Histological and morphometric changes in adrenal cortex of aged male albino rats

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Abstract: Background: Adrenal Cortical hormones play vital roles in a number of physiologic processes, including: electrolyte and fluid balance, cardiovascular homeostasis, carbohydrate, protein and lipid metabolism; immune and inflammatory responses, sexual development and reproductive function **Aim of the work:** The aim of this work to study the histological changes in different zones of aged adrenal gland and changes in the zonular pattern of the gland. **Material & Methods:** 20 male albino rats were used. The animals were divided into 2 groups, ten rats for each group. The animals were scarified at age (5months) and old age (2 years).The adrenal glands were processed for histological studies (light and electron microscope) and morphometric studies. **Results:** Variable histological and morphometrical changes were observed in the aged adrenal cortex compared to the adult one. **Conclusion:** Marked histological and morphometrical changes were observed in this study in aged adrenal cortex that could hinder its function, additional studies must be performed to do trials for attenuating these changes through addition of certain Food Supplements containing suitable antioxidants.
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Keywords: Adrenal gland, aging, albino rats

Introduction:

The adult human adrenal cortex is surrounded by a mesenchymal capsule and subdivided into three principal concentric biochemically and morphologically different zones of steroid synthesizing cells **(Jungueira and Carneiro, 2003).** The outer zona glomerulosa (zg) synthesizes the mineralocorticoid aldosterone; the middle zona fasciculata (zf) produces the glucocorticoid cortisol (corticosterone in mice and rats), and the inner zona reticularis (zr) bordering the medulla makes the so called adrenal androgens in humans and some primates, though not in rats and mice **(Wotus *et al.*, 1998).**

Adrenal Cortical hormones play vital roles in a number of physiologic processes, including: electrolyte and fluid balance, cardiovascular homeostasis, carbohydrate, protein and lipid metabolism; immune and inflammatory responses, sexual development and reproductive function **(Hart and Barton, 2011).**

Aging caused a notable lowering in the plasma aldosterone concentration and a marked decrease in both basal and ACTH- or angiotensin II -stimulated secretion of collagenase-dispersed zona glomerulosa (ZG) cells. Plasma renin activity underwent an age-dependent decrease, while the plasma level of ACTH displayed a significant rise. ZG and its parenchymal cells did not evidence an age-related morphologically demonstrable alteration in their growth, nor ZG cells showed any marked ultrastructural change, with the exception of a severe lipid-droplet repletion. This last finding is in keeping with the aging-induced decrease in the secretory activity of ZG cells, during aging **(Belloni et al., 1992).**

Aim of work:

The aim of this work to study the histological changes in different zones of aged adrenal gland and changes in the zonular pattern of the gland.

Material and methods

A total number of 20 male albino rats were used in this study.The animals were brought from the animal house of Assiut Faculty of Medicine. They were reared under the standard conditions of feeding, light-dark ratio and temperature. The animals were housed according to age and were divided into 2 groups, ten rats for each group. The animals were scarified at age (5months) and old age (2 years).

Preparation of material:

The rats were sacrificed.Adrenal glands of rats from each group were used. The adrenal glands were promptly removed, freed of pericapsular fat and immediately processed for histological studies.

Histological Methods for *Light* and *Electron Microscopy* (Bozzola and RusseL 1992):

Immediately after sacrificing animals, sliced pieces of the adrenal gland were fixed in 2.5% glutaraldehyde for 24 hours. The pH value (7.4) was adjusted by adding 2.7 of solution (B) to 50 ml ofsolution (A) and diluting to a total volume of 200 ml.The specimens were washed in sodium cacodylate buffer for one hour for3 changes, twenty minute each. One of them may be left overnight. The specimens were postfixed in 1% osmium tetraoxide in sodium I cacodylate buffer for two hours. The specimens were

washed in four changes of sodium cacodylate buffer (PH 7.2) for 20 minutes. The specimens were dehydrated in ascending grades of alcohol, ethanol 30% for five minutes, 50% for 15 minutes, 70% for 15 minutes, 95% twice 15 minutes each and absolute alcohol twice 20 minutes each. Then the specimens were put in propylene oxide for 30-60 minutes, propylene oxide/Mollenhauer's Epon-Araldite formulation 1:1 for 30-60 minutes. The specimens were embedded in Mollenhauer's Epon-Araldite. The tissue blocks were polymerized in an oven:- At 35, 45 and 60 centigrade for 24 hours before sectioning. Sections were stained by 2% aqueous Toluidine blue for light microscopic examination, then dried on a hot plate at about 40 centigrade and examined by light microscopy and photographed. Ultrathin sections (500-800Å) from selected areas of trimmed blocks were made by diamond knives and collected on copper grids. The ultrathin sections were then contrasted in urinyl acetate for 10 minutes and lead citrate for 5 minutes. They were examined and some areas were photographed by electron microscope (Jeol JEM 1010) in electron microscopic unit, Sohag University.

3- Morphometric measurements:

Quantitative measurements were done using image J program at Faculty of medicine, Sohag University. The measurements were done in 6 fields in 5 animals each group. The measurements included:

(1) Zonal width in adult and old rats.

(2) Lipid count in the three zones at adult and old age groups.

(3) Lipid diameter in the zones at adult and old age groups.

(4) Mitochondrial count in the zones at adult and old age groups.

(5) Mitochondrial diameter in the zones at adult and old age groups.

Results

1. Light microscopic examination of semithin sections

Adult adrenal cortex

adult rat adrenal cortex appeared covered with the capsule and the trabeculae extended from it, zona glomerulosa appeared with its polygonal cells with vacuolated cytoplasm and rounded vesicular nucleus The outer part of zona fasciculata were observed (Fig.1) medullary cells appeared as large pale cytoplasm and pale nucleus, in the subscapular part of the zona glomerulosa (Fig. 2).

The zona fasciculata appeared as polyhedral cells with clear cell membranes, vacuolated cytoplasm with lipid droplets and large rounded nuclei with prominent nuclei. Binucleated cells can be seen. Medullary cells could be observed in between cells of the zona fasciculate and characterized by its large size and pale cytoplasm containing chromaffin granules (Fig. 3). Anastomosing cords of the zona reticularis with wide blood sinusoids in between them was observed (Fig.4).

Old adrenal cortex

In old rats, cells of zona glomeruolosa appeared with foamy cytoplasm. packed cells of zona intermedia in between was observed. Som e cells appeared pyknotic (Fig.5). zona fasciculata cells appeared hypertrophic compared to adult group. Some nuclei have irregular outline (Fig.6).

Aggregation of fibroblasts were observed in zona fasciculata (Fig.7). Apart of large cytoplasmic vacuoles Zona reticularis of an old rat adrenal cortex appeared more or less as the adult group (Fig. 8).

