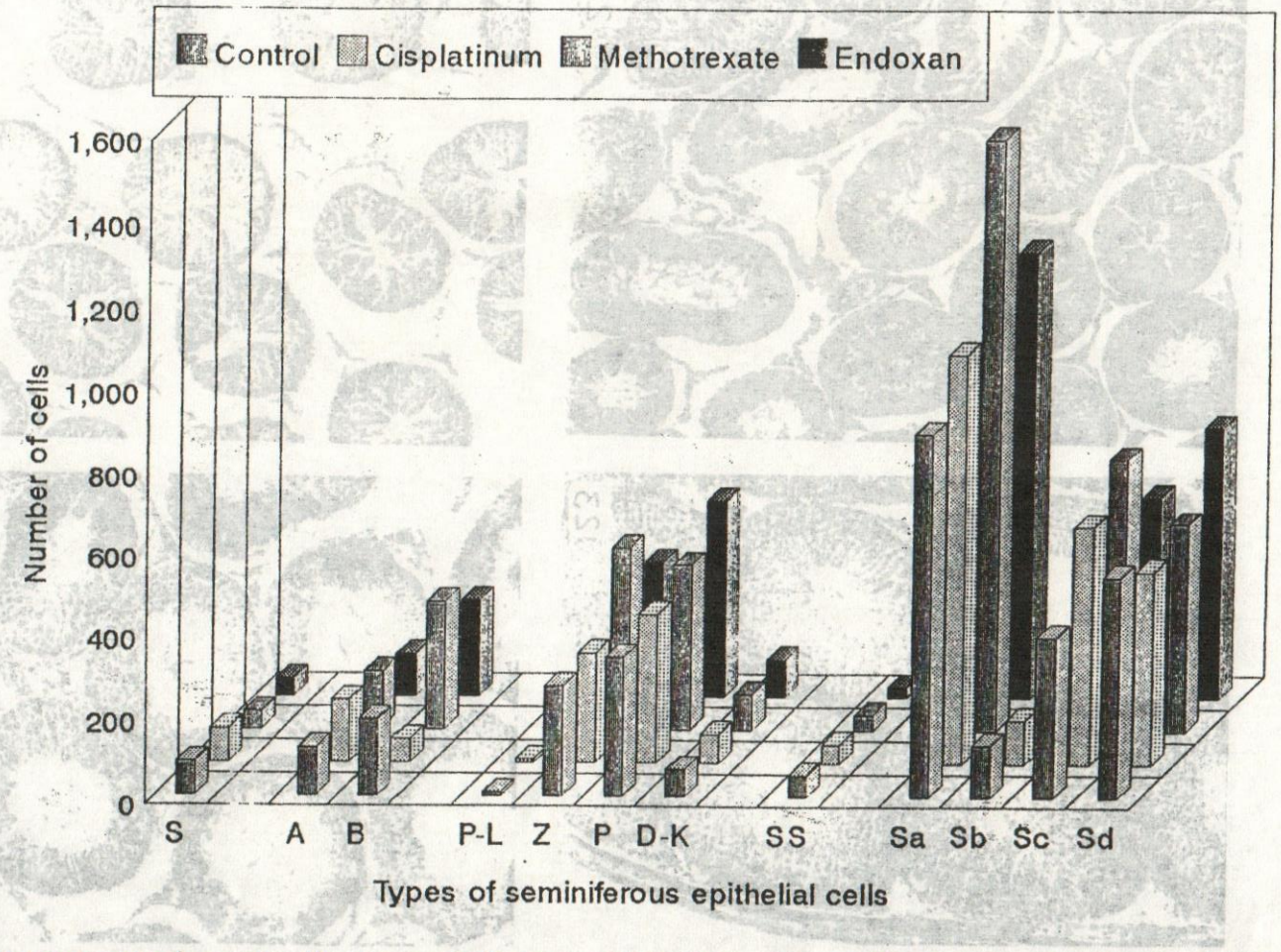


Fig.27.Average number of cells of seminiferous epithelial cycle of albino rats at different treatments with cytotoxic drugs(S,Sertoli cell;A,B,Spermatogonia;P-L,Z,P,D-K,Primary Spermatocytes;SS,Secondary Spermatocytes;Sa,Sb,Sc,Sd,Spermatids).

