

Histological and Ultrastructural Changes in Mammalian Testis under the Effect of Hydrocortisone

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Abstract: Hydrocortisone is a synthetic glucocorticoid currently utilized in the medical fields for the treatment of various types of diseases. The present study aimed to investigate the histological and ultrastructural changes induced in mammalian testis under the effect of hydrocortisone. Twenty adult male rats weighing 150-200g were divided into two groups; group I, injected i.m. with hydrocortisone sodium succinate (30mg/100g b.wt.) , daily for 15 days. Whereas, group II were kept as control. (injected with 0.6ml of bacteriostatic water). Histologically, testes of treated rats displayed thickening of tunica albuginea, disruption of spermatogenesis evident, marked reduction in germ cells caused dilatation of intercellular spaces, detachment of Sertoli cells from the irregular basal lamina, in addition to necrotic Leydig cells with infiltration of the interstitial tissues. Ultrastructurally, treated testes showed thickening and irregularity of the surrounding basal lamina, cytoplasmic vacuolation of atrophied Sertoli cells, shrinkage and pyknotic nuclei of spermatogonia and primary spermatocytes, condensed Golgi apparatus and detachment of the acrosomal granule from the anterior hemisphere of the nucleus of rounded spermatids, and disappearance of elongated spermatids and spermatozoa. Also, necrotic Leydig cells were observed in interstitial tissue. In conclusion, hydrocortisone administration into adult male rats exerts a clear effect on testicular structure and ultrastructure, which leads to much deficiency in their performance. So, it should be utilized under restricted precautions in the medical fields to protect the human health from its hazardous impact.

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1. Introduction:

Hydrocortisone is a synthetic glucocorticoid widely administered for the treatment of various types of diseases. It significantly reduced specific physical problems in prostate cancer patients and improved their emotional state (Kornblith *et al.*, 2001), it is used for the treatment of severe liver failure (Harry *et al.*, 2003), acute graft rejection in most forms of organ transplantation, some neoplastic conditions, collagen diseases, dermatological diseases, status asthmatics, allergic and anaphylactic reactions (Rigge and Jones, 2005), mild ulcerative colitis (Schimmer and Parker, 2006), acute pancreatitis (Wang *et al.*, 2007) and as bastion of control the brain swelling before, during, and after neurosurgical procedures, as well as during radiation and chemotherapy in the brain tumor patients (Da Silva and Schiff, 2007).

Jung and Inder (2008) reported that hydrocortisone administration is recommended in a wide scale for patients in the event of stress, severe illness, or surgical procedures; including minor surgery (i.e., hernia repair, laparoscopic cholecystectomy and knee surgery), moderate surgical stress (i.e., open cholecystectomy, partial colon resection, uncomplicated back surgery and hip

replacement), and major surgical stress (i.e., pancreateo-duodenectomy, esophagectomy, total colectomy, repair for perforated bowel, cardiopulmonary bypass, ileofemoral bypass and oral surgical stress).

Also, hydrocortisone is the drug of choice for glucocorticoid replacement therapy in adrenal insufficiency diseases (Mah *et al.*, 2004; Salvatori, 2005; Nieman *et al.*, 2006), it is also used in the treatment of congenital adrenal hyperplasia, a group of inherited disorders in which 21-hydroxylase enzyme involved in the biosynthesis of corticosteroids is deficient resulting in low production of cortisol or aldosterone (Schimmer and Parker, 2006) and for the treatment of acute adrenal crisis (Bornstein, 2009).

In 2010, Romer *et al.* elucidated that hydrocortisone has been shown to affect declarative memory. Recently, Wirth *et al.* (2011) declared that intravenous hydrocortisone administration to depressed patients produced mixed effects on mood and emotional processing.

In experimental animals, involvement of hydrocortisone has been studied in some biological aspects on different body organs rather than the testis (Nosenko and Mishunina, 2005; Mantzoros *et al.*,

2006; Tariq *et al.*, 2007; Li *et al.*, 2008; Casella *et al.*, 2010).

These studies indicate that almost no attention has been given for the influence of hydrocortisone administration on mammalian testicular tissues. Therefore, the present study aimed to investigate the effect of hydrocortisone on adult rat testis from the histological and ultrastructural point of view.

2. Materials and Methods:

2.1. Experimental animals

Twenty male Swiss albino rats (*Rattus norvegicus*) weighing 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, milk and water *ad-libitum*. They were kept under controlled environmental conditions, including a temperature of 25°C and a 12h light/dark cycle.

