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Anatomy of the Liver of Mizoram Local Pig (Zovawk)

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ABSTRACT

Background: The present study was aimed for the promotion and advancement of the anatomical knowledge at the gross, light microscopic and electron microscopic level in Zovawk (mizo local pig).

Methods: The current investigation was done at the Instructional livestock farm, Department of Veterinary Anatomy and Histology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram and Sophisticated Analytical Instrument Facility (SAIF), North Eastern Hill University (NEHU), Shillong, Meghalaya. Six liver samples were collected from six apparently healthy Zovawk animal of either sex and gross morphological observations were done directly after collection. Thereafter tissue samples were collected as such and were preserved in neutral buffer formalin (NBF) and in Karnovsky's fixative for routine histology and transmission electron microscopic examination, respectively.

Result: Zovawk liver shows four distinct borders, *i.e.* medial, lateral, dorsal and ventral border, two surfaces, *i.e.* parietal surface or diaphragmatic surface and caudal or visceral surface and six distinct lobes. The average weight of Zovawk liver was 1.402 kg. Weight of the liver was highly correlated with body weight of animal. Histologically, Zovawk liver was characterized with thick Glisson's capsule and thick connective tissue septa emerging from it, which gives the hepatic lobules its hexagonal shape. Sinusoids of adjacent hepatocytes were lined by stellate shaped Kupffer cells. The ultra-structural examination of liver shows that, the hepatocytes were rich in mitochondria, endoplasmic reticulum and glycogen granules. Free ribosome and well developed rER and dense lysosomal granules were common in those hepatocyte.

Key words: Gross, Liver, Ultrastructure, Zovawk.

INTRODUCTION

Indigenous pig breed of Mizoram, also known as Zovawk is dispersed in different parts of Mizoram, India. Lack of scientific breeding practice is responsible for the gradual decreasing of these pigs population. Age of puberty of these small sized animals is at 2.5 months with a body weight of 4.5 kg. They are very wary of their surrounding and can be used as watch animals because of their alert behavior (Hmar *et al*., 2010, Kalita *et al*., 2014, Prava *et al*., 2014). As a largest gland in animal body, liver has numerous multifaceted functions (Nickel *et al*., 1979). Six distinct lobes and two borders are characteristics features of pig liver. Average weight of an adult pig liver is about 1.5 to 2 kg (Getty, 2012). Pig liver is consists of 6 distinct lobes and two borders. Position of gall bladder in pigs is between the right medial lobe and quadrate lobe (Dyce *et al*., 1987). Microscopically, hepatic lobules and corresponding portal canals are seems to form the liver lobe. Contrary to other animals, Pig liver is characterized with distinct interlobular connective tissue septa emerges from Glisson's capsule. Because of the geographical position no standard work have been done on the liver of this unique animal therefore this investigation was performed to set a baseline data of the organ.

MATERIALS AND METHODS

Six apparently healthy Mizoram local pigs, irrespective of sex were utilized for this study. One liver samples from each animal were collected. Topographic study and gross measurements were taken right on the spot. Measurements of various physical parameters *i.e.* weight of the liver, length of the liver, width and thickness of the liver were evaluated. A physical balance was used to measure the weight of the liver. Length, width and thickness of the liver were measured by the measuring scale. Data obtained were then analyzed using statistical package SPSS version 20.

For histological examination, freshly collected liver samples were fixed in 10% NBF (Neutral buffer formalin) then standard tissue processing procedure were followed to make a tissue section of 6 µm thickness. Processed tissue sections were subsequently stained with H & E stain for observing normal tissue architecture.

For transmission electron microscopic examination multiple steps were followed to ensure proper ultra-structural viewing of the organ. Such as:

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i. Fixation: Tissue samples for TEM studies were fixed in Karnovsky's fixative (2.5% glutaraldehyde in 0.1M Sodium cacodylate or Phosphate buffer at pH 7.2) for 2-4 hours at 4°C. After washing in 0.1M buffer (3 changes of 15 minutes each).

ii. Washing: The fixed tissues were then washed in 0.1 M phosphate buffer saline (pH 7.4) for three times of 15 minutes duration each at 4°C.

iii. Post-fixation: These washed tissues were later post fixed for 2 hours in 1% osmium tetraoxide at 4°C.

iv. Washing: The osmium tetroxide fixed tissues were again washed in 0.1 M phosphate buffer saline (pH 7.4) for three times of 15 minutes duration each at 4°C.

v. Dehydration: Dehydration of tissues were done in graded acetone (*i.e.* 30, 50, 70, 80, 90% and dry acetone) for 30 minutes each at 4°C followed by dehydration in dry acetone once again for 30 minutes at room temperature.

vi. Clearing: The dehydrated tissues were then cleared in toluene-I and toluene-II for 30 minutes each.

vii. Infiltration: The infiltration of the cleared tissues was carried out as follows:

1. Part Embedding Medium and 3 parts of Toluene-12 hours.
2. Parts Embedding Medium and 2 parts of toluene-12 hours.
3. Parts Embedding Medium and 1 parts of Toluene-12 hours (under vacuum).

