

# Morpho-histological studies on the adrenal gland in male and female bat, *Taphozous Kachhensis* (Dobson)

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#### ABSTRACT

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It was aimed to study the size, weight and shape differences between right and left adrenal glands, the cortico-medullary ratio of the adrenal glands in bat Taphozous kachhensis. The specimen of Taphozous kachhensis were collected from Ambai Nimbai,45 kilometers from Bramhapuri (M.S.). Many collections were made during the breeding season so as to coincide with the time of reproductive cycle. These bats are very sluggish in nature after collection they were sexed and were brought to the laboratory. Weight recorded with digital balance before they were sacrificed. The Adrenal were dissect out and fixed in alcoholic Bouin's fluid. After fixation for 24 hr tissue were washed with 70% ethanol. For the histological examinations, after fixation, tissue samples were dehydrated, cleared, and embedded in paraffin. Haematoxylin and eosin staining method was used to examine tissue sections. The size and weight of the gland were different from male to female bats. The adrenal gland is composed of two distinct cell layers, the cortex and the medulla. The medulla is composed of chromaffin cells that produce the hormones epinephrine and norepinephrine. The mean diameter and the weight of the Right and left adrenal gland is varies during different phases of the reproductive cycle. The cortico-medullary ratio in male was 65.57% cortex and 34.43% medulla (1.9:1) and in female 58.84% cortex and 41.16% medulla (1.43:1). Medullary tissue of adrenal glands was more in female whereas the cortical tissue was more in male and this suggests that the adrenal medulla hormones production was more in female bat and similarly, cortical hormone secretion was more in male bat.

Key words: Adrenal gland, Cortex, Medulla, Cortico-medullary ratio, bat.

# **INTRODUCTION**

Adrenal gland is one of the most important glands of the endocrine system. These paired glands maintain the homeostasis and play the key roles in response the stress (Humayun *et al.*, 2012, Randall *et al.*, 2002, Freeman, 1985). The function of the adrenal gland is different according to their cortical and medullary cells. A variety of hormones are produced

from this gland which are very important to maintain the everyday life. Most of the mineralocorticoids and glucocorticoids are produced from adrenal cortex and medulla secretes norepinephrine and epinephrine (Humayun et al., 2012). Stress as well as hyper and hypofunction of the adrenal gland is known to suppress reproduction in mammals. Different factors are responsible for the weight, length, width and thickness of the adrenal glands. Unlike to the mammals, in chicken the cortico-medullary tissues are intermingled to each other (Ghosh *et al.*, 2001). The light microscopic observations of adrenal gland are known in few species of bat, Eptrsicus fuscus and Anatrozous pallidus; Megaderma lyra lyra (Bhima Rao Shankar 1975); Miniopterus schreibersii (Planel and Guliham, 1961);Rousettus leschenauti and Pteropus giganticus (Sapkal, 1977); Cynopterus sphinx and Taphozous longimanus; Taphozous melanopogon (Lawory and Lall, 1986); Hipposideros lankadiva (Seraphim, 2004), Hipposideros lankadiva (Dhamani, 2004) and Taphozous longimanus (Nerkar, 2007). The study of weight, size and shape of right and left adrenal gland in male and female bat show the significant difference in Taphozous melanopogon (Lawory and Lall, 1987). Therefore, this experiment was done to study the size and shape differences between right and left glands, the cortico-medullary ratio.

# **METHODOLOGY**

In the Bat, histomorphometric analysis was carried out at the light microscopic level on the right and left adrenal of male and female bat during various phases of reproductive cycle. The glands were fixed in Bouin's solution and embedded in paraffin wax. The entire gland was cut in serial section at 6µm. Three of these were selected, one from the middle of the gland and two from the poles. They were stained by Haematoxylin and eosin (H and E) to identify the main cell types. The histological sections were analyzed to determine the volume densities of the various components of the gland. The mean volume densities (Vv) were then calculated for the components of the gland. The analysis of the glandular components included the connective tissue, cortex, medulla, blood vessels and nerves. Since the counting was carried out under X10 objective lens, it was decided not to be considered chromaffin tissue and ganglion as two separate entities, but to consider both of them as adrenal medulla. Each complete section was analyzed field by field using an objective X10. Depending on the area of the section, the number of fields ranged from 5 to 15 field per sections.

