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Anatomical and Histochemical studies on the orbital glands (Lacrimal and Harderian glands) in Ostrich (*Strutheio camelus*)

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Abstract

Because of the clinical importance of the ocular diseases and its effect on the poultry productivity therefore we studied the macro anatomical and histochemical structure of both the harderian and lacrimal glands in ostriches in Egypt. Six male ostriches reared and slaughtered in local farms in Belbes region, El Sharkia Governorate Egypt at age from 10-12 months old during winter season. Both orbital glands of ostrich were compound tubuloalveolar type of apocrine mode of secretion. The H.G and L.G. secretions were drained by a duct system organized in the form of secondary ducts, primary ducts and main excretory duct respectively.The harderian gland is a large pyriform in shape while the lacrimal gland is a small, flat; quadrilateral in shape. H.G is positioned in the orbit, while L.G is positioned in the periorbital space and caudal to lateral canthus. The H.G in ostriches produces a mucoid secretion for moistening the cornea in addition to being a lymph epithelial organ asthe sub epithelial connective tissue in H.G. is highly cellular rich with densely packed lymphocytes and plasma cells. H.G. the main excretory duct epithelium is folded and demonstrated a stratified cuboidal type while the main excretory duct of L.G is not folded and lined with stratified squamous epithelium (5-7 cells thick).

Keywords: lacrimal gland, harderian gland, ostrich, anatomy and histochemistry.

Introduction

NAV (2012) recorded that the orbital glands were the lacrimal and harderian glands. In addition to, **Chieffi *et al. (*1993) and Payne (1994)** mentioned that the main functions of the orbital glands were the lubrication of the surface of the eyeball and nictitating membrane. Moreover, **Ohshima and Hiramatsu (2002)** documented that those glands have an immune response.

Sorgolu et al. (2003) and Stern et al. (2004) cited that the clinical importance of the orbital glands was that the ocular diseases have a significant impact on the condition and productivity of breeding animals.

The majority of dry eye symptoms are the result of chronic inflammation of the lacrimal gland, which decreases the ability of the eye to respond to environmental factors.

Burns (1992) and Dimitrov (2011a) in fowl and in turkey respectively, cited that the harderian glands were relatively large and had been much developed than the lacrimal gland. **The latter author** added that the harderian gland was the lymph epithelial organ.

O'Malley (2005) documented that the harderian gland was the largest gland, which laid craniomedial within the orbit behind the nictating membrane and produced a mucoid secretion that moistens the cornea. It emptied through dorsal and ventral puncta into the nasolacrimal duct.While, **Mobini (2012)** in nativechicken mentioned that the harderian gland is situated medioventral to the eyeball, near the inter-orbital septum**.**

Mobini (2014) in the domestic fowlreported that the paraorbital gland (Harderian) is an exocrine gland. It lies in the orbit ventral and posteromedial to the eyeball. 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.

Klećkowska et al. (2016) recorded that the harderian gland was located in the orbit near the inter-orbital septum, between the medial rectus muscle, the pyramidal muscle of the third eyelid, and the ventral oblique muscle. In the common pheasant, the gland was wider in the proximal and distal part. The common pheasant had more elongated lobes of the harderian gland than in the hybrid.

Reshag et al. (2016) cited that Harderian gland in pigeons was teardrop like in shape, light brown to pink in color, capsulated with thin connective tissue.Moreover, **Dyce et al. (2002)** mentioned that the harderian gland was loosely attached to the periorbital fascia.

O'Malley (2005) documented that the lacrimal gland was found at the caudolateral margins, it emptied through dorsal and ventral puncta into the nasolacrimal duct.

Williams (1994) revealed that the lacrimal gland was present inferior and lateral to the globe. The inferior and superior nasolacrimal puncta at the medial canthus drain the lacrimal secretions into the nasal cavities. The harderian gland acts as a second lacrimal gland at the base of the nictating membrane.While, **Harris *et al*. (2008**) stated thatthe lacrimal gland was situated in the periorbital space, in the dorsotemporal part of the orbit. Several excretory ducts evacuated their secretions into the conjunctival space beneath the lower eyelid.

Mohammadpour (2009) documented that the lacrimal gland lubricated the cornea which was very helpful for the eye of the bird in arid climates. Also, **Flanagan & Willcox (2009) and Kawashima et al. (2012)** recorded that the lacrimal gland secretory function, plays an important role in eye physiology and pathology.

Mohammadpour (2014) and **Joanna et al. (2015)** revealed that in ostrich, the lacrimal gland was located obliquely in dorsolateral part of orbit. **The former author** added that the lacrimal gland was dark brown to black color, while **the latter authors** added that the lacrimal gland of was bright red in color, moreover, it was a uniform, undivided, flattened, and oval in shape. It secreted through the multiple ducts that open into the conjunctival space beneath the lower eyelid. The nasolacrimal duct penetrated the lacrimal bone and rostrum maxillae premaxillae bone and opened into the nasal cavity.

Burns (1974, 1975), Aitken and Survashe (1977) reported that the avian harderian gland was classified into three different types according to acinar type and lobular epithelium structure. Type I is compound tubuloalveolar with one basic epithelial cells lining. Type II glands are compound tubular with two epithelial cells types. Finally type III glands are mixed gland from compound tubuloalveolar and compound tubular types.

