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Morphological Description and Histological Structure of Adrenal Gland in Black Iraqi Partridge *Francolinus francolinus*

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Abstract

The objective of this study was to investigate the morphological description and histological structure of adrenal gland in Iraqi black partridge *Francolinus francolinus* from Iraqi environment. Twenty adult healthy birds (male and female) were used in present study. They were measured and adrenal glands were removed, measured and fixed by 10% formalin and formalin and chromate fixative then stained with rotein histochemical stain (Harris hematoxylin and eosin stain (H&E), periodic acid Schiff (PAS), Azan and Mallory stain). The partridge had a small couple of adrenal glands of yellow colour they are located in abdominal cavity nearby the margin of superior kidney lobe. These are small, ovoid glands lying just cranial to the kidneys and gonads on either side of the aorta and posterior vena cava. There was no difference in shapes between adrenal gland of male and female. There were no significantly differences in the measurement of the right and left adrenal gland of the partridge. Histologically the adrenal gland of partridge was mainly compose capsule of dense connective tissue, the thickness of capsule increased according to the different point of blood vessels and large nerve trunk, nerve cell bodies and chromaffin cell embedded in connective tissue, and the cortex is composed of three zones: zona glomerulosa, zona fasciculata, and zona reticularis, these zones were interfered with others. The cells of Zona glomerulosa were arranged in irregular ovoid clusters, the nuclei stain strongly, and the cytoplasm is less pale than that of the next zone, zona fasciculata, The secretary cells were arranged in cords, the nuclei of these cells stain strongly, and the cytoplasm looks pale due to the presence of lipid droplets. The cytoplasm of Zona reticularis stained more darkly, and contains fewer lipid droplets. However the layer of gland where interfered together without clear zone contain sinusoid capillary blood vessels extend from capsular region to medulla whih contain basophilic staining cells, with a granular cytoplasm and no stored lipid. Medullary cells were found between the large and comparatively dark cells of the cortex

Keywords: adrenal gland, black Iraqi partridge, *Francolinus francolinus*, morphology, histochemistry

الوصف المظهري والتركيب النسيجي للغدة الكظرية في طائر الدراج العراقي الاسود *Francolinus francolinus*

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الخلاصة

أن الهدف من هذه الدراسة هو التعرف على الوصف الشكلي والتركيب النسيجي للغدة الكظرية للدراج العراقي الأسود *Francolinus francolinus* من البيئة العراقية. استخدم في هذه الدراسة 20 طائر بالغ من

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الذكور والإناث. تم تشريح الحيوانات و تم ازالة الغدة الكظرية وقياسها وثبتت الانسجة بالفورمالين 10% ومثبت الكرومات واستخدامات الصبغة الاعتيادية (الهيماتوكسيلين والايوسين، وصبغات خاصة -صبغة شيف وصبغة الازان وصبغة مالوري-). يحتوي الدراج على زوجين من الغدة الكظرية صغيرة الحجم ذات اللون الأصفر الواقعة في تجويف البطن بالقرب من الحافة العلوية للكلى. هذه الغدة بيضوية الشكل صغيرة تقع على الفص القحفي للكليتين والغدة التناسلية وكذلك على جانبي الأبهر والوريد الأجوف الخلفي. تشير النتائج الى عدم وجود اختلاف في اشكال الغدة الكظرية للذكور والإناث وكذلك عدم وجود اختلافات معنوية في القياسات للغدة الكظرية اليسرى واليمنى للدراج. اما تشريحا فالغدة الكظرية للدراج العراقي الاسود تتكون أساسا من كبسولة تحيط بالغدة من الخارج مؤلفة من النسيج الضام الكثيف وتتكون القشرة من ثلاث مناطق: المنطقة الكبيبية، المنطقة الحزمية، والمنطقة الشبكية، وتتداخل هذه المناطق مع بعضها. يتم ترتيب الخلايا من المنطقة الكبيبية بشكل مجاميع بيضوية غير منتظمة، تصطبغ النوى بشدة، والسييتوبلازم يصطبغ بصبغة فاتحة، اما المنطقة الحزمية، فتترتب الخلايا فيها بشكل حبال، ونواة هذه الخلايا تصطبغ بقوة، و السييتوبلازم يبدو شاحبا بسبب وجود قطرات الدهون اما السييتوبلازم من المنطقة الشبكية يصطبغ بشدة، ويحتوي على عدد أقل من قطرات الدهون. ومع ذلك فان طبقات الغدة تتداخل مع بعضها تحتوي على أوعية دموية شعرية جيبانية؛ تمتد من منطقة المحفظة إلى النخاع التي تحتوي على خلايا تصطبغ بصبغة قاعدية مع سايتوبلازم محب وتتوزع خلايا النخاع بين خلايا القشرة وتكون كبيرة وداكنة مقارنة بخلايا القشرة.

