Tropical Veterinarian
International Journal Devoted to All Aspects of Health and Diseases of Animals in the Tropics

SHORT COMMUNICATION

Tropical Vet. 42 (1) Pages 26 – 33, 2024

Histology and Histochemistry of the Adrenal Gland of the Four-Toed Hedgehog (*Atelerix albiventris* Wagner, 1841)

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ABSTRACT

This study investigated the histology of the adrenal gland of the four-toed hedgehog (*Atelerix albiventris*) using basic histological and histochemical techniques. The aim was to provide helpful scientific data on the structure and function of the adrenal gland of this animal. Four adult male four-toed hedgehogs (150-200g) were used for the study and the adrenal glands were processed for routine histology and histochemical procedures of Periodic acid Schiff (PAS) and Masson's Trichrome. The adrenal gland showed the histology of the outer cortex and the central medulla. The adrenal cortex has three zones, the cells of the outermost zona glomerulosa are columnar or pyramidal in shape with closely packed spherical nuclei arranged in curved columns. The middle zona fasciculata possessed large polyhedral cells with foamy cytoplasm, arranged in long parallel cords bounded by sinusoidal capillaries. The cells of the innermost zona reticularis displayed deeply staining nuclei arranged in interconnected cords delineated by fenestrated capillaries. The adrenal medulla was composed predominantly of large, pale-staining irregular or cuboidal medullary cells which formed clusters separated by numerous sinusoidal blood capillaries and connective tissue. However, other cell types were not distinguishable within the adrenal medulla. Only the adrenal capsule and zona fasciculata were positive for PAS reaction while the capsule, trabeculae and connective tissue elements within the adrenal medulla containing collagen fibres showed positive staining to Masson's Trichrome. The study therefore demonstrated that the histology and histochemical features of the adrenal gland of the four-toed hedgehog are similar to other mammals except for minor variations such as the absence of zona intermedia and predominance of the pale-staining cells in the adrenal medulla. This is perhaps the first report on the histology and histochemistry of the adrenal gland of the four-toed hedgehog.

Keywords: Adrenal gland, Four-toed hedgehog, Histology, Histochemistry

INTRODUCTION

The four-toed hedgehog (*Atelerix albiventris*) is the smallest (Santana *et al.*, 2010) out of the hedgehogs within the genus *Atelerix*, and is commonly kept as pets (Chaprazov *et al.*, 2014). They are found across much of the Eastern, Western and Central Africa (Hutterer, 2005) and predominantly inhabit the arid vegetation (Nicholas, 1999). In Nigeria, they have a wide distribution across different ecological zones,

though densely populated in the savannah region (Okorie-Kanu *et al.*, 2015). This species of hedgehog is currently classified as "least concern" by the International Union for Conservation of Nature (IUCN) due to its wide distribution and large population. However, questions are raised about their indiscriminate use as food and pets by humans. Okaeme and Osakwe (1988) reported that the four-toed hedgehog is commonly consumed as a game and hunted for fertility charms in Nigeria.

Over the past decade, the four-toed hedgehog has gained wide acceptance as a laboratory model for many biomedical research (Santana *et al.*, 2010). There have been reports of sporadic tumours in four-toed hedgehogs in which the gross, histopathological and immunohistochemical features of histiocytes and Langerhans cells were studied (Son *et al.,* 2020). Other studies about the parasites associated with hedgehogs have been reported due to their increased importance as pets and sentinels. Mariacher *et al*. (2021) and Rasmussen *et al*. (2021) investigated the occurrence of parasitic infections in *Erinaceus europaeus* in Italy and Denmark, respectively.

The adrenal glands are essential discrete endocrine organs, which secrete hormones (Rosol *et al*., 2001) and are located craniomedially to the kidneys (also known as suprarenal glands). Each gland, covered by a fibrous capsule, has an outer cortex and an inner medulla. The adrenal cortex produces steroid hormones, including glucocorticoids, while the medulla releases catecholamines (Rosol *et al*., 2001).

A couple of scientific reports on the four-toed hedgehog have been documented. Coker *et al.* (2018) studied the morphometry of some organs of the African four-toed hedgehog while Biu *et al.* (2018) described the incidence of the parasite and its associated haematological changes. However, there is no existing report on the histology and histochemical studies of the adrenal gland of the four-toed hedgehog. This study sought to investigate the histology and histochemistry of the adrenal gland of the four-toed hedgehog to provide helpful scientific data on the structure and function of the adrenal gland of the animal.

