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Light and Electron Microscopic Studies of the Effect of Tamoxifen on the Testes of Male Mice

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Abstract: The effect of oral administration of tamoxifen (a synthetic non-steroidal antiestrogen) at doses of 20 and 40 mg/kg/day for 60 days on the potency, the fecundity, the circulating concentrations of both Luteinizing hormone (LH) and testosterone and the ultrastructural changes of the male testicular tissues of mice were determined. Forty mg/kg/day tamoxifen was more effective than 20 mg/kg/day in reducing the male mice's ability to inseminate the female (potency), as well as its siring ability (fecundity). Both doses of tamoxifen had reduced the concentrations of testosterone in plasma while circulating LH levels were significantly increased in the blood of mice taken 40 mg/kg/day tamoxifen comparing to those taken 20 mg/kg/day and the control. Light microscopic results showed that sections of mice testes treated with tamoxifen revealed the presence of severely damaged and entirely normal seminiferous tubules adjacent to one another in the same section. However, most sections of mice testes showed disorganization of cytoarchitecture arrangement of seminiferous tubules with obliterated lumen. This was accompanied by an obvious occurrence of vacuolated tubules in most sections of these testes. 40 mg/kg/day tamoxifen had devoid the mature sperm in most tubules and the reduction in Leydig interstitial cells was parallel to the decrease in testosterone levels. At the electron microscopic level, wide intercellular vacuolation between the spermatogenic cells and large numerous cytoplasmic vacuoles in most spermatids were seen. Clear sign of karyolysis and karyorrhexis in degenerating spermatocytes were observed. The Sertoli cells in a number of sections had an increased electron density with marked dilated smooth endoplasmic reticulum and numerous dense bodies were dispersed throughout the cytoplasm. Thus, the study supports that the tamoxifen as a potent antiestrogen, has a direct effect in inhibiting the testosterone synthesis and hence altered spermatogenesis and testicular morphology at low and high doses. Thus, it can be used as a potential contraceptive agent.

Key words: Tamoxifen, male mice, testes

Introduction

The biological effects of tamoxifen, a synthetic nonsteroidal antiestrogen, are complex and range from complete oestrogen antagonism to pure oestrogen agonism depending on its concentration, the sex of the animal and the target organ. In humans and rats, tamoxifen is predominantly antioestrogenic with residual oestrogenic activity (Furr and Jordan, 1984). In the mice, tamoxifen appears to act as a pure estrogen (Harper and Walpole, 1966).

Tamoxifen is used extensively in the treatment of breast cancer (Jordan and Murphy, 1990) as well as it has been recommended for the treatment of oligozoospermia (Willis *et al.*, 1977; Vermeulen and Comhaire, 1978; Noci *et al.*, 1985; Ain-Melk *et al.*, 1987) and gynaecomastia (Parker *et al.*, 1986). Although, the contention that tamoxifen with its intrinsic oestrogen antagonist-against activities may interfere with male fertility is reasonable, many authors have postulated that tamoxifen possibly be developed into a male contraceptive agent (Gill-Sharma *et al.*, 1993; Gopalkrishnan *et al.*, 1998; Nakai *et al.*, 1999; Parte *et al.*, 2000). They found that oral administration of tamoxifen at dose levels of 40, 200 and 400 µg/ Kg/day for 90 days had reduced the fecundity, potency and the fertility index of male rats. They added that the testes of such rats revealed disorganization of the cytoarchitecture of seminiferous tubules with obliteration of their lumen. Also, Nakai *et al.* (1999) had reported that neonatal mice exposure to tamoxifen causes various abnormalities of the male reproductive organs in postpubertal mice, depending on the strains.

It is well documented that Leydig and Sertoli cell function is intimately involved with the initiation and maintenance of spermatogenesis. The major stimulus for their function involves the pituitary gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Among other functions, the sertoli cell establishes a "blood-testis" barrier by forming tight junction between opposing sertoli cell membranes which prevents toxic metabolites from entering the seminiferous tubule (Dyn and Fawcett, 1970).

Worthy to mention that, secretion of testosterone hormone by Leydig cells is under the influence of the anterior pituitary hormone, luteinizing hormone (LH). Steinberger (1971) had postulated that luteinizing hormone (LH) and testosterone are responsible for normal spermatogenesis in rat.