# RESULTS

**Gross anatomy of the adrenal glands of male and female bat:**The adrenal glands of the bat *Taphozous kachhensis* can be distinguished into two zones an outer cortex and inner medulla. The cortex is completely encircles the medulla. The medulla in this species consists of epitheloid cells arranged in smaller groups surrounded by blood capillaries. Adrenal glands of female bat, *Taphozous kachhensis* during pregnancy are larger than those of other phases. While in male bat, *Taphozous kachhensis* the weight is more during sexually active period. The weight of adrenal gland (mean ± SEM) during different phases of reproductive cycle is represented in Table.1

# A. Histology of male adrenal gland

Histology of adrenal gland during sexually quiescence period: Histomorphological findings show that left adrenal gland of male Taphozous kachhensis is always larger vascular than right. Light Microscopic studies of adrenal gland of sexually quiescence bat T. kachhensis shows that it is round to oval in shape. It is enclosed in collagenous capsule which sends variable deep trabeculae into cortex; the capsule shows enrich adrenal plexus supplying branches to the gland. Just below the capsule the gland has two distinct noticeable zonations, the cortex and medulla (Fig-1). The medulla is present centrally and completely surrounded by cortex. On the basis of histological observations of steroidogenic cells, the cortex is further divided into three major zones; zona glomerulosa, zona fasciculata and zona reticularis (Fig- 2).

Zona Glomerulosa: Zona glomerulosa is the smallest zone of the adrenal cortex present beneath the capsule measuring  $(73\mu)$  in thickness during the sexually quiescent period of reproductive cycle. This zone consists of polyhedral glomerular cells arranged in rounded cells and generally found in group of 3 - 7 cells or acini. These cells have darkly stained nucleus, either centrally or eccentrically placed. Chromatin clumps are seen in the nucleus scattered towards central or peripheral region. The cytoplasm takes acidic stain and eosinophilic in nature. A few lipid vacuoles are observed in the cytoplasmic matrix and blood capillaries are also observed between the clusters of acini. Zona glomerulosa is compactly associated with zona fasciculata and hence no identifying separation is observed in between these two zones (Fig- 3).

Reproductive Phase	Weight of the right adrenal gland of female bat Mean weight ± SEM ( n = 5)	Mean weight of right adrenal gland of male bat (mgs) ±SEM	Weight of the left adrenal gland of female bat Mean weight ± SEM (n = 5)	Mean weight of left adrenal gland of male bat (mgs) ±SEM
Sexually inactive	1.22±0.058	0.87±0.012	$1.43 \pm 0.045$	0.92± 0.009
Preparatory	1.50±0.071	1.04±0.009	1.87±0.051	1.12± 0.005
Sexually active	2.02±0.086	1.09±0.006	2.36±0.076	1.16± 0.034

Table. 1: Mean adrenal gland weight with SEM, diameter of various zones of adrenal gland during the different phases of reproductive cycle of male and female bat, *Taphozous kachhensis*.

Table 2: Mean adrenal gland diameter of various zones of adrenal gland during the different phases of reproductive cycle of Male and Female bat, *Taphozous kachhensis*.

	Diameter in (μ)						
	Whole	Zona	Zona	Zona	Medulla		
Period	Gland	glomerulosa	fasciculata	reticularis			
	Mean	Mean	Mean	Mean	Mean		
	diameter	diameter	diameter	diameter	diameter		
Female							
Sexually inactive	750 ± 9.82	90 ±1.96	300 ± 0.96	90 ± 2.10	270 ± 3.53		
Sexually active	930 ± 2.11	$105 \pm 2.54$	130 ±2.10	95 ± 1.10	600 ± 2.10		
Male							
Sexually quiescence	891 µ	73 μ	206 μ	69 µ	543 μ		
Sexually active	1072 μ	81 μ	221 μ	75 μ	695 μ		

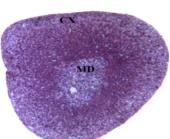


Fig-1

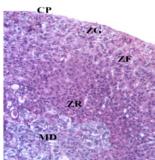


Fig-2

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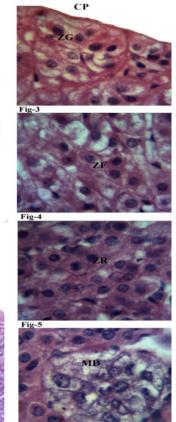


Fig-6

**Fig- 1**: Light micrograph of transverse section of adrenal gland during sexually quiescence period of reproductive cycle shows the presence of capsule (CP) covering the gland, outer cortex (CX) and inner medulla (MD). X 100

**Fig- 2**: Light micrograph of transverse section of adrenal gland during sexually quiescence period of reproductive cycle shows the outermost capsule (CP). Note the cortex differentiated in to outer small zone, zona glomerulosa (ZG), middle long cell cords of zona fasciculata (ZF) and inner zona reticularis (ZR) and innermost centrally placed medulla (MD). X 400

**Fig- 3 :** Light micrograph of transverse section of adrenal gland during sexually quiescence period of reproductive cycle shows the presence of outermost capsule (CP) followed by small acini like group of cells of zona glomerulosa (ZG). X 1000

**Fig- 4:** Light micrograph of transverse section of adrenal gland during sexually quiescence period of reproductive cycle showing cords of zona fasciculata (ZF). X 1000

**Fig- 5 :** Light micrograph of transverse section of adrenal gland during sexually quiescence period of reproductive cycle showing cords of zona reticularis (ZR). X 1000

