Preliminary Investigations on the Harderian Gland of the African Giant Rat (*Cricetomys gambianus,* Waterhouse, 1840)

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Abstract

Harderian gland (HG) is an orbital gland found in almost all vertebrae. Its function includes protection and lubrication of the corneal, site of immune responses, source of pheromones and lipid thermoregulation and also melatonin secretion and function. We described for the first time the HG of African giant rat (AGR), a nocturnal rodent belonging to the family *Muridae* and order *Rodentia* and typical to the arid and temperate areas of Africa. Ten adult African giant rats weighing between 850g-1200g of both sexes were used. They were sacrificed and the gland harvested for gross observations and sections were taken, fixed for routine histological examination. Grossly, the AGR HG appeared lobulated and lobated. It weighs 1±0.08g with length and width of 2.9±0.2cm and 1.1±0.7cm respectively. Histologically, the AGR HG showed extensive pleomorphism. The epithelium is simple columnar with rounded to oval nucleus. Interestingly, we showed that the HG of the African giant rat is binucleated. While the mean epithelial height is 129.98 ± 5.36µm, the mean luminal diameter and mean tubular diameter are 211.43 ± 13.98µm and 479.04 ± 17.03µm respectively. The mean nuclear diameters of the small and large sized nucleus are 24.97 ± 0.68µm and 17.78 ± 0.62µm. This study reports baseline research data

on the anatomy of AGR's HG and will have usefulness in theoretical and experimental studies of its vision, helping in domestication of this rodent.

Keywords
African giant rat, binucleated, domestication, Harderian gland, small nucleus

Introduction

Johann Jacob Harder while studying the *Dama vulgaris* deer, discovered a gland that he described as being of the lacrimal type, the *Harderian gland* (Reis *et al.,* 2005). This gland varies among species and plays roles in immune responses, pheromone production, photoreception, thermoregulation, lubrication of the eye and the nictating membrane and photo-protection by regulation of the incidence of light on the retina (Reis *et al.,* 2005; El-Leithy *et al.,* 2015).

Anatomically, the HG is located in the retro-orbital region towards the midpoint of the internal space between the eyeball and the extremity of the orbit and the caudal surface bordering the frontal bone (Reis *et al.,* 2005). In mammals, and especially in rodents such as rats, hamsters, gerbils, mice and guinea pigs the gland is well developed (Chieffi *et al.,* 1996). In several species of desert rodents, the anatomical structure of the HG has been studied in detail (Sakai and Yohro, 1981). In mouse however, the HG has an irregular shape, with three fissures (Watanabe 1980).One of these fissures contains the optic nerve while the other two contain extraocular muscle fibers (Johnston *et al.,* 1987; Djeridane, 1994).

Histologically, the HG is enveloped by a membrane that penetrates and divides it into several lobules with a proximal serous part of its excretory duct opening directly into the distal straight ampulla which in-turn is drained by a single narrow channels onto the concave surface of the nictating membrane (Johnston *et al.,* 1987; Djeridane, 1994; Reis 2005).The gland may be of the tubular, tubuloacinar or tubuloalveolar type (Hoffman 1971; Payne 1994; Chieffi *et al.,* 1996) with no significant structural variations existing in the gland among different species of animals (Reis *et al*., 2005). Most anatomical description of the Harderian gland has been on the Wistar rat and birds but Alawa *et al.,* (2016) reported the absence of this gland in AGR. We report for the first time research data on HG of AGR found in Gwagwalada, Abuja. Our study may open an array of investigation as to the possibility of HG being present in some AGR and absent in others possibly due to ecological changes and adaptation.

