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Morphological and Histological study of the Harderian gland in the Striated Scops Owl (Otus brucei)

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***Abstract*—** This research focused on examining the anatomical and histological features of the Harderian gland in seven Striated Scops Owls. Seven different Birds models were utilized, with the birds being euthanized following established protocols. The Harderian gland was subsequently removed for anatomical and histological analysis. The anatomical observations indicated that the gland is situated behind the eyeball in the dorsomedial area and has one central excretory duct that connects to the lacrimal duct.

Hematoxylin-eosin staining demonstrated the general architecture of the gland, whereas Masson's trichromatic stain illustrated the arrangement of collagen fibers and PAS-Alcian blue was utilized to identify the characteristics of the glandular secretions.

Histologically, the gland was observed to be encased in a fibrous capsule and made up of two primary lobes of different sizes: a smaller lobe and a larger lobe. The sizable lobe displayed thick glandular tissue with distinct intercellular connections partitioning it into smaller lobules. A significant density of collagen fibers was noted in the capsule encasing the gland, showcasing a blue hue, while the collagen concentration was reduced in the interstitial tissue, which appeared green.

The findings from the small lobe exhibited a positive response with Alcian blue, showing blue coloration, the secretion was of the acidic mucopolysaccharides variety. The large lobe exhibited a mixed response; regions of acini reacted with Alcian blue and turned blue, whereas the gland's center showed a positive reaction with PAS and appeared pink, the presence of neutral mucopolysaccharides. Cells in the central areas also displayed a purple hue from the combination of blue and pink, suggesting that their secretions were of a hybrid nature (acidic and neutral).

These findings suggest a distinct structural and functional verity between the two lobes of the horny gland in owls, highlighting a range in the characteristics of glandular secretions and their roles.

***Keywords*—** Histological, Harderian, Striated Scops, Morphology.

INTRODUCTION

Johann Jacob Harder in 1694 described the gland for first time, in red and fallow deer and described it as a large intra-orbital structure that probably moistens the surface of the eye, After the 1970 many others researcher was interested in studying the structure and functions of the Harder gland (1).
The Harderian gland has many important functions in animals; the excretion and the function of the gland vary with species (2). Since then, numerous studies have been conducted with the aim of describing the morphology and function of this gland in various animal species (3).

The Harderian gland is the major exocrine Para ocular gland of the domestic fowl. It lies in the orbit ventral and posteromedial to the eyeball (2). It extends rostrally from the region of the optic nerve and from its anterior extremity emerges its duct, which passes inferior to the origins of the superior and inferior oblique muscles (4(.

It is loosely attached to the periorbital fascia, so that when the eye is removed it usually remains in the cavity of the orbit, it is associate with third eyelid its secretion enters the conjunctiva bulbar on the surface of third eyelid (5,6,7).
Morphology avian Harderian glands vary in shape although the most usual form is similar to that found in the domestic fowl where it has been described as strap-like with hour-glass major borders, in some birds, such as the duck and pelican, it is almost hemispherical with a shallow concave face (7). The Harderian gland of the owl is flat and elongated flask-shaped gland with light pink color (8). While in pigeons were appeared oval flat with an irregular outline, in domestic fowl was appeared irregular in shape (9).

Histologically the Harderian gland is regarded as a lympho-epithelial gland and a site of immunological response, and the lacrimal system has been implicated in the head-associated lymphatic system and a site of immune response (10,11).

The Harderian gland is compound multilobular ocular gland It possess single duct that open in the inner angle of the eye at the base of the nictitating membrane (12).It is produces a thick, oily fluid (13).

Based on experimets in thirty-two bird species, Burns classified Harderian gland into three types according to their glandular structure (9).Type I has a compound tubulo-acinar structure with a lobule composed of a single type of epithelial cells with a large age-dependent population of plasma cells in the interstitium of the gland (7,14).

Type II has a compound tubular structure, lobule with two types of epithelial cells lining the tubule and much smaller population of plasma cells (9.15). Type III can be regarded as "mixed" structures (9).

The Harderian Gland produces a mucous secretion in amphibians, serous or sero-mucous in reptiles, mucous in birds, and lipid secretion in mammals. The Harderian gland primarily produces a muco-lipid secretion that lubricates the surface of the eyeball and the nictitating membrane, which is critical for corneal health and movement of the third eyelid, the secretion contains both neutral and acidic mucopolysaccharides, supporting its protective and lubricative functions (16).

