



Hormonal and histopathological changes in male reproductive system of albino mice after tamoxifen administration

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Abstract

Tamoxifen is a triphenylethylene derivative, nonsteroidal antiestrogen agent has been used to treatment for patients with all stages of breast cancer. This study was designed to evaluate the effects tamoxifen 0.25mg/kg daily for 14weeks on the reproductive system of male mice Twenty adult healthy male albino mice divided into two groups: treated group (n=10) and control group (n=10). Tamoxifen (0.25 mg/kg) was received orally to treated group daily for 14weeks. Sperm motility, count and morphology and sex hormonal levels were assessed. The testes and epididymis were isolated and histological section were prepared. The mice sacrificed at end of experiments. The serum testosterone, FSH, LH concentration, sperm count and sperm motility of treated group were insignificant decreased when compared to controls. The testes and epididymis treated mice showed several histopathological changes such as thickening in tunica albuginea, distortion and deformed in some seminiferous tubules and increased interstitial space and vacuolated cytoplasm of spermatogonia. Congestion of blood vessel, the germ cells were not detectable, multinucleated giant cell, many necrotic cells, degenerated Leydig cells. Some epididymal duct lost its normal shape with few or devoid sperms, other ducts destructed and decreased stereocilia.

Keywords: Tamoxifen, Mice, Testis, Epididymis, Sex hormones, Histological changes.

Introduction

Tamoxifen is a triphenylethylene derivative, nonsteroidal antiestrogen agent, selective estrogen receptor modulator (SERMs), binds to estrogen receptors (ERs) and elicits estrogen agonist or antagonistic responses, depending on the target tissue. Tamoxifen has been used endocrine treatment for patients with all stages of breast cancer (Jordan, 2003; Howell *et al.*, 2005). Tamoxifen used to manage and treatment of mania in patients with bipolar disorder by blocking kinase C (Yildiz *et al.*, 2008) and used to treatment of Riedel thyroiditis (Dabelic *et al.*, 2003). Tamoxifen, is the best hormone drug for breast cancer and metastatic breast cancer in men. Gynecomastia is the most common male breast disorder. Tamoxifen was much more effective to prevent gynecomastia in these men. Some athletes and bodybuilders used Tamoxifen in conjunction with anabolic steroids in an attempt to prevent gynecomastia. (Boccardo *et al.*, 2005; Parker *et al.*, 1986). Some studies showed that tamoxifen has adverse side effects on the systems of the body one of them the male reproductive system in human or animals such as

sexual dysfunction (Pemmaraju *et al.*, 2012.; Anelli *et al.*, 1994) and fertility (Gill-Sharma *et al.*, 1993) sex hormone in male rat (Gill-Sharma *et al.*, 2001). This study aimed to evaluate the effect of tamoxifen on the male reproductive system of mice.

Materials and Methods

Animals and experimental design: A total number of 20 adult healthy male albino mice weighing (24-27g) obtained from the higher institute of infertility diagnosis and assisted reproductive technology, Al-Nahrain University and used throughout this study. All animals were given free access to standard laboratory chow and tap water *Ad libitum*. The animals were divided randomly into two groups Ten mice for control (C) and ten mice for treated (T).

Group I normal control (C): Ten normal healthy mice, each received orally 0.25ml normal saline for 14weeks. Group II (T) Ten normal healthy mice, each received orally 0.25ml normal saline that containing the tamoxifen 0.25mg/kg daily for 14weeks, where this dose is considered equal to the human dose (20mg daily).

Blood collection and handling: At the end of the

experimental period, the animals from the experimental groups together with the normal control group were decapitated, and the blood was collected by heart puncture and immediately placed into non heparinized tubes to obtain the serum for analysis of hormones. Blood samples in the non-heparinized tubes were allowed to clot at room temperature for 1h. Serum samples were obtained by centrifugation of non heparinized tubes at 3000r.p.m. for 20min. Clear serum was aspired and stored at refrigerator until used in the same day. The kinetic measurement of testosterone, follicular stimulating hormone (FSH) and luteinizing hormone (LH) by using the ELISA with commercially available diagnostic.

