



Characterizing morphological heterogeneity in porcine hepatocytes

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ABSTRACT

The present study was conducted on liver of eight pigs collected from local slaughter house, for gross anatomical, histological, histochemical, immunohistochemical and histoenzymic studies. The paraffin sections of 3 µm were cut and stained with different histomorphochemical stains. The cryostat sections of fresh frozen tissues of 10 µm thickness were cut at -20°C and incubated in different substrates to localize phosphatases and Non-specific esterases. The left lateral lobe was significantly larger and quadrate lobe was shortest. The size of hepatocytes in periportal area was significantly more than size of hepatocytes in centrilobular area. The number of mononuclear hepatocytes was significantly more in periportal area than centrilobular area whereas number of binucleated hepatocytes didn't vary significantly between periportal and centrilobular areas. The average no. of Kupffer cells in periportal region was significantly more than centrilobular area. The reaction for neutral mucopolysaccharides and glycogen was more in centrilobular hepatocytes, proteins were distributed homogeneously and reaction for lipids was more in centrilobular hepatocytes. Periportal hepatocytes exhibited a strong phosphatases activity whereas non-specific esterases activity was more in centrilobular hepatocytes. This work will contribute to the corpus of knowledge regarding the heterogeneity of hepatocytes, which enables the liver to carry out a variety of functions and enhances its complexity and adaptability in preserving the body's homeostasis.

Keywords: Hepatocytes, Heterogeneity, Morphology, Pig

In comparison to other large animals, swine species serve as a superior model for several biological studies as pigs and human share a number of anatomical and physiological traits. Pigs are also used as an animal model for research and pharmaceutical development purposes (Swindle *et al.* 2012). Liver, the largest gland of body, has been ranked second in complexity next to brain due to its structural and functional heterogeneity. It has at least 12 different cell types, two separate blood supplies (nutritional supply by portal vein and oxygenated supply by hepatic artery), and is a primary site for biotransformation (especially rendering hydrophobic compounds water soluble) as well as defense against xenobiotics and foreign macromolecules (Burt and Day 2002). Studying the structure of the pig liver provides crucial context for instructing and preparing surgical teams for hepatectomy and partial hepatic transplantation (Swindle *et al.* 2012). Research on the morphology of pig liver is crucial for understanding its structure and function, providing insights into similarities and differences with human livers. This knowledge is essential for developing medical treatments and studying diseases, and ensuring the safety of xenotransplantation. Additionally, it contributes to veterinary medicine, livestock health, and optimization of

pig farming practices. Although many references are available on morphology of swine liver (Sasan *et al.* 2017, Kalita *et al.* 2019), a detailed review on morphological heterogeneity in centrilobular and periportal hepatocytes in pig is lacking. This research was planned to elucidate heterogeneity in morphological details of porcine hepatic architecture.

MATERIAL AND METHODS

Sample collection: The study was conducted on liver of 8 pigs collected from local slaughter house in Ludhiana. Before collection of samples the livers were thoroughly examined for any pathological lesions and rinsed with normal saline solution. These livers were used for gross anatomical, biometrical, histological, histochemical, immunohistochemical, histoenzymic and scanning electron microscopic studies.

Gross anatomical and biometrical study: The total weight of the liver was measured in grams. The biometrical measurements of length and width of different lobes were taken with inelastic thread and scale in centimeters.

Histomorphological and histochemical study: After taking gross and biometrical observations, the tissues were collected from different lobes of eight livers and fixed in 10% neutral buffered formalin. After fixation, the tissue samples were processed for paraffin block preparation by acetone-benzene schedule (Luna 1968). The paraffin sections of 3 µm were cut with rotary microtome and stained

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with Hematoxylin and Eosin for histological, Masson's trichrome for collagen fibres, Gridley's method for reticular fibres, Verhoeff's method for elastic fibres, Periodic acid Schiff for neutral mucopolysaccharides, Alcian blue for acid mucopolysaccharides, best carmine for glycogen and Bromophenol Blue stain for localization of total proteins (Luna 1968, Sheehan and Hrapchak 1973). Total lipids and phospholipids were demonstrated on cryosections (Chayen *et al.* 1969). The micrometrical observations were recorded on Hematoxylin and Eosin-stained sections with the help of Image J Software. The micrometrical observations of liver included the thickness of capsule, size of hepatocytes, diameter of nuclei of hepatocytes, number of total hepatocytes, number of mononuclear hepatocytes and number of binuclear hepatocytes in a specific area, size of central vein, portal vein, hepatic artery, and bile duct.