Introduction

The adrenal gland is an important gland in birds as it is in mammals; removal of the adrenal gland in birds eventually leads to death [1]. Completely adrenal ectomized birds will die within 60 hours, which depicts the importance of this gland [2]. The adrenal gland is a vitally important endocrine gland that occupies a central role in the regulatory mechanisms of the body metabolism. Environmental stress factors lead to permanent strain and overload of the body resulting in structural alterations of the adrenals that in turn are followed by hormonal imbalances [3]. The adrenal have a dual origin, the cortex arises from mesoderm whereas the medulla has a neuroectodermal origin [4]. The cortex develops as a proliferation of coelomic mesothelium into the underlying mesenchyme between the root of the dorsal mesogastrum (the root of the mesentery) and the urogenital ridge (the esonephros and the developing gonad). These cellular proliferations differentiate into large acidophilic organs that form the fetal or primitive cortex of the adrenal gland [5]. Shortly afterward, a second wave of cells from the mesothelium penetrates the mesenchyme and surrounds the original acidophilic cell mass; these cells, smaller than those of the first wave, later form the definitive cortex of the gland [4]. Several works have been done about the anatomy and histology of adrenal gland in different vertebrate [6-9]. Review of literatures revealed that there was a little information about the adrenal gland in Iraqi vertebrate especially wild vertebrates [10]. The present study aimed to investigate the morphological description and histological structure of adrenal gland in Iraqi black partridge (*Francolinus francolinus*). One of the major drawbacks for the present study was the almost lack information about the morphological description and histological structure of the adrenal gland in Iraqi wild birds, with hope to give detail information about the gland in species under investigation.

Materials and methods

Experimental animals: A total of 20 healthy Iraqi black partridge (male and female) were used in present study. The animals were obtained from local markets. Animals were kept under the laboratory conditions (12 h light: 12h dark photoperiod), with controlled room temperature 25-28°C, good ventilation and were feed normal pellets and tap water *ad Libitum*. Histological examination: After the animals were sacrificed, adrenal gland were removed and fixed in 10% formalin and chromate fixative for 48 hours, paraffin wax method were employed to prepare histological slides and different stains (Harris hematoxylin and eosin stain H&E, periodic acid Schiff (PAS), Azan stain, and Mallory stain) were used according to the methods of Kiernan [11].

Statistical analysis: Data are expressed as the mean \pm SE. The statistical significance was carried out using one- way analysis of variance test followed by Duncan's Multiple Range Test (SPSS statistical software package) [12]. A possibility of P value ($p < 0.05$) was considered as significant differences between means.

Result and Discussion

The black Iraqi partridge had a small couple of adrenal glands of yellow colour that were located in abdominal cavity nearby the kidney. These are small, ovoid glands lying just cranial to the kidneys and gonads on either side of the aorta and posterior vena cava. The surface of adrenal gland was wrapped with connective tissue and connected with kidney and gonads. There was a close relationship between adrenal glands and gonads Figure-1A, 1B. These results of present study was agree with King and McLelland [13] on a little number of species (greater Rhea and the common Loon) the left and adrenal gland fused together due to the body plane.

