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Histoarchitectural changes of Adrenal gland in Emballonurid male bat, *Taphozous longimanus* (Hardwicke) during the reproductive cycle

Shende Virendra Abaji[1*]

[1]*Department of Forensic Biology, Government Institute of Forensic Science, Nagpur, India.*

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Abstract

Taphozous longimanus has two adrenal glands, one on each side, situated retroperitoneally occupying an antero-mesial position abutting against the ipsilateral kidney. The left adrenal gland is pyramidal and the right adrenal gland is oval in shape. The diameter of the left adrenal gland is always higher than that of the right adrenal gland during different periods of reproductive activity. Each gland is duplex organ consisting of cortex and medulla. Cortex is divided into two zones which are poorly differentiated from each other. An outer zone is zona glomerulosa, and inner zone is zona fasciculata. The zona glomerulosa of left and right adrenal gland during the breeding period are larger than that of zona glomerulosa of left and right adrenal gland during the pre-breeding period respectively. The diameter of zona fasciculata of left and right adrenal gland during the breeding period is smaller than that of zona fasciculata of left and right adrenal gland of the pre-breeding period. There is no clear histological difference between medulla of right adrenal gland to that of left adrenal gland during the breeding period but the diameter of zona glomerulosa and medulla during the breeding period is larger than that of the pre-breeding period.

Keywords: adrenal Gland, histology, Histology, Bat, *Taphozous longimanus*, zona glomerulosa, zona fasciculata, Medulla, Light Microscopy

1 Introduction

Mammalian adrenal gland is unique among the vertebrate in that steroidogenic and chromaffin cells are clearly separated as cortex and medulla respectively. The light microscopic observations

*e-mail: virushende@gmail.com

of adrenal gland are known in few species of bats, viz., *Rousettus aegyptiacus* (Baranga 1980), *Rousettus leschenaulti* (Sapkal 1978), *Cynopterus sphix* and *Taphozous longimanus* (Karim et. al., 1969), *Miniopterus schreiberscii* (Planel et. al., 1961), *Taphozous Kachensis* (Chavhan, 2011). The adrenal glands (suprarenal glands) in human are closely associated with the kidneys. A gland sits atop each kidney like a cap and is embedded in the mass of adipose tissue that encloses the kidney. The adrenal glands are shaped like pyramids. Each adrenal gland is very vascular and consists of two parts. The central portion is the adrenal medulla, and the outer part is the adrenal cortex (Shier et al., 2007; Andreas et al., 2019). A primary function of the adrenal gland is to protect the organism against acute and chronic stress. In acute stress, catecholeamines of the medulla mobilize glucose and fatty acids for energy and prepare the heart, lungs and muscles for action. Glucocorticoids protect against over reactions of the body's responses to stress, and in the more chronic stress of food and fluid deprivation stimulate gluconeogenesis to maintain the supply of glucose. Stress as well as hyper- and hypo-function of the adrenal gland is known to suppress reproduction in mammals (Rivier et al., 1986).

The adrenal gland is composed of two distinct portions: an outer cortex (mesodermal origin) and an inner medulla (neuroectodermal origin) (Banks, 1993; Dellman, 1993; Gude et el., 1982; Junqueira et al., 1995). In porcupine (*Hystrix cristata*) paired adrenal glands were located to the cranio-medial of the kidneys and both right and left adrenals were flat. The adrenal cortex is subdivided into three zones. The outermost zone was formed as zona glomerulosa. It contained prismatic cells that formed irregular clusters or cords and contained lipid vacuoles. (Yilmaz & Girgin, 2005). The adrenal cortex of bats as in mammals is differentiated into three distinct zones: zona glomerulosa, zona fasciculata and zona reticularis. However, the zona reticularis is absent in the adrenal gland of *T. longimanus* but it is present in the form of islets of cortical cells in the medullary region (Lowry et al., 1980).

The present study dealt with histological changes and role of adrenal gland in reproduction of adrenal gland of a microchiropteran species, *Taphozous longimanus* during different phases of the reproductive cycle.