**Fig- 6 :**Light micrograph of transverse section of adrenal gland during sexually quiescence period of reproductive cycle showing inner medullary zone (MD) surrounded by many small blood vessels (BV) and capillaries. X 1000

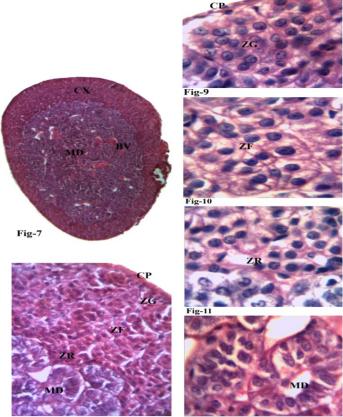


Fig-8

Fig-12

**Fig- 7:** Light micrograph of transverse section of adrenal gland during sexually active period of reproductive cycle shows the presence of capsule (CP) covering the gland followed by hypertrophoid cortex (CX) and inner medulla (MD). X 100

**Fig- 8** :Light micrograph of transverse section of adrenal gland during sexually active period of reproductive cycle shows the cortex differentiated in to outer small zona glomerulosa (ZG), middle hypertrophoid zona fasciculata (ZF) and inner zona reticularis (ZR) and innermost centrally placed medulla (MD) with blood vessel. X 400

**Fig- 9** :Light micrograph of transverse section of adrenal gland during sexually active period of reproductive cycle shows the presence of outermost capsule (CP) followed by small acini like group of cells of zona glomerulosa (ZG). X 1000

**Fig- 10 :** Light micrograph of transverse section of adrenal gland during sexually active period of reproductive cycle showing hypertrophoid cell cords of zona fasciculata (ZF) with vacuolations. X 1000

**Fig- 11 :** Light micrograph of transverse section of adrenal gland during sexually active period of reproductive cycle showing cells of zona reticularis (ZR). X 1000

**Fig- 12 :** Light micrograph of transverse section of adrenal gland during sexually active period of reproductive cycle showing enlarged medullary zone (MD) with blood vessels (BV). X 1000

**Zona Fasciculata:** Zona fasciculata is largest cortical zone present in between zona glomerulosa and zona reticularis measuring about ( $206\mu$ ) in width during the sexually inactive period of reproductive cycle. This zone shows presence of polyhedral cells arranged in straight column and forms a group of 2 - 5 cells. The cords present in this zone are arranged in a redial manner that runs towards the medulla in the center of the gland. The cords of the zona fasciculata are separated from each other by means of straight venous sinusoids. Comparatively the cells of this zone are larger and vacuolated as compare to the cells of zona glomerulosa and zona reticularis.

Each cell contain large nucleus with scattered chromatin clumps mostly near the peripheral region. Eccentrically, single nucleolus is prominently visible in nucleoplasm. Large numbers of lipid vacuoles are seen in this zone which gives spongy appearance to the cytoplasm, which is basophilic in nature. Formation of cords and spongy cytoplasm are thus characteristics feature of this zone (fig- 4). **Zona Reticularis:** Zona reticularis is the innermost zone of the cortex. It is present just below the zona fasciculata on one side and is adjacent to the medullary zone, on another side measures in  $(69\mu)$  in thickness.

In this zone, each cell disposed in anastomosing cords of varying shape and size. Each cell has basophilic cytoplasm with large vesicular nucleus either centrally or eccentrically placed. Dense chromatin material is seen near the peripheral region of nuclei. Cytoplasm shows few lipid vacuoles. Lipid droplets are very less comparative with zona fasciculata. Sinusoidal capillaries occupy the interstices of the cords of the zone (Fig- 5).

**Medulla:** Medulla is the central zone of adrenal gland, covered from all the sides by outer cortical zones having diameter (543 $\mu$ ). It shows distinct demarcation from the cortical zone. This zone consists of numerous short cords of 2-3 cells and few groups of 6-14 cells which are separated by sinusoids and capillaries. Each cells of the medulla are darkly stained, having large spherical to irregular shape nuclei. Single prominently visible, darkly stained nucleolus is also observed eccentrically in the

nucleoplasm. Chromaffin clumps are also seen towards the periphery of nucleus. Cytoplasm is basophilic and granular (Fig- 6).

ii) Histology of adrenal gland during sexually active **period**: The bat *T. kachhensis*, during sexually active period shows very typical and remarkable changes. Morphological studies reveal that, the size of adrenal gland during this period increases as compared to sexually quiescent period. The shape also changes from oval or circular during inactive period to oblong elliptical during active period (Fig- 7). The mean diameter of adrenal gland (1072 $\mu$ ) during sexually quiescence period (891 $\mu$ ) (Table- 6 and Histogram-10).

