Structural And Ultrastructural Alterations In Mammalian Adrenal Cortex Under Influence Of Steroidogenesis Inhibitor Drug

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Abstract: The risk of adverse human health effects due to endocrine-disrupting chemicals is of growing concern. In recent years, ketoconazole, an imidazole derivative has been developed and currently used in the medical fields as an anti-fungal and steroidogenesis inhibitor drug. The present study aimed to investigate the influence of ketoconazole in the structural and ultrastructural characteristics of albino rat adrenal cortex. Twenty adult male rats weighing 150-200 g. were divided into two even groups; group I were *i.p.* injected with 10mg/100g.b.wt. of ketoconazole dissolved in *1ml* physiological saline solution in a daily manner at 9am for 15 days. Whereas, group II were injected with *1ml* saline solution in the same manner. *Histologically*, adrenal cortex of treated rat displayed hypertrophy. Glomerulosa, fasciculata and reticularis cells were loaded with lipid droplets of variable sizes, occupying almost the cytoplasm thus displacing the nuclei eccentrically, which showed signs of pyknosis, karyorrhexis and karyolysis. *Ultrastructurally*, the three cortical zones displayed the presence of hypertrophied mitochondria filled with tightly packed tubular cristae, whereas the others having cavitaion results in a complete loss of cristae, and mitochondria are identified by the remainder cristae adjacent to the inner boundaries of the limiting membrane, in addition to extensive accumulation of variable sized lipid droplets and nuclei showing pyknosis and karyolysis. In conclusion, it is noticed that the destructive impacts of ketoconazole on the adrenocortical cells reflected on their functions leading to much deficiency in their performance. So, it should be taken in consideration and great concern that this drug must be utilized under restricted precautions in the medical fields to protect the human health from its hazardous impact.

1. Introduction:

The risk of adverse human health effects due to endocrine-disrupting chemicals is of growing concern. Despite that, the adrenal gland is an important organ yet it is neglectable in relation to effects of endocrine disruptors (Harvey *et al.,* 2007; Diamanti-Kandarakis *et al.,* 2009). This importance is being out from the fact that the adrenocortical zones synthesize and secrete steroid hormones, which fall into three major categories; mineralcorticoid, exemplified by aldosterone which is secreted by zona glomerulosa. Aldosterone is an important regulator of salt homeostasis and fluid balance and it is a major control unit of acid/base balance (Bielohuby *et al.,* 2007). While, zona fasciculata secrets gluco-corticoid, exemplified by cortisol, which is essential for life since it has a major role in responding to environmental stimuli; it decreases protein synthesis, thereby increasing the circulating level of amino acids; it elevates blood glucose by stimulating the enzymes involved in gluconeogenesis in the liver and it mobilizes fatty acids and glycerol from adipose cells. It has also anti-inflammatory effects (Fawcett and Jensh, 2002; Campbell, 2005). By zona reticularis, small amounts of androgens are secreted.

The two principal adrenal androgens are; androstenedione and dehydroepi-androsterone, which is far less potent than testosterone and has little physiological significance. Both hormones can serve as substrates for the conversion into testosterone and estradiol (Fawcett and Jensh, 2002; Keegan and Hammer, 2002). The hormone secretions of the adrenal cortex is dependent on steroidogenic cytochrome P450 (CYP) enzymes which possibly can be the target of endocrine-disrupting chemicals affecting them (Ohlsson *et al.,* 2010).

One of the groups that effects CYPs is the imidazoles, which are medically used as antifungal treatment. Of the imidazoles in current clinical use, ketoconazole which is a broad-spectrum anti-fungal and steroid hormones biosynthesis inhibitor drug (Marty *et al.,* 2001; Schimmer and Parcker, 2006*).* It has great activity against a variety of yeast, dimorphic fungal, fungal infections of the gastro-intestinal tract, dermatophytic infections of the skin and has been widely used in immunocompromised patients such as those with AIDS or those on chemotherapy (Vertzoni *et al.,* 2006*).* Also, it has 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).

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).

In the field of medicine, the great ability of ketoconazole to inhibit mineralcorticoid synthesis is used for palliative treatment of primary hyper-aldosteronism (Lionakis *et al.,* 2008). Also, in a treatment strategy of resistant and hyper-cortisolemic depressive patients as an inhibitor of glucocorticoid synthesis (Brown *et al.,* 2001; Dvorak, 2011). As well as, in treatment of ACTH-secreting adenomas, palliative treatment of Cushing disease, adrenal tumors, adrenocortical carcinoma and ectopic corticotrophin production by small-cell lung carcinoma or carcinoid tumors (Gordon, 2007; Lionakis *et al.,* 2008).

Ketoconazole as inhibitor of androgen has been used for treatment of prostate cancer with promising results (Peehl *et al.,* 2001; Kinobe *et al.,* 2006; Liebertz and Fox, 2006).

