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Anatomical, histological and histochemical study of orbital glands of the black winged kite, *Elanus caeruleus*, and their role in protection of the ocular system

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ABSTRACT

The present study discusses the immune function of the eye glands in a wild bird species. Various methodologies have been utilized in the current study to reveal the anatomical and histochemical qualities of the two glands in the black winged kite. The studied bird possesses small lacrimal and large Harderian glands. The lacrimal gland appears as a drop-like shape located on the ventrolateral surface of the sclera cartilage. Whereas, Harderian gland is flask shape and lies in the middle of the ventral oblique and pyramidalis muscles. Both eye glands of black winged kite are compound tubuloalveolar type. The alveoli of Harderian gland lined with simple columnar cells whilst the secretory acini of the lacrimal gland lined with simple cuboidal epithelium. Histochemically, the two glands contain neutral and acid mucosubstances. The Immunohistochemical investigation of the two orbital glands showed that the highest concentration population of CD138 positive plasma cells present in Harderian gland especially around the secretory acini and an increase in density around the main excretory duct, whereas, in lacrimal gland the plasma cells represent the lowest population and are found as diffuse singular cells between the secretory acini. This variability of the concentration of plasma cells around the secretory portion of the orbital glands confirms the critical immunity role of Harderian gland in protecting the ocular surface against any infection, as well as their acidity secretions may a significantly inhibit the growth of bacteria and fungi.

INTRODUCTION

The eye glands are a portion of the eye adnexa which are play a vital role in vision [1]. They lubricates the eye and provide conjunctiva with nutrients and antibodies to maintain eye‘s health [2][3] [4] [5].

In birds the main orbital glands are lacrimal and Harderian glands which belonging to the lacrimal apparatus [6] [7]. The eye glands have been studied by [8] [9] in many different classes of vertebrate as; amphibians, reptiles, Aves and in mammals.

Both glands are 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 [10, 11], and a site of immune response [12] [13].

The avian Harderian gland plays a significant role in the immunological defense of the eye surface [14]. Moreover, Harderian gland produces watery fluid; so, the gland has a vital role in moisten the eyeball surface and third eyelid [15]. This gland also acts as a source of growth factors and pheromones [16] [17]. Additionally, the avian Harderian gland is a lymphoepithelial organ and, act together with the bursa of Fabricius, spleen, and the caecal tonsils to determine general and local immunity [18].

The avian Harderian gland is a place of B-cells activation and differentiation, and manufacture of plasma cell. The avian Harderian gland participate in production of plasma cells which transmitted four classes of immunoglobulins: IgY, IgG, IgA and IgM [19].

The black winged kite *Elanus caeruleus* is a common wild bird species lives Egypt, in open land and semi-deserts. This bird species feeds on grasshoppers, crickets and other large insects, lizards, and rodents [20]. The eye of the black winged kite always exposed to the sand and dust during flight and hunting. The present study focuses on the importance of the orbital glands in protecting the eyes of wild birds during the hunting process.

MATERIALS AND METHODS

The specimens of the two eye glands were taken from six adult birds of the black winged kite. The birds were dissected out according to the recommendations made by the Assiut University research ethics committees (www.enrec.org). The eye glands were separated from the eyeball and fixed in 10% neutral puffer formalin, regularly handled and embedding in paraffin wax. The paraffin blocks were sectioned by microtome at 6μm thickness, the sections were stained by Masson's trichrome for the collagenous fiber’s detection [21]. For histochemical study, the sections were stained with PAS reaction to detect the general polysaccharides and by Alcian blue pH 2.5 characterized to the acid mucopolysaccharides detection [22]. The immunohistochemistry proceeded was performed according to [23]. The sections stained with the anti-CD138.

RESULTS

The eye of the black winged kite possesses two eye glands; the lacrimal and Harderian glands.

1. Macroscopical investigation

1.1 Macroscopical investigation of the Lacrimal gland

The lacrimal gland (Lg) is small and drop-like. It locates at ventrolateral surface of sclera cartilage. The Lg has a single narrow duct that draining its fluid near the posterior fissure in conjunctiva fornix of the lower eyelid (Fig. 1a).