During active phase, male bat shows vigorous spermatogenesis in their testis and seminiferous tubules are seen packed with spermatozoa. The histological study of adrenal gland during sexually active period shows that the gland is enclosed in a collagenous capsule which sends variable deep trabeculae into the cortex. Below the capsule gland shows two distinct zones, cortex and medulla. During this phase medulla occupies larger area as compare to the medulla of sexually inactive bat (Fig- 7). The cortex shows three distinct zones as seen in the sexually inactive bat; viz. zona glomerulosa, zona fasciculata and zona reticularis (Fig-8).

Zona Glomerulosa: Zona glomerulosa is the outermost small zone. The average diameter of zona glomerulosa of adrenal gland (81µ) during sexually active period is larger than the average diameter of zona glomerulosa of sexually quiescence period (73µ) (Table- 6 and Histogram- 10). Structurally similar cellular composition is observed in this zone as observed during sexually quiescence period of reproductive cycle. This zone is made up of thick radially arranged long cords or acini. The cell cords or acini are round to elongate in shape. Most of the acini are separated from each other by sinusoids. The acini consist of group of 7-10 large polyhedral cells. Plasma membrane is not clearly observed. The cells have large, eccentrically placed, darkly stained and rounded to oval shaped nucleus. Nucleolus is centric or eccentric in position. Cytoplasm is lightly stained and eosinophilic. The cytoplasm is vacuolated and lipid vacuoles are more than that observed in the cells of glomerulosa during the quiescence period (Fig- 9).

Zona Fasciculata: It is an intermediate zone present beneath the zona glomerulosa and is the largest zone of the cortex present between the zona glomerulosa and zona reticularis. The average diameter of zona fasciculata of adrenal gland (221µ) during sexually active period is larger than the average diameter of zona fasciculata of sexually quiescence period (206µ) (Table-6 and Histogram- 10). This zone consists of small columns of single or double row of alternately arranged and radially oriented cells. These cells are cuboidal and low columnar types. During this phase the hypertrophied cells of zona fasciculata show spongy cytoplasm with large number of lipid vacuoles. Nucleus is centrally situated, spherical in shape, darkly stained with well developed nuclear membrane having dense chromatin clumps at the periphery. Each cell shows spongy cytoplasm due to the presence of large number of lipid vacuoles. Darkly stained nucleolus is visible eccentrically in the nucleoplasm. Cytoplasm is eosinophilic. Lipid vacuoles in the cells of zona fasciculata are increased during sexually active phase than those found in the cells of sexually inactive phase. Many sinusoids and blood capillaries are seen in the cell cords of this zone (Fig-10).

Zona Reticularis: It is the innermost zone of the cortex without any clear demarcation. The zona reticularis is present below the zona fasciculata and above the medulla. The average diameter of zona fasciculata of adrenal gland (75µ) during sexually active period is larger than the average diameter of zona reticularis of sexually quiescence period  $(69\mu)$  (Table- 6 and Histogram- 10). This zone possesses polymorphic cells with lightly stained cytoplasm and vesicular nuclei. Chromatin clumps are seen at the periphery of the nuclei and a single nucleolus is also observed. The cytoplasm shows presence of few lipid vacuoles. The lipid vacuoles are also seen as vacuolations but these are less than those found in the cells of zona fasciculata of the active phase but more than the cells of zona reticularis of sexually quiescence phase of reproductive cycle. Few blood capillaries are also observed in this zone (Fig- 11).

**Medulla:** Medulla is the innermost region of the adrenal gland. Medullary zone of sexually active bat occupies largest area as compared to the medulla of sexually quiescent bat. During the active breeding period the diameter of medulla ( $695\mu$ ) is larger than the diameter of medulla during sexually quiescence period ( $543\mu$ ) (Table- 6 and Histogram- 10). Entire medulla is surrounded by cortex. It consists of many groups of 4-8

large cells which are separated from various sinusoids. The polymorphic cells of medulla contains spherical or irregular shaped, darkly stained, eccentric placed nucleus with one or two nucleoli in nucleoplasm. Nuclear membrane is well developed with chromatin clumps near periphery. Cytoplasm is granular and basophilic in nature (Fig-12).

#### B. Histology of female adrenal gland

The histological changes in the adrenal gland of the *Taphozous kachhensis* during different phases of reproductive cycle are presented in figure

#### i) Adrenal gland during sexually active period

Adrenal gland of *Taphozous kachhensis* is round to oval in shape and encloses in a connective capsule made up of spindle fibrous cells. (Fig.13). Thin strand of connective tissue or trabeculae extend from capsule between the columns of cell in the cortex. Beneath the capsule gland have two distinct zonations the cortex and medulla. On the basis of the observation on histology of the steroidogenic cells cortex is further divided in to three zones namely zona glomerulosa, zona fasciculata and zona reticularies. (fig.14)

**Zona glomerulosa:** This is the smallest zone of adrenal cortex measuring  $90\mu$  during the estrous period. This zone consists of small spherical cells that are compactly arranged and appear acinus like group of cells. These cells have lightly stained cytoplasm with small nuclei with clear nucleolus. Chromatin clumps are observed. Cytoplasm is eosinophilic and granular. Vaculation is observed in the cytoplasm of some cells. Acinar membrane is well defined. (Fig 15).