Wight et al. (1971a) and Frahmand and Mohammadpour (2015) in domestic fowl and in Canadian ostrich *(Strutheio camelus)*respectively, stated that the harderian gland was classified as type I compound tubuloalveolar gland.

Rothwell et al. (1972) and Frahmand and Mohammadpour (2015) revealed that avian harderian glands were considered as lymphoid organ with rich populations of lymphocytes and plasma cells in connective tissue stroma which increase by age.

***Rothwell* et al., (1972)** in domestic fowl stated that that harderian gland of domestic fowl have merocrine mode of secretion. **Frahmand and Mohammadpour (2015)**in ostrich documented that lacrimal gland as well as harderian gland have apocrine mode of secretion.

Materials and Methods

Six male ostriches reared and slaughtered in local farms in Belbes region- El Sharkia Governorate Egypt at age from 10-12 months old during winter season. For anatomical study, the specimens were fixed in 10% formalin for 3-4 days and then dissected. All the specimens were photographed by Sony camera h400, 20.1 megapixels, 63X optical zoom cyber shot. *Nomina Anatomica Avium* (***Baumel et al. 1993***) was used for nomenclature.

For histochemical studies, Tissue samples were fixed in 10% neutral buffered formalin for 72 hrs. Samples were trimmed and processed by serial grades of alcohols, cleared in Xylene, Paraplast synthetic wax infiltration and blocking out into Paraplast tissue embedding media. 3-5µ sections were cut by rotatory microtome. For microscopic examination; tissue sections were stained by: Harris Hematoxylinand Eosin as a general staining method, Masson's trichrome stain for demonstration of collagen fibers, Alcian blue pH 2.5 for demonstration of acidic non sulphated mucins, Alcian blue pH 1 for demonstration of acidic sulphated mucins, and PAS technique for demonstration of neutral mucins.All staining procedures as outlined by **Bancroft and Stevens (2010).**

Results

In ostriches, the eyes are large in size and are covered by the upper and lower eyelids, in addition to the nictating membrane. The lower eyelid performs more eye area coverage about two thirds of the eye as the lower eyelid is larger than the upper one. The upper eyelid carries more several rows of long eyelashes, while the lower one carries two rows of long eyelashes **(plate 1A)**. For more protection of the large, bulged globe, a third eyelid (nictating membrane) is found and moved over the cornea.

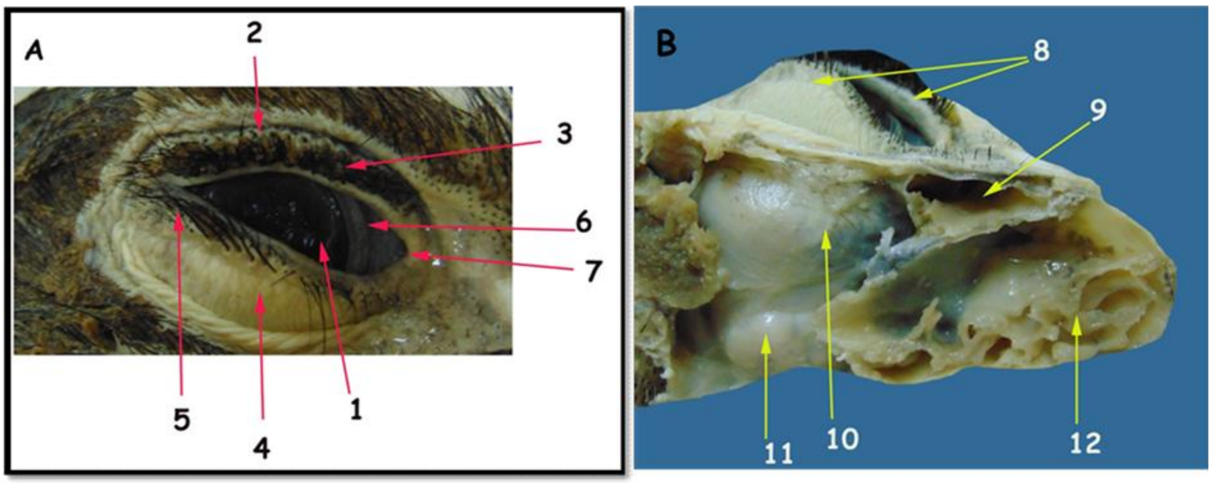

Plate 1: A; A photograph showing the right eye of ostrich
B; A photograph showing a longitudinal section in the ostrich head right half, which sectioned few centimeters ventral to the eye.

1. The cornea
2. Upper eyelid
3. Eyelashes
4. Lower eyelid
5. Double row of eyelashes
6. Third eyelid
7. Medial canthus of the eye
8. The eye lids
9. Infraorbital sinus
10. the eyeball with its periorbital fascia
11. Connective tissue capsule enclosing the harderian gland
12. Part of the nasal conchae

The orbital glands in ostriches are obtained as the harderian and lacrimal glands, where the harderian gland is much larger in size than the lacrimal gland. The lacrimal secretions descend through the dorsal and ventral puncta into the nasolacrimal duct, further way they passes to the nasal cavity.