African giant rat (AGR) (*Cricetomys gambianus,* Waterhouse, 1840) is found in Central and West African countries, including Nigeria and is known as a pouched rodent, belonging

to the family *Muridae*, and makes up a group of the order *Rodentia* (Ibe *et al.,* 2010). The AGR has been used to detect land mines in Mozambique (Mott, 2004) and diagnosis of tuberculosis in Europe (Maggie 2003). Its high acuity of olfaction is as a function of the well-developed rhinencephalon (Ibe *et al.,* 2014). The poor sight of the rodent which is a reason for its nocturnal behavior is due to its small rostral colliculi in relation to the caudal colliculi (Ibe *et al.,* 2014). Despite the wealth of information on the HG of rodents especially the Wister rats, there is limited information on the gross and microscopic anatomy of the AGR HG. This work therefore seeks to provide the baseline data on gross morphology, histology and histo-morphometry of the HG in the AGR found in Gwagwalada, North Central Nigeria.

Materials and methods

This study was conducted at Gwagwalada, Gwagwalada Area council in Abuja. Abuja is located between latitude 8° and 9° 25° North of the equator and longitude 6°45° and 7°45° East of Greenwich Meridian. The territory covers an area of 1,043km^3 and falls within the semi-seasonal equatorial climate zone with associated contrasting wet and dry periods (Ujof *et al.,* 2010).

The experimental protocol for this study was approved by the University of Abuja, Faculty of Veterinary Medicine Committee for Animal Studies. Ten adult male (6) and females (4) African giant rats weighing between 850g-1200g obtained from the wild in Gwagwalada, North Central part of Nigeria were used. They were sacrificed by lethal injection using a combination of xylazine (10mg/kg) and ketamine (100mg/kg) (Usende *et al.,* 2016) and the gland was harvested over ice for gross anatomical observation. The gland weight, length and width were taken and thereafter 4-6mm thick tissue was obtained and processed for histology and histomorphometrics.

Gross examination

For gross examination, a modified method by Usende *et al.,* (2014) was used. The length and width of the gland was obtained using thread and meter rule. The thread was place vertically on the gland from tip to tip and therefore the length of the thread measured with a meter rule. The same pattern was used for the width but now the thread placed horizontally at the mid-point. The weight of the gland was obtained using a bench top sensitive scale (LP 502A, China) with sensitivity of 0.1 to 5kg.

Histological examination

After gross examination, 4-6mm of tissue were taken from all samples and fixed in Bouin's fluid for 24 hours. Fixed tissues were prepared for light

microscopy as described by Luiz and Jose (2005). Paraffin blocks were sectioned at 5µm and stained with Haematoxylin and Eosin and Masson's trichome stains. High powered light microscope (Bio-microscope YJ 2015DN, Ningbo Yujie Optical Instrument Co., Ltd, Zhejiang, China) was used to study the stained sections at X40 to X 400. Photomicrographs were taken with the same microscope with inbuilt camera connected to a laptop (Samsung).

Histomorphometrics

This was done using analytic computer enabled software (Motic Images Plus 2.0). A total of twenty (20) tissue sections (two sections from each sample) were used for this study. The parameters measured include:

Epithelial height: A straight line was drawn using the software from epithelial basement membrane to the apical surface of the epithelial cell at X400. 10perfect acinar from each sample was observed.

Acinar diameter: A straight line was drawn both vertically and horizontally across the acinar from basement membrane to the opposite basement membrane and figures obtained were divided by two. Five perfect acinar from 10 fields at X100 were examined from all samples.

Nuclear diameter: A straight line was drawn both vertically and horizontally across the nucleus and figures obtained were divided by two. Five perfect acinar from 10 fields at x100 were examined from all samples.

Micronuclear diameter: A straight line was drawn both vertically and horizontally across the micronucleus and figures obtained were divided by 2. Five perfect acinar from 10 fields at x100 were examined from all samples.

Luminal area: This was obtained by drawing a circle with the computer software to cover the entire lumen as much as possible and figures were automatically generated. At least 5acinar from 10fields at X100 were examined from all samples.

Luminal diameter: A straight line was drawn both vertically and horizontally through the lumen and figures obtained were divided by 2. Five perfect acinar with lumen from 10 fields at X100 were examined from all samples.

Statistical analysis

Data generated from the gross and histological studies were analyzed using Graph pad prism version 6.0 and presented as mean±standard deviation.

