HISTOLOGICAL AND SCANNING STUDY ON THE LIVER OF NEWBORN AND ADULT WHITE SWISS MICE

AL-Zubaidi K.A.\textsuperscript{1} and Ahmed Ali K. Hirallah\textsuperscript{2*}

\textsuperscript{1}Department of Anatomy and Histology, College of Veterinary medicine, University of AL-Qasim Green, Babylon, Iraq.
\textsuperscript{2*}College of Medicine, University of AL-Ameed, Karbala, Iraq.

Abstract

The current study was carried out to investigate the histological and ultrastructural changes that occurred in the liver of Swiss mice (\textit{Mus musculus}) of different postnatal ages. To obtain this target, specimens from liver were collected from twenty mice at four different ages 1, 10, 30, 60 postnatal days, which were set as (1\textsuperscript{st}) day old (G1), 10\textsuperscript{th} day (G2), 30\textsuperscript{th} day (G3) and 60\textsuperscript{th} day (G4) respectively. Routine histological stain, Harris Hematoxyline and Eosin (H&E) and Periodic Acid Schiff (PAS) in addition to scanning electronic microscope, were conducted to achieve current targets. The histological examination of liver revealed many events occurred with progress of ages, hematopoietic elements were decreased in number, the capsule was decrease in thickness which in adult was thinner than that in young. Also, the hepatocytes and there nucleus increased in volume with acquired hexagonal-shape. The sinusoids were decreased in diameter from high expansion to very narrow in adult stage. Central vein showed increased in diameter. The portal area well-formed at 1\textsuperscript{st} day old with contain 1-4 of bile ducts and increased in number of portal area up to adult. The glycogen was absent through the 1\textsuperscript{st} day old and appeared at 10\textsuperscript{th} day old up to adult with PAS stain. Adipose droplets were seen for first time in hepatocellular cytoplasmic in 30\textsuperscript{th} day old with PAS. In the subsequent ages both their size and number of hematopoietic compartments were decrease. Hepatic liver lobules were initiated in 10\textsuperscript{th} day old and in 30\textsuperscript{th} day old the prominent boundaries were seen, Whereas, it well-formed at 60\textsuperscript{th} day old. The sub-capsular hematopoietic elements appeared from 30\textsuperscript{th} day old up to 60\textsuperscript{th} day old. Scanning electronic microscope examination of liver revealed that the shape of hepatocyte change from polyhedral in 1\textsuperscript{st} day old to hexagonal shape at the 30\textsuperscript{th} day old. The hematopoietic foci seen at (G1) scattered throughout the parenchyma and become very rare, as a small foci at the (G2). The triad surrounded by the periportal plates or “limiting plates” and the central vein was have not valves. The present study concluded a many of histological changes which referred to development in the structural and functional of liver throughout the life span.

Key words : Histology, Electronic microscope, Liver, Mice.

Introduction

The liver was the largest gland in the body and receives 25\% of the cardiac output (Lautt, 2010). It was the first site of processing for many of the body’s nutrients and metabolizes carbohydrates, lipids and proteins (Koeppen and Stanton, 2010). Exhibiting both endocrine and exocrine properties, endocrine functions include the secretion of several hormones such as (Insulin-like growth factors, Angiotensinogen and Thrombopoietin), while the major exocrine secretion was in the form of bile. The liver was also essential for glycogen storage (Si-Tayeb \textit{et al.}, 2010). The liver develops from an endodermal hepatic bud that arises from ventral aspect of the distal part of foregut, just at its junction with the midgut (Singh, 2012). The liver consists of a number of cells, hepatocytes, Endothelial cells, Kupffer cells, hepatic stellate/ltc cells and Fat storing cells (Gunasegaran, 2010). There were many studies on the liver development (Dawood and Khamas, 2017) in Indigenous Gazelle, (Hilmer \textit{et al.}, 2007) in rat, (Hashemnia \textit{et al.}, 2015) in chick embryo, (Schotanus \textit{et al.}, 2014) in canine, (Akat and Göçmen, 2014) in Amphibian. The use of animal models, such as the mouse, had identified many of the genes and molecular pathways regulating embryonic liver development (Zaret, 2008; Serup and Füchtbauer, 2010).