Many studies had reported that 40 and 200 µg/kg/day tamoxifen lower the concentration of testosterone in plasma of male rats (Gill-Sharma *et al.*, 1993; Gopalkrishnan *et al.*, 1998; Parte *et al.*, 2000); while only 200 µg tamoxifen had significantly decreased LH levels. However, plasma concentrations of LH and testosterone are increased in oligospermic patients responding to tamoxifen therapy (Willis *et al.*, 1977; Vermeulen and Comhaire, 1978; Noci *et al.*, 1985; Ain-Melk *et al.*, 1987).

The study reported here attempts to collate the effect of two different doses of tamoxifen, a potent antiestrogen on the profile of reproductive performance of male mice. Also, the histomorphologic and ultrastructural changes in seminiferous tubules of testes of these animals

were studied. The reproductive hormones, Luteinizing and testosterone hormones were tested in the blood of these animals.

Materials and Methods

This study was designed to evaluate the effect of two different dose levels of tamoxifen on the structure and the profile of the reproductive hormones of testes of male mice. Tamoxifen citrate tablets containing 10 mg were uniformly suspended in distilled water and administered daily via a bent stainless steel feeding tube at two different doses of 20 and 40 mg kg^{-1} body weight. The dose of tamoxifen was calculated from the therapeutic human dose according to the surface area (Pagat and Barners, 1964).

Experimental protocol

Forty five randomly bred male Swiss albino mice, *Mus musculus* of an average age of 25 days old and of an average body weight of 15-18 g were used in this study. The male mice were assigned to two different groups and the control, each of 15 mice and received either 20 and 40 mg/kg/day tamoxifen or saline, respectively. The following parameters were determined.

Percentage potency

The ability of male mice to inseminate females was expressed as the ratio of female mice inseminated to the number of female mice exposed for mating X 100.

Percentage fecundity

The measure of the ability of male mice to sire viable pups was expressed as the ratio of the number of males siring at least one viable pup to the total number of males exposed for mating X 100. The loss in fecundity indicated complete loss in the ability of spermatozoa to fertilize ova.

Mating studies

The tamoxifen-induced mice were allowed to mate with a true fertilized female mice (1 male X 3 females) and leave for 10 days.

Hormonal assay

At the end of the experiment, the animals were sacrificed where blood was collected from the trunk of the control and treated mice and tested for testosterone and luteinizing hormones as described by Passing and Bablok (1984).

Light and electron microscopic studies

The testes of control and experimental mice groups were fixed in an aqueous Bouin's fluid and subsequently embedded in paraffin wax, sectioned at 5-6 μm, stained with haematoxylin and eosin for histological studies. Small pieces (1- 2 mm^3) of these specimens were processed for the

electron microscopic studies.

Statistical analysis

The obtained data were statistically analyzed as complete randomized design (CRD). SAS computer was used (SAS, 1986).

Results

The present results showed that tamoxifen at 40 mg/kg/day for 60 days was more effective than 20 mg/kg/day in suppressing potency and fecundity of the male mice (Table 1). The level of LH in the blood of these animals was significantly increased than those taken 20 mg/kg/day and the control (Table 2). However, the level of testosterone was gradually decreased in the blood of male mice with the increase dose levels of tamoxifen (20 and 40 mg/kg/day, respectively) (Table 2).

Table 1: Effect of tamoxifen on the potency and fecundity of male mice after 60 days

Tamoxifen (mg/kg/day)	Mating design (male X female)	% Potency	% Fecundity
20	1 X 5	60	60
40	1 X 3	40	30
control	1 X 4	85	85

$$\text{Potency} = \frac{\text{Number of female mice inseminated}}{\text{number of female mice exposed to mating}} \times 100$$

$$\text{Facundity} = \frac{\text{number of siring at least one viable pup}}{\text{number of male exposed to mating}} \times 100$$

n = number of male mice

Table 2: Effect of tamoxifen on Plasma hormone profile after 60 days of tamoxifen in male mice.

		Tamoxifen (mg/kg/day)	
Hormone	Control	20	40
Luteinizing hormone (LH) (U L^{-1})	0.09b	0.11b	0.56a
Testesterone (ng L^{-1})	2.72a	1.93a	0.24a

*Values are expressed as means. *Means with the same letter are not significantly different.