Histochemical study: For histochemical localization, cryostat sections of fresh tissues of eight livers were taken before fixation. The cryosections of 10 μ m thickness was cut with cryostat microtome at -20°C . The sections were incubated with different substrates to localize histochemical activity of alkaline phosphatase, acid phosphatase and glucose 6 phosphatase (Pearse 1972).

Scanning electron microscopic study: For scanning electron microscopic study, fresh liver tissues were collected from four pig livers and fixed in 2.5% glutaraldehyde solution for overnight at 4°C , fixative was drained and tissue samples were thoroughly washed with 0.1M Cacodylate buffer. After draining, 1% Osmium tetroxide solution was added for 1-1.5 hours at 4°C . The tissues were again washed with 0.1M Cacodylate buffer and distilled water and dehydrated in graded series of ethanol as per protocol given by Bozzola and Russel (1999). The tissues were vacuum dried for 24 hours in vacuum desiccators. Dried specimens were mounted on an aluminum stub with double sided carbon tape and gold coated in an ion sputter coater (Hitachi E-1010). The specimen prepared were observed at different magnifications under the scanning electron microscope (Hitachi S-3400N).

Immunohistochemical study: The paraffin sections of 3 μ m thickness were mounted on positively charged microscopic slides. Sections were dewaxed with xylene and rehydrated with descending grades of alcohol to distilled water. Dipping of the tissue section in 3% H_2O_2 for 20 minutes at room temperature in a humidified chamber followed by washing with distilled water for 10 minutes resulted in quenching of the endogenous peroxidase activity. Heat induced antigen retrieval (HIAR) was carried out for Kupffer cell by boiling tissue with tris borate EDTA buffer using microwave for 30 minutes. Subsequently, the were sections cooled to room temperature for half an hour. The tissue sections were then washed with distilled water followed by one time washing in phosphate buffer saline (PBS) for three minutes and incubated in dark chamber with 1% bovine serum albumin (BSA) for an hour. Sections were incubated with primary antibody (1:500) for Kupffer cell (Santa Cruz Biotechnology) for 2 hours, washed thrice in PBS for three

minutes each and later incubated with secondary antibody (Ready to use, Vector Lab. USA) for 30 minutes followed by three washing in PBS for three minutes each. Freshly prepared Chromogen (Diaminobenzidine tetrachloride) solution was poured over the sections for 5-7 minutes and the reaction was stopped by rinsing slides in distilled water for 1 minute. The washing was followed by counterstaining with Mayer's Haematoxylin for 50 seconds and again washed in running tap water for 5 minutes. Finally, the slides were subjected to dehydration in ascending grades of alcohol, cleared in xylene and lastly mounted with Vecta mount for examination under the microscope.

RESULTS AND DISCUSSION

Gross anatomical observations

Pig liver had distinct lobation and was divided into six lobes i.e. right lateral, right medial, left medial, left lateral, quadrate lobe which did not reach up to ventral border of liver and a small caudate lobe (Fig. Supple 1). The liver was somewhat rectangular in shape and the weight ranged between 0.880 kg to 1.00 kg with an average of 0.924 ± 0.11 kg. The average length and width of different lobes have been summarized in table 1. The left lateral lobe was significantly longer and wider ($p < 0.05$) and quadrate lobe was significantly shortest and smallest ($p < 0.05$) than other lobes except caudate lobe.

