Comparative histological study of (*Hemiechinus auritus*) the Libyan Long-eared hedgehog liver.

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Abstract

Libya covers a massive area and as most of the different mammals found in it are small and nocturnal, active by night and hiding in the day, they are not easily recognized. The aim of this study is establishing national Libyan records on some morphological features of a wildlife animal, the long eared hedgehog. The investigation has been described the macro and micro morphology of an active organ involved in genesis of blood cells, the liver. The impartial has been accomplished by using one-day old and adult long-eared hedgehog from Benghazi province. The organs were defined in situ. Stained tissue sections were prepared to record the light microscopic features. Liver had a position in the abdominal cavity similar to that of other mammals. Only lobules of the new born animals showed hematopoietic activity.

keywords

Libyan long-eared hedgehog, Hedgehog Hematopoiesis, blood cells, liver of hedgehog.

Introduction

Geologist consider that most of the land which is now Libya emerged from the sea between 50 million years ago and much of the history of its mammals has still to be uncovered. Like many of the old mammals the hedgehogs have developed all the necessary morphological and functional modifications for a nocturnal and mostly insectivorous way of life. Feldhamer *et al*. (2004). Hedgehogs are available in many countries around the world. Hufnagl and Bennet (1972) and Al-Awami (1985) documented presence of three species of hedgehogs that belong to three genera in Libya. They are the long-eared hedgehog, *Hemiechinus auritus*, the Algerian hedgehog, *Erinaceus algirus* and the Ethiopian hedgehog, *Paraechinus aethopicus*. The most widely distributed species, especially in the eastern provinces is *Hemiechinus auritus* Gmelin, 1770.

Liver is the second largest organ after the skin. In human beings, it is located on the right side of the upper part of the abdomen below the diaphragm. It lies on the right side of the stomach and makes a suitable accommodation for the gallbladder (Bramstedt, 2006). It receives two vascular supplies. The first is the hepatic portal vein that brings blood which has previously passed through the intestine and spleen. The second is the hepatic artery that brings oxygenated blood from the aorta. Venous blood from the intestine and spleen and arterial blood from the aorta mix together in hepatic sinusoids before leaving the liver through the hepatic vein toward the inferior vena cava. The liver is divided into lobes whose number depends on the species. (Junqueira *et al*., 2003).

Most part of the liver is covered by a fibroconnective tissue capsule (Glisson's capsule) which in turn is covered by the visceral peritoneum tunica serosa. From the capsule

thin connective tissue septa enter the substance of the liver to divide it into lobes and lobules (Leeson *et al*., 1988). Each of the liver lobes is organized into lobules. Number of the liver lobes varies according to animal species. In the cow, sheep, camel and horse there are five lobes, in the pig six and in the dog seven. The lobule is mainly composed of plates of the polygonal hepatocytes. The classic or hepatic lobule is hexagonal in shape, at the corners of which the triads are located. The portal triad, or area, includes a branch of the portal vein, a branch of the hepatic artery and a bile duct. The bile canaliculi of the lobule are connected to the bile duct. At the central area of the lobule is a central vein that drains the sinusoids between the hepatocytes plates (Leeson *et al*., 1988; Martin, 2004). Structural variation in lobules of different animals has been recorded. Pig liver lobule has an envelope of fibrous connective tissue around each lobule. In human liver, the lobular organization is not immediately evident under the microscope and the lobules do not have distinct boundaries (Singh, 2002). Liver cells contain abundant rough endoplasmic reticulum and mitochondria with large deposits of glycogen and lipid droplets of various sizes. The sinusoids may contain phagocytic cells, known as Kupffer cells, derived from the agranulated monocytes. The Kupffer cells form part of the lining of the sinusoids, without formation of junctions with neighboring endothelial cells. They are recognized from the endothelial cells of the sinusoid boundary by their ovoid large nucleus and substantial amount of cytoplasm. The perisiusoid spaces contain lipocytes or adipose cells. (Leeson *et al*., 1988; Junqueira *et al*., 2003). Germann and Stanfield (2005) reported that human liver regeneration capability is due to the action of hepatocytes as unipotential stem cells. They also refer to the possible presence of a bipotential stem cells, named ova cells. They may differentiate either into hepatocytes or into cholangiocytes of the bile ducts.

