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ORIGINAL ARTICLE

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Morphological and functional relationship between the orbital gland and olfaction in *Upupa epops* (hoopoe) and *Bubulcus ibis* (cattle egret)

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Abstract

This study used both anatomical and histological techniques to investigate the orbital gland's topographic relationship with the surrounding system, using the hoopoe and cattle egret as biological models. Hoopoe has a spindle-shaped lacrimal gland that is suspended on the lateral edge of the frontal bone, whereas cattle egret has a tiny lacrimal gland that is embedded posteriorly within the periorbital fascia. The hoopoe's lacrimal gland has a single duct that runs parallel to the nasolacrimal duct and opens into the posterior nostril hole. In the cattle egret, the tubule-alveolar secretory components comprise neutral and acid glycosaminoglycan. In addition, the Harderian gland is found in both these species, but their draining ducts differ; the Harderian gland of the hoopoe opens into the anterodorsal to the conjunctival fornix, whereas the Harderian gland of the egret opens anteriorly. In both hoopoe and egret, the secretions of Harderian gland include neutral and acid glycosaminoglycan. The Harderian gland is categorized as type II in hoopoe and type I in cattle egrets. The present results concluded that both orbital glands of two bird species studied play an essential role in eye health, where cleaning and lubrication of the cornea surface. Furthermore, the lacrimal gland's location and secretory features may strengthen the olfactory sensitivity of hoopoe, which relies heavily on scent to locate their food, whereas egret relies heavily on visual cues.

KEYWORDS
anatomical and histological techniques, cattle egret, Harderian gland, hoopoe, olfactory, orbital glands

1 | INTRODUCTION

Bird's foraging strategies primarily rely on their vision. They typically have excellent eyesight that enables them to detect prey predators and other birds at great heights and at long distances. Many studies made the assumption that most birds do not make much of their sense of smell; however, other studies contradict this hypothesis. For example, Poteir (2020) reported that the raptor's sense of smell helps them locate food without having to see it. The study of the function of the orbital glands in relation to vision and other senses (in a manner like their role for olfaction) in various vertebrates has elicited new interest from the scientific community. This supposed function is based on the route of the nasolacrimal duct, which connects the anterior orbital region.

The major orbital glands that are a part of the eye accessory organs are the lacrimal and Harderian glands. To better understand the roles of both glands, several morphological, biochemical and physiological methods have been used to study them. Most previous studies have focused on the Harderian gland in many various vertebrate species (Altunay & Kozlu, 2004; Beheiry et al., 2020; Boydak & Aydin, 2009; Dubey et al., 2014; Mobini, 2012, 2014; Pradidarcheep et al., 2003; Reem & Khattab, 2018). However, there is little information about the structure and functions of the lacrimal glands; with only few publications available (i.e. Klećkowska-Nawrot et al., 2016; O'Malley, 2005; Shawki et al., 2019; Williams, 1994).

Many researchers have mentioned the main functions of the Harderian gland. For example, Baccari et al. (1993) demonstrated the osmoregulatory role of the Harderian gland and highlighted it as a source of salt in some turtles, while Chieffi et al. (1996) revealed its thermoregulatory role in some rodents. Rehorek et al. (2000, 2005) observed the relationship between the orbital gland and the vomeronasal organ in olfaction in reptiles. Ohshima and Hiramatsu (2002) reported that the avian Harderian gland is involved in mediating an immune response. Stern et al. (2004) highlighted the clinical importance of the orbital glands. Van Ginkel et al. (2009) hypothesized that ocular immunization with Ad5-H5 induces antigen-specific, humoral immune responses in the Harderian gland. Hirayama et al. (2013) demonstrated the potential of bioengineered organ replacement functionally restoring the lacrimal gland. Frahmand and Mohammadpour (2015) revealed that the avian Harderian gland is considered a lymphoid organ and that the plasma cells increase with age. Moreover, many recent studies have investigated the additional extraorbital function of the orbital glands. Nonetheless, knowledge of the normal anatomical and histological structures of the orbital glands remains insufficient in many avian species.

To clarify the relationship between the anatomical and histological features of the lacrimal and Harderian orbital glands and their other extraorbital functions, they should be investigated in multiple avian species. This study therefore aimed to describe and compare the anatomical and histological structure of the lacrimal and Harderian glands in two avian species, hoopoe and cattle egret, and determine whether these glands play a role in the visual system or have an extraorbital function.

2 | MATERIALS AND METHODS

2.1 | Collection of samples

We obtained ten specimens of adult hoopoe (*Upupa epops*, Linnaeus, 1758) and cattle egret (*Bubulcus ibis*, Linnaeus, 1758). They were brought, as live specimens, into the vertebrate comparative anatomy laboratory in preparation for this study, according to the guidelines of the Ethical Committee on Animal Experimentation of Assiut University. After anesthetization, each head was separated from the rest of the body and then prepared for the subsequent examinations.

2.2 | Anatomical investigation

Each bird head was preserved in 10% formalin for 3 weeks before being transferred into 2% phenoxyethanol. The orbital glands were carefully dissected under a Wild M3 Stero-microscope in preparation for photography, which used a digital camera (Samsung Galaxy A52, model number, SM-A525F/DS). Additionally, a similar procedure was utilized to prepare the cranium, which was photographed using a digital camera. The anatomical terminology of the skull and orbital tissue was used according to Nomina Anatomica Veterinaria (2012).

2.3 | Light microscopical investigation

For the histological investigation, the complete eyes and orbital glands of each species were fixed in 10% neutral formalin, dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene, embedded in paraffin wax at 58–62°C and then sectioned into 7 µm slices using a Leica rotatory microtome (RM 20352035; Leica Microsystems, Wetzlar, Germany). Slices were stained according to the staining procedures of Bancroft (1996): Masson's Trichrome stain to detect collagen fibres, and periodic acid Schiff's reagent (PAS) and Alcian blue pH 2.5 to demonstrate the neutral and acid glycosaminoglycan, respectively.