2.2. Drug used in an experiment.

Hydrocortisone Sodium Succinate available in Egypt under trade name; Solu-Cortef® in the form of 100mg/2ml bacteriostatic water for injection which is manufactured by EGYPTIAN INT. PHARMACEUTICAL INDUSTRIES (E.I.P.I.CO., under Licence of UPJOHN s.a. Puurs-Belgium). It is a highly water-soluble sodium succinate ester of hydrocortisone (Schimmer and Parker, 2006).

2.3. Experimental design

The rats were divided randomly into two even groups; Group I, were intramuscularly injected with hydrocortisone sodium succinate, with a dose equivalent to 30 mg/100g.b.wt. Dissolved in 0.6 ml of bacteriostatic water in a daily manner at 9am for 15 days. This dose was determined in accordance to the dose utilized in previous researches of experimental rats (Bogdanov and Yarushkina, 2004, 2006 & 2007; Yarushkina, 2008). Whereas, Group II (control), were intramuscularly injected only with 0.6 ml of bacteriostatic water in the same manner as group I.

2.4. Histological preparations

The excised testes were fixed in Bouin's fluid for 24 hours, were subjected to the normal procedures for paraffin sectioning, cut at the thickness of 4-6 µm 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 testes 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 study

Testes of control rats showed normal features of testicular tissue as illustrated 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 the 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.

Sections of testicular tissues obtained from hydrocortisone-treated rats displayed several histopathological changes as illustrated in figures (4-6). The seminiferous tubules surrounded by thickened tunica albuginea, showing deformed Sertoli cells,

being detached from the irregular basal lamina, marked reduction in the germ cells causing dilatation intercellular spaces, spermatogonia manifest vacuolated cytoplasm and pyknotic nuclei, primary spermatocytes reveal pyknotic nuclei, and the rounded spermatids having karyolytic nuclei. The effects are much severe in spermatid differentiation, whereas there is a complete loss of elongated

spermatids and accordingly of spermatozoa, which means that the spermatogenesis was arrested at the stage of rounded spermatids formation under the effect of hydrocortisone treatment.

The interstitial tissues between seminiferous tubules are infiltrated and the Leydig cells have pyknotic nuclei as clearly seen in figure (6).

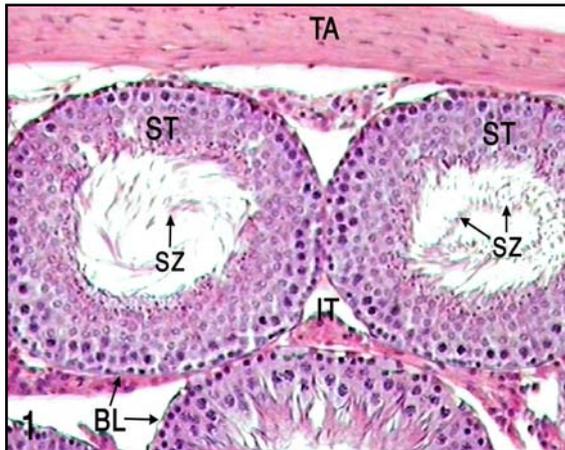


Figure (1): Normal testicular tissue architecture of control testis revealing tunica albuginea (TA), seminiferous tubules (ST) ensheathed with basal lamina (BL) and containing spermatozoa (SZ) in their lumens, in addition to the interstitial tissues (IT). (H&E, x330)

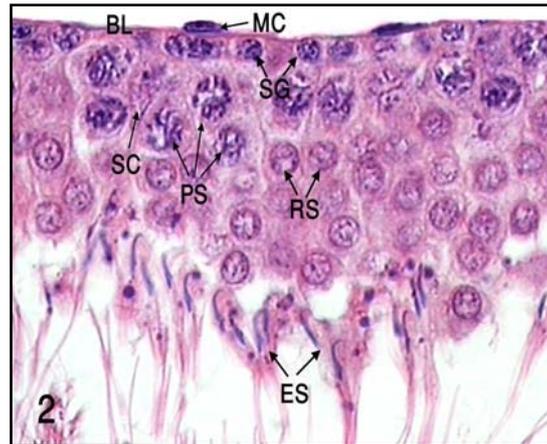


Figure (2): Normal seminiferous tubule of control testis surrounded by basal lamina (BL) with myoid cells (MC) showing Sertoli cells (SC) and germ cells including; spermatogonia (SG), primary spermatocytes (PS), rounded spermatids (RS) and elongated spermatids (ES). (H&E, x1320)