Preparation of Embedding Medium

Araldite cy212 - 10 ml.
DDSA (dodecenyl succinic anhydrite) - 10 ml.
DMP (2, 4, 6 tridimethylamino methyl phenol) - 0.4 ml.
Plasticizer (Dibutyl phthalate) - 1.0 ml.

The above gradients were added and stirred vigorously in order to mix them thoroughly. Then the air bubbles were allowed to settle down before use.

viii. Embedding: A pure embedding medium were use for embedding the tissue using gelatin capsule.

ix. Polymerization: The embedded blocks were kept at 50°C for 24 hours (polymerization) and then at 60°C for 48 hours.

x. Ultra-sectioning: Silver-to-gray (70-80 nm) ultrathin sections were cut with diamond knives using a ultra-microtome (Leica ultra-cut) UCT and mounted on copper grids.

xi. Negative staining: Uranyl acetate and lead citrate were used for staining the ultrathin sections which were then examined under a Transmission Electron Microscope, JEOL, JEM-2100, Japan which was operated at 80 KV.

xii. Photography: The photography was taken with the help of the digital camera which was inbuilt with the microscope.

xiii. Interpretation: Some of the interpretations were noted while viewing the tissues in the TEM and rest were done with the help of photomicrographs.

RESULTS AND DISCUSSION

Gross parameters

Position

In this present study liver of Zovawk was located in the cranial and right side of the median plane in the intra thoracic part of the abdominal cavity immediately after diaphragm as also reported by Dyce *et al.* (1996), Pareek (2000) in sheep, Dhoolappa *et al.* (2007) in Indian donkey and Stamatova *et al.* (2012) in rabbit. It was also found in this study that zovawk liver was directed obliquely downward and forward from the 12th ribs to 6th intercostal space where as Pareek (2000) found that sheep liver was extended from the ninth rib or eighth inter-costal space to the caudal border of the last rib.

Color and shape

In this present study the shape of Zovawk liver was irregularly rectangular in shape. Similar findings were also noticed by Pareek (2000) in sheep. Endo *et al.* (2000) observed that the liver of a two-humped camel (*Camelus bactrianus*) appeared characteristically as an enlarged triangle in visceral aspect. While Osman (2008) observed in the liver of the pig that it was divided into two independent segments, right and left. Color of the Zovawk liver was cherry red to brownish red. Miller (1965) stated in dog that the liver of dog was deep red in color and firm in consistency in fresh state. Oushine and Zguigal (1983) and Smuts and Bezuidenhout (1987) found dark brown liver in camel. Smuts and Bezuidenhout (1987) reported that in camel, the liver was dark brown in the fresh state.

Borders and surfaces

In this present study liver shows 4 distinct borders similar findings were found by Pareek (2000) in sheep and Bamaniya (2013) in Marwari goat. The medial border presented oesophageal notch and parts of caudal venacava similar findings were reported by Pareek (2000) in sheep and Bamaniya (2013) in Marwari goat. However the posterior venacava was partly embedded in the medial border above the esophageal notch as described by Nickel *et al*., (1979) in ruminants. Zovawk liver showed 2 surfaces *i.e.* parietal surface or diaphragmatic surface and caudal or visceral surface similar findings were also found by Pareek (2000) in sheep, Modekar, *et al.* (2003) in the goat. The visceral surface of Zovawk liver was related to the part of stomach, duodenum and gall bladder. However the visceral surface of the liver was related to the part of reticulum, omasum, duodenum and gall bladder in Marwari goat (Bamaniya, 2013).