Table 1. Biometrical observations on length and width of different lobes of pig liver

Lobe	Length (cm)	Width (cm)
Left medial	17.84 ± 0.14^a	7.9 ± 0.15^a
Left lateral	21.3 ± 0.35^b	13.3 ± 0.22^b
Right medial	14.28 ± 0.26^a	8.98 ± 0.18^a
Right lateral	18.4 ± 0.17^a	8.06 ± 0.21^a
Caudate	6.18 ± 0.19^c	3.5 ± 0.15^c
Quadrate	6.12 ± 0.10^c	3.38 ± 0.09^c

Mean value with same superscript, within column, do not differ significantly ($p > 0.05$)

Histomorphological observations

The liver was enclosed by simple squamous epithelium of visceral peritoneum. Below the epithelium there was a capsule called Glisson's capsule comprised of dense irregular connective tissue having collagen fibers, few reticular, elastic fibers and smooth muscle cells, fibroblast and fibrocytes. The average thickness of capsule was 18.126 ± 0.48 μ m. From capsule the trabeculae invaginated into parenchyma of liver and divided it into distinct lobules (Figs. Supple. 2, 10). Each lobule was surrounded by loose connective tissue septum. The thickness of interlobular septum was 49.19 ± 2.53 μ m. These hepatic lobules were polygonal and appeared hexagonal in cross section.

The average size of lobule and central vein diameter has been summarized in table 2. The size of lobule was smaller as compared to earlier reported by Sasan *et al.* (2017) who observed the size of lobule as 1403.25 ± 64.90 μ m. The diameter of central vein

in the present study was larger as compared to observations of Sasan *et al.* (2017) who reported it as $149.25 \pm 12.13 \mu\text{m}$. The thickness of interlobular septum was similar to earlier reports of Sasan *et al.* (2017). At periphery of lobule there was a distinct connective tissue area called portal triad area which comprised of a branch of hepatic artery, portal vein, bile duct, lymph vessel and nerves (Figs. Supple. 2, 13). Within the lobule the hepatocytes were arranged in a network of fine collagen and reticular fibers. The area near to central vein has been described as centrilobular area and near to portal area was described as periportal area. Different cell types in pig liver are described below:

Hepatocytes: Hepatocytes were large multifaceted polyhedral cells with prominent spherical nucleus and nucleolus (Fig.1). The surface of cell was smooth but blebbing could also be observed in few hepatocytes (Fig. Supple. 12). The cytoplasm was granular and acidophilic. Each hepatocyte had 3 surfaces; surface facing toward sinusoids, intercellular contact surface and canalicular surface bordering the bile canaliculi (Fig.1). The size of hepatocyte in centrilobular area and periportal area differed significantly ($p < 0.01$). Sasan *et al.* (2017) reported $12.86 \pm 0.49 \mu\text{m}$ size of hepatocytes in pig but they did not mention any difference in periportal and centrilobular hepatocytes whereas in present study a significant difference ($p < 0.01$) was observed in size of hepatocytes in two regions. White (1973) reported a larger hepatocyte–

$21 \mu\text{m}$ in the adult and $14.6 \mu\text{m}$ in the piglet. The diameter of nucleus was $5.51 \pm 0.07 \mu\text{m}$. Usually, the hepatocytes were mononuclear but binuclear hepatocytes were also observed. In an area of $76341.33 \pm 95.20 \mu\text{m}^2$, binucleated hepatocytes formed about 11.03% of total hepatocytes. The number of mononuclear hepatocytes was significantly more ($p < 0.01$) in periportal area than centrilobular area. The cell membrane of hepatocytes facing towards the portal triad area formed a clear-cut surface described as limiting membrane (Fig. Supple. 3). More number of binucleated cells in periportal area may be an indicative of better regeneration and proliferation power in this area as compared to centrilobular hepatocytes. Popescu *et al.* (2012) reported in mice that polyploidy occurs only in tissues with proliferation and differentiation capacity, and mammalian hepatocytes particularly contained one or more diploid nuclei with different DNA amounts. However, Tanami *et al.* (2016) mapped the zonation of liver polyploidy in rat and revealed that liver polyploidy proceeded in spatial waves advancing more rapidly in the mid lobule zone than the periportal and perivenous zones. Whereas Junatas *et al.* (2017) hypothesized that the peripheral parts of the liver lobes represented developmentally oldest hepatic regions and thus contained more large polyploidy cells.

