

Impact Of An Steroidogenesis Inhibitor Drug On Structure And Ultrastructure Of Mammalian Testis

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Abstract: ketoconazole, an imidazole derivative is currently used in the medical fields as an anti-fungal and steroidogenesis inhibitor drug. The present study aimed to investigate the effect of ketoconazole on the structural and ultrastructural characteristics of mammalian testis. So, twenty adult male rats weighing 150-200 g. were divided into two groups; group I were *i.p.* injected with 10mg/100g.b.wt. of ketoconazole dissolved in 1ml physiological saline solution daily for 15 days. Whereas, group II was injected with 1ml saline solution in the same manner. *Histologically*, testes of treated rats were surrounded by thickened tunica albuginea, and consisted of deformed seminiferous tubules ensheathed by irregular basal lamina and having deformed Sertoli cells, necrotic spermatogonia, primary spermatocytes and rounded spermatids, in addition to, deformed elongated spermatids exhibiting unusual amounts of residual cytoplasm extending from them into the lumen of the tubules. Deformed spermatozoa may be seen in the lumens of some of these affected tubules. Also, the interstitial tissue displayed vacuolation, necrotic Leydig cells and vasodilatation of the blood vessels engorged with stagnant blood cells are seen. *Ultrastructurally*, treated testes showed thickening and irregularity of the surrounding basal lamina, necrotic spermatogonia detached from the basal lamina and having pyknotic nuclei separated from the surrounding cytoplasm leaving clear zones. Primary spermatocytes and rounded spermatids exhibiting signs of necrosis, deformed elongated spermatids and malformed spermatozoa, in addition to, necrotic Leydig cells were frequently observed. In conclusion, the obtained results suggested that the testicular structural and ultrastructural alterations observed following ketoconazole administration may be responsible for the inhibition of the steroidogenesis. This decrease in steroidogenic activity has been suggested as the primary cause of spermatid production failure. Therefore, these destructive impacts of ketoconazole on the rat testes indicates that it should be used under strict medical care.

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Key words: histology, imidazole, ketoconazole, rat, steroidogenesis, testis, ultrastructure

1. Introduction:

Ketoconazole is an antifungal drug currently used in the medical field for the treatment of wide variety of yeast, dimorphic fungal, fungal infections of the gastro-intestinal tract, dermatophytic infections of the skin and fingernails and has been widely used in immunocompromised patients such as those with AIDS or those on chemotherapy (Vertzoni *et al.*, 2006). Also, it has an anti-inflammatory activity, it may prevent the development of acute respiratory distress syndrome and acute lung injury in critically ill patients (Wiedemann *et al.*, 2000).

Also, ketoconazole is an effective inhibitor of adrenal and gonadal steroidogenesis, primarily because of its inhibition of the activity of CYP17. At even higher doses, it also inhibits CYP11A1, effectively blocking steroidogenesis in all primary steroidogenic tissues (Cohen *et al.*, 2000; Schimmer and Parcker, 2006). Therefore, physicians have used ketoconazole for the treatment of resistant and hypercortisolemic depressive patients (Brown *et al.*, 2001), treatment of prostate cancer with promising results (Kinobe *et al.*, 2006; Liebertz and Fox, 2006),

treatment of ACTH-secreting adenomas (Gordon, 2007), for palliative treatment of primary hyperaldosteronism due to adrenal adenomas or hyperplasia, Cushing disease, adrenal tumors, adrenocortical carcinoma and ectopic corticotrophin production by small-cell lung carcinoma or carcinoid tumors autonomous, advanced breast cancer resistant to conventional chemotherapy and for ovarian hyperandrogenism syndrome, including polycystic ovarian syndrome and hyperthecosis (Lionakis *et al.*, 2008).

Experimentally, the impact of ketoconazole has been studied from some biological aspects on different body organs (Rodriguez and Buckholz, 2003; Braddock, 2003; Amin and Hamza, 2005; Furukawa *et al.*, 2008). Nevertheless, there seems to be an almost complete lack of information regarding its effect on testicular tissue in experimental animals. Therefore, the present work aimed to clarify the impact of ketoconazole on adult male rat testes.

2. Material and Methods

2.1. Experimental animals

Twenty male albino rats (*Rattus norvegicus*) ranging in weight from 150-200g, acquired from Schistosoma Biological Supply Program (SBSP) Theodor Bilharz Research Institute, were housed in clear plastic cages (2 animals/cage) with wood chips as bedding and given pellet rodent diet, in addition of milk and water *ad-libitum*. They kept under controlled environmental conditions, including a temperature of 25°C and a 12-h light/dark cycle.

2.2. Experimental drug used (Ketoconazole)

Ketoconazole is a synthetic imidazole of oral broad-spectrum antifungal agent (Vertzoni *et al.*, 2006; Dantas *et al.*, 2010). It is sold under trade name; Nizoral as a tablet of 200 mg which is manufactured by JANSSEN-CILAG Pharmaceutical N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium.

2.3. Experimental design

The rats were randomly divided into two even groups; **group I**, were *i.p.* injected with 10mg/100g.b.wt. of ketoconazole dissolved in 1 ml of physiological saline solution in a daily manner for 15 days. Whereas, **group II** was kept as a control group and rats were injected with 1 ml of physiological saline solution in the same manner.

This selected dose of ketoconazole and the route of administration have been previously used in different researches (O'Connor *et al.*, 2002; Amin and Hamza, 2005; Amin, 2008).

