



# Histomorphological Study of Major Organs in Mithun (*Bos frontalis*)

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## ABSTRACT

The study was conducted during (October–December) 2024 at ICAR National Research Centre on Mithun, Nagaland, India. This study investigated the histological features of skin, kidney, liver, lung, and heart tissues collected from adult mithun specimens. The mithun (*Bos frontalis*), a semi-domesticated bovine species native to mountainous regions of Northeast India, also known as Gayal or mountain cattle. Despite its cultural and economic significance, detailed histological data on mithun organs remained limited. Histological studies served as essential tools in veterinary science, providing detailed insights into tissue structure and function, which were fundamental for understanding normal physiology, identifying pathological changes, and supporting species-specific medical and conservation strategies. Post-mortem tissue samples were fixed in 10% neutral buffered formalin and processed using standard histological techniques. The skin displayed typical mammalian organization with epidermis (comprising stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale), dermis, and hypodermis. The kidney exhibited distinct cortical and medullary regions, with the cortex containing glomeruli, proximal convoluted tubules, and distal convoluted tubules. The liver was encapsulated by Glisson's capsule with hepatocytes forming the parenchymal structure. The lung parenchyma consisted of alveoli lined with simple squamous epithelium, interspersed with bronchioles lined by simple cuboidal epithelium. Cardiac tissue revealed branched cardiac muscle fibers containing one or two centrally located nuclei, with characteristic intercalated discs between adjacent fibers. This study established baseline histological parameters for major organs in mithun, contributing valuable information for comparative anatomy, veterinary diagnostics, and conservation efforts.

**KEYWORDS:** Histology, skin, kidney, liver, lung, heart, mithun

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**Data Availability Statement:** Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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## 1. INTRODUCTION

The mithun (*Bos frontalis*) is a robust bovine species endemic to the hilly terrains of Northeast India and parts of Southeast Asia. This semi-domesticated animal, commonly referred to as Gayal or mountain cattle, is believed to have descended from the wild Indian gaur or bison approximately 8,000 years ago (Dorji et al., 2021). The highest global population of mithun is found in the Northeastern region of India, particularly in Arunachal Pradesh, followed by Nagaland, Manipur, and Mizoram. These four states together account for the majority of India's mithun population, with Arunachal Pradesh alone contributing over 80% of the total. Animals in these regions are reared under free range system of management in a vast hilly pasture (Borkotoky et al., 2024). Additionally, mithun inhabits neighboring countries including Bangladesh, Myanmar, and Bhutan (Mukherjee et al., 2022). These animals are well-adapted to high-altitude environments, thriving in forested hills at elevations ranging from 300 to 3,000 m above sea level. Notably, the biomechanics of their vertebral column facilitate efficient movement on steep slopes, providing stability and agility in rugged terrains (Chungath and Kima, 2018).

Mithun plays a central role in social and cultural practices of many ethnic communities in the northeastern regions of India. It is used as an important commodity in marriage ceremonies and as compensation for social offenses and settling disputes (Chatterjee, 1995; Nemching, 2008; Moyong, 2012), represents wealth and status (Mahanta, 2008), and is considered a sacred animal for sacrifice, rituals, and festivals (Apum and Nimasow, 2015).

Despite its ecological and cultural significance, detailed anatomical and histological studies on mithun remain scarce. Understanding the normal histological architecture of major organs is essential for establishing reference standards that can aid in disease diagnosis, physiological studies, and conservation efforts. Previous studies have primarily focused on comparative histology of specific anatomical features such as nasal structures (Kalita et al., 2003), muzzle (Kalita and Kalita, 2004), and cranial morphology (Choudhary et al., 2020). These comparative studies with other ruminants including cattle and yak, revealed both conserved and species-specific anatomical features that may reflect evolutionary adaptations to environmental and geographical conditions. However, currently available data remains fragmented and insufficient to construct a reliable histological baseline for mithun.