1.2 Macroscopical investigation the Harderian gland

The Harderian gland has a flask shape and is located ventral to the ventral oblique muscle, and partially overlies the ventral rectus muscle. The gland extends dorsomedially where its anterior extremity emerges to its duct. This duct passes ventral to the origin of the ventral oblique muscle to be opened in the base of the nictitating membrane (Fig.1b).

2. Microscopical investigation

2. 1 Microscopical study of the lacrimal gland

The histological study of the Lg in black winged kite exhibits that it is a compound tubulo-alveoli gland. It is encased in thin collagenous connective tissue capsule (Fig. 2a). that capsule emerges which give of internally connective tissue septum dividing the gland into lobes and lobules in different sizes. The lobule composed of numerous secretory acini and intralobular ducts (Fig. 2a). The secretory acini of the Lg are lined with simple cuboidal cells which characterized by having round nuclei and a budding and exocytosis activities an irregular cell surface (Fig. 2b). The secretory cells are characterized by round nuclei and budding surfaces.

The histological study, illustrate the presence of myoepithelial cells distributed around the basal membrane of the acini and the secretory duct. These cells characterized by a spindle shaped and contains an elongated nucleus. Moreover, numerous plasma cells were scattered between the secretory acini (Fig. 2b).

The secretory acini of the Lg are weakly stained by PAS while, show a moderate reaction with Alcian blue pH 2.5. Whereas, the capsule and interlobular connective tissue show a purple and blue color with PAS reaction and Alcian blue pH 2.5, respectively (Fig. 2 c & d).

2. 2 Microscopical study of the Harderian gland

The Harderian gland of the black winged kite is enveloped in connective tissue capsule which formed of collagen fibers. That capsule gives of, internally, many septa which dividing the gland into numerous unequal lobules, each lobule consists of acini surrounded by interlobular connective tissue. (Fig. 3a).

The Harderian gland are compound tubulo-alveolar empties into a narrow and elongated central lumen it of simple columnar cells which characterized by budding cytoplasm. The lumen is irregular narrow and elongated. The secretory acini are composed of simple columnar epithelial cell with highly vacuolated cytoplasm (Fig. 3b).

Clusters of plasma cells surrounding the secretory vesicles. In addition, the secretory acini and the ductal epithelium are surrounded by myoepithelial cells. (Fig. 3b).

The acini of the Hg reacted positively with PAS in the center of the secretory acini but exhibits a moderate reaction with Alcian blue stain. on the other hand, the capsule and interlobular connective tissue show weakly purple and blue color with PAS reaction and Alcian blue pH 2.5, respectively (Fig. 3c &d).

3. Immunohistochemical investigation of the eye glands

Immunohistochemical study of the two eye glands of the black winged kite reveals that the population of CD138 positive plasma cells present in highest concentration in the Harderian gland especially around the secretory acini and increase in density around the main excretory duct, while, in the lacrimal gland the plasma cells represent the lowest population and found as diffuse singular cells between the secretory acini (Fig. 4b &c).

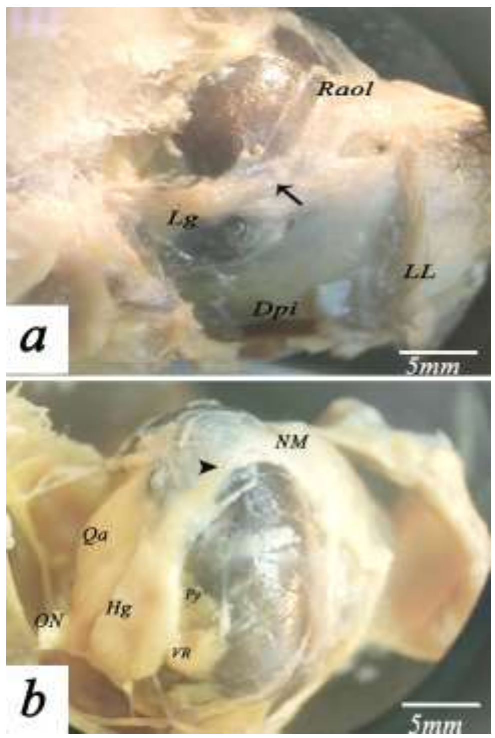

Figure 1. Photomacrograph of the eye of *Elanus caeruleus* showing**:** (a**)** The shape and position of lacrimal gland (Lg) on the posterior pole of the scleral ossicle opened with small duct (arrow) in posterior fornix (LL), Retractor anguli oculi lateralis muscle (Raol), depressor palpebral inferior (Dpi). (**b)** The Harderian gland (Hg) is flattened and has flask shaped, and has narrow duct (arrow head), open in the nictitating membrane (NM), optic nerve (ON), quadratus muscle (Qa), pyramidalis muscle (Py) ventral rectus muscles (Vr).