2.4. Histological preparations

The excised testes were fixed in Bouin's fluid for 24 hours, then subjected to the normal procedures for paraffin sectioning, and stained with haematoxylin & eosin stains. The stained sections were examined and photographed by light microscopy (BX-40 Olympus), fitted with 10x - 40x objective lenses with an adjustable numerical aperture (3.3). Images were captured using camera (Panasonic CD-220).

2.5. Ultrastructural preparations

For ultrastructural evaluation by transmission electron microscopy as described previously by Dykstra *et al.* (2002), freshly excised testis were cut into small blocks (1×1mm³), fixed directly in cold 4F1G (i.e. 4% formalin + 1% glutaraldehyde adjusted at pH 2.2) for 24 hours, then were post fixed in 1% osmium tetroxide in 0.1M phosphate buffer (pH 7.3), dehydrated in an ethanolic series culminating in 100% acetone, and infiltrated with epoxide resin. After polymerization overnight at 60°C, semithin sections (0.5 µm) were stained with 1% toluidine blue in 1% sodium borate and examined with light microscope. Areas of seminiferous tubules were selected and the blocks trimmed accordingly. Ultrathin sections (80-90 nm) were cut, mounted on 200 mesh copper grids, and stained with uranyl acetate and lead citrate. The stained grids were

examined and photographed by JEOL.JEM-1400-EX-ELECTRON MICROSCOPE at the Central Laboratory of Faculty of Science, Ain Shams University. The photographs were printed on KODABROMIDE F5s GLOSSY Black and White-Schwarzweib- Kodak.

3. Results

3.1. Histological studies

Histological examination of control testes displayed normal features of testicular tissues as seen in figures (1-3). The testis is enclosed in a thick fibrous capsule, the tunica albuginea. The seminiferous tubules are ensheathed by basal lamina formed of myoid cells. Each tubule possesses epithelial cells involved of Sertoli cells and the germ cells of various stages, covering the complete process of spermatogenesis. Sertoli cells exhibit typical irregular nuclei and well-defined cytoplasm. Spermatogonia are oval in shape, rest upon the basal lamina of the seminiferous tubule. Immediately above them are spherical primary spermatocytes, recognized by their copious cytoplasm and large nuclei containing coarse clumps of chromatin. Secondary spermatocytes are not seen in these sections due to rapid division processes. Therefore, above the primary spermatocytes, there are large clusters of small rounded spermatids with rounded nuclei devoid of coarse clumps of heterochromatin, followed by elongated spermatids which undergo dramatic shape changes, forming spermatozoa. The interstitium between seminiferous tubules contain distinct Leydig cells and blood vessels.

Whereas, ketoconazole-treated rat testes illustrated destructed features of testicular tissues. The testis is surrounded by thickened tunica albuginea, containing deformed seminiferous tubules and destructed interstitial tissues as displayed in figures (4-6). The seminiferous tubules are surrounded by irregular basal lamina and having spermatogonia, primary spermatocytes and rounded spermatids showing clear signs of pyknosis. One of the most common histopathological lesions observed after treatment with ketoconazole is the unusual amounts of residual cytoplasm that extend from the elongated spermatids into the lumen of the tubules. Deformed spermatozoa may be seen in the lumen of some of these affected tubules. Sertoli cells became irregular in shape. Large vacuoles are clearly observed in between germ cells.

The interstitial tissues between these deformed seminiferous tubules displayed vacuolation, necrotic Leydig cells and vasodilatation of the blood vessels that were engorged with stagnant blood cells as noticed in figures (4 & 6).

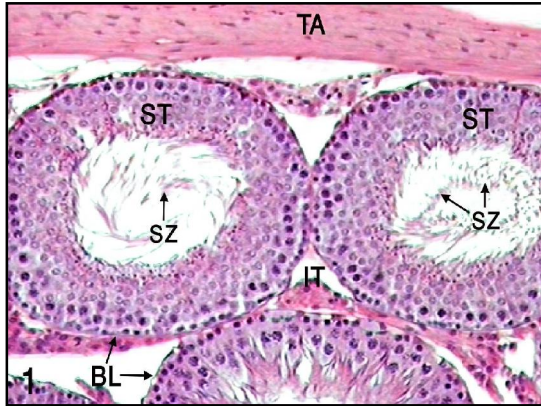


Fig.(1): Normal testicular tissue architecture of control testis revealing tunica albuginea (TA), seminiferous tubules (ST) ensheathed with basal lamina (BL) and containing spermatozoa (SZ) in their lumens, in addition to the interstitial tissues (IT). (H&E, x330)

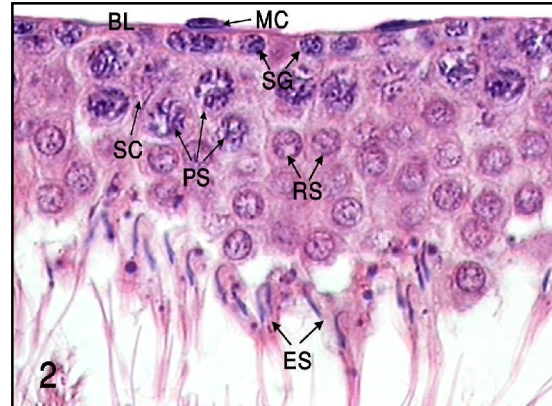


Fig.(2): Seminiferous tubule of control testis surrounded by basal lamina (BL) with myoid cells (MC) showing Sertoli cells (SC) and germ cells including; spermatogonia (SG), primary spermatocytes (PS), rounded spermatids (RS) and elongated spermatids (ES). (H&E, x1320)