Mithun populations are experiencing a threat, primarily due to habitat loss, inbreeding, and the absence of structured breeding programs. Although the 20<sup>th</sup> livestock census of India showed an overall increase of 29.93% of mithun

population (Anonymous, 2019), certain regions showed a decline of up to 33.69% that is mainly caused by foot and mouth disease and uncontrolled slaughtering for various purposes. Moreover, habitat fragmentation, driven by deforestation and human encroachment, has reduced the availability of grazing lands, forcing mithuns into smaller, isolated populations (Vikram et al., 2023). This isolation has led to increased inbreeding, resulting in reduced genetic diversity and potential inbreeding depression (Ponraj, 2018). Furthermore, the absence of scientific breeding strategies and limited access to quality breeding bulls compromise both the health and productivity of mithun populations (Faruque et al., 2015). Establishing histomorphological reference standards for vital organs is therefore critical for monitoring subclinical pathology, guiding health assessments, and supporting conservation breeding programs. The present investigation was hence designed to document and describe the skin, kidney, liver, lung, and heart of mithun, addressing a significant gap in the current knowledge base regarding this unique bovine species.

## 2. MATERIALS AND METHODS

The study was conducted during (October–December) 2024 at the institute farm of ICAR National Research Centre on Mithun, Nagaland, India. The present study was conducted on the skin, kidney, liver, lung and heart of mithun (n=6). Histopathological study was carried out at Department of Anatomy and Histology, College of Veterinary Science, Assam Agricultural University, Assam, India for a period of one year. Postmortem tissue samples were collected and fixed in 10% neutral buffered formalin solution and were processed as per the standard technique of procedure of Luna (1968). The paraffin blocks were sectioned in Shandon Finesse microtome at 5µm thickness, and the sections were stained with Mayer's Haematoxylin and Eosin staining technique for Cellular details as per the standard method of Luna (1968).

## 3. RESULTS AND DISCUSSION

### 3.1. Skin

The skin of Mithun exhibited a typical mammalian organization consisting of three distinct layers: epidermis, dermis, and hypodermis. The epidermis was further differentiated into five strata: stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale (Figure 1). The stratum basale was observed resting on a well-defined basement membrane. The dermis contained numerous dermal papillae projecting toward the epidermis. The hypodermis was characterized by the presence of dermal papillae, sebaceous glands, hair bulbs with distinct roots, sweat glands, and a subcutaneous fatty layer. Examination of hair follicles revealed a bi-

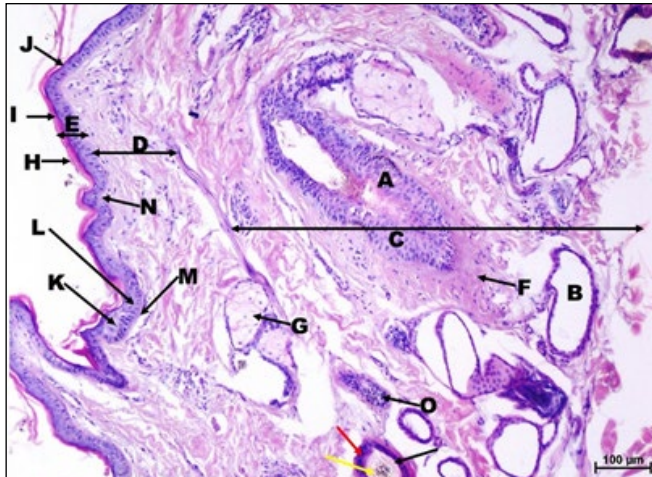


Figure 1: Photomicrograph showing the epidermis (E), dermis (D), hypodermis (C), stratum corneum (H), stratum lucidum (I), stratum granulosum (J), stratum spinosum (K), stratum basale (L), basement membrane (M), dermal papillae (N), sebaceous gland (G), hair bulb (A), root of the hair bulb (F), sweat gland (O), subcutaneous fatty layer (B), cuticle (red arrow), cortex (black arrow) and medulla (yellow arrow) of skin of mithun (*Bos frontalis*), H and E, 10×

compartmental structure composed of an outer cortex and central medulla, with the cortex being enveloped by a protective cuticle layer.

### 3.2. Kidney

The kidney displayed clear demarcation between the outer cortex and inner medulla. Within the cortical region, proximal convoluted tubules, distal convoluted tubules, and glomeruli were readily identifiable. Each glomerulus exhibited two distinct layers—parietal and visceral—and featured two poles: vascular and urinary (Figure 2). Morphometric observations revealed that proximal convoluted tubules possessed significantly larger diameters compared to distal convoluted tubules. Additional structures identified included extraglomerular mesangial cells, macula densa cells, juxtaglomerular apparatus, and medullary rays extending from the medulla into the cortex (Figure 3).