3 | RESULTS

3.1 | Anatomical investigations of the lacrimal gland of hoopoe and cattle egret

The hoopoe lacrimal gland is spindle-shaped with a narrow posterior tip that is situated within the lacrimal fossa (Figure 1a,b). The body of the lacrimal gland enlarges anteriorly as it emerges into the draining duct; this duct is unpaired and passes forward beneath the ventral surface of the lacrimal process, penetrating it and opening into the posterior nasal opening (Figure 1b). Moreover, the body of hoopoe lacrimal gland is securely attached to the lateral rim of the frontal bone via the fibrous connective tissue forming the periorbital sheath (Figure 2a,b).

In cattle egret, the lacrimal gland appears to be small and drop-like. It lies on the posterior pole of the scleral ossicles and is covered entirely by periorbital sheath (Figure 1c,d). After severing this sheath, the lacrimal gland appears between the adductor muscle of the jaw and anterior temporal crest, ventrally close to the origin site of the retractor anguli oculi muscle (Figure 1d,e). Upon careful separation of the gland from the adductor muscle, we noted that it is adhered to and penetrated by numerous nerve endings and blood arteries (Figure 1e). The lacrimal gland secretes through an unpaired draining duct, which opens into the posterior conjunctiva fornix of the upper eyelid (Figure 1d).

3.2 | Histological and histochemical investigations of the lacrimal gland

The histological investigation of the lacrimal glands in hoopoe and cattle egrets showed that the gland is a compound, tubulo-alveoli,

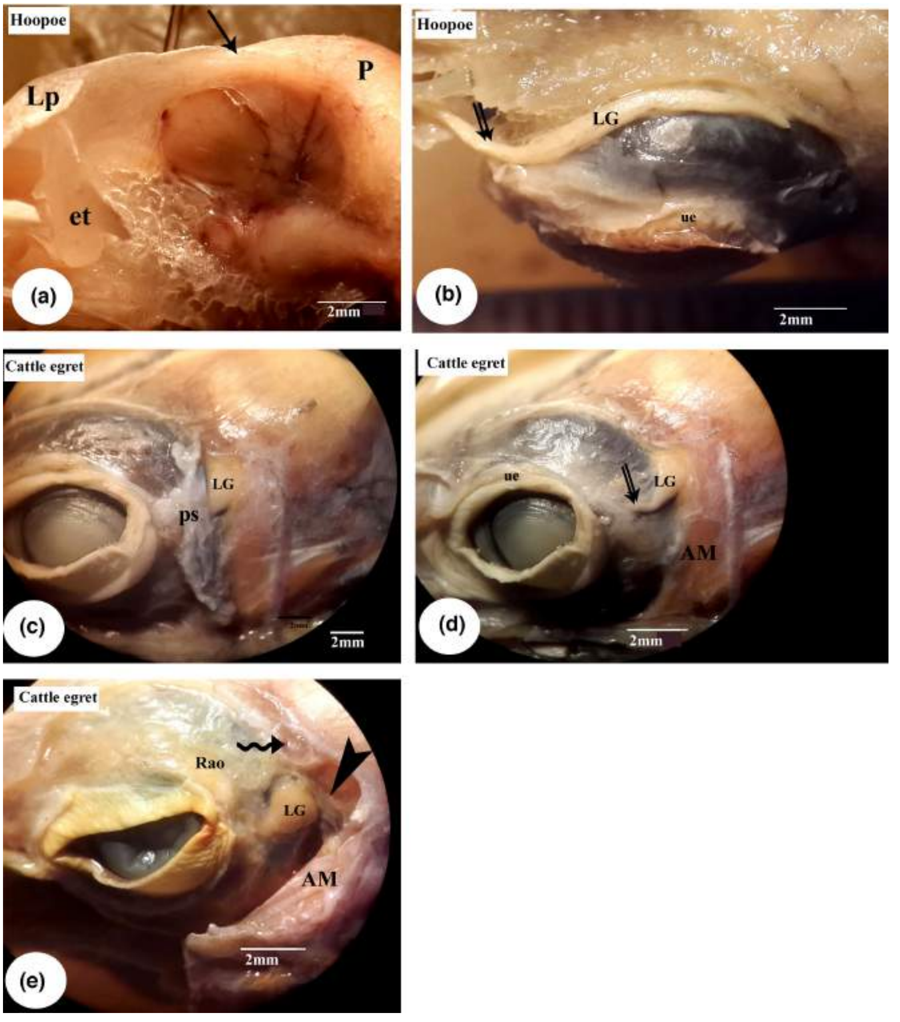

FIGURE 1 Steromicroscopic photographs of the lacrimal gland. (a, b) lateral view of the skull and eye of hoopoe *Upupa epops*, respectively, (c–e) in cattle egret, *Bubulcus ibis*, illustrating the shape of lacrimal gland and its location; lacrimal process (Lp), parietal bone (P), lacrimal fossa (arrow), ectoethmoid bone (et), anterior temporal crest (zigzag arrow), lacrimal gland (LG), lacrimal duct (double arrows), upper eyelid (Ue), periorbital sheath (Ps), adductor muscle of lower jaw (AM), retractor anguli oculi muscle (Rao), nerve ends penetrate the body of lacrimal gland (arrow head).

enveloped by a thin capsule of collagenous connective tissue. This capsule penetrates the gland and causes it to split into lobules (Figure 2). The interlobular tissue contains fibroblasts, blood vessels and nerve fibres. The plasma cells appear to be in a higher density in cattle egrets compared with hoopoe (Figure 2c,f). Each lacrimal lobule consists of many acini and intralobular ducts (Figure 2a). The alveoli of the hoopoe lacrimal gland are lined with cuboidal secretory cells with an irregular distal cell surface (Figure 2c,d). In cattle egret, the acini of the lacrimal gland are lined by cuboidal cells, which are characterized by the presence of large basophilic round nuclei and budding surfaces (Figure 2e,f).

The acini of the lacrimal glands of both bird species were positive to PAS and turned a bluish colour with Alcian blue pH 2.5. In hoopoe, the secretory cells of the gland were weakly positive to PAS but had a moderate reaction with Alcian blue pH 2.5. In contrast, the capsule and interlobular connective tissue exhibited a dark purple and blue colour with PAS reaction and Alcian blue pH 2.5, respectively (Figure 3a,b). In cattle egret, the secretory portion of the gland is filled with a large amount of neutral and acid glycosaminoglycans (Figure 3c,d).