2. **Electron microscopic examination**

Adult adrenal cortex

Ultra structurally,zona glomerulosa cells of an adult adrenal cortex had different cytoplasmic organelles as., Golgi, mitochondria,smooth endoplasmic reticulum, lipid droplets, free ribosomes and euchromatic nucleus with peripheral heterochromatin Microvilli appeared project from its surface (Fig.9) cells of the zona intermedia appeared as dark cells with irregular euchromatic nucleus (Fig.10).

Cells in the zona fasciculate appeared with rounded mitochondria having tubular cristae the cells were separated by blood capillaries which is lined by fenestrated endothelium. Microvilli of the cells project into the pericapillary space (Fig. 11) Cells of zona reticularis appeared with pyknotic nuclei and lysosomes. Lipid droplets and microvilli,mitochondria and smooth endoplasmic reticulum. (Fig. 12)

Old adrenal cortex

zona glomerulosa cells had shrunken nucleus with condensed peripheral heterochromatin,oval mitochondria with lamellar cristae some mitochondria contain lipid like inclusions and confluent lipid droplets. Collagen fibers were observed in the intercellular spaces. The pericapillary space contains microvilli and collagen fibers (Figs. 13 & 14). Zona intermedia cells had swollen mitochondria with accumulation of large sized lipid droplets nuclei with irregular nucler envelope and pyknotic nuclei were observed (Fig.15). In zona intermedia cells has increased amount of dilated smooth endoplasmic reticulum and lysosomes were observed (Figs.16 & 17). The nucleus appeared with condensed heterochromatain. (Fig.17). Lipofuscin pigments and mitochondria with lipid like inclusions were observed in other cells (Fig. 17). Cells zona fasciculata had pyknotic nuclei rounded mitochondria with disorganized cristae Lipofuscin pigments and excessive collagenous material were observed (Fig.18) fibroblast cell with branched cytoplasm and irregular nucleus was observed between the cells of the zona fasciculata also apoptotic body can be detected (Fig.19) zona reticularis cells had euchromatic nucleus with irregular nuclear

envelop. Mitochondria had distorted tubular cristae. There were abundant lipid droplets, lysosomes and lipofuscin pigments with s increase in the amount of the collagenous matrix (Fig.20).Macrophage cell in zona reticularis in an old rat showing; large size lipofuscin pigments which consisted of granular matrix and lipid like contents. (Fig.21)

3. Morphomrtric study:

The width of the four adrenocortical zones were measured from the H&E stained sections using magnification of 100 in the adult and old age groups. Adjacent sections stained for reticular fibers, which aided in distinguishing the columns of cells comprising the zona fasciculata from the compact irregularly arranged clusters of cells of the zona reticularis, were viewed at magnification of 100. The data of the two groups were compared by t test and the SD of the mean was calculated.

The average thickness of the zona glomerulosa (arbitrary units, pixels) of the old group (264.82), 14.38% of the cortical thickness, was slightly higher than that of the adult group (256.66), 13.93% with *P* value 0.24 (not significant).

There was significant ($P < 0.0001$) increase in the thickness of the zona intermedia in old age group (9.66%) than adult (6.99%).

There was increase in the thickness of zona fasciculate in older rats (54.07%) compared to that of the adult rats (46.99%). This increase approached statistical significance ($P < 0.0001$).

The median % of the cortex identifiable as zona reticularis in old rats was 48.78% while in the adult rats, it was 32.08% ($P < 0.0001$).

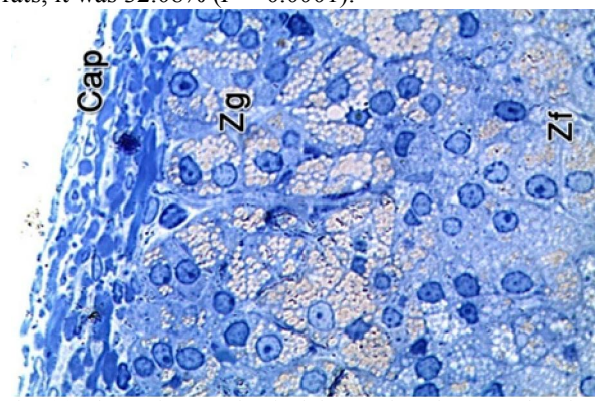


Fig. (1) Photomicrograph of adult rat adrenal cortex showing the capsule (cap) and the trabeculae extend from it, zona glomerulosa (zg) the cells of (z g) are poly gonal cells with vaculated cytoplasm with rounded vesicular nucleus. Outer part of zona fasciculata (zf)was observed Toluidine blue X 1000.

So there was significant hypertrophy in all cortical zones in older than adult groups except in zona glomerulosa the increase was not significant. Histogram (1).

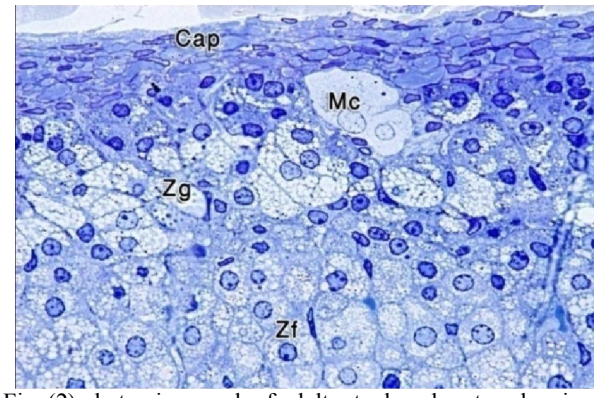


Fig. (2) photomicrograph of adult rat adrenal cortex showing; medullary cells (Mc), characterized by its large pale cytoplasm and pale nucleus, in the subscapular part of the zona glomerulosa (zg). Capsule (cap); zona fasciculata (zf). Toluidine blue X 1000.

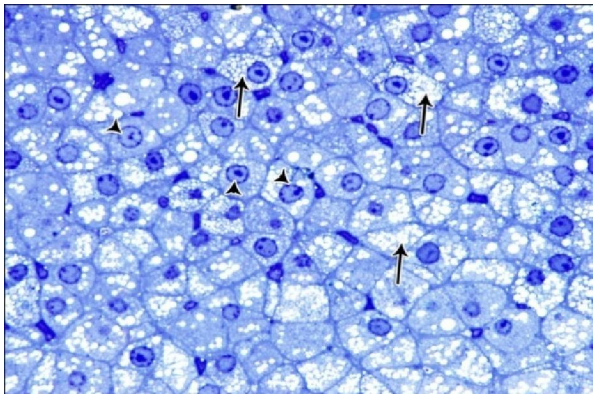


Fig. (3) A photomicrograph of adult rat adrenal cortex showing; the polyhedral cells of the zona fasciculata with excessive lipid droplets (arrows) in the cytoplasm and rounded vesicular nuclei with one or two prominent nucleoli (arrow heads). Note the distinct cell boundaries. Toluidine blue X 1000.