**Zona fasciculate:** This is the widest cortical zone observed in the adrenal cortex measuring  $300\mu$  during estrous period. Zona fasciculata consist of large polyhedral or cuboidal cells arrange in cords. Cytoplasm is eosinophilic and vacuolated due to the presence of lipid droplets. Nucleus is darkly stained. Zona fasciculata merges with zona glomerulosa above and zona reticularies below. The cords are separated from each other by connective tissue and blood capillaries. (Fig 16).

**Fig. 13** Transverse section of adrenal gland during sexually active period showing cortex (CX) and medulla (MD). Note the oblong shape of adrenal gland. X 40

**Fig. 14**Transverse section of adrenal gland during sexually active period showing zona glomerulosa (ZG), zona fasciculata (ZF), zona reticularies (ZR) and medulla (MD). X 1000

**Fig. 15** Transverse section of adrenal gland during sexually active period showing vacuolated cells of zona glomerulosa (ZG) with darkly stained spherical nuclei. X 1000

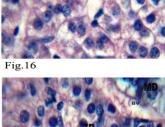
**Fig.16** Transverse section of adrenal gland during sexually active period showing elongated cell cords of zona fasciculata (ZF) with vacuolated cell cytoplasm. X 1000

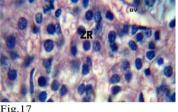
**Fig. 7** Transverse section of adrenal gland during sexually active period showing the cell of zona reticularis (ZR) are in the form anastomosing cords and having varying degree of shape and size. Each cell has eosinophilic cytoplasm with vesicular nucleus. Blood spaces are observed in the network of cell cords of zona reticularies. X 1000

**Fig. 18** Transverse section of adrenal gland during sexually active period showing medulla(MD) consist of cells arranged in irregular strands or short cords surrounded by blood capillaries. Cells contain darkly stained vesicular nucleus. Cytoplasm of cell is basophilic and granular and in some cells Vaculation is observed. X 1000

Fig.13

Fig.14





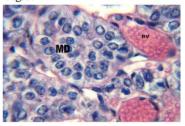
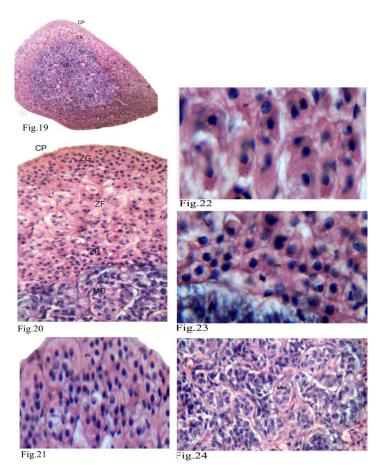


Fig.18

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**Fig. 19:** Transverse section of adrenal gland during sexually inactive showing cortex (CX) and medulla (MD). Note the triangular shape of adrenal gland. X 40 **Fig. 20:** Transverse section of adrenal gland during sexually inactive showing zona glomerulosa (ZG), zona fasciculata (ZF), zona reticularies (ZR) and medulla (MD). X 1000

**Fig. 21:** Transverse section of adrenal gland during sexually inactive showing elongated cells of zona glomerulosa (ZG) with darkly stained nuclei and vacuolation in cytoplasm. X 1000

**Fig.22:** Transverse section of adrenal gland during sexually inactive showing cells of zona fasciculata (ZF). cells are hypertrophied and nucleus is vesicular, round and darkly stained. The cytoplasm is eosinophilic and more vacuolated. X 1000

**Fig. 23:** Transverse section of adrenal gland during sexually inactive showing the cell of zona reticularis (ZR). Cell cytoplasm is eosinophilic with darkly stained nucleus and more vacuolated as compare to estrus. X 1000

**Fig. 24:** Transverse section of adrenal gland sexually inactive showing well developed medulla (MD) consists of cells arranged in acini surrounded by blood capillaries. X 1000

**Zona reticularis:** This zone is present just below the Zona fasciculata and measure about 90  $\mu$  during estrous period. The cell of Zona reticularis are in the form anastomosing cords and having varying degree of shape and size. Each cell has eosinophilic cytoplasm with vesicular nucleus. Blood spaces are observed in the network of cell cords of zona reticularies. (Fig 17).

**Medulla:** Medulla is clearly demarcated from the cortex in *Taphozous kachhensis.* It measure about  $270\mu$  during the estrous period of the reproductive cycle. Medulla consist of cells arranged in irregular strands or short cords surrounded by blood capillaries. Cells contain darkly stained vesicular nucleus. Cytoplasm of cell is basophilic and granular. In some cells Vaculation is observed (Fig 18).

