

## HYDROCORTISONE IMPACT ON THE STRUCTURAL AND ULTRASTRUCTURAL CHARACTERISTICS OF MAMMALIAN ADRENAL CORTEX

Waslat W. Elshennawy\* and Hanaa R. Aboelwafa

Department of Biology and Geology, Faculty of Education, Ain Shams University, Cairo, Egypt  
[dr-waslat@hotmail.com](mailto:dr-waslat@hotmail.com)

**Abstract:** Hydrocortisone is a synthetic glucocorticoid utilized in the medical fields for the treatment of different types of diseases in a very wide scale. The present study aimed to investigate the histological and ultrastructural alterations induced by administration of hydrocortisone on the testis of albino rat. Twenty adult male rats weighing 150-200g. were divided into two even groups; group I injected *i.m.* with 30mg/100g b.wt. of hydrocortisone sodium succinate dissolved in 0.6 ml of bacteriostatic water at 9am in a daily manner for 15 days. Whereas, group II were injected with 0.6ml of bacteriostatic water in the same manner. *Histologically*, adrenal cortex of treated rats displayed shrinkage in the thickness of its cortical zones, a mass of cortical cells projected out of its thickened capsule, beside zona glomerulosa, fasciculata and reticularis cells are compressed and lost their normal organization. Vacuolation and fibrotic areas are seen in the cytoplasm. The nuclei of some of these cells showing signs of pyknosis, karyorrhexis and karyolysis. *Ultrastructurally*, cortical cells are disarranged, compressed and possessed deformed mitochondria with abnormal type of cristae (i.e. lamelliform), lysosomes, in addition to numerous lipid droplets, collagen fibers and fingerprint-like configuration. Their nuclei showed clear signs of pyknosis, and engulfing blood cells were observed in blood sinusoids of the three zones. In conclusion, it seems that the destructive impacts of hydrocortisone sodium succinate 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 such drug must be utilized under restricted precautions in the medical fields to protect the human health from its hazardous impact.

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**Key Words:** adrenal cortex, glucocorticoids, histology, hydrocortisone, rat, ultrastructure.

### 1. Introduction:

Hydrocortisone is a synthetic glucocorticoid widely administered for the treatment of various types of diseases. It is used for the treatment of acute graft rejection in most forms of organ transplantation, some neoplastic conditions, collagen diseases, dermatological diseases, status asthmaticus, allergic and anaphylactic reactions (Rigge and Jones, 2005). It significantly reduced specific physical problems in prostate cancer patients and improved their emotional state (Kornblith *et al.*, 2001). In 2007, Da Silva and Schiff reported that hydrocortisone is commonly used as bastion for control of the brain swelling before, during, and after neurosurgical procedures, as well as during radiation and chemotherapy in the brain tumor patients. Also, in 2007, Wang *et al.* declared that, hydrocortisone decreased the severity of acute pancreatitis and restored the normal structure of the pancreas. 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.

Hydrocortisone is the drug of choice for glucocorticoid replacement therapy in adrenal

insufficiency diseases due to its property of short half-life which mimics the normal cortisol circadian rhythm (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). Immediate intravenous administration of hydrocortisone is used for the treatment of acute adrenal crisis (Bornstein, 2009). In 2010, Romer *et al.*, elucidated that glucocorticoids have been shown to affect declarative memory, an explicit form of memory for facts and events operated by medial temporal lobe structures. Recently, in 2011, Wirth *et al.*, declared that intravenous hydrocortisone administration to depressed patients was produced mixed effects on mood and emotional processing.

In experimental animals, involvement of hydrocortisone has been studied in some biological aspects in different body organs rather than the adrenal gland (Nosenko and Mishunina, 2005; Mantzoros *et al.*, 2006; Tariq *et al.*, 2007; Li *et al.*, 2008; Casella *et al.*, 2010).

It is clearly noticed from this previous literature, that hydrocortisone 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 cortical cells which are considered the main site in the body responsible for secreting the natural cortisol. Thus, the present study aimed to throw some light on the impact of hydrocortisone on the adrenal cortical tissues from the histological and ultrastructural point of view.

## 2. Materials and Methods:

### 2.1. Experimental animals

Twenty male Swiss albino rats (*Rattus norvegicus*) ranging in weights 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°C and a 12h light/darkness cycle.

### 2.2. Drugs used

Hydrocortisone Sodium Succinate available in Egypt under trade name; Solu-Cortef® in the form of 100mg/2ml bacteriostatic water(solvent) for injection which is manufactured by E.I.P.I.CO(Egyptian Int. Pharmaceutical Industries).It is a highly water-soluble sodium succinate ester of hydrocortisone permits the immediate intravenous and intramuscular administration of high doses of hydrocortisone in a small volume of diluent and is particularly useful where high blood levels of hydrocortisone are required rapidly (Rigge and Jones, 2005; 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 dealing with rats (Bogdanov and Yarushkina, 2004, 2006 & 2007; Yarushkina, 2008). Whereas, Group II, were intramuscularly injected only with 0.6 ml of bacteriostatic water in the same manner as group I.

### 2.4. Histological preparations

The excised adrenal glands were fixed in Bouin's fluid and in Zenker acetic for 24 hours. After fixation, they were subjected to the normal

procedures for paraffin sectioning. Sections, cut at the thickness of 4-6 µm were stained with haematoxylin & Eosin and Mallory's triple stains. The stained sections were dehydrated in ascending series of alcohol, cleared in xylene, mounted in DPX, 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 (1×1mm<sup>3</sup>), 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 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

#### 3.1.1. Haematoxylin and Eosin preparations

##### 3.1.1.1. Control adrenal cortex

Figure (1A) showing the three zones of adrenal cortex; zona glomerulosa, fasciculata and reticularis, respectively. As shown in *figure* (1B), 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. 1B).

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. 1C).

Zona reticularis 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. 1D).

### 3.1.1.2. Hydrocortisone-treated adrenal cortex

Generally, hydrocortisone induced degenerative changes involve shrinkage in the thickness of the three cortical zones according to the compression in their cells. A mass of the cortical cells projected out of the thickened fibrous connective tissue capsule as seen in figure (2A).

In zona glomerulosa an obviously clear fibrotic areas are seen in figure (2B), and in the three zones cytoplasmic vacuolation and nuclear pyknosis, karyorrhexis and karyolysis are frequently observed (Figs. 2B, C & D).

Blood sinusoids of zona reticularis became rounded in shape and loaded with stagnant blood in their lumina which are lined with pyknotic endothelial cells (Fig. 2D).

### 3.1.2. Mallory's triple stain preparations

Worthy to mention that, adrenal gland sections were stained with Mallory's triple stain to display more detailed histological features especially the fibrotic elements in the treated adrenal cortex.

#### 3.1.2.1. Control adrenal cortex

The capsular fibrous tissue exhibits a bluish colouration (Figs. 3A & B). The cortex – in general – apparently acquired varying grade of red to pinkish colouration (Fig. 3A).