*Author for correspondence : E-mail: ahmedali.8800@yahoo.com
Table 1: Showed, central vein diameter, hepatic cell diameter, nucleus diameter, sinusoids distance and liver capsule thickness of mice pups and adult in different ages.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Central vein diameter</th>
<th>Hepatic cell diameter</th>
<th>Nucleus diameter</th>
<th>Sinusoids distance</th>
<th>Liver capsule thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day old</td>
<td>640.8940±18.705</td>
<td>273.0886±12.896</td>
<td>98.7960±3.0824</td>
<td>158.4238±10.109</td>
<td>25.9820±0.832</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>10 day old</td>
<td>859.5148±23.443</td>
<td>358.8744±10.083</td>
<td>125.0146±4.2964</td>
<td>135.2886±6.718</td>
<td>22.1746±0.81</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>30 day old</td>
<td>1171.1023±43.870</td>
<td>438.8836±9.929</td>
<td>149.0652±7.9801</td>
<td>100.3842±4.008</td>
<td>19.5232±0.187</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>60 day old</td>
<td>1516.6722±42.353</td>
<td>532.8960±24.246</td>
<td>166.1610±7.7678</td>
<td>84.5078±9.460</td>
<td>15.3884±0.389</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.; different capital letters mean significant differences (P<0.05) between different age pups and adult.

Material and Methods

Thirty adult mice male and female (5 male and 25 female) were used and kept under laboratory conditions of temperature 20-25°C and allowed free access of food normal diet and tap water ad libitum. The mice were obtained from house of advisory office of the science collage, University of Babylon. These adult mice weighted 35-45 gram and aged between 60-90 days. Vaginal smears were useful in timed mating to determine the presence of sperms. Mating can be confirmed by the presence of capulatory plug in the vagina up to 24 hours post (Fletcher and Weber, 2004). The morning on which the vaginal plug was found was referred to as Zero embryonic day (E 0), (Swartley et al., 2016). The mice were divided into four groups: The newly born pups of one day age were set in the first group (G1). The second group comprised ten days aged of 5 suckling pups (G2). Whereas, the third group includes 5 mice of thirty days of age as post-weaned mice fed on solid pellets (G3). The fourth group includes five mice of sixty days of age (G4). Mice were euthanized by anesthetizing with chloroform dropped in cotton pad, inside a sealed glass box (AVMA, 2013). Animals were dissected, the abdominal cavity was opened by using a surgical scissor and the liver was viewed and removed from the abdomen. For the histological examination, the specimens were fixed in 10% neutral buffered formalin for 48 hours, then the specimen was trimmed and washed by tap water for 4-6 hours to remove the formalin solution and then the specimens were prepared for light microscope study by dehydrated, cleared by xylene and blocked in paraffin wax. Sectioning was made by using the rotary microtome thickness of sections (5-6µm). Two types of stains were used, the Harris Hematoxyline and Eosin (H&E) and Periodic Acid Schiff (PAS) (Suvarna et al., 2012). Micromorphometric measurements included, central vein diameter, hepatic cell diameter, nucleus diameter, sinusoids distance and liver capsule thickness, were measured using the color USB 2.0 digital image system (Scope Image 9.0-china). For scanning electron microscope (SEM), the specimens were washed in two changes of cold (0.1 ml) phosphate buffer solution (PBS), pH 7.2 at 4°C for one hour, the tissues were cut into approximately 1 mm² and put in fixative 3% Glutaraldehyde then incubate at 40°C for 12-24 hours, then pour of fixative and add the 1% osmium tetroxide solution incubate in the cold room overnight then pour of osmium solution and rinse 3 times with PBS, dehydrated in graded alcohol solution, and mounted on aluminums tube with silver conducting paint, coated with Carbone by routine methods (Kashi et al., 2014). The specimens examined under a Philips field emission scanning electron-microscope in collage of Pharmacy, university of Al- Basra. Computer package (sigma plot V12.0/systat software) was used to conduct the histomorphometrical analyses. Data were analyzed by using one way analysis of variance (ANOVA) with significant level set on p<0.05 and the differences among the groups were determined by D uncertain’s multiple range test (Systat Software Inc, 2016).

Result and Discussion

Histological examination

- At 1st day old (G1): The hepatic lobules were well-formed, composed of radialy arranged hepatocytes in plates separated by sinusoid, intervened by hematopoietic cells in a ratio 4:1, hepatocytes were small in size, polyhedral shape (Fig. 1) these results incompatible with that reported by (Leeson and Cutts, 1972) who mentioned that the rabbits liver at the first day postnatal appeared immature and within lobules the hepatocytes generally lacked organization into plates and with (Walthall et al., 2005) who they also claimed that the hepatic cords were loosely arranged and at least three cells thick of rat at birth. Hepatocytes were negative reaction for PAS (Fig. 2), this finding was coincides with (Russo, 2011) who also claimed that the glycogen rapidly...
disappears in the first day of life. Portal triad well formed, consists of: portal vein, 2-4 bile duct and hepatic artery (Fig. 1). (Faraj and Al-Bairuty, 2016) mentioned the structures in starling bird same, that the portal triad consists of: branch of the hepatic artery, one or more branches of hepatic portal vein and bile duct. Both small and large sizes of bile duct observed in the portal triad and this triad also contain 1-4 of bile ducts.