# ii) Adrenal gland during sexually inactive period

Adrenal gland of *Taphozous kachhensis* is oval to elongated in shape. Cortex is reduced in size and medulla extensively developed. (Fig 19&20).

**Zona glomerulosa:** Zona glomerulosa is made up of acinar structure. Vaculation are more pronounced in the cytoplasm. Zona glomerulosa measure about 105µ. Cell cytoplasm is lightly stained with round nucleus.(Fig 21).

**Zona fasciculate:** Zona fasciculata increases in diameter and measure about 130  $\mu$ . Zona fasciculata merge with zona glomerulosa above. It is in the form of cords which are not radially arranged and irregular in distribution. Cell cytoplasm is lightly stained. Cytoplasmic vacuolation is more pronounced.(Fig 22).

**Zona reticularis :** Zona reticularis is measure about  $95\mu$  in diameter. Cells are large with faintly stained nucleus observed in the center of the cell and shows prominent nucleolus. Lipid vacuoles are seen in the cells. (Fig 23).

**Medulla:** Medulla is very large and measure about  $600\mu$  in diameter. It is made up of group of cells and surrounds by blood capillaries. The compactly arranged group of cells during early pregnancy is seen scattered during late pregnancy. Cell cytoplasm appears granular and vacuolated. (Fig 24).

# DISCUSSION

Morphometric data on the adrenal gland of different species are scarce. In the camel the cortex occupied about 74% of the gland (Ali, 1987). According to Cronshow et al. (1974), the duck cortex constitutes 68.2%, the medulla 28.6% and vascular space 3.2% of the total area. This confirmed the present study in which it was observed that bat cortex occupies 64% of the gland during sexually active. In Taphozous kachhensis, the size and weight of left adrenal gland is always higher than the right adrenal gland during different phases of the reproductive cycle. Similar observations are reported in T. melanopogon (Lawory an Lall, 1987), H. lankadiva (Dhamani, 2004), Taphozous longimanus (Shende, 2009), T. kachhensis (Chavhan et al., 2011, Bansod and Dhamani, 2013) and *P. giganteus giganteus* (Papadkar and Dhamani, 2012). The weight of the adrenal gland increases during estrus and is maximum during early pregnancy in female, it decreases at midpregnancy. However, it again increases at late pregnancy. In male the weight of the adrenal gland is lowest during sexually quiescence period, increases from preparatory (recrudescence) period and the highest during sexually active period. Similar observations are reported in bat Herpestes auropunctatus (Tomich, 1965), T. melanopogon (Lowry and Lall, 1986) and P. giganteus giganteus (Papadkar and Dhamani, 2012), T. kachhensis (Chavhan et al., 2011, Bansod and Dhamani, 2013). The relative weight of adrenal gland remains more or less constant in adults in both the sexes of Indian mongoose Herpestes edwardsii, however the right adrenal gland is observed to be smaller than the left adrenal gland. This is also related with the present findings in T. kachhensis. The cortex and medulla of adrenal gland of bat, Taphozous kachhensis are clearly marked. Similar observations were made in the bat, M. schreibersii, V. pipistrellus (Saidapur and Nadkarni, 1976), R. leschenaulti (Sapkal, 1978), M. lyra lyra (Bhima Rao and Sarkar, 1975), Cynopterous sphinx and Taphozous longimanus, R. leschenaulti, T. melanopogon (Lowry and Lall, 1986), Taphozous longimanus (Nerkar, 2009), M. lyra lyra (Sonwane, 2010), P. giganteus giganteus (Sapkal, 1978; Papadkar and Dhamani, 2012), T. kachhensis (Chavhan et al., 2011, Bansod and Dhamani, 2013).

The adrenal cortex is divided into three distinct zones viz. zona glomerulosa, zona fasciculata and zona reticularis. A distinct zones of the cortex is observed in *M. schereibersii* (Planel et al., 1961), *M. lyra lyra* (Bhima

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Rao and Sarkar, 1975), V. pipistrellus (Saidapur and Nadkarni, 1976), P. giganteus giganteus and R. leschenaulti (Sapkal, 1978), Cynopterus sphinx, H. lankadiva (Dhamani, 2004) and P. giganteus giganteus (Papadkar and Dhamani, 2012). The zona reticularis is absent in the adrenal gland but it is present in the form of islets of cortical cells in the medullary region in T. melanopogon (Lowry and Lall, 1987) and Т. longimanus (Shende, 2009; Nerkar, 2009). The adrenal gland of T. kachhensis during sexually quiescence period is oval to elliptical in shape and is enclosed by thick capsule. Just below the capsule lies a small cortical zone, the zona glomerulosa. It consists of polyhedral glomerular cells which appear group of 3-7 acini or cells. Similar structure is also observed in the zona glomerulosa of T. longimanus, Hipposideros lankadiva (Widmaier and Kunz, 1993). In Cynopterus sphinx the cells are arranged in groups. Whereas, in Rousettus *leschenaulti* and *Pteropus giganteus* the cells are arrange in columns (Sapkal, 1978) and P. giganteus giganteus (Papadkar and Dhamani, 2012).