3.3 | Anatomical investigations of the Harderian gland

To see the Harderian gland in both bird species, the depressor eyelid muscle and loose periorbital fascia must be removed. In hoopoe, a thin transparent sheath envelopes the Harderian gland and the surrounding muscles to separate it from the depressor eyelid muscle. Meanwhile, in cattle egret, the Harderian gland is easily dissected from the loose capsule. Moreover, in both bird species, the Harderian gland can be differentiated into two halves: a nasal half and a temporal one. This gland has a convex surface that faces the periorbital fascia, whereas the surface facing the eyeball is concave (Figure 4).

In hoopoe, the Harderian gland has a tongue-like appearance. The middle isthmus is present between the two halves of gland. The temporal portion of the Harderian gland is broader and has a slightly median sulcus on its lateral surface (Figure 4b). It is located near the optic nerve, between the rectus medialis and oblique ventralis muscles, and covers the ventrolateral surface of the rectus ventralis muscle (Figures 4a and 5a). This portion of the gland turns anteromedially over the medial surface of the rectus medialis

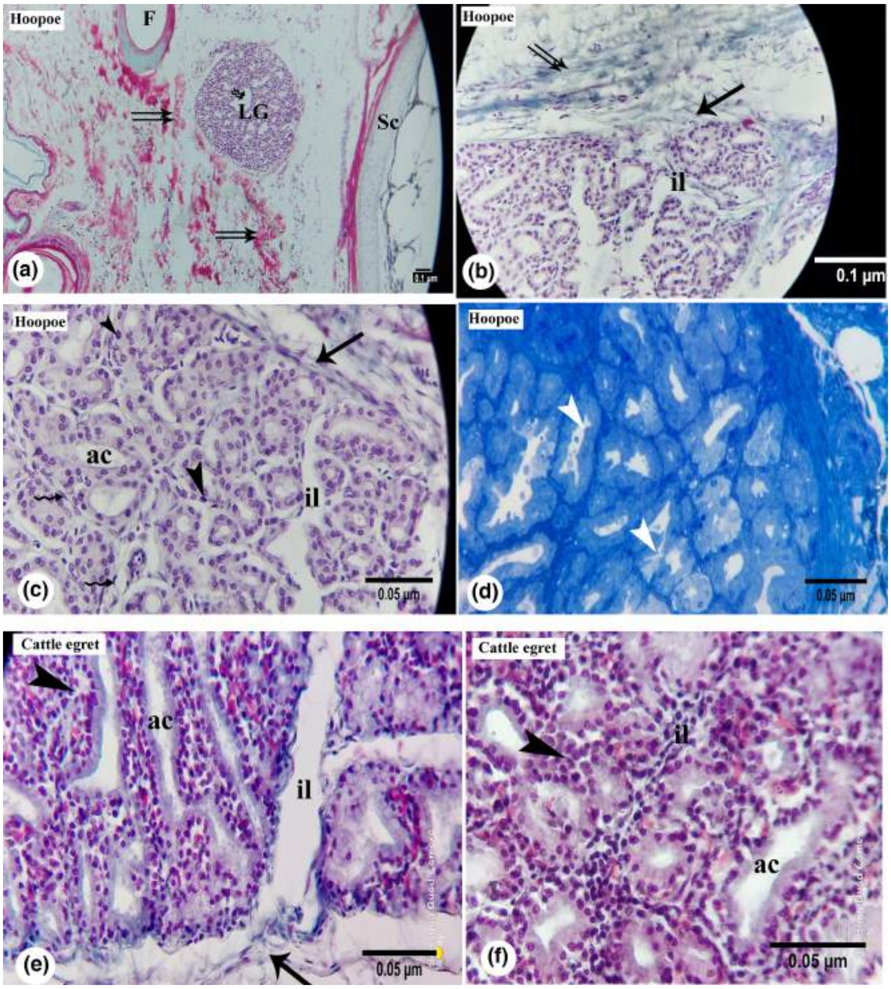

FIGURE 2 Photomicrograph of section of the lacrimal gland. (a–d) illustrate the lacrimal gland of hoopoe (LG), tubulo-alveolar acini (ac) with irregular lumen (whit arrow head), its connection with frontal bone (F), the periorbital sheath (double arrows), thin collagenous capsule (arrow), interlobular tissue (il), fibroblast cells (zigzag arrow), plasma cells (black arrow head) and interlobular duct (double zigzag arrow). (e, f) illustrate the lacrimal gland of cattle egret consist of tubulo-alveolar acini (ac) with budding surface and presence of high density of plasma cells within interlobular tissue (black arrow head). ((a–f) *by Masson's trichrme stain*, (d) *by toluidine blue scale bar, 0.1 and 0.05 μm*).

muscle, and narrows towards the antrodorsal surface of the eyeball. In hoopoe, the Harderian gland opens onto the anterodorsal surface of the conjunctival sac of the nictitating membrane via tiny ductules (Figure 4a). These ductules pass beneath the attachment site of the oblique dorsalis and rectus medialis muscles.

In cattle egret, the Harderian gland is an elongated blind sac with irregular borders (Figure 4e). The nasal half of the Harderian gland is broader, compared with the temporal half, and a narrow middle isthmus is present between the two halves of the gland (Figure 4e); this isthmus passes beneath the rectus medialis muscle (Figure 4c). The temporal half is located between the ventrolateral surface of the eyeball and the medial surface of the parasphenoid bone (Figure 4c). The nasal half of the Harderian gland runs dorsally to emerge into the draining duct, which opens into the anterior conjunctiva fornix (Figure 4c–e). Meanwhile, the temporal half of the Harderian gland extends posteriorly, nearly to the optic nerve.

3.4 | Histological and histochemical investigations of the Harderian gland

The Harderian gland of both bird species is a compound tubulo-alveolar type of gland. This gland is enveloped by a capsule of collagenous connective tissue, which penetrates the gland to form many lobules (Figure 5). This collagenous capsule is thinner in hoopoe than in cattle egret, and numerous plasma cells are evident within the interlobular tissue (Figure 5c,e).