Semen analysis: The left epididymis was removed and minced in 1ml phosphate buffered saline and mixed with 1% aqueous eosin Y. Sperm were counted with using Neubauer hemocytometer. Spermatozoa were assessed according to WHO laboratory manual for percentage dead/live spermatozoa, motility and abnormalities.

light microscope studies: All animals were sacrificed, the testes and epididymis of the control and treated rats removed and preserved in 10% formalin. Paraffin sections were prepared and stained with Haematoxylin and Eosin and assessed under light microscopy out for histological examinations.

Statistical analysis: The results are given as mean \pm standard error ($X \pm S.E.$). Significance of the differences was tested by analysis of variance (ANOVA) test. The levels of significance were taken at ($P < 0.01$).

Results and Discussion

Effect of tamoxifen on hormonal levels: Serum testosterone, FSH and LH concentration was significantly reduced ($P < 0.01$) when mice were treated with tamoxifen 0.25mg/kg daily for 14weeks as compared to the controls group for 14weeks (Table 1).

Effect of tamoxifen on sperm count and sperm characteristics: The results showed a significant decrease ($P < 0.01$) in sperm count and sperm motility of male mice treated with tamoxifen 0.25mg/kg daily for 14weeks compared with the control group. Also there was a significant increase ($p < 0.01$) in abnormal sperms and Dead sperms of male mice treated with tamoxifen 0.25 mg/kg daily for 14weeks compared with the control group (Table2).

Histological studies: In treated mice with tamoxifen 0.25mg/kg daily for 14weeks, many histopathological changes in testes were observed that included thickening in tunica albuginea, distortion

and deformed in some seminiferous tubules .the lumen of some seminiferous tubules are with few of sperms, some seminiferous tubules are separated from each other or increased interstitial space and vacuolated cytoplasm of spermatogonia (Figure 1 and 2). Besides the congestion of blood vessel, the germ cells were not detectable, multinucleated giant cell, many necrotic cells and degenerated Leydig cells (Figures 3, 4 and 5). In treated mice with tamoxifen 0.25mg/kg daily for 14weeks, many histopathological changes in epididymis were observed that included some epididymal duct lost its normal shape with few or devoid sperms, other ducts destructed and decreased stereocilia (6). The epithelial cells were distorted and nuclear pyknosis appeared among the epithelial cells and many vacuoles appeared in basal cells and principal cells (FigURES 7, 8 and 9). Various abnormalities in sperm morphology were observed in the treated mice with tamoxifen 0.25mg/kg daily for 14weeks (Figure 10).

Estrogens play key roles in the development and maintenance of reproductive function and fertility. Estrogens also have an important role in pathological processes observed in tissues of the reproductive system (O'Donnell *et al.*, 2001; Prins and Korach, 2008). The present study was investigated the effects of tamoxifen that is nonsteroidal antiestrogen agent and inhibits estrogen receptors, on changes occurring in reproductive system in adult male mice. Results showed a significant ($P < 0.01$) decrease in testosterone and LH levels in tamoxifen treated adult male mice compared control group. These results were in agreement with that reported (Attia, 2003) who demonstrated that tamoxifen administration to mice lowered LH levels and testosterone. Balasinor *et al.* (2006) reported that the administration of tamoxifen resulted in lower testosterone and LH levels in male rats . The decrease in serum testosterone levels of treated mice could be due to diminished responsiveness of Leydig cells to leutinizing hormone because LH acts exclusively on Leydig cells in the testis and is the primary regulator of testosterone secretion (Creasy *et al.*, 2002) while (Gill-Sharma *et al.*, 1993) suggest the decreasing in testosterone levels after tamoxifen treated in rat may be to tamoxifen has a direct effect at the testicular level, probably on Leydig cells and inhibits testosterone production. In the present study, tamoxifen treatment has been shown to cause an alteration in sperm motility, sperm counts and increased sperm abnormalities. These results were in agreement with many researches (D'Souza, 2004; Tunmise *et al.*, 2015) that described an increase in the number of sperm