So based on the present findings it may be hypothesized that as the hepatocytes near portal triad area are getting blood enriched in nutrition and oxygen most probably the cell proliferation is better in periportal area, near the portal triads, and lesser in centrilobular area in a hepatic lobule. Junatas *et al.* (2017) also reported that by proliferation, hepatocytes are initially piled, and then they rearrange themselves quickly, forming the classic cords. Thus, mature hepatic cells are involved in regeneration and undergo a division process and form daughter cells with higher degrees of polyploidy. So, it may be concluded that the greater number of binucleated hepatocytes in the periportal area may be contributing in the regeneration process in liver. In scanning electron micrographs, blebbing was observed in some of the hepatocytes (Fig. Supple. 12). Nicotera *et al.* (1986) reported that blebbing may be due to temporary detachment of the lipid bilayer from the submembranous cytoskeleton. This can be considered as an early sign of intracellular Ca^{2+} elevation, which indicates imminent or incipient membrane injury. Blebbing can be considered as a damage-control mechanism, which is triggered after the failure of plasmalemmal resealing.

Kupffer cells: These cells were stellate or sometimes oval shaped phagocytic cells with large nucleus and cytoplasm present in sinusoids (Fig. Supple. 4) similar to as described by Pareek (2000) in sheep, Mahata *et al.* (2003) in spotted deer, Sasan *et al.* (2017) and Kalita *et al.* (2019) in pig. The Kupffer cells closely resembled macrophages and their cell body showed numerous microvillous projections (Fig. 4). The number of Kupffer cells was significantly more ($p < 0.01$) in periportal area than centrilobular area. Bouwens *et al.* (1986) have also observed that kupffer cells were more frequently encountered in regions around the portal areas

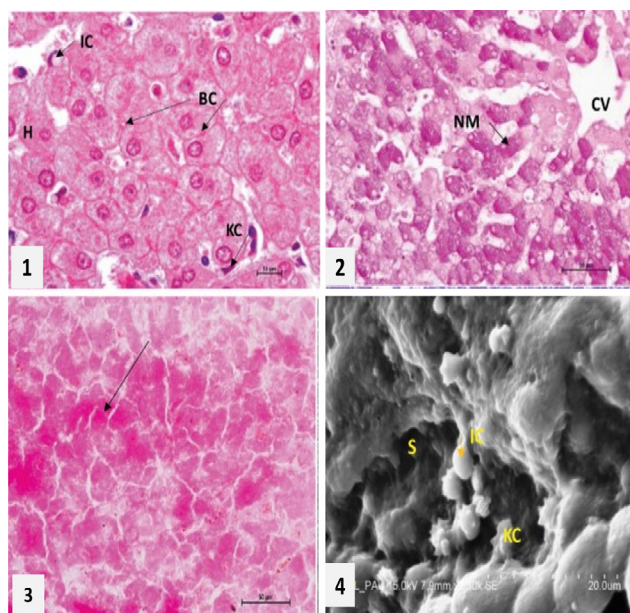


Fig. 1. Photomicrograph of paraffin section showing Kupffer cells (KC), hepatocyte (H), Ito cell (IC) and bile canaliculi (BC). H& E Bar = 1000, Fig 2: Photomicrograph of paraffin section showing strong neutral mucopolysaccharides (NM) reaction in hepatocytes around central vein (CV). Periodic Acid Schiff, Bar = 400, Fig. 3: Photomicrograph of cryostat section showing activity of ACPase (arrow) in periportal hepatocytes (H). Azo dye Method, Bar = 400, Fig. 4 Scanning electron micrograph of pig liver showing Kupffer cells (KC), Ito cells (IC), and sinusoids (S).

than around the central venules. These cells exhibit an important role in the regulation of many hepatocytic functions and in the immune biology of the liver in both normal and pathological conditions (Parker & Picut 2011).