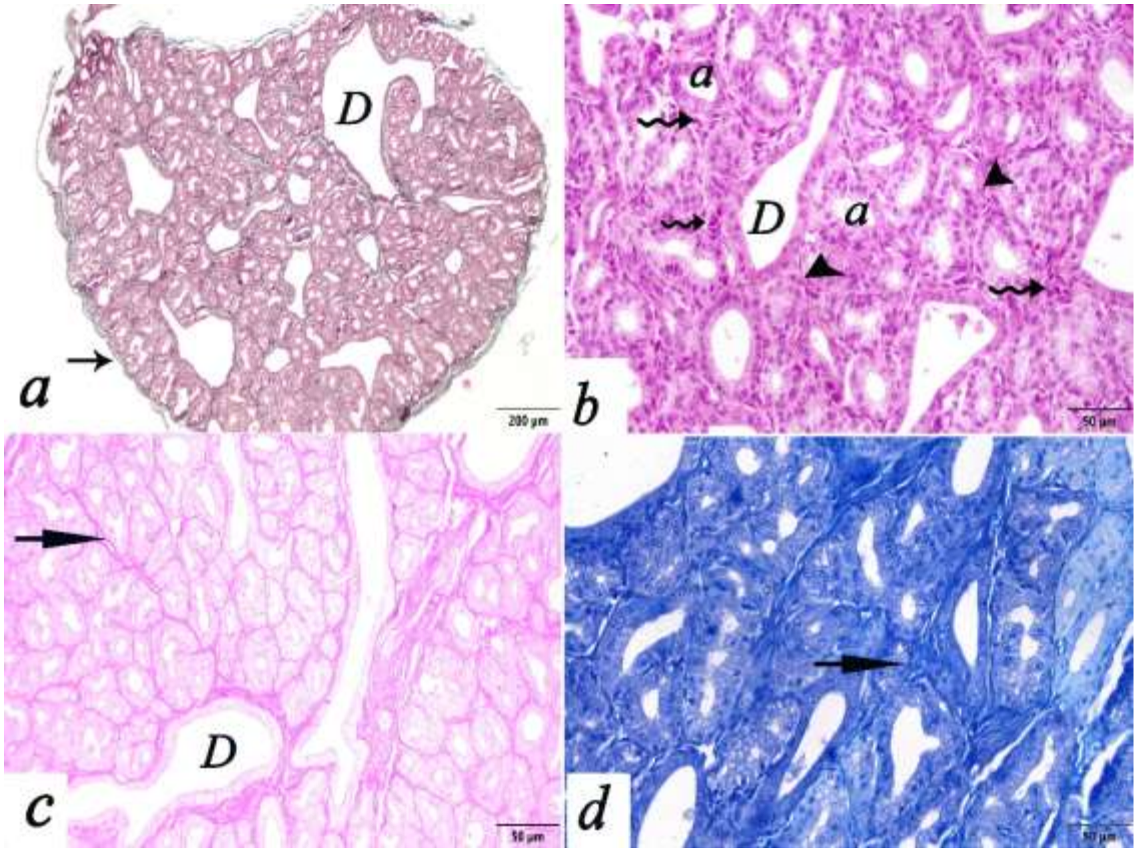

FIGURE 2: Photomicrographs of transverse sections of the lacrimal gland of *Elanus caeruleus* showing: **(a)** the gland, encased by a collagenous connective tissue capsule (arrow) (by Masson's trichromic scale bar 200 µm). **(b)** cuboidal secretory cells (a), myoepithelial cells (arrow head), plasma cells (zigzag arrow), duct (D) (by Hematoxylin& Eosin scale bar 50 µm). **(c)** the interlobular connective tissue (black arrow) reactive positively with PAS stain (by PAS, scale bar 50 µm). **(d)** interlobular connective tissue reactive positively with Alcian blue PH 2.5. (by Alcian blue, scale bar 50 µm).