Also, physicians use high-dose ketoconazole in women with advanced breast cancer resistant to conventional chemotherapy and for ovarian hyperandrogenism syndrome, including polycystic ovarian syndrome and hyperthecosis with considerable improvement in acne, hirsutism, and amenorrhea (Lionakis *et al.,* 2008).

In experimental animals, impact of ketoconazole has been studied in some biological aspects on different body organs rather than the adrenal gland (Rodriguez and Buckholz, 2003*;* Braddock, 2003; Amin and Hamza*,* 2005; Furukawa *et al.,* 2008).

It is clearly noticed from the previous literature, that ketoconazole has been widely utilized in the medical fields for the treatment of different types of diseases. But, unfortunately there is no attention for the influence of its administration on the adrenal cortex which is responsible for synthesis and secretion of different steroid hormones. Thus, the present study aimed to throw light on the influence of ketoconazole on adrenal cortical tissues from the histological and ultrastructural point of view
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2. Materials and Methods:
2.1. Experimental animals

Twenty male Swiss 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 were kept under controlled environmental conditions, including a temperature of 25^0C and a 12-h light/darkness cycle.

2.2. Drug used

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 Ketoconazole which is manufactured by JANSSEN-CILAG Pharmaceutica 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 were injected with *1ml* 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 adrenal glands were fixed in Bouin`s fluid for 24 hours, then subjected to the normal procedures for paraffin sectioning. Sections, of 4-6 μm were stained with haematoxylin & Eosin, dehydrated, cleared in xylene and mounted in DPX. The stained sections were examined and photographed by light microscopy (BX-40 Olympus), fitted with 4x - 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 adrenal glands were cut into small blocks ($1{\times}1mm^3$), 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^oC, semithin sections (0.5 μm) were stained with 1% toluidine blue in 1% sodium borate and examined with light microscope. Areas of cortical cells 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 (Haematoxylin and eosin preparations)
3.1.1. Control adrenal cortex

Figure (1) showing the three zones of adrenal cortex; zona glomerulosa, fasciculata and reticularis, respectively. As shown in figure (2), the adrenal gland is surrounded by a fibrous connective tissue capsule. Zona glomerulosa is formed of columnar or rather pyramidal cells arranged in glomeruli-like structure, which are separated by delicate trabeculae extending from the capsule. Its cells contain acidophilic cytoplasm with fairly large rounded to oval basophilic nuclei having distinct nucleoli (Fig. 2).

Zona fasciculata is composed of polyhedral or columnar cells arranged in one or two cell thick in long radial cords or fasciculae and they are separated by narrowed blood capillaries lined with endothelial cells. The cells have granulated eosinophilic cytoplasm embodying spherical basophilic nuclei showing distinct nucleoli. Binucleate cells are seen frequently (Fig. 3).

Zona reticularis is characterized by an irregular anastomosing network of intermingled cords separated by numerous wide blood sinusoids lined with endothelial cells. The cells of these cords are columnar cells having moderately eosinophilic cytoplasm, containing certain discrete granules and have rounded basophilic nuclei possessing centrally located nucleoli (Fig. 4).

3.1.2. Ketoconazole-treated adrenal cortex

Generally, the adrenal gland showed enlargement in size with its outer cortex showing hypertrophy as seen in figure (5). The fibrous connective tissue capsule being thickened with increased fibrous elements (Fig. 6).

Glomerulosa, fasciculata and reticularis cells exhibiting hypertrophy with accumulated variable sized lipid droplets in their cytoplasm. Some of these lipid droplets fused together and occupied almost the entire cytoplasm, thus displacing the nuclei eccentrically which showing clear signs of pyknosis, karyorrhexis and karyolysis as clearly observed in figures (6-8).

3.2. Ultrastructural Studies

3.2.1. Control adrenal cortex

Fine structure of zona glomerulosa cells reveal different mitochondrial configuration varying from oval to spherical shapes with a specific tubulo-saccular cristae. In addition, a fair amount of smooth endoplasmic reticulum, small Golgi vesicles and abundant number of lipid droplets are evident. The nuclei of these cells are rounded or oval in shape; sometimes wavy in appearance ensheathed by double nuclear envelopes and possessing nucleoli, peripheral dense heterochromatin and homogenous euchromatin material (Figs. 9-11).

Figures (12-14) exhibit the fine characteristic features of fasciculata cells including; abundance of rounded mitochondria with obvious tubular cristae, smooth endoplasmic reticulum in the form of branching tubules, scanty rough endoplasmic reticulum, fair amount of lysosomes and richness of lipid droplets. The nuclei are large, rounded, possessing prominent nucleoli, dense peripheral heterochromatin, lightly stained euchromatin and surrounded by double nuclear membranes. Blood capillaries lined with endothelial cells are noticed between these fasciculata cells (Fig. 12).