Zona glomerulosa and zona reticularis cells having relatively deep pink cytoplasm rather than zona fasciculata, and the nuclei of the three zones are possessing deep red colouration (Figs. 3B, C & D), while the delicate trabeculae between glomerulosa cells having bluish colouration (Fig. 3B).

Zona fasciculata and zona reticularis blood sinusoids are lined with obvious reddish endothelial cells (Figs. 3C & D).

### 3.1.2.2. Hydrocortisone-treated adrenal cortex

Generally, figure (4A) displays thickened bluish fibrous connective tissue capsule, beside an obvious mass of fibrotic tissue occupied the central area of the destructive cortical zones which appeared with a distinct colour of deep blue.

In figure (4B), a highly magnified part of the thick fibrous connective tissue capsule reveals

different degrees of blue colouration due to the variety of fibrotic elements. Also, an obvious invasion of medullary cells into zona glomerulosa with their characterized yellowish-pink colouration are seen.

An abnormal fibrotic mass is clearly observed in zona fasciculata exhibiting different degrees of bluish colouration. Slightly stagnant blood cells in orange colour are found in blood capillaries lumina (Fig. 4C).

Reticularis zone manifests numerous blood sinusoids engorged with haemorrhagic blood cells having orange-yellowish colouration (Fig. 4D).

## 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 in the form of rounded bodies are evident. The nuclei of these cells are rounded or oval in shape; sometimes wavy in appearance ensheathed by a double nuclear envelope and possessing nucleoli, peripheral dense heterochromatin and homogenous euchromatin material (Fig. 5).

Figure (6) exhibits 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 numbers of 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 membrane. Blood capillaries lined with endothelial cells are noticed between these fasciculata cells (Fig. 6A).

Zona reticularis cells are distinguished by their richness of rounded mitochondria with electron dense tubular cristae, smooth endoplasmic reticulum, lysosomes and lipid droplets with varying sizes. Their nuclei are spherical or ovoid in shape containing condensed heterochromatin, euchromatin and prominent nucleoli (Fig. 7). Widened and clear blood sinusoids lined with endothelial cells are manifested in figure (7A).

### 3.2.2. Hydrocortisone-treated adrenal cortex

Marked ultrastructural changes of zona glomerulosa cells are illustrated in figure (8); the cytoplasm contains deformed and dense mitochondria with loss of cristae configuration, abundant lipid

droplets and collagen fibers which reveal prominent features of fibrosis. Their nuclei became shrunken, showing clear signs of pyknosis, surrounded by broken nuclear envelope and containing an obvious electron dense nucleoli, peripheral heterochromatin and euchromatin. Engulfing blood cells are observed in between glomerulosa cells (Fig. 8A).

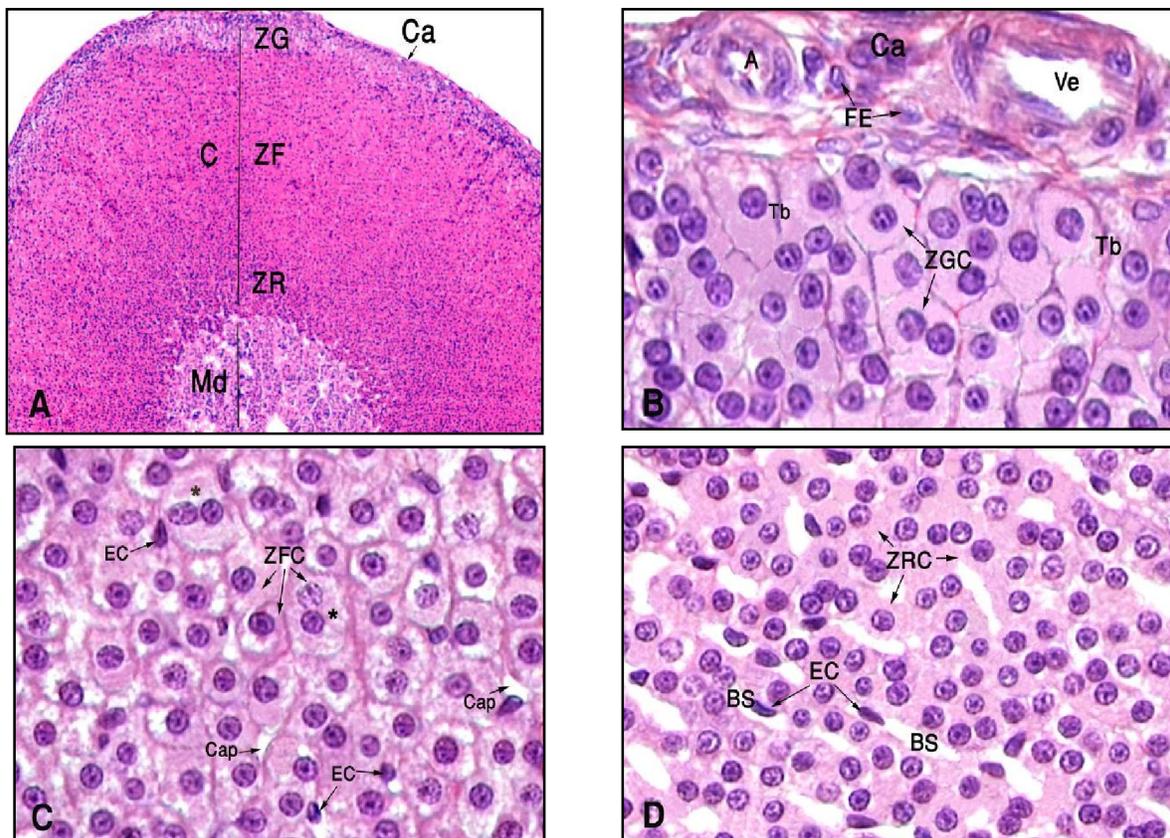
Cells of zona fasciculata are characterized by prominent deformed mitochondria with abnormal type of cristae (i.e. lamelliform cristae), as well as extensive accumulation of lipid droplets and collagen fibers throughout the cytoplasm. The nuclei acquired apparently a highly deformed irregular shape, surrounded by ruptured nuclear membrane and containing an obviously condensed nucleoli, heterochromatin and euchromatin manifesting clear signs of pyknosis (Fig. 9). Intense hypertrophied blood cells are seen in figure (9A).