The harderian gland is a large pyriform in shape, with a rounded body and pointed rostral end **(Plate 3 E and F),** leading to the harderian gland duct**(Plate 2 D)**. This duct passes medial to the eyeball till it opened at the medial canthus of the eye **(Plate 3 E)**.

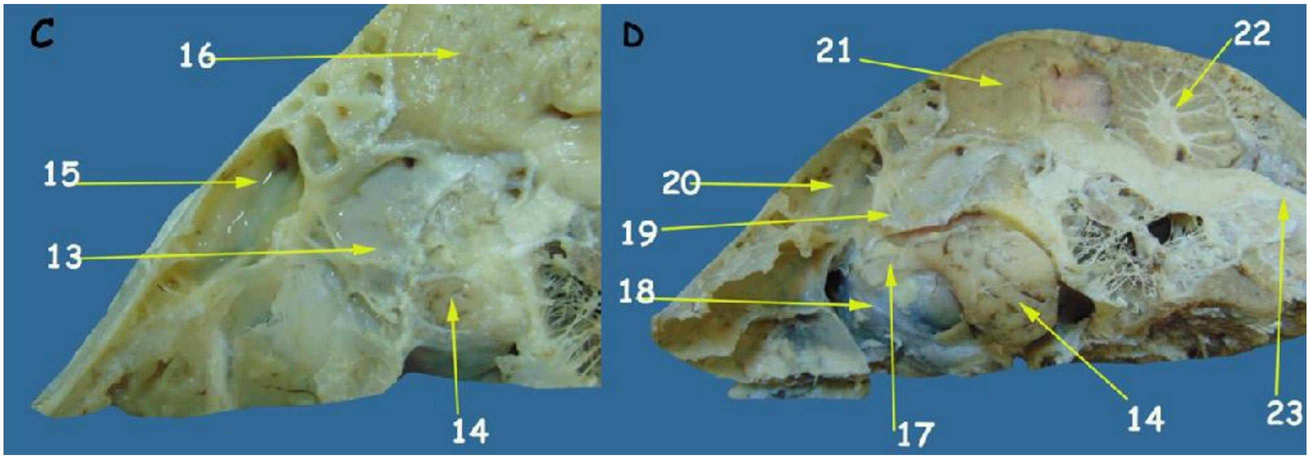

Plate 2; C;A photograph showing paramedian section in the upper half of the ostrich head
D; A photograph showing the partial removal of the Interorbital septum, in the paramedian section of the head ostrich half

13. Interorbital septum
14. Harderian gland
15. Frontal sinus
16. Cerebral hemisphere
17. Harderian gland duct
18. Eyeball
19. Remained part of the Interorbital septum
20. Frontal sinus
21. Cerebrum
22. Cerebellum
23. Spinal cord

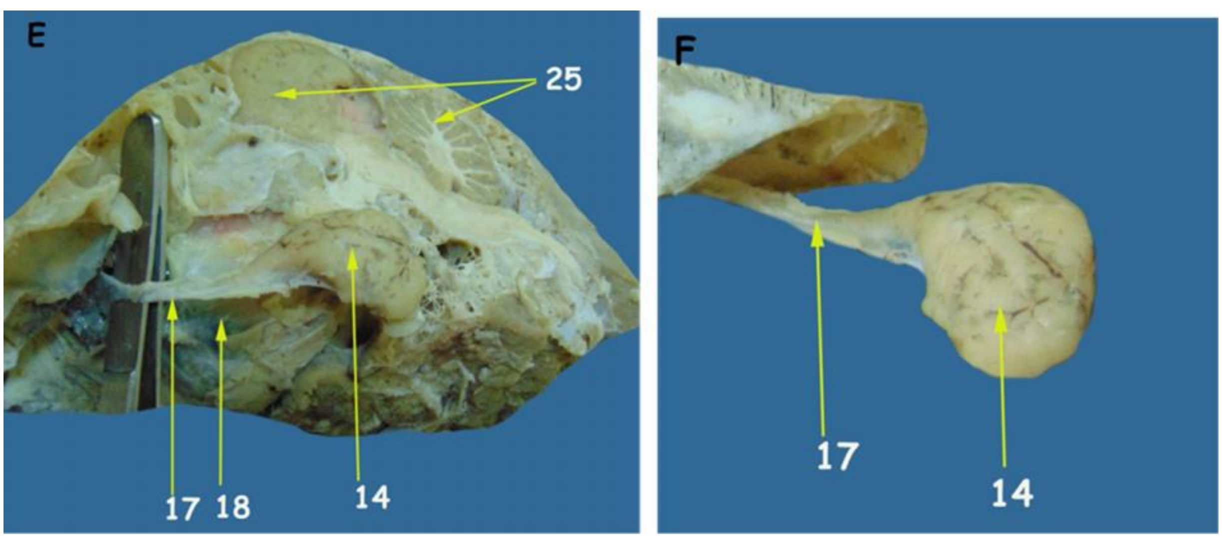

Plate 3; E;A photograph showing the harderian gland duct in the medioventral view of the paramedian section of the upper half of the ostrich head
F; A photograph viewing the isolated harderian gland of ostrich with its duct alone