There was no different in shapes of adrenal gland of male and female. The right adrenal gland (R) was often triangle or flat-ellipse, Figure-1C. The mean length of right adrenal gland is (6.167 ± 0.601 mm), and the weight is (0.044 ± 0.002 g), while the left adrenal gland (L) was oblong in shape, with length of left adrenal gland is (7.333 ± 0.760 mm), and the weight of (0.052 ± 0.009 g). There were no significant differences in measurements between right and left adrenal gland Iraqi black partridge. These results were agree with the results of Bozakova and Popova-Ralcheva [14] who were confirmed that the weight of the adrenal gland of the domestic fowl can be used as reliable indicators for the worsening of the poultry welfare that's due to the varies considerably breed, age, health and environment[15,2]. Histological and Histochemical structure: Adrenal of the Iraqi black partridge was mainly composed of capsule, cortex and medulla, Cortex occupied most part of the adrenal gland. Its colour was comparatively light, while medulla had a small size, with a comparatively dark colour Figure-2. Results of the present study showed that the cortex is not well differentiated from the medulla Figure-3 a, this result agree with Hodges [16] and Rae [17]. In some male birds, like the domestic fowl, they are firmly attached to the appendix of the epididymis by connective tissue [13]. Birds are unique among homeothermic vertebrates, in which cortical and medullary tissues of the adrenal gland are found always intermingled and the zonation of the internal tissue into sub-capsular zone and inner zone was not evident in the adrenal of three days-old Japanese quail [18].

Capsule: The adrenal gland of the experimental animals was surrounded by a thin capsule of dense fibro-elastic connective tissues essentially consist of a thin layer of collagen fibres with little amount of elastic fibres interspersed with fibroblast Figure- 3a,b. In addition, the trabeculi contain blood vessel, nerve cells and extended to the second layer of cortex Figure-3c. However, the thickness of capsule may be increase according to the different point of blood vessels and large nerve trunk, nerve cell bodies and chromaffin cell embedded in connective tissue Figure-3d. Numerous fine trabeculi pass from the capsule between the cord and groups of parenchymal cell and within the substance of the gland become reduced to very fine strands of tissue compose of collagen and reticular fibbers. Glandular structure surrounded by fibrous connective tissue contain chromaffin cell and thick muscular layers of blood vessels as well as blood vessels in capsular. Results of the present study were disagree with Hartman and Brownell [19] who found that in some birds the chromaffin tissue tends to concentrate towards the outer region of the glands, and show some differences due to environments.

On other hand the results of the present study agree with Bacha and Bacha [20] who revealed that the adrenal gland of chick is enclosed with capsule of dense connective tissue, and the thickness of capsule increase considerably at different point over the surface of gland particularly in places where arteries, large vein, and nerve trunk run through or within and where the capsule underlies the supra renal ganglia. The disagreement is probably due to the different environmental or perhaps due to the differences in the diet.

Adrenal cortex: The adult adrenal cortex (internal tissue) is composed of three zones: zona glomerulosa, zona fasciculata, and zona reticularis Figure-2 these zones are interfered with others. Zona glomerulosa: is the outermost zone of the adrenal cortex. The cells are arranged in irregular ovoid clusters that are surrounded by trabeculae, which contain capillaries. The nuclei stain strongly, and the cytoplasm is less pale than that of the next zone, the zona fasciculata, The nucleus of glomerulosa with vaculated of their cell which appear only dark vesicle nuclei with clear nucleolus Figure-2 ZG, the main cells were acidophilic and they are significantly different between right and left adrenal gland, they showed (31.4 ± 1.12) in right and (23.80 ± 1.2) in left adrenal, while the basophilic cells in right adrenal is (19.20 ± 1.24) and significantly differences from that of left (12.00 ± 0.87). Table-1. These results agree with Elbajory [9] who was found the same results, Similar differences were also noted in the chicken adrenals [21]. In chicken, cortical cells are columnar. When longitudinal cuts have been made through cords of cortical cells, the cells form a bilayer, while cords