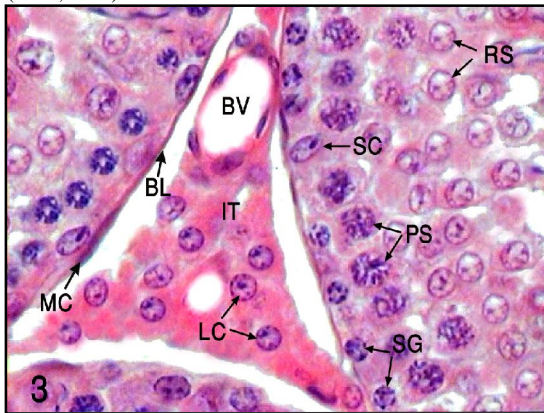


Fig.(3): Normal architecture of interstitial tissue (IT) of control testis embodying clusters of Leydig cells (LC) and blood vessel (BV) and located in between three seminiferous tubules being surrounded by basal lamina (BL) containing myoid cells (MC), having spermatogonia (SG), primary spermatocytes (PS), rounded spermatids (RS) and Sertoli cells (SC). (H&E, x1320)

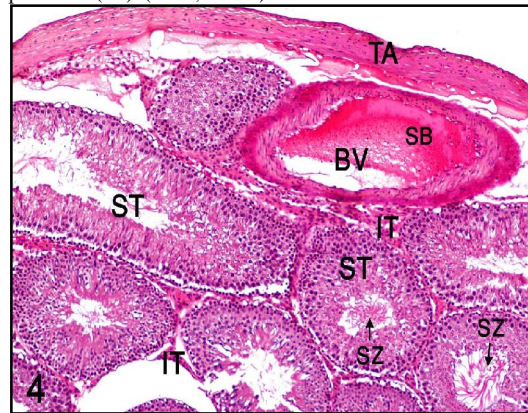


Fig.(4): Destroyed testicular tissues of ketoconazole-treated rat illustrating thickened tunica albuginea (TA), deformed seminiferous tubules (ST) containing spermatozoa (SZ) in their lumens, and interstitial tissue (IT) with dilated blood vessel (BV) engorged with stagnant blood cells (SB). (H&E, x330)

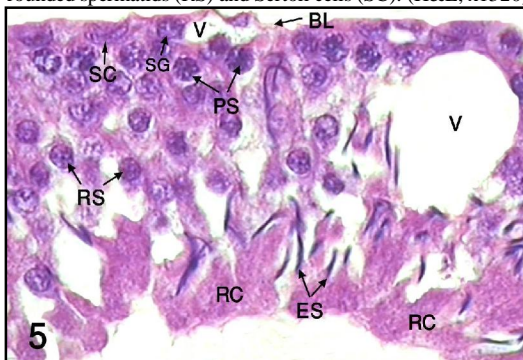


Fig.(5): Destroyed seminiferous tubule of ketoconazole-treated testis surrounded by irregular basal lamina (BL) and possessing necrotic spermatogonia (SG), primary spermatocytes (PS) and rounded spermatids (RS). In addition to elongated spermatids (ES) with residual cytoplasm (RC). Also, Sertoli cells (SC) with irregular shapes and large vacuole (V) between germ cells are clearly seen. (H&E, x1320)

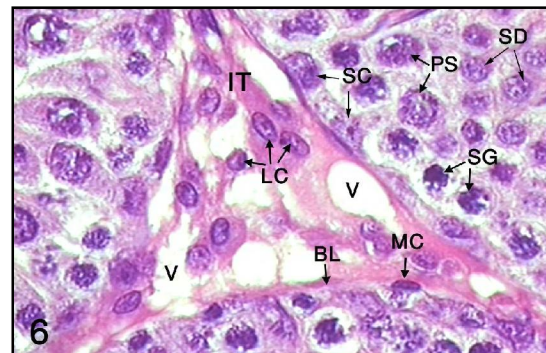


Fig.(6): Destroyed interstitial tissue (IT) of ketoconazole-treated testis showing vacuoles (V) and necrotic Leydig cells (LC). Parts of three deformed seminiferous tubules surrounded by ruptured basal lamina (BL) with myoid cells (MC) and possessing necrotic spermatogonia (SG), primary spermatocytes (PS) and round spermatids (RS), in addition to irregular shaped Sertoli cells (SC). (H&E, x1320)

3.2. Ultrastructural study

Electron micrographs of control rat testes showed Sertoli cells and germ cells with cellular characteristics typical of those seen in active spermatogenesis. The germ cells in various developmental stages arranged orderly as illustrated in figure (7). The spermatogonia rest upon the basal lamina of the tubule possessing mitochondria and nuclei with one or two nucleoli, euchromatin and dense clumps of marginated heterochromatin (Fig.7). However, ketoconazole-treated testis showed necrotic spermatogonia which are detached from the underlying thickened basal lamina and having pyknotic nuclei separated from the surrounding cytoplasm leaving clear zones (Fig. 8).

The primary spermatocytes of control testes are rounded in configuration with prominent large rounded nuclei having distinct nucleoli, homogenous chromatin materials including both heterochromatin and euchromatin, and surrounded by nuclear membrane. The cytoplasm appear granular, characterized by dispersed oval mitochondria, cisternae of smooth endoplasmic reticulum, lysosomes and Golgi apparatus (Figs. 7&9). While, ketoconazole-treated testes showing primary spermatocytes possessing irregular shaped nuclei surrounded by nuclear membranes, containing nucleoli and fragmented chromatin materials. The surrounding cytoplasm showing deformed mitochondria, condensed Golgi apparatus and lysosomes as observed in figure (10).