MATERIALS AND METHODS

Experimental Animals

Four adult male African four-toed hedgehogs, weighing between 150-200g, sourced from the local rodent market at Oranyan, Ibadan were used for the study. They were acclimatised in their cages for 72 hours at the Department of Veterinary Anatomy animal facility, University of Ibadan. Feed (smoked fish) and water were provided *ad libitum*.

Sample Collection

The animals were euthanized after a 72-hour acclimatisation period. The hedgehogs were anaesthetised with intramuscular injection of ketamine at 25 mg/kg BW as described by Olukole *et al*. (2018) before slaughter Thereafter, their body weights were measured with the use of Microvar® weighing balance at 0.1 g sensitivity. An incision was made on the linea alba cranially to the xiphoid cartilage to expose the heart for intracardial perfusion with 10% neutral buffered formalin by gravity feed method. The kidneys, with the attached adrenal glands, were carefully harvested and immediately fixed in 10% formalin solution.

Histological and histochemical procedures

The tissues were allowed to fix in formalin for 2 days after which the adrenal glands were trimmed for further tissue processing as previously described by Olukole *et al*. (2016). Dehydration was done in 50%, 70%, 90% and absolute alcohol after which they were cleared in three jars of xylene and then embedded in paraffin wax after infiltration in the same medium at 60°C. The paraffin sections of 5 microns thick were cut on a microtome, mounted on clear slides after floating on a warm bath and

allowed to air dry. The slides were stained with H&E dyes for routine cellular architecture, and with histochemical stains, PAS and Masson's Trichrome, for polysaccharides localisation and collagen fibres with smooth muscle identification, respectively.

RESULTS

Histology of the adrenal gland of the four-toed hedgehog

The gland showed a clear demarcation of the outer cortex from the inner medulla (Fig. 1). The whole organ was ensheathed in a capsule containing adipose tissue, some blood vessels, and a few smooth muscle fibres.

Histology of the capsule and adrenal cortex of the four-toed hedgehog

With routine staining, the capsule was composed of dense connective tissue containing smooth muscle fibres, and blood vessels with thin trabeculae from the capsule projected partially into the cortical parenchyma (Fig. 2A). The cortical region of the gland, located just beneath the capsule, showed three zones; the outermost zona glomerulosa, the middle zona fasciculata and the innermost zona reticularis. The cellular elements of the outermost zone were arranged in round clusters and curved columns continuous with the cords in the middle zone. Cells of this zone appeared small and columnar or pyramidal with closely packed and densely stained nuclei (Fig. 2B). Zona fasciculata cells were large and polyhedral in shape. They were arranged in long parallel cords, one or two cells thick, delineated by sinusoids. Most cells of this zone possessed pale staining round nuclei and their cytoplasm contained numerous vacuoles, hence referred to as spongiform cells (Fig. 2C). The cells of the deepest cortical zone had deep staining nuclei and were smaller compared to the zona fasciculata. They appeared as interlaced cords sandwiched with fenestrated capillaries (Fig. 2D).

The images on the stained slides were captured using a light microscope (Olympus BX63 coupled with DP72 camera) attached with software, TSview and sections were observed at different magnifications (x4 and x40).

Histology of the adrenal medulla of the four-toed hedgehog

The central region of the organ, the adrenal medulla, possessed large, lightly stained irregular or cuboidal cells as its parenchyma, connective tissue and numerous sinusoidal blood capillaries (Fig. 3).

The pale-staining medullary cells were predominant in this region and they formed clusters separated by the sinusoids. However, other cell types were not distinguishable within the adrenal medulla of the four-toed hedgehog. Some cells from the adjacent zona reticularis also project into the adrenal medulla (Fig. 3).

Reaction to Periodic Acid-Schiff (PAS) staining

The adrenal capsule was strongly positive for PAS reaction and stained bright purple, though the smooth muscle fibres were not distinct from its collagen component (Fig. 4A). Within the adrenal cortex, the cells of zona fasciculata were moderately positive for PAS reaction, while zona glomerulosa and zona reticularis showed minimal reactions to PAS with the connective tissue trabeculae showing much PAS reactivity (Fig. 4B and C). The cells of the adrenal medulla were also slightly positive to PAS reaction with the connective tissue component which surrounds the clusters of medullary cells being moderately positive to PAS (Fig. 4D).