Materials and methods

Animals

Twenty adult (12 females and 8 males) long-eared hedgehogs were collected from several localities of Benghazi city. A pregnant female was obtained from the wilderness and brought to the laboratory. It gave birth to four pups, hedgehoglets. The adult hedgehogs were examined for abnormalities. Physical examination revealed absence of apparent disease symptoms.

Chemicals

Component of formalin- acetic acid-alcohol (F.A.A.) fixative solution, Zenker fixative solution and Harris hematoxylin and eosin stains were of technical grade. Anesthetic ether, Sodium chloride of the normal physiological saline solution, ethyl alcohol, methyl alcohol and xylene were also of technical grade.

Dissecting out of organs

After recording notes about the site and macromorphology of the liver, the organs were then dissected out. They were washed with normal physiological saline solution. Half of the organs were transferred into glass containers of formalin-acetic acid-alcohol fixative solution and the other set of organs was transferred into Zenker fixative solution.

Preparation of histological sections

At the time of sections preparation, the organs tissues that were fixed in Zenker solution were washed with tap water for 24 hours. Then, all of the fixed tissues of both fixatives were dehydrated by transferring them into ascending grades of ethyl alcohol: 70% → 80% → 90% → 95% → 100 % → 100%. Duration of each transfer was one hour. The processed tissues were cleared by three transfers, for one hour each, into absolute ethanol-xylene, xylene and xylene. Infiltration with melted paraffin wax (melting point: 58) was carried out by three transfers, one and a half an hour each. The glass containers of the paraffin wax and tissues were put into an oven at 65^{o} C. Embedding was achieved by placing the infiltrated tissues into suitable molds containing melting paraffin wax. The molds were left for 24 hours at room temperature for hardening. The prepared paraffin wax blocks were then stored in a refrigerator at 4^{o}C until sectioning step. A rotary microtome (Shandon, U.K.) was used to prepare ribbon of six micrometers thick sections. The sections were flattened by floating the ribbon on warm water (48C^{o}) in a water bath (B. Braun, Germany). Five to six histological sections were mounted on a precleaned glass slide whose surface was smeared with Mayer's albumin adhering mixture. The slides were transferred into an incubator oven (37^{o}C) for 24 hours.

Staining

Deparaffination of the mounted sections was ensured by two transfers, five minutes each, in xylene. The following step of hydration was then carried out by transfers, for two minute each, through descending concentrations of ethanol: 100% → 90% → 80% → 70% → 50%. Slides were then washed with distilled water for one minute. The hydrated section was transferred into Harris' Hematoxylin jar for five minutes. After that, the slides were kept in distilled water for one minute. Differentiation step was carried out by dipping the slides in 1% HCl for 30 seconds, and then placed for ten minutes in jars containing tap water. The slides were then kept for two minutes in the eosin stain. Later on, the stained sections were placed for two minute each through the ascending concentrations of ethyl alcohol 70% → 80% → 90% and two changes, five minutes each, in absolute alcohol. The dehydrated sections were cleared by two transfers, five minutes each, in xylene. The next step was mounting the sections with Distrene-Plasticizer-Xylene (D.P.X). Glass cover slips were placed on the mounted sections and the slides were transferred for 24 hours into an incubation oven at 37^{o}C. Steps of preparation and staining of the histological sections were modified from Gray (1964) and Humason (1981).

Microphotography

The stained sections were closely examined under the light microscope to investigate developing micro morphological aspects of the liver. Selected fields of histological sections were microphotographed with the aid of a light microscope- digital camera unit. A personal computer was connected to the digital computer. With the aid of a T-V capture built-in card and a Movie Maker software program (Microsoft, USA), the selected photographs were captured and saved in a portable storage device. Photo Manager program (Microsoft, USA) was employed to adjust color, brightness and contrast of the captured microphotographs. The magnification power of the adjusted figures was determined by using a micrometer stage slide to measure the distance between lines as appeared with the used objective lens.

Results

Figure 1 shows the abdominal and thoracic viscera of a long-eared hedgehog. The liver takes a transverse position below the diaphragm and to the right side of the stomach. It has a reddish brown color. The surface facing the abdominal wall is smooth and has no connections with blood vessels or ducts. The inner side facing other abdominal viscera shows connections of blood vessels and bile ducts. The gall bladder, which is connected to the main bile duct, is on the left marginal area of this side of the liver. With the exception of the blood vessels and bile ducts region, a fibro-connective capsule surrounds the liver. Liver of this mammalian species has five distinguished lobes (Figure 2). Gross morphology of the new born hedgehog's liver did not show differences from that of the adult hedgehogs.