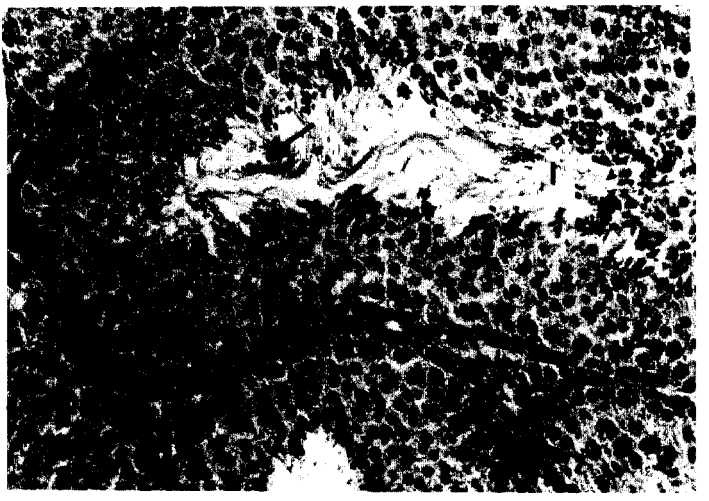
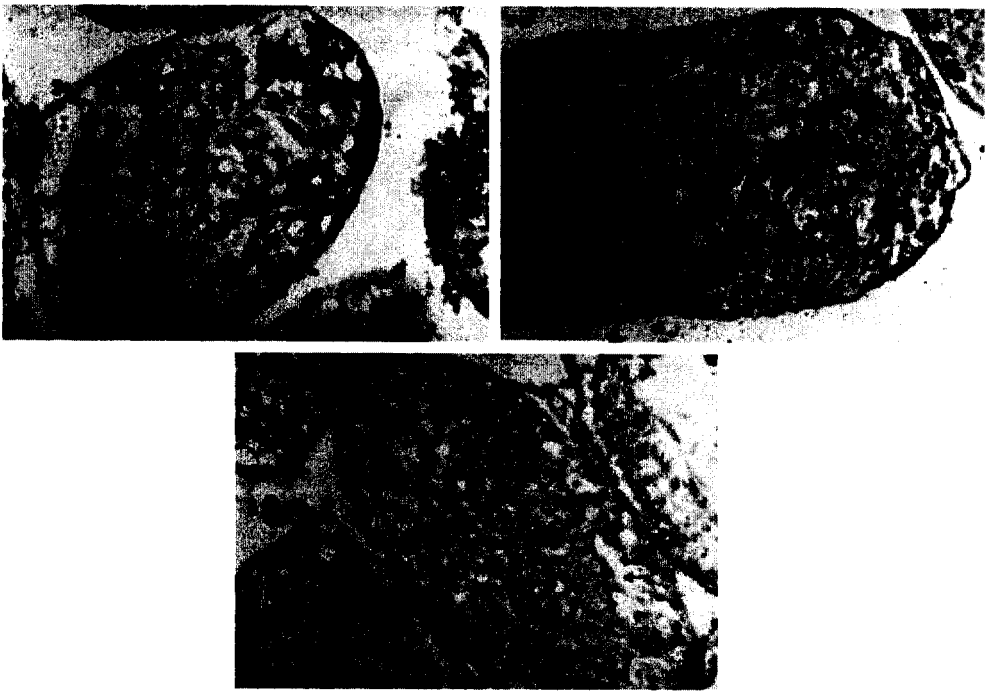


Fig. 1: Light micrograph of testis of control mice showing the different stages of spermatogenic cells in seminiferous tubules (St); Note: round spermatids (sp); spermatozoa (arrow); the interstitial Leydig cells (L); tubular lumen (l). H and E, (X400)



Figs. 2-4: Light micrograph of testis of mice taken 20 mg/kg/day tamoxifen, showing disorganization of the spermatogenic lineage in seminiferous tubules (St); arrows point at the vacuoles in the tubules; Note: the tubules show no lumen; reduction in Leydig cells. H and E, (X400)

Fig. 5: Light micrograph of testis of mice taken 20 mg/kg/day tamoxifen showing karyorrhexis (arrows) in the nuclei of spermatogenic cell. H and E, (X400)

Fig. 6: Light micrograph of testis of mice taken 40 mg/kg/day tamoxifen showing the decrease in spermatozoa; irregularly-shaped seminiferous tubules (arrows); intertubular spaces (arrowheads). H and E, (X400).