Reaction to Masson's Trichrome (MT) staining

The adrenal capsule showed positive reactivity to MT staining with the collagen fibres staining greenish-blue (Fig. 5A). The three zones of the adrenal cortex were negative to MT staining, however, the trabeculae around the zona glomerulosa showed minimal collagen presence as they also stained greenish-blue (Fig. 5A). The stromal elements within the adrenal medulla

demonstrated some collagen fibres as there were distinct boundaries of the cell clusters (Fig. 5B).

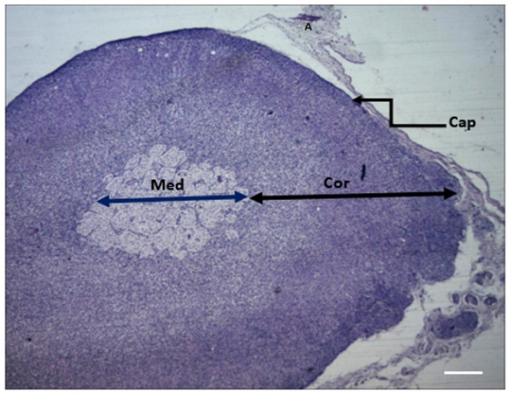

Figure 1. Photomicrograph of the adrenal gland of the four-toed hedgehog (*Atelerix albiventris*) showing the two major divisions of the gland into the outer cortex (Cor) and the inner medulla (Med). Note the presence of the adrenal capsule (Cap) and the surrounding adipose tissue (A). (H&E; Scale bar 200µm)

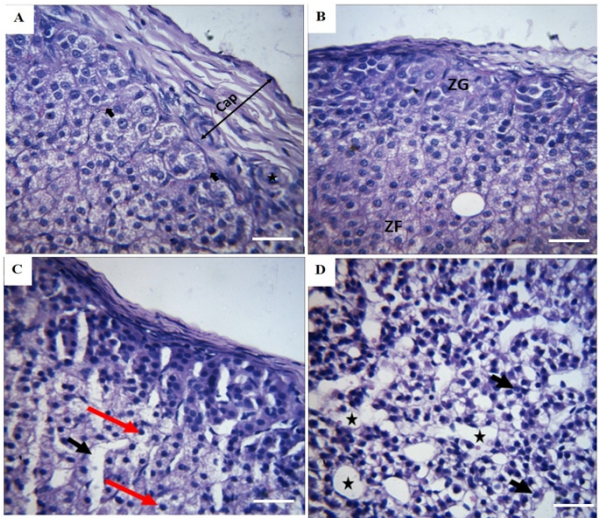

Figure 2. Photomicrographs of the adrenal gland of the four-toed hedgehog (*Atelerix albiventris*) showing **(a)** the capsule (Cap), together with the trabeculae (block arrows) and blood vessel (star), **(b)** the zona glomerulosa (ZG) as well as the zona fasciculata (ZF). The cells of the zona glomerulosa are columnar and pyramidal in shape (arrows), **(c)** the zona fasciculata (ZF). The cells (spongiocytes) are large, cuboidal or polyhedral in shape with foamy cytoplasm (red arrows). The sinusoidal capillaries are abundant in this zone of the adrenal cortex (black arrows) and **(d)** the zona reticularis. The cells are smaller compared to the zona fasciculata (arrowheads)

An overview of the regional reactivity of the adrenal of the four-toed hedgehog to PAS and MT stains is shown in Table 1

arranged in an irregular network with fenestrated capillaries (star). (H&E; Scale bar 50µm).

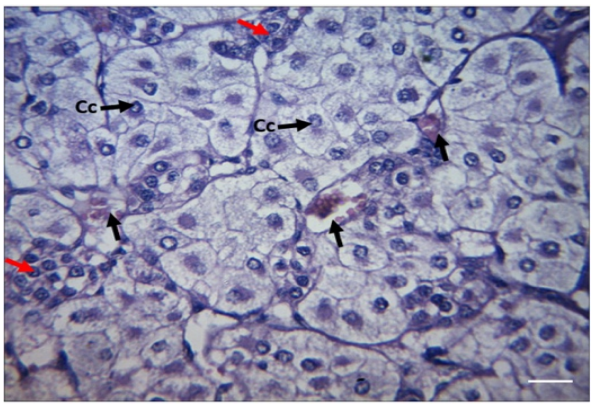

Figure 3. Photomicrograph of the adrenal medulla of the four-toed hedgehog (*Atelerix albiventris*). There are clusters of pale-staining cells (Cc) surrounded by sinusoidal blood capillaries (black arrows) with some cells from the zona reticularis extending into the medullary zone (red arrows) (H&E; Scale bar 50µm).

