Original Research

Histomorphological Studies of Liver in Buffalo (*Bubalus bubalis*)

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Abstract

The histomorphological studies on the 24 buffalo livers (12 male and 12 female) were carried out. The liver was externally covered by dense white fibrous connective tissue (capsule). From the capsule originate the interlobular septae. The parenchyma of the liver was formed of hepatic lobules. Each lobule comprised of the cords of hepatocytes, which separated from each other by sinusoids. In each hepatic lobule, the plates of hepatocytes and sinusoids were appeared to be radiated outward from the central vein to the periphery of the liver lobule. Hepatocytes were polyhedral shaped cells, characterized by a centrally located spherical nucleus with chromatin granules and distinct cell membrane. The cytoplasm showed the dirty stained mitochondria. The lumen of sinusoids was lined by Von-Kupffer cells and endothelial cells. Bile canaliculi were observed in between two adjacent hepatocytes. As the bile duct enlarges from bile canaliculi to the hepatic duct, the epithelium appeared more columnar. The portal triad consisted of interlobular branches of hepatic artery, portal veins, interlobular bile duct, small lymphatic vessels and nerves. Collagen fibers were observed in the capsule, interlobular connective tissue and blood vessels, elastic fibers were observed in the capsule, interlobular connective tissue and blood vessels and the reticular fibers were observed in the capsule, interlobular septa and sinusoids of the liver in both male and female buffaloes.

Key words: Buffalo, Histology, Liver

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Introduction

Liver is one of the vital organs for mammalian species actively involved in various metabolic function of the body such as metabolism of amino acids, protein, lipids and carbohydrate (Choudhury and Singh, 2016). Histological studies on the liver of domestic animals like cow, sheep, goat and camel have been carried out (Chen *et al.*, 1996 and Madhan & Raju, 2013). Some prenatal anatomical studies have been carried on the

liver of Non-descript Indian buffaloes (Doley *et al.*, 2006) and sheep (Choudhury and Singh, 2016, 2017). However, to the best of our knowledge no such work has been carried out on the histology of postnatal liver in buffaloes. The present study was therefore designed with the aim to study the postnatal histology of liver in buffaloes.

Materials and Methods

The present study was carried out on normal livers 12 male and 12 female adult buffaloes (*Bubalus bubalis*) of Murrah breed. The liver was examined *In-situ* and all attachment and other particulars were recorded. The specimens were collected immediately after slaughter at deonar abattoir, Mumbai. The livers were separated from the pluck and cleared by removing the fascia, blood vessels, nerves etc. in order to facilitate the observations. These separated livers were washed under running tap water to remove all blood clot exudate and tissue debris. The organs were brought to the laboratory in ice- cooled box for further study. For the histological study of the liver, five tissue pieces of 4 to 5 mm. size in all dimension was collected from upper third of parietal surface, middle of the liver at center, just below the portal vein, from the neck of gall bladder and apex of caudate lobe. These tissue pieces were fixed in 10% neutral buffered formalin solution for 24 hours. The tissue pieces were treated with routine method of dehydration in in ascending grades of alcohol, cleared in cedar wood oil and embedded in paraffin wax as per the method of Drury *et al.* (1967) each prepared paraffin block was sectioned at 3 to 5 µm.

The prepared slides were stained with Mayer's haematoxylin and eosin stain as described by Singh and Sulochana (1996-97) for general histological structure and micrometry of the various components from different regions of the liver.

Special Staining Techniques

1. Van Gieson stain for collagen fibers as described by Drury *et al.* (1967).
2. Weigert's stain for elastic fibers as per method of Luna (1968).
3. Wilder's stain for reticular fibers described by Singh and Sulochana (1996-97).

Result and Discussion

In the present study, the histo-architecture of the liver presented a capsule, hepatic lobules, hepatocytes, hepatic sinusoids, biliary and lymphatic system and portal triads (Fig. 1, 2 & 3). The observations of the present study are similar to those reported by Dellmann (1993), Banks (1993) and Frandson (1986). The liver was covered by visceral peritoneum forming the fibrous capsule (capsule of Gilsson). Singh *et al.* (2014) reported in buffalo fetus that the liver was enclosed by single layer of connective tissue capsule. It was composed of dense white fibrous connective tissue and elastic fibers. Doley *et al.* (2006) in buffalo fetus, Mandal *et al*. (2006) in sheep & goat and Singh *et al.* (2014) in buffalo fetus reported that the capsule

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of liver was made up of collagen and reticular fibers. These connective tissue from the capsule was extended into the interlobular spaces that act as a supportive storma for the parenchyma. The interlobular connective tissue was scanty in the present study. These observations were similar to those reported by Dellmann (1993), Banks (1993) and Frandson (1986). Chen *et al.* (1996) reported that the capsule was thicker in Bactrian camel than that of horse or cattle, whereas Dyce *et al.* (1996) reported that the liver was enclosed within a tough fibrous tissue capsule.