In hoopoe, the secretory portion of the Harderian gland is lined by two distinct epithelia: The broad portion (temporal) of the gland is lined with pyramidal cells with oval basal nuclei, whereas the narrow portion (nasal) is lined with tall cuboidal cells with round basal nuclei (Figure 5b–d). Thus, it can be classified as a type II gland.

In cattle egret, the secretory portion of this gland is lined with pyramid cells with oval and round nuclei (Figure 5e). Thus, it is classified as a type I gland in cattle egrets.

The secretory acini of the Harderian gland in both bird species reacts positively to PAS and Alcian blue pH 2.5. In hoopoe, the acini of the Harderian gland exhibit variability in the presence of neutral glycosaminoglycan; the granules of glycosaminoglycan are distributed uniformly throughout the cytoplasm of pyramidal cells, whereas they are concentrated in the distal portion of cuboidal cells (Figure 6a–c). In contrast, the acini of the Harderian gland swell with intense purple granules in cattle egrets (Figure 6d,e).

4 | DISCUSSION

The lacrimal and Harderian gland play an essential role in eye health. The lacrimal gland in mammals, particularly in humans, has been the focus of extensive research, with clinical trials attempting to develop

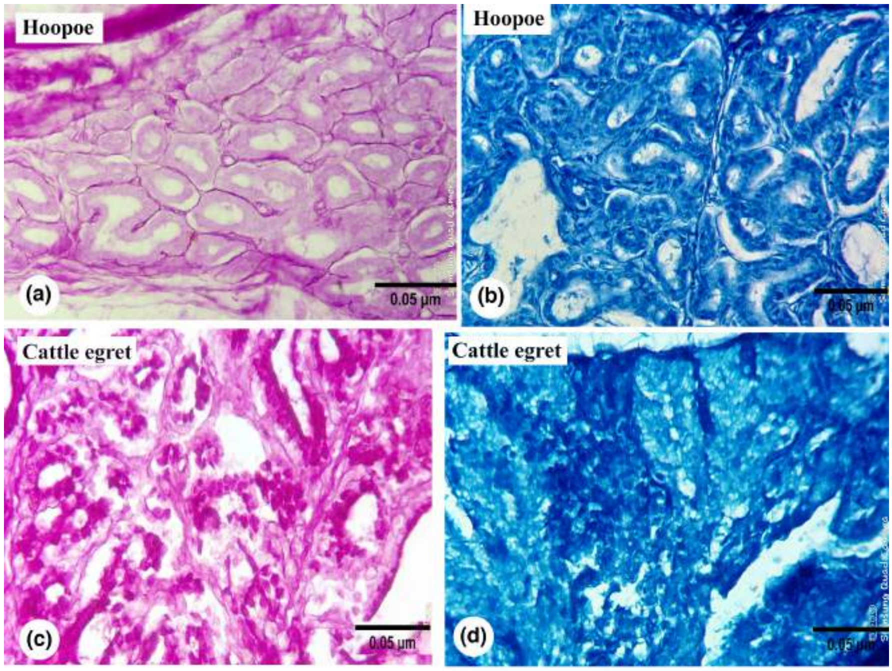

FIGURE 3 Photomicrograph of section of the lacrimal gland stained by PAS and Alcian blue PH 2.5. (a, b) in hoopoe and (c, d) in cattle egret. The magenta colour with PAS and bluish colour with Alcian blue indicates the presence of neutral and acid mucopolysaccarides respectively (*scale bar 0.05 μm*).