Fig. 7: Light micrograph of testis of mice taken 40 mg/kg/day tamoxifen showing the marked decrease in spermatozoa; irregularly-shaped seminiferous tubules (arrows); wide lumen (l); arrowheads point at occluded lumen of certain tubules. H and E, (X400)

Fig. 8: Light micrograph of testis of mice taken 40 mg/kg/day tamoxifen showing irregularly-shaped seminiferous tubules with disintegration of spermatogenic layers (arrows); wide lumen (l); and marked decrease in spermatozoa. H and E, (X400)

Fig. 9: Electron micrograph of control testis of mice revealing the different stages of spermatogenic cells (arrows); the spermatogonia rest upon basement membrane (arrowhead); myoid cell (m). (X5000)

Fig. 10: Electron micrograph of control testis of mice showing the acrosomal cap spermatids (cap-phase) (arrows); the developing tail (t) of spermatozoa; mitochondria (M). (X 5000)

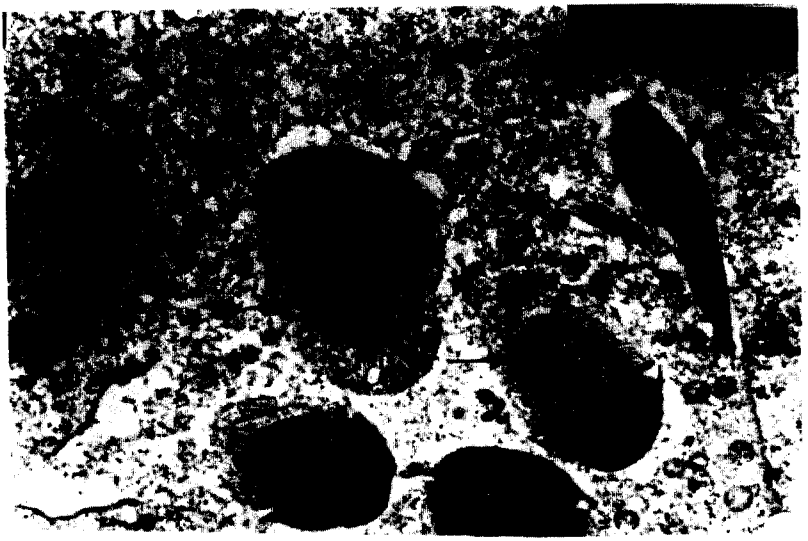


Fig. 11: Electron micrograph of control testis of mice showing the acrosomal cap spermatids (cap-phase) (arrow); the developing head and tail of spermatozoa (arrowhead) are seen. (X 5000)

Fig. 12: Electron micrograph of testis of mice taken 20 mg/kg/day tamoxifen showing intercellular space (I) between the spermatogenic cells; spermatogonia (arrowhead); large Sertoli cell (S) with folded outline (arrow); myoid cell (m). (X 4000)

Fig. 13: Electron micrograph of testis of mice taken 20 mg/kg/day tamoxifen showing intercellular space (I) between the spermatogenic cells; Note: the presence of vacuoles (V) in most spermatids (arrow); Golgi zone (G); mitochondria (M); the developing spermatozoa (arrowheads). (X 4000)

Fig. 14: Electron micrograph of testis of mice taken 20 mg/kg/day tamoxifen showing sign of karyolysis in degenerating spermatocyte (arrow); Note that: the mitochondria (M) appear as empty vesicles with disintegrated cristae; many dense bodies (d) and vacuolated smooth endoplasmic reticulum (sER) in a part of Sertoli cell (S). (X 4000)

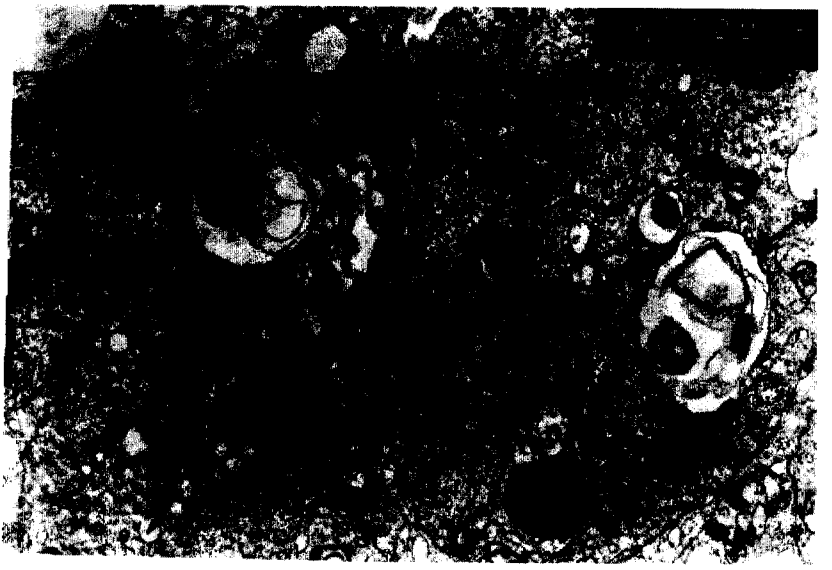


Fig. 15: Electron micrograph of testis of mice taken 20 mg/kg/day tamoxifen showing parts of spermatids with phagolysosomes (arrows); Golgi zone (G); mitochondria (M); small vesicles (v); Note: the distorted acrosomic system (arrowhead). (X6700)

