



Histological, and Transmission Electron Microscopic Investigation of Adrenal Gland of Iraqi Local Squirrel (*Sciurus anomalous*)

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Abstract

The adrenal gland is one the main endocrine gland, which is responsible for producing important hormones in relation to regulating functions of the nervous system, metabolism and balance of ions, and reproduction of the animals. The present study was designed to identify histological contents and structures of adrenal glands in the Iraqi wild squirrel. Electronic microscopic, routine and special stains were used to recognize the main cells and major histological structures. Twelfth healthy animals were dissected for this study following the animal ethics regulations of the college of veterinary, university of Al-Qadisiyah. The histological study identified that the adrenal gland has two main layers' the medulla and cortex, also, the cortex has three layers including zona glomerulosa, zona fasciculata and zona reticularis. Cells of the cortex and medulla of the gland were negative with PAS stain, but Alcian blue was positive with cortex cells and negative with medulla. The features and distributions of cells of the cortex were in the zona glomerulosa was paved together cells and constituted a thin layer under the capsule, and zona fasciculata was extended as pillars in shape and a thicker layer of the cortex and zona reticularis, also medulla cells were clusters cells in shape and found in the centre of gland surrounded the central vein. Electronic transmission study was focused on the contents of cells, as a result, large secretory granules were detected as dark in colour and were very large amounts in zona glomerulosa, and zona reticularis, and a limited amount in zona fasciculata. Also, the medulla displayed many dense small secretory granules in the cytoplasm and limited moderately secretory granules. The cells of the layers' cortex and medulla revealed that the nucleus was polyhedral in shape, but cells of zona fasciculata was single or diploid nuclei. Our finding concluded that special and routine stains clarified many structures of the adrenal gland, but electron transmission microscopic was the better technique to distinguish different types of granules in the adrenal gland of the squirrel.

Keywords: Adrenal gland, zona glomerulosa, zona fasciculata and zona reticularis, and Secretory.

Introduction

Adrenal glands or suprarenal glands are paired endocrine glands located at the cranial poles of the left and right kidneys, it has triangular-shaped glands (1, 2). Adrenal glands are very important glands and produce different hormones that regulate essential functions of the body such as the balance of ions, immune system, nervous system, metabolism of carbohydrates, immune system, blood pressure

and reproduction (3-5). The adrenal glands are embedded with adipose tissue of kidneys and received blood supply from the superior suprarenal artery, a branch of the inferior phrenic artery, the middle suprarenal artery, a direct branch of the abdominal aorta, the inferior suprarenal artery, and a branch of the renal artery (6, 7), while veins supply is by the right suprarenal vein drains into the inferior



vena cava, whereas, the left suprarenal vein drains into the left renal vein or the left inferior phrenic vein (8, 9). The medullary veins were distributed in the medulla region and constituted the central vein in the middle of the medulla (10-12). Histologically, the adrenal gland has two layers cortex and medulla(13), cortex consists of three layers, zona glomerulosa, zona fasciculata and zona reticularis (14, 15). Also, adrenal gland produced different hormones, subsequently, zona glomerulosa produces mineralocorticoid hormone which is regulated blood pressure and ions (sodium and potassium) (16, 17). The zona fasciculata produces cortisol (18, 19) which regulates several functions in the body including the metabolism of fats, proteins and carbohydrates, stress, suppresses inflammation, blood pressure; blood sugar; and reduces bone formation(18, 20).The dehydroepiandrosterone (DHEA) and androgenic precursor steroid hormones are produced by zona reticularis that are converted estrogens in females and androgens in males (21, 22). In addition, the adrenal medulla is produced epinephrine (adrenaline) and norepinephrine (noradrenaline) which are associated with the sympathetic response of the body (23, 24). Among other things, many secretory granules and different cells are distributed between the adrenal cortex and medulla (25).This study was designed to investigate the electronic, histological and histochemical structures of the adrenal gland of the Iraqi Squirrel (*Sciurus anomalus*) and detect the particular secretory granules in adrenal regions to increase knowledge about functions of the adrenal gland.