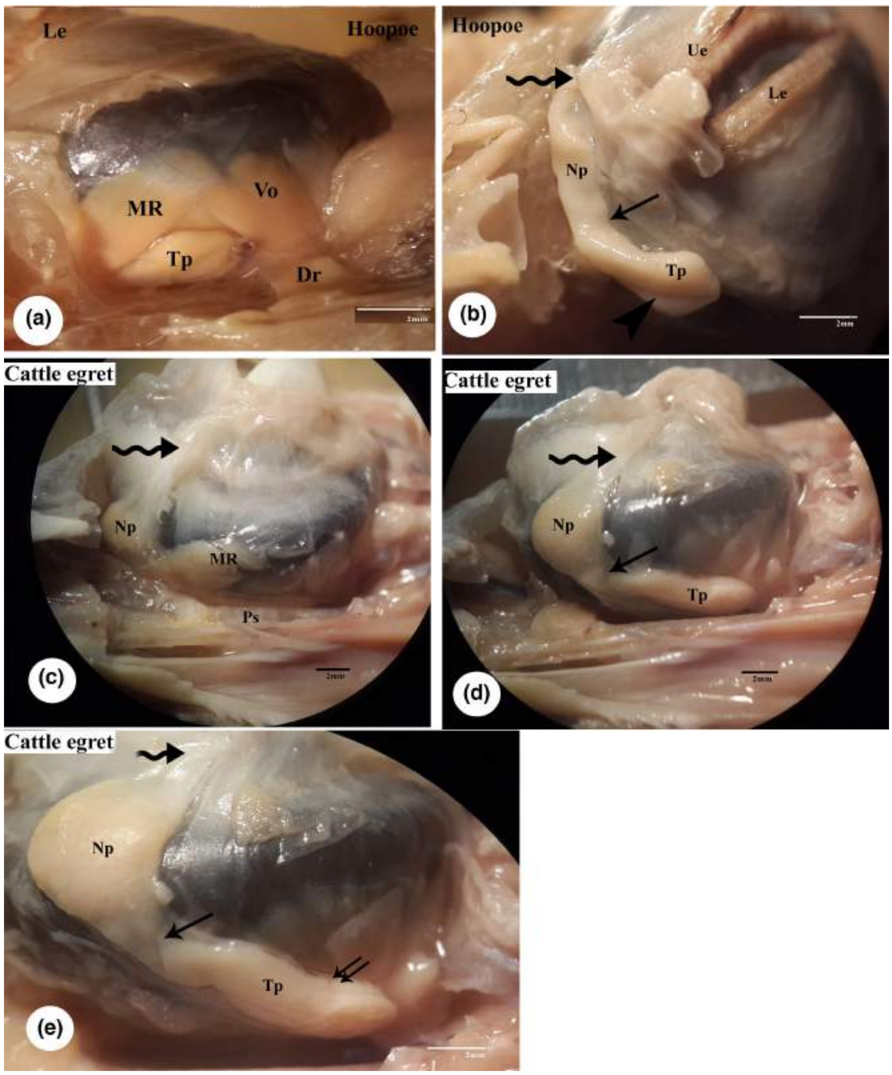

FIGURE 4 Steromicroscopic photographs of the Harderian gland. (a, b) in hoopoe, (c–e) in cattle egret illustrating the anterior half of gland (np), temporal half (Tp), draining duct of gland (zigzag arrow), middle isthmus (arrow), median sulcus (arrow head), irregular borders of gland (double arrows), upper eyelid (Ue) lower eyelid (Le), Parasphenoid bone (Ps), rectus medialis muscle (Mr), rectus Ventralis muscle (Vo), depressor muscle of eye (Dr), ectoethmoid bone (et).

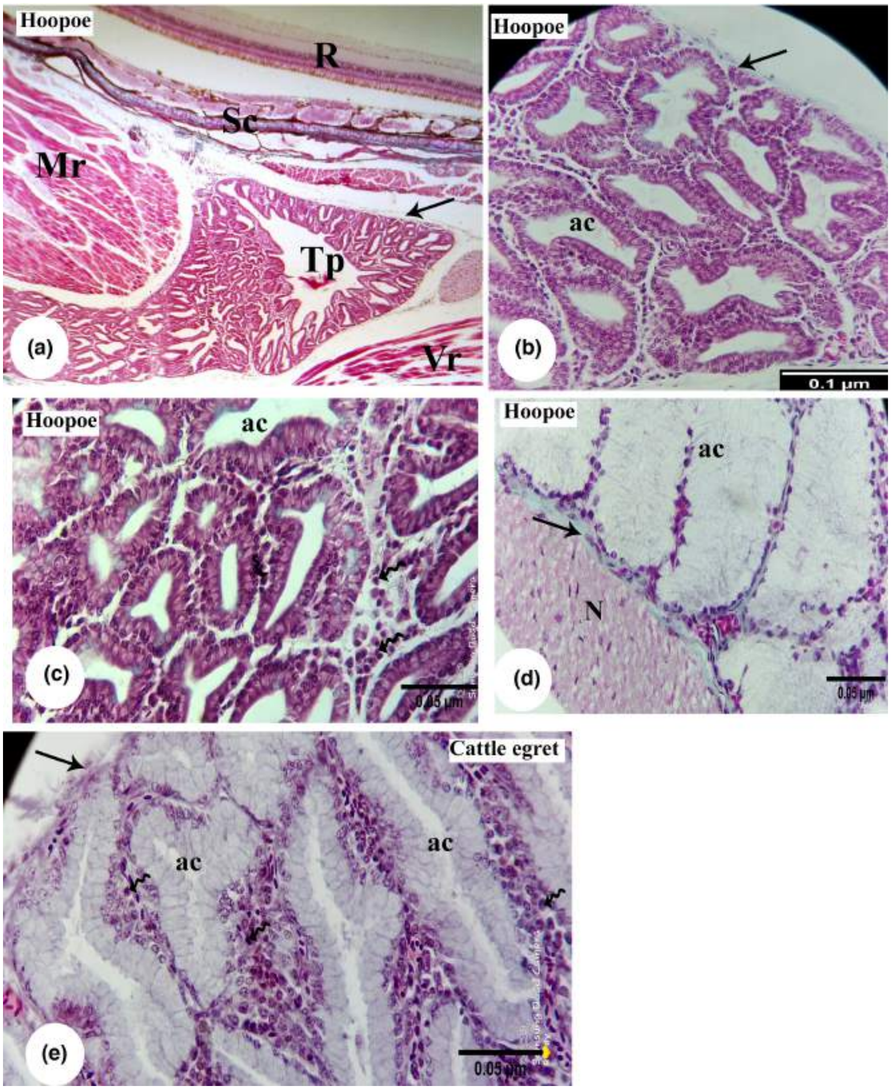

FIGURE 5 Photomicrograph of section of the Harderian gland. (a–d) in hoopoe illustrating the location of temporal half (Tp) between the rectus medialis muscle (Mr), rectus ventralis muscle (Vo), sclera cartilage (Sc), retina (R), tubulo-alveolar acini (ac) enveloped by thin collagenous capsule (arrow), plasma cells (zigzag arrow), (black arrow head). (e) Illustrate the structure of Harderian gland of cattle egret consist of tubulo-alveolar acini (ac) and aggregation of plasma cells within interlobular tissue (zigzag arrow). *Masson's trichrme stain, scale bar, 0.1 and 0.05 µm*).

several numerous innovative products for the functional restoration of the lacrimal gland as a potential dry eye therapy (Hirayama, 2018; Butovich et al., 2021). However, in other vertebrates such as birds, the lacrimal gland has not received sufficient attention as evident from the current ornithology literature. Several lines of evidence suggest that the Harderian gland is the dominant gland in birds possibly owing to its association with the nictitating membrane. Burns and Maxwell (1979) provided an excellent account of the anatomical and histological structure of the lacrimal gland in three bird species: *Gallus domesticus L* (fowl), *Meleagris gallopavo L* (turkeys) and *Anas platyrhynchos L* (ducks); in all three species, the lacrimal gland has the same anatomical location, i. e., on the dorsolateral angle of the orbit.

Some birds species, such as *Meleagris gallopavo* (turkey) (Dimitrov, 2011), capercaillie (Klećkowska-Nawrot et al., 2015, 2016), ostrich, *Strutheio camelus* (Reem & Khattab, 2018) and *Athene noctua* (little owl) (Shawki et al., 2019) have the same location of the lacrimal gland; this is consistent with the findings of the present study. The location of the lacrimal gland in hoopoe is consistent with that of little owl, fowl and capercaillie. Conversely, in cattle egret, the lacrimal gland is in a different location, which is nearly identical to the location found in ostrich as reported by Reem and Khattab (2018); it is located caudal to the canthus of the eye.