### 3.3. Liver

The liver was encapsulated by Glisson's capsule (Figure 5), a fibrous connective tissue sheath. The parenchyma predominantly consisted of hepatocytes arranged in anastomosing cords (hepatic cords) (Figure 4). Between these cords, hepatic sinusoids lined with von Kupffer cells were observed. Portal triads, comprising the hepatic vein, hepatic artery, and bile duct, were distributed throughout the parenchyma at regular intervals.

### 3.4. Lung

The pulmonary parenchyma was characterized by numerous alveoli lined with simple squamous epithelium. Interspersed

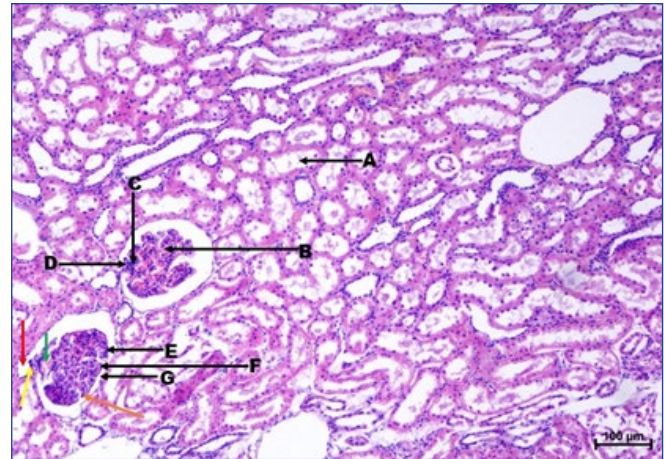


Figure 2: Photomicrograph showing the proximal convoluted tubules (A), glomerulus (B), vascular pole (D), Extraglomerular mesangial cells (C), visceral layer of glomerular capsule (E), parietal layer of glomerulus (G), urinary pole (F), distal convoluted tubules (red arrow), macula densa (yellow arrow) and juxtaglomerular apparatus (green arrow) of kidney of mithun (*Bos frontalis*), H and E, 10×

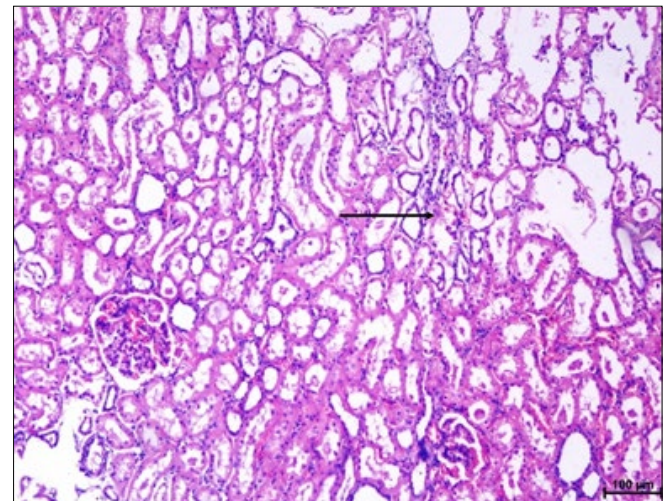


Figure 3: Photomicrograph showing the medullary ray (black arrow) of kidney of mithun (*Bos frontalis*), H and E, 10×

among the alveoli were bronchioles lined with simple cuboidal epithelium. In cross-section, these bronchioles exhibited a distinctive stellate configuration, surrounded by a layer of smooth muscle. Bronchus-Associated Lymphoid Tissue (BALT) was identified in close proximity to bronchioles (Figure 6). Cross-sections of pulmonary blood vessels were also observed throughout the lung tissue.

### 3.5. Heart

Cardiac tissue examination revealed branched cardiac muscle fibers, with each fiber containing one or two centrally positioned nuclei. Characteristic intercalated discs were observed between adjacent cardiac muscle fibers (Figure 7), representing the specialized junctional complexes unique