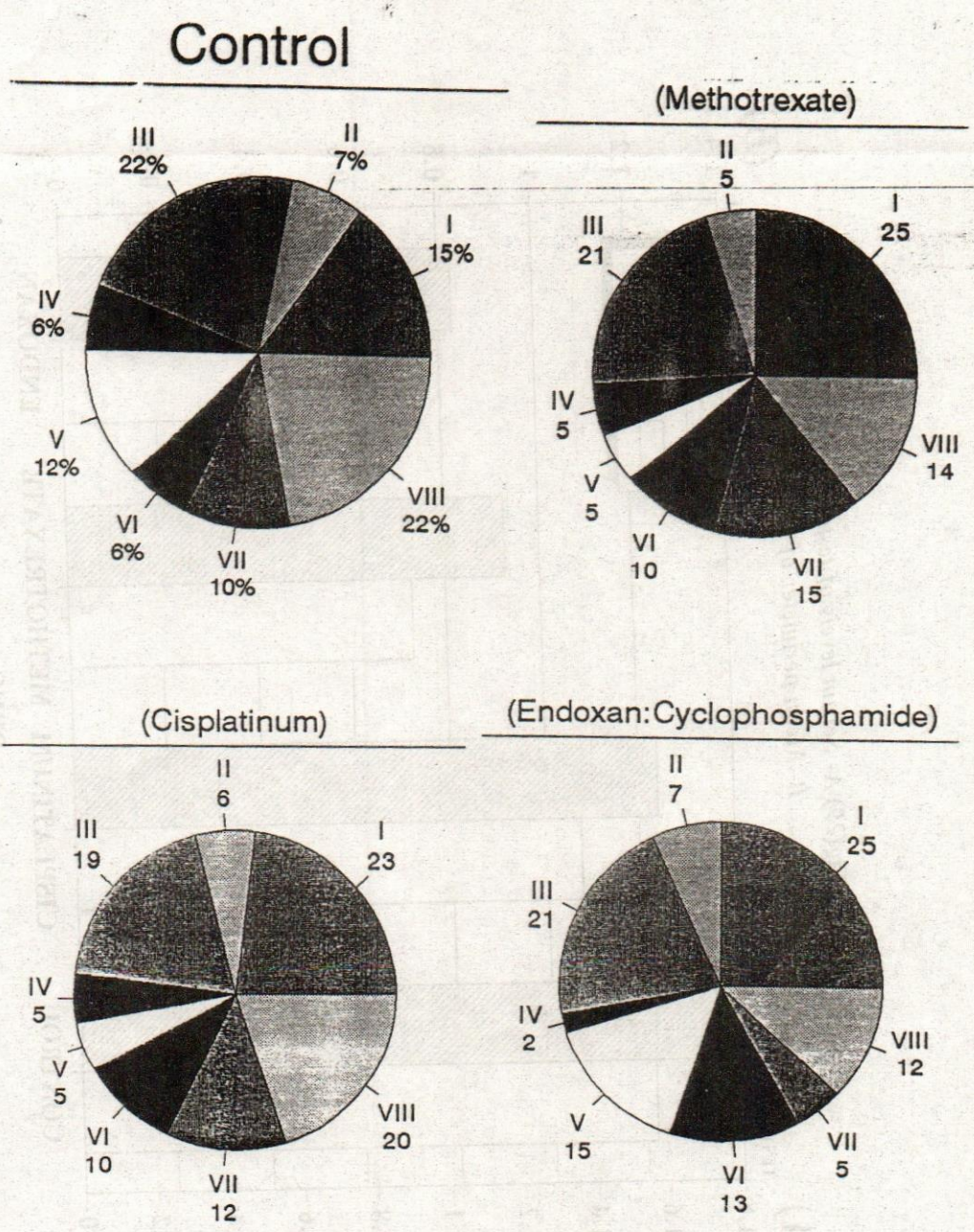
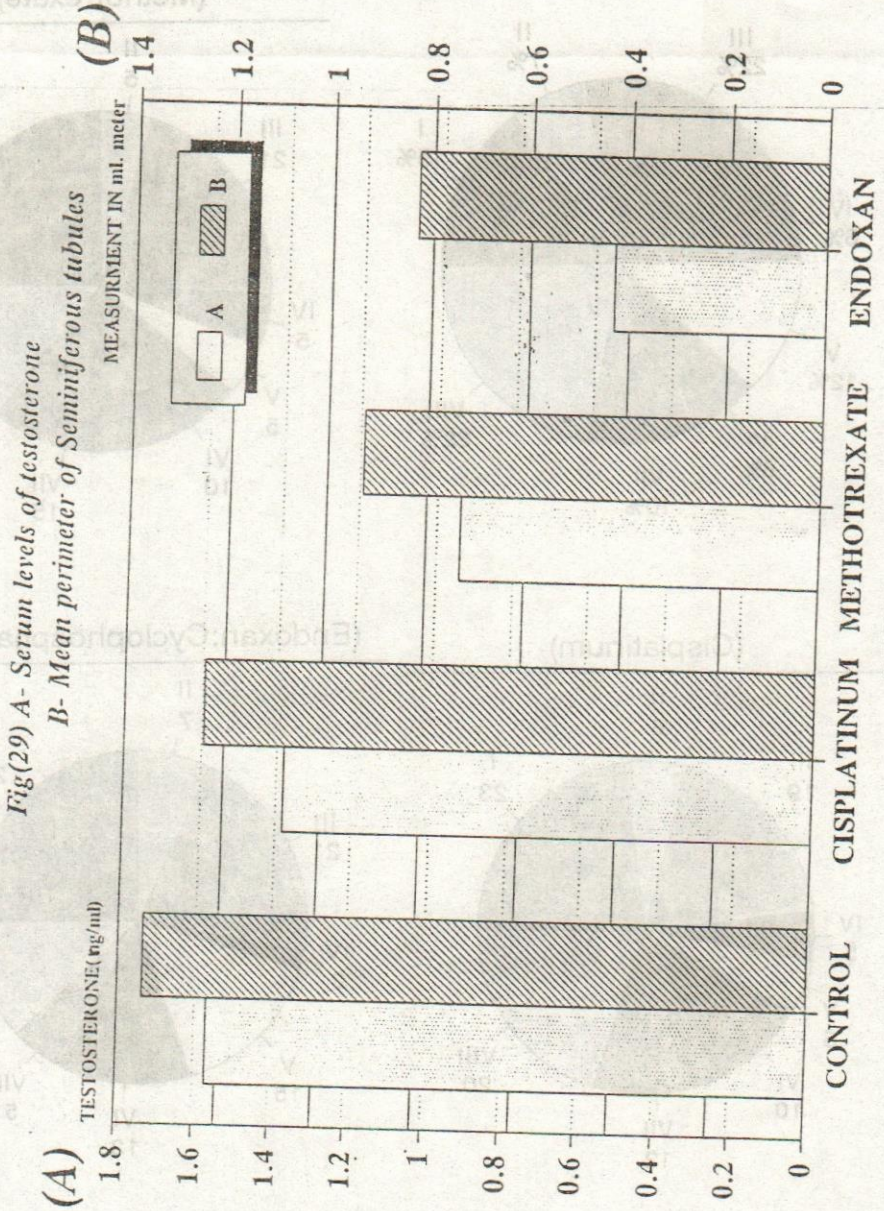


Fig.28:Frequency of the stages of the seminiferous epithelial cycle of albino rats at different treatments with cytotoxic drugs.



Fig(29) A- Serum levels of testosterone
B- Mean perimeter of Seminiferous tubules