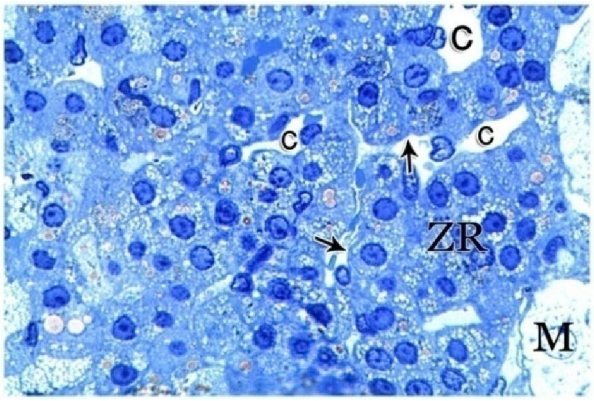


Fig. (4) photomicrograph of adult rat adrenal gland showing the anastomosing cords (arrows) of the zona reticularis (ZR) with wide blood sinusoids (c) Not., adrenal Medulla (M) Toluidine blue X 1000.

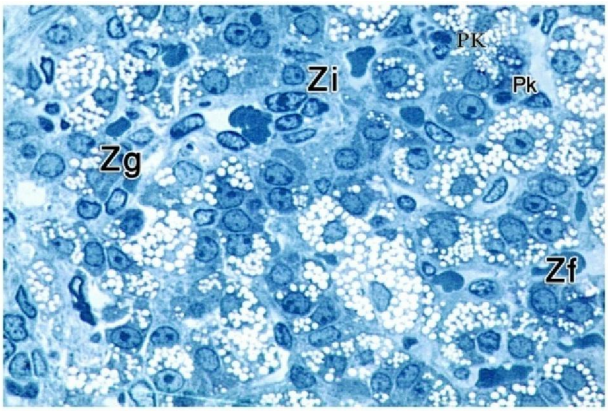


Fig. (5) A photomicrograph of an old rat adrenal cortex showing; the foamy appearance of zonae glomerulosa (zg) and fasciculata (zf) with packed cells of zona intermedia (zi) in between. pyknotic cells (pk). Toluidine blue X 1000.

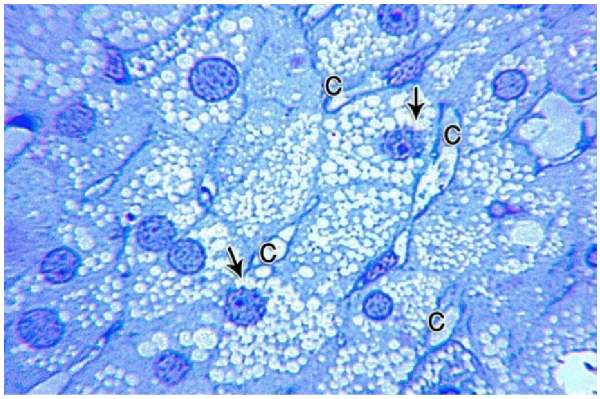


Fig. (6) A photomicrograph of zona fasciculata (ZF) of an old rat showing; the polyhedral cells which are hypertrophic compared to the adult group. The cells are arranged in radially oriented cords. They have foamy cytoplasm and rounded nuclei with prominent nucleoli. Some nuclei have irregular outline (arrows). Toluidine blue X 1000.

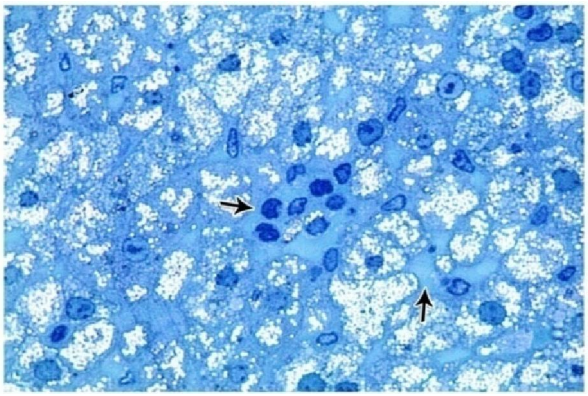


Fig. (7) Aphotomicrograph of an old rat adrenal cortex showing; the aggregation of a group of fibroblasts (arrow) in zona fasciculata. Tolidine blue X 1000.

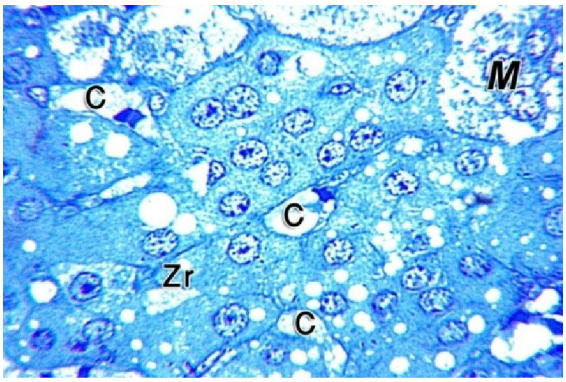


Fig. (8) A photomicrograph of zona reticularis (zr) of an old rat adrenal cortex showing; its columnar cells forming irregular anastomosing network of intermingled cords separated by wide blood capillaries (c). The cells have large cytoplasmic vacuoles, rounded nuclei possessing centrally located nucleoli. Toluidine blue X 1000.

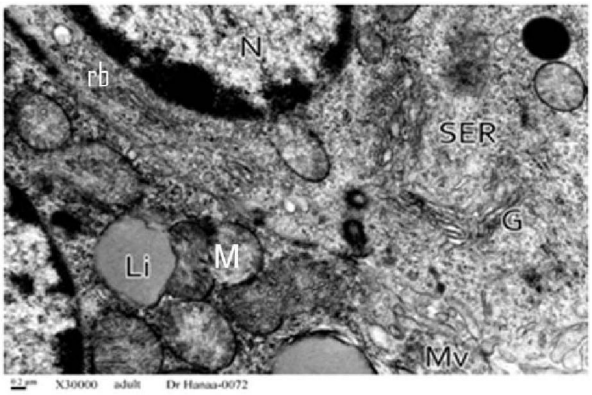


Fig. (9) Electronmicrograph of cells in the zona glomerulosa of an adult rat adrenal cortex showing the cytoplasmic organelles at higher magnification. Golgi (G); mitochondria (M); smooth endoplasmic reticulum (SER); lipid (Li); free ribosomes (rb) and euchromatic nucleus with peripheral heterochromatin (N) Note., microvelli (MV) project from the cell surface X 30000.