Fig. 16: Electron micrograph of testis of mice taken 40 mg/kg/day tamoxifen showing a part of spermatogenic cell and an extension of an electron dense Sertoli cell (arrow) with dilated smooth endoplasmic reticulum (sER) and numerous dense bodies (d); Golgi zone (G); intercellular spaces (I). (X5000)

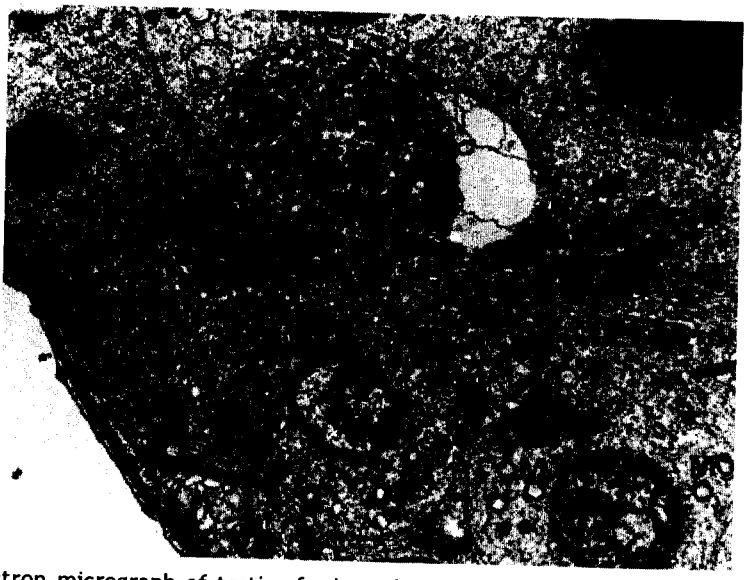


Fig. 17: Electron micrograph of testis of mice taken 40 mg/kg/day tamoxifen showing sign of karyorrhexis in degenerating spermatocyte (arrow); Note that: the mitochondria (M) appear as empty vesicles with disintegrated cristae in most spermatogenic cells; large folded Sertoli cell (S). (X2700)

Fig. 18: Electron micrograph of testis of mice taken 40 mg/kg/day tamoxifen showing the presence of large cytoplasmic vacuoles (V) in most spermatids (sp); the developing spermatozoa (*); Note: the distorted acrosomic system (arrow) and the sloughed section of sperm tail (arrowhead) in the lumen (l) of the tubules. (X2700)

Fig. 19: Electron micrograph of testis of mice taken 40 mg/kg/day tamoxifen showing the presence of many spermatids (sp); Note: the distorted acrosomic system (arrow); sections of sperm tails are sloughed in the lumen (l) of seminiferous tubules; the developing spermatozoa (arrowheads) toward the extended part of Sertoli cell (S); Golgi zone (G); mitochondria (M). (X5000)

The histological examination of sections of testis of the control mice showed the presence of different stages of spermatogenic cells in seminiferous tubules separated by intertubular or interstitial tissue. The spermatids are the smallest cells and represent the final stage of spermatogenesis (Fig. 1). Daily administration of 20 mg/kg/day of tamoxifen for 60 days revealed that the cytoarchitecture of most of seminiferous tubules was disorganized. The spermatogenic cells had lost their arrangement into layers (Fig. 2- 4) and their nuclei exhibited karyorrhexis (Fig. 5). Moreover, there is an obvious increase in the occurrence of vacuolated tubules and their lumen were occluded and fewer tubules were contained a lumen (Fig. 2-4). In addition, a reduction in the interstitial (Leydig) cells were seen between the tubules (Fig. 2-4). Results revealed that mice taken 40 mg/kg/day tamoxifen for 60 days (Figs. 6-8) showed that most of seminiferous tubules were irregular in shape and devoid of mature sperms. A large decrease in the numbers of late spermatids was manifested by a scarcity of germ cells with condensed nuclei in the seminiferous epithelium, and the lumen of the tubules appeared to be wide. In addition, marked decrease in Leydig cells was observed in most sections of these testes (Figs. 6-8).

At the ultrastructural level, the electron micrographs of control mice testis revealed the different stages of spermatogenic cells and that spermatogonia rest upon the basement membrane beneath which is a slender smooth muscle-myoid cells where they surround the seminiferous tubules (Fig. 9). Late stages of spermatids (cap-phase) have developing acrosomal cap spermatids and the developing head and tail of spermatozoa were observed (Figs. 10 and 11).