Zona reticularis cells are distinguished by their richness of rounded mitochondria with intensely tubular cristae, smooth endoplasmic reticulum, lysosomes and lipid droplets with varying sizes. Their nuclei are spherical or ovoid in shape contained condensed heterochromatin, euchromatin and prominent nucleoli (Figs. 15-17). Widened and clear blood sinusoids lined with endothelial cells are manifested in figure (15).

3.2.2. Ketoconazole- treated adrenal cortex

Marked ultrastructural changes of zona glomerulosa cells are illustrated in figures (18-20); their cytoplasm contain hypertrophied mitochondria with more electron dense matrices and some of them possessing small vacuolar degenerations, in addition to lysosomes and lipid droplets of variable sizes. The nuclei being electron dens, showing shrinkage, and signs of pyknosis. They are surrounded by irregular nuclear envelopes and containing electron dense nucleoli, heterochromatin and euchromatin.

Zona fasciculata cells showing hypertrophied mitochondria filled with tightly packed tubular cristae, some of them having cavitaion and finally these mitochondria are identified only by the remainder cristae adjacent to the inner boundaries of the limiting membrane. Extensive accumulation of various sized lipid droplets, some of them became so large thus occupying almost the entire cytoplasm, masking the organelles and distending the cells. Fair amounts of lysosomes are seen, beside, the nuclei which displayed signs of pyknosis and karyolysis. Some

blood cells are shown in between fasciculata cells (Figs. 21-23).

Similarly, zona reticularis cells having hypertrophied mitochondria with some of them showing ruptured mitochondrial membrane, degenerated cristae and cavitation, in addition to accumulated lipid droplets of different sizes, and pyknotic nuclei surrounded by irregular nuclear membrane and containing electron dense heterochromatin and euchromatin. As well as, blood sinusoids containing stagnant blood cells are observed (Figs. 24-26).

It is worthy to mention that smooth endoplasmic reticulum, was scanty, sometimes almost absent in all examined cells of the adrenocortical zones, suggesting that it may be disintegrated under the influence of ketoconazole.

