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Morphological and histochemical investigation of Adrenal Gland in Eagle-owl (Bubo ascalaphus)

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***Abstract*—** The aim of this study to investigate the morphological and histological characteristics of the adrenal gland in the Eagle-owl (Bubo ascalaphus). Adrenal glands from seven specimens were collected, located dorsomedial near the kidneys. Morphologically, the gland was oval-shaped. It was a capsulated gland. Morphometric measurements showed similarities between the right and left glands; there was no noticeable difference between them in terms of weight, length, and width, and the proportionality was directly proportional to the bird's weight and size. Histologically, after performing the histological section and staining it with routine staining, we observed the three layers that make up the cortex (The zona Glomerulosa, zona fasciculata, and zona reticulares). The three layers with no obvious tissue distinctions between these; we also performed histological measurements using the Imaging program to determine the thickness of each of the capsule, cortex, and medulla in the gland, and used the arithmetic mean to determine the final value. The result was that the cortex is one and a half times the size of the medulla due to its multiple layers and secretion.

***Keywords* —** Morphological, Histochemical, Adrenal Gland, Eagle-owl.

INTRODUCTION

One of the most significant endocrine glands in birds is the adrenal gland. It is essential for metabolism, homeostasis, immunological response, stress response, and blood pressure regulation. (1, 2, 3, 4).

A paired organ is the poultry adrenal gland. The left and right glands are located on either side of the dorsal aorta and inferior vena cava, cranio-medially to the kidneys. The chicken's accessory adrenal glands are located in the capsule or next to the main adrenal gland (5).

Adrenal gland accessories frequently grow in fish and mammals. It has been suggested that the formation of neoplasms is centered on auxiliary adrenal glands (6, 7, 8)

The adrenal gland in a duck that is just one day old is spherical. The left adrenal gland takes on an oval shape around 24 weeks, while the right adrenal gland takes on a pyramidal structure (2). The chicken's left adrenal gland is long and oval in shape, whereas the right adrenal gland is oval or triangular (1, 5), Ostriches, on the other hand, have oblong and ellipsoid shapes (9). Japanese quail glands are triangular in shape on the right and elongated on the left (10).

Poultry adrenal glands range in hue from gray to cream in pigeons and cream-yellow in quails (11).

Guinea fowl can turn yellow or yellow-red. According to (2), the adrenal gland's color changes from cream or yellow in young ducks to brown in adults. According to (12), the degree to which the adrenal gland's tissues are saturated with carotenoids determines how yellow the gland appears.

Mammals and birds both have adrenal glands. These paired organs, which are implanted in adipose tissue and have a pear or triangle form and are colored yellow or orange, are located close to the kidneys' superior poles. One of the main characteristics of the avian adrenal medulla is the interfering nature of the cortex and medulla components (1,13).

A capsule made of dense fibrous connective tissue, rich in collagen, reticular fibers, and blood vessels with limited elastic components, covers the outside of the fowl adrenal gland. (5,6). Female chickens have thicker adrenal capsules (22.09 ± 2.17 μm) than male chickens (18.14 ± 1.82 μm) (5). The pigeon adrenal capsule's thickness is limited to 13.46 ± 0.67 μm (7). The quail's adrenal capsule contains chromaffin cells and autonomic nervous system ganglia (11), geese (14), chickens (5,6, 15)

The two separate parts of the adrenal gland are the inner medulla (neuroectodermal origin) and the outer cortex (mesodermal origin). There are three or four zones in the adrenal cortex. In ruminants, humans, and a few rodents, the outer layer of the cortex is known as the zonaglomerulosa. In horses, donkeys, pigs, and carnivores, it is composed of irregular cell clusters and cords (16) revealed that the subcapsular, peripheral, and central zones comprised the interrenal, cortical structure of the chicken adrenal gland (17).

Mineralocorticoids and glucocorticoids are produced by the avian adrenal cortex, whereas the medulla secretes Epinephrine and Norepinephrine (3). The bird dies when this gland is removed because these hormones control a number of essential physiological processes (2, 18),

In order to help physiologists, comprehend how the gland carries out its duties, the current work attempts to give a succinct description of the morphology and histological architecture of the adrenal gland in adult owls using macroscopic, microscopic, and histochemical studies.

MATERIALS AND MTHODS

The specimens were obtained from commercial bird markets and hunting in the deserts of Karbala and Najaf. Seven Bubo Owl birds were selected for morphological and histological examination. According to a prior study, the avian animals received an intravenous injection of a combination of 5 mg/kg xylazine and 25 mg/kg ketamine into the alar vein to induce anesthesia (19).