The observation made on ultrathin sections of testis of mice treated with 20 mg/kg/day tamoxifen were similar to those taken 40 mg/kg/day for 60 days. The results revealed large wide intercellular vacuolation between the spermatogenic cells (Fig. 12, 13, 16 and 18). Clear sign of karyolysis and karyorrhexis in degenerating spermatocyes were observed (Fig. 14 and 17). The majority of the mitochondria of both spermatocytes and spermatids appeared as empty vesicles with disintegrated cristae (Fig. 14 and 17). In addition, large numerous cytoplasmic vacuoles and large phagolysosomes and Golgi zone were observed in most spermatids (Fig. 13, 15 and 18). Moreover, the acrosomic system became distorted and was inverted into the nucleus instead of lying in its normal position on the surface (Fig. 15, 18 and 19). The heads of late spermatids were detached from the middle piece where they were sloughed into the lumen of seminiferous tubules without heads (Fig. 18 and 19). The Sertoli cells in a number of sections had an increased electron density and the cisternae of the agranular endoplasmic reticulum were markedly dilated and numerous dense bodies were dispersed throughout the cytoplasm (Fig. 14 and 16).

Discussion

The present study revealed that daily administration of 20 and 40 mg/kg tamoxifen for 60 days had decreased the fecundity and the potency of male mice than the control. This was followed by the decrease in the levels of testosterone while luteinizing hormone (LH) concentratoins were increased in plasma of these mice compared to the control. These two hormones are responsible for normal spermatogenesis in male rats (Kliesch *et al.*, 1992). Parte *et al.* (2000) found that administration of tamoxifen had reduced the potency and the fecundity of adult male rats as well as it reduced the level of circulating testosterone. The present results showed that the decrease in testosterone concentrations is parallel with the decrease in Leydig cells between most seminiferous tubules. This contention is supported by earlier observations that tamoxifen has a direct effect on reducing testosterone production by Leydig cells *in vitro* (Lin *et al.*, 1981; Gill-Sharma *et al.*,1993; Gopalkrishnan *et al.*,1998). As the sperm fertilizing potential depends on circulating testosterone (Orgebin-Crist *et al.*, 1975), a lower concentration of circulating testosterone may have affected the sperm fertilizing potential. Moreover, the present histological results revealed disorganization in the normal arrangement of germ cells and obliteration of the lumen of seminiferous tubules of sections of mice testes taken both doses of tamoxifen (20 and 40 mg/kg/day). This indicates that the process of classification of germ cells into discrete stages is lost. Also, Nakai *et al.* (1999) found that the testes of the neonatal mice taken tamoxifen were often necrotic and highly disorganized with severe inflammation. Steinberger (1971) and Russell *et al.* (1981) had reported that the alterations in seminiferous epithelium are probably the results of an increase in LH level subsequently lowering testosterone level. Although, Gill-Sharma *et al.* (1993) and Gopalkrishnan *et al.* (1998) found the same features of testicular injury in male rats following a significant decrease in testosterone level after administration of tamoxifen, they had reported that tamoxifen at 40 μg/kg/day was insufficient in modifying circulating levels of LH. However, 200 μg/kg/day tamoxifen had inhibited serum LH levels significantly from day 10 to 90 of treatment. Thus the present results contradict their observations in increasing LH levels after 20 and 40 mg/kg/day tamoxifen in the blood of male

mice. Furthermore, Gill-Sharma *et al.* (1993) explained that if the effects of tamoxifen were limited to testicular tissue, lower testosterone concentrations would have activated regulatory "long-loop" feedback mechanisms to increase the secretion of LH.

Moreover, the present results showed that the majority of seminiferous tubules of mice testes treated with 40 mg/kg/day tamoxifen suffered from vanishing of sperms. This was accompanied by the presence of many large vacuoles in spermatids. Flickinger and Loving (1976) had suggested that in the presence of tamoxifen, germ cells develop up to cap-phase spermatids and then begin to undergo degeneration and death. This alteration may have an important role in the antifertility effect of the drug. Also, Gopalkrishnan *et al.* (1998) had reported that the decrease in the number of spermatids and spermatozoa suggested that germ cells developed up to stages VI and VII of spermatogenesis and then degenerated. Furthermore, the electron micrographs of testes of mice taken any dose of tamoxifen (20 and 40 mg/kg/day) showed the presence of degenerating primary spermatocytes suggesting that the earlier stage cells are affected.