14. Harderian gland
17. Harderian gland duct
18. Eyeball
25. Brain

The harderian gland is light brown in color, and is positioned in the orbit, lateral to the caudal portion of the interorbital septum and medial to the caudal third of the eyeball **(Plate 2C).** Moreover, the harderian gland is surrounded by a connective tissue capsule and also tightly attaches to the eyeball **(Plate 1B).**

The lacrimal gland is a flat, narrow in thickness and irregular quadrilateral in shape **(Plate 4).** Its color is light brown with black patches. This gland is positioned in the periorbital space, in the dorsotemporal part of the orbit and also caudal to the lateral canthus of the eye **(Plate 4 G and H)**.

The lacrimal gland evacuates its secretions through several excretory ducts **(Plate 4 G and H)** into the lateral canthus at the conjunctival space of the lower eyelid.

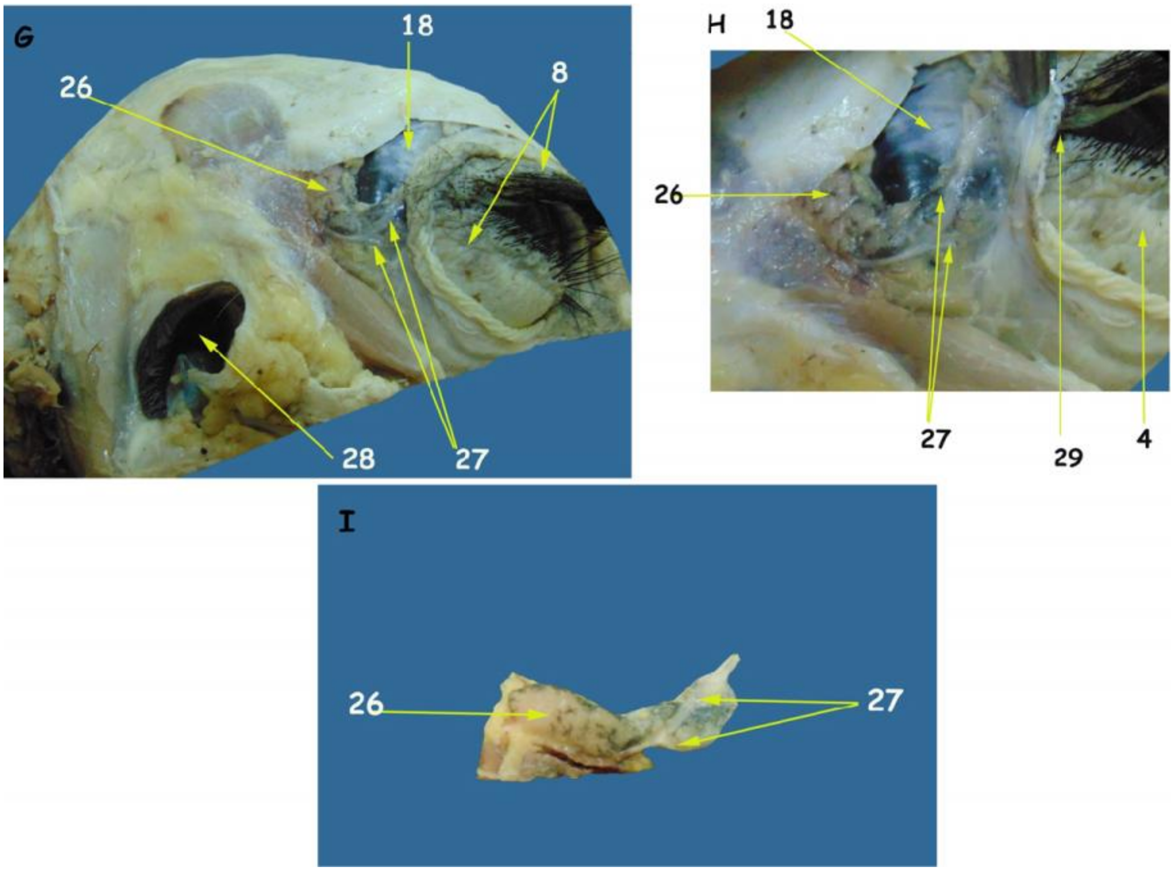

Plate 4; G; A photograph showing the dorsolateral view of the dissected ostich head
H; A photograph showing the magnified view of the lacrimal gland in situ
I; A photograph viewing the isolated lacrimal gland of ostrich

4. Lower eyelid
8. Eyelids
18. Eyeball
26. Lacrimal gland
27. Lacrimal gland ducts
28. Ear opening
29. Lateral canthus of the eye

Microscopic examination of tissue sections from different levels of H.G and L.G revealed that; both orbital glands of ostrich were compound tubuloalveolar type of apocrine mode of secretion. H.G. as well as L.G was surrounded externally with a thick fibrous connective tissue capsule sending several trabeculae which divide the gland into irregularly organized lobes and lobules. The connective tissue septa were rich in collagen fibers as well as fibroblasts and housing several blood vessels. Each lobule was composed of several secretory unites **(Plate 5 A, B, C and D)**. It was evident that the secretory adenomeres of both glands were lined by tall columnar cells with oval or small round nuclei basally situated **(Figs 5A and 1B).** However; the secretory cells in H.G. were either vacuolated with lightly stained cytoplasm or non-vacuolated with more eosinophilic cytoplasm especially in Masson's Trichrome stained sections. In contrast to L.G. the sub epithelial connective tissue in H.G. was highly cellular rich with densely packed lymphocytes and plasma cells **(Figs 5A and 5B)**.