Materials and Methods

Ethical approval and samples of tissue

The twelfth healthy mature Iraqi Squirrels (*Sciurus anomalus*) were sacrificed for this

Results

experiment by following regulations of animal ethics at the College of Veterinary, University of Al-Qadisiyah. Animals were divided into nine animals for histology study using routine stains and special stains including Periodic acid–Schiff (PAS) and Alcian blue, and three animals for transmission electron microscopic.

Histology of tissue

Specimens were prepared for histology study after fixation of specimens in the 10% formalin buffer for 48 hours. After that specimens were washed for two hours then different serial dilutions of ethanol from 50%, 70%, 90% and 100%, then specimens were embedded in wax, and sections were made and loaded on slides for the next steps. Afterwards, sections of tissue were stained with histological stain following protocol for (26, 27).

Preparation of Specimens for Transmission Electron Microscopic

Specimens of the adrenal gland of Iraqi Squirrel were directly fixed in 2.5% glutaraldehyde for 24 hours and then were rinsed in 0.1 M cacodylate buffer (PH7.4). The specimens were then post-fixed in 1% osmium tetroxide at room temperature for one hour. Next, specimens were dehydrated in different dilutions of ethanol which started with 70% ethanol for 10 minutes, 100% ethanol for 10 minutes, 100% ethanol for 15 minutes, 100% propylene Oxide for 15 minutes, and finally 100% propylene oxide for 15 minutes. Finally, specimens were embedded in EPON 812 (28) and blocks. Semi-thin sections were obtained from prepared blocks, stained with eosin examined by light microscopy to select the desired areas. Ultra-thin sections were cut and stained with a saturated solution of uranyl acetate dehydrate and lead citrate (29). The stained ultrathin sections were examined and photographed by JEM-1400 TEM at the faculty of agriculture, university of Cairo, Egypt.



Histological study was explained that the adrenal gland surrounded by thin adipose tissue and collagen fibers capsule, and internally comprised of the adrenal cortex and adrenal medulla, and the central vein was in the middle of the medulla. Afferent blood vessels penetrated the capsule and parenchyma and then branched into sinusoids to supply the cortex and medulla. Moreover, the adrenal cortex displayed three layers involved with zona glomerulosa, fasciculata, and reticularis, figure (1, and 5). The zona glomerulosa was a thin layer located under the capsule and cells were compressed together, and circled polyhedral cells arranged in acini in shape with a prominent basophilic nucleus, narrow acidophilic cytoplasm and noticed fewer lipid droplets in these cells. Many trabeculae were extended from capsules between the acini of the parenchyma of zona granulosa, figure (1, and 5). Too, zona fasciculata was the underneath and middle zone of the adrenal cortex and constituted the majority of cells of this region. Cells of this layer were the largest cells and arranged in cord-like rows from zona granulosa toward the next layer zona reticularis and venous sinuses were detected between these cells. Also, cells have central nuclei, some of these cells were diploid nuclei and the cytoplasm of these was foamy due to the presence of droplets of lipids and pale in colour because it contained numerous small lipid vacuoles figure (2, and 6). The zona reticularis was the innermost zone of the cortex, this layer was thinner than zona fasciculata and thicker than zona granulosa. Cells were irregular in shape with vesicular nuclei and wide anastomosing rows between cells. The cytoplasm showed many irregulars and smaller sizes. Few capillaries were observed and sinusoids as well. Finally, the medulla was established centre of the adrenal gland, the chromaffin cells were seen as columnar in shape or polygonal with prominent nuclei with

basophilic granular cytoplasm. Additionally, small numbers of sinusoids between cells were seen, (4, and 7). The special stains were constructed for the adrenal gland to recognize the type of mucopolysaccharides contents of cells, thus, cells have appeared negative reactions with the PAS stain which confirmed that carbohydrates were not neutral sugar, but alcian blue(AB) was positive colour with the adrenal cortex and negative colour with adrenal medulla, figures (1,2, 3, and 4). Importantly, adrenal cortex cells were totally stained with AB. However, zona granulosa were strongly stained with AB stain, while zona fasciculata was less stained with AB stain compared to zona granulosa, but it was more intensity in comparison with zona reticularis figures (1,2, 3, and 4). This result confirmed that mucopolysaccharides were acidic sugar types in these cells.

