

Original Research Article

Histomorphology of certain internal organs of Mithun (*Bos frontalis*) in Eastern Himalayan region of Arunachal Pradesh

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Abstract: Mithun is found in eastern Himalayan region of India covering Arunachal Pradesh, Mizoram, Nagaland and Manipur. In Arunachal Pradesh it is reared under free grazing system at an altitude of 1000-3000m (msl). Mithun has indispensable role in socio-economic and cultural practices in local tribal population. Mithun is considered as the semi domesticated animal and found grazing in open fields. It has got links with environment, agricultural practices, ecology and overall economy of the state. Because of gradual denudation of free grazing area and sacrifice of Mithuns in festive occasions, existence of this animal is at stake. The animal being vulnerable and flagship species of Arunachal Pradesh, there is a need of detailed study and documentation of pure breed in every scientific field like pathology, histology, blood profile, hormone profile and genetics etc. for sustainable management of Mithun. To make it helpful for the scientific community, detailed histological structure of Mithun liver, kidney, adrenal gland and testis is reported for the first time in this paper. Formalin fixed, paraffin embedded sections were used for routine Eosin-Hematoxylin staining. Results showed that the Mithun liver lobule is not hexagonal in shape unlike other bovine species. Cross section of kidney showed renal corpuscle, proximal convoluted tubule and distal convoluted tubule. Well demarcated cortex and medulla is observed in section of adrenal gland of Mithun. Sperms at various stages of development are observed inside the Seminiferous tubule in cross section of Mithun testis.

Key words : Adrenal gland, Arunachal Pradesh, Kidney, Liver, Mithun, Testis

Introduction

The eastern Himalayan region having a number of endemic species is one of the biodiversity hot spots of the world (Meyers *et al.*, 2000; Brooks *et al.*, 2006). Arunachal Pradesh is situated in eastern Himalayan region which ultimately merges with the Patkai range of Himalaya. Altitudinal variation, different climatic zone, forest pattern, lakes and rivers and high rainfall is the characteristics of this biodiversity hot spot of Indian subcontinent. The forest pattern ranges from tropical humid rain forest to snow covered alpine zone at high altitude. According to the forest pattern and temperature variation, floral and faunal distribution varies in this biodiversity hot

spot. Thus, this biodiversity hot spot is enriched with endemic floral and faunal population (Gurumayum *et al.*, 2016). Mithun is one of such species of Arunachal Pradesh which is found grazing in low altitudinal hilly terrain. The state of Arunachal Pradesh is inhabited by a large numbers of tribal groups of populations. They live in different pockets of the state and depend mainly on the neighboring forest resources for livelihood (Fathima *et al.*, 2015). Certain tribal groups of the state raise Mithun (*Bos frontalis*) that has been attached with the community as indispensable part of the society (Moyong Otem, 2012). In India, total population of Mithun is 0.29

million according to the All India 19th Livestock Census (2012). Arunachal Pradesh has the highest population of Mithun, 83.48% of the total population of the country. In Arunachal Pradesh, highest population density of Mithun is recorded in Papum Pare district (Tayo *et al.*, 2013). Associated with culture, Mithun is considered as symbol of honour, respect and status in society of certain tribal population of the state (Shisode *et al.*, 2009). The history of this robust animal is as old as the tribal community of the state. The animal is offered to new relatives especially at the marriage of son and daughters. Moreover, Mithun meat is most preferred non vegetarian delicacy among certain group of tribal community of the state.

Four strains of Mithun have been identified by Bhusan *et al.* (2000). They are Arunachalee strain found in Arunachal Pradesh, Mizorami strain in Mizoram, Nagami strain in Nagaland, Manipuri strain in Manipur. These different strains differ in phenotypic and genetic characters (Mondal *et al.*, 2014). However, a detailed study of all these different strains of Mithun available in north eastern states is yet to be documented. The conservation strategy of this robust mammal needs data on its behaviour, physiology, food and feeding habitat, diseases as well as its treatments. A little data on biology of Arunachalee strain of Mithun is available. Histological study of internal organ is one of the methods for understanding cellular physiology of organism. Techniques based on histological studies provide the information of cellular functions and gene expression in particular tissue.