An interesting observation is seen in the resulted electron micrographs, that there are dense particles participated allover the cells of these three zones, which may have occurred as a result of a chemical reaction between ketoconazole and the chemical components of the cells.
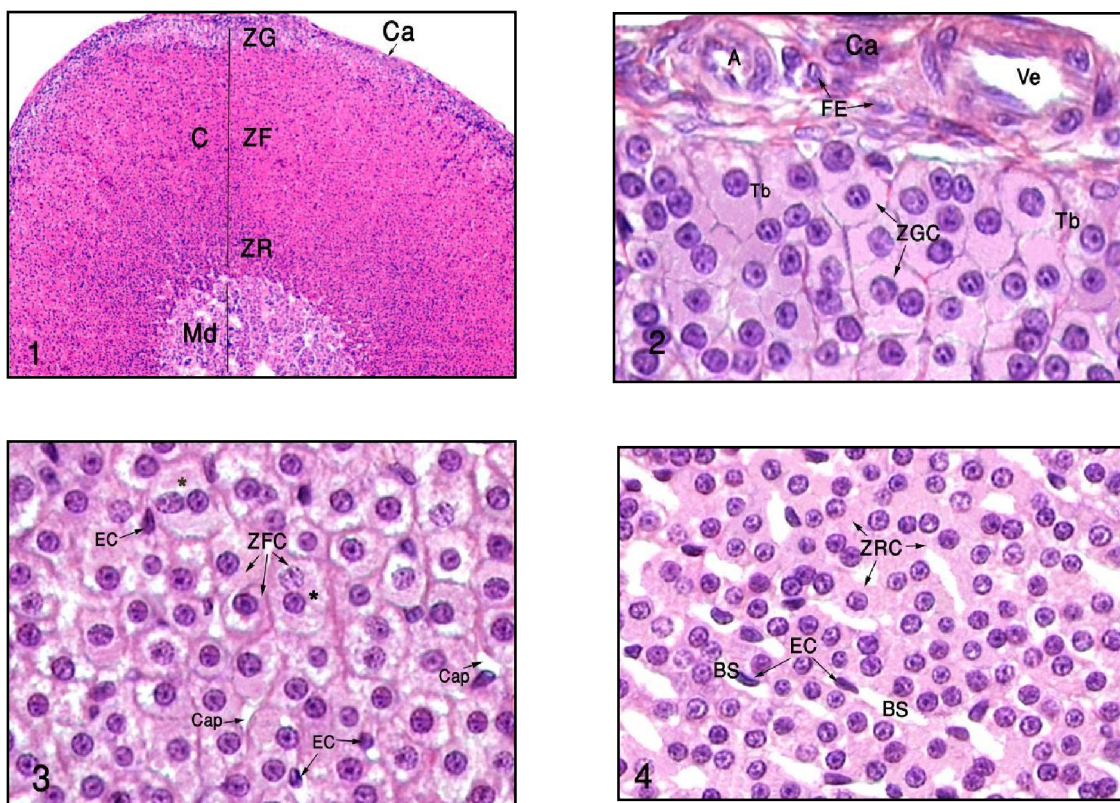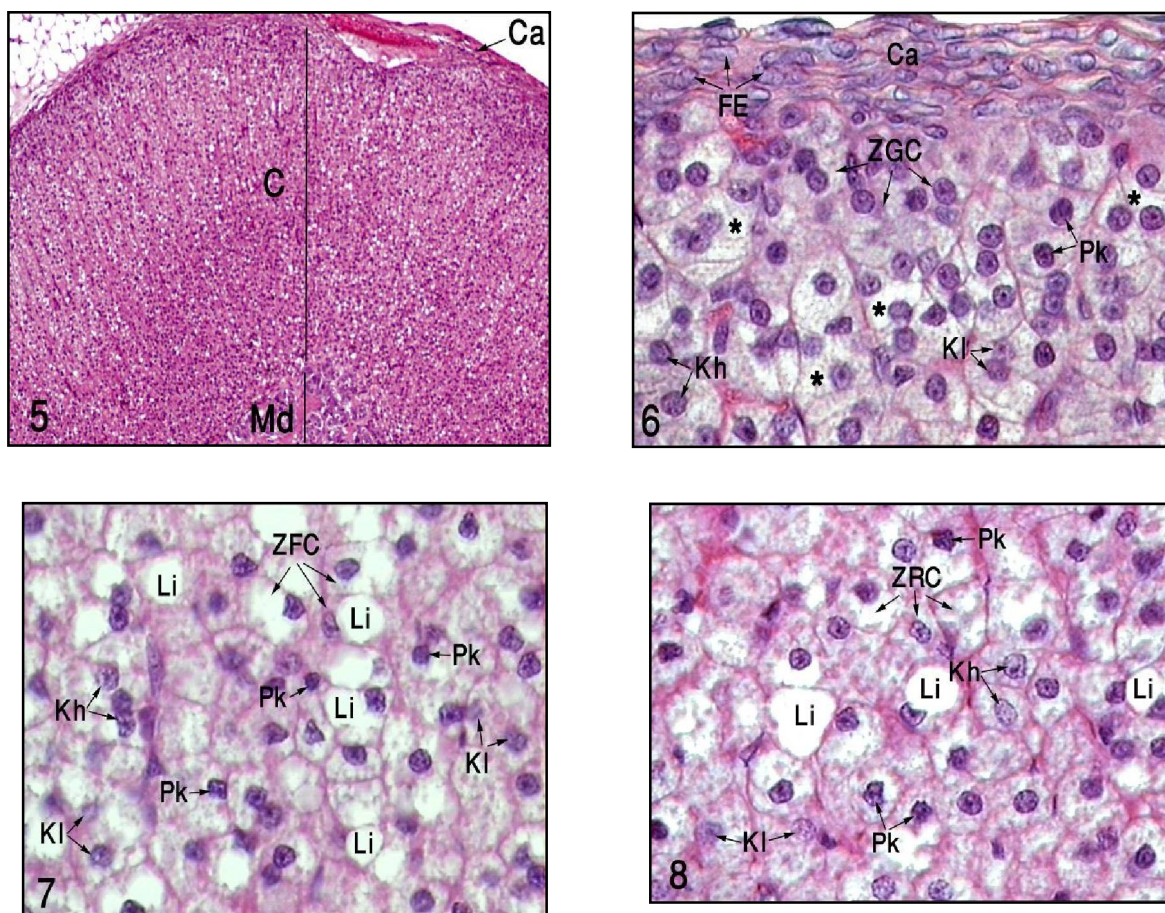

Figures 1-4: Light micrographs of H&E stained sections of control adrenal gland.
Figure 1: General structure of adrenal gland illustrating the capsule (Ca), the cortex (C) which is differentiated into zona   glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR), and the medulla (Md) (x132).
Figure 2: Glomerular organization of zona glomerulosa cells (ZGC), being separated by trabeculae (Tb) extended from the capsule (Ca), which is formed of fibrous elements (FE), an arteriole (A) and a veinule (Ve) (x1320).
Figure 3: Zona fasciculata cells (ZFC) arranged in long radial cords, separated by narrowed blood capillaries (Cap) lined with endothelial cells (EC). Binucleate cells (*) are also seen (x1320).
Figure 4: Zona reticularis cells (ZRC) arranged in irregular network of intermingled cords, separated by numerous wide blood sinusoids (BS) lined with endothelial cells (EC) (x1320).