2 Materials and Methods

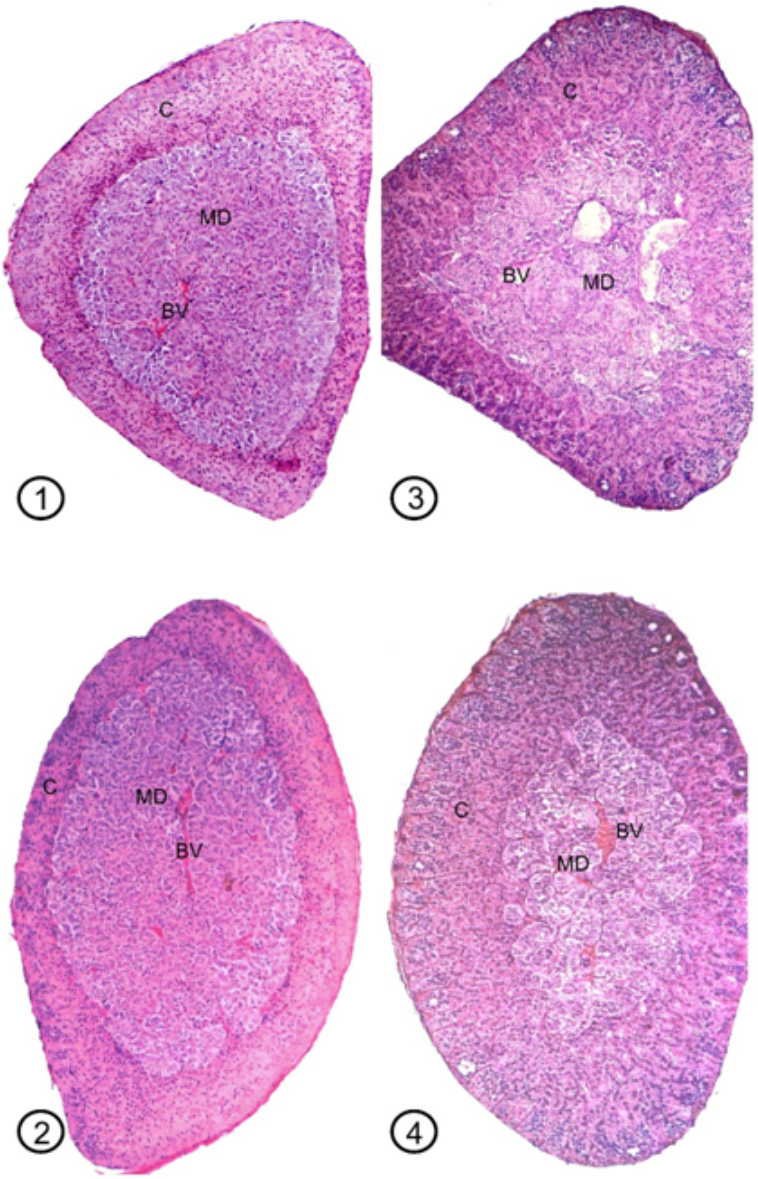
Adrenal glands of *Taphozous longimanus* bat were fixed in the aqueous Bouin's. After fixation, materials were washed in running tap water for 12 hrs. and dehydrated with upgraded series of ethyl alcohol, cleared in xylene and embedded in molten paraffin wax. Blocks were prepared, trimmed and cut into thin sections of 5-6 µ with the help of Leica 2417 microtome. The ribbon containing sections were spread and used for Hematoxylin-Eosin staining. For staining, slides were dewaxed in xylene, hydrated by downgraded series of ethyl alcohol upto the water, stained with Hematoxylin, washed in water and dehydrated by upgraded series of alcohol upto 70% alcohol. Slides were stained with eosin, washed in 90% alcohol, transferred in absolute alcohol, cleared in xylene and mount in DPX. The desired stained slides were observed under light microscope and micro-photographed at different magnifications.

3 Results

Observations

Taphozous longimanus has two adrenal glands one on each side situated retroperitoneally occupy-

ing an antero-mesial position abutting against the ipsilateral kidney. These glands are attached to the kidney by fibrous connective tissue. The left adrenal gland is pyramidal and the right adrenal gland is oval in shape (figs. 1-4). The diameter of the left adrenal gland is always higher than that of the right adrenal gland during different periods of reproductive activity. The average weight of adrenal gland during the pre-breeding period and the breeding period is more or less same.
Histology of left and right adrenal gland is studied during different phases of reproductive cycle in bat, *Taphozous longimanus*.