are cut transversely, the cells are seen to be arranged radially [20]. Zona fasciculata is represented the middle zone of the adrenal cortex. The secretory cells are arranged in cords, often one cell thick, surrounded by fine strands of supporting tissue when stained with PAS. The nuclei of these cells stain strongly, and the cytoplasm looks pale due to the presence of lipid droplets. Some cells of this layer have no lipid droplet, characterized by dark nuclei, and condense their cellular. These layers reach to the capsule region and replace the zone with less lipid droplet. Diluted sinusoids filled with blood were severing in zona fasciculata and some cell express with the red vasculature. The main cells were acidophilic and they are significantly different between right and left adrenal gland, they showed (19.60 ± 0.75) in right and (22.40 ± 0.87) in left adrenal gland, while the basophilic cells in right adrenal gland is (25.80 ± 1.6) and no significantly differences with the basophilic cells in left (25.60 ± 0.60) . Table-1. The present results agree with those of Aire [15] and Hodges [16], who suggested that adrenal cortical cells in the chicken are arranged in columns with a small, round to slightly oval, eccentrically placed nucleus with eosinophilic staining. While, Humayu *et al.*, [22] measured the diameters of cell nuclei of cortical cells in the different zones of the chicken adrenal and they found that There were variations in the diameters of cell nuclei within the zone, It is possible that adrenal cortical tissue synthesizes and secretes at least two types of cells.

Zona reticularis : The innermost layer of the cortex, Some brown pigment is seen in some of these cells. The cytoplasm of the cells in this region stains more darkly, and contains fewer lipid droplets. However the layer of gland where confused together without clear zone contain sinusoid capillary blood vessels extend from capsular region to medulla. In addition, the sinusoid lined by chromate cell with characterized by brownish the chromate. The main cells were basophilic and they are significantly different between right and left adrenal gland, they showed (24.20 ± 1.59) in right and (22.20 ± 1.16) in left adrenal, while the acidophilic cells in right adrenal is (11.4 ± 0.40) and significantly differences with the acidophilic cells in left (18.00 ± 0.32) . Table-1. Chromate cell was increased in numbers in the reticular zone than in the glomerulosa. According to Cronshaw *et al.* [23], the duck cortex constitutes 68.2%, the medulla 28.6% and vascular space 3.2% of the total area. Results of the present study was agree with the results of Aire [15] which were revealed that the duck cortex occupies 68% of the gland, that of the chicken occupied a smaller volume amounting to 48%, the proportion of the interrenal tissue or cortex, was significantly greater in the guinea fowl than in the domestic fowl, but the medullary tissue, blood vessels and connective tissue were not significantly different. Medulla region of the adrenal glands contains basophilic staining cells, with a granular cytoplasm and no stored lipid. Medullary cells were found between the large and comparatively dark cells of the cortex, the results of this study agree with Smitten [24]. The cells are partly arranged in a net-like structure, and are small with little cytoplasm. The nuclei contain moderate amounts of chromatin, are oval and much smaller than the cortical cell nuclei Figure-2M. The main cells were basophilic and they are significantly different between right and left adrenal gland, they showed (18.8 ± 0.85) in right and (17.80 ± 0.73) in left adrenal, while the acidophilic cells in right adrenal is (8.80 ± 1.1) and significantly differences with the basophilic cells in left (7.00 ± 0.32) . Table-1. As compared with the mature medullary cells of adult animals, the cytoplasm is low in amount, basophilic and does not display a distinctly positive chromaffin reaction [25]. In birds, the medullary tissue forms a pattern that varies from scattered islets to an interwoven network, depending upon the amount of tissue present. The pattern of the medullary tissue in our study was similar to those of Hartman *et al.* (1947) and Holmes [26] and Cronshaw [27]. They stated that medullary tissue forms an attractive meshwork pattern, seeming to take the form of islets in the Florida bobwhite. Moreover, the present results correspond with those of Hodges [16] and Ohmori [28]; Similarly, they suggested that adrenal medulla cells in the chicken are arranged in a polygonal shape, with a large, spherical, centrally placed nucleus. The basophilic staining of the cytoplasm is due to its content of numerous small, basophilic granules [22]. The interrenal columnar cells had no vacuolations. The chromaffin cells had the tendency to form smaller islets among the interrenal cells. The chromaffin cells were very few in the subcapsular zone. Among the two types of chromaffin cells, the noradrenaline cells were found to be more predominant than the adrenaline cells. Sinusoids were small and very few in numbers. (Figure 3 c,d). Arrangement of the interrenal cell cords formed two definite zones that are subcapsular zone and inner zone. Aire [15] reported the existence of two clear zones of internal tissue in Nigerian fowl. On the contrary, Knouff and Hartmann [29] observed three zones of internal tissue in Brown pelican just as in mammals. Medullary cells also formed a discontinuous subcapsular layer. Presences of ganglion