In order to examine the Adrenal glands shape, spatial orientation, and anatomical relationships. After being removed, the specimens were fixed for 24 hours in a fixative solution that contained 10% formalin. After that, they were treated with alcohol, which is gradually upgraded from different concentrations. This technique takes two hours for each step. Following that, the sample underwent two rounds of xylene treatment, each lasting five minutes. During the continuous filtering process, Tissue sections of a thickness of roughly 5 to 6 µm are produced by using a rotary microtome. (H and E) stain were used to stain the sections. The studied slides were photographed using a Microscopic camera mounted to a light microscope. Histometrical measurements were also performed using the Image J program to determine the capsule thickness in the gland, as well as the size of the cortex and medulla. And using a statistical analysis program SPSS.

Ethics-approved

This study was conducted in the anatomical laboratory of the University of Kerbala's College of Veterinary Medicine under reference number UOK.VET.AN.2025.137.

RESULT AND DISCUSSION

The adrenal glands were revealed in a dorsomedial location to the kidneys (figure 1) following anatomical examination and excision. They had an elongated oval shape for both gland and were 15±1 mm in length, 10±1.2 mm in width, 0.40 ± 0.05weight which is the typical size of all Eagle-owl (Bubo ascalaphus) show in (table 1).this may be directly proportional to the weight of the bird. As for the color, the color of the glands was gray.

Histological analysis showed there was a clear capsule around the gland. based on the order and form of the cells inside each zone, that the gland was separated into two separate regions: the medulla, whose cells secreted noradrenaline, and the cortex, whose cells secreted steroid hormones (figure 2).

The cortical cells were separated into three zones (figure3) Cells were arranged in parallel cords of columnar cells in the first zone, known as the zona granulosa (figure 4). Larger, polygonal cells were grouped in clusters in the second zone (figure 5). The third zone was called the reticular zone because its cells had complicated, entangled ends.

Histometrical measurements determine the thickness of each of the capsule, cortex, and medulla in the gland, and used mean to determine the final value. The result was that the cortex is one and a half times the size of the medulla due to its multiple layers and secretion. The measurements were within the range mentioned in the (Table 2).

Table 1. it illustrate the different measurements (weight, length, width) of the seven owls.

No. of bird	Wight of bird (kg)	Weight of adrenal gland (g)		Length of adrenal gland(mm)		Width of adrenal gland	
		R	L	R	L	R	L
1	3.5	0.41	0.46	16	16.2	11.3	11.4
2	3.2	0.35	0.38	15.9	16	10.5	10.6
3	3	0.30	0.35	15.6	15.8	9.2	9.3
4	2.9	0.29	0.32	15.4	15.5	8.7	8.7
5	3.1	0.33	0.36	15.7	15.9	10.3	10.3
6	2.7	0.27	0.32	13.8	14	8	8
7	3.3	0.40	0.43	16	16.1	11	11

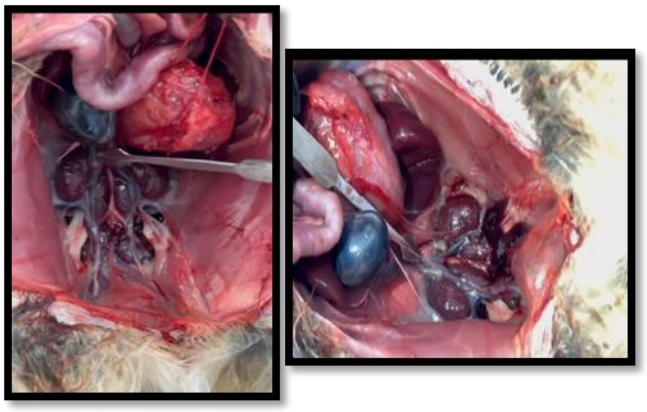

Figure 1 . Photomacrograph show the location of the adrenal gland of Eagle-owl (Bubo ascalaphus)

Table 2. It illustrates the different measurements used mean (capsule Thickness, cortex Thickness, medulla Thickness) in Eagle-owl (Bubo ascalaphus)

Thickness of capsule	Thickness of cortex	Thickness of medulla
12.19 ± 1.15 µm	63.07 ± 2.19 µm	39.30 ± 1.54 µm

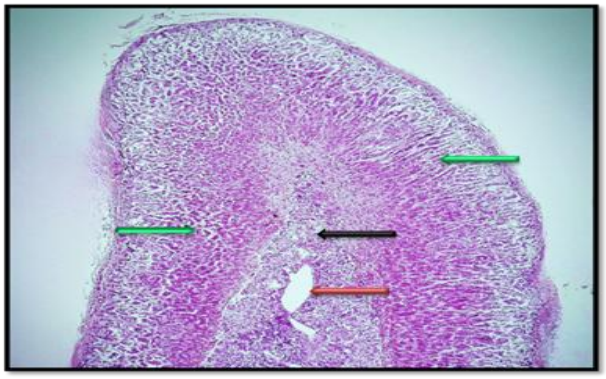

Figure 2. Photomicrograph of the adrenal gland of Eagle-owl show: (red arrow) vein, 9black arrow) medulla, and (green arrow) cortex, with H&E stain.4X,