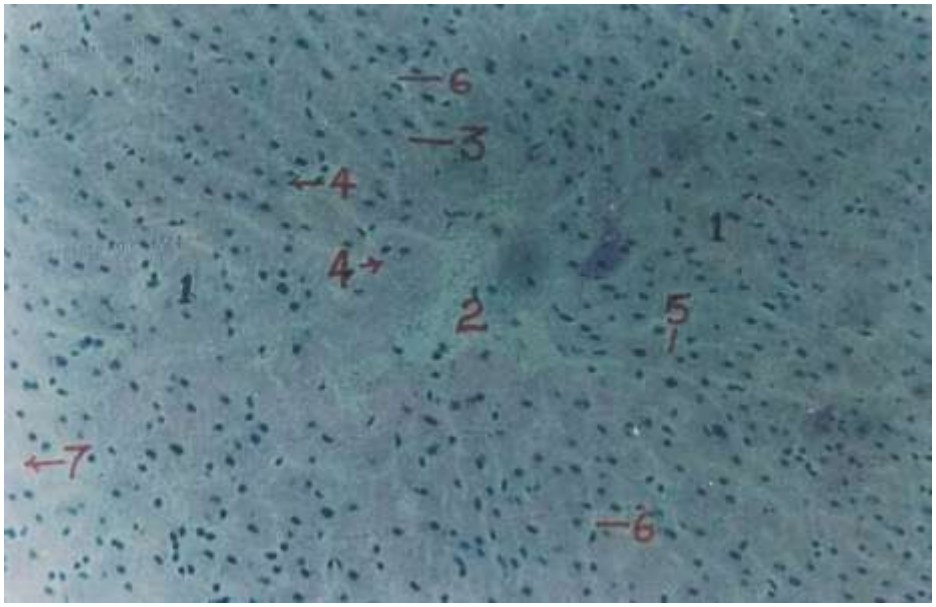

Fig. 1: Photomicrograph of the liver showing: 1. Hepatocyte, 2. Central vein, 3. Cords of Hepatocyte, 4. Bi-nucleate hepatocyte, 5. Rows of hepatocytes, 6. Sinusoid and 7. Bile canaliculi. Haematoxylin and Eosin 66X

The interlobular septa were less conspicuous since the connective tissue was very scanty. The hepatic cords of the one lobule were appeared to be blend into adjacent lobules without a clear line of demarcation. In each hepatic lobule, the plates of hepatocytes and sinusoids were appeared to be radiate outward from the centrally positioned central vein to the periphery of the liver lobule (Fig. 2 &3). Chen *et al.* (1996) reported that the parenchyma was composed of lobules with a radial pattern of the cell plates and sinusoids. In the perilobular region, the connective tissue was in small amount.

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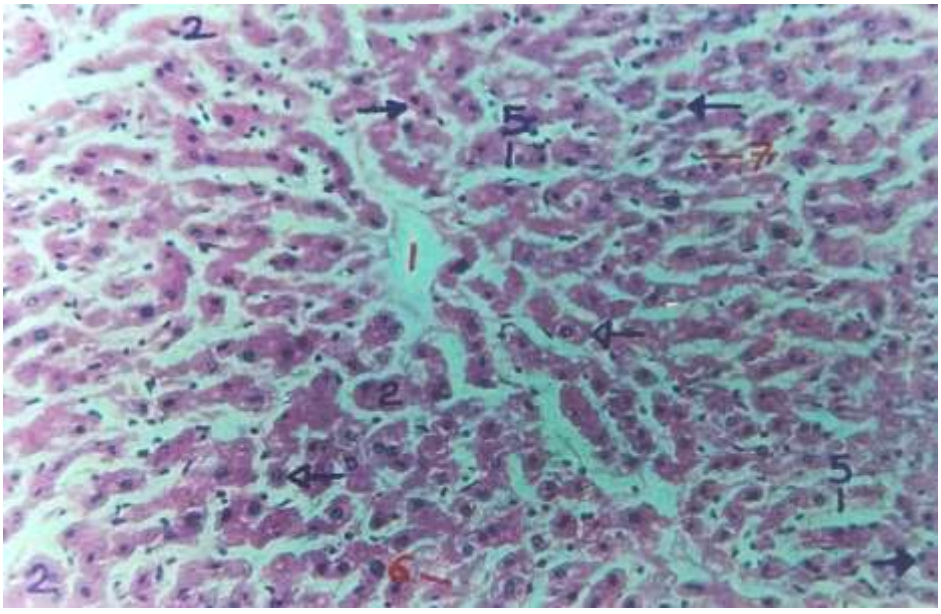

