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MORPHOLOGICAL AND HISTOCHEMICAL STUDY OF HARDERIAN GLAND OF DOMESTIC PIGEON (*COLUMBA LIVIA DOMESTICA*)

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ABSTRACT

The aim of this work was to study the histological and histochemical structure of the Harderian gland in indigenous pigeons. Samples were obtained from 10 males and 10 females of adult healthy pigeons. Hematoxylin and eosin, Alcian blue (pH 2.5), periodic acid-Schiff and promo phenol blue, stains were used for paraffin section examination. The gland was teardrop like in shape, light brown to pink in color, capsulated with thin connective tissue. It was multilobular compound acinotubular in structure and lined by columnar epithelial cells. Lymphocyte, plasma cells and plasma cells with Russell bodies were present underneath the epithelia of central collecting duct and around the secretory unite. Histochemically; the epithelial cells of both secretory unites and their ducts contained acidic mucins. Russell bodies gave positive reaction with periodic acid Schiff reagent. The present of immunoglobulin was proved in the cytoplasm of plasma cells with Russell body. There were no differences in Harderian gland in both sexes. In conclusion, the Harderian gland of pigeon was histologically and histochemically well developed, and it is immunological effective secondary lymphatic organ, and it had few differences from the gland of other birds.

Key word: *Columba*, Harderian gland, Histochemical study, Russell bodies, Pigeon.

INTRODUCTION

The Harderian gland is compound multilobular ocular gland that located at the orbital cavity. It possess single duct that open in the inner angle of the eye at the base of the nictitating membrane. It present in most terrestrial vertebrates such as amphibian, reptiles, birds and some mammals (Weaker, 1981; Baccari *et al*., 1990; Payne, 1994; Shirama *et al*., 1996). The Harderian gland in some animal is large and more developed than the lacrimal gland (Sakai, 1989).The functions of the gland are diverse with animal species (Buzzell, 1996). It is involved in the protection of the eye against bright light and play role in photodynamic process (Reis *et al*., 2005; Funasaka *et al*., 2010). The gland may have endocrine function (Pradidarcheep *et al*., 2003). In avian the gland has great role in response to infection and vaccination (Salam *et al*., 2003; Zakeri and Kashefi, 2011).

MATERIALS AND METHODS

Twenty of pigeon Harderian glands were used in this study that included 10 females and 10 males. The birds were sacrificed by decapitation and their heads were quickly removed and the glands were fixed direct by injection of fixative solution (10%formalin, Bouin´s solution) in to the orbital cavity, then the heads as whole were immersed in the same fixative solution

for 48 hours. For anatomical study of the eyeball, including Harderian gland and muscles were carefully dissected after removing of the cranial bone, brain and the lower jaw. Each head was divided into two equal parts by using a sharp blade; the cut line was passed sagittal through the septa between the orbital cavities. For microscopic study the Harderian gland was prepared for paraffin embedding and sectioned serially at 6µm. The tissue sections were stained with Hematoxylin & eosin and Alcian blue (pH 2.5), periodic acid-Schiff and bromophenol blue for basic protein (Vacca, 1985).

RESULTS AND DISCUSSION

Macroscopic observations:

The Harderian gland of pigeon located at the orbital cavity lying at the ventromedial aspect of eye behind the eyeball. It was extended from the origin of the optic nerve passing inferiorly covered with the external ocular muscles (Fig.1). It is in agreement with the results of Wight *et al*. (1971a) in domestic fowl and the result of Dimitrov (2012) in pheasants. The gross observations revealed that gland was light brown to pink in color, and look like tear drop in shape had narrow anterior end and wide posterior end with curved flat body and had single main duct (Fig.1). The gross features of the gland in pigeon were similar to those observed by Kozlu *et al*. (2010) in osprey. Where in domestic fowl and sparrow Harderian glands were different in which the gland appeared irregular in shape (Wight *et al*., 1971a; Payne, 1994).