Results

Gross findings

The findings reported are first time research data on HG of AGR found in

Gwagwalada, Abuja. The AGR HG was light brown in colour and appeared lobulated and lobated ventrally. It was a large retrobulbar gland and was located in the medio-caudal aspect of the orbital wrapping round the optic nerve. It is irregularly shaped, appearing thin at one end, wider at the middle and narrower and segmented at the other end. Dorsally, it is less lobulated (Fig. 1A and B).The measurements of the length, width and weight of the Harderian gland were presented in table 1. Whereas the mean length of the HG of AGR was 2.9±0.2cm and the width was 1.1±0.7cm, the gland weight was recorded to be 1.0±0.08g.

Histologically, the AGR HG is covered by a capsule which its trabeculae extend into the parenchyma of the gland dividing it into lobes and lobules. The gland also showed extensive pleomorphism ranging from acinar to tubular to tubuloacinar forms (Fig 2). Whereas some have large lumen, others have small lumen (Fig 2 and 3). The epithelium is of the simple columnar type with round to oval nucleus with the longest diameter being horizontal (Fig 3). Clumps of exfoliated epithelia are found in the lumina making their secretion holocrine in nature (Fig 3). These clumps together form a thick cellular adhesion. The apical (supranuclear part of the glandular epithelia) appeared vacuolated. Secretory vesicles emerged from the level of the nucleus. Sometimes globulated (fatty clump) are found in the lumen (Fig 2 and 3). The scanty connective tissues in the periacinar interstitium are of the fibrocytes type with abundant collagen fibers (Fig 4). Blood vessels are also found in the interstitium (Fig 2). The apical margins of the glandular epithelium are rough. There are well developed fusiform or elongated and highly stained myoepithelial cells (Fig 3). The HG of AGR also possesses myoepithelial cells between the basal laminae and the secretory cells (Fig 3). There are also presences of interstitial cells between the tubules (Fig 2 and 3). There is no specialized duct system seen in the HG of AGR. At various stages, there are binucleated cells (Fig 3). Binuclear cells appeared smaller. There is also evidence of mitosis. The results of the histomorphometric studies of the AGR HG are presented in table 2. The nuclear diameter was higher (24.97±0.68µm) compare to the micronucleus diameter (17.78±0.62). The epithelial height, luminal and acinar diameters were 129.98±5.36µm, 211.43±13.98µm and 228.35±10.2µm respectively. The luminal area on the other hand was 426.57±9.86µm^2.

Discussion

The present study revealed that the HG of AGR appeared generally light brown in colour. Sohair (2009) and El-Leithy *et al.,* (2015) reported that the HG of rabbit is bilobed, having a small

white lobe and a larger pink lobed. Our finding that the gland is a large orbital structure simulates earlier reports of Sohair (2009) and El-Leithy*et al.,* (2015). HG of AGR is covered by a capsule which trabeculae extends into the parenchyma dividing the gland into lobes and lobules similar to earlier work of Sohair (2009) with female rat and rabbit HG (El-Leithy*et al.,* 2015). We also reported that the parenchymatous tissues of AGR HG has no distinct duct system similar to what Sohair (2009) reported.

The secretory end piece of the HG of AGR in the present study was tubuloacinar corroborating works of Khan *et al*., (2007) in chicken, Murat and Mehmet(2009) in domestic geese and Sohair (2009) and El-Leithy*et al.,* (2015) in rabbit.

The present study also showed that the AGR HG can be mononucleated or binucleated similar to that seen in gerbil (Sakai and Yohro 1981; Johnston *et al.,* 1983). We also showed that some of the cells are mitotic in appearance. Vianna *et al.,* (1975) have earlier reported mitotic activities in the HG of rat only during the first few days of postnatal life. However, in our work adult AGR were used. This implies that the HG of AGR has a way that even at old age; the HG continues to replace its cells. However, absence of binucleated and mitotic cells has been reported in the HG of albino rats (Brownscheidle and Niewenhuis 1978).