Furthermore, the results showed that the presence of intercellular vacuolation separating spermatogonia from the cytoplasm of Sertoli cells in the tubules of mice testes treated with tamoxifen might be a sign of testicular toxicity and cell degeneration.

In consisting with the study of Creasy and Foster (1991) the appearance of dark condensed Sertoli cells in most tubules of the treated mice testes with tamoxifen. They interpreted that as a chemically induced ultrastructural changes probably reflecting altered membrane permeability. Furthermore, the appearance of many vacuoles and an increase in the number of lysosomal bodies together with autophagic vacuoles in the Sertoli cells indicating the phagocytic function of such cells (Haschek and Rousseaux, 1991; Gartner and Hiatt, 1997; Junqueira *et al.*, 1998; Burkitt *et al.*,1999). In addition, Creasy and Foster (1991) added that the necrotic cells are rapidly sloughed into the lumen and phagocytized by the Sertoli cells. Sertoli cells play a crucial role in the organization and differentiation of germ cells which in turn, modulate Sertoli cell and seminiferous tubule functions (Sharpe *et al.*, 1993). Accordingly vacuolization in the Sertoli cells may lead to decreased production of testosterone hormone and hence be considered as an additional factor for the diminished spermatogenesis. Moreover, Allard *et al.* (1993) had reported that the microtubules of Sertoli cells play an important role in the maintenance of the normal cytoskeleton of rat testis seminiferous epithelium and spermatogenesis.

Furthermore, the results contradict the suggestion of many authors (Comhaire,1976; Willis *et al.*, 1977; Buvat *et al.*, 1982) who reported that tamoxifen might use in the treatment of idiopathic oligozoospermia through elevating testosterone levels which is probably secondary to the increased effects of gonadotropins on the testis (Gill-Sharma *et al.*, 1993).

It is inferred that sperm fertilizing potential is sensitive to concentrations of testosterone in plasma and is impaired on its reduction. The sperm fertilizing potential is virtually zero in the presence of low circulating testosterone (Gill-Sharma *et al.*, 1993).

In summary, evidence is presented that tamoxifen is a potent antiestrogen, inhibits the effects of exogenous and endogenous estrogen probably by binding to cytoplasmic estrogen receptors (Jordan and Koerner, 1975). Estrogens are known to exert a negative feedback action

on the synthesis and secretion of gonadotropins via receptors present in the pituitary and hypothalamus (McEwen, 1975; Muldoon, 1977). Yanaihara and Troen (1974) and Bartke *et al.* (1977) had reported that estrogens have a direct inhibiting effects on testosterone synthesis.

In conclusion, the treatment of tamoxifen altered spermatogenesis and testicular morphology at low and high doses (20 and 40 mg/kg/day), which in turn affected fertility of male mice through elevating LH level and then decreasing the testosterone.

References

Ain-Melk,Y., S, Belisle, M. Carmel and T. Jean-Pierre, 1987. Fertility and Sterility, 48: 113-116.

Allard, E.K., K.I. Johnson and K. Bochetheirde, 1993. Colchicines disrupts the cytoskeleton of rat testis seminiferous epithelium in a stage - dependent manner. Biology of Reproduction, 48: 143-153.

Bartke, A., K.L.H. Williams and S. Dalterio, 1977. Effects of estrogen on testicular testosterone production in vitro. Biol. Reprod., 17: 645 - 649.

Burkitt, H. G., B. Young and J. W. Health, 1999. Wheater's functional Histology. 3rd ed., churchill Livingstone, London.

Buvat, J., A. Gautheir, K. Ardaens, M. Buvat-Herbaut and A. Lemaire, 1982. Effects du tamoxifen sur les hormones et le sperme de 80 sujets oligospermiques et asthenospermiques. J. Gynecol. Obstet. Biol. Reprod., (Paris), 11: 407 - 412.

Comhaire, F., 1976. Treatment of oligospermia with tamoxifen. Int. J. Fertil., 21: 232 - 237.

Creasy, D.M. and P.M.D. Foster, 1991. Male reproductive system. In: Handbook of Toxicologic Pathology. (Haschek, W.M. and C.G. Rousseaux). Academic Press, London and New York, pp: 829 - 874.

Dyn, M. and D. Fawcett, 1970. The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. J. Biol. Reprod., 3: 308 - 316.

Flickinger, C.J. and C.K. Loving, 1976. Fine structure of the testis and epididymis of rats treated with cyproterone acetate. Am. J. Anat., 146: 359 - 384.