Microscopic results:

The histological examination revealed that the Harderian gland of pigeon was compounded acinotubular gland. The glandular acini were at the peripheral region of the lobes and continued with the tubular portions of the secretary units. Many of ducts were drained in to central collecting duct of the wide irregular lumen. The glandular units were lined with simple columnar epithelium which possessed round to oval darkly stained nuclei. The gland was covered with thin connective tissue capsule which sends many septa dividing the gland into many lobes (Fig.2, 3). The general histological structure of the pigeon Harderian gland was incompatible with those of domestic fowl and turkey (Burns and Maxwell, 1979; Maxwell *et al*., 1986). It was also compounded acinotubular and lined with high columnar cells. Similarly the Harderian gland of fowl, duck and turkey possessed central collecting duct that lined with simple columnar secretory epithelium (Burns and Maxwell, 1979). Where in domestic duck the gland was compound tubular (Brobby, 1972), the gland observed compound tubuloalveolar in domestic geese, osprey and quail (Boydak and Aydin, 2009; Kozlu *et al*., 2010; Kozlu and Altunay, 2011). Myoepithelial cells with darkly stained flat nucleus were noticed, it surrounding the secretary cells (Fig.4). The presence of these cells was mentioned by Cacho *et al.* (1991) and Mobin (2012) in the Harderian gland of chicken and by Altunay and Kozlu, (2004) in ostrich.

Histochemical results:

Most of the epithelial cells that lined the secretary units and ducts had positive reaction towered Alcian blue (PH 2.5), the cytoplasm of columnar cells stained blue which indicating the presence of acid mucopolysaccharides (Fig.4). The current histochemical finding of this study are in parallel with the results of Wight *et al.* (1971b) in domestic fowl and with results of Boydak and Aydin (2009) in domestic geese, they detected acidic mucous secretions in Harderian gland of those birds. On other hand, in domestic duck and native chicken the glandular secretion of this gland have given positive result to Alcian blue PH 2.5 and periodic acid Schiff reagent that mean the cells contained both acidic and neutral mucopolysaccharids (Brobby, 1972; Mobini, 2012). In osprey and quail the Harderian gland secretion found mainly neutral mucopolysaccharid (Kozlu, *et al*., 2010; Kozlu and Altuna, 2011).

The current result showed the presence of lymphocytes, plasma cells, as well as polymorph cells in the structure of Harderian gland. These cells were densely aggregated in the central regions of the lobules underneath the lining columnar cell epithelium in addition to individual cells in the interstitial connective tissue that surrounding the acini and tubules (Fig.5), This result was in agreement with those of Schramm (1980) and the histological structures of Harderian gland in different birds such as, Burns and Maxwell (1979) in fowl ,duck and turkey, Altuany and kozlu (2004) in ostrich, Boyak and Aydin (2009) in geese, kozlu and Altuany (2011) in quail, Mobini (2012) in chicken. The Harderian gland plasma cells were proliferated from the lymphocytes as a result for the elaboration of a factor which acted like a lymphokine (Scott and Savage, 1996).

The present result showed the presence of plasma cells with different sizes .Some of these cells, which contained Russell bodies (Mott cells) were large with a small, darkly stained nucleus with pink cytoplasm (Fig.5). The Russell bodies were in different size and occupied the majority of the cell. The Russell bodies give a positive reaction with periodic acid Schiff reagent (Fig.6); these findings were in accordance with those of Wight *et al*., (1971a) in a domestic fowl, Burns (1975) in rook and Bejdic *et al*. (2014) in laying hens.

The Russell bodies in the plasma cells have stained deep blue with bromophenol blue stain (Fig.7), and such observation proved that the cytoplasm of the plasma cells with Russell bodies contained globulin as basic protein, which gave the Harderian gland an important role in immune response, the latter issue was in agreement with what reported by Matthews (1983), Baba *et al*. (1990), Oliveira *et al*. (2006), Khan *et al*. (2007) and Ginkel *et al*. (2009), this investigation revealed that there were no differences between genders. Which was not parallel with the results of Boydak and Aydin (2009) in domestic geese, Kozlu and Altunay (2011) in quail and Mobini (2012) in native chicken.

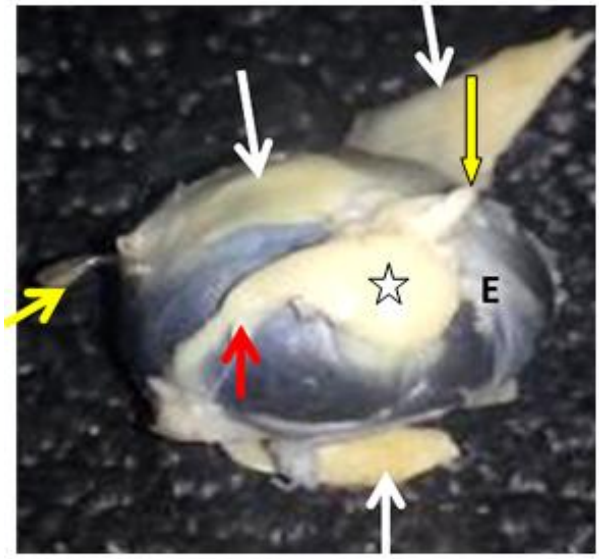

Figure (1): The eye of pigeon (posterior view) showing: Harderian gland (star), external ocular muscles (white arrows), optic nerve (yellow arrow), eyeball (E), main duct (red arrow).