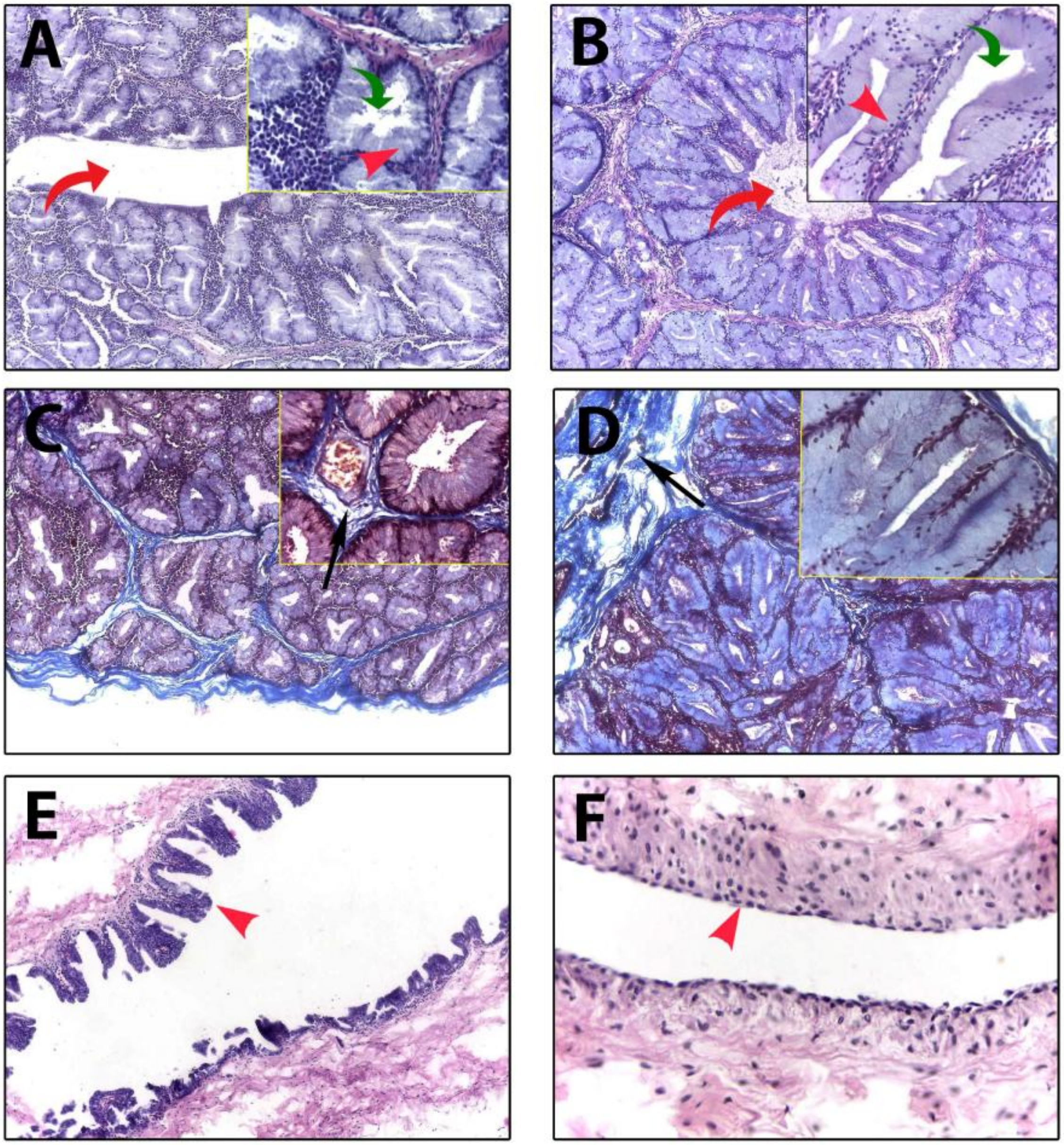

Plate 5; A&B; H.G and L.G respectively (H&E 100X - inset box at 400X). C&D; H.G and L.G respectively (Masson's Trichrome stain 100X - inset box at 400X). E&F; Main excretory duct of H.G and L.G respectively (H&E 400X).

Black arrows: connective tissue capsule and septa.
Red arrow heads: lining epithelium.
Green curved arrow: primary excretory duct.
Red curved arrow: secondary excretory duct.