In this paper, we present our results on histological structure of liver, kidney, adrenal gland and testis of Mithun. This study was undertaken as there is no information on basic histology of internal organs of Mithun from Arunachal Pradesh. It has been reported that similar kind of study was done in bull, goat, cow and sheep organs. Therefore, the present study has been carried out to throw light on the histology of certain important organs of Mithun which will provide valuable information to the anatomist, pathologist and theriogenologist for management of this species in eastern Himalayan region.

Materials and methods

Sample collection

Tissue sample of internal organs (Liver, Kidney, Adrenal and testis) was collected from the animals scarified during social celebration. Animals are adult and healthy which is very often sacrificed for purpose of societal rituals as well as source of meat. During the study, samples were collected from ten different animals from three different districts viz., East Kameng, Papum Pare and East Siang of the state. Collected tissues were immediately fixed in 10% formaldehyde and brought to the laboratory for further study. Fixation was done in small pieces for 72 - 96 hours with occasional change of the fixative. Tissues were processed for routine histological study following the method of Culling (1974). Fixed tissues were washed in running tap water for 3-4 hours for removal of fixatives. The tissues were dehydrated thoroughly passing through graded alcohol and subsequently embedded in paraffin.

The paraffin embedded tissue blocks were cut in a rotary microtome in 5 ~ thick sections and stretched on poly-L-lysine coated slides. Routine Eosin Hematoxyline stain was used for staining the tissue sections. The tissue sections were observed under DM5000B Leica microscope and photomicrographed the relevant part of the tissues.

Results

In the present study, we did the histological study of liver, kidney, adrenal gland and testis of Mithun. Observations were made on internal structural organization of the different cell types, blood vessels and connective tissues in the respective tissue. Each organ has specific structural cellular organization which is well known. However, there is some variation in different species.

Liver

Liver is the largest organ of the body with varying sizes in different animals. The liver performs diverse metabolic functions. The liver lobule is the functional unit of liver which

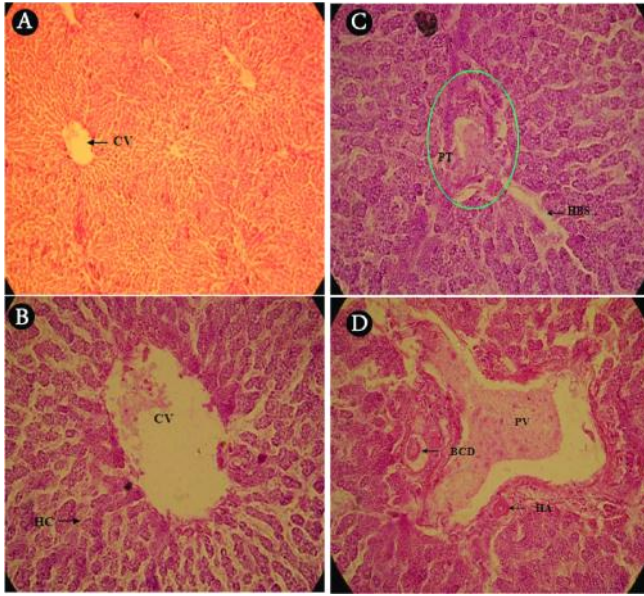


Fig. 1: Photomicrographs of histological structures of liver of Mithun. The hepatic lobule of Mithun liver is not hexagonal in shape. Hepatocytes (HC) form anastomosing plates which radiate from Central vein (CV). These anastomosing plates are separated by Hepatic Biliary Sinusoids (HBS) (Fig.1A,B). Portal tract (PT) contains part of portal vein (PV), part of bile collecting duct (BCD) and part of hepatic artery (HA) (Fig.1C,D). Original magnification: A 10x; B 20x; C 20x; D 40x.