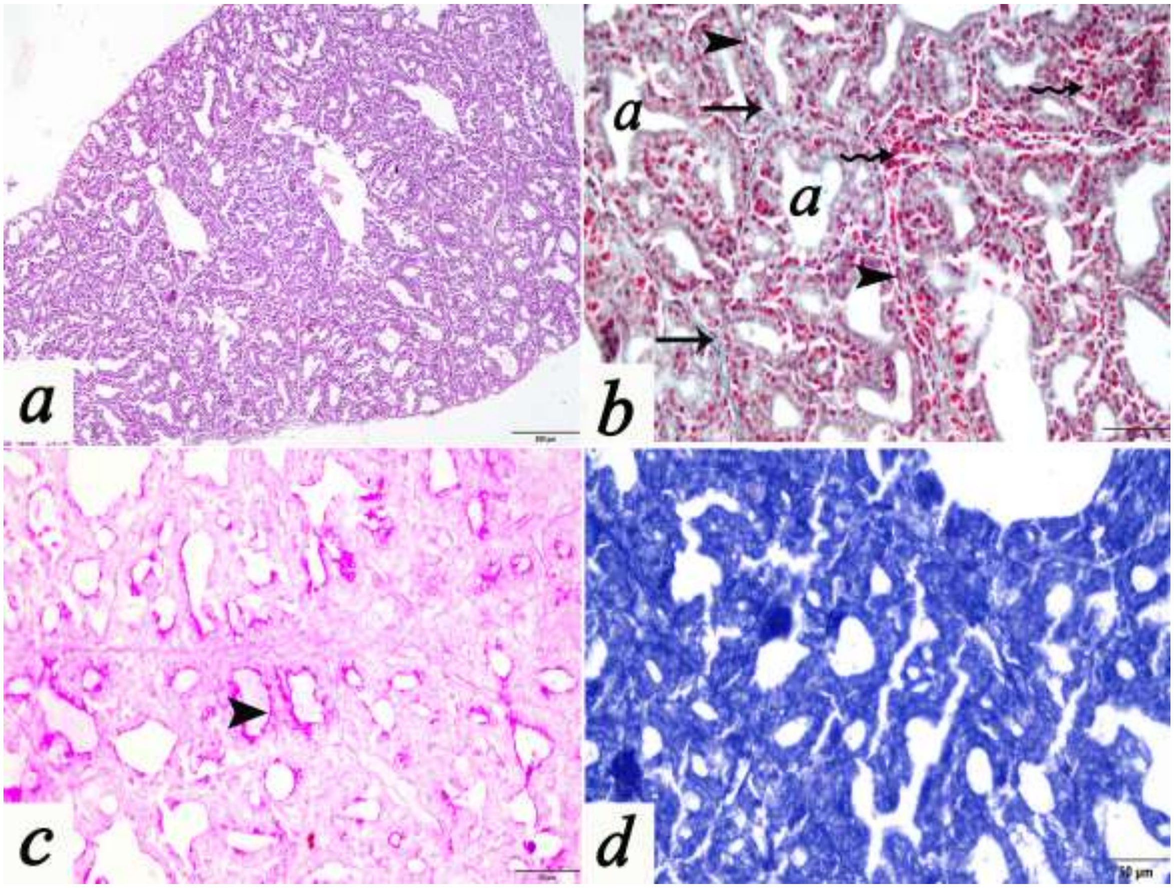

FIGURE 3: Photomicrographs of transverse sections of the Harderian gland of *Elanus caeruleus* showing (**a**) the connective tissue capsule encased the gland and its septa divided the gland to lobules. (by Hematoxylin & Eosin scale bar 200 µm). **(b)** secretory acini (a), connective tissue septa (arrow), myoepithelial cells (arrow head), plasma cell (zigzag arrow) (trichromic scale bar 50 µm). **(c)** the secretory cells positive with PAS stain (by PAS, scale bar 50 µm). **(d)** secretory cells of the gland give a moderate reaction with Alcian blue pH 2.5. (by Alcian blue, scale bar 50 µm)