Rounded spermatids of control rat testes (Fig. 11) displayed the formation of the acrosome in developing spermatid. This formation starts with the appearance of a proacrosomal granule in an acrosomal vesicle associated with the Golgi complex, then the acrosomal vesicle enlarged and adhered to the anterior pole of the nucleus, spreading over the hemisphere of the nucleus to form an acrosomal cap. Also, the cytoplasm possesses few stacks of rough endoplasmic reticulum, vacuolated mitochondria and lysosomes. The nucleus is well defined characterized by a single nucleolus, chromatin networks, and surrounded by distinct nuclear membrane. Contrarily, rounded spermatids of ketoconazole-treated testes were atrophied, surrounded by irregular plasma membrane and possessing nuclei ensheathed by ruptured nuclear membranes, with condensed chromatin materials and lacking nucleoli. The cytoplasm exhibiting deeply electron dense shrunken mitochondria, condensed Golgi apparatus and numerous vacuoles (Fig. 12).

As illustrated in figure (13), elongated spermatids of control testes is formed of elongated nuclei, covered by the acrosomal caps and forming a structure known as the acrosomal head cap.

Cylindrical bundles of microtubules –the manchette– limit the nuclei laterally, and extend caudally from a circumferential specialization of the nuclear envelopes at the posterior margin of the acrosomal caps. With the formation of the manchette, there is a marked elongation of the spermatid, with the bulk of the cytoplasm moving behind the posterior pole of the nucleus where it surrounds the proximal portion of the flagellum. This migration of cytoplasm thus concentrates mitochondria in the flagellar region. Controversely, ketoconazole-treated testes were possessing deformed elongated spermatids showing deformed acrosomal head cap with the nuclei exhibiting distortion and the acrosome revealing discontinuation. Also, the manchette occurred as a continuous patch connected to one side of the nucleus. The flagellum having disarranged mitochondria as revealed in figure (14).

The electron micrograph (15) displayed the ultrastructural features of spermatozoa. Each one is formed of the head, composed of the nucleus, which occupies most of the head and is formed of very condensed chromatin. Surrounding the anterior two-thirds of the nucleus is the acrosomal cap. Following the head is the neck, a short linking segment between the sperm head with the flagellum. It is composed of the segmented columns and a dense fibrous structure –the capitulum-. The tail is the longest part of the sperm and consists of midpiece, principal piece and end piece. Only, the middle piece is seen, it is characterized by the mitochondrial sheath. Malformed spermatozoa are seen post ketoconazole treatment, as seen in electron micrograph (16), the spermatozoon exhibiting head size abnormality, including macrocephalic head formed of nucleus and surrounded by the acrosomal cap, which was partially lost, while the midpiece included vacuolated mitochondria (Fig. 16).

Sertoli cells of control testes showed distinct nuclei and cytoplasmic characteristics consistent with an active secretory state. They rest on the basal lamina of the tubule, extending towards the lumen of the tubule, filling the narrow spaces between the cells of the spermatogenic series. Their cytoplasm contain mitochondria with distinct tubular cristae, cisternae of smooth endoplasmic reticulum, few stacks of rough endoplasmic reticulum, Golgi apparatus and lysosomes. The nuclei appear irregular in shape, devoid of heterochromatin, containing prominent nucleoli, surrounded by nuclear envelopes and exhibit deep indentation as clearly observed in figure (17). Whereas, Sertoli cells of treated testes with their nuclei exhibiting plate-like or elongated configuration, having nucleoli and surrounded by irregular nuclear membranes. The surrounding

cytoplasm contained deformed mitochondria and large vacuoles (Figs. 8 & 18).

The interstitial tissues of control testes manifested normal Leydig cells having large spherical nuclei with distinct nucleoli, euchromatin and coarse clumps of peripheral heterochromatin, in addition to the cytoplasm containing cisternae of smooth endoplasmic reticulum, mitochondria and lipid droplets. Monocyte is also found in the interstitial tissue; it is characterized by a large eccentrically placed bilobed nucleus. Numerous small pseudopodia extend from the monocyte for its phagocytic role and amoeboid movement (Fig. 19). While, the interstitial tissues of treated testes showed remarkable decrease in the number of Leydig cells, most of which were deformed and characterized by electron dense mitochondria, disappearance of smooth endoplasmic reticulum and pyknotic nuclei as illustrated in figure (20).

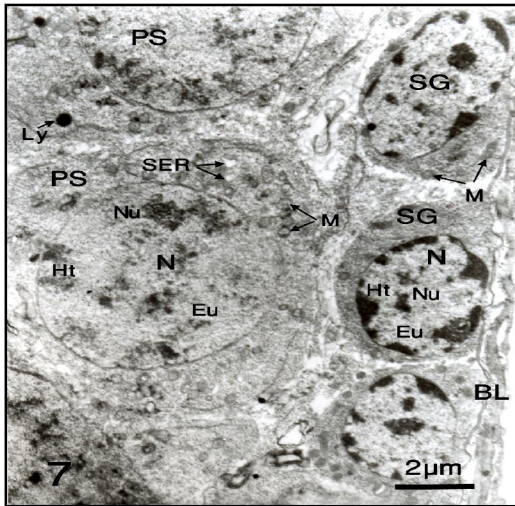


Fig.(7): Seminiferous tubule of control rat testis surrounded by thin basal lamina (BL) and formed of; spermatogonia (SG) that possessing rounded to oval nuclei containing nucleoli (Nu), euchromatin (Eu) and marginated heterochromatin (Ht), and the cytoplasm containing mitochondria (M). Primary spermatocytes (PS) which are rounded in shape having large rounded nucleus (N) containing nucleoli (Nu), heterochromatin (Ht) and euchromatin (Eu) and the cytoplasm involving mitochondria (M), smooth endoplasmic reticulum (SER) and lysosomes (Ly). (x4000)