Several studies confirm that the Harderian gland is an important site of local immune response, functioning as a lymphoepithelial organ. It contributes to the activation and differentiation of B cells and the production of plasma cells, this immune capacity helps protect the eye against pathogens and supports vaccine responses in birds The Harderian gland can form one main eye-associated lymphoid structure (17,18).

MATERIALS AND MTHODS

The specimens were gathered from commercial bird markets and hunting in the deserts of Karbala and Najaf. Seven 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 2mg/kg xylazine and 25 mg/kg ketamine into the alar vein to induce anesthesia (19). In order to examine the Harderian glands shape, spatial orientation, and anatomical relationships. After being removed, the specimens were submerged for 24 hours in a fixative solution that contained 10% formalin. After that, they were treated with Alcohol, which is gradually upgraded from deferent 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, Cutting slices of a thickness of roughly 5 to 6 µm are produced by using a rotary microtome. H and E stain, Masson trichrome stain, and PAS& Alcian blue, were used to stain the sections. The studied slides were photographed using a digital camera mounted to a light microscope.

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.2025.134.

RESULT AND DISCUSSION

Morphological description

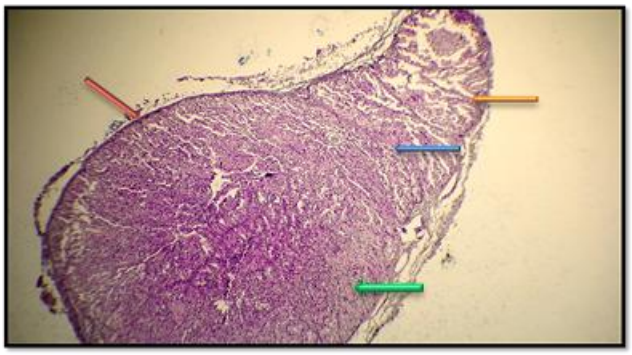
The only isolated and independent gland in this owl was the Harderian gland. It was discovered to be flask-like, positioned dorso-medially of the eye on the inner side of the eye. Its primary secretory duct was lengthy and reached the tear duct, where it opened.

Histological description

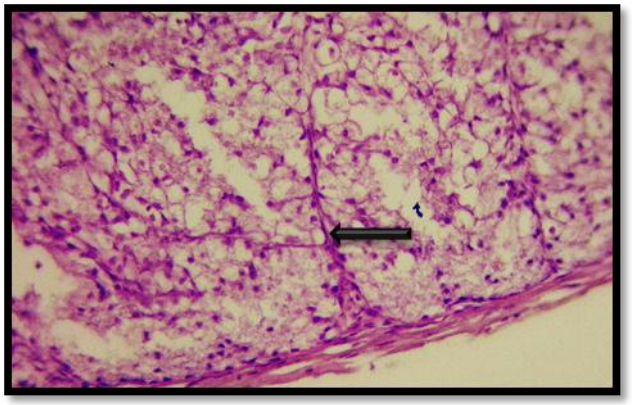
The gland was encased in a capsule and split into two lobes, one minor and the other the primary, larger one, as shown (Figure 1). The two lobes were separated by septa that extended from the outer capsule, haphazardly in the smaller lobe and systematically in the larger lobe (Figure 2, 3).

The capsule encasing the gland was composed of connective tissue with a high concentration of collagen, which caused it to appear blue, while the trabeculae that extended inward and partitioned the gland contained less collagen, resulting in a green appearance by Masson stain. The secretory cells remained stable on those septa, leaning against one another toward the center (Figure 4).

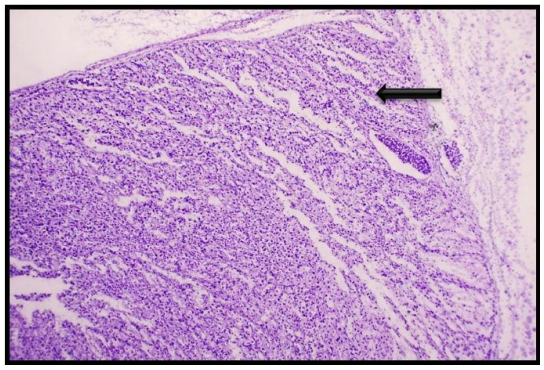
The Harderian gland was regarded as a distinctive gland because it produces multiple types of secretion. The smaller lobe had a secretory-mucous characteristic. This was emphasized by applying Alcian blue along with PAS stain, where the smaller lobe displayed the blue hue entirely (Figure 5), while the bigger lobe consisted of secretory-mucous cells, additional serous cells, and a third type that was mixed, as indicated by the reaction observed when the two stains, Alcian blue and PAS, were used in combination (Figures 6).