An obvious strange pathological changes occurred in zona reticularis characterized by the appearance of fingerprint-like configuration composed of concentric whorls of membranes

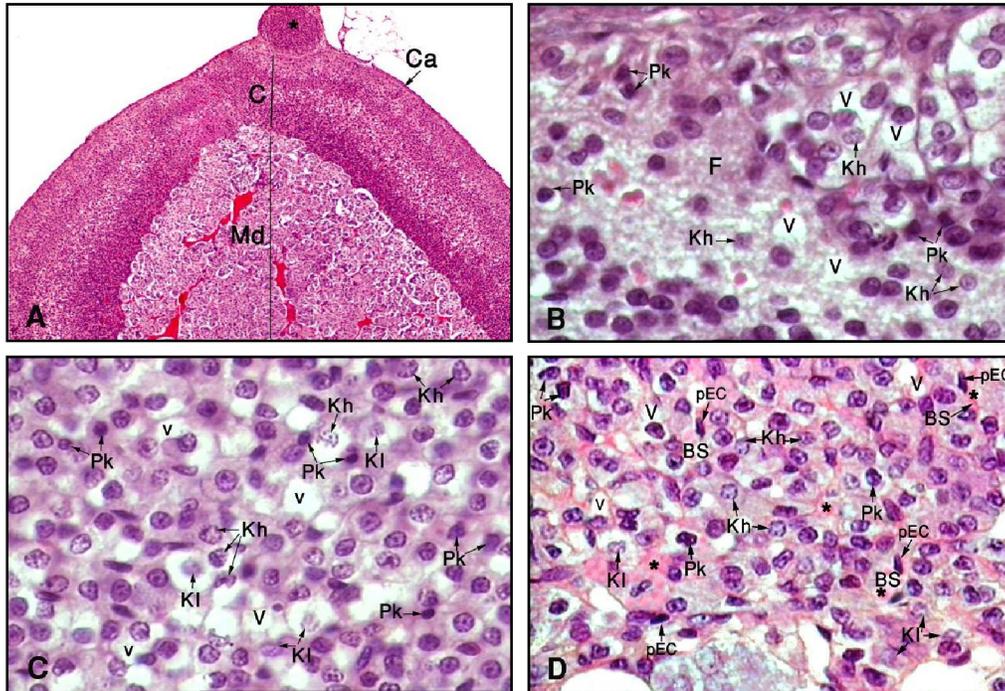
(myelin-like membranes) and intense collagen fibers. Beside, the abnormally lamelliform cristae of mitochondria, numerous lipid droplets and cytoplasmic vacuoles are seen in figure (10). The nuclei lost their rounded shape being shrunken, surrounded by ruptured nuclear envelope and containing more electron dense nucleoli, clumps of heterochromatin and euchromatin thus revealing clear signs of pyknosis (Fig. 10). The blood sinusoids in between these cells embodying intense hypertrophied blood cells (Fig. 10A).

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 hydrocortisone.

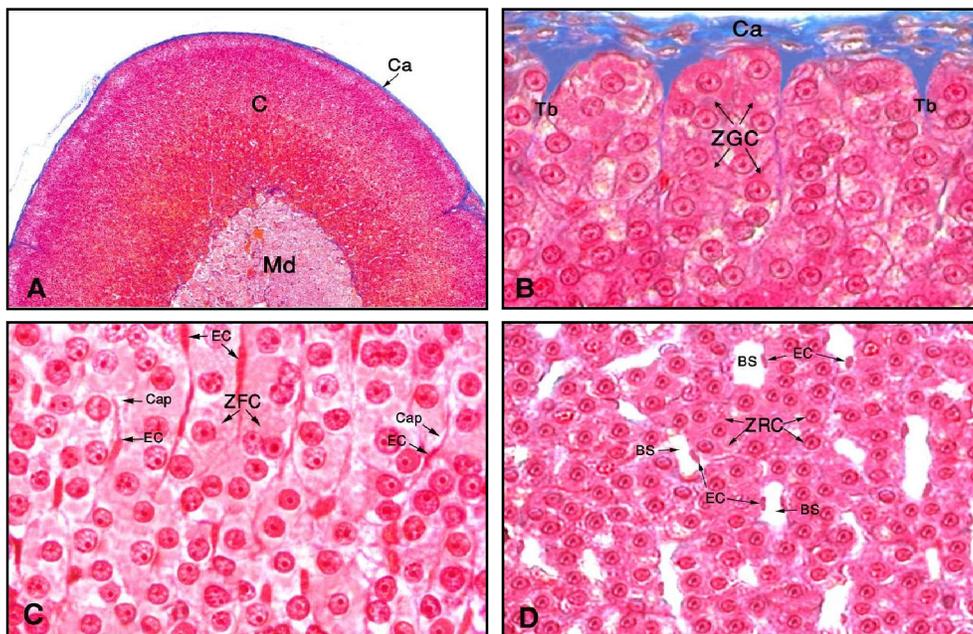
An interesting observation is seen in the resulted electron micrographs, that there are dense particles participated all over the cells of these three zones, which may be occurred as a result of a chemical reaction between hydrocortisone and the chemical components of the cells.



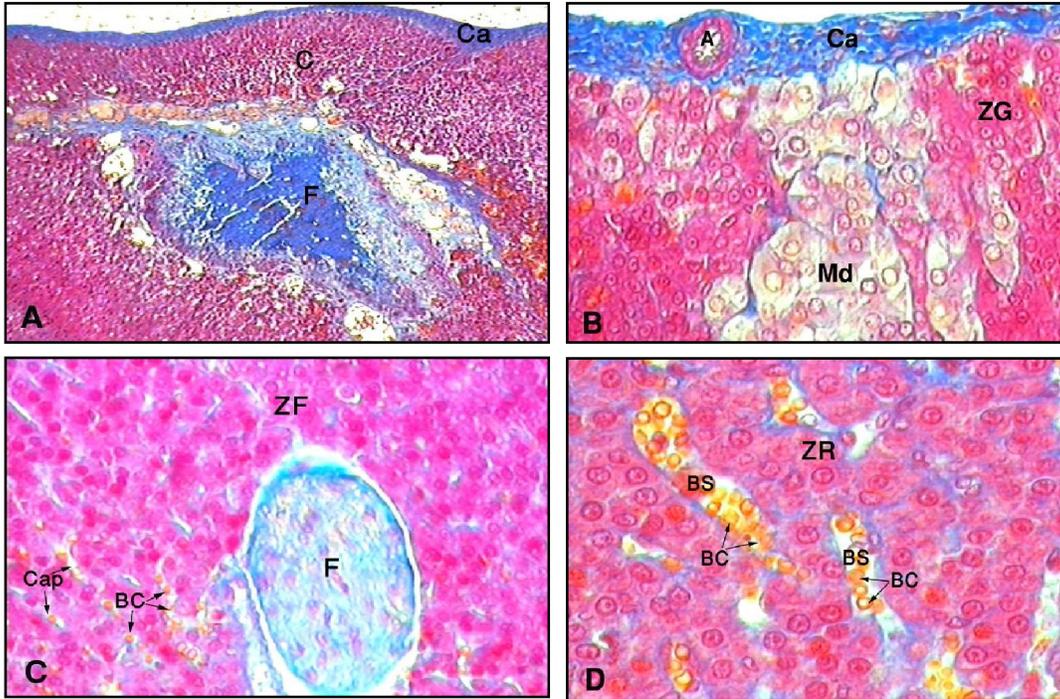
**Figure 1:** Light micrographs of H&E stained sections of control adrenal gland. **(A)** General structure of adrenal gland illustrating the capsule (Ca), the cortex (C) which is differentiated into zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR), and the medulla (Md) (x132). **(B)** 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 venule (Ve) (x1320). **(C)** 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). **(D)** Zona reticularis cells (ZRC) arranged in irregular network of intermingled cords, separated by numerous wide blood sinusoids (BS) lined with endothelial cells (EC) (x1320).