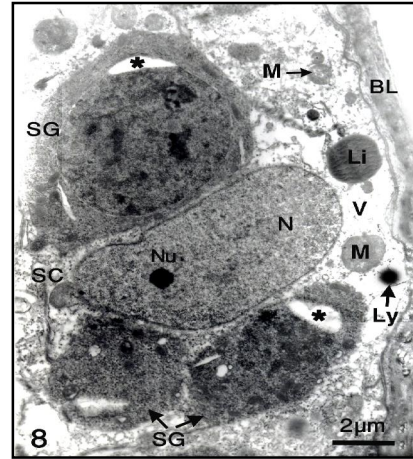


Fig.(8): Seminiferous tubule of ketoconazole-treated testis illustrating necrotic spermatogonia (SG) which are detached from the underlying thickened basal lamina (BL) and having pyknotic nuclei (N) separated from the surrounding cytoplasm leaving clear zones (*). In between these degenerated spermatogonia lying Sertoli cell (SC) having an elongated nucleus (N) with a nucleolus (Nu) and ensheathed by nuclear membrane with a notch and the surrounding cytoplasm showing deformed mitochondria (M), lysosomes (Ly), large electron dense lipid droplet (Li) and vacuoles (V). (x4000).

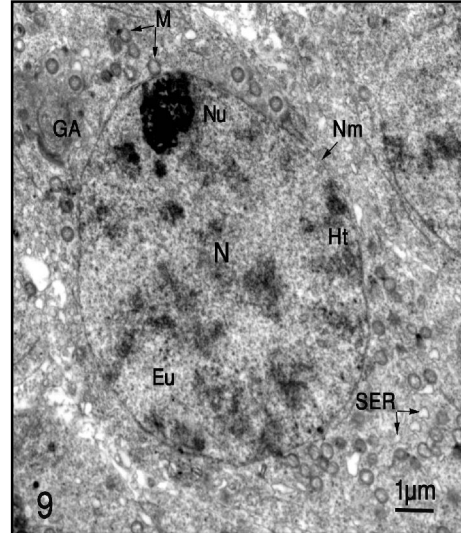


Fig.(9): Primary spermatocyte of control testis having large rounded nucleus (N) containing nucleolus (Nu), heterochromatin (Ht) and euchromatin (Eu) and surrounded by nuclear membrane (Nm). The cytoplasm containing oval mitochondria (M), smooth endoplasmic reticulum (SER) and Golgi apparatus (GA). (x6000)

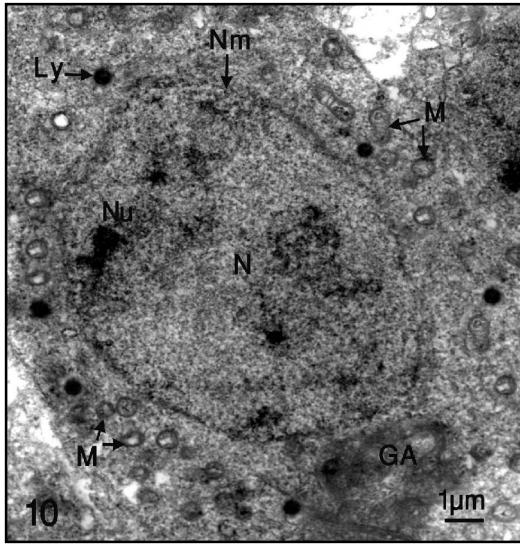


Fig.(10): Primary spermatocyte of treated testis possessing irregular shaped nucleus (N) surrounded by nuclear membrane (Nm) and containing nucleolus (Nu), fragmented chromatin materials. The surrounding cytoplasm showing deformed mitochondria, condensed Golgi apparatus (GA) and lysosomes (Ly). (x6000)

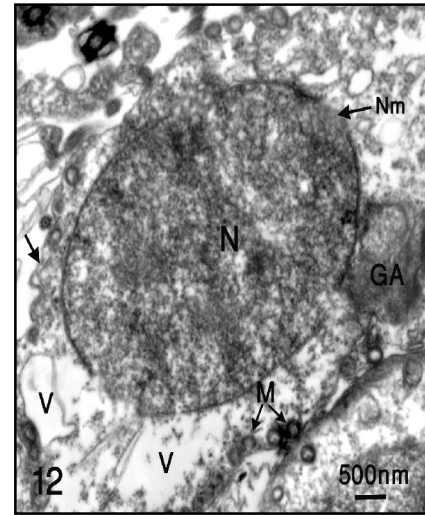


Fig.(12): Atrophied rounded spermatid of ketoconazole-treated testis surrounded by irregular plasma membrane (arrows) possessing nucleus (N) ensheathed by ruptured nuclear membrane (Nm), showing condensed chromatin material and disappearance of the nucleolus. The cytoplasm exhibiting deeply electron dense mitochondria (M) with reduced sizes, condensed Golgi apparatus (GA) and numerous vacuoles (V). (x10,000)