Ultrastructural examination of adrenal gland by electron microscopic

Ultrastructural scanning by electron microscopic of the parenchyma of the adrenal gland revealed that zona granulosa was enveloped by a capsule which had different directions of fibers, these fibers were given trabeculae extended toward the cortex for different distances. Also, zona granulosa were firmly attached and had fewer spaces, also consisted of many columnar cells which contained large central nuclei and were surrounded by cytoplasm. Most of the cytoplasm of these cells was rich in large dark secretory granules, in addition to pale granules that were different in size and shape which might be represented by mitochondria with dense matrices, smooth endoplasmic reticulum and many scattered ribosomes. Besides, sinusoids were distributed around and between cells of the zona granulosa figure (5). Also, zona fasciculata was columnar polyhedral in shape with large nuclei, and the nucleolus is not clear, cytoplasm contained large and middle-dense



secretory granules, in addition, there are a lot of pale granules, and through these cells there were sinusoids. However, these cells showed fewer granules than zona granulosa and reticularis figure (6). The zona reticularis were illustrated columnar cells with a large nucleus, and the cytoplasm was rich dense large secretory granules, and pale granules, which confirmed

the role of this layer figure (7). The adrenal medulla region displayed chromaffin cells that were polyhedral in shape with nuclei poor in chromatin and small and dense secretory granules around large nucleolus. The cytoplasm was very rich in pale multivesicular bodies, and electron-dense secretory granules figure (8).

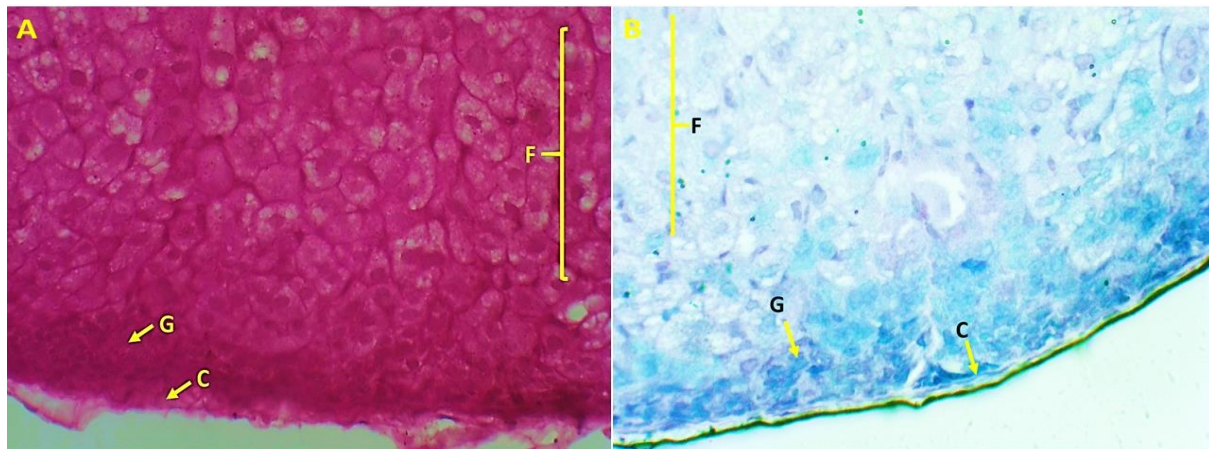


Figure (1): Arrangement of the zona granulosa adrenal cortex. Image A stained with PAS: C: Capsule, G: Zona granulosa F: Fasciculata. Image A stained with AB: B: C: Capsule, G: Zona granulosa F: Fasciculata. Magnificent 400x.