• At 10th day old (G2): The microscopic study of this age have been shown a hematopoietic element scattered within mass of the liver less than previous ages, it concentrated in the periphery of hepatic lobe more than core of lobe at this age (Fig. 3 & 4). This result was

Fig. 1: Photomicrograph of liver at 1st day old showed: Portal vein (PV). Bile duct (BD). Hepatocytes plates (Hp). Sinusoids (S). Erythroblasts (Er). (H&E. 200X).

Fig. 2: Photomicrograph of liver at 1st day old showed: Hepatocytes (H) negative for PAS. Capsule (Ca). Nucleus of mesothelium cells (N). (PAS. 400X).

Fig. 3: Photomicrograph of liver at 10th day old showed: Portal triads (PT). Central vein (CV). Hematopoietic elements (He). (H&E. 100X).

Fig. 4: Photomicrograph of liver at 10th day old showed: Portal vein (PV). Hepatic artery (Ha). Bile duct (Bd). Central vein (CV). Hematopoietic foci (Hf). Sinusoids (S). Hepatic cords (Hc). (H&E. 200X).

Fig. 5: Photomicrograph of liver at 10th day old showed: Portal vein (PV). Bile ducts (Bd). Deposited glycogen (G). (PAS. 400X).
In accordance with (Apte et al., 2007) who documented that the extensive proliferation of biliary cells was observed along with an increase in portal triads during 10th old day postnatal. The ratio of hepatocyte to hematopoietic (15:1), (Fig. 3) according to the observations of (Baratta, 2009). Hepatocytes stained with PAS mainly in the (zone 1 of liver acinus) and around the portal triads (Fig. 5). This result explained by (Leslie and James, 2001) who showed that the blood comes through the portal vein was rich in nutrients, which leads deposition of glycogen in this region. In this age, the hepatic cords began to establishment from small hepatocytes, pale-staining nuclei, around central veins, initiated the liver lobules, its boundaries not clear (Fig. 6). This description also recorded by (Grossi et al., 1985) who claimed that liver paranchymal cells (hepatocytes) began to organize into well-defined hepatic plates beginning around one week postnatal.

Fig. 6: Photomicrograph of liver at 10th day old showed: Central vein (CV). Hepatic cords (Hc). Sinusoids (S). (H&E. 200X).

Fig. 7: Photomicrograph of liver at 30th day old showed: Adipose droplets (Ad). Sinusoids (S). Megakaryocyte (Me). Endothelial cells (Ec). Binucleated cells (Bi). (PAS. 400X).

Fig. 8: Photomicrograph of liver at 30th day old showed: Liver lobules boundaries (Lb). Central vein (CV). Hepatic cords (Hc). Portal vein (PV). (H&E. 100X).

Fig. 9: Photomicrograph of liver at 30th day old showed: Central vein (CV). Deposited glycogen (G). (PAS. 400X).

Fig. 10 a,b: Photomicrograph of liver at 30th day old showed: Hepatic cords (Hc). Sinusoids (S). Central vein (CV). Hematopoietic elements (He). (H&E. a,100X. b, 400X).
• **At 30th day old (G3):** Histological study of this age showed that the hepatocytes gained the final shape (polyhedral), large in size, many of binucleated hepatocyte were seen, formation of the hepatic cords for the hepatic lobules around the central vein (Fig. 8). Sinusoids was narrow with prominent endothelial cells (Fig. 7). The liver architecture like the adult liver showed at this age (Fig. 8). These results agreement with (Apte et al., 2007) who mentioned that the hepatic architecture began to resemble the adult liver after 20th day old and supported that (Leeson and Cutts, 1972) in rabbits, when they explained, an adult appearance of the liver architecture was achieved by 28 days. These results on the other hand, incompatible with (Wong And Cavey, 1992) who reported that the avian liver, was a mass of branching, hollow cords and the bile canaliculi were represented by the lumina of the cords and there was little or no evidence of lobulation in the avian liver. Positive reactions to PAS especially around the central veins (Fig. 9). A very few hematopoietic element scattered within liver parenchyma mainly around central vein (Fig. 10 a,b). Portal triad (consisted of portal vein, bile duct and branch of hepatic artery) (Fig. 11). Accumulation of adipose droplet in hepatic cells (Fig. 7), due to the liver converts fatty acids and glycerol into phospholipids, for the formation of cell membranes and to cholesterol for bile salts (Kulkarni et al., 2013) and they discovered that the liver also associated with synthesis of lipoproteins, cholesterol and phospholipids. Excess carbohydrates and proteins get converted into fatty acids and triglyceride in liver. The septa between hepatic lobules not obvious, this coincides with (Madhan and Raju, 2014) in human, who revealed that the connective tissue septum between portal triad in human

---

**Fig. 11:** Photomicrograph of liver at 30th day old showed:
- Portal vein (PV).
- Hepatic artery (Ha).
- Bile duct (Bd).
- Lymphatic vessel (Lv).
- Red blood cells (RBC). (H&E. 200X).
liver was scanty or less, but they mentioned these septum were present in sheep’s, goat’s and cow’s liver.