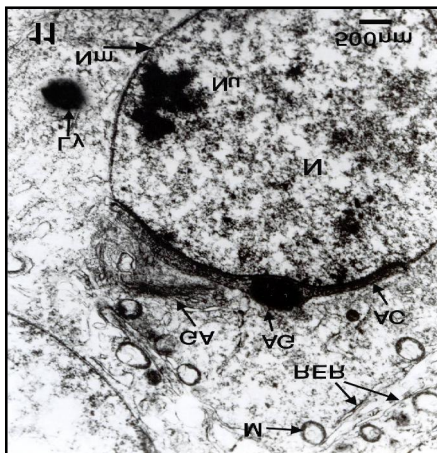


Fig.(11): Rounded spermatid of control testis illustrating the formation of the acrosome, indicated by the presence of a proacrosomal granule (AG), acrosomal cap (AC), Golgi apparatus (GA) over the anterior hemisphere of the nucleus (N) which is rounded in shape, containing distinct nucleolus (Nu), homogenous chromatin materials and surrounded by nuclear membrane (Nm). The cytoplasm containing vacuolated mitochondria (M), few stacks of rough endoplasmic reticulum (RER) and lysosome (Ly). (x10,000)

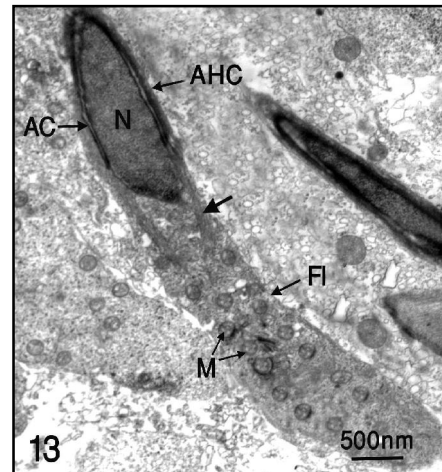


Fig.(13): Elongated spermatid of control testis illustrating the elongated nucleus (N) in the center, covered by the acrosomal cap (AC) forming a structure known as the acrosomal head cap (AHC), in addition to bundles of microtubules –the manchette- (arrow) limit the nucleus laterally. Part of the flagellum containing numerous numbers of rounded mitochondria (M) is clearly noticed. (X12,000)

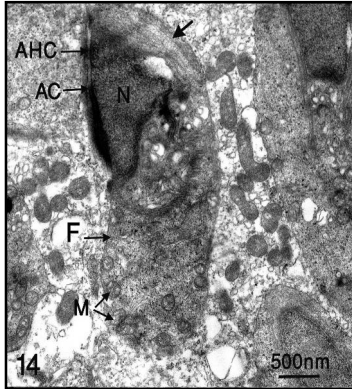


Fig.(14): Deformed elongated spermatid of treated testis showing the deformed acrosomal head cap (AHC) with the nucleus (N) exhibited distortion and the acrosome (AC) revealing discontinuation. Also, the manchette (arrow) occurred as a continuous patch connected to one side of the nucleus. The flagellum (F) having disarranged mitochondria (M). (x12,000)

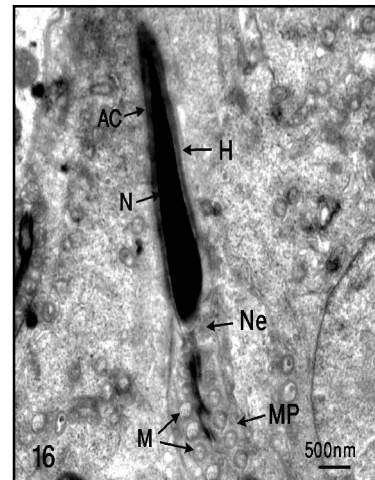


Fig. (16): Deformed spermatozoon of treated testis displaying long head (H), formed of the nucleus (N) and surrounded by the acrosomal cap (AC) that partially lost, the neck (Ne) and the midpiece (MP) containing vacuolated mitochondria (M). (x15,000)

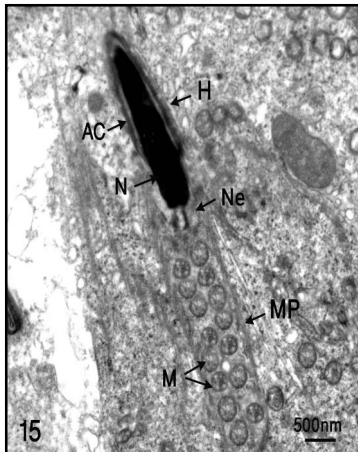


Fig. (15): Spermatozoon of control testis showing the head (H), formed of the nucleus (N) which occupies most of the head, composed of condensed chromatin and surrounded by the acrosomal cap (AC). The neck (Ne) is a very short segment connecting the head with the midpiece (MP) - the first part of the tail - which is characterized by the mitochondrial sheath formed of elongated mitochondria (M). (x15,000)

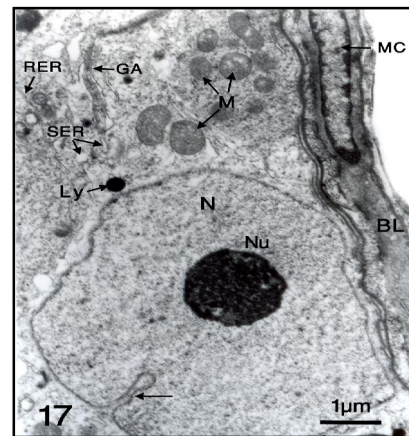


Fig. (17): Sertoli cell of control testis revealing irregularly shaped nucleus (N), devoid of heterochromatin, containing one prominent nucleolus (Nu), and enclosed with nuclear envelope that exhibiting a deep indentation (arrow). The cytoplasm containing mitochondria with distinct tubular cristae (M), cisternae of smooth endoplasmic reticulum (SER), few stacks of rough endoplasmic reticulum (RER), Golgi apparatus (GA) and lysosomes (Ly). Part of the basal lamina (BL), with myoid cell (MC) are noticed. (x7500)