Hepatic stellate cells/Ito cells: These cells were located mainly in peri sinusoidal space between hepatocytes and sinusoids. These cells were oval in shape with ellipsoidal or oval nucleus. The stellate cells were distributed homogeneously throughout the liver and no consistent pattern was observed between periportal and pericentral regions. These cells appeared as spherical and round cells with smooth surface (Fig. 4). Krishna (2013) has reported that hepatic stellate cells were closely related to small nerve endings in human liver and played an important role in the control of sinusoidal blood flow, fibrogenesis and storage of vitamin A. These cells are known to have a major role in regeneration and hepatic fibrogenesis and cirrhosis (Ratzliff and Friedman 1997, Fehrenbach *et al.* 2001). In present study no significant difference was observed in distribution of HSC in hepatic parenchyma but Bleser *et al.* (1991) reported that stellate cells were found more frequently in periportal areas than in pericentral areas in man. Morphologically, these cells appeared as spherical and round cells with smooth surface. Malarkey *et al.* (2005) reported that HSCs get activated to transform into myofibroblasts which typically expressed desmin and smooth muscle actin filaments in human. Grigoraş *et al.* (2016) reported that these cells store and mobilize vitamin A which played a key role in fibrosis progression specific for chronic hepatic pathology, regeneration, metabolism regulation, modulation of local inflammatory and immune reactions.

Lymphocytes: One or two lymphocyte aggregations and a few lymphocytes were observed in the connective tissue of portal triad area. Swindle *et al.* (2012) also observed these small random foci of lymphocytes but without any toxicological significance. These lymphocytes have also been described as Pit cells by Malarkey *et al.* (2005). Dimitrios *et al.* (2013) have described that the liver facilitates tolerance rather than immunoreactivity in human, which protects the host from antigenic overload of dietary components and drugs derived from the gut. Liver-related lymphoid subpopulations also act as critical antigen-presenting cells such as the gut and liver and consist of cells whose primary function may not be immunological but nonetheless still perform essential immune tasks. Within a normal liver, the lymphocyte population is largely resident in the portal tract but can be also scattered throughout the parenchyma. Hepatocytes and biliary epithelial cells (BECs) are sites of immune-mediated destruction, induced by a variety of infectious xenobiotic and tumor-originating sources (Kimura *et al.* 2005).

Central vein and Sinusoids: The sinusoids were the thin-walled blood vessels present between hepatic cords which communicated with central veins. The difference between size of periportal sinusoids and size of centrilobular sinusoids was insignificant ($p > 0.01\%$). The central veins

and sinusoids were lined by endothelium i.e. simple squamous epithelium. Large no. of sinusoids openings in the central veins were observed (Fig. Supple. 11). Large no. of RBCs and other blood cells were also frequently observed in the sinusoids. The little difference in size may be due to large size of hepatocytes in the periportal areas. The size of central vein was lesser in current study whereas Sasan *et al.* (2017) have reported larger size of central vein i.e. $149.25 \pm 12.13 \mu\text{m}$ in pig.

Portal vein and Hepatic arteriole: The portal vein conveyed the nutritional blood to the hepatocytes. They were thin walled with average diameter of $81.380 \pm 3.44 \mu\text{m}$ in portal triad area. The hepatic arterioles were the interlobular branches of hepatic artery, which either joined the capillaries within the portal area or directly entered into the sinusoids. In the current study two or three sections of arterioles of $33.141 \pm 1.14 \mu\text{m}$ diameter were occasionally seen in portal triad area. The tunica media of arteriole consisted of 3-5 layers of smooth muscles depending upon size of arteriole.

Bile canaliculi and bile ductules: The bile canaliculi appeared as expanded intercellular spaces between apposing hepatocytes with size of $1.31 \pm 0.08 \mu\text{m}$ (Fig. 1). Bile canaliculi drained into bile ducts present in portal triad area. The lining cells of bile duct were either cuboidal or columnar cells with round nucleus. A very few goblet cells were also observed at some places among lining epithelial cells of ducts. The average diameter of bile duct was $30.00 \pm 1.48 \mu\text{m}$.

Lymphatics and Nerve fibres: Besides portal vein, hepatic artery and bile duct, thin walled lymphatic vessels and nerve fibres were also seen in the portal area. The lymph vessels were lined by simple squamous epithelium and were surrounded by loose connective tissue of portal triad area.