The position of the lacrimal gland varies considerably among bird species, as does its size, shape and mucins type. The lacrimal gland in hoopoe was larger than in cattle egret. In hoopoe, it was elongated, spindle-shaped and suspended on the lateral edge of the skull, whereas in cattle egret, it was small, drop shaped and embedded within periorbital fascia. Similar results as those for hoopoe were observed during an anatomical investigation of the location of the lacrimal gland in little owl (Shawki et al., 2019) and common moorhens (in preparation).

O'Malley (2005) reported that the lacrimal gland empties into the nasolacrimal duct via dorsal and ventral puncta. In certain bird species such as capercaillie and ostrich (Klećkowska-Nawrot et al., 2015, 2016), the lacrimal gland secretes their tears through multiple ducts and opens into the conjunctival space beneath the lower eyelid. The present study found that the lacrimal gland of hoopoe empties its secretions through a single duct that opens into the posterior nasal aperture through nasolacrimal duct. This pathway

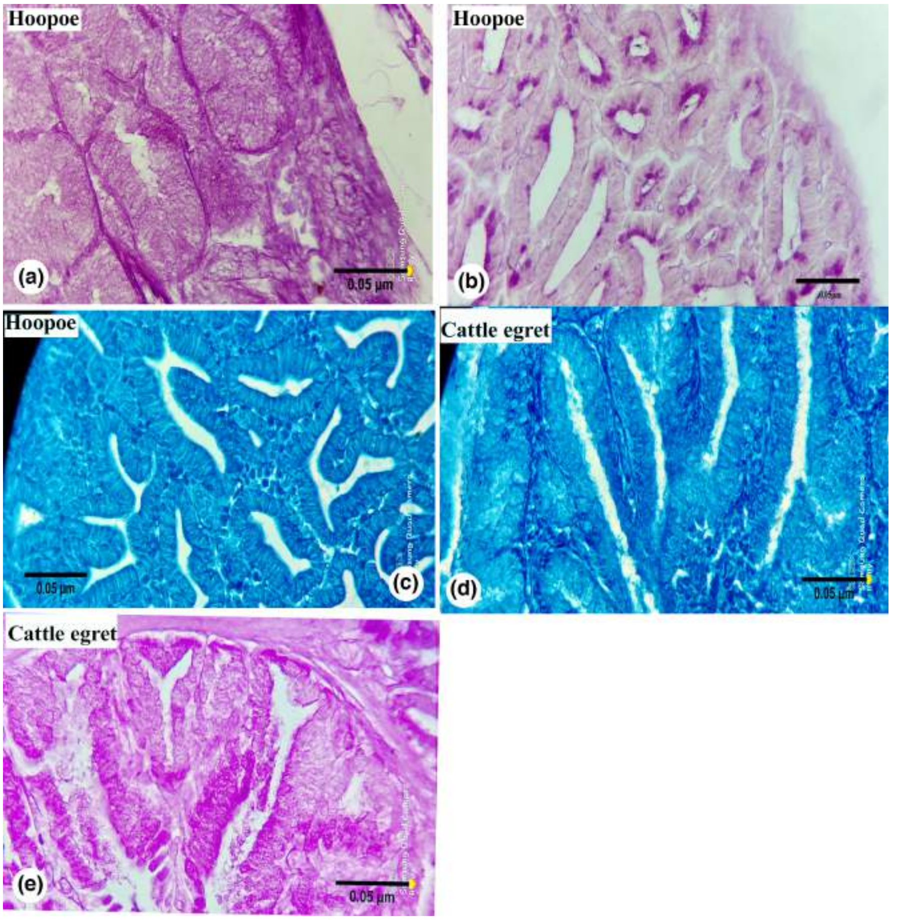

FIGURE 6 Photomicrograph of section of the Harderian gland stained by PAS and Alcian blue PH 2.5. (a–c) in hoopoe and (d, e) in cattle egret. The magenta colour with PAS and bluish colour with Alcian blue indicates the presence of neutral and acid mucopolysaccarides respectively. (*scale bar, 0.05 μm*).

has also been observed in little owl (Shawki et al., 2019). In contrast, in cattle egret, the postero-lateral anatomical position of the lacrimal gland results in a different pathway for secretions: which are emptied directly into the conjunctival space of the upper eyelid through a single duct.

The explanation of the pathway of the lacrimal duct's secretory pathway is fascinating, as it describes how the gland's tears empty into the external nasal opening which is close to the nasolacrimal duct's outlet. This pathway perhaps relates to the lacrimal gland's cooperative function in olfaction. Rehorek et al. (2000, 2005) discussed about how some reptiles, such as alligators and snakes, use their orbit glands to enhance vomeronasal sense.

Anatomically, the nasolacrimal duct located ventral and parallel to the lacrimal duct in hoopoe. This anatomical pattern facilitates the tear delivery directly to the nasal cavity, and allows for drainage into the conjunctival space to cover the anterior surface of the cornea. The present authors speculate that lacrimal secretions are mainly responsible in lubrication of the ocular surface. Additionally, these secretions may aid in the olfaction or in deglutition.

The nasal cavity of birds passes through a series of mucous-covered chambers called nasal conchae; these conchae influence airflow dynamics and direct odours to the caudal-most chambers (Scanes, 2014). Odour molecules diffuse through a mucous membrane. The most rostral region of the nasal cavity is the vestibule; the nares open into this region (Farouk et al., 2017). In laughing dove, the stratified squamous epithelium of the nasal vestibule varies from keratinized to non-keratinized; this suggests no glandular activity in this region; however, because it is the first chamber to receive airflow, it must be wet. In hoopoe, lacrimal secretions spread over the nasal vestibular epithelium to lubricate and allow odour molecules to diffuse to it; this explains the ability of hoopoe to find hidden prey by detecting their odour Rubene et al. (2018) investigated birds' preferences for olfactory and visual cues and demonstrated that birds prioritize visual information when visual and olfactory cues are separated, but use odours to guide their foraging choices when visual information is held constant.