The capsule of Glisson that surrounds liver lobes of the new born animals is thin (Figure 3). On the contrary, the Glisson's capsule of the adult's hepatic lobes is thick (Figure 4). The liver lobules of the new born that are formed by the trabeculae of the capsule show areas of hemopoietic activity. The trabeculae are very thin to the extent that lobulation is not easily recognized. The epithelial parenchymal cells, the hepatocytes, are not well defined as cords. Sinusoids are also observed connecting to the central vein (Figure 5). Some Kupffer cells are observed at lining sites of the sinusoids. Many stem cells could be noticed in sections of the new born hedgehog (Figure 6). Portal vein and bile ducts are situated at the periphery of the lobules (Figure 7). In liver sections of the adult hedgehogs, lobules do not contain hemopoiesis areas. The parenchymal cells are well organized in anastomosing cords surrounding the central vein. The sinusoids are drained into the central vein. Some phagocytic Kupffer cells are observed lining the sinusoids at interrupted sites (Figures 8 and 9). As in the new born, liver sections of the adult hedgehog show portal vein and bile ducts at junctions between adjacent lobules (Figure 10).

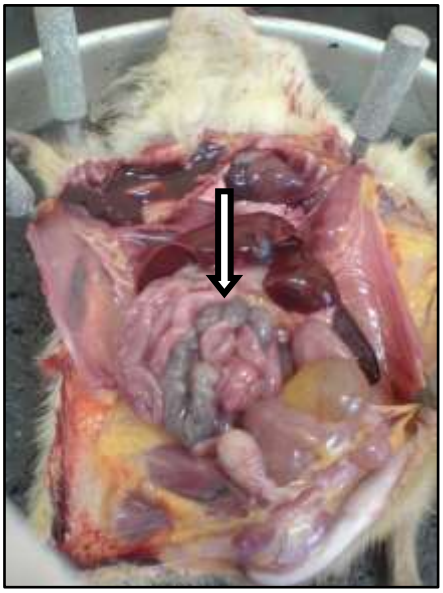

Fig. 1. General view of the long-eared hedgehog viscera. liver (long thick arrow) is observed.

Fig. 2. Gross morphology of the adult long-eared hedgehog liver. The organ has five lobes.

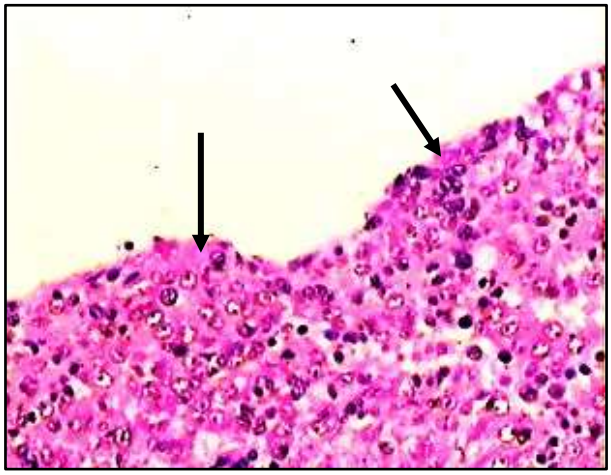

Fig. 3. Hematoxylin-eosin stained section in liver of new born *Hemiechinus auritus*. Thin fibroconnective tissue capsule of Glisson surrounds most of the liver (arrows). (X 150)

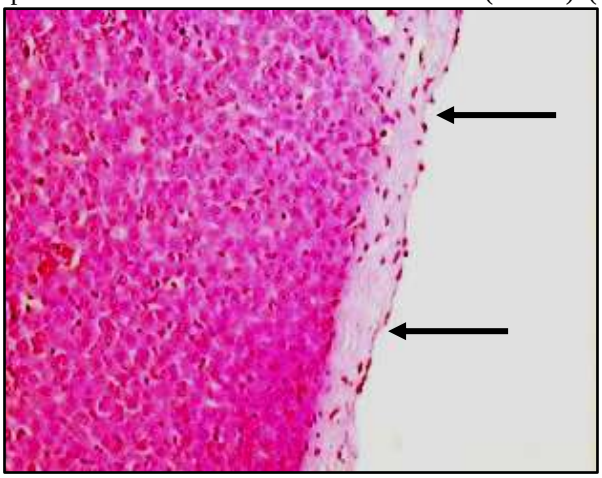

Fig. 4. The thick fibroconnective tissue capsule (Glisson's capsule)

of an adult hedgehog liver (arrows). (H-E, X 150)