comprises of radially organized hepatocytes around central vein. In the section of Mithun liver, circular and elliptical shaped central vein (CV) is observed (Fig. 1 A, B). The principal cells of liver are hepatocytes (HC) which are arranged to form lobules. Hepatocytes are large polyhedral cells having variable cytoplasmic appearance. Hepatocytes have nucleus in the centre and form anastomosing plates which radiate from central vein. The liver lobule of Mithun is not hexagonal in shape. There is no well defined boundary of connective tissue around the liver lobule. In certain parts of the organ connective tissue is thick while, in other part it is not visible. There is presence of some inter-lobular blood vessels in the connective tissue.

At the angles of lobular margins portal tracts are present. More than one lobule is connected to each portal tract. Each portal tract has three vessels, one large and two small vessels. The whole composite structure containing the blood vessels is known as portal triad (PT). The larger one having thin wall is the part of portal vein (PV). The portal vein possesses a thin connective tissue layer and surrounded by the hepatocytes. In histological sections, unlike the central

vein (CV), portal vein appears irregular in shape. Attached to the wall of the portal vein, the hepatic artery (HA) is present. Hepatic artery is thick walled and smaller in diameter than that of the portal vein. The bile collecting duct (BCD) lined by simple cuboidal and columnar epithelial cells is smaller in size in comparison to the portal vein appears in the portal triad (Fig. 1 C, D). The hepatocytes (HC) which are radially arranged in the hepatic lobule are clearly visible surrounding the central vein (CV) and portal triad. The radial arrangement of the hepatocytes forms the hepatic biliary sinusoids (HBS) as shown in Fig. 1C,D. These sinusoids are seen which originate from lobule margin run through anastomosing plates of hepatocytes to converge upon the central vein (Fig. 1 B).

Kidney

The kidneys are well vascularised organs whose main function is to remove toxic waste products from blood. The main functional unit of kidney is nephron. Nephron consists of two components renal corpuscle and renal tubule. In the section of Mithun kidney, numerous renal corpuscles (RC) are visible in the cortex area. Most of the part surrounding the renal corpuscle consists of proximal convoluted tubule (PCT) and distal convoluted tubule (DCT) as shown in Fig. 2A,B,C. Renal corpuscle consists of a dense, rounded structure, the glomeruli, surrounded by narrow spaces called Bowman's spaces. The structure as a whole is termed as Bowman's capsule (BC) which is prominently observed in the histological section of mithun kidney. The numerous nuclei observed in the glomerulus (G) are those of capillary endothelial cells, mesangial cells of supporting mesangium and podocytes (P) (Fig. 2 C). The afferent and efferent arterioles are seen entering and leaving the glomerulus. The afferent arteriole (Aa) has greater diameter than efferent arteriole (Fig. 2 C).

Podocytes are seen which envelop glomerular capillaries. The podocytes have pale stained cytoplasm and large rounded pale stained nucleus (Fig. 2 C). The proximal convoluted tubules are seen having prominent brush border (BB) which completely fills the lumen. Distal convoluted tubules are also seen which do not have brush border. They