Light microphotograph of transverse section of adrenal gland (WM) during the pre-breeding and breeding period showing, outer cortex (C) and blood vessels (BV) in inner medulla (MD) X 100; Fig. 1- Left adrenal gland during the pre-breeding period showing triangular shape; Fig. 2- Oval shaped Right adrenal gland during the pre-breeding period; Fig. 3- Left adrenal gland during the breeding period showing large triangular to pyramidal shape; Fig. 4- Oval shaped Right adrenal gland during the breeding period.

Adrenal gland during the pre-breeding period

During the pre-breeding period, weight and diameter of the left adrenal gland is slightly higher than that of the right adrenal gland. Each adrenal gland is encased in a thick capsule of connective tissue in which branches of main arteries are observed. The capsule – glomerulosa border is distinct. Each gland is duplex organ consisting of cortex and medulla. Cortex is divided into two zones which are poorly differentiated from each other. An outer zone is zona glomerulosa and inner zone is zona fasciculata. The zona reticularis is absent in this species of bat. In the centre large medulla is seen (figs. 5 and 6).

This is outermost zone of cortex covered by thick fibrous capsule. The average diameter of zona glomerulosa of the left adrenal gland is less than the diameter of zona glomerulosa of the right adrenal gland. This zone consists of small polyhedral cells arranged in acini by short cords of 2-3 cells thick. Each acinus is separated by sinusoid capillaries. The plasma membrane of cell is not well distinct. The nucleus is darkly stained, spherical to oval in shape present in the central or eccentric in position. The nucleus has darkly stained nucleolus. Cytoplasm is eosinophilic.

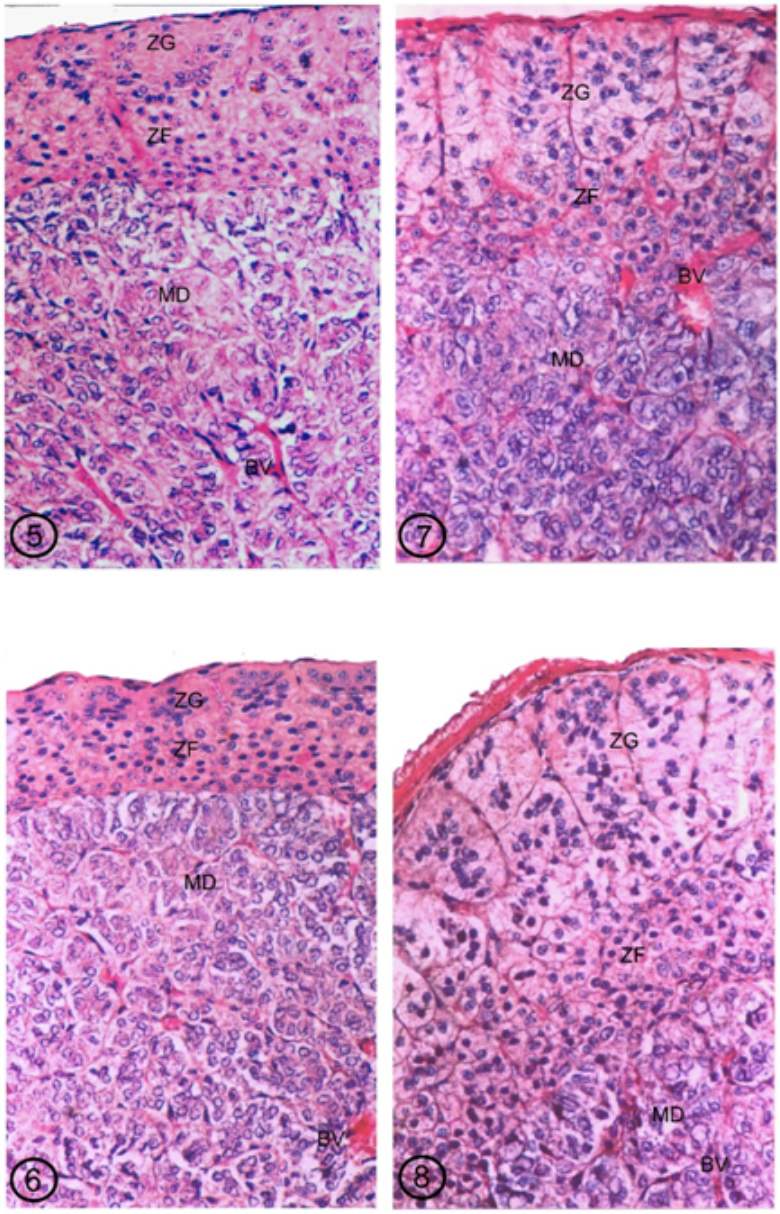
The diameter and cell height of zona fasciculata of the left adrenal gland is larger than the diameter and cell height of zona fasciculata of right adrenal gland. The diameter of zona fasciculata of the left and the right adrenal gland is larger than the diameter of zona glomerulosa of the left and the right adrenal gland respectively. This zone is made up of small cuboidal cells arranged into small columns oriented in radial direction towards medulla. The nucleus is darkly stained. It is spherical to oval in shape, eccentrically placed with well developed nuclear membrane. Nucleolus is single and well marked. The cytoplasm of fasciculata cells is slightly basophilic. Blood vessels and capillaries are quite less in this zone. This zone is separated from zone glomerulosa and medulla by venous sinusoids.