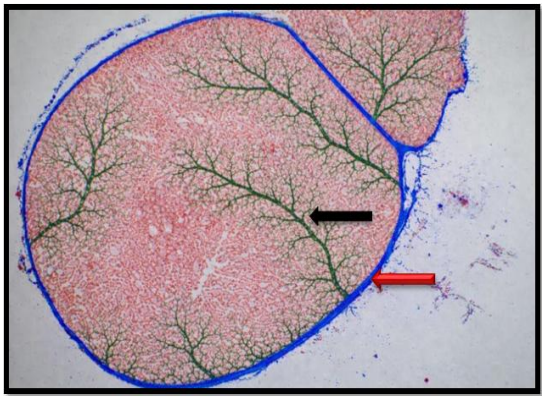
Figures 1. show the histological structure of an Harderian gland red row capsule (red arrow), septa (blue arrow), minor lobe (orange arrow), and major lobe (green arrow). Use H&E stain.40X,



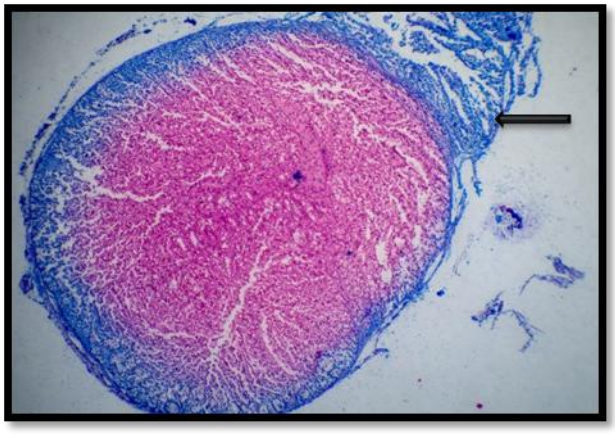
Figures 2. show the histological structure of Harderian gland trabeculae in Major lobe (black arrow). With H&E stain.100X



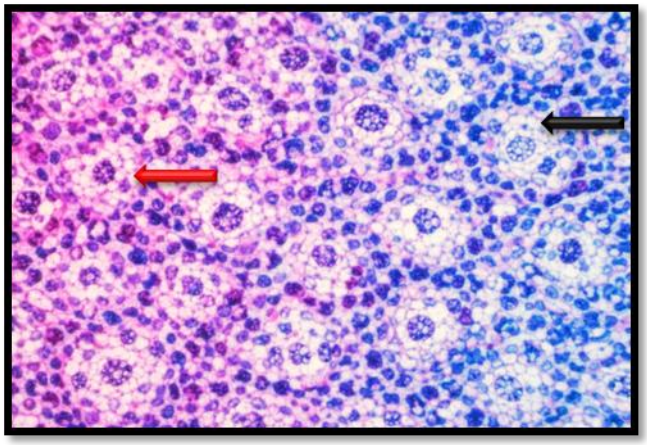
Figures 3. show the histological structure of Harderian gland trabeculae in Miner lobe (black arrow) with H&E stain.100X



Figures 4. show the histological structure of a Harderian gland, trabeculae (black arrow), Capsule of gland (red arrow) with Masson stain.40X.



Figures 5. show the histological structure of a Harderian gland, show the smaller lobe had a secretory-mucous characteristic (black arrow). This was emphasized by applying Alcian blue along with PAS stain 40x.



Figures 6. show the histological structure of a Harderian gland, show secretory-mucous cells (black arrow), mixed secretory cells (red arrow) use Alcian blue and PAS, 1000x .

In this research, the Harderian glands of all assessed adult birds were located dorsomedially relative to the eye on its inner side. These results were corroborated by (7) in chickens, whereas (9) in Capercaillies noted the gland positioned in the orbit, adjacent to the rear part of the interorbital septum and toward the inner side of the back third of the eyeball. Our study found that the Harderiangland of all investigated adult birds possess a single excretory duct that originates from the anterior end and then opens in the medial section of the conjunctival membrane. This aligns with the findings presented by Wight, (20) regarding osprey.

The current study showed that the histological examination of the HG in all the birds analyzed aligns with findings by (15) regarding geese. The gland was encased in a connective tissue capsule that was compound tubuloacinar and was lined with simple columnar cells. In our research, the central collecting duct was covered with simple columnar secretory epithelium in the HG of all observed birds; these findings resembled those of (21) in ostriches, (15) in geeseand (7) in chickens

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