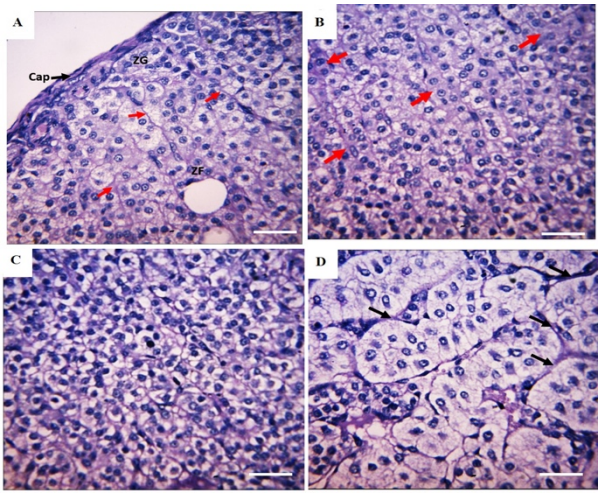

Figure 4. Photomicrograph of the adrenal gland of the four-toed hedgehog (*Atelerix albiventris*) showing **(a)** the capsule (Cap) as well as the trabeculae (red arrows) was positive to PAS reaction while the cells of zona glomerulosa (ZG) had minimal reactivity, **(b)** the cells of zona fasciculata were moderately positive for PAS reactivity (red arrows), **(c)** the cells of zona reticularis had a slightly positive reaction to PAS and **(d)** The connective tissue components within the medulla cell cluster were positive to PAS (black arrows) though the medullary cells had minimal reactivity (PAS; Scale bar 50µm).

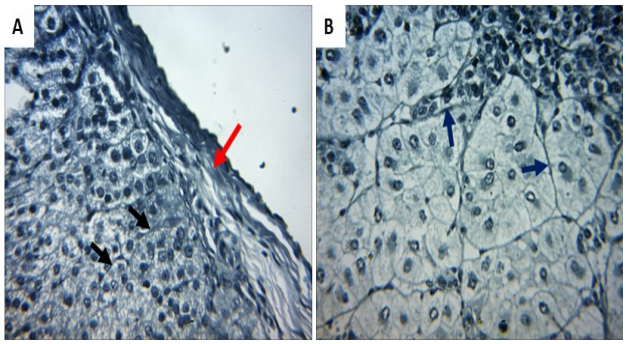

Figure 5. Photomicrographs of the adrenal cortex and medulla of the four-toed hedgehog (*Atelerix albiventris*) stained with Masson's Trichrome (MT). **(a)** The capsule contains large collagen fibres (red arrow) with some minimal presence within the trabeculae (black arrows) and **(b)** the boundaries of cell clusters within the adrenal medulla (blue arrows) also shows some collagen investment. (MT; Scale bar 50µm).

Table 1. Summary of the reactions of different regions of the adrenal gland of the four-toed hedgehog

ADRENAL GLAND		MT	PAS
CAPSULE	COLLAGEN	++	++
	SMOOTH MUSCLE	+	++
ZONA GLOMERULOSA		-	+
ZONA FASCICULATA		-	++
ZONA RETICULARIS		-	+
ADRENAL MEDULLA		+	+

DISCUSSION

The adrenal gland is of critical importance in several homeostatic processes such as stress response, cardiovascular physiology and general metabolism. The adrenal gland of the four-toed hedgehog possesses a thick capsule composed of dense irregular connective tissue with well-developed collagen fibre and peripheral adipose tissue. The capsule of four-toed hedgehog was positive to Masson's Trichrome and Periodic Acid-Schiff implying its abundance of collagen as well as polysaccharides, respectively. This agrees with previous studies in the African giant rat by Olukole *et al*. (2016) and in other domestic mammals (Bacha and Bacha, 2012).