This is a central zone of adrenal gland. In this zone blood vessels and capillaries are noticed. The diameter of medulla of the left adrenal gland is less than the diameter of the right adrenal gland. This zone consists of numerous short cords of 2-3 cells and few groups of 6-12 cells which are separated by sinusoid and capillaries. These polymorphic cells have a large darkly stained, spherical to irregular shaped nuclei. Nucleolus is not clearly seen. Cytoplasm is basophilic in nature.

Adrenal gland during the breeding period

The mean weight and diameter of adrenal gland during, the breeding period is more than that of the pre-breeding period and the mean weight and diameter of the left adrenal gland is higher than that of the right adrenal gland during the breeding period. The left adrenal is pyramidal to triangular and the right adrenal is more elliptical in shape than the pre-breeding period. The adrenal gland is enclosed by capsule made up of thick connective tissue. It consists of outer cortex and inner medulla. The cortex is divided into two zones, i.e. zona glomerulosa and zona fasciculata (figs. 7 and 8).

The average diameter of zona glomerulosa of left adrenal gland is less than average diameter of zona glomerulosa of the right adrenal gland; but the height of zona glomerulosa cells of the left adrenal gland is higher than the height of zona glomerulosa of the right adrenal gland. The zona glomerulosa of left and right adrenal gland during the breeding period are larger than that of zona glomerulosa of left and right adrenal gland during the pre-breeding period respectively. This zone is made up of thick radially arranged long cords or acini. Most of the acini are separated from each other by sinusoids. Each acinus consists of group of 12-20 large polyhedral cells. Few vacuolated cells are also noticed. Plasma membrane is not clearly demarcated. Each cell has large sized, eccentrically placed, rounded to oval shaped darkly stained nucleus. Nucleolus is centric or eccentric in position. Cytoplasm is eosinophilic.



Light microphotograph of transverse sections of adrenal gland during the pre-breeding and breeding stage showing outermost layer zona glomerulosa (ZG), middle zona fasciculata (ZF) and inner medulla (MD). Blood capillaries (BV) are in zona fasciculata and medullary region. X400; Fig. 5- Left adrenal gland during the pre-breeding stage; Fig.6- Right adrenal gland during the pre-breeding stage; Fig. 7- Left adrenal glands during the breeding stage; Fig. 8- Right adrenal glands during the breeding stage.

Zona fasciculata is an intermediate zone between zona glomerulosa and medulla. The diameter of zona fasciculata of the left and the right adrenal gland during pre-breeding period is smaller than the diameter of zona glomerulosa of the left and the right adrenal gland during breeding period respectively. The diameter and cell height of zona fasciculata of the left adrenal gland is larger than the diameter and cell height of zona fasciculata of the right adrenal gland during the breeding

period. This zone consists of small columns of single or double row of alternately arranged and radially oriented cells. The zona fasciculata of the right adrenal gland has slightly larger cells; outer zone contains more vacuoles and less sinusoid than that of zona fasciculata of left adrenal gland. These cells are cuboidal and low columnar types. Nucleus is centrally situated, spherical in shape, darkly stained and covered by well developed nuclear membrane. The nucleolus is mostly situated at the centre or sometimes in the peripheral region of the nucleus. Few binucleate cells are also observed in this region. The cytoplasm is eosinophilic. Many large sinusoids and blood capillaries are seen in this region. This zone is separated from zona glomerulosa and medulla by sinusoids.