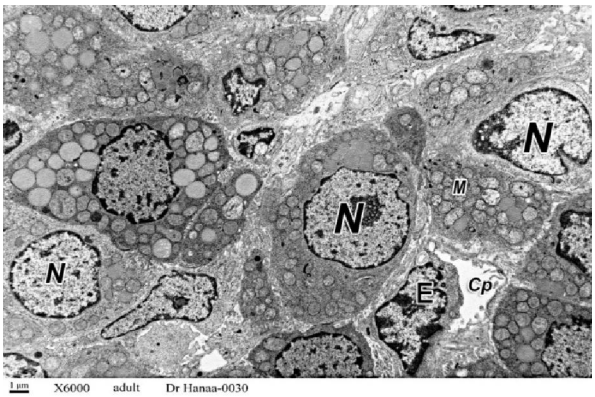


Fig. (10) Electromicrograph of cells in the zona intermedia of an adult rat adrenal cortex showing; oval and rounded mitochondria (M); irregular euchromatic nucleus (N); sinusoidal capillary (cp) lined by endothelial cell (E). X6000

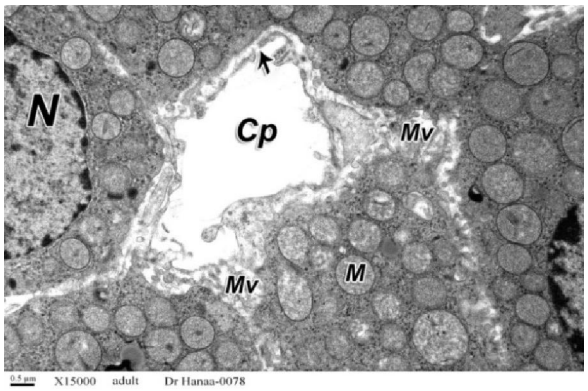


Fig. (11) Electronmicrograph of a cell in the zona fasciculata in an adult rat showing; polyhedral cells with rounded mitochondria having tubular cristae, a blood capillary (cp) in between its cells lined by fenestrated endothelium (arrow). The pericapillary space is clear containing only microvilli (Mv). X 15000.

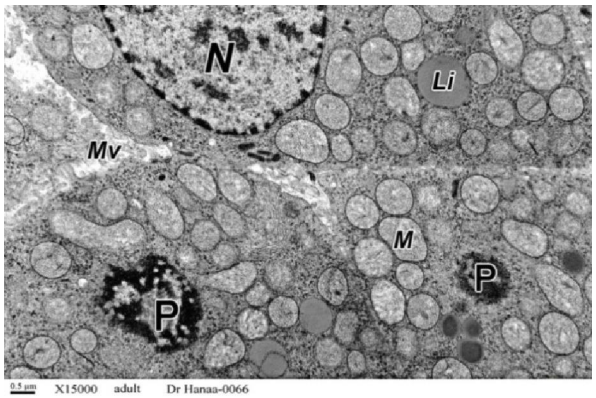


Fig. (12) Electromicrograph of cells of an adult rat zona reticularis showing; pyknotic nuclei (p) and lysosomes (Ly).Lipid droplets (Li) and microvilli (Mv). mitochondria (M). smooth endoplasmic reticulum (SER) X 15000.

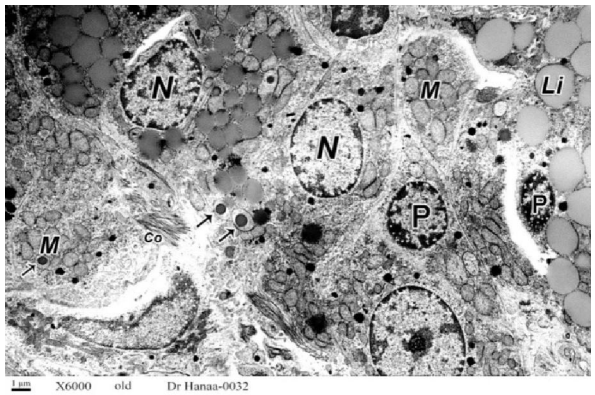


Fig. (13) Electronmicrograph of zona glomerulosa cells in an old rat adrenal cortex showing; shrunken nucleus with condensed peripheral heterochromatin (pyknotic nucleus, p), oval mitochondria with lamellar cristae (M), some mitochondria contain lipid like inclusions (arrows) and confluent lipid droplets (Li) and presence of collagen fibers (co) in the intercellular spaces. X 6000.

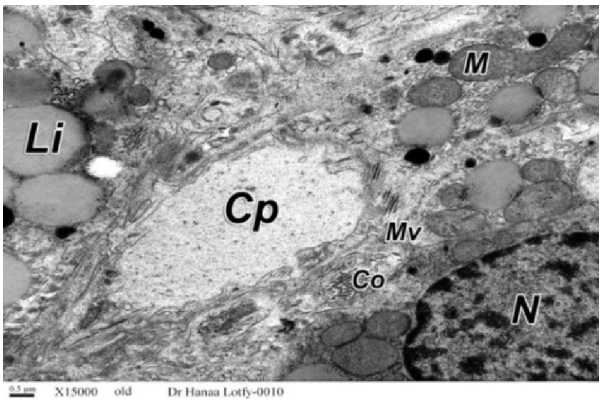


Fig. (14) Electromicrograph of zona glomerulosa of an old rat adrenal cortex showing; euchromatic nucleus with clumps of peripheral heterochromatin. The pericapillary space contains microvilli (Mv) and collagen fibers (Co). Mitochondria (M); lipid droplets (Li). X 15000.

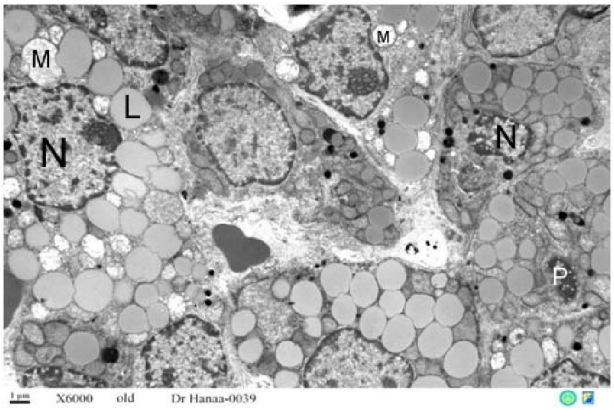


Fig. (15) Electronmicrograph of zona intermedia cells of an old rat adrenal cortex showing; swollen mitochondria (M),, accumulation of large sized lipid droplets (Li), nuclei with irregular nucler envelope (N) and pyknotic nuclei (p). blood capillary (c). X 6000.