Zona intermedia, a zone located between the zona glomerulosa and zona fasciculata, is lacking in the four-toed hedgehog. It has been reported in carnivores, horses and bottlenose dolphins (Banks, 1993; Bacha and Bacha, 2012). This zone has been postulated to be a site of adrenocortical stem cell development in those animals and since it is absent in the four-toed hedgehog, the role is played, possibly, by another zone (Nicolaides *et al*., 2023). The pyramidal or columnar cells of the zona glomerulosa are arranged in irregular clusters or cords. This pattern is a common feature in most mammals, though differs in the shape formed by the clusters of cells. The cells of the zona glomerulosa assume a columnar shape and are grouped into arcs in the carnivore and horse, with the cells being tall columnar cells in the latter (Bacha and Bacha, 2012). This zone is the site of secretion of aldosterone which acts on the distal convoluted tubule and collecting duct of the kidney (Curnow *et al*., 1991; Marieb and Hoehn, 2012). The cells of zona glomerulosa of four-toed hedgehog being slightly positive to PAS and non-reactive to Masson's Trichrome positive implied that the cells probably do not store abundant glycogen within their cytoplasm and possibly make use of an alternative energy source for their metabolic activities. However, the trabeculae which traverse the zona glomerulosa from the capsule showed some reaction with MT due to their collagen investment.

The appearance of the zona fasciculata of the four-toed hedgehog agrees with previous studies that reported it as the widest of adrenocortical zones making up to 60-80% of the cortical parenchyma in domestic mammals such as the dog and cattle (Junqueira and Carneiro, 2005; Bacha and Bacha, 2012). The zona fasciculata possesses parallel cords of cuboidal or polyhedral cells separated by sinusoids (Vukovic *et al*., 2010). The foamy appearance of the cytoplasm

of the cells of this zone is attributable to their high lipid storage ability as they produce steroid hormones, and glucocorticoids (Young *et al*., 2013). Cortisol, the main glucocorticoid produced from this zone functions in the mobilisation of fats, proteins and carbohydrates from the body stores for immediate use during stress responses (Dunn *et al*., 2011). The findings from the present study are similar to the histological features of the zone in most domestic and some captive mammalian species (Vukovic *et al*., 2010; Olukole *et al*., 2016). The positive reaction of the zona fasciculata to PAS indicates that the parenchyma of the zone is rich in glycogen.

The subtle change from zona fasciculata to zona reticularis with the cells of the latter arranged in anastomosing cords is identical to earlier reports in ruminants, carnivores and pigs (Banks, 1993). This zone is contiguous with the medulla its function is to produce androgens, mainly dehydroepiandrosterone (DHEA). The cellular elements appear as clusters or uneven cords intertwined with capillaries and connective tissue. They also contain relatively small amounts of cytoplasm and lipid droplets and sometimes display brown lipofuscin pigment. Oftentimes, they extend into the adrenal medulla and are seen among the clusters of medullary cells. The hormones synthesized by the zona reticularis augment the gonadal secretions. They are released in response to ACTH from the adenohypophysis and are modified in the tissues to testosterone or oestrogens. The cells of this zone showed weak PAS reactivity and no reaction to MT which is suggestive of reduced storage of cytoplasmic polysaccharide, unlike the cells of zona fasciculata. This is at variance with the report of Olukole *et al*. (2016) in which the zona reticularis of the African giant rat showed no PAS reactivity.

The adrenal medulla of the four-toed hedgehog forms the central portion of the organ enclosed by the adrenal cortex. There was no connective tissue layer separating these two layers and this finding is similar to the reports in most domestic mammals (Bacha and Bacha, 2012; Olukole *et al*., 2016) but differs from the dolphin (Clark *et al*., 2008). The adrenal medulla was composed of columnar or polyhedral, pale-staining epithelioid cells arranged in rounded groups or clusters separated by large sinusoids. The large pale-staining cells, similar to chromaffin cells in other mammalian species, are the predominant cells of the medulla. These cells secrete the catecholamines (Dunn *et al*., 2011). The cells showed weak reactivity to PAS staining, which could indicate that the cells do not actively store glycogen but make use of readily available energy sources for their metabolic activities. This finding differs from the considerable positive reaction to PAS observed in the African giant rat (Olukole *et al*., 2016). However, the stromal elements which surround the medullary cell clusters possess some collagen fibres as they stained greenish-blue to MT. This makes each cluster of cells within the adrenal medulla of the four-toed hedgehog distinguishable from the other.

CONCLUSION

The microscopic anatomy of the adrenal gland of the four-toed hedgehog showed similarities to other mammals except for the presence of a relatively thick dense connective tissue capsule, the absence of zona intermedia and the predominance of the pale-staining cells in the adrenal medulla. In addition, the adrenal capsule and the zona fasciculata of the four-toed hedgehog were positive for Periodic Acid-Schiff reaction. Also, the adrenal capsule with the medullary stromal elements possessed considerable collagen fibres indicative of their contractility.

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