The diameter of zona fasciculata of left and right adrenal gland during the breeding period is smaller than that of zona fasciculata of left and right adrenal gland of the pre-breeding period. During the pre-breeding and breeding period, zona fasciculata cell height of the left adrenal gland is more than that of the cell height of right adrenal gland.

During the pre-breeding period, the diameter of the medulla of the left adrenal gland is smaller than that of the medulla of the right adrenal gland and during the breeding period the diameter of the medulla of left and right adrenal gland is similar. The medulla of the adrenal gland during the pre-breeding and the breeding period is more or less similar in diameter and rich in large sinusoids and capillaries. It consists of many groups of 5-10 large cells which are separated from venous sinusoids. The plasma membrane is not well developed. These polymorphic cells contain spherical or irregular shaped, eccentric, darkly stained nucleus. Nucleolus is not clearly seen. Nuclear membrane is well developed. Cytoplasm is basophilic in nature.

There is no clear histological difference between medulla of right adrenal gland to that of left adrenal gland during the breeding period but the diameter of zona glomerulosa and medulla during the breeding period is larger than that of the pre-breeding period.

4 Discussion

The left adrenal gland of *Taphozous longimanus* is pyramidal and the right adrenal gland is oval in shape. The diameter and weight of left adrenal gland is always higher than that of right adrenal gland during different periods of reproductive activity (Shende et al., 2014). The average weight of adrenal gland during the pre-breeding and the breeding period is more or less same. Similar observations are reported in bat, *Taphozous melanopogon* (Lowry & Lall, 1986), *Herpestes auropunctatus* (Tomich, 1965) and *Pteropus giganteus giganteus* (Papadkar & Dhamani, 2012). The relative weight of adrenal gland remains more or less constant in adults in both the sexes of Indian gray mongoose, *Herpestes edwardsii*, however the right adrenal gland is observed to be uniformly smaller than the left adrenal gland (Shetty et al., 1984). This is in agreement with the present findings.

The two main components of the adrenal gland, the cortex and medulla differ in their structure, function and origin (Shelton & Jones, 1970). The cortex and medulla are clearly demarcated in adrenal gland of *Taphozous longimanus*. Similar observations are reported in other bats, *Megaderma lyra lyra* (Bhima Rao and Devaraj Sarkar, 1975); *Miniopterus schreibersii* (Planel et al., 1961); *Pteropus giganteus giganteus* and *Rousettus leschenaulti* (Sapkal, 1978; Rao & Maiti, 1981); *Cynopterus sphinx gangeticus* and *Taphozous longimanus* (Karim et al., 1979).

In *Taphozous longimanus* adrenal cortex is divided into two distinct zones, outer- zona glomerulosa and inner- zona fasciculata. The central medulla is large and extensive. The zona glomerulosa

consists of small polyhedral cells arranged in a group of short cords of 2-3 cells thick. The zona fasciculata is made up of small cuboidal cells arranged into small columns oriented in radial direction towards medulla. The medulla consists of numerous short cords of 2-3 cells and few groups of 6-12 cells which are separated by sinusoid and capillaries (Shende et al., 2014).

There are considerable differences in the details of the structure of the adrenals among the few bats so far studied. True zonation is not present in the adrenal cortex of *Eptesicus fuscus* (Gorbman, 1962), a distinct zonation of the adrenal cortex was noticed in *Megaderma lyra lyra* (BhimaRao and Devaraj Sarkar, 1975), *Miniopterus schreibersii* (Planel et. al., 1961), *Vesperugo pipistrellus* (Saidapur and Nadkarni, 1976) and *Pteropus giganteus* and *Rousettus leschenaulti* (Sapkal, 1978). In present bat, *Taphozous longimanus* only two zones, the zona glomerulosa and the zona fasciculata are identified as similar reported by Karim et al. (1979).

During the breeding period, there are no remarkable structural differences between the left and right adrenal gland of the bat, *Taphozous longimanus*. But the diameter of zona fasciculata of left and right adrenal gland during the pre-breeding period is larger than that of zona fasciculata of left and right adrenal gland of the breeding period. This shows, during the pre-breeding period Zona Fasciculata secrete more glucocorticoids Cortisol and Corticosterone hormones which regulate carbohydrate metabolism helps the body manage and use carbohydrates, protein and fat (Andreas et al., 2019) which is required to transform from pre-breeding to breeding stage.

Conflict of interests

There are no conflicts of interest.

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