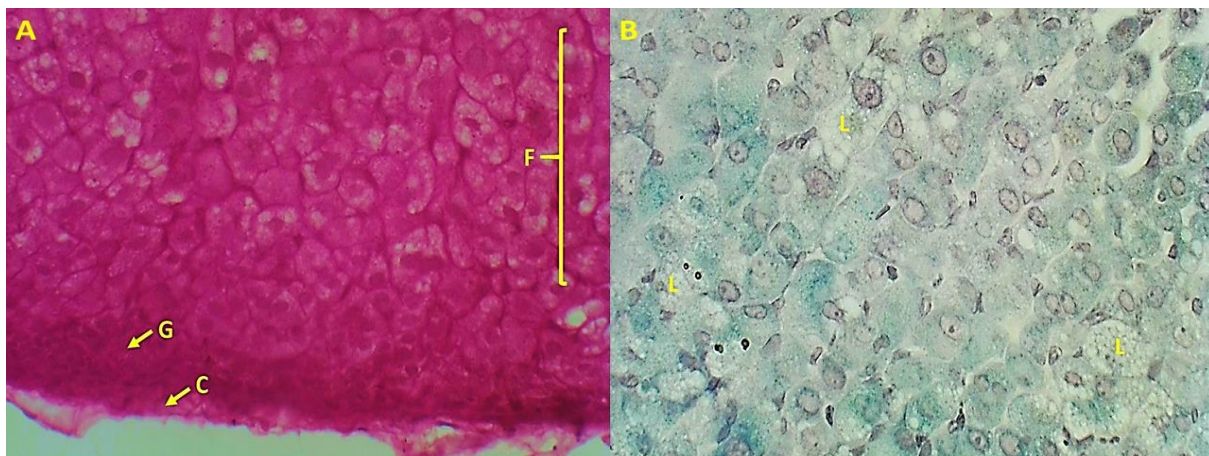


Figure (2): Arrangement of zona fasciculata of the adrenal cortex. Image A stained with PAS: C: Capsule, G: Zona granulosa F: Fasciculata. Arrangement of adrenal cortex. Image B stained with AB: L: droplets lipids. Magnificent 400x.

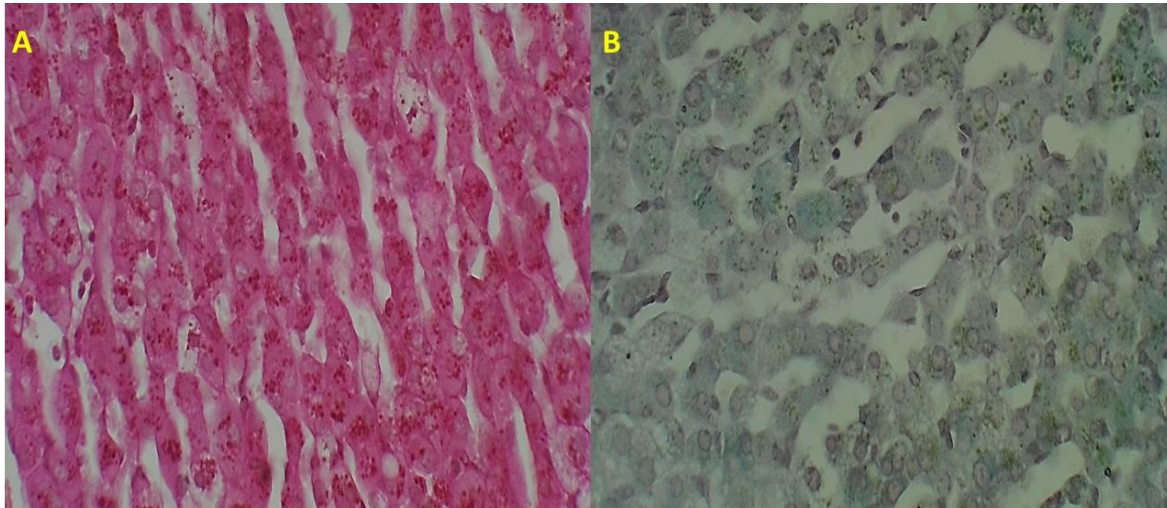


Figure (3): Arrangement of the zona reticularis of the adrenal cortex. Image A stained with PAS. Image B stained with AB. Magnificent 400x.