cells were common in the medullary islets. The noradrenaline cells outnumbered the adrenaline cells in the immature age groups of both the sexes studied as observed by Sivaram [21] in fowl. The histomorphology of the adrenal gland in the mature age groups of Japanese quail revealed characteristic structural features attributable to sexual maturity. The cells of the subcapsular zone had lesser lipid accumulation comparatively [30]. PAS positive material was at its minimum in the cortical and medullary tissue of the adrenal gland in the Iraqi black partridge than the remaining phases of its physiological activity similar to chicken and Nigerian fowl [15].

Table 1- the numbers of cells (basophilic and acidophilic) in the layers of the adrenal gland:

Zones		Basophilic (mean \pm S.E)		Acidophilic (mean \pm S.E)	
		Right adrenal	Left adrenal	Right adrenal	Left adrenal
Cortex	Glomeruloza	31.4 \pm 1.12 a	23.80 \pm 1.2 a	19.20 \pm 1.24 b	12.00 \pm 0.8 b
	Fasiculata	19.60 \pm 0.75 a	22.40 \pm 0.87 a	25.80 \pm 1.6 b	25.60 \pm 0.60 b
	Reticularis	24.20 \pm 1.59 a	22.20 \pm 1.16 a	11.4 \pm 0.40 b	18.00 \pm 0.32b
Medulla	adrenal and noradrenal	18.8 \pm 0.85 a	17.80 \pm 0.73 a	8.80 \pm 1.1 b	7.00 \pm 0.32 b

Values with different letter is significant, and the same letters is no significant \pm SE: Standard error