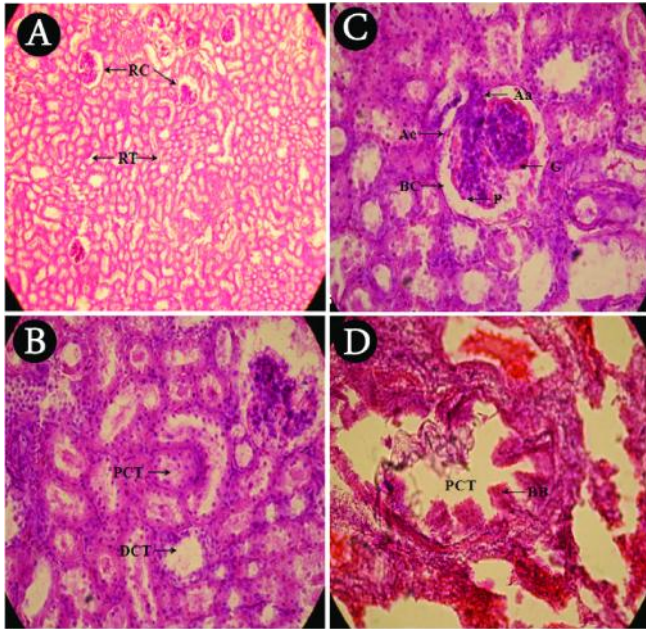


Fig.2 Photomicrographs of histological structures of Kidney of Mithun. Nephron, the basic unit of kidney consists of two parts- the renal corpuscle (RC) and renal tubule (RT) (Fig. 2A). Renal corpuscle is made up of glomerulus and Bowman's capsule (BC). The numerous nuclei observed in the glomerulus (G) are those of capillary endothelial cells, mesangial cells of supporting mesangium and podocytes (P). The afferent arteriole (Aa) having larger diameter enters the renal corpuscle, whereas, efferent arteriole (Ae) having smaller diameter leaves the corpuscle (Fig. 2C). Proximal Convoluted Tubule (PCT) and Distal Convoluted Tubule (DCT) are part of renal tubule and can be well distinguished on the basis of brush border (BB). PCT is having brush border, on the other hand DCT does not have (Fig. 2B, D). Original magnification : A 10x; B 20x; C 20x; D 40x.

have large, clearly defined lumen, pale stained cytoplasm and more nuclei in the transverse section than proximal convoluted tubule (Fig. 2 B). The number of proximal convoluted tubule is more than distal convoluted tubule.

Adrenal gland

The adrenal glands also known as supra-renal glands are a pair of endocrine glands located above kidney. A variety of hormones are secreted by adrenal glands which are beneficial for the maintenance of normal body function. The adrenal gland of Mithun is divided into two parts anatomically as well as histologically. The outer part of the gland, the cortex (AC) is distinctly different in appearance both in structural organization and staining characters, from the inner medulla portion (AM) as shown in Fig. 3 A, B. The outer cortex zone

covers more area than the inner medulla zone. Adrenal cortex of Mithun is distinctly separated into three zones viz. Zona glomerulosa, Zona fasciculata and Zona reticularis according to the arrangement of secretory cells (Fig. 3 B). Histologically, the outermost part of the adrenal cortex is a thinner zone of connective tissue called the capsule (C) of the gland (Fig. 3C). Below the capsule, the secretory cells of Zona glomerulosa (ZG) are arranged in irregular, ovoid clumps (Fig. 3C). The cells possess round and darkly stained nuclei just beneath the capsule. Zona fasciculata (ZF) is the middle of the three zones of cortical layers. This zone consists of narrow cords of secretory cells and sinusoids within spaces of cellular cords. The nucleus of these cells is also prominent like that of glomerulosa, but the cells are large with poorly stained cytoplasm. The cytoplasm of the cells of Zona glomerulosa and Zona fasciculata contain eosinophilic granules. The innermost part of the adrenal cortex is Zona reticularis (ZR). This zone is thin and is attached to the central medulla. The cords and glandular cells here are irregularly arranged and the cells are smaller in size with strongly stained cytoplasm (Fig. 3B). The adrenal gland is highly vascularised having a central vein in medulla. This central medullary vein (MV)