with abnormal morphology, dead sperm and a decrease in motility and sperm counts, in rat after a treated with tamoxifen. They reported that significant decline in sperm count is due to the genotoxic activity of tamoxifen while (Padmalatha and Vijayalaxmi, 2001) explain The changes in the sperm density number and abnormal sperm is an evidence of interference of tamoxifen or its metabolites with the genetically controlled differentiation of spermatozoa indicating the genotoxicity of this drug in vivo at higher dose

levels. In this study was observed the decrease in the number of spermatozoa in testis and epididymis with a significant deterioration in the histology. This observation indicates that degeneration of spermatocytes and spermatids occurs after tamoxifen treatment are in agreement with (Motrich *et al.*, 2007) who explained the exposure to tamoxifen caused a significant increase in sperm abnormalities. Tamoxifen induced the formation of abnormal sperms indicating its genotoxicity to germ cells.

Table (1): Levels of testosterone, FSH and LH of male mice in control and treatment group.

Parameters	Group 1 (n=10) Normal (Control)	Group II (n=10) Tamoxifen treated
Testosterone (ng/ml serum)	2 2.4±0.34	1.42* ±0.51
% change		40.8%
FSH (IU/ml)	0.84 ± 0.027	0.57* ±0.019
% change		32.1%
LH (IU/ml)	0.69+ 0.0 67	0.36 * ±0.072
% change		47.8%

Levels of significance values are (mean ± SEM; n = 10). *p<0,01

Table (2): Sperm count, percentages of abnormal sperm, motility and dead sperm of male mice in control and treatment group .

Parameters	Group 1(n=10) Normal (Control)	Group II (n=10) Tamoxifen treated
Sperm count (x10 ⁶)	1.64 ± 0.09	0.62* ± 0.08
Abnormal sperm (%)	13.8 ± 2.37	63.42*± 4.62
Sperm motility(%)	83.6 ± 4.82	41.8*± 4.93
Dead sperms (%)	20.3 ± 5.49	51.6*± 6,18

Levels of significance values are (mean ± SEM; n = 10). *p<0,01

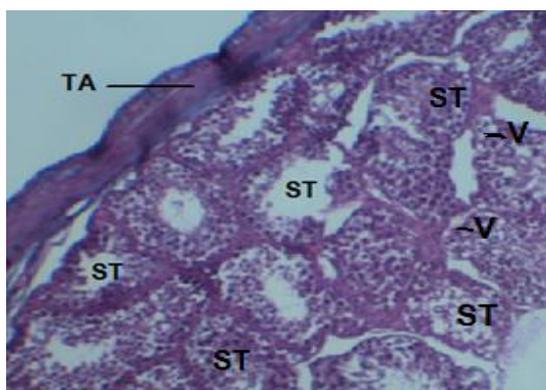


Figure (1): Section of testis tissues of mice after 14weeks from treated with tamoxifen showing thick tunica albuginea (TA), deformed seminiferous tubules (ST), cytoplasmic vacuolization in spermatogonia (V) (H & E, X100).



Figure (2): Section of testis tissues of mice after 14weeks from treated with tamoxifen showing some histopathological changes in the seminiferous tubules (ST), increased interstitial space (IS), cytoplasmic vacuolization in spermatogonia (V) congested blood vessel (Bv) (H & E, X100).