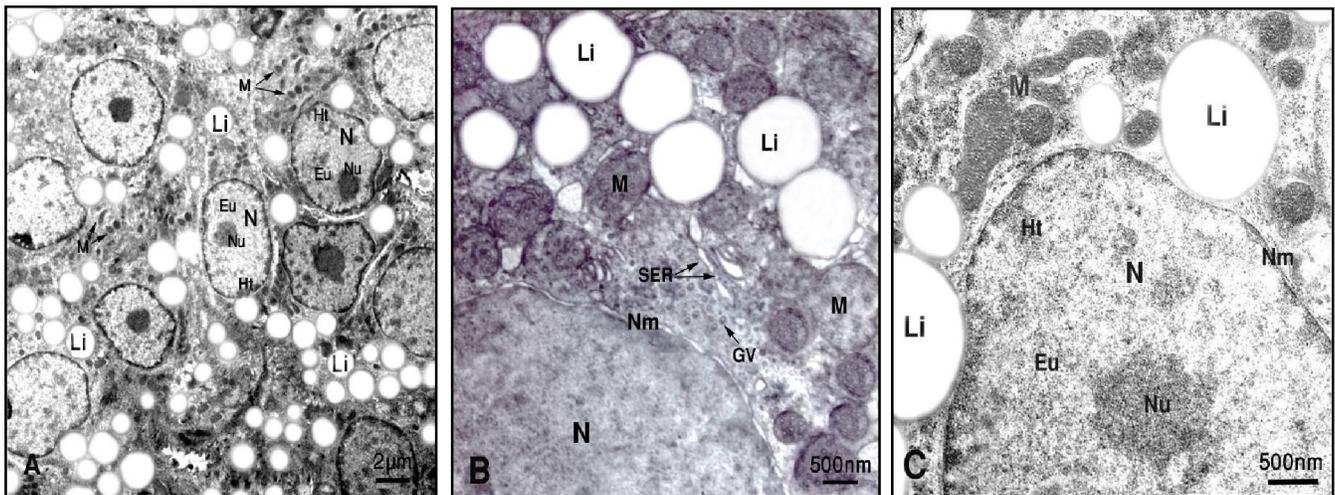
**Figure 2:** Light micrographs of Hx&E stained sections of hydrocortisone-treated adrenal gland. (A) Adrenal gland showing shrinkage of cortical zones (C), an extended inner medulla (Md) and an obvious mass of cortical cells (\*) projected out of the capsule (Ca) (x132). (B) Zona glomerulosa cells designating clear fibrotic area (F) (x1320). (B, C & D) Zona glomerulosa, fasciculata and reticularis cells displaying vacuolated (V) cytoplasm and necrotic nuclei showing pyknosis (Pk), karyorrhexis (Kh) and karyolysis (Kl) (x1320). (D) Some of blood sinusoids (BS) between reticularis cells became rounded in shape and loaded with stagnant blood (\*) in their lumina which are lined with pyknotic endothelial cells (pEC).



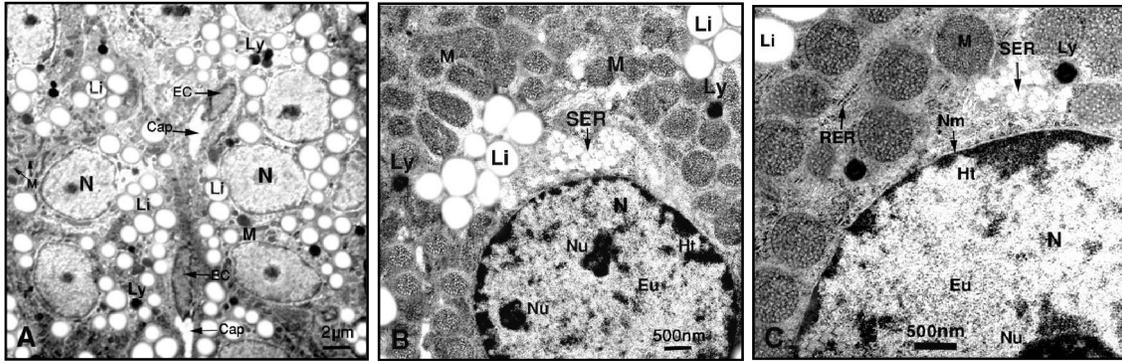
**Figure 3:** Light micrographs of Mallory's triple stained sections of control adrenal gland. (A) Adrenal gland exhibiting an obvious bluish capsule (Ca), reddish cortex (C) and pinkish medulla (Md) (x132). (B) The capsule (Ca) and the extended trabeculae (Tb) revealing bluish colouration, zona glomerulosa cells (ZGC) displaying relatively deep pinkish cytoplasm (x1320). (C & D) Zona fasciculata cells (ZFC) showing relatively lighter pinkish cytoplasm rather than zona reticularis cells (ZRC), a deep reddish endothelial cells (EC) are seen. The nuclei of cells of the three zones possessing deep red colouration (x1320).



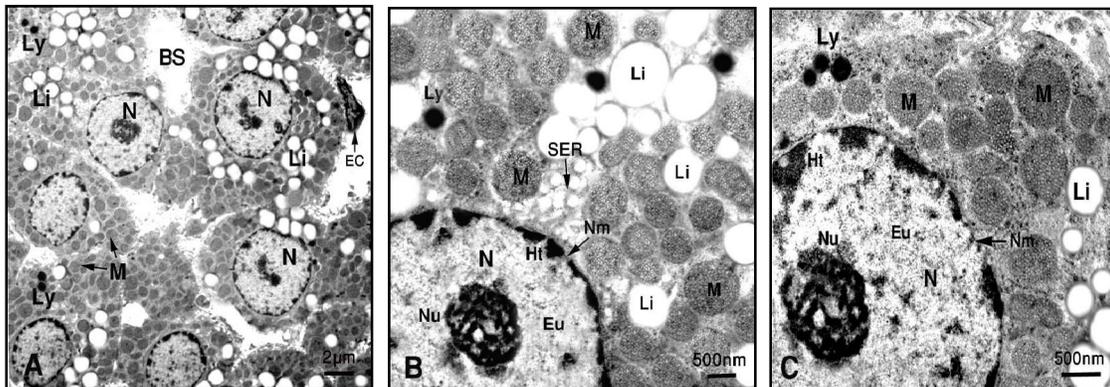
**Figure 4:** Light micrographs of Mallory's triple stained sections of hydrocortisone-treated adrenal gland. (A) Thickened bluish capsule (Ca), beside an obvious mass of bluish fibrotic tissue (F) occupying the central area of the destructive cortical zones (C) (x132). (B) A highly magnified part of the bluish thick capsule (Ca) with a reddish boundary of an arteriole (A), as well as an obvious invasion of yellowish-pink medullary cells into zona glomerulosa (ZG) (x1320). (C) Zona fasciculata (ZF) exhibiting an abnormal fibrotic mass (F), as well as orange stagnant blood cells (BC) in the lumina of the blood capillaries (Cap) (x1320). (D) Reticularis zone (ZR) manifesting numerous blood sinusoids (BS) engorged with stagnant blood cells (BC) in orange-yellowish colouration (x1320).