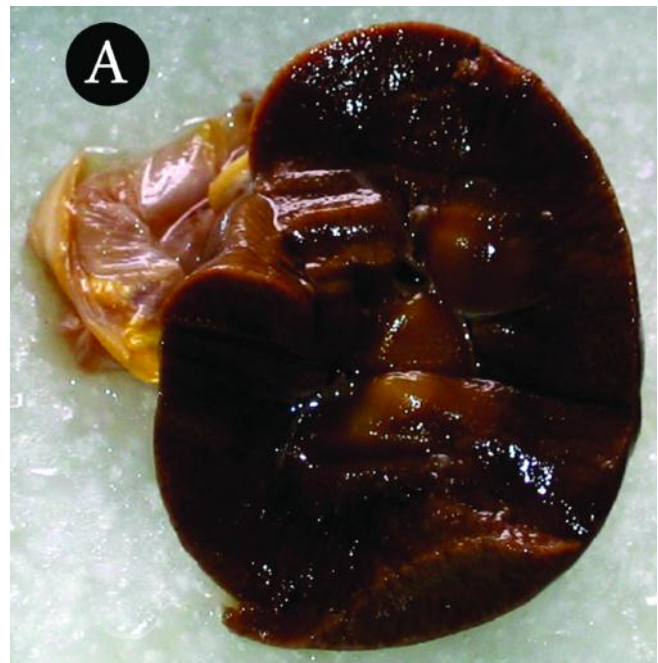


Fig. 3 A. Anatomy of adrenal gland of Mithun (transverse section). The inner medulla (AM) is surrounded by the outer cortex (AC).

expands the branches to the cortical region creating cortical sinusoids (CS) among the cords of cells (Fig. 3C)

The innermost part of the gland is the medullary zone (Fig. 3 D,E) with its distinctive pattern of cells. The boundary between medulla and cortex is not uniform in Mithun adrenal gland. Anatomically, the medulla looks to be lobular in structure (Fig. 3A). This uneven demarcation is reflected in the distribution of medullary cells in histological tissue sections. The secretory cells of adrenal medulla also known as chromaffin cells, form closely packed clumps. The staining property of adrenal medullary cells is distinctly different than that of cortical cells. In Mithun adrenal gland, two different cell types are observed. The outer medullary cells, also termed as “A” cells and inner “N” cells. The “N” cells showed a pale stain than that of “A” cells. The medulla is extremely vascularised having the central medullary vein and its anastomosing branches. The cells of this region have large nuclei. Blood vessels are significantly visible in this region.

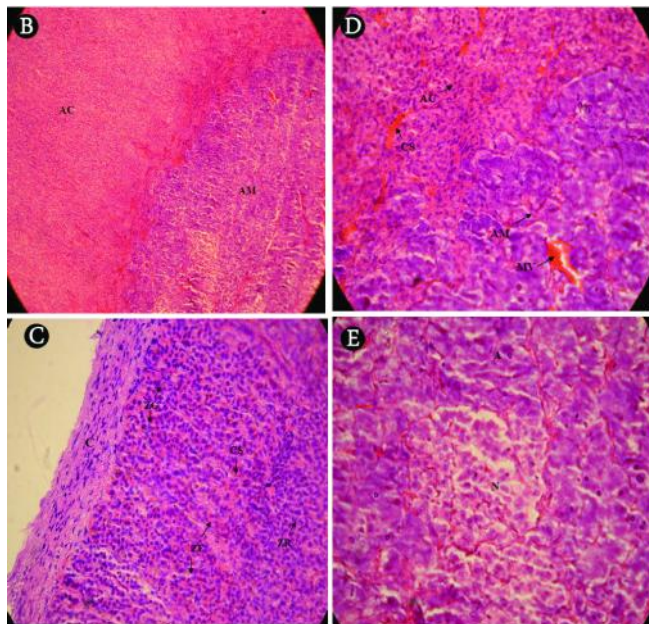


Fig. 3 B, C, D, E. Photomicrograph of histological structures of Adrenal Gland of Mithun. Cortex (AC) and Medulla (AM) can be well differentiated in the section of adrenal gland of Mithun (figure 3B). The gland is covered by capsule (C). Cortex is divided into Zona glomerulosa (ZG), Zona fasciculata (ZF) and Zona reticularis (ZR) (Fig. 3C). The medullary portion consists of outer dark stained cells termed as “A” cells and inner pale stained “N” cells. Blood vessels are also visible in medullary region (Fig. 3 D,E). Original magnification : B 10x; C 20x; D 40x and E 40x