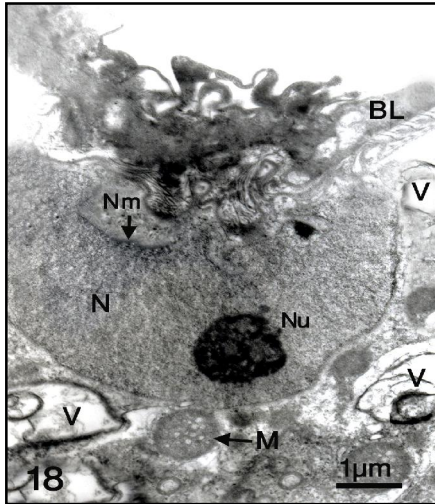


Fig.(18): Sertoli cell of treated testis illustrating the nucleus (N) with plate-like configuration, possessing nucleolus (Nu) and surrounded by irregular nuclear membrane (Nm). The cytoplasm containing deformed mitochondria (M) and large vacuoles (V). In addition, part of irregular, thickened basal lamina (BL) is also seen. (x7500)

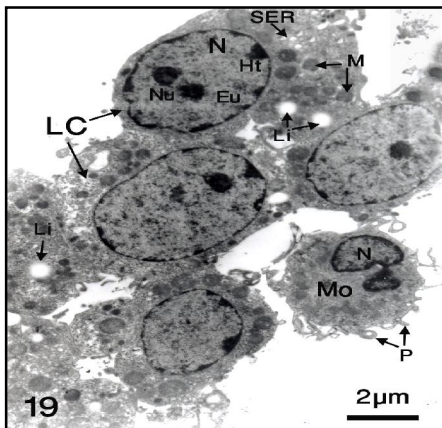


Fig.(19): Interstitial tissue of control testis revealing normal fine structure of Leydig cells (LC), characterized by cytoplasm containing mitochondria (M), smooth endoplasmic reticulum (SER) and lipid droplets (Li), in addition to oval to rounded nuclei (N) containing one or two nucleoli (Nu), heterochromatin (Ht) and euchromatin (Eu). A monocyte cell (Mo) is also noticed with its eccentric nucleus (N) and numerous small pseudopodia (P) extending from it. (x4000)

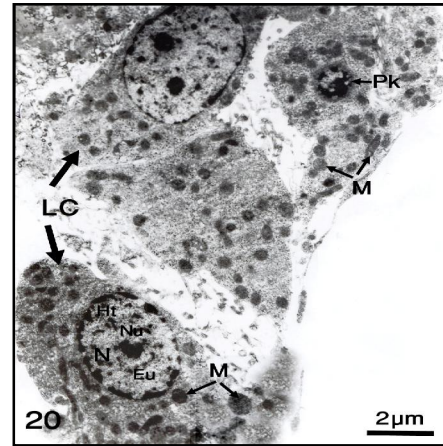


Fig.(20): Destroyed interstitial tissue from treated rat testis illustrating remarkable decrease in the number of Leydig cells (LC) which possess electron dense mitochondria (M), and nuclei (N) including electron dense nucleoli, euchromatin and heterochromatin, pyknotic nucleus is also seen. (x4000)

4. Discussion

Reproductive toxicology has been receiving interest and concern in recent years (**Mangelsdorf et al., 2003**). Recently, it has been shown that environmental chemicals may cause serious effects on human reproductive organs (**Shin et al., 2006**). One of such chemicals is ketoconazole, an imidazole derivative currently used in the medical fields as an antifungal drug with a broad spectrum of activity for the treatment of a number of superficial and systemic infections (**Schimmer and Parker, 2006**). Ketoconazole directly interferes with steroidogenesis by acting as potent inhibitors of steroidogenic enzymes and are known to cause endocrine disruption mainly via this mechanism (**Sanderson, 2006**).

The roles of the testis are producing fertile sperm for procreation and synthesizing and secreting steroid hormones for sexual and reproductive function. The testis specializes in de novo steroid production, which starts with the conversion of cholesterol to pregnenolone by CYP11A (cholesterol side-chain cleavage) that is bound to the inner membranes of the mitochondria in Leydig cells. Pregnenolone is converted to progesterone by 3-hydroxysteroid dehydrogenase (3-HSD), which is found in both mitochondria and smooth endoplasmic reticulum. Pregnenolone and progesterone form the precursors for all other steroid hormones (**Gingras et al., 2001; Simard et al., 2005**). Normal spermatogenesis depends on the testosterone secretion and gonadotropic hormonal action of Follicle-stimulating hormone (FSH) and Luteinizing

hormone (LH). FSH stimulates spermatogonial cells to develop into mature sperm, a process called spermatogenesis. FSH binds to FSH receptors on Sertoli cells and stimulates the cAMP-mediated second messenger pathway resulting in the activation of various factors required for successful spermatid production (MacLachlan *et al.*, 2002). While, LH binds to its receptor on the Leydig cell membrane, which is also coupled to the cAMP signaling pathway, to stimulate the production of testosterone de novo from cholesterol. In concert with the actions of FSH, this testosterone is required for optimal sperm production, as well as for sexual function. LH induces the various cytochrome P450 enzymes and dehydrogenases involved in testosterone synthesis in Leydig cells, including CYP17 17,20-lyase, the key activity directing the biosynthesis of steroids toward the sex hormones (Dharia *et al.*, 2004, Spaliviero *et al.*, 2004). Leydig, Sertoli, and germ cells further express low levels of aromatase, which converts testosterone originating from the Leydig cells into estradiol, a step that appears to be necessary for the successful inhibition of spermatogenesis and mitosis of spermatogonia (Carreau *et al.*, 2003).