The micrometrical observations can help to understand Liver Zonation and Functions. Since centrilobular and periportal areas are differentially affected by certain diseases, studying these areas microscopically can help understand the pathophysiology of liver diseases. This zonal differentiation in pigs can offer insights applicable to other species, including humans.

Histochemical observations

Neutral mucopolysaccharides, acid mucopolysaccharides and glycogen: A weak to moderate reaction of neutral mucopolysaccharides was observed in capsule, connective tissue and periportal hepatocytes but hepatocytes towards central vein exhibited a strong reaction for neutral mucopolysaccharides (Fig. 2). A moderate reaction in hepatic artery, weak reaction in central vein and negligible to weak reaction in portal vein and weak to moderate reaction was observed in bile duct for neutral mucopolysaccharides. A moderate to strong reaction of acid mucopolysaccharides was demonstrated in capsule and connective tissue whereas periportal and centrilobular hepatocytes, bile duct, hepatic artery and portal vein showed a weak to moderate reaction (Fig. 5). The central

Table 2. Micrometrical observations on different parameters in pig liver

Structure	Average	Centrilobular Area	Periportal Area
No. of Hepatocytes/76341.33 ± 95.20 µm ²	228.3±1.74	—	—
No. of mononuclear hepatocyte /76341.33 ± 95.20 µm ²	—	160.1±1.99 ^a	170.5±2.68 ^b
No. of binuclear hepatocyte /76341.33 ± 95.20 µm ²	25.2 ± 0.77	13.16±0.60 ^a	15±0.57 ^a
No. of Kupffer cells/76341.33 ± 95.20 µm ²	—	17.1±0.72 ^a	27.7±0.76 ^b
Size of Hepatocytes	15.07±0.26 µm	12.36±0.25 ^a µm	15.74±0.30 ^b µm
Size of Nucleus of Hepatocyte	—	5.33±0.08 ^a µm	5.72±0.08 ^a µm
Size of Sinusoids	—	9.87±0.34 ^a µm	9.26±0.21 ^a µm
Capsule Thickness	18.12±0.48 µm	—	—
Size Of Lobule	1288.5±40.29 µm	—	—
Size of Central vein	81.74±11.58 µm	—	—
Interlobular Septum Thickness	49.19±2.53 µm	—	—
Size of Nucleus	5.51±0.074 µm	—	—
Size of Portal Vein	81.38±3.44 µm	—	—
Size of Hepatic Artery	33.14±1.14 µm	—	—
Size of Bile Duct	30.00 ± 1.48 µm	—	—

Mean value with at least one same superscript within row do not differ significantly ($p > 0.01$).

vein was weakly positive. The capsule, connective tissue, bile duct, hepatic artery and portal vein demonstrated weak reaction and periportal hepatocytes showed weak to moderate reaction whereas centrilobular hepatocytes showed a strong reaction for glycogen deposition (Fig. 7). Aziz (1984) demonstrated a strong PAS reaction for neutral mucopolysaccharides in periportal hepatocytes and moderate reaction in centrilobular hepatocytes in sheep. The intensity of PAS staining may indicate a degree of accumulation or transformation of products associated with biosynthetic activity of the cells (Ramos *et al.* 1980). During this study, it was observed that reaction for glycogen was more in centrilobular hepatocytes but less in the periportal hepatocytes but in camel, Siddig *et al.* (2015) had reported that the glycogen content of hepatocytes varied from cell to cell. The periportal hepatic cells showed intensely stained masses of glycogen and the area of the peripheral zone varied among lobules, depending on the amount of glycogen. In some lobules, the mass of glycogen appeared as a thin strand while in other lobules it was thick. Researchers have observed that when rats were fed a carbohydrate-rich diet the glycogen accumulation was more intense in hepatocytes in periportal area but when it was after a long starving period, the reaction of glycogen was more intense in hepatocytes around central vein and confirmed that during starvation the glycogen depletion starts first from hepatocytes in periportal and then proceeds towards centrilobular hepatocytes (Garrido *et al.* 1996, Surina-Baumgartner *et al.* 1996). In present study, the tissue samples were collected from slaughter house where the animals a fasted for about 24 hrs, so it may be correlated that during starvation period glycogen first depleted from periportal hepatocytes and later on from centrilobular hepatocytes as the glycogen and PAS reaction was more in the hepatocytes around central vein. Contrary to the findings of present study, Gumucio and Miller (1981) reported that amount of glycogen in hepatocytes of