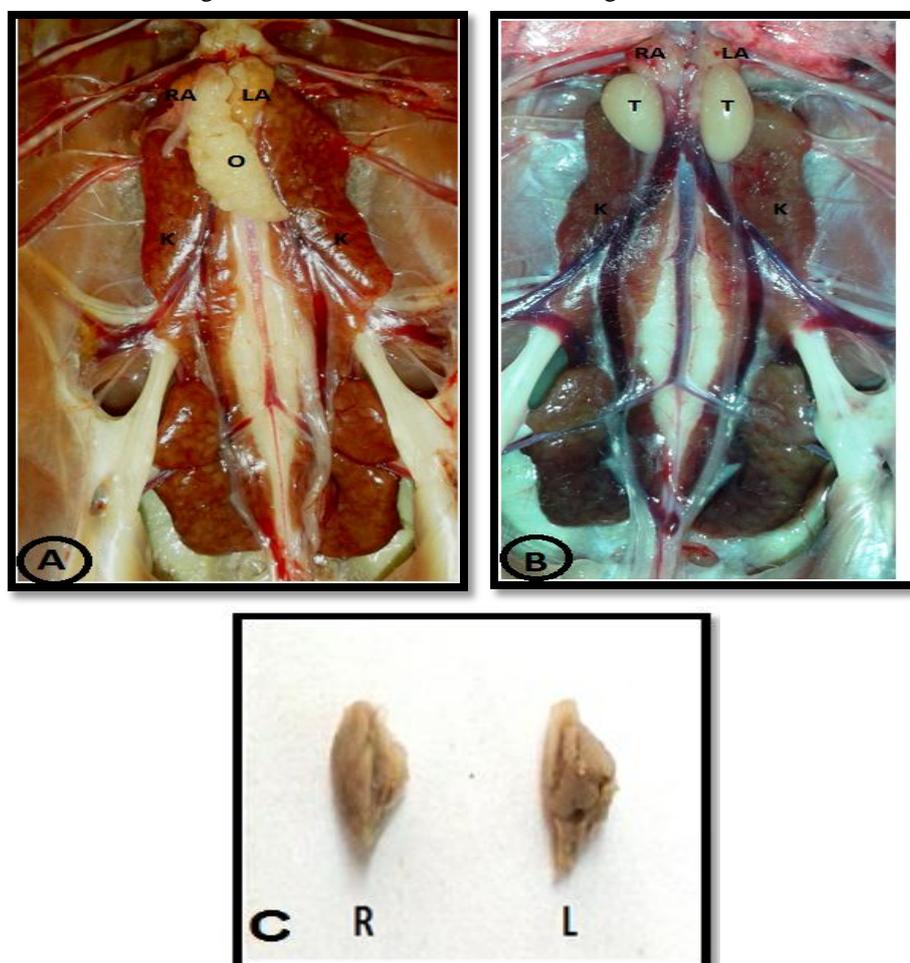


Figure 1- General morphology of adrenal gland of Iraqi black partridge *Francolinus francolinus*. (A) The location of adrenal gland in female. (B) The location of adrenal gland in male. Two glands situated at the anterior poles of the kidneys, the right adrenal gland (RA) was partly adherent to the anteriorly cranial of the right kidney (K) and the left gland (LA) located on the medial border of 1/2 cranial of the left kidney (K), and testes (T). (C) The shape of the adrenal gland. The right adrenal gland (R) was often triangle or flat-ellipse, and the left adrenal gland (LA) was oblong in shape.

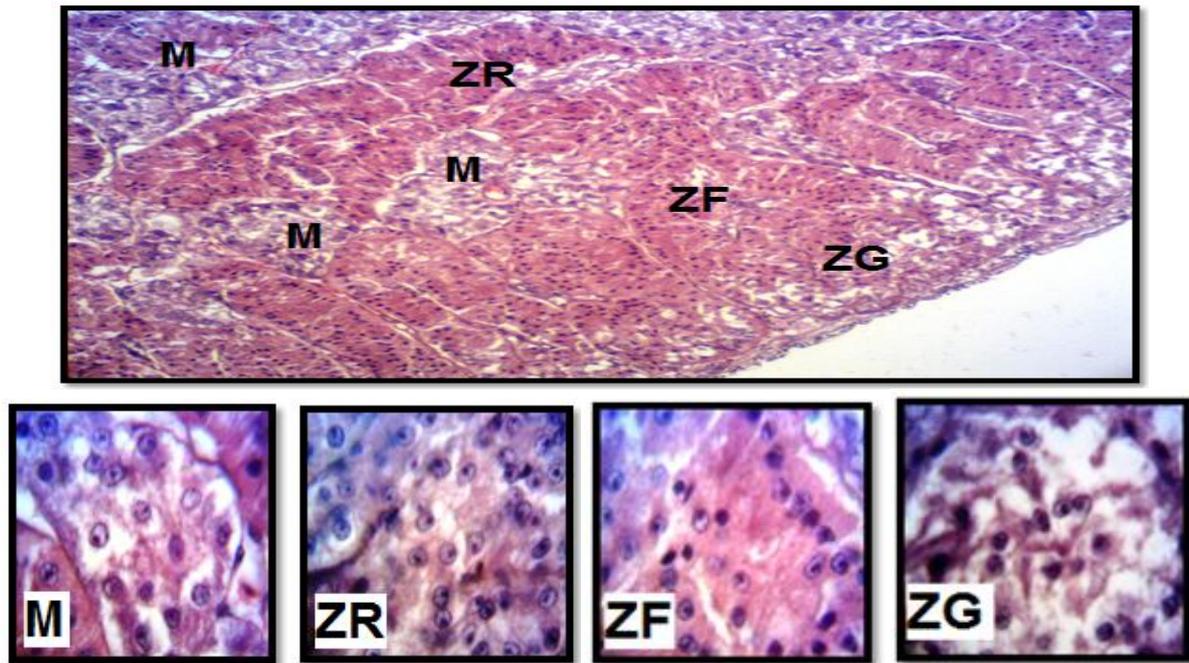


Figure 2- Cross section of cortex layers in Iraqi black partridge adrenal gland showing three zones of cortex, zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR), H&E stain (100X, 1000X).

