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HISTOMORPHOLOGICAL OVERLOOK OF LIVER IN KING COBRA (*OPHIOPHAGUS HANNAH*)

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ABSTRACT

King cobra (*Ophiophagus hannah*), the world's longest venomous snake, belonging to the family Elapidae, is endemic to forests from India through Southeast Asia. Gross and histological structure of liver in King cobra were studied using a specimen brought for post-mortem at Veterinary Hospital, Kannur. Specimens for histological study were fixed in 10 per cent neutral buffered formalin (NBF) for 48 hours, after recording the gross features. The fixed specimens were processed using routine procedure. Sections of 5 µm were stained using Haematoxylin and Eosin, and Masson's trichrome. Grossly, liver was elongated with narrower ends and dark brown in colour. The gland was encapsulated in a glistening connective tissue capsule. The liver weighed 400 g and the measured values of length, width and thickness were 61.0 cm, 5.5 cm and 1.5 cm respectively. Microscopic examination did not reveal the typical hexagonal hepatic lobules. The connective tissue in the hepatic parenchyma supported branches of portal vein, hepatic artery and bile duct. Vessels and ducts representing central vein and portal triad were arranged randomly. Arrangement of hepatocytes varied between that of glandular alveoli and tubules. Further, hepatocytes were separated by capillary sinusoids. Each alveolus presented three to eight hepatocytes. The sinusoidal wall presented endothelial cells with flattened nucleus and occasional Kupffer cells. Sinusoids contained oval and nucleated red blood corpuscles. The hepatocytes were polyhedral with vacuolated cytoplasm and presented round nucleus with distinct nucleolus. Parenchyma also presented melanomacrophages and heterophils.

Keywords: Liver, Hepatocytes, Kupffer cells, Melanomacrophages

INTRODUCTION

King cobra (*Ophiophagus hannah*), the world's longest venomous

snake, belonging to the family Elapidae, is endemic to forests from India through Southeast Asia. It is the national reptile of India. Despite the word "Cobra" in its common name, this species does not belong to genus Naja but it is the sole member of its own species Hannah. The word *Ophiophagus* means "snake eater". The presence of chevron and a pair of occipital scales are its special features. It feeds upon other snakes and occasionally on lizards and rodents. Adaptation of snakes to wide range of habitat is enabled through a special metabolic system, in which the liver plays a significant role. Functions of liver including glycogen storage, detoxification and plasma protein synthesis as reviewed by Ahmed *et al*. (2018), imparts importance to the studies on snake liver. Deforestation and extensive killing of king cobra for traditional medicine preparation limited its number, and presently the species are classified under IUCN vulnerable list. In ecosystem, it acts as bio-control agent for other venomous snakes. There is a gap in our understanding of the comparative liver histology between mammalian and non-mammalian vertebrates due to the lack of morphological studies of the liver from non-mammalian species, especially reptiles. Hence, the present study was undertaken with the aim of exploring the gross and histological features of the liver in the king cobra (*Ophiophagus hannah*).

MATERIALS AND METHODS

The liver was collected from a king cobra brought for post mortem at Veterinary Hospital, Kannur. Specimens for histological purpose were fixed in 10 per cent neutral buffered formalin (NBF), for 48 hours, after recording the gross features. The fixed specimens were washed, dehydrated and embedded in high melting paraffin (Melting Point 58-60°C). Serial sections of 5µm thickness were made and stained using Haematoxylin and Eosin (H&E) for routine studies and Masson's trichrome method for connective tissue.

RESULTS AND DISCUSSION

The liver of king cobra was elongated (Fig. 1) with narrower ends and had dark brown colour in fresh state. This is in partial agreement with the observations of Ahmed *et al.* (2018) in Nile monitor. Liver was covered by a glistening connective tissue capsule. The liver weighed 400 g and the measured values of length, width and thickness were 61.0 cm, 5.5 cm and 1.5 cm respectively. Liver is considered as the largest internal organ filling the space between heart and stomach adjacent to lungs (Ahmed *et al*., 2018).

Histologically, cross section of liver showed atypical organisation of hepatocytes. The hepatic parenchyma was surrounded by a connective tissue capsule