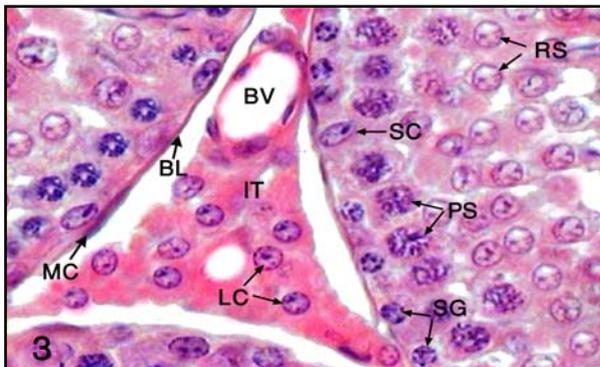


Figure (3): Normal architecture of interstitial tissue (IT) of control testis embodying clusters of Leydig cells (LC) and blood vessel (BV) and located in between three seminiferous tubules being surrounded by basal lamina (BL) containing myoid cells (MC), having spermatogonia (SG), primary spermatocytes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

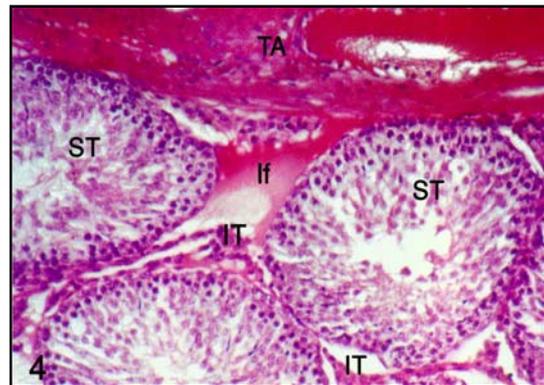


Figure (4): Destructed testicular tissues of treated testis elucidating thickened tunica albuginea (TA), deformed seminiferous tubules (ST) devoid of spermatozoa and infiltration (If) of the interstitial tissue (IT). (H&E, x330)



Figure (5): Destroyed seminiferous tubule of treated testis displaying necrotic spermatogonia (SG) and primary spermatocytes (PS). Detachment of Sertoli cells (SC) from the basement membrane. Dilated intercellular spaces (*) between germ cells are also noticed. (H&E, x1320)

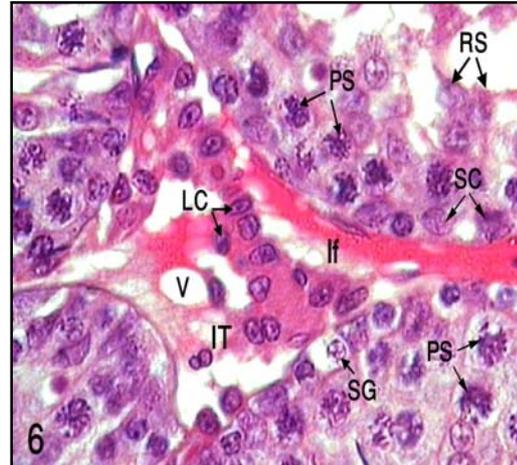


Figure (6): Destroyed interstitial tissue (IT) of treated testis in between four seminiferous tubules revealing necrotic Leydig cells (LC), vacuolation (V) and infiltrated tissue (If). The seminiferous tubules showing vacuolated spermatogonia (SG), necrotic primary spermatocytes (PS) and rounded spermatids. Beside, deformed Sertoli cells (SC) are clearly seen. (H&E, x1320)

3.2. Ultrastructural study

Ultrastructural examination of control rat testis showed Sertoli cells and germ cells with cellular characteristics typical of those seen in active spermatogenesis. The germ cells in various developmental stages are arranged orderly as illustrate in figure (7). The spermatogonia rest upon the basal lamina of the tubules possessing mitochondria and nuclei with one or two nucleoli, euchromatin and dense clumps of marginated heterochromatin (Fig.7). while, in hydrocortisone-treated rats, the spermatogonia lost their normal architecture, being irregular or pyramidal in shape possessing all the features of necrotic cells as; shrinkage with pyknotic nuclei characterized by chromatin condensation. Accordingly, the intercellular spaces between these necrotic spermatogonia are dilated (Figs. 8 & 9).