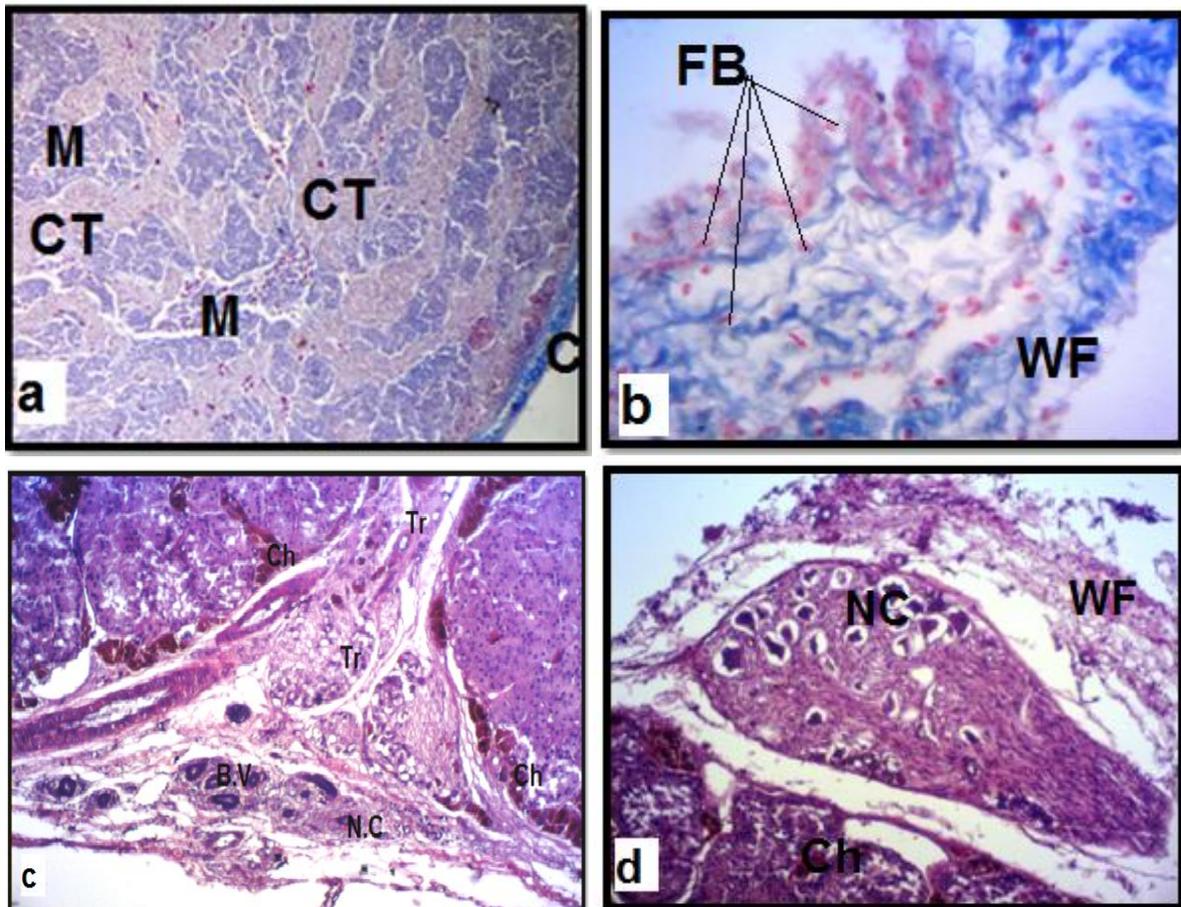


Figure 3- Cross section of capsule in Iraqi black partridge adrenal gland showing **a-** adrenal layers. **b-** The white fibers (WF) with fibroblast (FB), Azan stain (400X). **c-** The blood vessels (BV), trabeculi (Tr), and chromaffin cell (CH). PAS stain (400X). **d-** Nerve supply (NS), nerve cell (NC). PAS stain (400X), fibroblast (FB) and chromaffin cell (CH). PAS stain (400X).