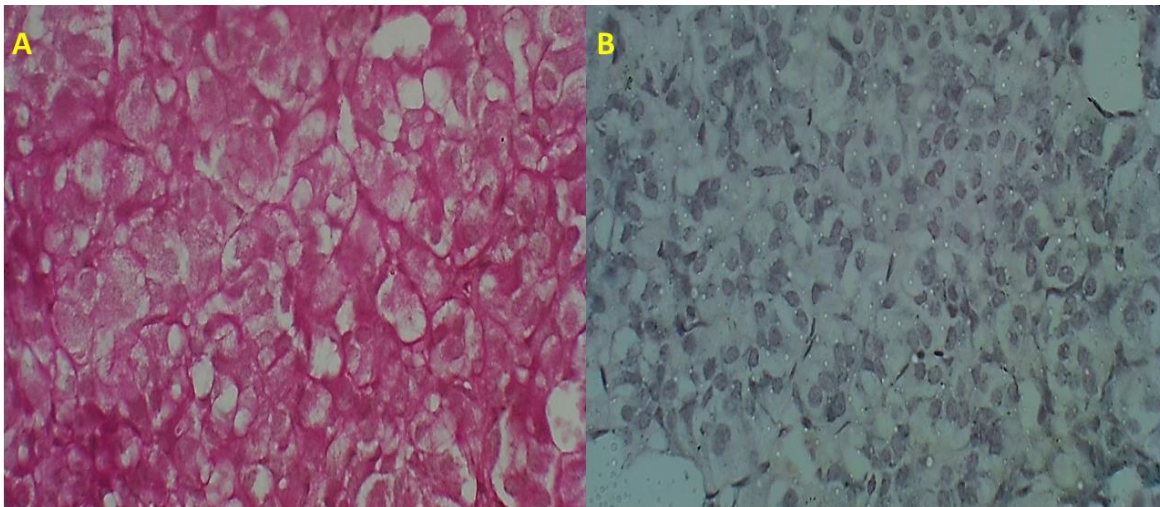


Figure (4): Arrangement of the medulla of the adrenal cortex. Image A stained with PAS. Image B stained with AB. Magnificent 400x.

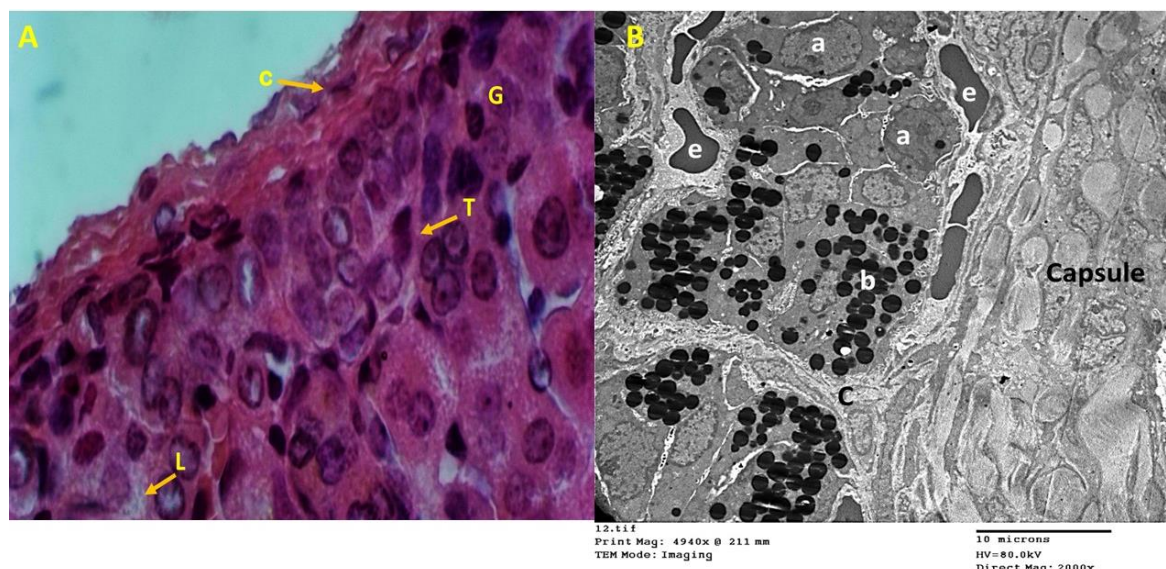


Figure (5): Arrangement of the zona granulosa and capsule of the adrenal cortex. Image A Stained H&E, C: Capsule, G: Zona granulosa, T: trabeculae, L: lipid droplets. Magnificent 400x. Image B transmission electron micrograph from the zona granulosa and capsule adrenal gland showing: a: nucleus, b: secretory granule, c: trabeculae, and e: sinusoids. x 2000.