The H.G and L.G. secretions were drained by a duct system organized in the form of secondary ducts, primary ducts and main excretory duct respectively. The lining epithelium of secondary and primary ducts was alternating simple cuboidal or columnar cells. However; in H.G. the main excretory duct epithelium was folded and demonstrated a stratified cuboidal type and occasionally columnar cells were evident **(Plate5, E).** The main excretory duct of L.G wasn't folded and lined with stratified squamous epithelium

(5-7 cells thick) **(Plate5, F)**. The sub epithelial connective tissue of the main excretory duct was rich in fibroblasts, collagen fibers and blood vessels in both glands.

It was evident that the H.G secretory epithelium and mucins have weak positive reactivity to Alcian blue pH 1. However; the reactivity was stronger to Alcian blue pH 2.5. Unlike L.G demonstrated a moderate strong reactivity to both Alcian blue pH 1 and pH 2.5 stains.

Regarding PAS reactivity, H.G secretory unites demonstrated a moderate reactivity to PAS technique unlike L.G revealed a very strong positive reactivity to PAS technique **(plate 6).**

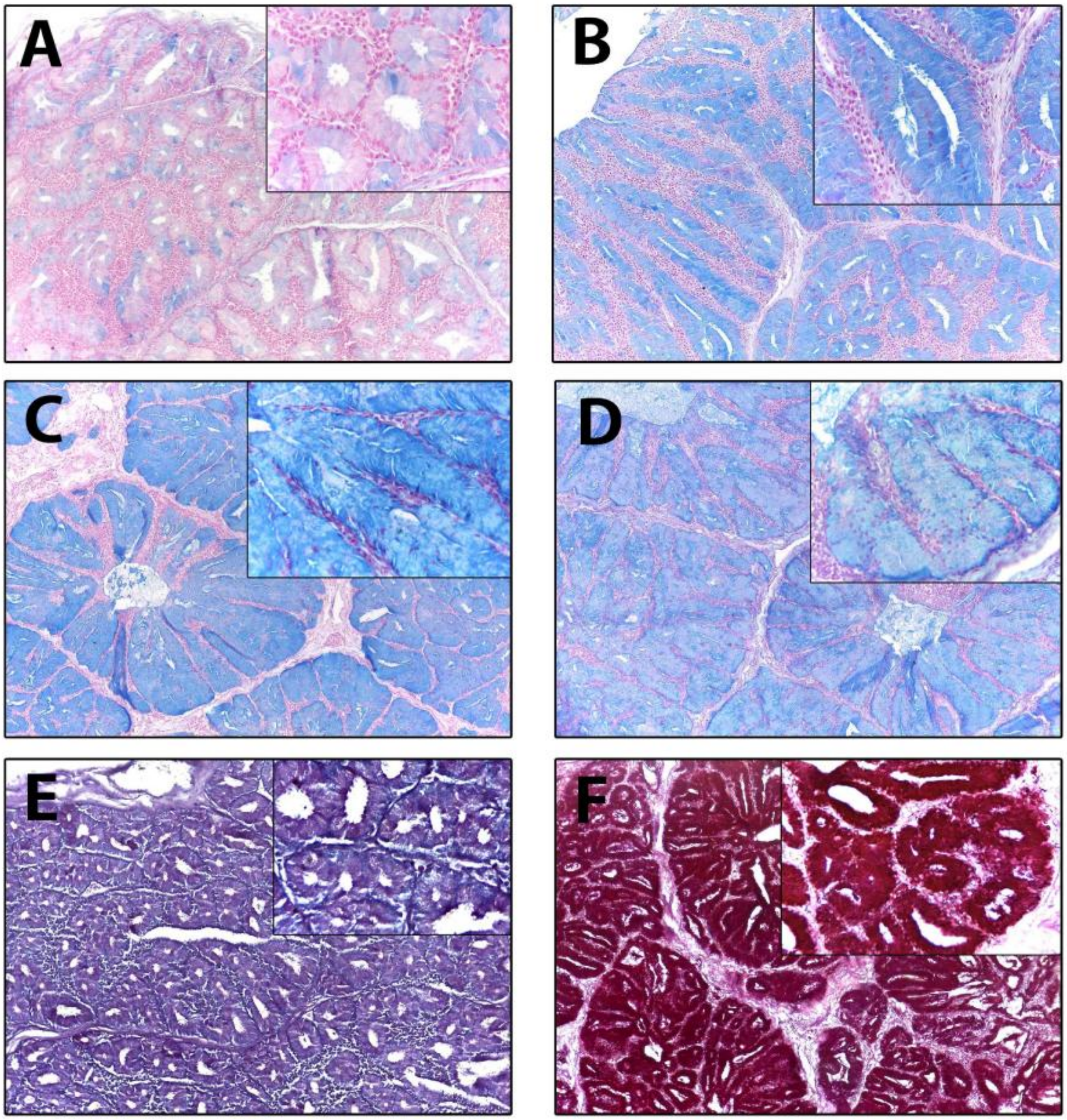

Plate 6;
A&B: showing H.G reactivity to Alcian blue pH 1 & pH 2.5 respectively. (100X & inset box 400X)
C&D: showing L.G reactivity to Alcian blue pH 1 & pH 2.5 respectively. (100X & inset box 400X)
E&F: demonstrating PAS reactivity in H.G and L.G respectively. (100X & inset box 400X)

In contrast to the glandular epithelium reactivity; the main excretory duct epithelium of H.G. revealed a strong positive reactivity to both types of Alcian blue pH 1 and 2.5. However; negative reactivity for both stains were demonstrated in L.G. lining epithelium of the main excretory duct. It was evident that PAS reactivity was stronger in H.G main excretory duct epithelium than that of the L.G **(Plate 7 A, B,C and D)**.