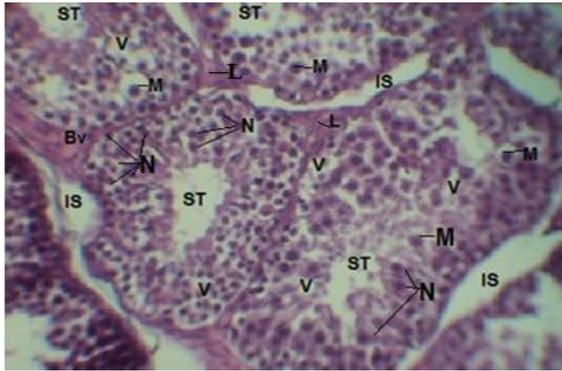


Figure (3): Section of testis tissues of mice after 14weeks from treated with tamoxifen showing some histopathological changes in the seminiferous tubules (ST), increased interstitial space(IS), cytoplasmic vacuolization in spermatogonia (V), Necrotic cells (N) and multinucleated giant cells (M) (H & E, X100).



Figure (6): Section of epididymis tissues of mice after 14weeks from treated with tamoxifen showing the duct(D) of epididymis with few or devoid sperms(S), congested blood vessel (Bv) and irregular layer of smooth muscle(SM) (H & E, X100).

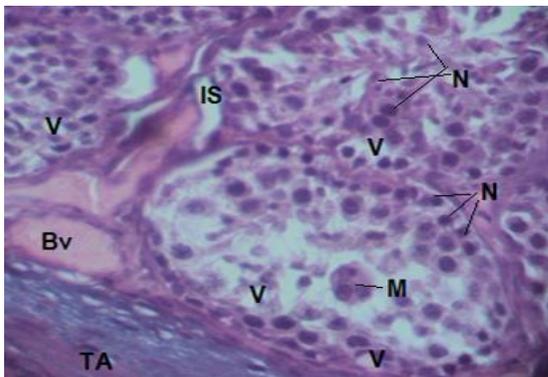


Figure (4): Section of testis tissues of mice after 14weeks from treated with tamoxifen showing thick tunica albuginea (TA), increased interstitial space (IS), cytoplasmic vacuolization in spermatogonia (V), multinucleated giant cells(M), Necrotic Cells (N) and congested blood vessel (Bv) (H & E, X200).



Figure (7): Section of epididymis tissues of mice after 14weeks from treated with tamoxifen showing the duct(D) of epididymis with few sperms(S), congested blood vessel (Bv), irregular layer of smooth muscle(SM) and increased cytoplasmic vacuolation in basal cells (Bc) and principal cells(Pc) (H & E, X200).

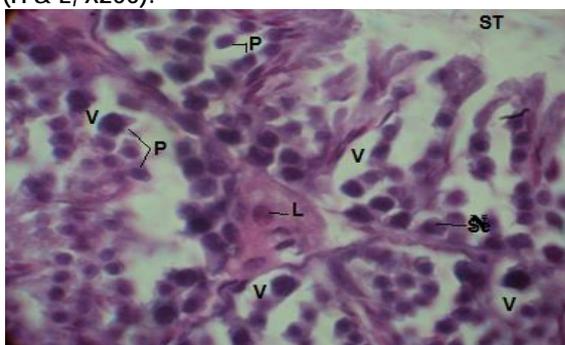


Figure (5): Section of testis tissues of mice after 14weeks from treated with tamoxifen showing seminiferous tubules with few spermatids (ST), cytoplasmic vacuolization in spermatogonia (V), pyknotic nucleus of some cell (P) and degenerated Leydig cell(L) (H & E, X200).

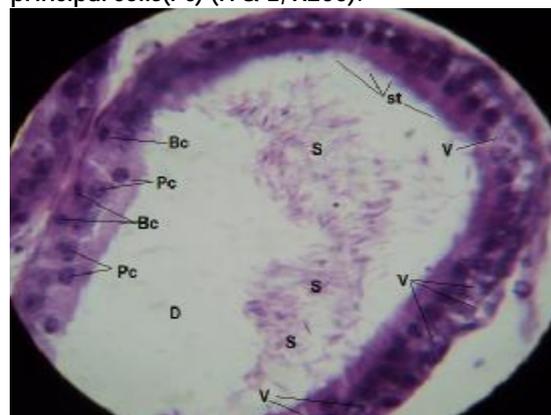


Figure (8): Section of epididymis tissues of mice after 14weeks from treated with tamoxifen showing the duct (D) of epididymis with few sperms (S), irregular formed of stereocilia and increased cytoplasmic vacuolation(v) in basal cells (Bc) and principal cells(Pc) (H & E, X200).