The zona fasciculata shows presence of polyhedral cells arranged in straight column and forms a group of 2-5 cells. The cords are arranged in a redial manner that runs towards the medulla. Similar pattern of arrangement in zona fasciculata is observed in *M. lyra lyra* (Bhima Rao and Sarkar, 1975), *P. giganteus* (Sapkal, 1978, Papadkar and Dhamani, 2012), *H. lankadiva* (Dhamani, 2004) and *T. longimanus* (Shende, 2009). The irregular short cords with one or two radially arranged merge cells observed in *R. leschenaulti* (Sapkal, 1978)

The zona reticularis with anastomosing cords of varying shape and size is present just below the zona fasciculata and adjacent to the medulla. Similar morphological observations are reported in *M. lyra lyra* (Bhima Rao and Sarkar, 1975), *P. giganteus* (Sapkal, 1977; Papadkar and Dhamani, 2012), *C. sphinx* and *H. lankadiva* (Dhamani, 2004). However in *R. leschenaulti* zona reticularis is not distinctly demarcated from zona fasciculata (Sapkal, 1977). In *T. melanopogon*, the reticulum occurs in the form of islets of cells (Lowry and Lall, 1986) but is absent in *T. longimanus* (Shende, 2009).

Medulla of *T. kachhensis* shows well developed distinct demarcation. This zone consists of numerous irregular stands or short cords of 4-8 cells and few groups of 6-10 cells which are separated by sinusoids and capillaries. Similar structure of medulla is reported in *M. lyra lyra* (Bhima Rao and Sarkar, 1975), *R. leschenaulti* and *P.* 

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*giganteus* (Sapkal, 1978; Papadkar and Dhamani, 2012) supporting present observations. Two types of medullary cells in *P. giganteus* and *R.leschenaulti* on the basis of staining with basic dyes were reported (Sapkal, 1978). However, in the present study adrenal gland is fixed in Bouin's fixative followed by double staining using haematoxylin-eosin, could not produce similar results. Therefore, the present study is unable to give an account of the two types of cells in medulla.

During sexually active period there is an increase in the size of adrenal gland; it is oval to circular during sexually quiescence period and becomes oblong to elliptical in shape during sexually active period. Histoarchitectural study of adrenal gland during sexually active period is advanced over that of adrenal gland of sexually quiescence period.

The cells of glomerulosa are elongated consists of group of 7-10 large polyhedral cells with large eccentrically placed nucleus. More lipid vacuoles are observed during sexually active period than those of sexually quiescence period. During sexually active period, the zona fasciculata is well developed than the zona glomerulosa. Lipid vacuoles in the cells of zona fasciculata are increased during sexually active phase than those found in the cells of sexually quiescence period. Similar structures are observed in *H. lankadiva & T. longimanus* (Sapkal, 1978) The cells of zona reticularis are polymorphic with few vacuolations. These lipid vacuoles are less than those found in the cells of zona fasciculata of the active phase but more than the cells of zona reticularis of sexually quiescence period.

The medulla is well developed, spherical or irregular in shaped and occupies largest area as compared to the medulla of sexually quiescent bat with granular and basophilic cytoplasm. Similar morphological findings are reported in *H. lankadiva* (Dhamani, 2004).

In the present study, during lactation and post lactation, the proportion of interrenal tissue was less, which depicts that the bat were more comfortable and less stressed at this stage. Active growth and comfortable environmental temperature during this period also added the effect. However, in the subcapsular, inner and central zones, the overall mean percentage of interrenal tissue was the highest during estrus and pregnancy. This increase might be due to higher demand of the interrenal hormones for the ovulation and formation of eggs. In this bat the uterus is bicornuate and the contralateral continuous ovary shows the

folliculogenesis. Hydroxycorticosterone and desoxycorticosterone are reported to be essential for ovulation in bat. In pigeons, adrenal hypertrophy began at 108 hours before ovulation and disappeared after another 108 hour period. This also supported the role of interrenal hormones in ovulation.

During estrus the zona fasciculata is well developed and made up of polygonal cells with vesicular nuclei and vaculations are more pronounced due to lipid droplets. Thus zona fasciculata is more developed than two other zones. Such distinct features are also observed in *Taphozous melanopogon* (Lawry and Lall, 1987) during the non pregnant state. There is an increase in the size of adrenal gland during pregnancy, it is oval to elongate in shape.

Histoarchitecture of adrenal gland during early pregnancy is advanced over that of adrenal gland of estrus female bat. Adrenal gland is oval to elongated in shape. Cortex and medulla is well developed. Zona glomerulosa increase in size and measure about  $120\mu$  in diameter. This zone is wider than that of zona glomerulosa of estrus stage. Cells of glomerulosa are elongated with darkly stained nuclei. Cytoplasm is eosinophilic and Vacuolations are observed in cytoplasm.