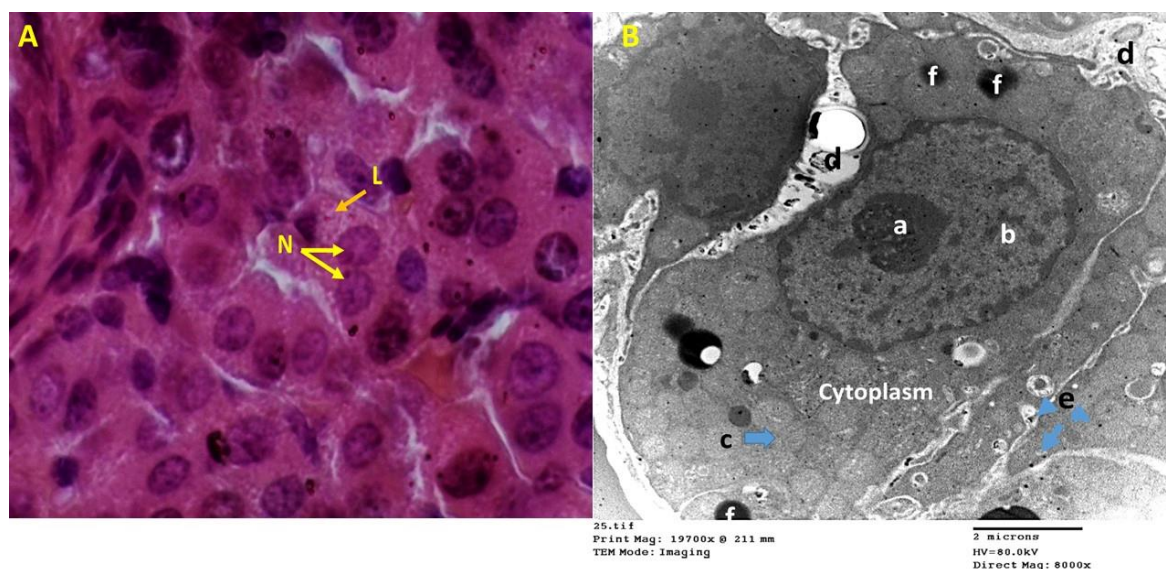


Figure (6): Arrangement of the zona fasciculata of the adrenal cortex. Image A Stained H&E, L: droplets lipids, and N: Nucleus. Magnificent 400x. Image B transmission electron micrograph from the zona fasciculata of the adrenal gland showing: a: nucleolus, b: nucleus, c: droplets lipids, d: sinusoids e: small secretory granule, and f: middle secretory granules. x 8000.