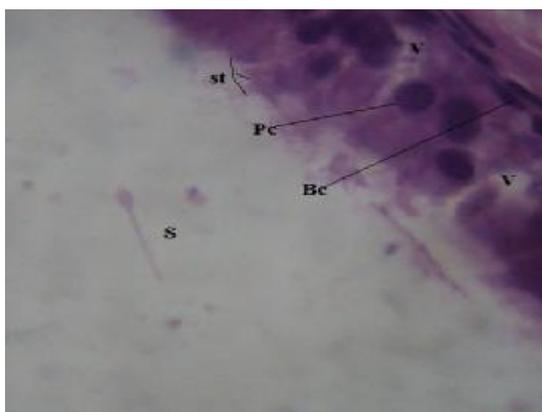


Figure (9): Section of epididymis tissues of mice after 14weeks from treated with tamoxifen showing the duct (D) of epididymis with one sperm (S), irregular formed of stereocilia and increased cytoplasmic vacuolation (v) in basal cells (Bc) and principal cells(Pc) (H & E, X400).

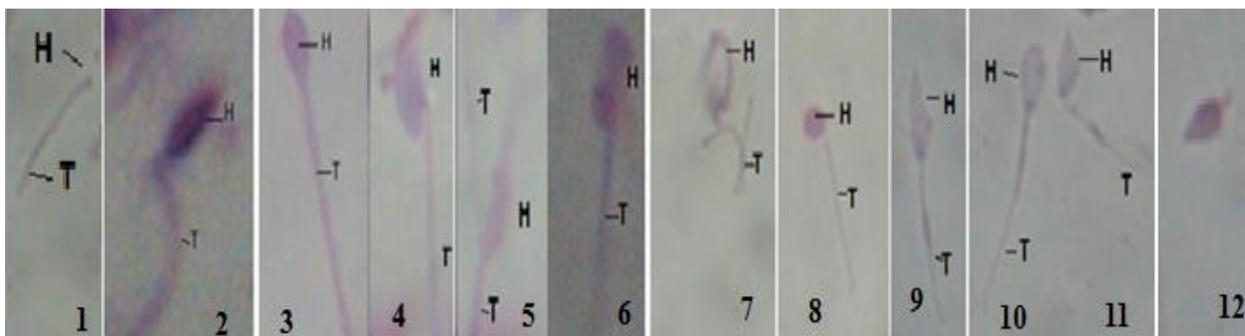


Figure (10): Types of sperm abnormalities in mice treatment with tamoxifen: Head (H) and Tail (T) (H & E, X500).

Tamoxifen induced the histopathological changes in the testis and epididymis by inducing the vacuoles in the seminiferous epithelium and epithelial cells. The presence of vacuoles and sloughing of the seminiferous epithelium and epithelial cells, sertoli cells damage and reduction in germinal epithelium be due to the inhibition of spermatogenesis, which must also be a contributor to decrease the sperm count. All these histological changes might be sign of testicular toxicity, cell degeneration and atrophied and collapsed seminiferous tubules with oligospermia (Badawy *et al.*, 2002; Oryan *et al.*, 2008). In present study, the tamoxifen induced the formation of multinucleated giant cells in the seminiferous tubules (Figures 3 and 4), that indicated the degeneration process in germinal cell of the seminiferous tubules (D'Souza, 2003).

Conclusion

In conclusion, the results of this study indicate the tamoxifen 0.25 mg / kg daily for 14weeks induced

histological change in testes and epididymis besides decreased in serum testosterone, FSH, LH concentration, sperm count and sperm motility. The tamoxifen induced to increased in sperms abnormality.

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