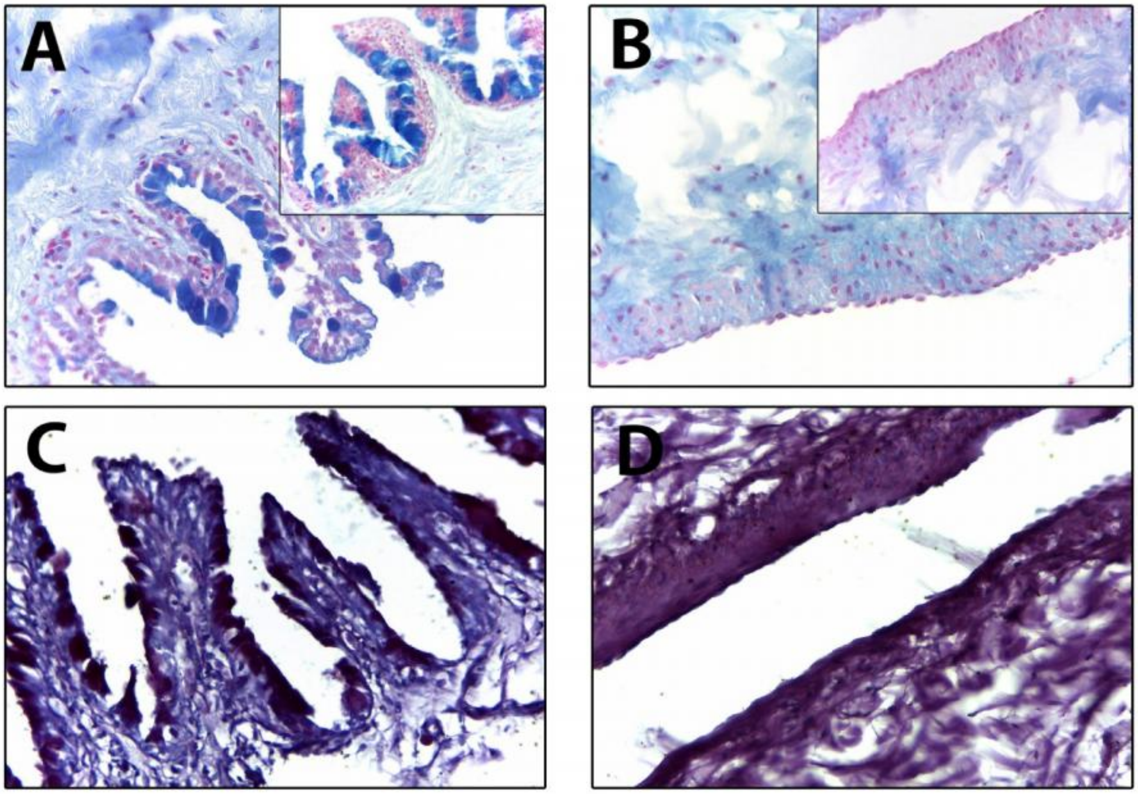

Plate 7;
A) Showing main excretory duct epithelium of H.G reactivity to Alcian blue pH 2.5 - inset box demonstrating reactivity to Alcian blue pH 1. (400X)
B) Showing main excretory duct epithelium of L.G reactivity to Alcian blue pH 2.5 - inset box demonstrating reactivity to Alcian blue pH 1. (400X)
C) Showing main excretory duct epithelium of H.G reactivity to PAS. (400X)
D) Showing main excretory duct epithelium of L.G reactivity to PAS. (400X)

Discussion

The orbital glands in ostriches were obtained as the harderian and lacrimal glands. Similar observations were given by **NAV (2012)**

The harderian gland was much larger in size than the lacrimal gland, in the present investigation was similar to the description of **Burns (1992), O'Malley (2005) and Dimitrov (2011a)**

The present work revealed that the lacrimal secretions were descended through the dorsal and ventral puncta into the nasolacrimal duct, further way they passed to the nasal cavity. This result was in accordance with the observation of **O'Malley (2005).**

In the present study, the harderian gland was a large pyriform in shape, with a rounded body and pointed rostral end, leading to the harderian gland duct. On the other hand, **Reshag et al. (2016)** cited that Harderian gland in pigeons was teardrop like in shape, light brown to pink in color. Meanwhile, **Klećkowska et al. (2016)** recorded that the harderian gland, in the common pheasant, was wider in the proximal and distal part. The common pheasant had more elongated lobes of the harderian gland than in the hybrid.

Reshag et al. (2016) cited that Harderian gland in pigeons was light brown to pink in color. A condition which was simulated the present study.