The primary spermatocytes of control testis are rounded in configurations 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 & 10). Whereas, primary spermatocytes of hydrocortisone-treated testis decreased in size, revealing condensed chromatin materials in the nucleus and in the

cytoplasm electron dense mitochondria and vacuoles are seen (Fig. 11).

As shown in figure (12), the electron micrograph of rounded spermatids of control rat testis manifested 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. While, hydrocortisone-treated rat revealed rounded spermatids with ruptured plasma membrane in some positions, vacuolated cytoplasm, electron dense mitochondria, condensed Golgi apparatus and acrosomal granule detached from the anterior hemisphere of an obviously atrophied nucleus with disappearance of the nucleolus reflecting stage of karyolysis (Figs. 9&13). As previously mentioned, in the treated rats, the spermatogenesis was arrested at the stage of rounded spermatids formation as revealed by the disappearance of elongated spermatids and spermatozoa in these electron micrographs which means that they were severely affected post hydrocortisone treatment.

In electron micrograph of control testis, Sertoli cells showed distinct nucleus and cytoplasmic characteristics consistent with an active secretory state. It rests 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. The cytoplasm contains mitochondria with distinct tubular cristae, cisternae of smooth endoplasmic reticulum, few stacks of rough endoplasmic reticulum, Golgi apparatus and lysosome. The nucleus appears irregular in shape, devoid of heterochromatin, containing a prominent nucleolus, surrounded by nuclear envelop and exhibits deep indentation as clearly observed in figure (14). While, Sertoli cells of hydrocortisone-treated rats are shrunk in size, containing electron dense mitochondria, fragmented smooth endoplasmic reticulum, lysosomes and vacuoles. The nuclei are irregular in shape with deep indentation and containing distinct electron dense nucleoli and prominent electron dense chromatin bodies (Figs 9 & 15). It is clearly noticed in figure (9) that, Sertoli cells detached from the basal lamina and moved towards the lumen of the tubule.

Normal Leydig cells of control rat possess 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. Monocytes are also found in the interstitial tissue. This cell is the largest white blood cells, highly motile, phagocytic cell and is the precursor of macrophages. 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. 16).

Examination of the interstitial tissue from hydrocortisone-treated testis showed necrotic Leydig cells having deformed mitochondria, reduced smooth endoplasmic reticulum and few lipid droplets. Blood capillary lined with endothelial cells is also seen (Fig. 17).

4. Discussion

The testis is considered to be the most important organ in the male reproductive system. It is characterized by two main functions, synthesis of steroid hormones and production of spermatozoa (Carreau *et al.*, 2002). Various factors could affect spermatogenesis, among these factors are chemical agents, such as medicines and toxic elements in environmental pollution (Yano and Dolder, 2002).

Several studies had been carried out to investigate the severity of adverse effects of hydrocortisone as a synthetic glucocorticoid drug on

different body organs such as the pancreas (Gloor *et al.*, 2001), reproductive aspects of female rats (Piffer and Pereira, 2004), the thymus (Rodrigues-Mascarenhas *et al.*, 2006), the liver (Gevorgyan *et al.*, 2008), the hypothalamo-hypophyseal-adrenocortical system (Yarushkina, 2008) and the hippocampus (Tata and Anderson, 2010). Unfortunately, no literatures can be detected on the impacts of hydrocortisone on the structures and ultrastructures of mammalian testis. So, the present work aimed to throw the light on such studies in adult albino rat testis.

The results of the present study revealed various histological and ultrastructural alterations of the testicular tissues. The surrounding basal lamina of the tubules was thickened with irregular wavy appearance. The basal lamina plays an important role in maintaining substance transportation between interstitial tissue and spermatogenic epithelium and in maintaining the structural and functional integrity of tissues (Richardson *et al.*, 1998).