acinar zone is subject to diurnal and metabolic variation. After eating, glycogen is stored initially in the “periportal” area, and later more centrally; during glycogenolysis, however, glycogen disappears from the centrilobular region first. Earlier, Sasse *et al.* (1979) have also reported that hepatocytes of acinar 1, near to portal region, are predominantly involved in gluconeogenesis, while the cells of zone 3, near to central vein, participate mainly in glycolysis. However, the cells of both zones do contain all of the gluconeogenic and glycolytic enzymes, but in different proportions. Therefore, the metabolic zones of the acinus should be viewed as a dynamic reaction of hepatocytes to the micro- environmental conditions extant in each zone rather than as an example of differing intrinsic cellular capabilities. Pareek (2000) observed that both centrilobular and periportal hepatocytes were rich in glycogen content in Magra sheep. Bamaniya (2013) observed that the glycogen exhibited an irregular distribution of glycogen within the cytoplasm of the hepatic cells in Marwari goat.

Basic protein: Capsule of liver, connective tissue and hepatic artery showed a moderate reaction for basic proteins whereas strong reaction was observed in periportal and centrilobular hepatocyte (Fig. 6). Central vein and portal vein showed weak reaction and bile duct showed weak to moderate reaction for basic proteins. A strong reaction for basic proteins was observed in periportal and centrilobular hepatocyte as reported by Gumucio and Miller (1981) in man. Gumucio and Miller (1981) had reported that immunocytochemical studies have shown that under physiologic conditions, between 15% and 35% of human hepatocytes contain detectable amounts of albumin, and about 1% of them contain fibrinogen (Schreiber *et al.* 1970). They further reported that these albumin- or fibrinogen-containing hepatocytes were distributed randomly throughout the acinus. Jensen *et al.* (1967) had observed an increment in the synthesis of albumin in nephrotic rats and this protein was present in all hepatocytes. Thus, it

has been suggested that all hepatocytes have the capacity for albumin synthesis, but for some reason, only a small proportion of them are actively engaged in synthesis at a given time.

Total lipid and Phospholipids: A weak reaction was seen in capsule, connective tissue, central vein, bile duct, hepatic artery and portal vein and weak to moderate reaction was seen in periportal hepatocyte but centrilobular hepatocyte showed moderate to strong reaction for total lipids in pig liver. The lipid droplets varied in size and located in the cytoplasm of hepatocytes adjacent to the sinusoids.

The current study revealed weak to moderate reaction for phospholipids in capsule, connective tissue, hepatic artery and portal vein, moderate reaction in centrilobular hepatocyte but periportal hepatocytes exhibited a strong reaction for phospholipids (Fig. 8). Central vein and bile duct showed a weak reaction. Gumucio and Miller (1981) in human who reported active participation of centrilobular hepatocytes in lipid metabolism. These cells also contain a greater number of microbodies that presumably play some role in lipid metabolism. In addition, the amount of stainable lipid was also more concentrated in the cells of this zone. Siddig *et al.* (2015) suggested that lipid droplets showed wide distribution in the overall lobule with the tendency to concentrate in the cells located at peripheral zone as compared to central zone. In normal rat liver, the lipid content were low and mainly localized peripherally in the lobules (Van Noorden *et al.* 1994). In pig and camel liver, large cytoplasmic vacuoles in the hepatic cells presumed to be lipid droplets have been reported by Bojan (1971) and Abdalla *et al.* (1971) respectively. However, Lalla and Drommer (1997) described a mild to moderate lipid content in normal hepatocytes. According to Shahien *et al.* (1977), the hepatocytes contained small to medium-sized lipid droplets which were concentrated in the peripheral part of the cells along the sinusoids. Natarajan *et al.* (2007) have reported that earlier lipid droplets were considered as an inert bag of lipid for storage of energy-rich fat molecules but later on they have been considered as an active subcellular organelle especially designed for assembling, storing and subsequently supplying lipids for generating energy and membrane synthesis and in the case of hepatocytes for VLDL secretion. These droplets also play a central role in many other cellular functions such as viral assembly and protein degradation.