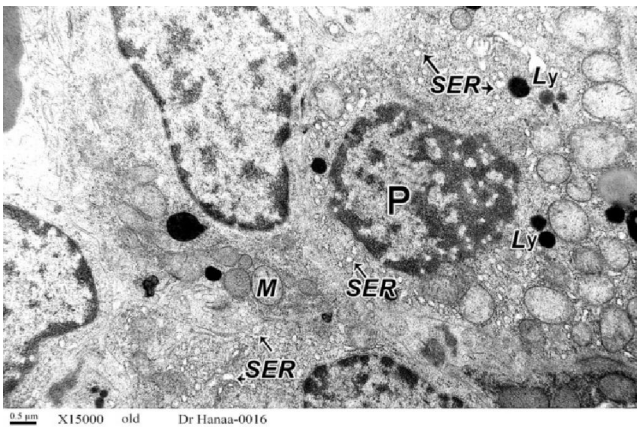


Fig. (16) Electronmichrograph of zona intermedia cells of an old rat adrenal cortex showing; oval mitochondria (M), increased amount of dilated smooth endoplasmic reticulum (SER), lysosomes (Ly) and nucleus with condensed heterochromatain(N). X15000.

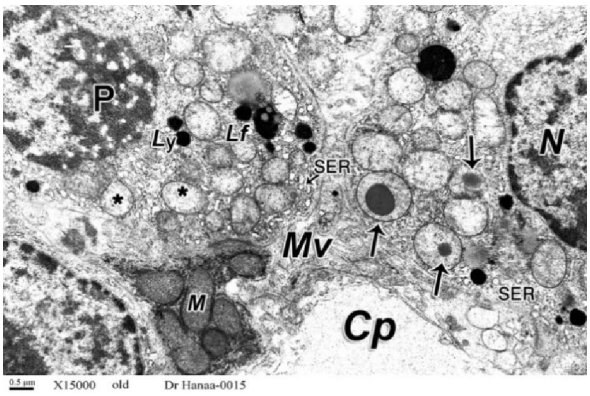


Fig. (17) Electronmicrograph of zona intermedia of old rat showing, lysosomes (Ly), lipofuscin pigments (Lf), normal mitochondria (M) others with lipid like inclusions (arrows); some mitochondria with disrupted organelles; dilated smooth endoplasmic reticulum (SER). Nucleus (N); nucleus with condensed heterochromatin (p); microvilli (Mv). X 15000.

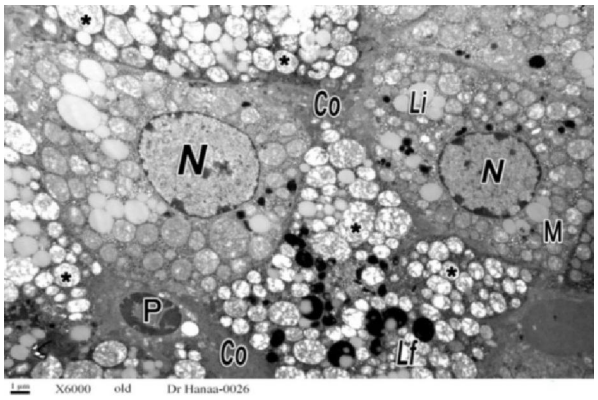


Fig. (18) Electromicrograph of zona fasciculata in an old rat adrenal cortex showing; euchromatic (N) and pyknotic (p) nuclei rounded mitochondria with disorganized cristae (*), lipofuscin pigments (Lf) and excessive collagenous material (co). X 6000.

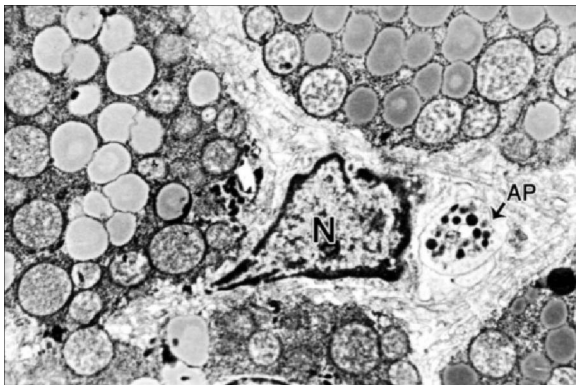


Fig. (19) Electronmicrograph of zona fasciculata in an old rat showing; fibroblast cell between the cells of the zone with irregular nucleus (N) and branched cytoplasm. Note the apoptotic body (ap). X 15000.

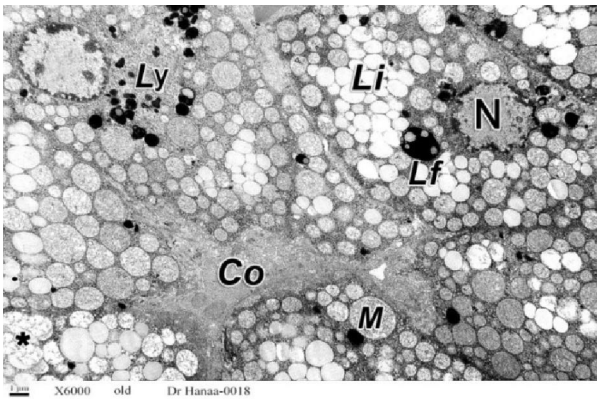


Fig. (20) Electronmicrograph of cells of the zona reticularis in an old rat showing; euchromatic nucleus with irregular nuclear envelop (N), mitochondria with distorted tubular cristae (*), abundant lipid droplets (Li), lysosomes (Ly) and lipofuscin pigments (Lf). There is increase in the amount of the collagenous matrix (co). X 6000.

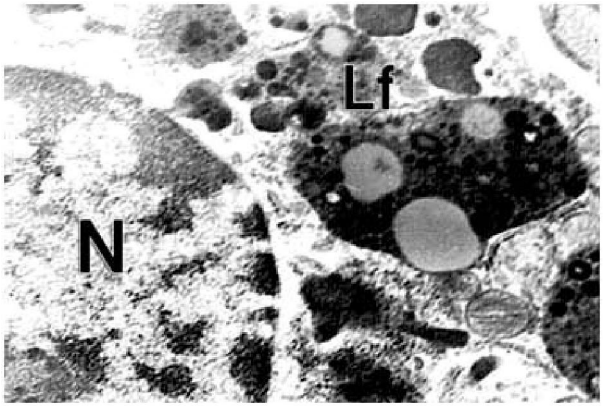


Fig. (21) Electronmicrograph of a macrophage cell in zona reticularis in an old rat showing; large size lipofuscin pigments which consisted of granular matrix and lipid like contents. X 15000.