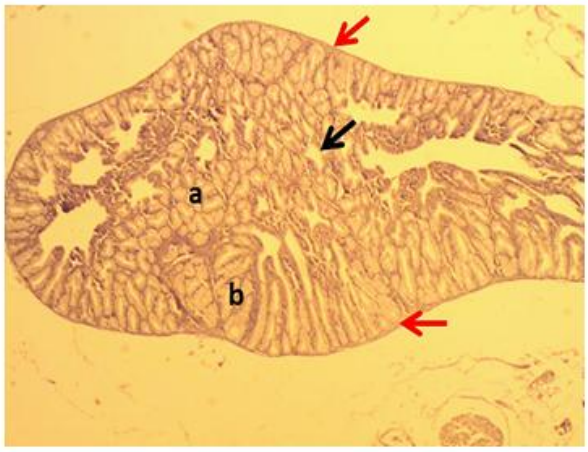

Figure (2): Longitudinal histological section of Harderian gland showing: Thin connective tissue capsule (Red arrows). Central collecting duct (Black arrow).Glandular acini (a) Lobe (b). (H& E stain X4)

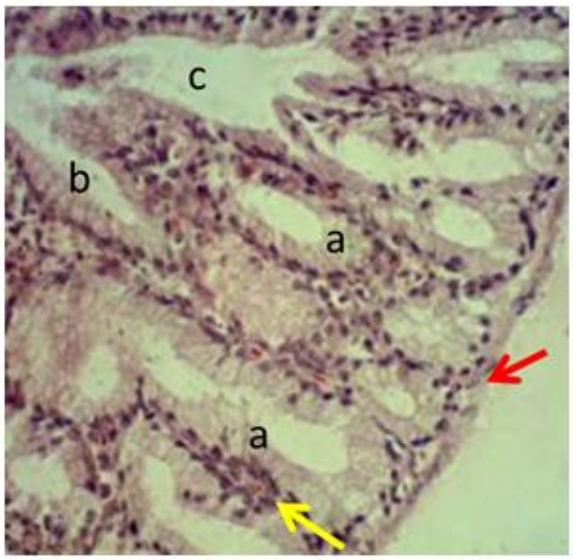

Figure (3): Longitudinal histological section of Harderian gland shows: Thin connective tissue capsule (Red arrow). Secretory acini (a). Secretory tubule (b). Main duct (c). (H&E. 40X)

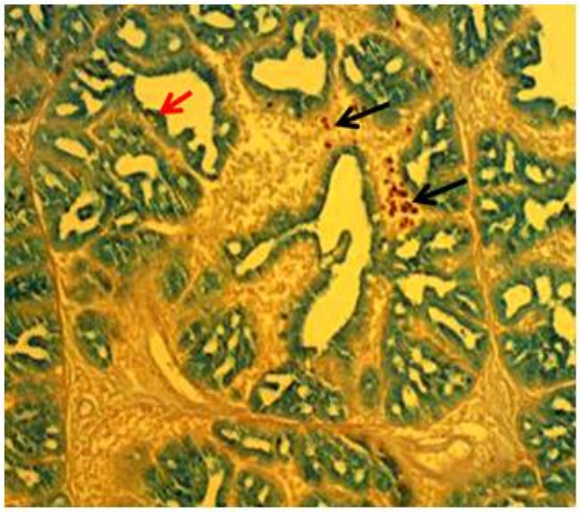

Figure (4): Histological section shows: Russell bodies PAS- Positive (purple red) (Black arrows). Lining epithelia AB-positive (blue) (Red arrow). PAS-AB stain.x4o

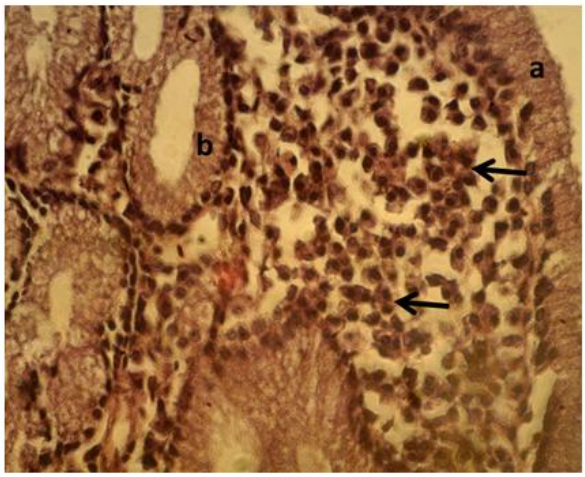

Figure (5): Histological section shows, a-columnar cells lining the central canal (a). Secretory unite (b).Plasma cells with Russell bodies (Arrows) (H & E stain. X100).