Zona fasciculata decreases in size during pregnancy as compare to estrus period of reproductive cycle and measure about 113  $\mu$  in diameter. The cells are hypertrophied and nucleus is vesicular, round and darkly stained. The cytoplasm is eosinophilic and more vacuolated as compare to estrus period because of high lipid droplets.

During early pregnancy zona reticularis is also increase in size and measures  $105 \mu$  in diameter. The cells are hypertrophied. Cytoplasm is eosinophilic with darkly stained nuclei and more vacuolated as compare to estrus. Zona fasciculata merges with Zona reticularis without any clear demarcation.

Medulla is more developed during early pregnancy and measure about  $580\mu$  in diameter. Hypertrophied medullary cells are arranged in the acini encircling the blood spaces. Cell cytoplasm is lightly stained and granular with vaculation. Blood supply is also increased and blood vessels are observed encircling the cells. The hyper activity of adrenal gland during pregnancy in this species of female bat correlates with the findings of

Sonwane, 2010 in *Megaderma lyra lyra* and Nerkar, 2007 in *Taphozous longimanus*.

The adrenal gland in this species of female bat is oval to elongated in shape during late pregnancy. Cortex is reduced in size and medulla is extensively developed. Zona glomerulosa is made up of acinus like cells with round nucleus. Vacuolations are more pronounced in the cytoplasm. Zona glomerulosa measures about  $105\mu$  in diameter. Cell cytoplasm is lightly stained.

Zona fasciculata increases in diameter and measures about 130  $\mu$ . Zona fasciculata merge with zona glomerulosa above and zona reticularis below without any demarcation. It is in the form of cords which are not radially arranged and irregular in distribution. Cell cytoplasm is lightly stained and cytoplasmic vacuolation are more pronounced.

Zona reticularis is smaller cortical zone measuring about  $95\mu$  in diameter. Cells are large with faintly stained centrally placed nucleus and shows prominent nucleolus. Few lipid vacuoles are also seen in the cell.

Medulla is the largest zone measuring about  $600\mu$  in diameter. It is made up of group of a cells surrounded by blood capillaries. The compactly arranged group of cells observed during early pregnancy are now seen scattered during late pregnancy. Cell cytoplasm appears granular and vacuolated. The present observations are in conformity with observation reported on adrenal gland of *Megaderma lyra lyra* (Sonwane, 2010) and *Hipposideros lankadiva* (Seraphim, 2004).

Adrenal gland during lactation is oval to triangular in shape. During this stage the cortex and medulla is clearly demarcated. Zona glomerulosa is increases in size as compared to late pregnancy and measure about 136µ in diameter. A hypertrophied cell of zona glomerulosa shows granular cytoplasm and small number of lipid vacuoles as compared to the cells observed during pregnancy.

Zona fasciculata is also hypertrophied and measured about 174  $\mu$  in diameter. Cell cytoplasm is lightly stained with centrally placed nucleus and clear nucleolus. The cell cytoplasm shows large number lipid vacuoles.

Zona reticularis is smaller in diameter as compared to late pregnancy and measure about  $80\mu$  in diameter. These cells have deeply stained granular cytoplasm and

darkly stained nucleus. Blood sinuses are also seen in between the network of cells. Lipid vacuoles are seen in the cytoplasm of the cells. Size of medulla decreases and measure about  $510\mu$  in diameter. Cytoplasm is deeply stained with vesicular nucleus. The nucleolus is eccentric in position.

The acini like cell cords are seen with presence of blood sinuses at the center (Chavhan et. al., 2011). The adrenal medulla in this species of female bat composed of two type of cells. One type of cell are enveloped by the membrane and found in cluster, and the second type is lightly stained. Two type of cell is also reported in many species of bats, *Megaderma lyra lyra* (Sonwane, 2010). *Hipposideros lankadiva* (Seraphim, 2004). *Rousettus leschenaulti, Pteropus giganteus* (Sapkal, 1978), *Taphozous longimanus, Cynopterus sphinx*.

The adrenal gland produced steroids such as cortisol and cortisone during embryonic and prenatal periods in bat *Megaderma lyra lyra* (Sonwane, 2010). The functional significance of higher proportion of cortex is not fully understood, but one possible explanation could be the increased need to regulate water and electrolyte balance in ducks. It is known that the adrenal cortex produces glucocorticoids and mineralocorticoids that aid in water and electrolyte balance; thus a larger cortex is the adaptation to its habitat.

The highest IC ratio of 2:1 attained during anestrous and estrus in this study can be correlated to the physiological status. Search in the literature has shown no morphometric data on the adrenal gland of any of the chiropteran bat species in India. These structural differences in the adrenal gland of the *T. kachhensis* during reproductive period seem to indicate some physiological relationship.

The hypertrophy of cortical cells suggests that steroid secretion and elaboration from them is involved with at least some aspect of male and female production.

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