Fig. 2: Photomicrograph of the liver showing: 1. Central vein, 2. Plate of hepatocytes, 3. Uni-nucleated hepatocyte (open arrow), 4. Bi-nucleated hepatocyte (solid arrow), 5. Sinusoid, 6. Bile canaliculi and 7. Nucleus of endothelial cell. Haematoxylin and Eosin 66X

The parenchyma of the liver comprised the hepatocytes. Similar findings have been reported by (Choudhury and Singh, 2013) in prenatal of sheep and Singh *et al.* (2014) in fetus of buffalo. Hepatocytes were polyhedral in shaped with distinct boundaries. They were characterized by a centrally located spherical nucleus with one or more prominent chromatin granules. The cytoplasm was consisted of darkly stained mitochondria. Binucleate cells were also observed occasionally. The appearance of the cytoplasm and shape of hepatocytes varied during observations (Fig. 2). This might be due to physiological, state of animal at the time of sampling. Fasted animals have small, turbid and indistinctly outlined hepatocytes. After feeding, the hepatocytes enlarged with distinct outline. These cells were filled with numerous glycogen and lipid inclusions, causing a foamy or honey combed appearance as reported by Banks (1993). Dellmann (1993) reported that, the appearance of the cytoplasm of hepatocytes varies depending on nutritional and functional cellular changes. The present observations are in agreement with Dellmann (1993), Banks (1993) and Frandson (1986) whereas; Chen *et al.* (1996) reported the bilirubin granules in the cytoplasm of hepatocytes, which were no observed in the present study.

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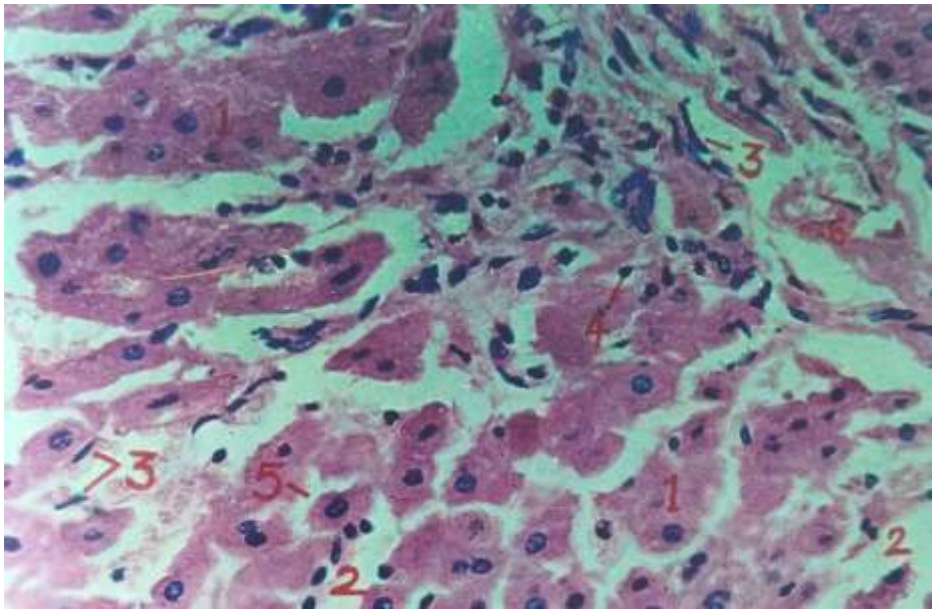

Fig. 3: Photomicrograph of the Liver showing: 1. Plate of hepatocytes, 2. Sinusoid, 3. Nucleus of endothelial cell, 4. Von-Kuffer cells, 5. Bile canaliculi and 6. Interlobular connective tissue. Haematoxylin and Eosin 132X

The hepatic sinusoids were observed between the hepatic cords. These were large spaces within the liver lobule, which were formed from small distributing branches of the interlobular vein. The arterial and venous blood mixed in the sinusoids carried from the periphery of the lobule and directed toward the central veins of the lobule (Fig. 2). This observation is in accordance with Dyce *et al*. (1996). Von-Kupffer cell were present in the lumen of sinusoids (Fig. 3). These cells were large in size, irregular or stellate in shaped with several processes. Similar findings were reported by Choudhury and Singh (2016) during ultrastructural study of postnatal sheep. These cells were prominent in the sinusoids due to presence of foreign material like carbon particles in the cytoplasm. The nucleus of the Von-Kupffer cell was difficult to observe because of the accumulation of the carbon particles. The sinusoids were lined by the endothelial cells which were smaller in size with visible nucleus. Similar observations were reported by Banks (1993), Dellmann (1993) and Chen *et al.* (1996) except the perisinusoidal space.