Lobation

In this study, Zovawk liver was distinctly lobulated on the visceral surface. Similar findings were reported by Pareek (2000) in sheep and Bamaniya (2013) in Marwari goat. Zovawk liver was divided in to 6 distinct lobes. Whereas Miller *et al.* (1965) reported that dog liver was divided into

five lobes, Raghavan (1964) reported that in ox the liver was divided in four lobes, Getty (1977) reported that there was horse liver was divided in to 4 lobes and there was presence of 3 liver lobes in sheep and goat reported by Raghavan (1964) and Getty (1977) respectively. An imaginary midline divides the Zovawk liver in to 2 halves, left and right liver. On the left side there is a presence of large left lateral and small left medial lobes. On the right side there is a larger right medial lobe and smaller right lateral lobe. Above the right lateral lobe there is a presence of caudate process. In Zovawk only caudate process was present in caudate lobe, whereas papillary process was not found. In live caudate process was not in relation with kidney so no renal impression was seen in the caudate process. In the midline smaller quadrate lobe was seen. Between the quadrate lobe and right medial lobe there was presence of gallbladder. Near the imaginary midline, below the caudate process there was presence of porta through which portal vein, hepatic artery pass (Fig 1 and 2).

Weight

In this study, average weight of Zovawk liver was 1.402 kg. Whereas Nickel *et al.* (1979) recorded weight of liver as 0.775 kg in sheep. Miller, (1965) described that the average weight of liver of dog was 450 gm. It was highly correlated with body weight of animal (Table 2).

Length

In this study, average length of the Zovawk liver was 25.65 cm. While Pareek (2000) found that the average length was 8.97 inches in sheep.

Width

The width of the dorsal margin was ranged from 8.9 cm to 12 cm on an average of 11.22 cm. The width of middle margin was ranged from 21.4 cm to 30.2 cm on an average of 20.205 cm. The width of ventral margin was ranged from 13.5 cm to 18.3 cm on an average of 16.23 cm (Table 1). Width of middle margin was maximum and width of dorsal margin was minimum. which is similar with the findings of the May (1955) in sheep but was contrary with the findings of Pareek (2000) in sheep where he observed that that the dorsal margin of the liver was significantly larger than that of the ventral margin. Correlation between width of dorsal margin and width of middle margin with body weight is significant @ 5%, whereas correlation between widths of ventral margin body weight of animal is non-significant (Table 2).

Thickness

Thickness of dorsal lobe was ranged from 2.3 cm to 4.1 cm on an average of 3.06 cm. Thickness of ventral lobe was ranged from 1.6 cm to 3.3 cm on an average of 2.2 cm. Thickness of caudate lobe was ranged from 0.9 cm to 1.15 cm

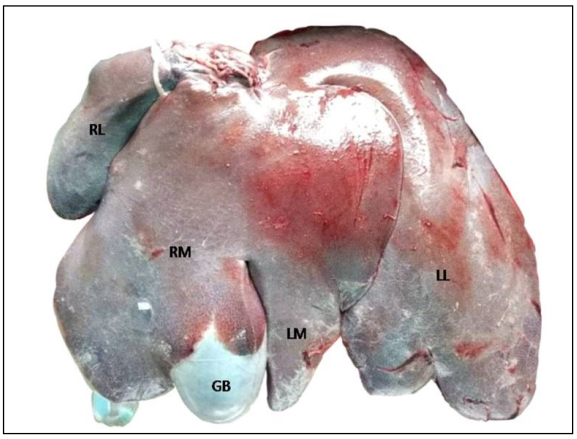

Fig 1: Diaphragmatic surface of liver. LL= Left lateral, LM= Left medial, RM= Right medial, RL= Right lateral, GB= Gall bladder.

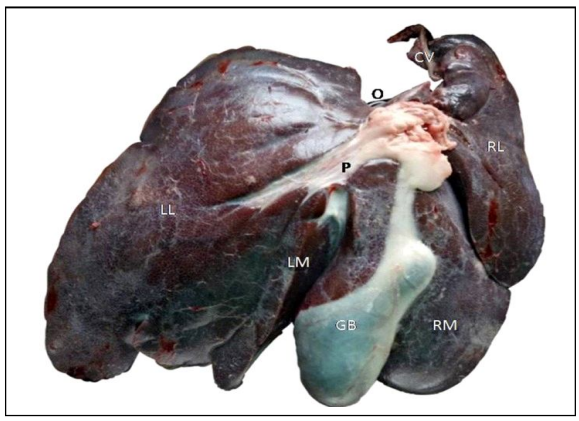

Fig 2: Visceral surface of the liver. LL= Left lateral, LM= Left medial, RM= Right medial, RL= Right lateral, GB= Gall bladder, P= Porta, O= Oesophageal notch, CV= Caudal venacava.