Myoepithelial cells seen in the AGR HG is used for contractions of the gland. The gland used both holocrine and apocrine modes of secretion similar to the reports of Johnson *et al.,* (1983), Johnson *et al.,* (1987) and Djeridane (1994). In the apocrine mechanism, microscopic fragments of cellular cytoplasm are found in the lumina of the acini while in the holocrine mechanism, dead epithelial cells are the main component of the gland secretion. The end-piece of armadillo HG has also been described as been of the apocrine secretory type (Aldana Marcos and Affinni 2005). Sohair (2009) has shown numerous mucous secretory cells in female rat and rabbit HG. The mucous secretion of the gland may aid in lubrication and protection of the eye. Again, the collagen fibers identified with the Masson's trichome stain could be responsible for the strength and cushioning effects to the gland.

In conclusion, we have shown that AGR HG is light brown and showed extensive pleomorphism. The epithelium is simple columnar with rounded to oval nucleus and sometimes binucleated. More anatomical work using immunohistochemistry and electron microscope should be done on the HG of AGR which will serve as a useful model for theoretical and experimental

studies on the photodynamic process of the vision and help in domestication of this rodent.

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Table (1): Gross parameters of the Harderian gland (HG) of African giant rat (AGR)

Parameter	Mean ± S.D
Length(cm)	2.9±0.2
Width(cm)	1.1±0.7
Weight(g)	1±0.08

Table (2): Histomorphometric parameters of the HG of AGR

Parameter	Mean ± S.D
Epithelial height(µm)	129.98 ± 5.36
Acinar diameter(µm)	228.35 ±10.2
Nuclear diameter(µm)	24.97 ± 0.68
Micronucleus diameter(µm)	17.78 ± 0.62
Luminal area (μm^2)	426.57 ± 9.86
Luminal diameter(µm	211.43 ± 13.98

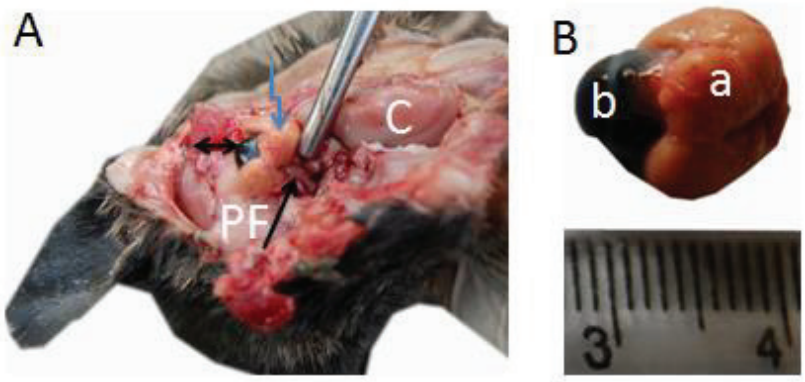

Fig (1): A; A photograph of dissected head of AGR showing the eyeball (two edged black arrow) and the Harderian gland (zigzag blue arrow). Note that the Harderian gland wrapped round the optic nerve (single edge black arrow). Also note the position of the brain (C) and peri-orbital fats (PF).
B; A photograph of dissected Harderian gland (a) attached to the eyeball (b).