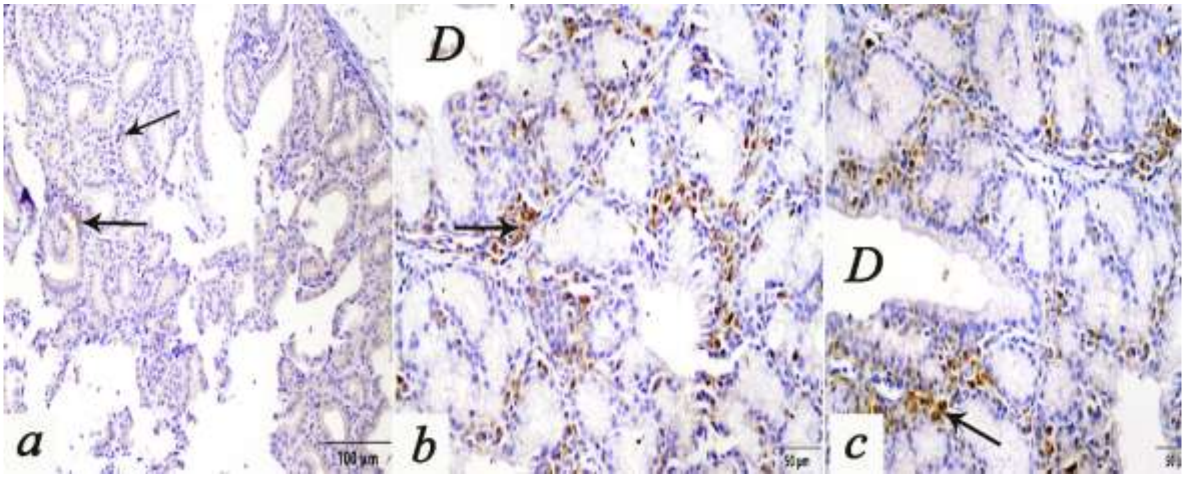

FIGURE 4: Immunohistochemistry of CD138-positive plasma cells showing: **(a)** single diffused plasma cell (black arrow) in Lacrimal gland. **(b & c)** high concentration of plasma cell (black arrow) in Harderian gland (scale bar, 50 µm).

DISCUSSION

The orbital glands play an important role in ocular safety. The Lg in mammalian eye, especially in humans, has got attention a thorough inquiry, clinical trials seeking to create a number of novel items for the functional restoration of the lacrimal gland as a possible treatment for dry eyes [24] [25]. However, as the current ornithology literature makes clear, the eye glands has not gotten enough attention.

The Harderian gland of the black winged kite lies in antero-ventral part of the orbit in the middle of the ventral oblique and pyramidalis muscles. This result different to that documented by [26] who reported that the ostrich Harderian gland was located in the posterior portion of the interorbital septum. The author of this work leans towards the idea that the location of the Harderian gland underlies the ventral oblique muscle helps the gland to release its secretion easily during the contraction and relaxation of the ventral oblique muscle. [27] discussed the site of the harderian gland in *Athen noctua*, which also located between the two muscles. They concluded that this location helps to exert pressure on the body of the gland, thereby helping to the release of its secretion.

Meanwhile, the lacrimal gland of the black winged kite appears small compared to the Harderian gland and positioned on the ventro lateral surface of the scleral cartilage. The same localization had been observed in cattle egret, *Bubulcus ibis* [28], but [27] found the lacrimal gland of the Athen *noctua* locating on the dorsal edge of the orbit. The place of the lacrimal gland is not fixed among different avian species, thereby, its draining duct change related to the location of the gland. In the black winged kite, the duct of lacrimal gland opens directly in the lower fornix of the eye. In contrast, the anatomical study of Harderian gland had been recorded same locations in almost bird species and its duct opened at the fixed medial base of the third eyelid. The position of the lacrimal and Harderian glands in the black winged kite help to increase the tear fluid on the ocular surface consequently during the opening and retrograde movement of nictitating membrane, the secretions spread across the cornea. Also, the secretion from the two glands reduces the friction occurs during the nictitating membrane movement across the surface of the cornea.

Histochemical analysis illustrates that the two eye glands of the black winged kite reacted positively to PAS, and Alcian blue; which indicates that the secretion of the two glands contains carboxylate acidic mucopolysaccharides. The secretion of both eye glands in black winged kite appropriate to their preventative action in the ocular disease. Moreover, the two glands' typical purpose of moisturizing the nictitating membrane and the surface of the eyeball [14]. This result disagrees that of [27] who pointed that the lacrimal gland of little owl gives weak reaction with PAS, but positive to Alcian blue.