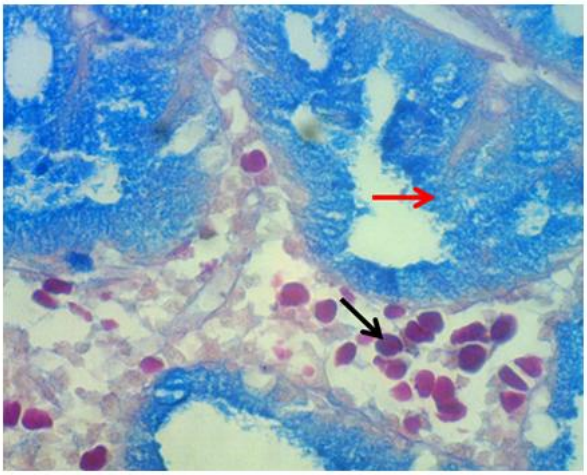

Figure (6): Histological section shows Russell bodies (black Arrow) which gives positive reaction to PAS Stain. Acidic mucopolysaccharid (red arrow) (PAS-AB stain.x400)

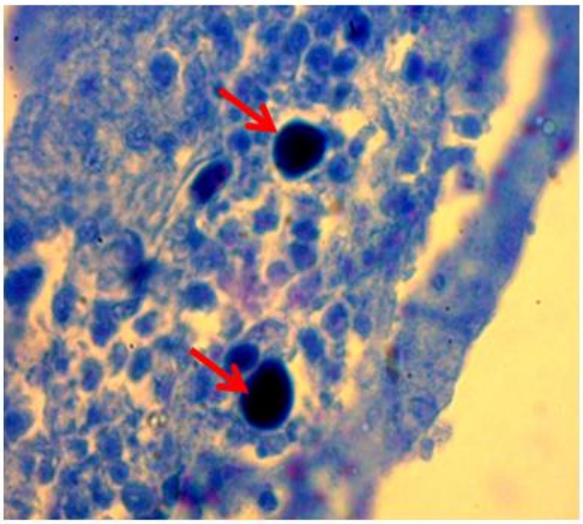

Figure (7): Histological section shows the globulin protein in the cytoplasm of Plasma cells (Russell bodies) stains deep blue Bromophenol blue stain.X1000.

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دراسة شكلانية وكيمياء- نسجية لغدة الهاردريان في الحمام الداجن *Columba livia domestica*

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الخلاصة

الهدف من البحث لدراسة التركيب النسجي والكيمياء النسجية لغدة هارديان لطيور الحمام لعينات جمعت من عشرة ذكور و عشرة اناث من الحمام البالغ. تم استخدام صبغة الهيماتوكسيلين والايوزين والشيان الأزرق (الاس الهادروجيني 2.5)، وكاشف حامض شيف وصبغة البرموفينول الأزرق. كانت الغدة تشبه الدمعة في الشكل، ذات لون يتراوح بين الوردي والبني، مفصصة مبطنة بخلايا الظهارية عمودية مختلفة الارتفاع ومحاطة بمحفظة رقيقة من النسيج الضام .

الخلايا اللمفاوية، خلايا البلازما وخلايا البلازما الحاوية على جسيمات راسل تتجمع تحت الظهارة المبطنة للعنبات الإفرازية و القناة الافرازية المركزي ة؛ و ا ظهرت الصابغات الكيمياء النسجية ان الخلايا المبطنة للعنبات الافرازية والقنوات جميع ها حاوية على مخاط حامضي، اما جسيمات راسل اظهرت تفاعل موجب مع كاشف شيف الحامض ؛ علاوة على ذلك تم اثبات احتواء جسيمات راسل على الكلوبيولين المناعي ، كما لم يلاحظ اي اختلاف في شكل وتركيب غدة الهادرين اعتمادا على الجنس.

استنتجت الدراسة ان غدة الهادرين في طائر الحمام جيدة التطور وتعد عضولمفاوي ثانوي مؤثر في مناعة الطائر ، وان هناك بعض الاختلاف عن الغدة في الطيور الاخرى.