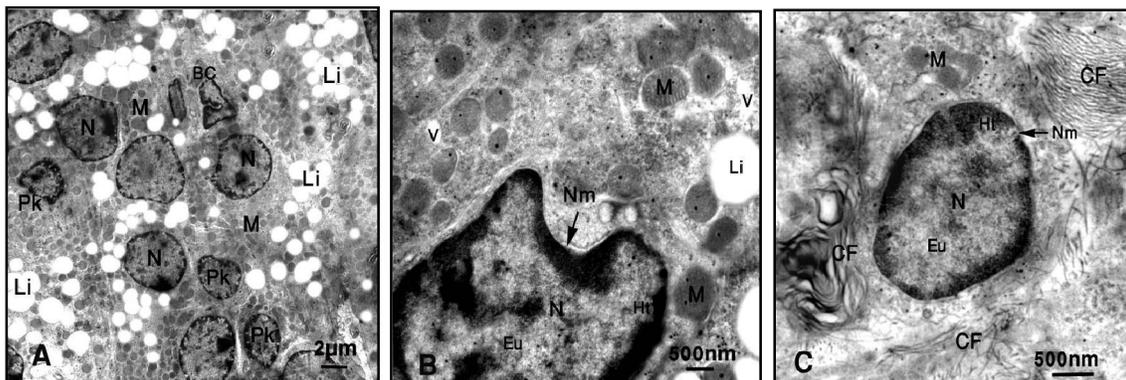
**Figure 5:** Transmission electron micrographs of control zona glomerulosa. (A) Zona glomerulosa cells displaying numerous lipid droplets (Li), mitochondria (M) and oval or rounded shaped nuclei (N) (x2000). (B & C) A highly magnified part of glomerulosa cells showing mitochondria (M) with tubulo-saccular cristae, smooth endoplasmic reticulum (SER), small Golgi vesicles (GV), lipid droplets (Li) and part of the nucleus (N) ensheathed by a double nuclear envelope and involving nucleolus (Nu), marginated dense clumps of heterochromatin (Ht) and homogenous euchromatin (Eu) (x10,000 & x12,000, respectively).



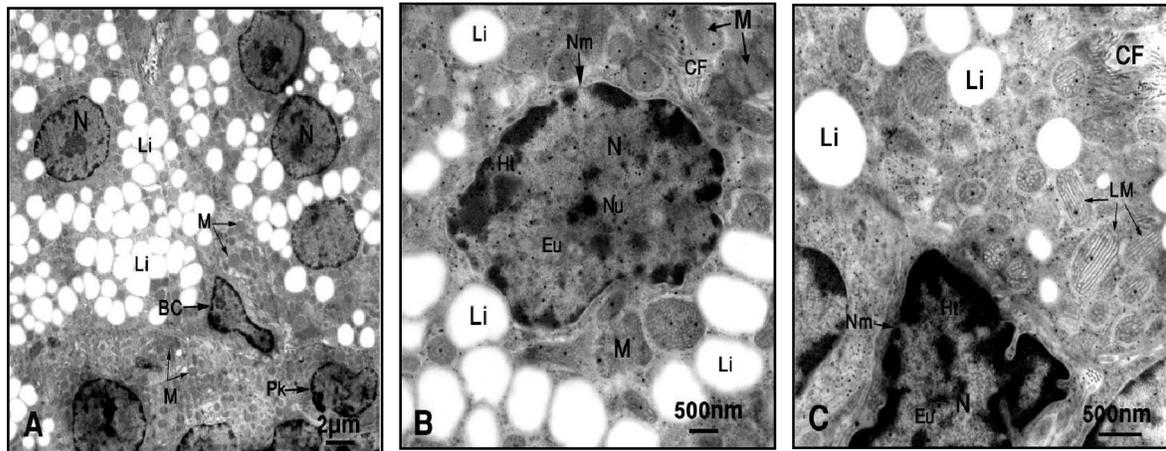
**Figure 6:** Transmission electron micrographs of control zona fasciculata. (A) Zona fasciculata cells having cytoplasm loaded with lipid droplets (Li), mitochondria (M), lysosomes (Ly), and possessing oval to rounded nuclei (N). In between them, narrowed blood capillaries (Cap) are noticed (x2000). (B & C) Part of fasciculata cells showing tubular cristae of mitochondria (M), smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER), lysosomes (Ly), lipid droplets (Li), in addition to rounded nucleus (N) surrounded by double nuclear membrane (Nm) and containing nucleoli (Nu), heterochromatin (Ht) and euchromatin (Eu) (x10,000 & x12,000, respectively).



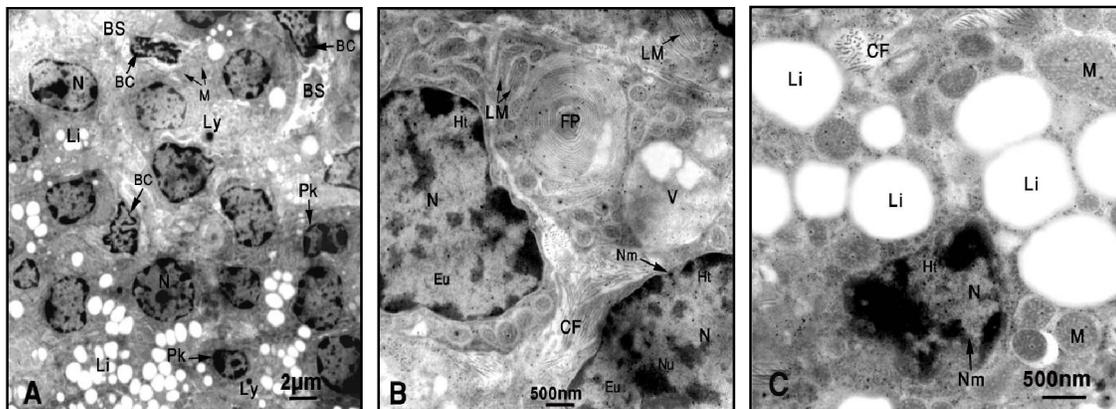
**Figure 7:** Transmission electron micrographs of control zona reticularis. (A) Reticularis cells containing numerous mitochondria (M), lipid droplets (Li) and spherical or ovoid nuclei (N). Widened blood sinusoids (BS) lined with endothelial cell (EC) are obviously seen (x2000). (B & C) Magnified part of reticularis cells illustrating rounded mitochondria (M), smooth endoplasmic reticulum (SER), lysosomes (Ly), lipid droplets (Li) and part of the nucleus (N) which is surrounded by nuclear membrane (Nm) and possessing distinct nucleolus (Nu), peripheral heterochromatin (Ht) and euchromatin (Eu) (x10,000 & x12,000, respectively).