Histochemical analysis exhibits the importance of neutral and acid mucopolysaccharide of the lacrimal gland to lubrication the cornea and immune protection of the eye. [29] discus the antimicrobial activity role of uropygial gland and documented that the releasing secretions of this gland remarkably repress the growing of different types of bacteria and fungi.

[30] discussed the head-associated lymphatic system in birds which involved the Harderian gland, build on the presence of different immune cells such interstitial plasma cells. Additionally, [17] cite the fact that distinct types of immunoglobulins are not produced by the lacrimal gland. These scientific viewpoints are supported by the current findings. Where, the histological and immunohistochemical investigation demonstrated the existence of high concentration of interstitial plasma cells between the acini of the Harderian gland than that present in the lacrimal gland, these results agree with opinion of [28]. So, the Harderian gland is a vital structure for the immune defense of the eye.

CONCLUSION

The lacrimal and Harderian glands' structures and secretions attest to their significance in the tear production process, which lubricates the nictitating membrane and the surface of the eye. Additionally, the elevated plasma cell concentration strengthened the Harderian gland's protective function over the ocular surface as well as its ability to avert ocular disorders.

REFERENCES

[1] A. Bayon, R.M. Almela, and J. Talavera, Avian ophthalmology, EJCAP ,17 (2007). Practice:1–13.

[2] D. J. Maggs, Third eyelid. In: D. J. Maggs, P. E. Miller, P. E. Ofri, 5th ed editors, Slatter's fundamentals of veterinary ophthalmology. St. Louis, MO: Saunders Elsevier, (2013) pp. 151–156.

[3] D. A. Dartt, Neural regulation of lacrimal gland secretory processes, Relevance in dry eye diseases Progress in Retinal and Eye Research, 28 (2009) :155–177. doi: 10.1016/j.preteyeres.2009.04.003.

[4] B. Jochems, T. E. Phillips, Histological and ultrastructural studies on the conjunctiva of the barred owl (*Strix varia*), PloS One 10: e0142783, (2015). doi: 10.1371/journal.pone.0142783.

[5] J. Klećkowska-Nawrot, R. Nowaczyk, K. Goździewska-Harłajczuk, K. Barszcz, A. Kowalczyk, E. Łukaszewicz, Light and electron microscopic study of the eyelids, conjunctiva-associated lymphoid tissue and lacrimal gland in Bilgorajska goose (*Anser anser*), ASI, 91(2016). 74–88. doi:10.1007/s12565-015-0274-1.

[6] R. A. Paynter, *Handbook of avian anatomy: Nomina Anatomica Avium*, 2nd edn., Jnr. (ed.), Nuttall Ornithological Club, Cambridge, Massachusetts, (1993).

[7] D. Jordan, Accessory lacrimal glands, *Ophthalmic Surgery*, 2 (1990) 146–147.

[8] T. Sakai, Major ocular glands (Harderian gland and lacrimal gland) of the musk shrew *Suncus murinus* with a review on the comparative anatomy and histology of the mammalian lacrimal glands', *Journal of Morphology*, 201(1989). 39–57. http://dx.doi.org/10.1002/jmor.1052010105.

[9] K. Shirama & M. Hokano, Harderian glands and their development in laboratory rats and mice, in M. Webb, R.A. Hoffman, M. L. Puig-Domingo & R.J. Reiter (eds.), *Harderian Gland*, Springer-Verlag, Berlin, (1992) pp. 25–51, Heidelberg: http://dx.doi.org/10.1007/978-3-642-76685-5_3.

[10] D. S. Dimitrov and A.G. Genchev, Comparative morphometric investigations of intraorbital glands in Japanese quails, *Coturnix coturnix japonica*, BJVM, *14* (2011). *124–127.*

[11] K. Ohshima and K. Hiramatsu, Immunohistochemical localization of three different immunoglobulin classes in the Harderian gland of young chickens, Tissue and Cell ,34 (2002) 129–133. http://dx.doi.org/10.1016/S0040-8166(02)00030-7.

[12] T. Kozlu and H. Altunay, Light and electron microscopic studies of the quail (*Coturnix coturnix*) Harderian gland, JAVA, 10 (2011). 932–938. http://dx.doi.org/10.3923/javaa.2011.932.938.

[13] A. P. Payne, The Harderian gland: A tercentennial review, Journal of Anatomy, 185 (1994). 1–49.