Table 1: Different gross measurements of Zovawk liver.

Sl. no	1	2	3	4	5	6	Average
Body weight	250 kg	250 kg	165 kg	220 kg	240 kg	250 kg	
Weight of liver	1.44 kg	1.6 kg	0.943 kg	1.205 kg	1.72 kg	1.54 kg	1.402 kg
Length	24.6 cm	26.6 cm	21.9 cm	27.1 cm	28.9 cm	24.79 cm	25.65 cm
Width (Dorsal Margin)	11.7 cm	10.9 cm	8.9 cm	11.92 cm	11.92 cm	12 cm	11.22 cm
Width (Ventral Margin)	16.3 cm	16.5 cm	13.5 cm	18.3 cm	16.9 cm	15.9 cm	16.23 cm
Width (Middle Margin)	29.4 cm	28.8 cm	21.4 cm	30.2 cm	29.89 cm	29.54 cm	28.205 cm
Thickness (Dorsal Lobe)	2.9 cm	3 cm	2.3 cm	3.01 cm	4.1 cm	3.1 cm	3.06 cm
Thickness (Ventral Lobe)	1.7 cm	2.05 cm	1.6 cm	2.92 cm	3.3 cm	1.8 cm	2.22 cm
Thickness (Caudate Lobe)	0.9 cm	1.15 cm	0.5 cm	1.07 cm	1.8 cm	1.3 cm	1.12 cm

on an average of 1.12 cm (Table 1). Whereas Miller, (1965) described that the average thickness of ventral lobe of liver was 6 cm in dog.

Histological examination

Externally Zovawk liver was covered by a thick, distinct connective tissue capsule (Fig 3). This connective tissue capsule is known as Glisson's capsule; same was also reported by Kalita *et al.* (2019) in Zovawk and also by Copenhaver *et al.* (1967) in pig. This connective tissue capsule runs inside as well-defined connective tissue septa and divides the pig liver in to many distinct hexagonal liver lobules. These hexagonal liver lobules (Fig 4) were characterized by centrally placed central vein and a portal area in the periphery. Similar findings were reported by Kalita *et al.* (2019) in Zovawk whereas Pareek (2000) in sheep and Bamaniya (2013) in Marwari goat reported that, there were no distinct hexagonal shaped liver lobules in sheep and goat with scanty connective tissue septa. The central veins were connected with sub lobular veins, ran in the middle of the lobule and communicated directly, with sinusoids as also reported by Kalita *et al.* (2019) in Zovawk. Polygonal Hepatocytes (Fig 5) with centrally placed large, rounded nucleus with one or more nucleoli were seen. Hepatocytes were arranged in the form of one cell thick cord. These cords anastomosed with one another, enclosing spaces which contained the sinusoids. These findings compare well with the reports of Kalita *et al.* (2019) in Zovawk, Copenhaver *et al.* (1967) in pig and camel and Adibmoradi (2007) in horse. The sinusoids were formed between the hepatic cell cords and separated from the hepatocytes by a

Table 2: Correlation between different parameters of liver with body weight of the animals.

Parameters	Number of animals	Body weight of animal (Pearson Correlation)
Weight of liver	6	0.885*
Length	6	0.596
Width (Dorsal margin)	6	0.837*
Width (Ventral margin)	6	0.621
Width (Middle margin)	6	0.889*
Thickness (Dorsal lobe)	6	0.582
Thickness (Ventral lobe)	6	0.185
Thickness (Caudate lobe)	6	0.655

*: Correlation is significant at the 0.05 level (2-tailed).

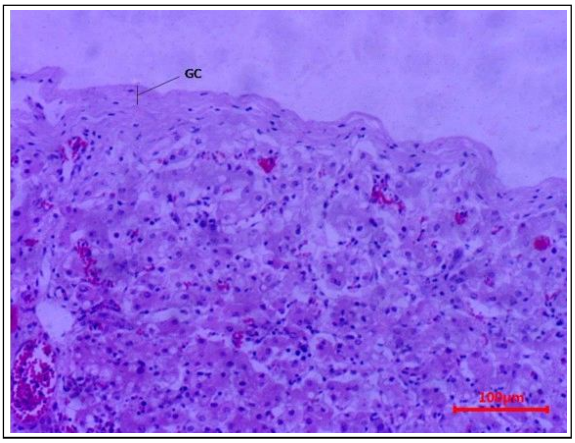

Fig 3: Liver capsule; GC= Glisson's capsule. H & E, 10X.