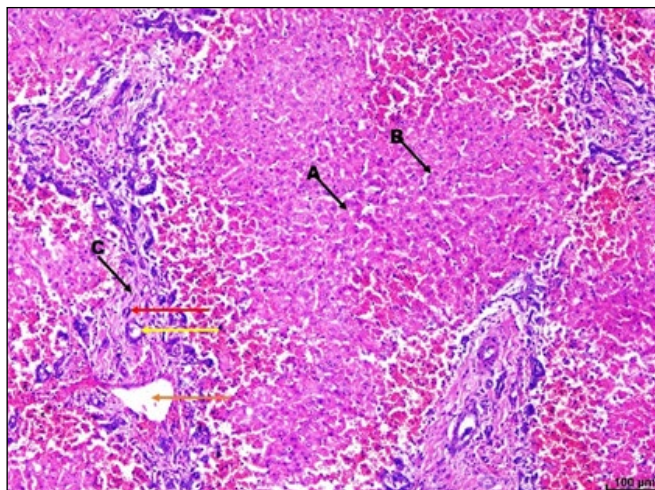


Figure 4: Photomicrograph showing the hepatic cord (A), hepatocytes (B), hepatic vein (orange arrow), bile duct (yellow arrow), hepatic artery (red arrow) and connective tissue (C) of liver of mithun (*Bos frontalis*), H and E, 10×

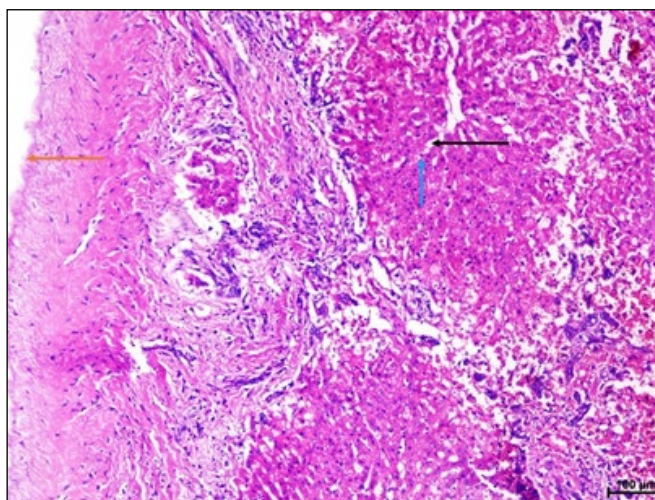


Figure 5: Photomicrograph showing the Glisson's capsule (red arrow), hepatic sinusoids (black arrow), Van Kupffer cells (blue arrow) of liver of mithun (*Bos frontalis*), H and E, 10×

to cardiac tissue.

The histological organization of mithun skin demonstrated the classical three-layered structure typical of mammalian integument. This finding aligned with observations reported by Kapadnis and Thakur (2018) in crossbred cattle. The five-layered stratification of the epidermis-stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale-mirrors the epidermal organization described by Alsodany et al. (2019) in Jenubi cattle and their crossbreeds. Similar findings were reported by Mainde et al. (2018) in goats. The presence of dermal papillae, sebaceous glands, and sweat glands in the dermis and hypodermis reflected the typical organization of bovine skin, suggesting functional adaptations for thermoregulation. In

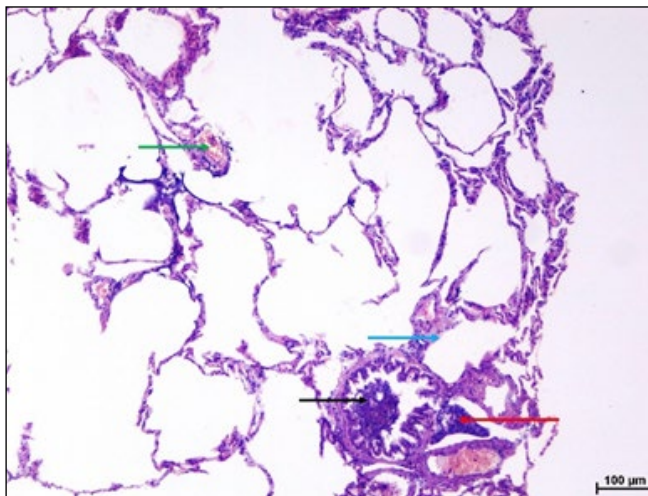


Figure 6: Photomicrograph showing the bronchiole (black arrow), Bronchus Associated Lymphoid Tissue (red arrow), simple squamous epithelium of alveoli (blue arrow) and blood vessels (green arrow) of lung of mithun (*Bos frontalis*), H and E, 10×