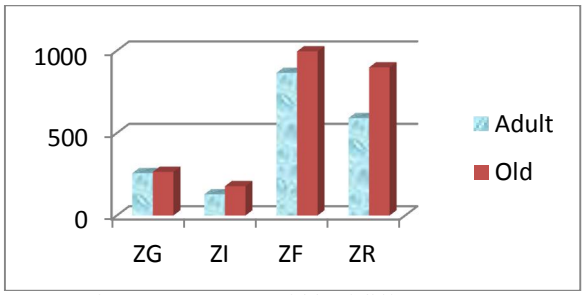
On electron micrographs at a magnification of 6000, number and diameter of the lipid droplets in the main three zones were estimated.

The data obtained were averaged per group, and the SD of the mean was calculated. The statistical comparison of the data was performed by use of the t test.

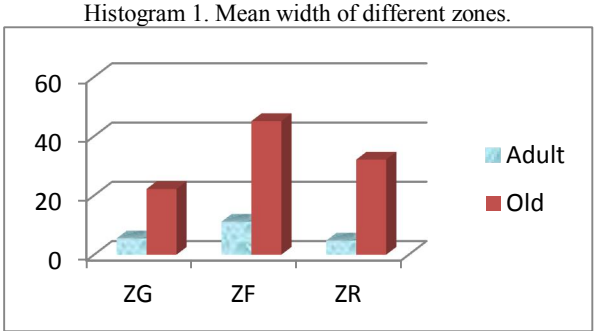
There was significant increase in the number Histogram (2).and diameter Histogram (3) of the lipid droplets in the main three cortical zones ($P< 0.0001$).

There was significant increase in the number Histogram (3). and width Histogram (5).of the mitochondria in the zona fasciculate and zona reticularis in old group than adult one.

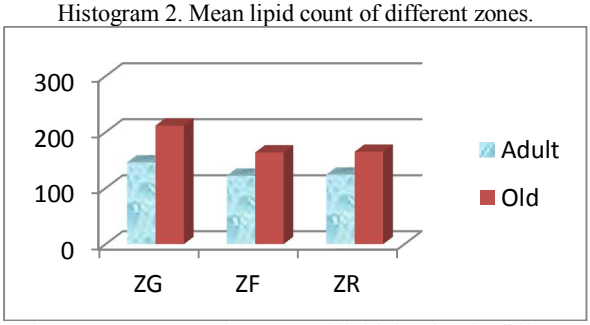
There was non significant increase in the number of the mitochondria in the zona glomerulosa ($P = 0.11$) Histogram (4). But there was significant increase in the diameter of the mitochondria in this zone ($P < 0.0001$) Histogram (5).



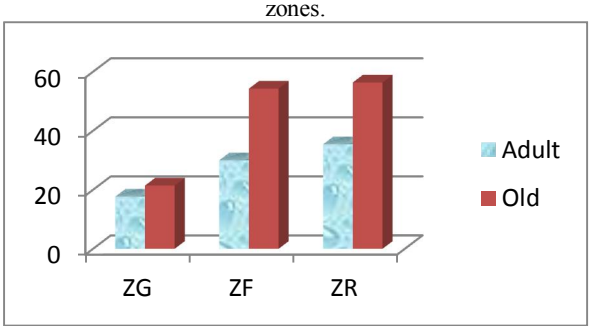
Histogram 1. Mean width of different zones.



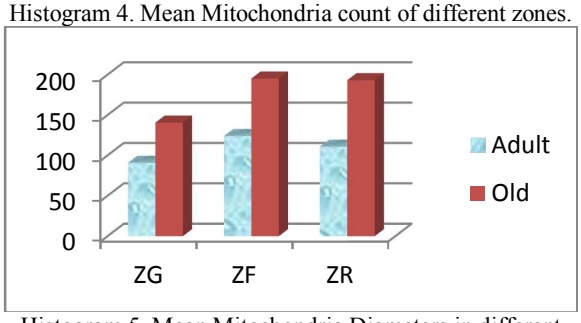
Histogram 2. Mean lipid count of different zones.



Histogram 3. Mean Diameters of lipid droplets in different zones.



Histogram 4. Mean Mitochondria count of different zones.



Histogram 5. Mean Mitochondria Diameters in different zones.

Discussion

In the present study we examined the detailed morphological features of the adult rat adrenal cortex as well as the effects of ageing on the gland.

Regarding the parenchymal adrenocortical cells in the adult rats, the present work showed that:

The zona glomerulosa cells (13.93% of the cortical thickness) were arranged in rounded groups or glomeruli of vacuolated cells. The cells of the zona fasciculata (46.99% of the cortical thickness) arranged in fascicles or radial columns with well defined cellular outlines. Its cells

part of the zona fasciculata. This result was in agreement with that of **Mirko Spiroski., 2010.** The reticular zone was composed of small irregularly arranged cells surrounded by wider sinusoidal capillaries which, in contrast to the radial capillaries of the zona fascisulata, form anastomosing system. Many apoptotic cells with pyknotic nuclei were evident in the reticularis. These observations are in accordance with those of **Nussdorfer, 1986 and Otis *et al.*, 2007.** The presence of the apoptotic cells exclusively in this zone without the other two zones fits well with the migration theory of adrenal cortical cell renewal.

Bielohuby *et al.*, 2007 described three hypothetical theories on zonation of the adrenal cortex that could be invoked toexplain the growth and differentiation that occur within theadrenal cortex: 1) the cell migration theory; 2) the transformation field theory; and 3) the zonal theory.The zonation theory hypothesizes that each zone develops independentlyof the other. An increasing number of studies, as **Zajicek *et al.*, 1986; Mitani *et al.*, 1994** supported the cell migration theory of adrenal cortex cytogenesis. In this theory, each is derived from a common pool of progenitor cells located in the periphery of the postnatal adrenal gland (i.e., zona glomerulosa/zona fasciculata boundary and/or subcapsular layer). **Pawlikowski, 2005** stated that in the adrenal cortex the cell proliferation indices are lower when we pass from zona glomerulosa to the inner zones and are the lowest in ZR. In contrast, the apoptotic index is the highest in ZR. Consequently, each adrenocortical cell sequentially adopts the phenotypes of glomerulosa, fasciculate and reticularis cells during its migration through the gland.