The mucins of the lacrimal gland of hoopoe, which include neutral and acid glycosaminoglycan, may also play an important role as inflammatory and antibacterial agents, preventing microbial growth in the moist chamber of the nasal cavity and protecting the eye from inflammation. Because it is tiny and opens directly onto the ocular surface, the lacrimal gland in cattle egrets is less efficient for this purpose than it is in the hoopoe because it is rather tiny and opens directly onto the ocular surface. Braun et al. (2018) pointed out the antimicrobial activity of other glands like preen gland (uropygial gland) and found that their secretions significantly inhibited the growth of broad spectrum of bacteria and fungi.

The present study demonstrated that the tubule-alveolar acinus of the lacrimal gland of both bird species secrete neutral and acid glycosaminoglycan; however, the degree of secretions is considerably more dense in cattle egret than hoopoe. Shawki et al. (2019) noted that the lacrimal gland of little owl reacted weakly to PAS, but positively to Alcian blue; similar results were observed in capercaillies (Klećkowska-Nawrot et al., 2015) and ostriches (Klećkowska-Nawrot et al., 2015; Reem & Khattab, 2018). These results reveal the biological importance of acidic sulphated and neutral mucins in the lacrimal gland with respect to corneal lubrication and protection dynamics. Numerous studies (i.e. Beheiry et al., 2020; Dubey et al., 2014; van Ginkel et al., 2009; Shawki et al., 2019) have reported that the head-associated lymphatic tissue system in bird includes the Harderian gland, based on the presence of interstitial lymphatic tissue (plasma cells). Additionally, Klećkowska-Nawrot et al. (2015) reported that the lacrimal gland is not responsible for immunoglobulin production. The present results support these scientific perspectives: although the Harderian gland is a critical component of the immune defence of the eye, this does not indicate that the lacrimal gland does not also contribute to this function.

The function of the lacrimal gland of both bird species was established in this study via histological and histochemical investigation. The histological investigation demonstrated the presence of plasma cells between the acini of this gland in both species. Cattle egret had a higher density of interstitial plasma cells than hoopoe, as well as more acidic secretion.

Moreover, the laboratory observation of cattle egret eyes during dissection revealed the existence of several of parasitic worms lying under the eyelids. These anatomical and histochemical observations of cattle egret suggested the role of lacrimal secretions may share in defending the eye against parasitic infestation (infectious disease), thereby maintaining conjunctival and corneal integrity and visual acuity.

In 1979, Burns removed the Harderian gland surgically in domestic fowl, which resulted in enhanced secretory activity of the lacrimal gland and an increase in the number of goblet cells throughout the length of the lacrimal duct. Furthermore, the plasma cells became more abundant in the lacrimal gland. The present authors agree with the view of Burns. The activity of the lacrimal gland is determined by the habitat and feeding behaviours of the bird species, which confirmed in the present study. The hoopoe and cattle egret are different bird species with different feeding behaviours. Hoopoe detects their prey using visual and olfactory cues; thus, they increase their olfactory performance via lacrimal secretion. On the contrary, cattle egret depend more on visual cues than odours, thereby the function of the lacrimal gland of cattle egret is restricted to lubrication and protection of the ocular surface.

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CONFLICT OF INTEREST

No conflict of interest is declared for this work.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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REFERENCES

Altunay, H., & Kozlu, T. (2004). The fine structure of the Harderian gland in the ostrich (*Struthio camelus*). *Anatomia, Histologia, Embryologia*, *33*, 141–145. https://doi.org/10.1111/j.1439-0264.2004.00510.x

Baccari, G. C., Minucci, S., & Di Matteo, L. (1993). The orbital glands of the terrapin PseudemysScripta in response to osmotic stress: A light and electron microscope study. *Journal of Anatomy*, *183*, 21–33.

Bancroft, J. D., & Stevens, A. (1996). *Theory and practice of histological techniques* (4th ed., 190–191). Churchill Livingstone. Chap 10.

Beheiry, R. R., Ali, S. A., Aref, M., & Emam, H. (2020). Harderian gland of flying and non-flying birds: Morphological, histological, and histochemical studies. *The Journal of Basic and Applied Zoology*, *81*, 35. https://doi.org/10.1186/s41936-020-00175-x

Boydak, M., & Aydin, M. F. (2009). Histology of the Harderian gland of domestic geese (Anser anser domesticus). *Acta Veterinaria*, *78*, 199–204. https://doi.org/10.2754/avb200978020199

Braun, M. S., Sporer, F., Zimmermann, S., & Wink, M. (2018). Birds, feather- degrading bacteria and preen glands; the antimicrobial activity of preen gland secretions from turkeys (*Meleagris gallopavo*) is amplified by keratinase. *FSMS Microbiology Ecology*, *94*(9), fiy117. https://doi.org/10.1093/femsec/fiy117

Burns, R. B., & Maxwell, M. H. (1979). The structure of the Harderian and lacrimal gland ducts of Turkey, fowl and duck. A light microscopic study. *Journal of Anatomy*, *128*, 285–292.

Butovich, I. A., Yuksel, S., Leonard, B., Gadek, T., Polans, A. S., & Albert, D. M. (2021). Novel lipids of the rabbit Harderian gland improve tear stability in an animal model of dry eye disease. *Journal of Ocular Pharmacology and Therapeutics*, *37*, 545–555. https://doi.org/10.1089/jop.2021.0015

Chieffi, G., Baccari, G. C., Di Matteo, L., D'Istria, M., Minucci, S., & Varriale, B. (1996). Cell biology of the Harderian gland. *International Review of Cytology*, *168*, 1–79. https://doi.org/10.1016/s0074-7696(08)60882-7

Dimitrov, D. (2011). Histometrical investigation on the Turkey broiler's third eyelid (Harderian) gland. *Journal of Agricultural Science and Technology*, *3*, 246–248.

Dubey, S., Verma, R., & Haldar, C. (2014). Electron microscopic study of Harderian gland of tropical bird Indian jungle bush quail (Perdicula asiatica). *Journal of Endocrinology and Reproduction*, *18*(1), 41–46.