Fig. 1 Liver of King cobra. 1. Caudal Venacava

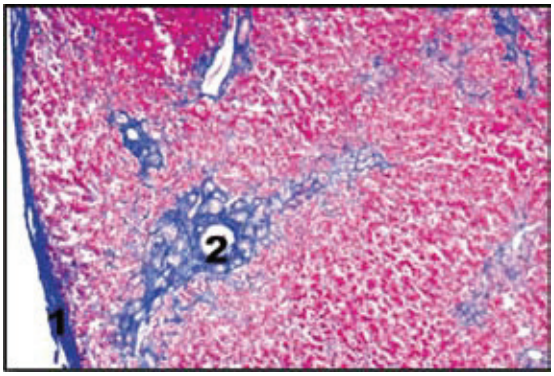

Fig. 2 Liver of King Cobra. Masson's trichrome x 40 1. Capsule 2. Portal triad

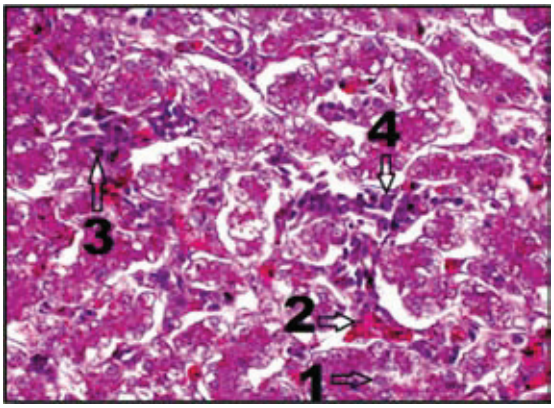

Fig. 3 Liver of King cobra. H& E x 400
1. Hepatocyte 2. Melano-macrophage
3. Kupffer cells 4. Heterophils

interlaid by smooth muscle fibres (Fig. 2). The parenchyma did not conform to that of typical hexagonal hepatic lobule. According to Boonyoung *et al.* (2017) hepatic parenchyma in water snakes, consisted mainly of hepatocytes and hepatic sinusoids. The connective tissue in portal area contained branches of the portal vein, hepatic artery and bile duct (Fig. 2). The bile ducts were lined with simple columnar epithelium. The vessels and ducts corresponding to central vein and portal triad were randomly distributed throughout the hepatic parenchyma. Hepatocytes were disposed in singles and in small clusters which in turn were separated by thin fibrovascular stroma as per the microscopic outline of liver described by Odokuma and Omokaro (2015) on the vertebrate liver. Arrangement of hepatocytes varied between that of glandular alveoli and tubules. Hepatocytes were separated by capillary sinusoids. Each alveolus presented three to eight hepatocytes which was in accordance to the findings in Nile monitor by Ahmed *et al.* (2018). The earlier reports described that the hepatic parenchyma of mammalian liver contained one layer of hepatocytes arranged in plates or cords radiating from the central vein and lining the sinusoids. However, the hepatocytes appeared in clusters and cords in birds or groups of single and clustered cells in reptiles (Odokuma and Omokaro, 2015). This peculiar distribution of the hepatocytes may be attributed to phylogeny owing to a primitive distribution of liver cells.

The hepatocytes were polyhedral with abundant vacuolated cytoplasm and single, large, rounded, eccentric and vesicular nucleus with prominent dark nucleolus (Fig. 3). Ahmed *et al.* (2018) reported that these vacuoles stained positive for lipids in semithin sections fixed with osmium tetroxide. The hepatocytes contained variable amounts of granules, which were confirmed to be glycogen as per the reports by Ahmed *et al.* (2018) but in the present study these granules were not found due to failure of food intake for two weeks. The sinusoidal walls were lined with endothelial cells having flattened nucleus. Sinusoids contained oval and nucleated red blood corpuscles. The differences exhibited by histologic architecture of the reptilian liver in the present study may be attributed to species specific metabolic activities, adaptational changes, mode of nutrition and phylogenic organizational peculiarities.

The hepatic tissue in the present study contained highly pigmented melano-macrophages in the hepatic parenchyma (Fig. 3), which appeared as clumps of cells with dark brown pigments. These cells were reported to be phagocytic and were peculiar in reptiles (Johnson *et al*., 1999). The cells were not found in the liver of mammalian or avian species (Firmiano *et al.*, 2011) and thought to be useful as monitors for environmental pollution (Agius and Roberts, 2003). Stellate Kupffer cells were seen lining the sinusoidal lumen (Fig. 3), which was also considered to be phagocytic. Heterophils were also found between the hepatocytes and were characterized by acidophilic granulated-cytoplasm and eccentric nucleus (Fig. 3).

SUMMARY

Morphohistological observations of liver of king cobra (*Ophiophagus hannah*) was studied using a specimen brought for post mortem at Veterinary Hospital, Kannur. The organ was elongated and was encapsulated by a glistening connective tissue capsule externally. Parenchyma was devoid of distinct lobulation and was flooded with clusters of hepatocytes separated by sinusoids. A random arrangement of the vessels and ducts corresponding to portal triad and central vein were seen inside the parenchyma. The connective tissue stroma supported hepatic parenchyma as well as the vessels and ducts. The sinusoidal wall presented endothelial cells with flattened nucleus and occasional Kupffer cells. Sinusoids were filled with oval and nucleated red blood corpuscles. The parenchyma also presented melano-macrophages and heterophils.

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