The present study revealed that chromaffin cells occurred in the cortex of the adrenal gland in adult rats which frequently spread into the subcapsular region of the zona glomerulosa or in the form of finger like projection from the medulla. They were in close contact with the adrenocortical cells with no separating CT in between. Similar rays of chromaffin cells have also been observed by **Gallo-Payet *et al.*, 1987** and **Bornstein & Rutkowski 2002**

On the basis of morphological data of the present study, it is clear to demonstrate the existence of intimate cellular contacts between adrenal medullary cells and cortical cells. This contact may be important in regulation of adrenocortical function since increasing evidence exists for a functional interaction of adrenal medulla and adrenal cortex. The classical perspective of the adrenal gland has in general, treated the cortex and medulla as functionally independent tissues which, by chance are located together. Recent data challenge this outlook and there is now evidence of regulatory mechanisms which are common to both the cortex and the medulla (**Hinson, 1990**).Examples of functional interactions include the maintenance of chromaffin cell phenotype by corticosteroids and the modulation of steroidogenesis by the neurotransmitters and neuropeptides expressed by chromaffin cells **(Claude Colomer *et al.,* 2012**). The mammalian adrenal is organized such that a central medulla is surrounded by a zonated adrenal cortex with centripetally directed blood flow (**Rosal *et al.,* 2001**), providing the mechanism by which adrenocortical cell products directly affected medullary cells. On the basis of this organization, however, it is unlikely that medullary products affect adrenocortical cells via vascular perfusion. Alternatively, neuronal and paracrine interactions are the likely mechanisms for medullary control of adrenocortical function in mammals (**Hinson, 1990** and **Holzwarth *et al.,* 1987**).

Regarding the ultrastructural features of the adult rat adrenal cortex, the present study showed that four regions differentiated mainly by the mitochondrial morphology, the lipid droplets, and the structure of the ground cytoplasm were recognized. In the zona glomerulosa, mitochondria were thin and elongated with lamellar cristae. In the zona intermedia, mitochondria were larger and elongated or irregular. The matrix was filled with lamellar as well as vesicular cristae. The lipid droplets were numerous, larger and irregular in the glomerulosa comparing to less and smaller lipid droplets in the intermedia. The present study showed that the mitochondria of the ZF had the usual vesicular cristae and dense matrix. The lipid droplets were abundant, scattered and displayed low, These observations accord well with **Black *et al.,* 2005**. Golgi zones were seldom seen in the zona fasciculata in the present work, may be due to the compact arrangement of the intercellular organelles which obscure this structure from view.

In the present study we recognized that, in addition to the basic three layers of the cortex, a cell layer existed between the zona Glomerulosa and the zona fasciculata, zona intermedia (ZI) (6.99% of cortical width). A similar layer has been visualized in rats & sheep (**Peterson *et al.,* 2001**) and marmoset adrenal (**Bird and Pattison 2004**). This zone is not present in the mouse (**Guasti *et al.,* 2011**). In this zone the cells were darker and the nuclei were closely packed than in the adjacent zones (ZG, ZF). The mitochondria in this zone had both lamellar and vesicular cristae. These finding in accordance with **Bornstein *et al.,* 2002** who stated that this zone contained neither P450 aldo nor P450 11B, it is considered inert in producing both corticosterone and aldosterone. As the rat adrenal cortex has been known not to contain steroid 17α hydroxylase (**Nishihara *et al.,* 1989**), so this zone is presumed to be incapable of synthesizing adrenal androgens as well. They reported also that the ZI may be the product of rapid cell division at the inner edge of the ZG or outer edge of the ZF must be considered, particularly as these are the regions of maximum mitotic activity in the cortex. If an inward passage of cells from the zona glomerulosa is responsible for the presence of ZI, this would support the popular theory of a centripetal migration of cortical cells (**Hu *et al.,* 1999**). **Mitani *et al.,* 2003** reported that in this zone, DNA synthesizing cells (which labeled by Brdu, 5-bromo-2'-deoxyuridine, a thymidine analogue) were abundant in and around this zone and they migrated towards the center of the adrenal gland i.e. to the border between the cortex and the medulla. Based on these findings, they suggested that the zone contained the stem cells for the adreno-cortical cells.

The present work declared that the cells of the ZR were smaller, their nuclei contained more condensed chromatin, few lipid droplets were present, abundant profiles of SER and elongated mitochondria with vesicular or tubulo-vesicular cristae could be seen. These finding were observed by **Suzuki *et al.,* 2007.** In the present study we noticed that the macrophages were especially numerous among zona reticularis cells. This is in agreements with **Almeida *et al.,* 2004** who stated that this distribution pattern, which may be related to different functions, agreed with the migration theory. Also the present observation is in accordance with ***David*, *2003*** stated that in addition to phagocytic activity of the macrophages, they produce and secrete cytokines which interact with adrenocortical cells and influence their functions. **Currie *et al.,* 2000** identified a novel paracrine signaling pathway between macrophages and adrenal chromaffin cells that regulate catecholamine release. It is also becoming apparent that resident macrophages can participate in paracrine signaling within the adrenal gland; locally produced cytokines act on the adrenal cortex to stimulate steroid production (**Mazzocchi and Nussdorfer. 1998**).

Steroid secreting cells are characterized by abundant SER which contains many of the enzymes for sterol and steroid synthesis **(Junqueira. 2003)** Changes in SER volume and enzyme content with functional state indicate that there must be fluctuations in synthesis and degradation of HMGR (3-hydroxy-3-

methylglutaryl-coenzyme A) which is a key enzyme in cholesterol synthesis. So morphologically identifiable RER, free and bound ribosomes, which were demonstrated in the present study in cells of adrenal cortex, were presumably required for these functions. This RER increases when cells are treated by ACTH (**Black *et al.,* 2005**). We observed microvilli in the pericapillary and intercellular spaces around the parenchymal adrenocortical cells in all zones. This result is in consistent with the previous studies as that carried by **Feinmesser *et al.,* 1992**. The microvilli had a role in adrenocortical secretion and the increase in the number and size of the microvilli has been thought to lead to an increase in the surface area of the adrenocortical cells, thereby facilitating hormone discharge. Also the detachment of microvilli from adrenocortical cells may represent a form of apocrine secretion and contribute to hypercorticosteronaemia in corticotrophin releasing hormone excess.

Regarding the old rats, we observed that there was non-significant increase in the width of the zona glomerulosa (P=0.24) in the old rats. There was an age dependent significant increase in the width of the zona intermedia (P< 0.0001) zona fasciculata (P<0.0001) and reticularis (P<0.0001) in old rats. These observations are in accordance with **Rebuffat *et al.,* 1992** who stated that this increase was due to both hyperplasia and hypertrophy of their parenchymal cells, which in turn may be caused by the chronic exposure to elevated plasma concentrations of ACTH, and as a compensating response for the age dependent decrease in glucocorticoid secretory activity of ZF and ZR cells **(Deepashree.2014).** The present findings suggested that there was altered zonation with aging with increased irregularity of the cortical zones and the border between the zona reticularis and the zona fasciculata becomes increasingly tortuous with age, this is in agreement with **Pawlikowski, 2005**

The present results revealed that there was significant increase in the number (P< 0.0001) and diameter (P< 0.0001) of the lipid droplet in the three main functioning cortical zones in old rats, this in accordance **Rebuffat *et al.,* 1992.** As the lipid droplets are the intercellular stores of cholesterol esters, the obligate precursors of steroid hormones in rats,this finding is explained as impairment of the utilization of intracellular cholesterol-esters stores ina non-metabolizable form of steroidogenesis.