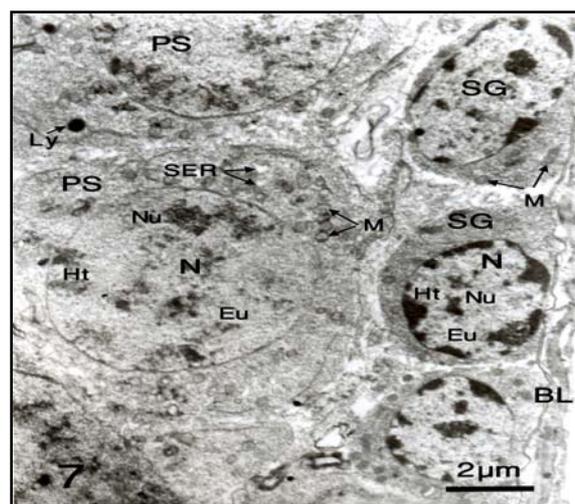


Figure (7): Seminiferous tubule of control rat surrounding by thin basal lamina (BL) and formed of; spermatogonia (SG) possessing rounded to oval nuclei containing nucleoli (Nu), euchromatin (Eu) and margined heterochromatin (Ht). The cytoplasm containing mitochondria (M), and primary spermatocytes (PS) which are rounded in shape having large rounded nucleus (N) containing; nucleoli (Nu), heterochromatin (Ht) and euchromatin (Eu). Mitochondria (M), smooth endoplasmic reticulum (SER) and lysosomes (Ly) are seen clearly in the cytoplasm. (x4000).

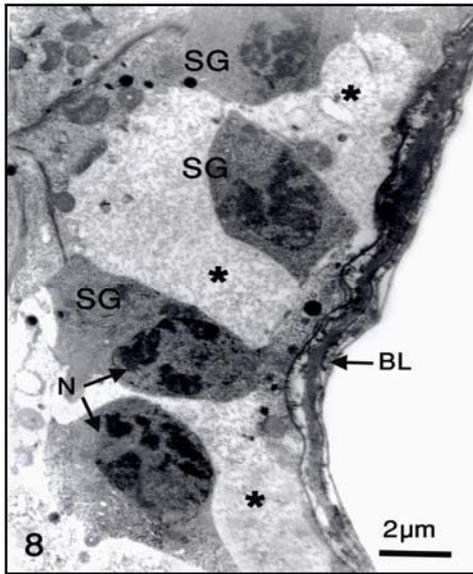


Figure (8): Seminiferous tubule of hydrocortisone-treated rat displaying degenerated spermatogonia (SG) with irregular shapes, detached from the thickened basal lamina (BL) and having pyknotic nuclei (N) with condensed chromatin, in addition to increased intercellular spaces (*) between them. (x4000)

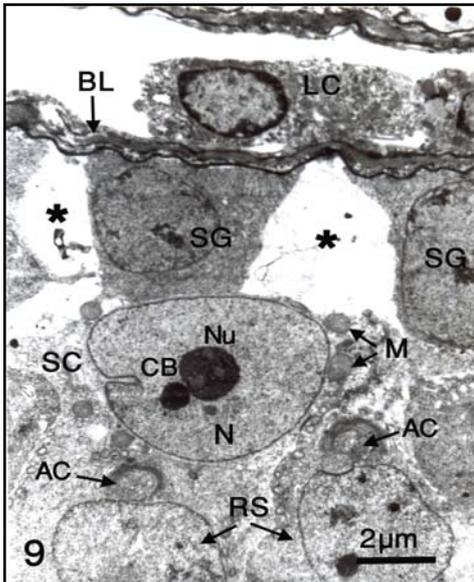


Figure (9): Another tubule of treated rat surrounded by an irregular, thickened basal lamina (BL) and having degenerated spermatogonia (SG) with trapezoidal shape and increased intercellular spaces (*) between them, round spermatids (RS) with detached acrosomal cap (AC) of their nuclei, in addition to Sertoli cell (SC) detached from the basal lamina, resting in front of the spermatogonia with destructed cytoplasm having few mitochondria (M). Besides, Leydig cell (LC) with pyknotic nucleus is also noticed. (x4000)