Figures 5-8 Light micrographs of H&E stained sections of ketoconazole-treated adrenal gland.

Figure 5: Adrenal gland with an enlarged outer cortex (C), an inner medulla (Md) and a thick fibrous capsule (Ca) (x132).

Figure 6: Hypertrophied glomerulosa cells (ZGC), containing lipid droplets (*) and their nuclei showing signs of pyknosis (Pk), karyorrhexis (Kh) and karyolysis (Ki), in addition to part of thickened capsule (Ca) with increased fibrous elements (FE) (x1320).

Figure 7: Hypertrophied fasciculata cells (ZFC), overloaded with lipid droplets (Li) of variable size and possessing necrotic nuclei revealing signs of pyknosis (Pk), karyorrhexis (Kh) and karyolysis (Kl) (x1320).

Figure 8: Enlarged reticularis cells (ZRC) containing lipid droplets (Li) with different sizes and necrotic nuclei revealing pyknosis (Pk), karyorrhexis (Kh) and karyolysis (Kl) (x1320).
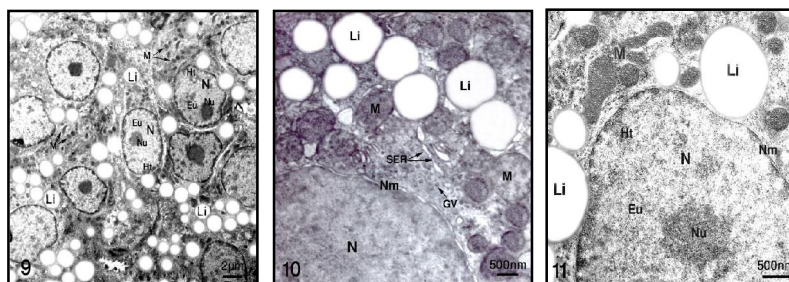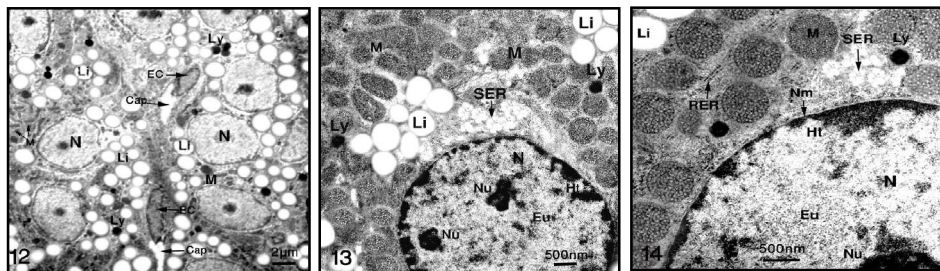

Figures 9-11: Transmission electron micrographs of control zona glomerulosa.

Figure 9: Zona glomerulosa cells displaying numerous lipid droplets (Li), mitochondria (M) and oval or round shaped nuclei (N) (x2000).

Figure 10: Glomerulosa cell having mitochondria (M) with tubulo-saccular cristae, smooth endoplasmic reticulum (SER), small Golgi vesicles (GV), lipid droplets (Li) and part of the nucleus (N) ensheathed by a double nuclear envelope (x10,000).

Figure 11: Another glomerulosa cell possessing mitochondria (M) with tubulo-saccular cristae, lipid droplets (Li) and part of the nucleus (N) ensheathed by a double nuclear envelope and involving nucleolus (Nu), marginated dense clumps of heterochromatin (Ht) and homogenous euchromatin (Eu) (x12,000).
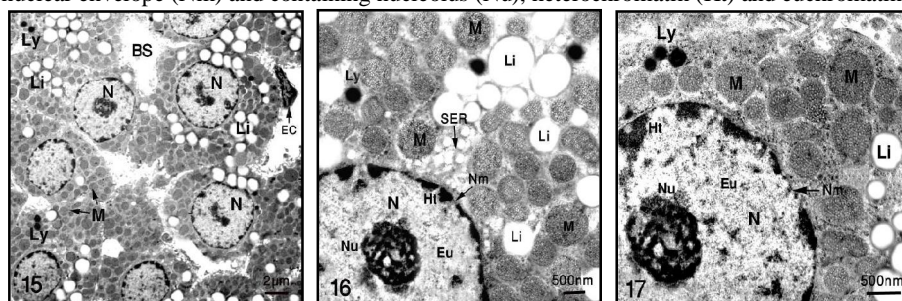
Figures 12-14: Transmission electron micrographs of control zona fasciculata.
Figure 12: Zona fasciculata cells loaded with lipid droplets (Li), mitochondria (M), lysosomes (Ly), and possessing oval to rounded nuclei (N). In between them, narrowed blood capillaries (Cap) lined with endothelial cells (EC) are noticed (x2000).
Figure 13: Fasciculata cell containing mitochondria (M) with tubular cristae, smooth endoplasmic reticulum (SER), lysosomes (Ly), lipid droplets (Li), rounded nucleus (N) containing nucleoli (Nu), peripheral heterochromatin (Ht) and euchromatin (Eu) (x10,000).
Figure 14: Another part of fasciculata cell revealing rounded mitochondria (M) with tubular cristae, smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER), lysosomes (Ly), lipid droplets (Li), nucleus (N) surrounded by double nuclear envelope (Nm) and containing nucleolus (Nu), heterochromatin (Ht) and euchromatin (Eu) (x12,000).
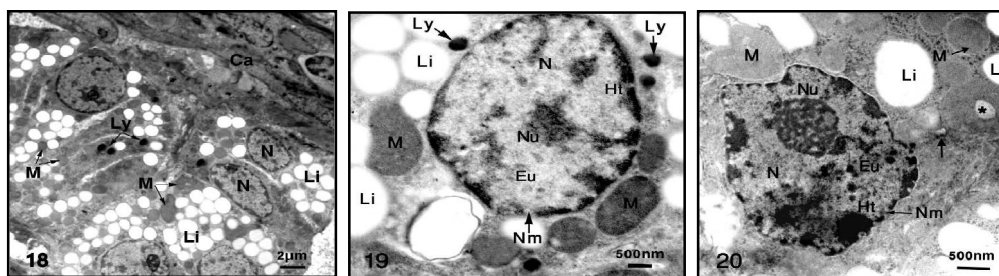