In addition to lipid droplets repletion, swollen mitochondria with disorganized cristae and lipid like contents inside it in the ZG of old rats. There was significant increase in the mitochondrial size (P< 0.0001) but non-significant increase in the mitochondrial number (P= 0.11). These observations were in close conformity with **Belloni *et al.,* 1992. Nussdorfer, 1986** reported that the plasma concentration of aldosterone is decreased and ZG doesn't evidence any sign of hypertrophy or hyperplasia; though the plasma levels of ACTH which is well known to stimulate the growth of the ZG are significantly increased. **Pignatelli *et al.,* 1998** could argue in favor of the independence of ZG in relation to the inner zone with the following reason: In normal conditions, ZG is known to be regulated independently from the inner zones, particularly by the rennin-angiotensin system. The same occurs upon direct stimulation with angiotensin infusion with both sodium restriction and angiotensin infusion an increase in cell proliferation was demonstrated (**McEwan *et al.,* 1999**).

We demonstrated a marked proliferation of SER and a significant (P< 0.0001) increase in the size and number of the mitochondria (the two organelles responsible for steroidogenesis) in ZF cells in old rats. The mitochondria were swollen and contained disorganized and disrupted cristae. This is consistent with **Murakoshi *et al.,* 1985**. These morphologic changes, conceivably due to the chronic exposure to high levels of circulating ACTH which occur during aging, are interpreted as a response enabling ZF and ZR to compensate for their age-dependent lowering in glucocorticoid secretion (**Rebuffat *et al.,* 1992**).

We demonstrated age dependent accumulation of primary and secondary lysosomes and lipofuscin pigments in the zona fasciculata with electron microscope as well as with the light microscope examination. This contention accords well with the demonstration carried by **Rebuffat *et al.,* 1992 and Almeida *et al.,* 1998.**

IN ZR the most obvious finding was the numerous lipofuscin pigments which displaying similar features to those observed in ZF. Increased numbers of macrophages and features of phagocytosis of debris of parenchyma cells were common in this zone. This is in accordance with **Almeida *et al.,* 1995**. We reported nearly the same mitochondrial alterations that were seen in the ZF. These observations are in accordance with **Almeida *et al.,* 1995**, they reported that giant mitochondria suggest abnormal permeability of the membrane mainly of the outer membrane.

Obvious increase in macrophages in all zones especially zona reticularis was observed. These observation are in accordance with **Almeida *et al.,* 2004**, they explained this finding as macrophage may operate as paracrine regulators in an attempt to enhance corticosterone production and secretion. Also **Ehrhart-Bornstein *et al.,* 1998** reported that macrophages secrete interleukin-1 which causes corticosterone release in the rat adrenal, whether directly or indirectly.

Lipofuscin pigments, a common structure in aged cells of ZF and ZR, are frequently observed as a classic marker of ageing. **Ward and Reznik- Schuller, 1980** stated that lipofuscin pigments may have originated

from degradation of lipid droplets of epithelial cells. Lipofuscin does not exert any obvious damaging effect on cells but it is possible that, at a certain moment, this accumulation may interfere with cellular function (**Almeida *et al.*, 1998**). Although quantitative measurements were not made, it was obvious in the present study that the number of pigment bearing cells and the total amount of pigment varied between adult and old age group and with narrow limits among animals of the same age group. The nuclei of some cells in ZR showed signs of pyknosis, karyorrhexis and karyolysis which were characteristic to this zone. Again these finding may support the cell migration theory which consider that the outermost part of the cortex is the major site of adrenal cell proliferation, and the inner zones are the site of cell death (**Wolkersdorfer and Bornstein 1998; Mitani *et al.*, 2003**).

Definite changes in the structure and zonular pattern of the adrenal glands have been observed with ageing. The most prominent structural changes in the adrenal cortex were the fibrosis in zona reticularis accumulation of lipofuscin pigments associated with some degree of cellular degeneration in the inner two zones of the cortex. There were significant increase in the thickness of both zona fascilculata and zona reticularis. From the previous finding we can concluded Marked histological and morphometrical changes were observed in this study in aged adrenal cortex that could hinder its function, additional studies must be performed to do trials for attenuating these changes through addition of certain Food Supplements containing suitable antioxidants.

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العربى الملخص

الغده قشرة فى والقياسيه النسيجيه التغيرات المسنه الفئران لذكور كلويه الفوق

، محمد صابر ضحى صادق لطفى هناء***

معة جا بنين طب كلية التشريح قسم * مصر.سوهاج

مصر.سوهاج معة جا كليةالطب الهستولوجى قسم **

القشرية الكظرية الغدة هرمونات تلعب **الخلفية** في بما ،الفسيولوجية العمليات من عدد في حيويا دورا القلب توازن ،السوائل وتوازن الاملاح نسبة :ذلك والتمثيل والبروتين والكربوهيدرات ،الدموية والأوعية ،والألتهابات المناعية الاستجابات .للدهون الغذائي **:البحث من الغرض**.بيهالإنجا والوظيفة الجنسي والنمو الغدة من مختلفة مناطق في النسيجية التغيرات دراسة البالغه والقئران المسنه الفئران فى الكظرية **:والطرق المواد** .للغدة النطيقي نمط في والتغيرات تقسيم تم .البيضاء الجرذان ذكور من20 استخدمت .مجموعة لكل فئران عشرة ، مجموعتين إلى الحيوانات اشهر خمسة سن (البالغه الحيوانات) الأولى المجموعه تشريح تم .عامان (المسنه الحيوانات) الثانيه المجموعه للدراسات الكظرية الغدد على والحصول الحيوانات (الإلكترونى والمجهر الضوئى بالمجهر) النسيجية **:النتائج** .الكظرية الغدة قشرة في القياسيه والدراسات الحيوانات فى وقياسيه نسيجيه اتتغير لوحظ ولقد الحيوانات مع بالمقارنة بين أعمارهم تتراوح المسنه الغدة قشرة في الدراسة هذه في :**الخلاصه**.البالغه يجب ،وظيفتها تعيق أن يمكن التي القديمة الكظرية هذه تخفيف لمحاولة للقيام إضافية دراسات إجراء التي غذائيةال المكملات بعض إضافة خلال من التغيرات .مناسبة للاكسدة المضادة المواد على تحتوي

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