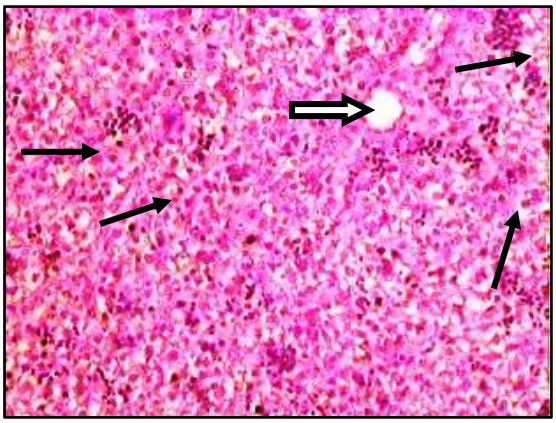

Fig. 5. Central vein (thick arrow) and areas of hemopoietic activity (thin arrows) in a hepatic lobule of a new born hedgehog. (H-E, X 150)

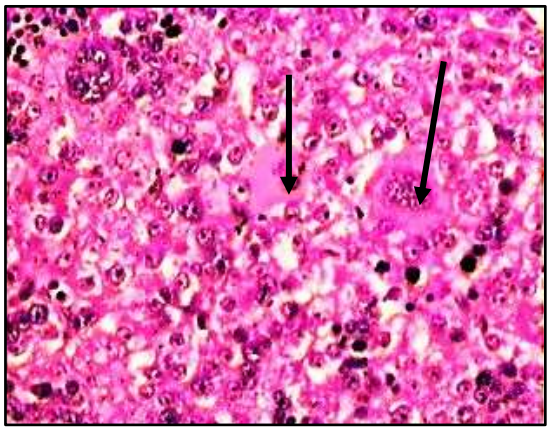

Fig. 6. Stem cells in stained liver section of a new born long-eared hedgehog (arrows). (H-E, X 375)

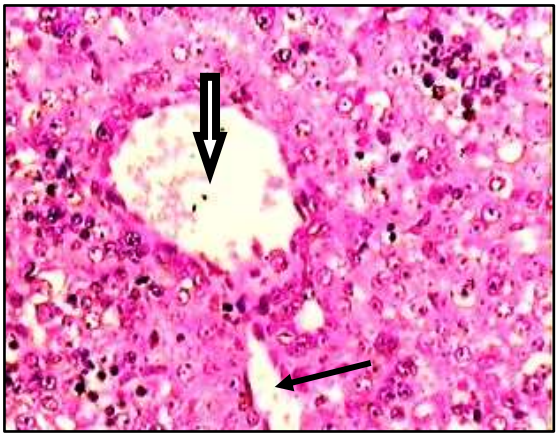

Fig. 7. Portal vein (thick arrow) and bile duct (thin arrow)

in liver section of a new born hedgehog. (H-E, X 375)

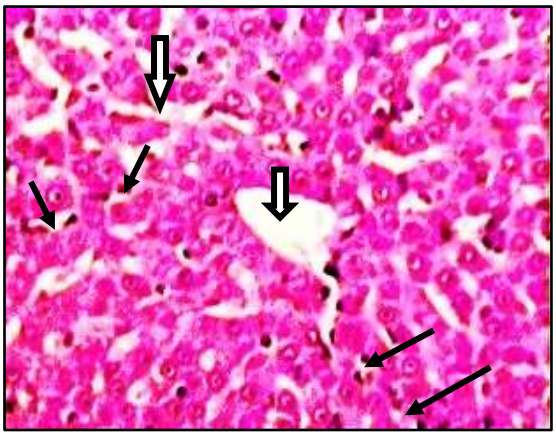

Fig. 8. Central vein (short thick arrow), hepatocytes (short thin arrows) sinusoid (long thick arrow) and Kupffer cells (long thin arrows) lining
the sinusoids in liver section of an adult hedgehog. (H-E, X 375)