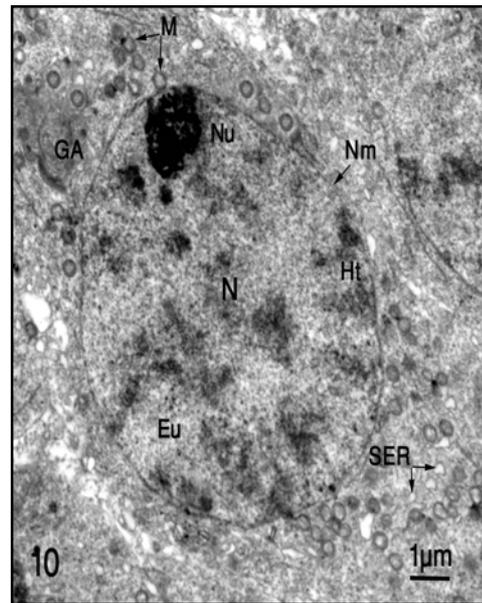


Figure (10): Primary spermatocyte of control testis having large rounded nucleus (N) containing nucleolus (Nu), heterochromatin (Ht) and euchromatin (Eu) and surrounded by nuclear membrane (Nm). The cytoplasm containing oval mitochondria (M), smooth endoplasmic reticulum (SER) and Golgi apparatus (GA). (x6000)

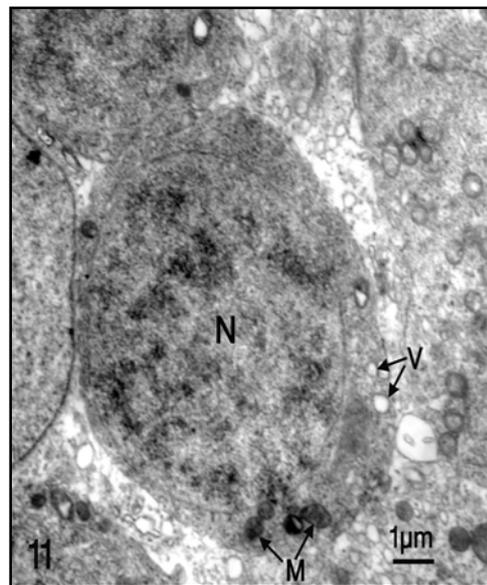


Figure (11): Primary spermatocyte of hydrocortisone-treated testis showing an atrophy in size, pyknotic nucleus (N) having condensed chromatin materials and the surrounding cytoplasm containing deformed mitochondria and vacuoles (V). (x6000)

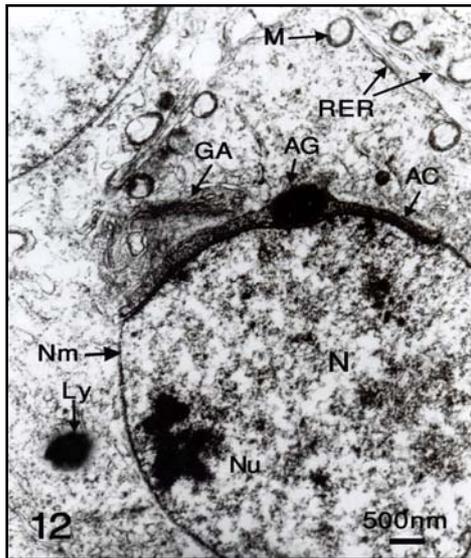


Figure (12): Rounded spermatid of control testis illustrating the formation of the acrosome, indicated by the presence of a proacrosomal granule (AG), acrosomal cap (AC), Golgi apparatus (GA) over the anterior hemisphere of the nucleus (N) which is rounded in shape, containing distinct nucleolus (Nu), 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)

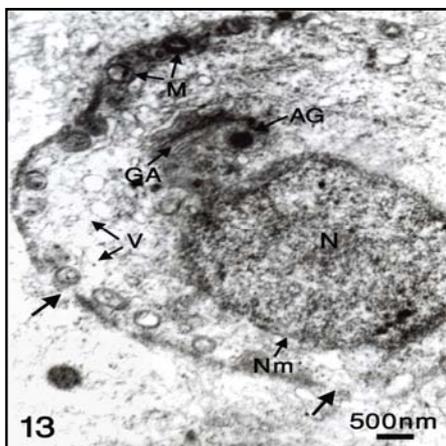


Figure (13): Rounded spermatid of hydrocortisone-treated testis exhibiting ruptured plasma membrane (arrows→), detached acrosomal granule (AG) from the nucleus (N), condensed Golgi apparatus (GA), destructed stacks of rough endoplasmic reticulum (RER), mitochondria (M) in which some of them revealed deep electron density and numerous vacuoles (V). The nucleus showing an obvious atrophy in size with disappearance of the nucleolus reflecting stage of karyolysis. (x10,000)