Concerning the harderian gland position, the present study revealed that it was laid in the orbit, lateral to the caudal portion of the interorbital septum and medial to the caudal third of the eyeball. Similar observations were simulated by **Klećkowska et al. (2016).** While, **Mobini (2012) and Mobini (2014)** inchicken mentioned that the harderian gland is situated ventral to the eyeball, near the inter-orbital septum**.**

The harderian gland was surrounded by a connective tissue capsule as simulated by **Reshag et al. (2016)** and also tightly attached to the eyeball which disagreed with **Dyce et al. (2002)** whose mentioned that the harderian gland was loosely attached to the periorbital fascia.

In accordance with **Joanna et al. (2015)** in ostrich the lacrimal gland was flat, narrow in thickness; also the present study added that its shape was irregular quadrilateral.

The present study was in line with **Mohammadpour (2014)** and **Joanna et al. (2015)** that the lacrimal gland was dark brown to black color. In addition that it had black patches with its light brown color as mentioned in our work. This result was disagreed with **Joanna et al. (2015)**, described that the lacrimal gland of ostrich of was bright red in color.

Similar findings were observed by **Harris *et al*. (2008**) that the lacrimal gland was positioned in the periorbital space, in the dorsotemporal part of the orbit. In addition to, the lacrimal gland in ostrich was positioned caudal to the lateral canthus of the eye which added in our study and **O'Malley (2005)** whodocumented that the lacrimal gland was found at the caudolateral margins. On the other hand, **Williams (1994)** revealed that the lacrimal gland was present inferior and lateral to the globe. Meanwhile, **Mohammadpour (2014)** and **Joanna et al. (2015)** revealed that in ostrich, the lacrimal gland was located obliquely in dorsolateral part of orbit.

The present study as well as **Harris *et al*. (2008**) and **Joanna et al. (2015)** documented that the lacrimal gland evacuated its secretions through several excretory ducts into the lateral canthus at the conjunctival space of the lower eyelid. While, **Williams (1994) and O'Malley (2005)** reported that the lacrimal gland emptied through dorsal and ventral puncta into the nasolacrimal duct.

According to **Burns (1974, 1975) and Aitken and Survashe (1977)** the avian harderian glands was classified into three different types according to acinar type and lobular epithelium structure. Type I is compound tubuloalveolar with one basic epithelial cells lining. Type II glands are compound tubular with two epithelial cells types. Finally type III glands are mixed gland from compound tubuloalveolar and compound tubular types. The current study revealed that H.G of ostrich could be classified as type I compound tubuloalveolar gland lined with tall columnar epithelial cells with basally oval situated nuclei in agreement with **Wight et al. (1971a)** in domestic fowl and **Frahmand and Mohammadpour (2015)** in Canadian ostrich ***(Strutheio camelus).***

In the present study unlike lacrimal glands, Densely packed populations of lymphocytes and plasma cells were recorded in harderian gland as recorded by **Rothwell *et al., 1972* and Frahmand and Mohammadpour, 2015).**The same author stated that avian harderian glands were considered as lymphoid organ with rich populations of lymphocytes and plasma cells in connective tissue stroma which increase by age.

It was recorded that harderian gland of domestic fowl have merocrine mode of secretion ***(Rothwell et al., 1972).*** However; present study revealed that in ostrich lacrimal gland as well as harderian gland have apocrine mode of secretion in accordance with **Frahmand and Mohammadpour (2015).**

The current study records revealed that mucins expression and types are variable between both glands regions. Carboxylated acidic mucopolysaccharides were predominated in H.G secretory unites as demonstrated by Alcian blue 2.5 reactivity in agreement with **(Klećkowska- Nawrot, Chęć, Goździewska-Harłajczuk, Nowaczyk and Barszcz, 2015).** These finding may suggests an immune protective role of carboxylated acidic mucopolysaccharides in avian ocular disorders. In contrast; sulphated acidic mucins as well as neutral mucins were expressed in lesser amounts by Alcian blue 1 and PAS respectively in the present study. L.G secretory unites demonstrated a stronger expression of acidic mucins generally and acidic sulphated mucins as well as neutral mucins specifically than H.G. These records make a focus on the biological importance of acidic sulphated mucins and neutral one in lacrimal apparatus, corneal lubrication and protection dynamics in ostrich. Previous records regarding excretory duct mucins dynamics for both glands in ostrich are rarely found. Current findings demonstrated that H.G excretory duct epithelium produce different mucins types including acidic as well as neutral mucins. Unlike L.G excretory duct which have negative expression for most of surveyed mucins type.

In Conclusion: The harderian gland is a large pyriform in shape while the lacrimal gland is a flat; quadrilateral in shape. H.G is positioned in the orbit, while L.G is positioned in the periorbital space and caudal to lateral canthus. The H.G in ostriches produces a mucoid secretion for moistening the cornea in addition to being a lymph epithelial organ asthe sub epithelial connective tissue in H.G. is highly cellular rich with densely packed lymphocytes and plasma cells. H.G. the main excretory duct epithelium is folded and demonstrated a stratified cuboidal type while the main excretory duct of L.G is not folded and lined with stratified squamous epithelium (5-7 cells thick).

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