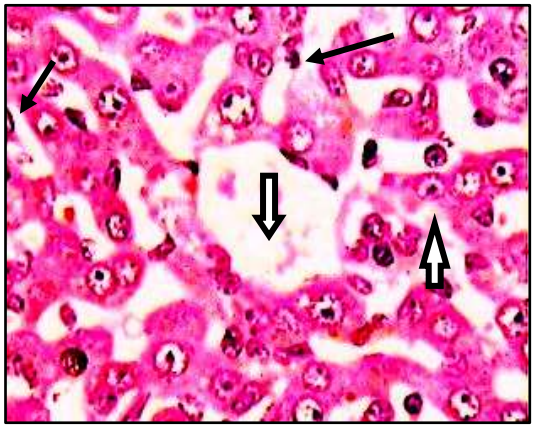

Fig. 9. Central vein (thick short arrow) in liver section of adult hedgehog into which sinusoids (long thick arrow) are drained. Some Kupffer cells (thin arrows) lining the sinusoids
are observed. (H-E, X 600)

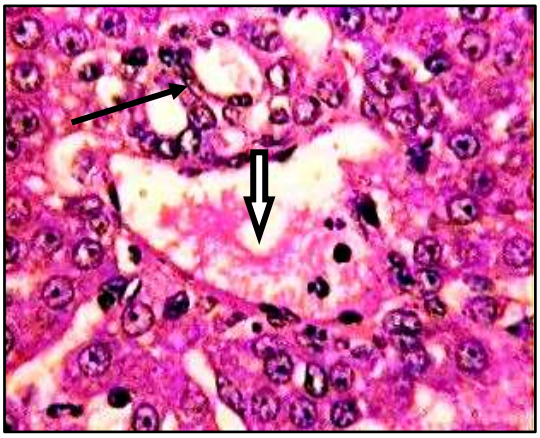

Fig. 10. Section in liver of adult animal showing portal vein (thick arrow) and bile duct (thin arrow). (H-E, X 600)

Discussion

Liver position of the new born and adult *Hemiechinus auritus* within the abdominal cavity and its relationship with the diaphragm are almost the same as in other mammals (Hebel and Stromberg, 1976; Kardong, 1998). Number of the hepatic lobes in the investigated animals was five. Liver of the hedgehog *Erinaceus europaeus* has five distinct lobes (Pelleqrini, 1974). The rat has a liver with five lobes, one of them is small in comparison with the other four (Hebel and Stromberg, 1976). Description of the gross morphology of the rabbit's liver has been outlined and referred to the presence of three lobes, namely the right ventral, right lateral and caudal (Perez *et al*., 2005; Özparlak *et al*.,2011). Liver of the bovine, ovine, equine and camelidae species has five lobes, whereas that of the pig has six lobes and that of the dog has seven lobes (Martin, 2004).

Liver of all mammalian species is surrounded by the Glisson's capsule. The fibroconnective tissue capsule of the adult long eared hedgehog liver was thick in comparison with that of the new born. In adult human beings (Leeson *et al*., 1988; Junqueira *et al*., 2003) and domestic mammals (Dellman and Brown, 1981) the capsule has been described as thick at the hilum as compared with the capsule thickness on other parts of the liver.

Thin trabeculae that the results show in livers of the new born and adult hedgehogs, have also been observed in other mammals with the exception of the pig whose liver trabeculae have dense fibrous tissue mass (Dellman and Brown, 1981; Leeson *et al.*, 1988). Results of the present investigation reveal hematopoiesic activity only in hepatic lobules of the newborn hedgehogs. Similar areas of hemopoiesis have been noticed in hepatic lobules of one week old mice (Grossi *et al*., 1985; Trulson *et al*., 2007).

The well organization of the parenchymal cells was limited to the adult hedgehog liver. Presence of the organized parenchymal cells cords surrounding the central vein has been encountered in livers of other mammalian species. Furthermore, sinsusoids that are lined at interrupted intervals with the phagocytic Kupffer cells have also been described in addition to the portal veins and bile ducts at the periphery of the lobules (Andrew and Hickman, 1974; Dellman and Brown, 1981; Junqueira *et al*., 2003).

Conclusion

Liver of the studied hedgehogs had a position in the abdominal cavity similar to that of other mammals. Five lobes could be identified. Trabeculae of the fibroconnective capsule divided the lobes into lobules. Only lobules of the new born animals showed hematopoietic activity. Well organization of the hepatocytes into cords was observed in adult hedgehogs. Sinusoids in liver of both groups of animals were lined at intervals with the Kupffer cells.

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