Ketoconazole is reported by O'Connor *et al.* (2002) to inhibit testosterone biosynthesis by binding to the heme iron of cytochrome P450 isozymes of testosterone biosynthetic pathway. Therefore, Leydig cells are unable to produce sufficient quantities of testosterone. As a result, LH and FSH release from the pituitary are increased to stimulate testosterone production. Secondary to the decreases in testosterone, serum levels of DHT (dihydrotestosterone, the major metabolite of testosterone), and E₂ (estradiol, the aromatization product of testosterone) are also decreased. As a result of the decreased androgen levels, the weights for the androgen-dependent tissues are also decreased.

Ketoconazole steroidogenesis inhibition property has been physiologically studied in intact male rats following its oral administration for 15-days by O'Connor *et al.* (2002). They declared that, ketoconazole decreased weights of all androgen-dependent tissues and caused hormonal alterations. Also, Shin *et al.* (2006) studied the toxicity of ketoconazole in male rats post 28-days of oral administration. They also reported that ketoconazole showed reduction of epididymis and accessory sex organ weights, alteration of hormonal patterns including; decrease of testosterone and increases of estradiol, luteinizing hormone (LH) and follicular stimulating hormone (FSH).

These presently reported alterations included; thickening and irregularity of basal lamina, and degenerated changes of germ cells, Sertoli cells, and Leydig cells.

The basal lamina plays an important role in maintaining the structural and functional integrity of tissues. It provides structural stability of organs and sends signals to cells through cell surface receptors. Altered basal lamina structure has been associated with severe functional impairment of the testis. It contains several proteins including laminin, type IV collagen, various heparin sulfate proteoglycans and ectatin/nidogen. Type IV collagen is a major constituent of mammalian basal lamina that is secreted by myoid cells and Sertoli cells (Dobashi *et al.*, 2003). Interactions between Sertoli cells, myoid cells, Leydig cells, and germ cells are thought to be essential for spermatogenesis. Each of these interactions must be communicated through the basal lamina. Many reports have demonstrated that over-expression of the subtypes of type IV collagen correlates with abnormally thickened basal lamina and is related to spermatogenic dysfunction in human and other mammals (Mattias *et al.*, 2005).

Sertoli cells are essential for the development and maintenance of testicular functions (McLaren, 2000). Spermatogenesis is a complex and dynamic process that results in the continual production of spermatozoa. Sertoli cells are largely responsible for orchestrating the germ cells through sequential phases of mitosis, meiosis, and differentiation. Sertoli cells accomplish this task by providing hormonal, nutritional, and physical support (Mesbah *et al.*, 2008). Sertoli cells have been reported to be the targets for various toxicants (Krishnamoorthy *et al.*, 2005). Therefore, any agent that impairs the viability and function of Sertoli cells may have profound effects on spermatogenesis. Ketoconazole has been shown in the present study to injure Sertoli cells leading to disruption of their functions which are reflected on the development and maintaining of spermatogenesis.

Necrotic spermatogonia were observed in the present work after ketoconazole treatment. Spermatogonia were reported by De Rooij and Russell (2000) to be particularly vulnerable to toxicants and physical agents. In particular, because of their mitotic activity. They have three major roles; first, spermatogenesis is initiated via spermatogonia. Second, the population of germ cells is greatly increased via the mitotic activity of spermatogonia, as one spermatogonium on average goes through 8 to 9 divisions before differentiating into a spermatocytes. Third, regulation of germ cell numbers is accomplished in the spermatogonial population of cells. Thus, alterations of spermatogonia will be reflected on the development of the following stages of spermatogenesis.

Different defects in spermatocytes and spermatids post ketoconazole administration may be

due to the disturbances in the microenvironment of Sertoli cells, that affect the protein synthesis machinery essential for germ cell differentiation. Proteins necessary for the differentiation of germ cells are secreted at their highest rates in the testis during spermatid elongation and spermiation (**Manivannan et al., 2009**).

The changes observed in the spermatids as a result of ketoconazole administration may cause delayed spermiation as elucidated by **Yano and Dolder (2002)** post paracetamol treatment. These authors displayed that delayed spermiation is the consequence of the large quantity of residual cytoplasm that remains connected to the spermatids and may delay the entire process of spermiogenesis. With modifications of spermiogenesis, the resulting spermatozoa may be immature or functionally defective.

The abnormalities in the size of sperm head are the result of alterations in the pattern of chromatin condensation and/or in the development of the acrosome in the developing rounded spermatids (**Pinart et al., 1998**). Such malformed spermatozoa have a reduced ability to bind and penetrate the zona pellucida (**Pesch and Bergmann, 2006**).

The results of the present study showed a remarkable decrease in the number of Leydig cells after ketoconazole treatment. These results were consistent with **Mesbah et al., (2008)** who found pyknotic and severe depletion of Leydig cells following treatment with anabolic androgenic steroids. There is a close relationship between Leydig cells and blood vessels suggesting that these cells are at high risk of exogenous toxicants. Leydig cells are known to have receptors for LH that stimulates these cells to produce testosterone. Both LH and testosterone are responsible for normal spermatogenesis in male rats (**Dharia et al., 2004, Spaliviero et al., 2004**). Thus, depletion of LH receptors and decrease in peripheral LH post ketoconazole treatment result in the reduction of testosterone secretion.

In conclusion, the obtained results suggested that the testicular structural and ultrastructural alterations observed following ketoconazole administration may be responsible for the inhibition of the steroidogenesis. This decrease in steroidogenic activity has been suggested as the primary cause of spermiatic production failure. Therefore, it may be recommended that ketoconazole must be used only under strict medical follow up.

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5. References

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