The interlobular bile ducts were lined by simple cuboidal epithelium in agreement with the reports of Singh *et al.* (2014) in buffalo fetus. The bile duct joined with larger intrahepatic duct in the portal areas and finally left the liver through the hepatic duct. Bile canaliculi were the smallest component of biliary system and were observed in between the hepatocytes (Fig. 3). It was observed that the ducts were gradually enlarged from bile canliculi to hepatic duct. The epithelium was gradually increased in height and finally became simple columnar cells in the large hepatic ducts. These observations are in accordance with Banks (1993) and Dellmann (1993). Whereas, Weyrauch (1979) recorded various cell types during the study of

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epithelium of the main excretory duct of the liver in ruminants. He also observed the filament bundles of microvilli, perinuclear spaces, and electron dense granules in distal part and around the nucleus. Such type of components was not observed in the present study. The interlobular blood vessels entered a space of portal area through the connective tissue and liver parenchyma. Further, the lymph was carried from portal area by large lymph vessels. These lymphatic vessels were found in the connective tissue capsule, connective tissue capsule, connective tissue around portal veins and hepatic veins. These observations are in agreement with the observations made by Banks (1993) and Dellmann (1993).

Portal triad was situated between three or more hepatic lobules composed of connective tissues forming the portal area (Fig. 3). It was consisted of interlobular branches of the hepatic artery, portal veins, interlobular bile ducts, small lymphatic vessels and nerves. The group of these vessels formed the portal triad. Similar observations were recorded by Banks (1993) and Dellann (1993), whereas Chen *et al.* (1996) recorded that within the area of portal canal, connective tissue formed a common sheath around the branches of portal vein, hepatic artery and bile duct.

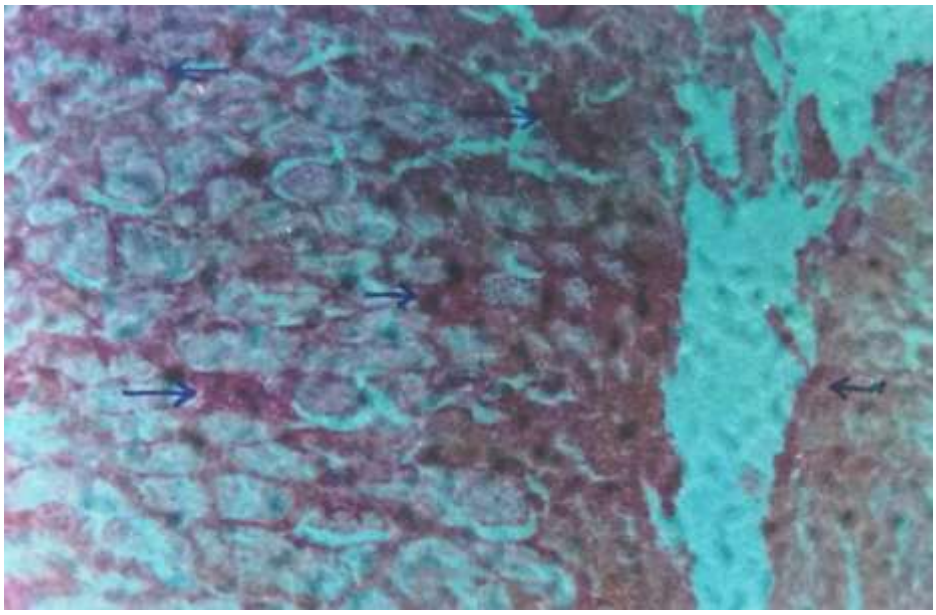

Fig. 4: Photomicrograph of the Liver showing: Arrow showing the red colored collagen fibers. Van-Gieson Stain 33X

In the present study, collagen, in addition to few elastic fibers, were observed in the capsule, interlobular connective tissue and blood vessels in male and female buffaloes (Fig. 4). Chen *et al.* (1996) also reported the small bundles of collagen fibers around the central vein. This variation in the distribution of collagen fibers in liver components might be due to species difference. Dellmann (1993) recorded that the sinusoids were surrounded by a very fine network of reticular fibers as well as peri-sinusoidal space contained reticular fibers while, Chen *et al.* (1996) also recorded the network of reticular fibers between sinusoids

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and hepatic cell plates. The present observations are in agreement with Dellmann (1993) and Chen *et al.* (1996).

Conclusion

From the present study we concluded that the histology of liver in buffalo was somewhat similar to other ruminants and did not present any significant variations. There was no significant difference in between male and female liver.

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