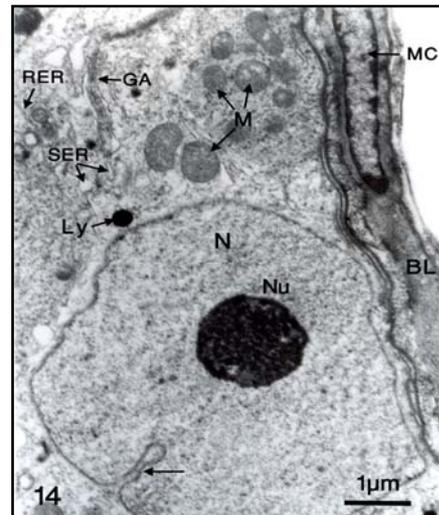


Figure (14): Sertoli cell of control testis revealing irregularly shaped nucleus (N), devoid of heterochromatin, containing one prominent nucleolus (Nu), and enclosed with nuclear envelope that exhibiting a deep indentation (arrow →). The cytoplasm containing mitochondria with distinct tubular cristae (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 seen. (x7500)

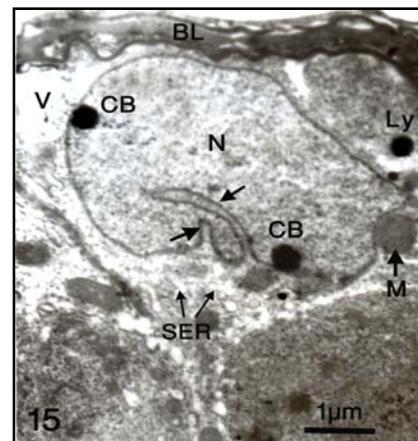


Figure (15): Sertoli cell of hydrocortisone-treated testis having cytoplasm containing electron dense mitochondria (M), lysosome (Ly), fragmented smooth endoplasmic reticulum (SER) and vacuoles (V). The nucleus (N) lacking of nucleolus, but containing two dense chromatin bodies (CB) adjacent to its inner nuclear membrane which is characterized by the presence of two deep indentations (arrows→). Part of irregular, thickened basal lamina (BL) is also clearly observed. (x7500)

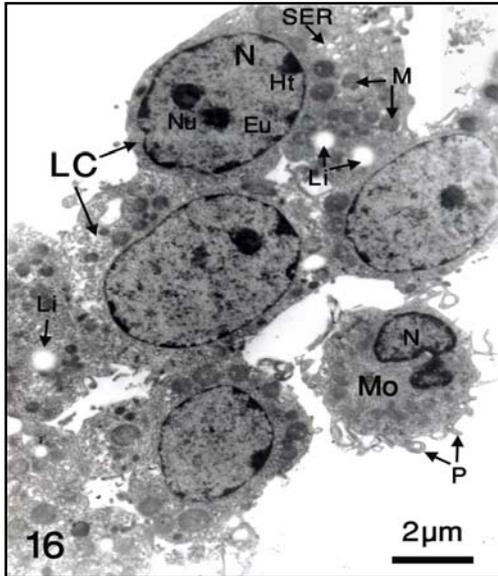


Figure (16): Interstitial tissue of control testis revealing normal fine structure of Leydig cells (LC), characterized by cytoplasm contain mitochondria (M), smooth endoplasmic reticulum (SER) and lipid droplets (Li), in addition to oval to rounded nuclei (N) containing one or two nucleoli (Nu), heterochromatin (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)

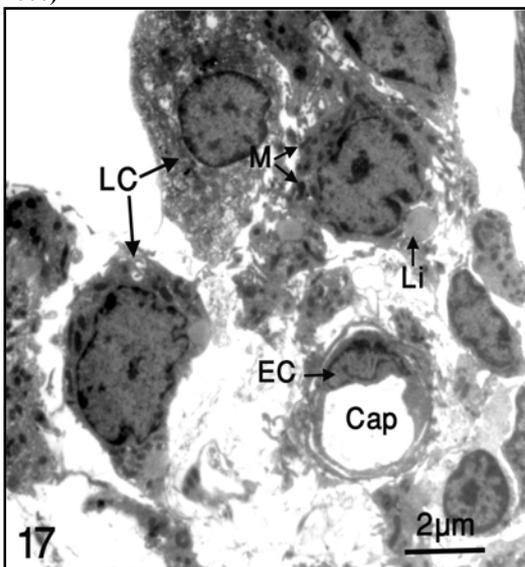


Figure (17): Interstitial tissue of hydrocortisone-treated testis displaying atrophied Leydig cells (LC) cytoplasm containing electron dense mitochondria (M), few lipid droplets (Li) and the nuclei (N) are irregular in shape showing signs of pyknosis. Blood capillary (Cap) lined by endothelial cell (EC) is also noticed. (x4000)