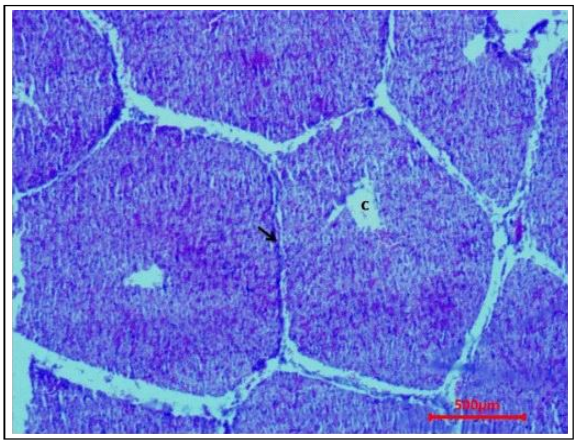

Fig 4: Liver lobulation, C= Central vein, arrow head showing connective tissue septa. H & E, 4x.

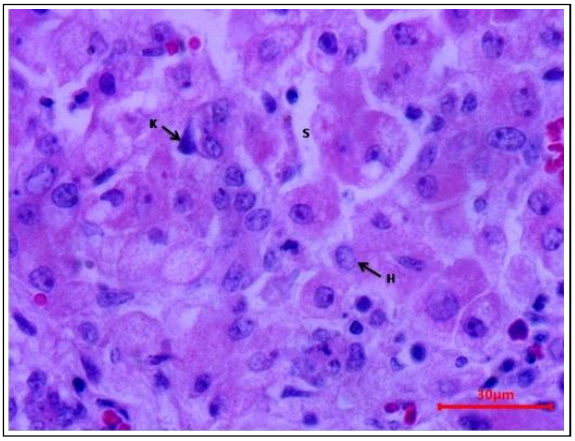

Fig 5: Parenchyma of liver showing, H= hepatocytes, S= sinusoids, K= Kupffer's cells.

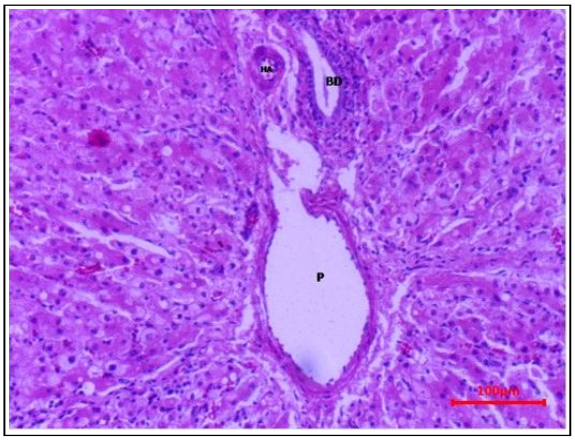

Fig 6: Portal area of liver showing, P= portal vein, HA= hepatic artery, BD= Bile duct.

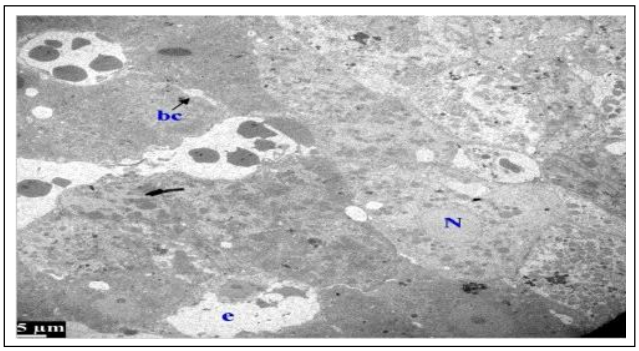

Fig 7: Parenchyma of liver showing N= Nucleus, bc= Bile canaliculi, e= endothelial cells. TEM, 720X (Liver).

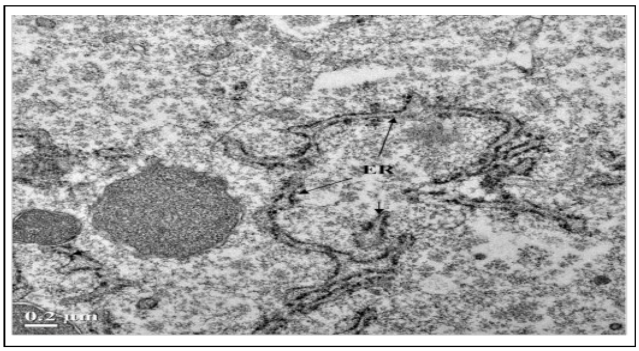

Fig 9: Cytoplasm of hepatocytes showing ER= Endoplasmic reticulum. TEM, 90000X (Liver).