The current study revealed moderate reaction of phospholipids in centrilobular hepatocyte but periportal hepatocytes exhibited a strong reaction. Central vein and bile duct showed a weak reaction. Phospholipids are known to form the double layer of cellular and subcellular membranes and precondition their fluidity and biological activity. They also play an important role as anti-inflammatory, antioxidant, antifibrogenic, antiapoptotic, membrane protective and lipid regulating effects (Gundermann *et al.* 2016).

Histoenzymic observations

Alkaline phosphatase (AKPase), Acid phosphatase

(ACPase) and Glucose -6- phosphatase (G-6-Pase): The capsule, connective tissue and central vein in the liver showed negligible to weak activity of AKPase and ACPase whereas periportal hepatocytes showed strong activity and centrilobular hepatocytes demonstrated weak to moderate activity (Fig. Supple. 9). The bile duct demonstrated moderate activity while hepatic artery and portal vein showed moderate to strong activity of Alkaline phosphatase (AKPase) and Acid phosphatase (ACPase) (Fig. 3).

Capsule, connective tissue, central vein, bile duct, hepatic artery and portal vein showed negligible to weak activity, periportal hepatocytes showed moderate to strong activity and weak to moderate activity of Glucose -6-phosphatase (G-6-Pase) was demonstrated in centrilobular hepatocytes.

Alkaline phosphatase enzyme represents a barrier function and is observed in structure with directed transport function (Meier-Ruge *et al.* 2008). ACPase enzyme is a lysosomal enzyme therefore its strong activity in periportal hepatocytes is indicative of better phagocytic capability.

Periportal hepatocyte showed moderate to strong activity of Glucose -6- phosphatase (G-6-Pase) as reported by Gumucios and Miller (1981) in pig which correlates with the findings of Gebhardt (1992) and MacSween *et al.* (2002) who also reported strong Glucose-6-phosphatase (G-6-Pase) in periportal hepatocytes. Glucose-6- phosphatase has a greater gradient of expression in the periportal hepatocytes as compared to centrilobular hepatocytes in rat (Malarkey *et al.* 2005). The overall organization of human hepatic lobule shows resemblance with that of rat hepatic lobule with maximal Glucose -6- phosphatase (G-6-Pase) activity in periportal zone (Racine-Samson *et al.* 1996).

So, it is concluded that expression of activity of different phosphatases was more in periportal hepatocytes as compared to centrilobular hepatocytes indicative of more active physiologically than centrilobular hepatocytes. Earlier also, Singh *et al.* (2019) observed a better activity of different oxidoreductases (SDH, MDH, LDH, ALC, GLD, NADH, G-6-PD) in periportal hepatocytes as compared to centrilobular hepatocytes.

Nonspecific Esterases (NSE): Negligible to weak activity was demonstrated by capsule, connective tissue, central vein, bile duct, hepatic artery and portal vein, periportal hepatocyte showed weak to moderate activity and centrilobular hepatocyte showed moderate to strong activity of non-specific esterases as reported by Gumucio and Miller (1981) in human and Bhattacharya *et al.* (1986) in goat. Non-specific esterases are a group of enzymes associated with lipid metabolism. The presence of intense NSE activity suggested that these played a role in synthesis of lipids and phospholipids which are used for the formation of cell membranes.

The present study showed that the number of mononuclear hepatocytes and the average number of Kupffer cells were significantly more in periportal region than the centrilobular area. Histochemical reactions for neutral mucopolysaccharides glycogen and lipids were

more in centrilobular hepatocytes whereas the distribution of proteins was homogeneous. Moreover, periportal hepatocytes exhibited a strong activity of phosphatases whereas non-specific esterases activity was more in centri-lobular hepatocytes.

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