Testis

The testis is the main organ of male reproductive system. It is enclosed by connective tissue capsule, tunica albuginea. Most part of testis is made up of seminiferous tubule from which spermatozoa are produced. A cross section of Mithun’s testis (Figure 4A-C) shows several seminiferous tubules that are circular, elliptical and elongated in shape. The tubules are separated by connective tissue septum from each other. Inter-tubular connective tissue lining is prominent in Mithun testis (Fig.4B). The Leydig cells (LC) appears at the inter-tubular space of testis (Fig.4 B). The tunica vasculosa (TV) appears at the surface of testis extends its branches into the inter-tubular space forming small blood vessels (V). A single seminiferous

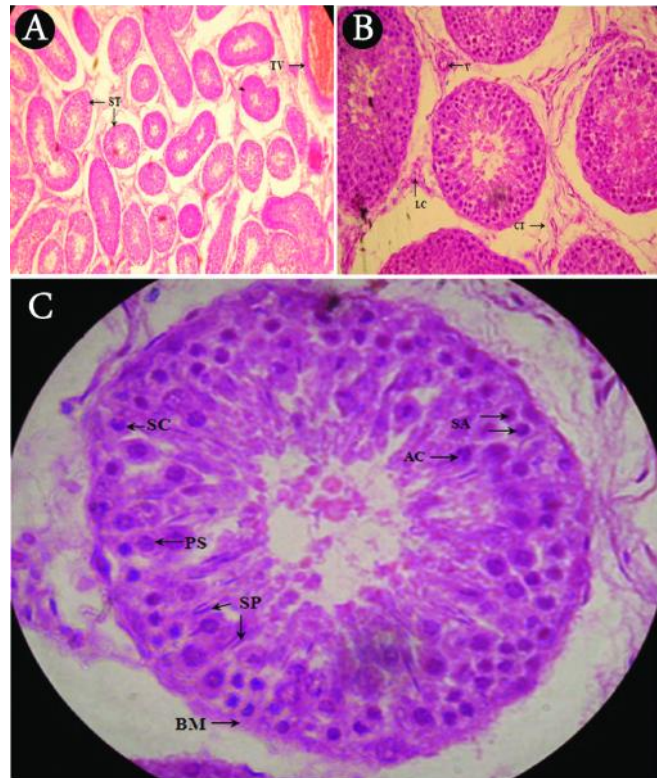


Fig. 4 Photomicrograph of histological structures of Testis of Mithun. The testis of Mithun is packed with numerous coiled Seminiferous tubule (ST). Leydig cells (LC), blood vessel (V) are found in the connective tissue (CT) between seminiferous tubule. The tunica vasculosa (TV) is present beneath the surface of testis and extends its branches into the inter-tubular space forming small blood vessels (Fig. 4A,B). The spermatogonia (SA) are found in the basal layer of seminiferous epithelium which further divides to primary spermatocyte (PS) and finally the elongated sperms (SP). The Acrosomal Cap (AC) is present at the tip of spermatid. The Sertoli cells (SC) are found towards the basement membrane (BM) of seminiferous tubule (Fig. 4C). Original magnification : A 10x; B 20x; C 40x.