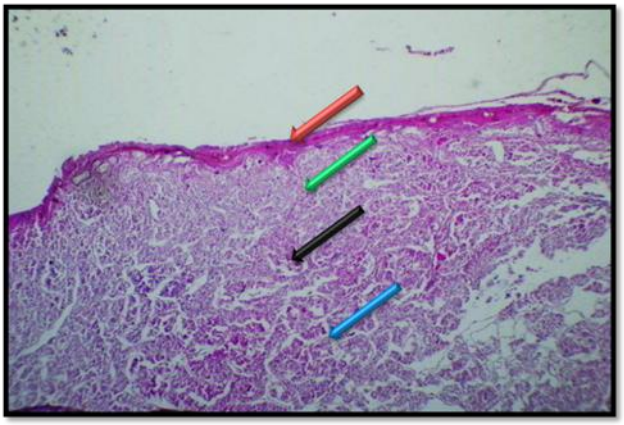

Figure 3. Photomicrograph of the adrenal gland of Eagle-owl show: (red arrow) capsule, (green arrow) zona glomerulosa, (black arrow) zona fasciculata, and (blue arrow) zona reticularis with H&E stain.10X.

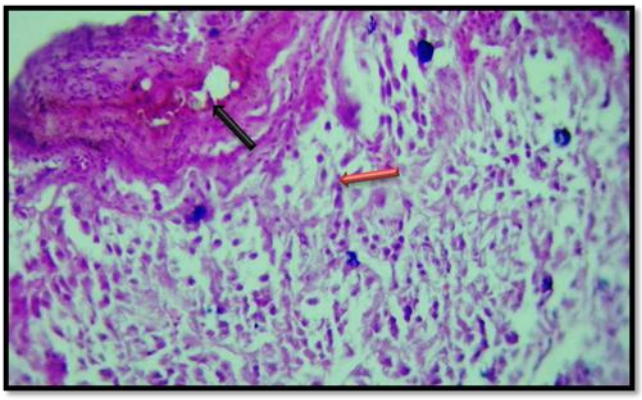

Figure 4. Photomicrograph of the adrenal gland of Eagle-owl show: (black arrow) blood vessel, (red arrow) zona glomerulosa, with H&E stain.40X.

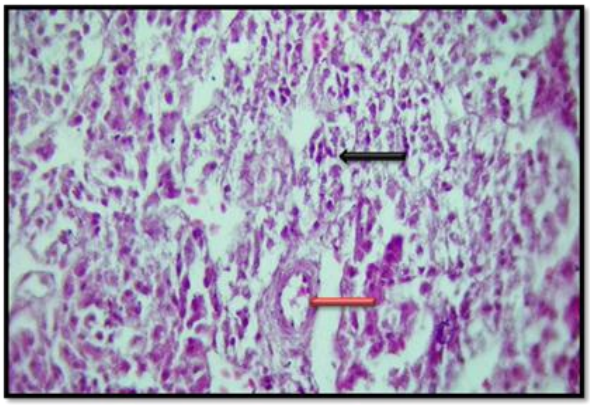

Figure 5. Photomicrograph of the adrenal gland of Eagle-owl show: zona fasciculate (black arrow), blood vessels (red arrow). H&E stain. 40x

Our morphological findings were consistent with those of the researchers, in that the shape of the gland was elongated oval (5). But this contradicts the findings of other researchers who proved in their experiments, Ostriches have oblong and ellipsoid shapes (9). Japanese quail glands are triangular in shape on the right and elongated on the left (10). And they stated that the adrenal gland in mammals and birds is pear-shaped or triangular (1,13).

As for color, the glands in the eagle owl were gray, a finding supported by the researcher (11) who conducted his study on hue. While other researchers have reported different findings regarding adrenal gland color in their studies, they are cream-yellow in quails. Guinea fowl can turn yellow or yellow-red. according to (2), the adrenal gland's color changes from cream or yellow in young ducks to brown in adults. According to (12), the degree to which the adrenal gland's tissues are saturated with carotenoids determines how yellow the gland appears.

The thickness of the capsule surrounding the gland was consistent with findings in pigeons (The pigeon adrenal capsule's thickness is limited to 13.46 ± 0.67 µm (7).

And contradicted those in Female chickens have thicker adrenal capsules (22.09 ± 2.17 µm) than male chickens (18.14 ± 1.82 µm) (5). This proves that the capsule is thicker in the adrenal gland of this

We also observed that the two main layers of this gland are separated by clear histological structures. This agree with (16) study proved, as he demonstrated that the two sections are separate from each other, while the cortex region of this gland was divided into three regions in the same order established by (17).

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