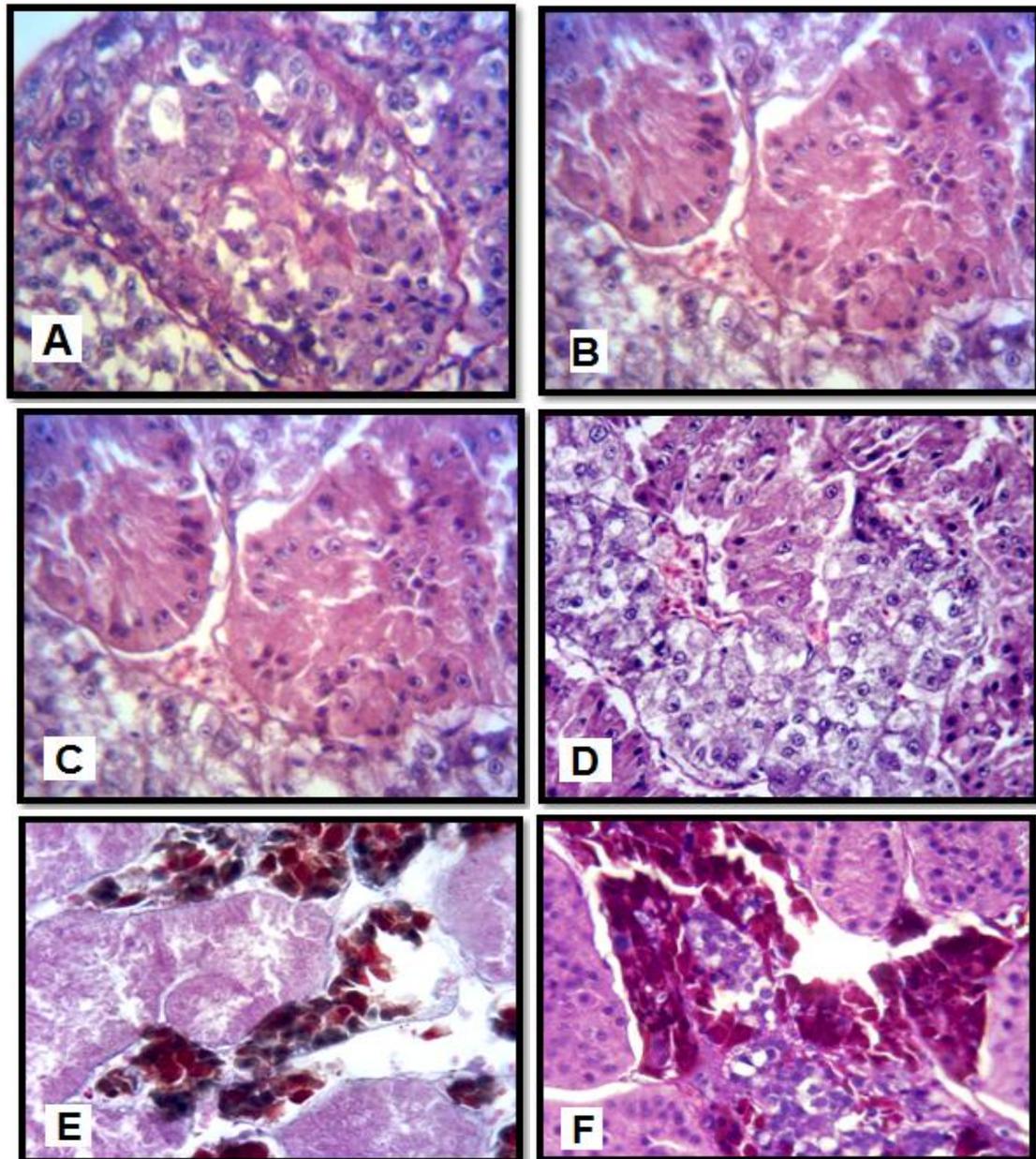


Figure 4- Cross section of cortex layers in Iraqi black partridge s gland showing (A) zona glomerulosa (ZG) of the cortex (PAS stain), (B) zona fasciculata (ZF) of the cortex (PAS stain), (C) zones of cortex reticularis (ZR),(H&E stain). (D) Zones of cortex reticularis (ZR), and (E) medulla layer, azan stain (400x). (F) medulla layer, PAS stain (400x).

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