**Figure 8:** Transmission electron micrographs of hydrocortisone-treated zona glomerulosa. (A) Zona glomerulosa cells loaded with lipid droplets (Li) and mitochondria (M). Some cells having electron dense nuclei (N), whereas the others showing pyknotic nuclei (Pk). Engulfing blood cells (BC) are observed (x2000). (B & C) Part of glomerulosa cells illustrating deformed and dense mitochondria (M) with loss of cristae configuration, lipid droplets (Li), vacuoles (V) and intense collagenous fibers (CF). The nuclei (N) revealing clear signs of pyknosis, surrounded by ruptured nuclear membrane (Nm) and containing electron dense peripheral heterochromatin (Ht) and euchromatin (Eu) (x10,000 & 12,000, respectively).



**Figure 9:** Transmission electron micrographs of hydrocortisone-treated zona fasciculata. (A) Zona fasciculata cells loaded with numerous lipid droplets (Li) and mitochondria (M). Some nuclei showing pyknosis (Pk). Hypertrophied blood cell (BC) is seen (x2000). (B & C) Magnified part of fasciculata cells showing mitochondria with lamelliform cristae (LM), lipid droplets (Li), collagenous fibers (CF) and part of highly deformed irregular shaped nucleus (N) surrounded by ruptured nuclear membrane (Nm) and containing dense clumps of heterochromatin (Ht) and euchromatin (Eu) (x10,000 & x12,000, respectively).



**Figure 10:** Transmission electron micrographs of hydrocortisone-treated zona reticularis. (A) Zona reticularis cells having plenty of lipid droplets (Li), deformed mitochondria (M), lysosomes (Ly) and electron dense nuclei showing pyknosis (Pk). Blood sinusoids (BS) containing phagocytic blood cells (BC) are noticed (x2000). (B) Destroyed reticularis cells showing mitochondria with lamelliform cristae (LM), fingerprint-like configuration (FP), collagenous fibers (CF) and vacuoles (V) (x10,000). (C) Highly magnified part of reticularis cell loaded with deformed mitochondria (M), lipid droplets (Li) and collagenous fibers (CF) (x12,000). The nuclei showing clear signs of pyknosis, surrounded by ruptured irregular nuclear membrane (Nm) and involving electron dense heterochromatin (Ht) and euchromatin (Eu).

#### 4. Discussion

The adrenal cortex plays a tremendous number of vital activities in the human body. This importance is being out from the fact that the adrenocortical zones synthesize and secrete steroid hormones, which fall into three major categories; mineralocorticoid, exemplified by aldosterone which is secreted by zona glomerulosa. Aldosterone is an important regulator of salt homeostasis and fluid balance, it can also potently influence the blood pressure, and it is a major control unit of acid/base balance (Bielohuby *et al.*, 2007). While, zona fasciculata secretes

glucocorticoid, 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; increasing the activity of the urea cycle, and it mobilizes fatty acids and glycerol from adipose cells. It has also anti-inflammatory effects: it stabilizes lysosomal membrane, reducing release of damaging proteolytic enzymes at sites of inflammation; and it decreases capillary permeability,

minimizing local swelling. These attributes make cortisol a valuable medication (Fawcett and Jensch, 2002; Campbell, 2005). By zona reticularis, small amounts of androgens are secreted. The two principal adrenal androgens are; androstenedione (Andro) hormone and dehydroepiandrosterone (DHEA), 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 Jensch, 2002; Keegan and Hammer, 2002).

The fact behind carrying out this work is that hydrocortisone, as a synthetic glucocorticoid, has been widely utilized in the medical fields for the treatment of different types of diseases as previously mentioned in the literature. Hydrocortisone has been studied in certain serious alterations of some biological aspects by many researchers on different body organs rather than the adrenal gland, 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 (HHACA) (Yarushkina, 2008), the development of the neuroendocrine system related to reproduction and stress responses both in male and female (Reznikov *et al.*, 2008) and the hippocampus (Tata and Anderson, 2010).

The effects of different treatments on the adrenal gland has been investigated by different researchers, as Lorente *et al.* (2002) who elucidated the changes in the rat adrenal zona glomerulosa under the effect of chronic hypoxia, Pereira *et al.* (2007) studied the toxicity of Clophen A60 and diethyl phthalate on the adrenal cortex, Hermenean *et al.* (2008) demonstrated the adrenal glands morpho-functional damages in rats post chemotherapy administration, Dogaru *et al.* (2009) examined the impacts of pulsed short wave on the rat adrenal glands, Guerrero *et al.* (2010) detected the changes caused by the brown widow spider venom in mice adrenal glands, Isola *et al.* (2010) revealed that the 3-D structure of mitochondrial cristae in rat adrenal cortex varies after acute stimulation with ACTH and CRH, as well as Florea and Craciun (2011) who evaluated the abnormal mitochondrial cristae generated by high doses of *Apis mellifera* (honey bee) venom (AmV) in rat adrenal cortex.

The majority of severe degenerative changes induced in the adrenal gland following pathological disorders are often judged by the physiological tools. Thus, the present study throws the light on the impact of hydrocortisone administration on the adrenal cortex histologically and ultrastructurally, although

these types of studies did not receive marked attention in spite of its importance.

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 destructive structural changes of the adrenal cortex that resulted in the present study are in accordance with those reported in other previous physiological and pathological studies. Whereas Coburn-Litvak *et al.* (2004) found that corticosterone (CORT), subcutaneously injected into rats significantly reduced adrenal gland weight. The influence of dexamethasone (DEX), a potent synthetic glucocorticoid on the rats adrenal gland was investigated by different researchers (Almeida *et al.*, 2001; Illera *et al.*, 2007; Silvan *et al.*, 2007; Ye *et al.*, 2008). These studies demonstrated that dexamethasone reduced the adrenal gland weight as a result of the atrophy in the layers of adrenal cortex, causing a suppressive steroid secretion from the adrenal cortical cells. Also, vacuolation and degeneration were seen in zona fasciculata of rats treated with Clophen A60 and diethyl phthalate (Pereira *et al.*, 2007). In addition, cytoplasmic vacuolation was noticed in the rat adrenal cortical cells after chemotherapy administration (Hermenean *et al.*, 2008).

Ultrastructurally, conspicuous deleterious alterations in all cytoplasmic organelles of the three zones are seen, specially, the occurrence of an abnormal type of mitochondrial cristae (i.e. lamelliform cristae) and increased density of its matrix. Such abnormalities were also demonstrated in the rat adrenal cortex after the acute treatment with *Apis mellifera* (honeybee) venom (AmV) as reported by Florea and Craciun (2011). These authors mentioned that, this abnormal lamellar mitochondrial cristae resulted by a complex fusion of the tubular mitochondrial cristae, and it may represent a first level of alteration of the cristae, which can be next, either destroyed, or closed into circular, concentric mitochondrial cristae causing a severe mitochondrial dysfunction. The profound lesions observed in mitochondria and endoplasmic reticulum by Guerrero

*et al.* (2010) might be sufficient to cause impairment of steroid synthesis, in accordance of the fact 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 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ 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.