Furr, B.J.A. and V.C. Jordan, 1984. The pharmacologiy and clinical uses of tamoxifen. Pharmacology and Therapeutics, 25: 127-205.

Gartner, I.P. and J. L. Hiatt, 1997. Histology. W.B. Saunders Company, USA.

Gill-Sharma, M. K., K. Gopalkrishnan, N. Balasinor and P. Parte, 1993. J. of Reproduction and Fertility, 99: 395 - 402.

Gopalkrishnan, K., M.K. Gill-Sharma, N. Balasinor, V. Padwal, S. D'Souza, P. P. Parte, S. Jayaraman and S. Juneja, 1998. Contraception, 57: 261 - 269.

Harper, M.J.K. and A. L. Walpole, 1966. Contrasting endocrine activities of cis-and trans- isomers in a series of substituted triphenylethylene. Nature, 212: 87 - 94.

Haschek, W.M. and C.G. Rousseaux, 1991. Handbook of Toxicologic Pathology. Academic Press, London and New York.

Jordan, V.C. and S. Koerner, 1975. Tamoxifen (ICI46,474) and the human carcinoma 8S oestrogen receptor. Eur. J. Cancer, 205 - 206.

Jordan, V.C. and C.S. Murphy, 1990. Endocrine pharmacology of antiestrogens as antitumor agents. Endocrine Research, 11: 578 - 610.

Junqueira, L.C., J. Cameiro, R.O. Kelley, 1998. Basic Histology. 9th ed. Appleton and Langue, USA.

Kliesch, G., P.T.K. Penttila, E. Saunder, A. Nieschlag and M. Parvinen, 1992. FSH receptor mRNA is expressed stage-dependently during spermatogenesis. Mol. Cell Endocrinolo., 84: 45-49.

Lin, T., E.P. Muronon, J. Osterman and H. R. Nankin, 1981. Metabolism, 30: 150-159.

McEwen, B.S., 1975. Hormones, receptors and brain function. Adv. Pathol., 1: 56-59.

Muldoon, T.H.G., 1977. Pituitary estrogen receptors. In: M.B. Allen and V.B. Mahesh Editors, Pituitary Estroggen Receptors Academic Press, New York, pp: 295-326.

Nakai, M., K. Uchida, C. Teuscher, 1999. J. Androl., 20: 626-634.

Noci, I., E. Chelo, O. Salataresili, G. Donaticori and G. Scarselli, 1985. Archives of Andrology, 15: 83-88.

Orgebin-Crist, M.C., B.J. Danzo and J. Davies, 1975. Endocrine control of the development and maintenance of sperm fertilizing ability in the epididymis. In: handbook of physiol., Vol. 5, pp: 319 - 338. Eds RO Greep and EB Astwood American Physiological Society Washington, DC.

Pagat, T. and M. Barners, 1964. Evaluation of Drug Activities, Vol. I. Academic Press.

Parker, L.N., D.R. Gray, M.K.Lai, E.R. Levin and V.C. Jordan, 1986. Metabolism, 35: 705-708.

Parte, P.P., N. Balasinor, M.K.Gill-Sharma, H.S. Juneja, 2000. J. Androl., 21: 525-533.

Passing, H. and W. Bablok, 1984. Comparison of several regression procedures for method comparison studies and determination of sample sizes. J. Clin. Chem. Clin. Biochem., 22: 431-445.

Russell, L., J. Malone, S. Karpas, 1981. Morphological pattern elicited by agents affecting spermatogenesis by disruption of its hormonal stimulation. Tissue Cell, 13: 296-380.

SAS., 1986. User's Guide Statistics, SAS Institute, statistical Analysis System, Cary, N.C., USA.

Sharpe, R.H., M. Millar and C. McKinnel, 1993. Relative roles of testosterone and germ cell complement in determining stage-dependent changes in protein secretion by isolated rat seminiferous tubule. Int. J. Androl., 16: 71-81. EMBASE.

Steinberger, E.C., 1971. Hormonal control of mammalian spermatogenesis. Physiol. Rev., 51: 1-22.

Vermeulen, A. and F. Comhaire, 1978. Hormonal effects of an antiestrogen tamoxifen in normal and oligospermic men. Fertil. Sreril., 29: 320-327.

Willis, K.J., D.R. London, M.A. Bevis, W.R. Butt, S.S. Lynch and G. Holder, 1977. J. Endocrinol., 73: 171-178.

Yanaihara, T. and P. Troen, 1974. Studies of human testis: III Effect of estrogen on testosterone formation in human testis in vitro. J. Clin. Endocrinol. Metab., 34: 968-973.