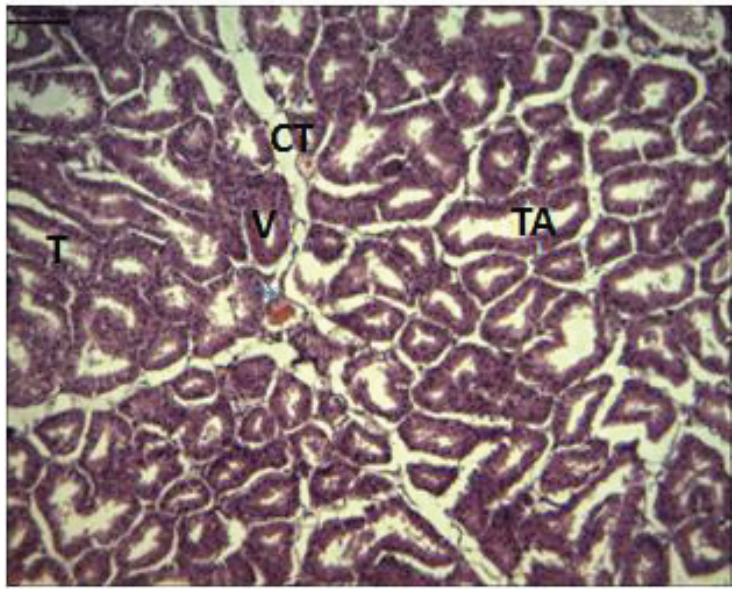

Fig (2): A photomicrograph of paraffin section of adult AGR HG.
Note the tubular (T), alveoli (V) and tubuloalveoli (TA) types of gland, blood vessel (arrow) and CT septa separating the lobules and few interstitium between the gland. H&E, x40

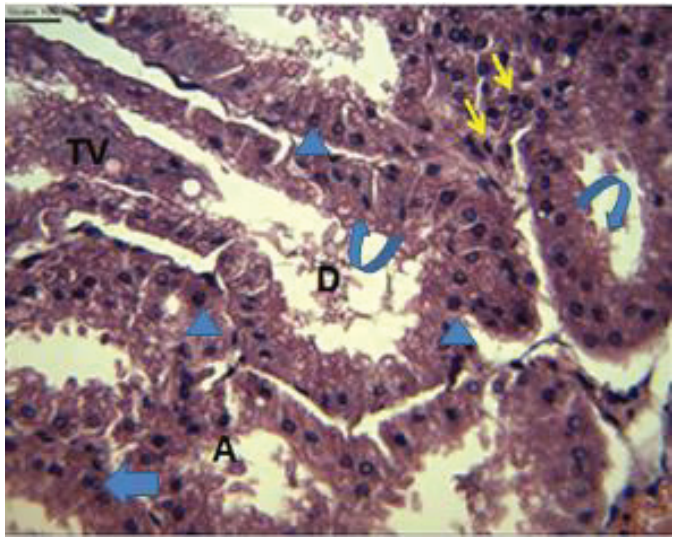

Fig (3): A photomicrograph of paraffin section of adult AGR HG.
Note the tubuloalveoli (TV) type of gland, with cellular debris (D) (Holocrine secretion) mixed with porphyrin secretion in the lumen. Also note the alveoli type of gland (A), columnar glandular epithelium (bent arrow) and nuclei (arrow head), myoepithelial cells (yellow thin arrow), binucleated cell (thick arrow). H&E, x400

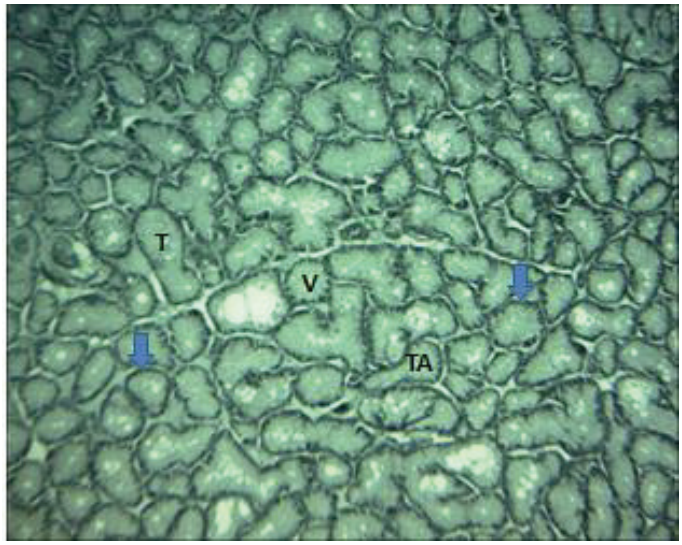

Fig (4): A photomicrograph of paraffin section of adult AGR HG.
Note the presence of collagen fibers at the basement membrane and few in the interstitium between the aveoli (arrow). Also note the tubular (T), alveoli (V) and tubuloalveoli (TV) gland types. MT, x40