tubule with spermatogenic cells and sertoli cells is shown in Fig. 4 C. The tubule is surrounded by the basement membrane (BM). The histological study of the seminiferous tubule showed multiple layers of different stages of spermatogonial cells starting from the basement membrane to the innermost luminal part. The first layer of spermatogonia (SA) appears with darkly stained nuclei and round in shape attached to the basement membrane of the tubule. The spermatogonia divides to form primary spermatocytes (PS) which are larger than spermatogonia and recognized by their extensive cytoplasm and their large nuclei containing either coarse clumps or thin threads of chromatin (Fig.4C). During this developmental stages of the spermatogonia, the spermatocytes and the spermatids are observed with the deposition of acrosome granules forming the acrosomal cap (AC) surrounding the nucleus of the spermatids. The sertoli cells are found supporting the spermatogenic cells. Sertoli cells (SC) which are little elongated in shape appear lightly stained. Very often, elongated spermatozoa are observed attached to the Sertoli cells. However, the elongated spermatozoa are commonly observed towards the lumen of the seminiferous tubules. The lumen of the seminiferous tubules is seen to be filled with lightly stained granular structures, the component of the seminiferous fluid.

Discussion

The Eastern Himalayan region comprising Arunachal Pradesh is one of the global biodiversity hotspots. In this zone, Mithun is one of the unique herbivorous bovine species which is available only in four (Arunachal Pradesh, Nagaland, Manipur and Mizoram) out of eight North-East states (Mukherjee *et al.*, 2010) of India. This animal is not completely domesticated as the animal is reared in wild condition with no human input except occasional salt lick. Mithun is extremely docile animal and prefers cold and mild climate (Das *et al.*, 2011). This is an underutilized animal as the quality of Mithun milk and leather is good in quality as reported by the owner of animals in the state. Due to the slaughtering of Mithun, declining feed or fodder resources, inadequate Mithun health coverage, there

is a need to conserve the mighty species. To conserve this animal, adequate measure like conservation of natural habitat, cultivation of natural fodders, improvement of breeding, adequate health coverage is the need of the hour in the state. A detailed scientific evaluation of the food, habit and habitat, physiology and genetics is unavoidable to achieve the goal of conservation of the species. Basic histological study of internal organs is one of such approaches to understand the physiology of the animal.

In this paper, histological structural organization of some of the organs of Mithun was studied. Histology of liver, kidney, adrenal gland and testis as well as other internal organs of various domesticated and farm animals have been elaborated in various literatures (Schatten, 2007; Paul *et al.*, 1991). Gross anatomy and structural organization of respective organs in various mammalian species are found to be common. Mithun is available in hilly region of eastern Himalayan and Indo-Burma biodiversity hotspot of Indian subcontinent. Its physiological adaptations are restricted to this humid temperate forest of north east India. In such situation a fine organizational variation of cell types in respective organs can't be ignored.

The liver is composed of multiple lobes in common domesticated animal. The functional subunit of liver is hepatic lobule separated by septa of connective tissue. In Mithun, the connective tissue boundary is not well defined, so the shape of liver lobule is not hexagonal. On the other hand, in cattle like cow, sheep and goat, hepatic lobule is hexagonal in shape (Madhan and Raju, 2014). Central vein is located approximately at the centre of hepatic lobule. Another common structure in hepatic tissue is the formation of triad which is observed in Mithun liver too. 70-80 % of liver mass is the hepatocytes which are organized in cords creating the hepatic biliary sinusoids for draining the bile to hepatic duct. Invasion of macrophages is an indispensable cellular character of hepatic tissue in mammal. In present investigation, neutrophil invasion in the hepatic tissue was not detected. It has been speculated that macrophages in the Mithun liver could be detected by other methodology of staining of hepatic tissues.

Like other bovine species, the Mithun kidney consists of two main components-renal corpuscle and renal tubule (Qureshi *et al.*, 2013). Renal corpuscle is made up of two structures, Bowman's capsule and glomeruli. A single layer of flattened cells form the Bowman's capsule. Glomerulus is round structure which is formed by coiled network of anastomosing capillaries which invaginates Bowman's capsule. The afferent arteriole having large diameter enters the glomerulus and efferent arteriole with smaller diameter leaves the glomerulus. Proximal convoluted tubules are longer than distal convoluted tubule, hence cover major portion of the cortex of kidney. Proximal convoluted tubules have well developed brush border which can be seen in other bovine species also. Distal convoluted tubules do not possess brush border.