- **At 60th day old (G4):** The superficial layer in liver of mice covered with hematopoietic sub-capsular elements, the hepatic lobule well-formed, it composed of radially in arranged hepatocytes (polyhedral in shape, contained large, round, centrally situated nucleus) in plates separated by sinusoids (Fig. 12). Boundaries of sinusoid prominent and contained the endothelial cells and Kupffer cell (Fig. 13), all these results agreement with (Akat and Göçmen, 2014) in Amphibian, who reported that the superficial liver covered with hematopoietic subcapsular tissue, which was responsible for its support and protection the liver. Whereas the position of portal area at the lobule periphery, it consists of interlobular artery, vein and bile duct, the lumen of artery was smaller than vein, the interlobular bile duct was composed of simple cuboidal epithelium (Fig. 14), such finding were similar to (Apte et al., 2007) in mice. Many of hepatocytes were binucleated, positive reaction to PAS, large glycogen deposits were determined in the clusters of melanin granules, after Periodic acid-Schiff (PAS) staining (Fig. 15). Many interpretations of large glycogen deposits were mentioned that the carbohydrate metabolism, presence of excess glucose was stored as glycogen (glycogenesis) in the liver (Strader et al., 2004).

**Micromorphometrical Mesurements**

Micromorphometric measurement of the liver at G1, G2, G3 and G4 of present study revealed that the capsule thickness were decrease significantly (P<0.05) with
progress of the ages respectively. Sawada et al., (1989) in rat demonstrated that the age-related with these changes of liver capsule thickness, due to the hepatocyte proliferative which leads to a superficial tightening of the capsule and dilate and become thin. The central veins diameter were increase significantly (P<0.05) with progress of the ages from (1st day up to 60th day old). Similar obvious changes of central veins diameter which were recorded in rat and goat with increasing age by (Vollmar et al., 2002 and Al-Hameary, 2013) respectively. These increase in diameter to keep up with the increase in the size of the organ and organism. Hepatocyte diameter was increase significantly (P<0.05) from G1 to G4 respectively. Similar hepatocyte growth was described previously in the liver of rodent and human by (Schmucker, 1990; Schmucker and Sachs, 2002), they indicated a change in the functioning of the cell and increase in volume through maturity. The table referred to increased significantly (P<0.05) of nucleus diameter to the G1 up to G4 respectively. Similar to (Vassy et al., 1988) were revealed a same developed in rat liver. From G1 to G4 respectively, the sinusoids diameter were decrease significantly (P<0.05). In fact, researchers, such as (Braet and Wisse, 2002), (Kikuchi and Kondo, 2006) reported through the progress of age, the hematopoietic cells continued to decline, resulting in narrowing of the sinusoids and a decrease in their diameter. All above parameters were set in table to achieve comparison among different of liver in each age.

Electron Microscopic Study

Through the 1st day old, the hematopoietic foci were scattered throughout the liver parenchyma (Fig. 16).

Fig. 19: Scanning electronic micrograph, at 30th day old, illustrated the hexagonal shape of hepatocytes (H), that encircled the sinusoids (S) which lined by endothelial cells (En). Were can see bile canaliculi (Bc) on the intercellular face of hepatocyte. The erythrocytes (Er) stuffed in sinusoids. 5300X. Scale bar 2µm.

Histological and Scanning Study on the Liver of Newborn and Adult White Swiss Mice

Hepatocytes polyhedral in shape and intervened by hematopoietic element, with prominent sinusoids (Fig. 16). These finding were accordance with (Grossi et al., 1985) who reported small foci of hematopoietic cells could be seen. The hepatocytes at 10th day old acquired semi-hexagonal shape near and surrounded the central vein (Fig. 17). Hematopoietic elements were very rare throughout the liver parenchymal as a small foci (Fig. 18). Similar description also recorded by (Grossi et al., 1985). At 30th day old the hepatocytes acquired the hexagonal shape, it had six facets, two or three facets are in contact with the sinusoids, while the remaining facets make contact with adjacent hepatocytes or portal tract, bile canaliculi was seen on the intercellular face (Fig. 19). The portal triad contained very wide portal vein,
with branch of hepatic artery and bile duct. The triad surrounded by the periportal plates or “limiting plates” (Fig. 20). These results coincides with (Warren et al., 2008) in rat. All the erythrocytes in portal veins were anucleated (Fig. 21). Sinusoids branches encircled by hepatocytes (Fig. 19) those views were also seen by (Grisham et al., 1975) in rat.

References


Si-Tayeb, K., F.P. Lemaigre and S.A. Duncan (2010).