Many testicular disorders are associated with a thickened aspect of the tubular wall of the seminiferous tubules, which impairs the relationship between the inner tubular population and the interstitium. During the pathological thickening processes, Sertoli cell functions are progressively altered and eventually suppressed. According to the disturbance progresses, the germ cells display a progressive arrest of the spermatogenetic processes. Subsequently, the lamina propria begins to separate, then thicken and finally shrunken. At the end of this process the basal lamina is absorbed, and leaves the so-called ghost tubules in the tissue (Anniballo *et al.*, 2000). Altered basement membrane structure has been associated with severe functional impairment of the testis. This destruction might subsequently affect transportation of oxygen, hormone, nutrition, and metabolites (Zheng *et al.*, 2008).

Interactions between Sertoli cells, peritubular myoid cells, Leydig cells, and germ cells are thought to be essential for spermatogenesis. Each of these interactions must be communicated through the extracellular matrix of the basement membrane. Many reports have demonstrated that over expression of the subtypes of type IV collagen correlates with abnormal thickened basement membrane and it is related to spermatogenic dysfunction in human and other mammals, since type IV collagen is a major constituent of mammalian basement membrane and is secreted by myoid cells and Sertoli cells (Dobashi *et al.*, 2003; Mattias *et al.*, 2005).

Sertoli cells have been shown to be the target for various toxicants (Krishnamoorthy *et al.*, 2005). In the present work, damage of Sertoli cells was evident following hydrocortisone administration. Dilated intercellular spaces and loss of contact between germ cells is apparently due to Sertoli cell disturbances which also lead to loss of these germ cells and finally to the destruction of testicular tissue and infertility (Monsees *et al.*, 2002). Sertoli cells foster the development and maintain the viability of germ cells by secreting hormonal and nutritive factors into a specialized compartment (blood-testis barrier), formed by tight junctions between the adjacent Sertoli cells and the germ cells. Also, it forms the sites of attachment of germ cells and provide physical support to them (Richburg, 2000; Sawada and Esaki, 2003). During spermatogenesis, spermatogonia differentiate into spermatocytes that cross through the blood-testis barrier as they mature and traverse the tubular lumen (Cook and Saunders, 2002; Siu and Cheng, 2004).

Spermatogonia demonstrated severe defects post hydrocortisone administration, they lost their

normal shapes, possessing features of necrotic cells. As De Rooij and Russell (2000) described, spermatogonia are particularly vulnerable to toxicants and physical agents. In particular, because of their mitotic activity, they are more vulnerable than Sertoli, Leydig cells and spermatids. Spermatogonia 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 hydrocortisone treatment may reflect the disturbances in the microenvironment of the 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 present results showed that hydrocortisone treatment also exerts an effect on Leydig cells; showing remarkable decrease in number and size. Close relationship between Leydig cells and blood vessels suggests that these cells are at high risk of exogenous toxicants. In normal testicular function, Leydig cells are the centers of fertility regulation and reproductive health (Boekelheide, 1993). Within the testis, steroidogenesis occurs in the Leydig cells. Inside these cells, the steroidogenic pathway begins in the cytoplasm and includes chemical reactions that occur in the mitochondria and smooth endoplasmic reticulum, where the final end product –testosterone- is produced (Dharia *et al.*, 2004).

The results of the present study are in accordance with other previous studies using other drugs, such as those reported by Yano and Dolder, (2002) after paracetamol treatment into rats, after injection of rats with Nandrolone decanote -an anabolic androgenic steroid drug- (Mesbah *et al.*, 2008), and post long-term treatment with the methanol subfraction of Carica papaya seeds in rats (Manivannan *et al.*, 2009).

In conclusion, data from the present study showed that hydrocortisone administration into adult male rats exerts a clear effect on testicular structures and ultrastructures including degenerated changes of germ cells, Sertoli cells, and Leydig cells. These changes reflect on their functions exerting deficiency in their performance. So, it should be utilized under

restricted precautions in the medical fields to protect the human health from its hazardous impact.

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