Figures 15-17: Transmission electron micrographs of control zona reticularis.
Figure 15: Reticularis cells containing numerous mitochondria (M), lipid droplets (Li) and spherical or ovoid nuclei (N). Widened blood sinusoids (BS) lined with endothelial cell (EC) are obviously seen (x2000).
Figure 16: Reticularis cell having mitochondria (M), smooth endoplasmic reticulum (SER), lysosomes (Ly), lipid droplets (Li) and nucleus (N) which is surrounded by nuclear membrane (Nm) and possessing distinct nucleolus (Nu), peripheral heterochromatin (Ht) and euchromatin (Eu) (x10,000).
Figure 17: Another reticularis cell possessing nucleus (N) containing distinct nucleolus (Nu), peripheral heterochromatin (Ht) and euchromatin (Eu) and is surrounded by nuclear envelope (Nm), in addition to numerous rounded mitochondria (M), aggregation of three lysosomes (Ly) and lipid droplets (Li) (x12,000).
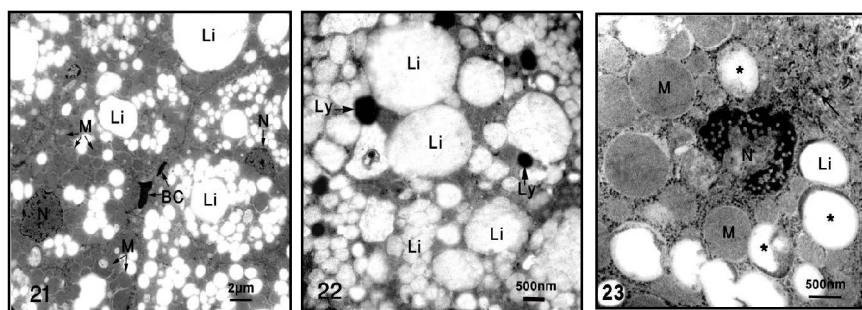

Figures 18-20: Transmission electron micrographs of ketoconazole-treated zona glomerulosa.
Figure 18: Zona glomerulosa cells having mitochondria (M), lipid droplets (Li), lysosomes (Ly) and deformed nuclei (N), in addition to deformed capsule (Ca) (x2000).
Figure 19: Glomerulosa cell illustrating electron dense hypertrophied mitochondria (M), lysosomes (Ly), lipid droplets (Li) and nucleus (N) surrounded by nuclear membrane (Nm) and containing electron dense peripheral heterochromatin (Ht) and euchromatin (Eu) (x10,000).
Figure 20: Another glomerulosa cell exhibiting hypertrophied mitochondria (M), some of them having vacuolar degeneration (*), lipid droplets (Li), dense particles participate (arrow   ) through the matrix and pyknotic nucleus (N), being shrunken ensheathed by irregular nuclear membrane (Nm) and having electron dense heterochromatin (Ht) and euchromatin (Eu) (12,000).
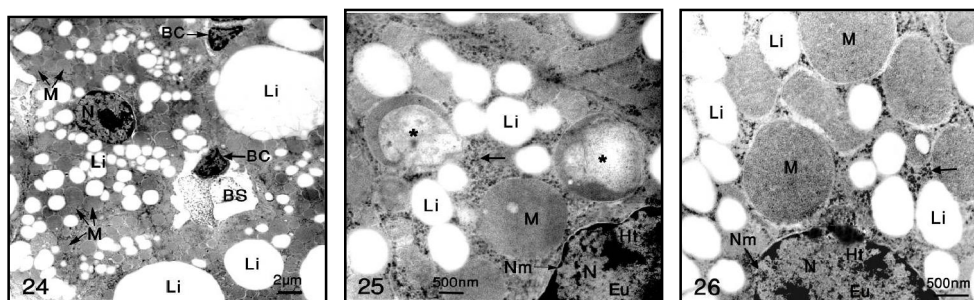
Figures 21-23: Transmission electron micrographs of ketoconazole-treated zona fasciculata.
Figure 21: Zona fasciculata cells overloaded with lipid droplets (Li) of variable sizes, in addition to deformed mitochondria (M) and pyknotic nuclei (N). Blood cell (BC) are seen (x2000).
Figure 22: Fasciculata cell exhibiting progressive accumulation of various sized lipid droplets (Li), in addition to numerous lysosomes (Ly) (x10,000).
Figure 23: Another fasciculata cell revealing hypertrophied mitochondria (M), some of them possessing cavitation with progressive loss of their cristae (*), in addition to lipid droplets (Li), dense particles participate (arrow   ) through the matrix and nucleus (N) with signs of karyolysis (X12,000).