[14] B. Mobini, Histological and histochemical studies on the Harderian gland in native chickens. Veterinary Medicine, 57 (2012). 404–409. http://dx.doi.org/ 10.17221/6308.

[15] T. Baba, T. Kawata, K. Matsumoto & T. Kajiwara, Role of the Harderian gland in immunoglobulin A production in chicken lacrimal fluid, Research in Veterinary Science, 49 (1990). 20–2

[16] S. Frahmand & A. A. Mohammadpour, Role of the Harderian gland in immunoglobulin A production in chicken lacrimal fluid, *R V S* 49, Harderian gland in Canadian ostrich (*Struthio camelus*): A morphological and histochemical study. Anatomia, Histologia, Embryologia, 44(3) (2015). 178–185. https://doi.org/10.1111/ahe.12123.

[17] J. Klećkowska-Nawrot, A. Chęć, K. Goździewska-Harłajczuk, R. Nowaczyk, Light and electron microscopic studies of the Harderian gland in Bilgorajska goose (*Anser anser*), Acta Biol Hung 66(3) (2015). 249-57. doi: 10.1556/018.66.2015.3.1.

[18] M. K. Nasrin, M. Z. I. Khan, M.N.H. Siddiqi & M. A. Masum, Mobilization of immunoglobulin (Ig)-containing plasma cells in Harderian gland, cecal tonsil and trachea of broilers vaccinated with Newcastle disease vaccine, *Tissue and Cell* 45, (2013) 191–197. http://dx.doi.org/10.1016/j.tice.2012.12.001.

[19] P. Bejdic, R. Avdic, L. Amidzic, V. Cutahija, F. Tandir, N. Hadziomerovic. Developmental changes of lymphoid tissue in the Harderian gland of laying hens, Macedonian Veterinary Review, 47 (2014). 83-88.

[20] S. Ali, S. D. Ripley, Handbook of the birds of India and Pakistan, Vol. 1 (2nd ed.), Oxford University Press, (1978). pp. 212–214. ISBN 978-0-19-562063-4

[21] A. R. B Drury and E. A. Wallington, Carleton's histological technique. 5th ed., Oxford University Press, London, (1980).

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

[23] N. K Ramadan, G. Badr, H. S. Abdel-Tawab, S. F Ahmed, M. H. Mahmoud, Camel whey protein enhances lymphocyte survival by modulating the expression of survivin, bim/bax, and cytochrome C and restores heat stress-mediated pathological alteration in lymphoid organs. IJBMS, 21(9) (2018). 896. https:// doi. org/ 10. 22038/ 27584. 6729.

[24] M. Hirayama, Advances in functional restoration of the lacrimal glands. *IOVS*

, 59(14) (2018). DES174–DES182. https://doi.org/10.1167/iovs.17-23528

[25] I. A. Butovich, S. Yuksel, B. Leonard, T. Gadek, A. S. Polans, & D. M. Albert, 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 (2021). 545–555. https://doi.org/10.1089/jop.2021.0015.

[26] R. T. Reem and M. A. Khattab, Anatomical and Histochemical studies on the orbital glands (Lacrimal and Harderian glands) in Ostrich (*Strutheio camelus*). *IJARBS*, 5(1) (2018). 125-135. doi.org/10.22192/ ijarbs.2018.05.01.019.

[27] N. A. Shawki, F. A. Mahmoud, & A. M. Abdel-Megeed, Anatomical, histological and histochemical studies of orbital glands of little owl Athene noctua. *AUNJMSR* 48(2) (2019). 171–200.

[28] F. A. Al-Nefeiy, N. A Shawki, & F. A Mahmoud, Morphological and functional relationship between the orbital gland and olfaction in *Upupa epops*

(hoopoe) and *Bubulcus ibis* (cattle egret). Anatomia, Histologia, Embryologia, 51 (2022). 793–801. https://doi.org/10.1111/ahe.12855.

[29] M. S Braun, F. Sporer, S. Zimmermann, & M. Wink, 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) (2018). fiy117. https://doi.org/10.1093/femse c/fiy117.

[30] R. R. Beheiry, S. A. Ali, M. Aref, & H. Emam, Harderian gland of flying and non-flying birds: Morphological, histological, and histochemical studies. *JOBAZ*, 81 (2020). 35