The adrenal gland is covered by capsule made up of connective tissue. Two different cellular zones viz. outer cortex and inner medulla is anatomically visible in transverse section of the gland. The differential staining properties of adrenal cortex and medulla is well known (Qureshi *et al.*, 2013). In Mithun, the gland is covered by thin connective tissue layer. Below the capsule, three layers of cortical tissues are present which is similar to adrenal cortex of domestic and farm animals of herbivorous nature like cow, bull, heifers (Jelinek and Konecny, 2011) etc. In Mithun adrenal histology, the central zone of adrenal cortex shows higher sinusoids among the cords of the Zona fasciculata. The cells of Zona glomerulosa and Zona fasciculata are responsible for synthesis of mineralocorticoids and glucocorticoids. However, there is no symptom of formation of nodular hyperplasia in Mithun unlike many other domesticated animals (Capen, 2007). The Zona reticularis, which is the innermost zone of cortex, is known to synthesize the androgens. Similar to many other ruminants, the Mithun adrenal reticularis cells are arranged in more compact manner in comparison to the glomerulosa and fasciculata. The cells are arranged in discrete form of cortical parenchyma and extend to the medullary chromaffin tissues. The adrenal medulla of Mithun comprises of polygonal chromaffin cells. The medulla of cattle has an outer region

having darkly stained cells ("A cells") known to synthesize adrenaline while the inner lightly stained cells ("N cells") synthesize nor-adrenaline (Smollich, 1992; Dellmann, 1993, Bacha and Bacha, 2000; Hullinger and Andrisani, 2006; Greco and Stabenfeld, 2007). Similar to other animals like sheep, pig and horse, Mithun adrenal medulla showed the "A" cell at the periphery and "N" cell at the innermost part of medulla.

Study of testicular development and its histology is inevitable to understand the reproductive biology of the species. Bovine testicular studies on measurements (Coulter and Foote, 1979), testicular development and spermatogenesis in Holstein bull (Curtis and Amann, 1981), biometry of bull testis (Gofur *et al.*, 2007) provides fundamental information of cattle testis. Similar study of Mithun of Arunachal Pradesh is required to understand base level information on male reproduction of the animal. The testicular parenchyma which is mainly composed of seminiferous tubules and the Leydig cells which are responsible for testosterone biosynthesis is known for decades (Copenhaver *et al.*, 1978; Dellmann and Eurell, 1998; Hafez, 2000). Seminiferous tubules are separated by connective tissue septa with the presence of Leydig cells and blood vessels at the intertubular space. The testicular histoarchitecture of Arunachalee Mithun has been found to possess similarities with that of *Bos indicus* (Gofur *et al.*, 2008). The periphery of seminiferous tubule is a connective tissue layer along with smooth muscle cells in Mithun testis. Multiple layers of spermatogonial cells are orderly arranged in the seminiferous tubules. Spermatogonia are arranged at the periphery of the tubules; accordingly the primary spermatocytes, spermatids and elongated sperms appear towards the lumen of the tubules. A quantitative analysis of the germ cells inside the seminiferous tubules is quite important to determine the reproductive potentiality of the animal. Quantitative histology of testis for study of sperm production has been reported in boar (Kennelly and Foote, 1964) and short horn bulls (Swienstra, 1966). It is reported that reduction in the number any type of spermatogonial cells may cause infertility in males (Paulsen, 1968)

The present study on Arunachalee Mithun has forwarded a base level data on certain internal organs of the animal. However, histological study using routine H&E staining is not sufficient to understand cellular organizational detail of the organs. Studies at cellular and molecular level targeting specific molecules in the cell shall provide information to understand the physiology of the animal.

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