Figures 24-26: Transmission electron micrographs of ketoconazole-treated zona reticularis.
Figure 24: Zona reticularis cells having deformed mitochondria (M), lipid droplets (Li) of different sizes and deformed nuclei (N), in addition to blood sinusoids (BS) containing blood cell (BC) (x2000).
Figure 25: Reticularis cell containing hypertrophied mitochondria (M), some of them showing cavitation with ruptured limiting membranes and degenerated cristae (*), in addition to several lipid droplets (Li), dense particles participate (arrow   ) through the matrix and part of the nucleus (N) surrounded by nuclear membrane (Nm) and containing electron dense heterochromatin (Ht) and euchromatin (Eu) (x10,000).
Figure 26: Another reticularis cell having extensive accumulation of hypertrophied mitochondria (M) and lipid droplets (Li), in addition to dense particles participate (arrow   ) through the matrix and   nucleus (N) possessing electron dense heterochromatin (Ht) and euchromatin (Eu) and surrounded by nuclear envelope (Nm) (X12,000).

4. Discussion

There is increasing evidence that various chemicals introduced into the environment have the potential to disrupt the endocrine system in humans and wildlife. Increasingly, the enzymes involved in the steroid biosynthesis pathway are being recognized as important targets for the actions of the endocrine-disrupting chemicals. Steroidogenic enzymes are responsible for the biosynthesis from cholesterol of various steroid hormones including glucocorticoids, mineralcorticoids, androgens, and estrogens. They consist of several specific cytochrome P450 enzymes (CYPs), hydroxysteroid dehydrogenases (HSDs), and steroid reductases (Sanderson, 2006).

In recent years, a number of imidazole derivatives have been developed and used as an anti-fungal agents. Ketoconazole is one of the imidazole derivatives currently used in the clinical fields. It is an effective inhibitor of adrenal and gonadal steroidogenesis (Schimmer and Parker, 2006). For this property, it has been used for the treatment of different types of diseases including; Cushing disease, hyper-cortisolemic depressive patients, adrenal tumors, adrenocortical carcinoma, adrenal adenomas, prostate cancer, advanced breast cancer and various cancer cell lines such as hepatic metastasis and pulmonary metastasis (Lionakis *et al.,* 2008).

The majority of severe degenerative changes induced in the adrenal gland following pathological disorders are often judged by the physiological tools. Steroidogenesis inhibition of ketoconazole has also been studied in experimental animals by O`Connor

et al., (2002), which evaluated a 15-days screening assay using intact male rats for identifying physiologically steroid biosynthesis inhibition of ketoconazole, also Shin *et al.* (2006) elucidated a 28-days repeated dose toxicity study of ketoconazole in rats. These authors reported in their interesting researches that ketoconazole caused increase of adrenal's weights, reflecting on the level of these hormones; decrease of testosterone, increase of estradiol, luteinizing hormone (LH) and follicular stimulating hormone (FSH). They suggested that ketoconazole should be identified as an impairment of endocrine-related compound.

The adrenal gland is the most important steroidogenic tissue in the human body and essential for survival. All steroidogenic processes take place in the adrenal cortex (Bielohuby *et al.,* 2007). The adrenal cortex, and in particular zona fasciculata has been reported by Rosol *et al.* (2001) to be among the most common site lesions in the endocrine system. The factors which predispose this organ to such lesions include: its disproportionately large blood supply per unit mass; its high content of lipids and the susceptibility of its unsaturated fatty acids to peroxidation damage; and its high levels of cytochrome P450 which metabolize xenobiotics to reactive intermediates. In addition, the adrenal expresses several of the pathways for steroid production present in the testes and ovaries. Therefore, toxic chemicals can affect the adrenal or its axis directly or indirectly in a manner similar to the testes and ovaries.