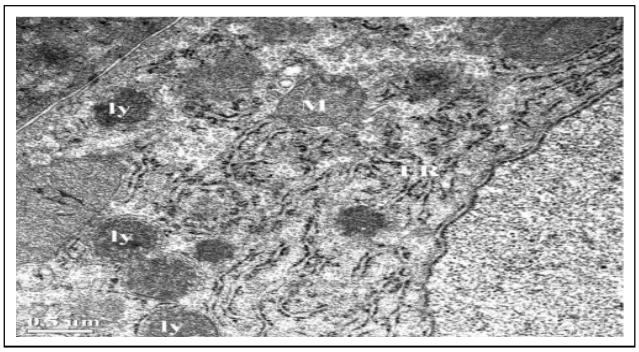

Fig 8: Demonstration of cytoplasm of hepatocytes showing ly= Dense lysosomal granules (ly), M= Mitochondria, ER= Endoplasmic reticulum. TEM 3000X (Liver).

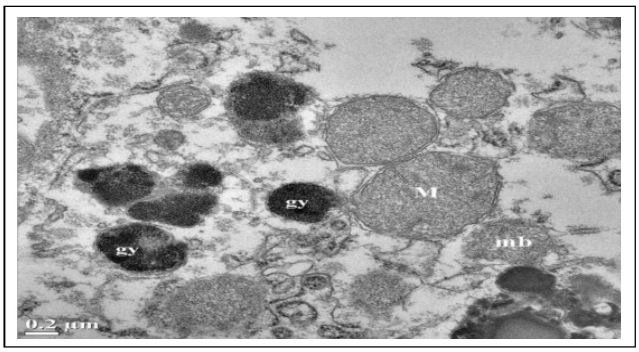

Fig 10: Demonstration of M= Mitochondria, gy= Glycogen granules, mb= Microbodies. TEM, 80,000X (Liver).

perisinusoidal. Sinusoids were lined by stellate shaped cells known as Kupffer's cells. These cells were also observed by Kalita *et al.* (2019) in Zovawk, Pareek (2000) in sheep and Mahata *et al.* (2003) in spotted deer. Portal area was characterized by the presences of various vessels and ducts, *i.e.* portal vein, hepatic artery, bile duct (Fig 6).

Transmission electron microscopic examination

The hepatocyte has a large, rounded nucleus, one or more prominent nucleoli and abundant cytoplasm. Very dense lysosomes bodies or lipofuscin granules were found. The sinusoidal endothelial cells were seen (Fig 7). The hepatocyte is rich in mitochondria, endoplasmic reticulum and glycogen cluster of free ribosome and well developed rER and sER (Fig 8) as reported by Lalla (1997) in camel. The mitochondria of the liver have a dense matrix and transversely or occasionally longitudinally oriented crests. The granular and agranular endoplasmic reticulum are in continuity in several locations. The cisternae are flattened with some content. The endoplasmic reticulum is in close association with the mitochondria (Fig 9). Dense lysosomal granules are common in the hepatocyte (Fig 8).

The sinusoidal endothelial cells were observed in widely separated areas. Very thin, fenestrated cytoplasmic extensions from the endothelial cells encompass the lumen of the sinusoidal blood vessel. Bile canaliculi appear as irregular pores between cells in the cords (Fig 7). Filamentous or tubular stems were seemed to interconnect some of the organelles. The microbodies generally appear somewhat smaller than the mitochondria. Shahien *et al.,* (1977) reported that in camels, small to medium sized lipid droplets are mainly concentrated in the peripheral part of the hepatocytes along the sinusoids. The microbody is surrounded by a single membrane and this opportune section has passed through a dense nucleoid. This microbody is located among a number of glycogen granules. Glycogen granules were seen as dense granules in a rosette configuration (Fig 10).

CONCLUSION

In conclusion it can be said that, even though gross structure, overall cell morphologies, organelles within cells, appeared typical, there are few important ultrastuctural features which were quite different than other species such as Very dense lysosomes bodies or lipofuscin granules were found in the cytoplasm of hepatocytes which enhance the excretion properties of the liver. Apart from that this present investigation helps to set a baseline data about the gross and ultrastuctural features of the liver of this rear porcine breed of Mizoram.

Conflict of interest

All authors declare that they have no conflict of interest.

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