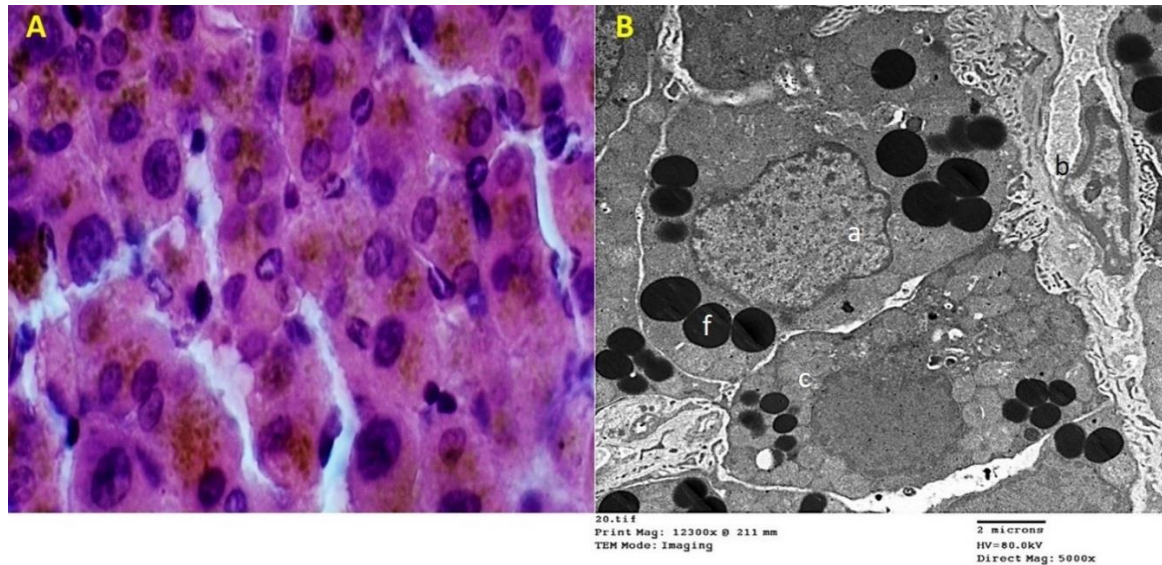


Figure (7): Arrangement of the zona reticularis of the adrenal cortex. Image A Stained H&E, Magnificent 400x. Image B transmission electron micrograph from the zona reticularis of the adrenal gland showing: a:nucleus, c: droplets lipids, d: sinusoids, f:middle and large secretory granule. x 3000.

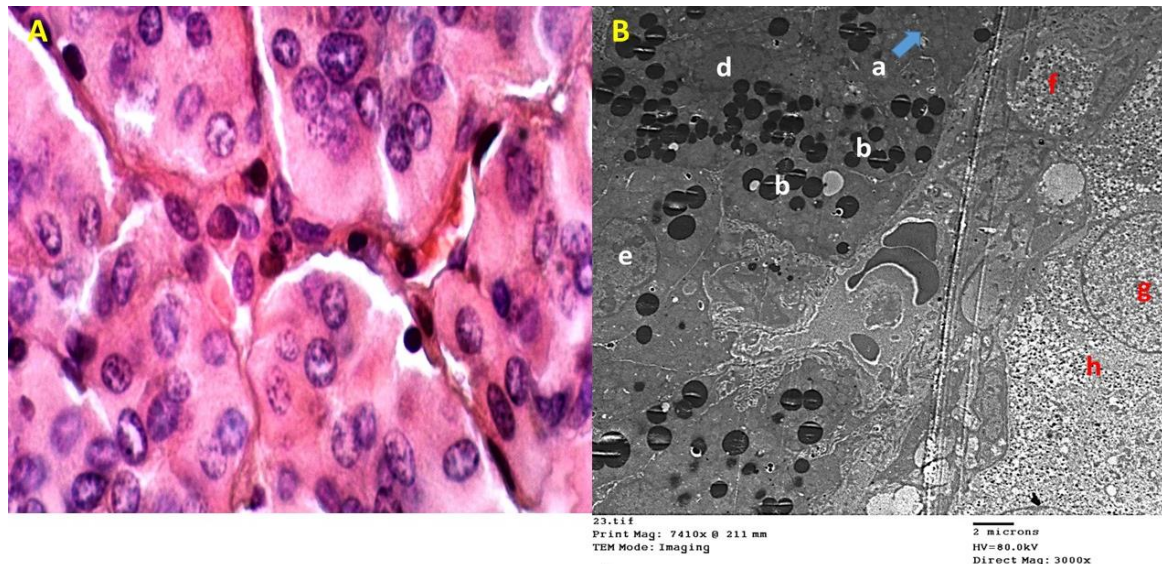


Figure (8): Arrangement of the zona medulla of the adrenal cortex. Image A Stained H&E, magnificent 400x. Image B transmission electron micrograph from the zona medulla of the adrenal gland showing: a: droplets lipids, b: large secretory granules of the zona reticularis, d, and e: nucleus of the cells of the zona reticularis, g: nucleolus of cells of the zona medulla, f and h: tiny secretory granules. x 3000.