The present study throws the light on the impact of ketoconazole on adrenal cortex from the histological and ultrastructural point of view, because these types of studies did not receive marked attention in spite of its importance to characterize lesions that may suppress the function of the adrenocortical cells.

In the present investigation, ketoconazole is found to have destructive structural and ultrastructural alterations in rat adrenal cortex. The most striking change is the enlargement and extensive accumulation of lipid droplets throughout the cytoplasm of the three zones cells. This alteration was also reported in the influence of chronic treatment with aminoglutethimide, an inhibitor of the cholesterol enzyme $P450_{SCC}$ (Szabo *et al.,* 1996), congenital lipoid adrenal hyperplasia in humans and mice, a disorder caused by hereditary deficiency of the StAR protein (Miller and Strauss, 1999), hormone-sensitive lipase (HSL) deficiency in mice (Li *et al.,* 2002) and after chemotherapy administration (Hermenean *et al.,* 2008).

In this work, distinct alterations are evidenced in the mitochondria displayed by hypertrophy with degeneration of the cristae resulting in cavitation of their matrix. These lesions are not reported previously in rat adrenal cortex. In our opinion this hypertrophy and cavitations in mitochondria probably resulted from inhibition of the conversion of cholesterol to pregnenolone, cholesterol may accumulate within the mitochondria. Consequently, it undergo considerable hypertrophy and vacuolation. Also, similar results were observed by Ishihara *et al.* (1974) in human adrenal cortex in Cushing's syndrome. They explained that the intra-mitochondrial vacuoles might be associated with the deposition of steroids or their related compounds in the cristae.

The profound lesions observed in mitochondria and smooth endoplasmic reticulum might be sufficient to cause impairment of steroid synthesis in accordance with Guerrero *et al.* (2010), who reported that these organelles play a great role in steroidogenesis within the cortex, though, they involve in the coordinated actions of cytochrome P450 and the enzyme 3 -hydroxysteroid dehydrogenase (3 HSD), which are distributed between the mitochondria and the smooth endoplasmic reticulum. The rate-limiting step in steroid hormone biosynthesis is the translocation of substrate cholesterol from the outer mitochondrial membrane to cholesterol side-chain cleavage enzyme (CYP11A), the first enzyme in the steroidogenic pathway, which is located inside the mitochondria as Rainey *et al.* (2004) and Isola *et al.* (2010) elucidated in their interesting studies.

It is well known that, adrenocortical cells require constant supply of cholesterol as a precursor for the conversion of steroid hormones. Cholesterol delivery in the adrenal glands involves three major processes: uptake of lipoprotein-derived cholesterol via low density lipoprotein receptor (LDLR) mediated endocytic pathways and scavenger receptor class B member 1 (SCARB1)-mediated "selective" uptake pathways; endogenous cholesterol biosynthesis in endoplasmic reticulum; and cholesterol mobilization from intracellular cholesterol esters (CEs) stored in lipid droplets (Kraemer, 2007). The delivered CEs should be hydrolyzed to be utilized for steroidogenesis by non-lysosomal neutral lipases. Therefore, CE hydrolysis plays a pivotal role not only in the break down of stored lipids but also in the lipoprotein uptake and utilization. The resultant unesterified cholesterol is transported to mitochondria by the steroidogenic acute regulatory protein (StAR), where it is converted into the different steroid hormones by a battery of oxidative enzymes (Miller, 2007).

Ketoconazole was found to inhibit cholesterol synthesis in a dose-dependent fashion by blocking

conversion of lanosterol to cholesterol. Other lipid-modifying properties of ketoconazole include decreasing of lipoprotein lipase and 3-hydroxy-3-methylglutaryl coenzyme A reductase activities, inhibition of intestinal cholesterol absorption and bile acid synthesis, and upregulation of LDL-C receptor activity (Lionakis *et al.,* 2008).

Ultimately, It seems, that the impaired steroidogenesis is an important mechanism of toxicity in the adrenal cortex. It may have occurred due to disruption of cytochrome P450 enzymes, accordingly the cholesterol biosynthesis will be suppressed. This will lead to the accumulation of lipid droplets that seen in the electron micrographs, indicating that the target organ of ketoconazole on adrenocortical cells are mitochondria since the treated animals had destructive mitochondria on which cytochrome P450 enzymes are enclosed.

In conclusion, it is noticed now that the destructive impacts of ketoconazole on the adrenocortical cells reflected on their functions leading to much deficiency in their performance. So, it should be taken in consideration and great concern that this drug must be utilized under restricted precautions in the medical fields to protect the human health from its hazardous impact.

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