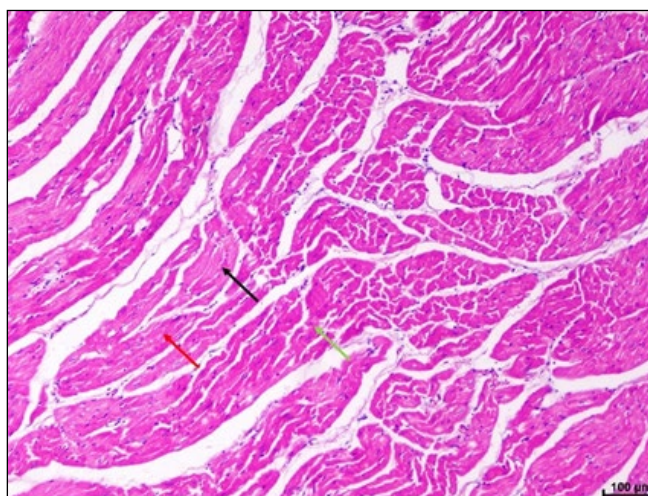


Figure 7: Photomicrograph showing the cardiac muscle fibres (black arrow), nucleus of cardiac muscle fibres (red arrow) and intercalated disc (green arrow) of cardiac muscle of mithun (*Bos frontalis*), H and E, 10×

Brahman cattle, for instance, the dermis is characterized by a thinner structure and larger sweat gland areas compared to Angus cattle, indicating breed-specific adaptations for thermoregulation (Mateescu et al., 2023).

The renal architecture of mithun displayed the characteristic corticomedullary differentiation typical of mammalian kidneys. Our observations of proximal convoluted tubules, distal convoluted tubules, and glomeruli in the cortical region corroborated the findings of Talukdar et al. (2016) in mithun from the Eastern Himalayan Region of Arunachal Pradesh and Jabbar et al. (2018) in goat. The notable difference in diameter between proximal and

distal convoluted tubules suggested specialized functional adaptations in the nephron segments for reabsorption and secretion. The identification of extraglomerular mesangial cells, macula densa, and juxtaglomerular apparatus indicated a well-developed renal regulatory system for controlling glomerular filtration.

The hepatic organization in mithun, with its Glisson's capsule and parenchymal arrangement of hepatocytes in cords, demonstrated the classical lobular structure of mammalian liver. The presence of hepatic sinusoids lined with von Kupffer cells and regularly distributed portal triads containing hepatic vein, hepatic artery, and bile duct aligned with previous findings in mithun (Talukdar et al., 2016) as well as in goat and sheep (Madhan and Raju, 2014). This arrangement facilitated efficient hepatic blood flow and bile transport, essential for the liver's metabolic and detoxification functions.

The pulmonary histoarchitecture of mithun, characterized by alveoli lined with simple squamous epithelium, corresponded with observations reported by Sinha et al. (2022) in Black Bengal Goat and Garole Sheep. The stellate appearance of bronchioles in cross-section, with their cuboidal epithelium and surrounding smooth muscle layer, indicated structural adaptations for airway control. These findings were in total agreement with the findings of Yousif (2021) in goat of Baghdad province. The identification of Bronchus-Associated Lymphoid Tissue (BALT) near bronchioles suggested a robust local immune defence system in the respiratory tract of Mithun, potentially providing protection against inhaled pathogens and particulates.

The cardiac musculature of mithun exhibited the characteristic branched fiber arrangement with one to two nuclei and distinctive intercalated discs between adjacent fibers. Our observations aligned with findings reported by Bacha and Wood (1990) in domestic animals. The presence of intercalated discs was particularly significant, as these specialized junctional complexes facilitated rapid electrical impulse propagation essential for coordinated myocardial contraction. It was also noticed that the cardiac myocytes were irregular polygonal cells of different sizes with centrally placed large round nucleus and showed striations in longitudinal section. Similar findings were opined by Chaurasia and Menaka (2023) in goat.

The histological findings across all five organs examined provided a comprehensive baseline for normal tissue architecture in Mithun. These observations not only contributed to our understanding of this semi-domesticated bovine species but also established reference standards that could aid in disease diagnosis and comparative anatomical studies. Future research incorporating histochemical and immunohistochemical techniques would further elucidate

the functional specializations of these organs in mithun.

#### 4. CONCLUSION

A comprehensive histological characterization of five major organs in mithun, establishing baseline microanatomical features. The findings served as reference standards for normal tissue architecture, supporting future research in veterinary diagnostics and conservation. Observed structures were consistent with known ruminant histology, highlighting the species' typical mammalian organ organization. These results offered valuable insights into the biology of this culturally and economically important bovine, encouraging further studies using advanced techniques to deepen understanding.

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