Discussion

Many previous studies for squirrels and other species have detected that the capsule of the adrenal gland is a thin layer and consisted of collagen fibers and adipose tissue, and firmly attaches to the adrenal cortex (30, 31). These

findings were distinguished in Iraqi local squirrels and this layer was given many trabeculae toward the adrenal cortex. The adrenal gland of the Iraqi local squirrel mainly consisted of two major layers including the



adrenal cortex and medulla, which agreed with many researchers' results (32). Other researchers in squirrels have found that the adrenal cortex is directly located underneath the capsule and consists of three main layers involved with zona granulosa, zona fasciculata and zona reticularis, same these pattern of results is consistent with the previous studies (33, 34). Our findings highlight that zona glomerulosa was a thin dark layer and compressed polyhedral cells without spaces, and arranged in acini with acidic cytoplasm and fewer lipid droplets, so these results were potentially identified by other research articles (35). Also, zona fasciculata constituted the largest layer of the adrenal cortex and cells were single or diploid nuclei, and extended as cords from the adrenal cortex to the zona fasciculata, also cells were foamy because it was very rich with lipid droplets in comparison with other layers and parts of the adrenal gland, similarly this pattern was noticed in squirrel and other species (31, 35, 36). On the other hand, the zona reticularis is directly located beneath the zona fasciculata and surrounded the adrenal medulla and is thicker than the zona granulosa. Also, cells have appeared as anastomosing rows and irregular in shape, (33, 37) Iraqi squirrels appeared same frame structure and features. Moreover, the adrenal medulla constituted the core and middle of the adrenal gland, and chromaffin cells were columnar or polygonal cells. Also, it has large nuclei with basophilic granular cytoplasm. Same this result was described by (31, 38). Also, the special stain was used to identify the type of mucopolysaccharides, this article showed that PAS was negative in tissue sections of the adrenal gland, which was confirmed by other researchers, (39), this consensus of researchers with our study has been that mucopolysaccharides and glycoproteins are absolutely not neutral sugar. While much attention has been drawn to AB stain was

positive with the adrenal cortex, and negative with the adrenal medulla. However, zona granulosa was more intense with AB and less intense with zona fasciculata and zona reticularis. This study was disagreeing with advancing the study of the squirrel (39). Blood supply was branched into the parenchyma of the adrenal cortex and medulla, and consisted of many capillaries and sinusoids between tissue, so prior research generally confirms that distributions of capillaries and sinusoids in squirrels and other species (39, 40). A number of authors have studied the parenchyma of the adrenal gland and scanned the parenchyma of the adrenal gland (41-43). These results proved that capsule, consisted of that projected trabeculae toward the cortex for different distances. Furthermore, zona granulosa consisted of many columnar cells which contained large central nuclei and were surrounded by cytoplasm which were similar to most recent research (41, 42). Our investigation was observed cytoplasm of zona granulosa cells was very rich in large dark secretory granules, and lipid droplets, this study evidenced by other authors (41, 42), and is considered the activity of cells of the zona granulosa. Similarly, zona fasciculata was generally a polyhedral cell cytoplasm containing large and middle-dense secretory granules, also this result was performed in other studies in the squirrel and other species (41, 42). What's more, prior research has thoroughly investigated zona reticularis and remarked dense large secretory granules in the cytoplasm of zona reticularis (41-43) that confirmed our research. In addition, this study covered the chromaffin cells of the adrenal medulla region and polyhedral cells and cytoplasm have small electron-dense secretory granules, which is agreed with (41, 42, 40, 44) these dense secretory granules have appeared role of these cells in producing epinephrine and norepinephrine. To be brief, this study concluded that the adrenal gland consisted of



two main layers' the adrenal cortex and medulla, and the adrenal cortex comprised zona granulosa, zona fasciculate, and zona reticularis. The mucopolysaccharides and glycoproteins are acidic sugar, which might be related to the function of the adrenal cortex.

Also, many multivesicular granules were detected in the cytoplasm of cells of the adrenal gland.

Conflict of interest

The study has no conflict of interest.

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