Farouk, S. M., Hassan, S. A., & Emam, M. A. (2017). Histochemical and surface ultrastructural characteristics of the nasal cavity of laughing dove. *Anatomia, Histologia, Embryologia*, *46*, 592–599. https://doi.org/10.1111/ahe.12317

Frahmand, S., & Mohammadpour, A. A. (2015). Harderian gland in Canadian ostrich (Struthio camelus): A morphological and histochemical study. *Anatomia, Histologia, Embryologia*, *44*(3), 178–185. https://doi.org/10.1111/ahe.12123

Hirayama, M. (2018). Advances in functional restoration of the lacrimal glands. *Investigative Ophthalmology & Visual Science*, *59*(14), DES174–DES182. https://doi.org/10.1167/iovs.17-23528

Hirayama, M., Ogawa, M., Oshima, M., Sekine, Y., Ishida, K., Yamashita, K., Ikeda, K., Shimmura, S., Kawakita, T., Tsubota, K., & Tsuji, T. (2013). Functional lacrimal gland regeneration by transplantation of a bioengineered organ germ. *Nature Communications*, *4*, 2497. https://doi.org/10.1038/ncomms3497

Klećkowska-Nawrot, J., Goździewska-Harłajczuk, K., Barszcz, K., & Kowalczyk, A. (2015). Morphological studies on the harderian gland in the ostrich (*Struthio camelus domesticus*) on the embryonic and postnatal period. *Anatomia, Histologia, Embryologia*, *44*(2), 146–156. https://doi.org/10.1111/ahe.12124

Klećkowska-Nawrot, J., Goździewska-Harłajczuk, K., Kowalczyk, A., Łukaszewicz, E., & Nowaczyk, R. (2016). Structural differences of the Harderian gland between common pheasants (*Phasianus colchicus talischensis*) and hybrids of Italian Amber and common pheasants. *Brazilian Journal of Poultry Science. Revista Brasileira de CiênciaAvícola*, *18*(2), 309–318. https://doi.org/10.1590/1806-9061-2015-0059

Mobini, B. (2012). Histological and histochemical studies on the Harderian gland in native chicken. *Veterinary Medicine*, *57*, 404–409.

Mobini, B. (2014). Histological and histochemical studies on the paraorbital gland in broilers. *Journal of Animal Production Advances*, *4*(11), 527–533. https://doi.org/10.5455/japa.20141122084241

Nomina Anatomica Veterinaria. (2012). 5th edition prepared by the International Committe on Veterinary Gross Anatomical Nomenclature (I.C.V.G.A.N.) and authorized by the General assembly of the World Association of Veterinary Anatomists (W.A.V.A.), konxville, T.N (USA). Published by the Editorial Committee, Hannover, Columbia, Gent and Sapporo. 1–160.

Ohshima, K., & Hiramatsu, K. (2002). Immunohistochemical localization of three different immunoglobulin classes in the Harderian gland of young chickens. *Tissue and Cell*, *34*, 129–133. https://doi.org/10.1016/s0040-8166(02)00030-7

O'Malley, B. (2005). *Clinical anatomy and physiology of exotic species structure and function of mammals, birds, reptiles and amphibias*. Elsevier Saunders.

Poteir, S. (2020). Review olfaction in raptors. *Zoological Journal of the Linnean Society*, *189*, 713–721.

Pradidarcheep, W., Asavapongpatana, S., Mingsakul, T., Poonkhum, R., Nilbunga, S., & Somana, R. (2003). Microscpic anatomy of the orbital Harderian gland in the common tree shrew (*Tupaia glis*). *Journal of Morphology*, *255*(3), 328–336. https://doi.org/10.1002/jmor.10066

Reem, R. T., & Khattab, M. A. (2018). Anatomical and histochemical studies on the orbital glands (lacrimal and Harderian glands) in ostrich (*Strutheio camelus*). *International Journal of Advanced Research in Biological Science*, *5*(1), 125–135. https://doi.org/10.22192/ijarbs.2018.05.01.019

Rehorek, S. J., Hillenius, W. J., Quan, W., & Halpern, M. (2000). Passage of the Harderian gland secretions to the vomeronasal organ of the snake, *Thamnophis sirtalis*. *Canadian Journal of Zoology*, *78*, 1284–1288. https://doi.org/10.1139/z00-047

Rehorek, S. J., Legenzoff, E. J., Carmody, K., Smith, T. D., & Sedlmayr, J. C. (2005). Alligator tears: A re-evaluation of the lacrimal apparatus of the crocodilians. *Journal of Morphology*, *266*, 298–308. https://doi.org/10.1002/jmor.10378

Rubene, D., Leidefors, M., Ninkovic, V., Eggers, S., & Low, M. (2018). Disentangling olfactory and visual information used by filed foraging birds. *Ecology and Evolution*, *9*, 545–552. https://doi.org/10.1002/ece3.4773

Scanes, C. G. (2014). *Sturkie's avian physiology* (6th ed.). Elsevier/Academic Press.

Shawki, N. A., Mahmoud, F. A., & Abdel-Megeed, A. M. (2019). Anatomical, histological and histochemical studies of orbital glands of little owl *Athene noctua*. *Assiut University Journal of Zoology. Assiut, Egypt*, *48*(2), 171–200.

Stern, M. E., Gao, J., Siemasko, K. F., Beuerman, R. W., & Pflugfelder, S. C. (2004). The role of the lacrimal functional unit in the pathophysiology of dry eye. *Experimental Eye Research*, *78*, 409–416. https://doi.org/10.1016/j.exer.2003.09.003

van Ginkel, F. W., Tang, D. C., Gulley, S. L., & Toro, H. (2009). Induction of mucosal immunity in the avian Harderian gland with a replication-deficient Ad5 vector expressing avian influenza H5 hemagglutinin. *Developmental & Comparative Immunology*, *33*(1), 28–34. https://doi.org/10.1016/j.dci.2008.07.018

Williams, D. (1994). *In avian medicine: Principles and application: Ophthalmomlogy*. By Ritchie, B. W., Harrison, G. J. and Harrison, L. R Section four, Chapter 26 (pp. 673–677). Wingers Publishing. http://avianmedicine.net/wp-content/uploads/2013/03/con1.pdf

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