The accumulation of lipid droplets observed in this study has also been reported in dexamethasone-treated rats particularly in cells of zona fasciculata and zona reticularis (Almeida *et al.*, 2001). Much of the cholesterol used in steroid synthesis is stored in lipid droplets of the steroid-forming cells. The cholesterol ester in these droplets is transported to the inner mitochondrial membrane where it enters the pathway to steroid hormones as free cholesterol (Hall, 1995). Lipid accumulation inside adrenocortical cells may be considered as a secondary phenomenon due to the inhibition of the sequence of reactions leading from cholesterol to progesterone (Hemmaid, 2009). According to these mentioned reports, hydrocortisone may prevent the translocation of cholesterol leading to accumulation of lipid droplets in the cytoplasm of adrenal cortical cells.

Almeida *et al.* (2006) found nearly the same results of destructive alterations in the ultrastructural features of the nuclei in their study of dexamethasone influence.

The finding of fibrotic phenomena here were emphasized previously in the research of Kovacs and his colleagues (1970) on the rat adrenal cortex following aniline administration, specially the fibrotic fingerprint-like configuration. Beside, the same results were obtained in a valuable investigation at 2010 by Guerrero and his team, when they treated the mice adrenal cortex with brown widow spider venom.

Finally, it is obvious, that the impaired steroidogenesis is an important mechanism of toxicity in the adrenal cortex. It may have occurred due to the appearance of fingerprint-like configuration composed of concentric whorls of membranes (myelin-like membranes) and intense collagen fibers. Beside, the abnormally lamelliform cristae of mitochondria, and the disintegration of smooth endoplasmic reticulum- the sites of steroidogenesis- in the cortical cells, as a result of hydrocortisone treatment.

In conclusion, it seems that the destructive impacts of hydrocortisone sodium succinate 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 such drug must be utilized under restricted precautions in the medical fields to protect the human health from its hazardous impact.

#### Corresponding author

Waslat W. Elshennawy  
Department of Biology and Geology, Faculty of Education, Ain Shams University, Cairo, Egypt  
dr-waslat@hotmail.com

#### References

- Almeida H, Matos L, Ferreira J. and Neves D.,2006. Age-related effects of dexamethasone administration in adrenal zona reticularis. *Ann NY Acad Sci.*, 1067:: 354-60.
- Almeida H, Ros-Dominguez S, Ribeiro N, Magalhaes MC. and Magalhaes MM.,2001. Dexamethasone administration during ageing - a structural and biochemical study on rat adrenal cortex. *Biol Cell*, 93: 372-87.
- Bielohuby M, Herbach N, Rudiger W, Maser-Gluth C, Beuschlein F, Wolf E. and Hoeflich A.,2007. Growth analysis of the mouse adrenal gland from weaning to adulthood: time- and gender-dependent alterations of cell size and number in the cortical compartment. *Am J Physiol Endocrinol Metab.*, 293: 139-46.
- Bogdanov AI. and Yarushkina NI.,2004. The role of adrenocorticotrophic hormone in the inhibition of pain reactions in conscious rats. *Neurosci and Behav Physiol.*, 34: 575-78.
- Bogdanov AI. and Yarushkina NI.,2006. Analgesic effect of corticotrophin-releasing hormone: involvement of the hypothalamic-pituitary-adrenocortical axis into its realization. *Bull Exp Biol and Med.*, 141: 184-86.
- Bogdanov AI. and Yarushkina NI.,2007. The role of the hormones of the hypothalamo-hypophyseal-adrenocortical system in the analgesic effect of corticotrophin-releasing hormone. *Neurosci and Behav Physiol.*, 34 : 363-67.
- Bornstein SR., 2009. Predisposing factors for adrenal insufficiency. *N. Engl Med.*, 360: 2328-39.
- Campbell I., 2005 . Adrenocortical hormones. *Anesth and Intensive Care Med.*, 6: 326-29.
- Casella S, Giudice E, Alberghina D; Giannetto C, Marafioti S. and Piccione G., 2010. Hydrocortisone inhibition of adenosine diphosphate (ADP)-induced platelet aggregation in horse. *Comp Clin Pathol.*,19 (3):225-237.

- Coburn-Litvak PS, Tata DA, Gorby HE, McCloskey DP, Richardson G. and Anderson BJ., 2004. Chronic corticosterone affects brain weight, and mitochondrial, but not glial volume fraction in hippocampal area CA3. *Neuroscience*, 124: 429-38.
- Da Silva AN. and Schiff D., 2007. Adrenal insufficiency secondary to glucocorticoid withdrawal in patients with brain tumor. *Surgical Neurol.*, 67: 508-10.
- Dogaru GB, Craciun C, Toader S, Tripon S. and Pop L., 2009. Structural and ultrastructural changes in the adrenal glands of rats undergoing pulsed short wave treatment. *Annals of RSCB-Archives*, 14: 80-86.
- Dykstra MJ, Mann PC, Eiwel MR. and Ching SV., 2002. Suggested standard operating procedures (SOPs) for the preparation of electron microscopy samples for toxicology/pathology studies in a GLP environment. *Toxicol Pathol.*, 30: 735-43.
- Fawcett DW. and Jensch RP., 2002. *Bloom & Fawcett's Concise Histology*. 2<sup>nd</sup> ed., London, New York, New Delhi
- Florea A. and Craciun C., 2011. Abnormal mitochondrial cristae were experimentally generated by high doses of *Apis mellifera* venom in the rat adrenal cortex. *Micron*, 42: 434-42.
- Gevorgyan ES, Yavroyan ZV, Galstyan AG. and Demirkhanyan LO., 2008. Content of some phospholipid fractions in rat liver nuclei after the in vivo action of hydrocortisone and insulin. *Biophysics*, 1: 18-24.
- Gloor B, Uhl W, Tcholakov O, Roggo A, Muller CA, Worni M. and Buchler MW., 2001. Hydrocortisone treatment of early SIRS in acute experimental pancreatitis. *Dig Dis and Sci.*, 46:2154-61.
- Guerrero B, Finol HJ, Reyes-Lugo M, Salazar AM, Sanchez EE, Estrella A, Roschman-Gonzalez A, Ibarra C, Salvi I. and Rodriguez-Acosta A. ,2010. Activities against hemostatic proteins and adrenal gland ultrastructural changes caused by the brown widow spider *Latrodectus geometricus* (Araneae: Theridiidae) venom. *Comp Biochem and Physiol.*, 151: 113-21.
- Hall PF., 1995. The roles of microfilaments and intermediate filaments in the regulation of steroid synthesis. *J. Steroid Biochem Mol Biol.*, 55: 601-5.
- Hemmaid KZ., 2009. Ultrastructural patterns of the adrenal cortical cells of rats during suppression of secretion by dexamethasone injection. Fourth Environment Conference, Faculty of Science, Zagazig University; p: 65-83.
- Hermenean A, Ardelean A. and Craciun C., 2008. Adrenal glands morpho-functional damages at Wistar rats after chemotherapy administration. *Studia Universitatis*, 18: 251-53.
- Illera JC, Pena L, Martinez-Mateos MM, Camacho L, Blass A, Garcia-Partida P, Illera MJ. and Silvan G., 2007. The effect of long-term exposure to combinations of growth promoters in Long Evans rats. Part 2: adrenal morphology (histopathology and immunochemical studies). *Analytic Chim Acta*, 586: 252-258.
- Isola R, Solinas P, Loy F, Mariotti S. and Riva A., 2010. 3-D structure of mitochondrial cristae in rat adrenal cortex varies after acute stimulation with ACTH and CRH. *Mitochondrion*, 10: 472-78.
- Jung C. and Inder WJ., 2008. Management of adrenal insufficiency during the stress of medical illness and surgery. *MJA*, 188: 409-13.
- Keegan CE. and Hammer GD., 2002. Recent insights into organogenesis of the adrenal cortex. *Trends Endocrinol Metab.*, 13: 200-8.
- Kornblith AB, Herndon JE, Zuckerman E, Godley PA, Savarese D, Vogelzang NJ. and Holland JC., 2001. The impact of docetaxel, estramustine, and low dose hydrocortisone on the quality of life of men with hormone refractory prostate cancer and their partners: a feasibility study. *Ann of Oncol.*, 12: 633-41.
- Kovaca K, Blascheck JA, Yeghiayan E, Hatakeyama S. and Gardell C., 1970. Adrenocortical lipid hyperplasia induced in rats by aniline. A histologic and electron microscopic study. *Am J Pathol.*, 62:17-34.
- Li Y, Cui X, Li X, Solomon SB, Danner RL, Banks SM, Fitz Y, Annane D, Natanson C. and Eichacker P., 2008. Risk of death does not alter the efficacy of hydrocortisone therapy in a mouse *E. coli* pneumonia model risk and corticosteroids in sepsis. *Intensive Care Med.*, 34: 568-77
- Lorente M, Mirapeix RM, Miguel M, Longmei W, Volk D. and Cervos-Navarro J. (2002). Chronic hypoxia induced ultrastructural changes in the rat adrenal zona glomerulosa. *Histol Histopathol.*, 17: 185-90.
- Mah PM, Jeenkins RC, Rostami-Hodjegan A, Newell-Price J, Doane A, Ibbotson V, Tuckner GT. and Ross RJ., 2004. Weight related dosing, timing and monitoring hydrocortisone replacement therapy in patients with adrenal insufficiency. *Clin Endocrinol.*, 61: 367-75.
- Mantzoros I, Kanellos I, Angelopoulos S, Koliakos G, Pramateftakis M, G, Kanellos D, Zacharakis E, Zaraboukas T. and Betsis D., 2006. The effect of insulin-like growth factor I on healing of colonic anastomoses in cortisone-treated rats. *Dis Colon Rectum*, 49: 1431-38.

- Nieman LK, Maria L. and Turner C., 2006. Addison's disease. *Clinics in Dermatol.*, 24: 276-80.
- Nosenko ND. and Mishunina TM., 2005. Glucocorticoid-induced prenatal modification of GABA-ergic regulation of the responsiveness of the hypothalamo-hypophyseal-adrenocortical system to stress in rats. *Neurophysiology*, 37: 213-218.
- Pereira C, Mapuskar K. and Rao CV., 2007. A two-generation chronic mixture toxicity study of Clophen A60 and diethyl phthalate on histology of adrenal cortex and thyroid of rats. *Acta Histochemica*, 109: 29-36.
- Piffer RC. and Pereira OC., 2004. Reproductive aspects in female rats exposed prenatally to hydrocortisone. *Comp Biochem and Physiol.*, 139: 11-16.
- Rainey WE, Saner K. and Schimmer BP., 2004. Adrenocortical cell lines. *Mol and Cell Endocrinol.*, 228: 23-38.
- Reznikov A, Nosenko N, Tarasenko L, Sinitsyn P, Polyakova L. and Mishunina T., 2008. Neuroendocrine disorders in adult rats treated prenatally with hydrocortisone acetate. *Exp and Toxicol Pathol.*, 60: 489-97.
- Rigge DC. and Jones MF., 2005. Shelf lives of aseptically prepared medicines-stability of hydrocortisone sodium succinate in PVC and non-PVC bags and in polypropylene syringes. *J. Pharm and Biomed Anal.*, 38: 332-36.
- Rodrigues-Mascarenhas S, Fernandes dos Santos N. and Rumjanek VM., 2006. Synergistic effect between ouabain and glucocorticoids for the induction of thymic atrophy. *Biosci Rep.*, 26:159-69.
- Romer S, Schulz A, Richter S, Lass-Hennemann J. and Schachinger H., 2010. Oral cortisol impairs implicit sequence learning. *Psychopharmacology*, 215: 33-40.
- Rosol TJ, Yarrington JT, Latendresse J. and Capen CC. (2001). Adrenal gland: structure, function, and mechanisms of toxicity. *Toxicol. Pathol.*, 29: 41-48.
- Salvatori R., 2005. Adrenal insufficiency. *J. Am Med Ass.*, 294: 2481-88.
- Schimmer BP. and Parker KL., 2006. Adrenocorticotrophic hormone; adrenocortical steroids and their synthetic analogs; inhibitors of the synthesis and actions of adrenocortical hormones. Chapter 60. In: Goodman & Gilman's the pharmacological basis of therapeutics. 11<sup>th</sup> ed., McGraw-Hill, New York; p. 1649-77.
- Silvan G, Martinez-Mateos MM, Blass A, Camacho L, Gonzalez-Gil A, Garcia-Partida P. and Illera JC., 2007. The effect of long-term exposure to combinations of growth promoters in Long Evans rats. Part 1: Endocrine adrenal function. *Analy Chim Acta*, 586: 246-51.
- Tariq M, Khan HA, Siddiquei MM, Al Moutaery K. and Al Deeb S., 2007. Protective effect of hydrocortisone on imidipropionitrile-induced neurotoxicity in rats. *Basic Clin Pharmacol Toxicol.*, 100: 176-81.
- Tata DA. and Anderson BJ., 2010. The effects of chronic glucocorticoid exposure on dendritic length synapse numbers and glial volume in animal models: implications for hippocampal volume reductions in depression. *Physiol Behav.*, 99: 186-93.
- Wang JD, Shu SG, Chi CS. and Lin CC., 2007. Successful treatment of acute pancreatitis with hydrocortisone in a patient post adrenalectomy. *Dig Dis and Sci.*, 52 : 3355-57.
- Wirth MM, Scherer SM, Hoks RM. and Abercrombie HC. (2011). The effect of cortisol on emotional responses depends on order of cortisol and placebo administration in a within-subject design. *Psychoneuro-endocrinology*, 12:269-282.
- Yarushkina NI., 2008. The role of hypothalamo-hypophyseal-adrenocortical system hormones in controlling pain sensitivity. *Neurosci Behav Physiol*, 38: 759-66.
- Ye P, Kenyon CJ, Mackenzie SM, Nichol K, Seckl JR., Fraser R, Connell JM. and Davies E., 2008. Effects of ACTH, dexamethasone and adrenalectomy on 11  $\beta$ -hydroxylase (CYP11B1) and aldosterone synthase (CYP11B2) gene expression in the rat central nervous system. *J. Endocrinol.*, 196: 305-11.

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