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Histological study of liver in Dhub lizard *Uromastyx aegyptius microlepis* (Blanford, 1874) during the period of hibernation

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ABSTRACT

The Iraqi dhub-lizard enters the hibernation when the temperature decreases in the winter. During this period, light microscopic analysis showed that the hepatic tissue is surrounded by a thin capsule composed mainly of collagen fibres. Trabeculae extended from this capsule towards the parenchyma were restricted to the subcapsular region and the liver tissue had no clear lobulation. Hepatocytes were arranged into one or two cell thick cords or acini contained three to five cells. Hepatocytes were polyhedral, oval and pyramidal in shape, and some of these cells demonstrated degenerative changes. Sinusoids appeared either as narrow irregular shape channels separating the hepatic cords with two layers of cells or wide channels separating the cords with one layer of cells. At the beginning of hibernation, application of the PAS technique revealed that glycogen granules spread heterogeneously within the hepatocyte cytoplasm and appeared dark pink in colour while at the end of hibernation these granules decreased in quantity and appeared light pink in colour. Large quantities of various-sized melano-macrophage aggregations were present in the liver tissue. The liver contained a very dense vascular system represented by the vessels of portal areas, the central veins, and the presence of hematopoietic tissue.

Keywords: Liver, Dhub lizard, *Uromastyx aegyptius microlepis*, Hibernation.
Article type: Research Article.

INTRODUCTION

The lizard, *Uromastyx aegyptius microlepis* belongs to Class Reptile, Order Squamata, Family Agamidae, Genus, *Uromastyx* (Afrasiab *et al.* 2018). This lizard enters hibernation at the beginning of winter in November, when the temperature reaches 20 °C or less in most parts of the day, and remains throughout the winter months until the end of March inside the burrow (Kotpal 2010). The liver varies morphologically in lizards and it is located in some types of lizards on the right side of the body cavity (Wyneken 2011). McClellan-Green *et al*. (2006) and Jacobson (2007) documented a brief overview of reptile liver tissues. Schaffner (1998) stated that the histological structure and hepatocytes in reptiles can be compared with those of other vertebrates. This researcher also indicated that the liver in reptiles as in the rest of the vertebrates are surrounded by a capsule of connective tissue known as Glisson's capsule. Jacobson (2007) reported that the connective tissue septa separating the adjacent lobules of the liver in some mammals are not clear in reptiles. There are some reports about histological studies in different organisms (Abbaszadeh & Şişman 2021; Al-Hadidy & Mostafa 2022; Aziz Mahdi Al-badry 2022). However, review of the literature showed that a few studies have attempted to describe the structure of digestive organs in the Iraqi reptilian species such as the study of Al-Shuaily (2012) on the gecko and Mezyad (2015) on the turtles. In this literature no work has been reported on the digestive organs of the dhub lizard, *Uromastyx aegyptius microlepis* that inhabited the Iraqi desert land. So, this work aimed to study the liver structure of this lizard by light microscopy.

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MATERIALS AND METHODS

The study was carried out on ten healthy adult male of *U. aegyptius microlepis* weighting 803.4 ± 109.9 g. These lizards collected during October and November 2019 from Al-Najaf province and placed in cages suitable for the animal during hibernation season. The lizards were anesthetized by inhalation of chloroform and then dissected. Samples of the duhb liver lobes were fixed with 10% formaldehyde for 48 h. After fixation, the duhb liver were dehydrated and then embedded in pure wax paraffin. Sections of 7 µm thickness were stained with Harris Haematoxylin and Eosin (HE); Periodic acid Schiff (PAS) stain; Alcian blue stain (AB; 2.5 pH) and Masson's trichrome stain according to Suvarna *et al.* (2019), and Gomori's methenamine silver stain according to Luna (1968). The microscopic slides were examined with Compound Light Microscope (XSZ-N107 ROYA) and then the sections photographed with a digital microscope camera (MC500) connected to compound microscope.

RESULTS

Examination of the liver histologic structure in male lizard, *U. aegyptius microlepis* during hibernation showed that the liver parenchyma is surrounded by a thin layer of loose connective tissue forming Glisson's capsule, that mainly contains collagen fibres appeared in blue colour with Masson's trichrome stain. Also, an outer covering of mesothelium, consisting of simple squamous epithelium was noticed over this capsule (Fig. 1-A). Trabeculae extended from the capsule restricted to the sub-capsular zone of the liver parenchyma and lobular fashion not recognized in the liver of the male dhub lizard.

The hepatocytes constitute the basic structural units of the hepatic parenchymal tissue, and these cells are mainly arranged as plates or hepatic cords, the thickness of which is one or two layers of cells, or in the form of acini containing three to five cells. The hepatic cords or plates were often not arranged radially around the central vein, but rather appeared in the form of a network of cells around the central vein and the portal area or between them. Narrow sinusoidal capillaries of irregular shape were noticed between the hepatic cords with two layers of cells (Fig. 1-B), while wide sinusoidal capillaries were seen surrounding the cords with one layer of cells in most cases. The use of Gomori's methenamine silver stain showed that the liver parenchyma is supported by a dense and highly branched reticular fibres network surrounding hepatocytes, the portal area and sinusoidal vessels (Fig. 1-C). The male dhub lizard hepatocytes appeared as polyhedral, oval, or pyramidal-shaped cells and some of these cells showed degenerative changes during the winter season. One nucleus was observed in the majority of the hepatocytes, which was located either in the centre of the cell or peripheral, on the side of the cell facing the sinusoidal blood vessel in most cases. In addition, binucleated hepatocytes sometimes were observed in the liver sections. Using routine stain (Hematoxylin-eosin), the hepatocyte showed granular acidophilic as well as vacuolated cytoplasm in which granules are scattered heterogeneously in terms of distribution and size (Fig. 2-A). On the other hand, application of PAS reaction revealed that these cells contained dark pink-coloured glycogen granules that spread heterogeneously within the cell, and usually aggregated on one side of the cell at the beginning of hibernation (Fig. 2-B). However, at the end of hibernation, it was noticed that these granules decreased in quantity and appeared in light pink colour (Fig. 2-C). The cytoplasm of hepatocytes gave a negative response to alcian blue and not coloured in blue with this stain (Fig. 2-D). Microscopic examination also revealed the presence of other cells in the liver parenchyma such as endothelial cells that found in the discontinuous lining of the hepatic sinusoids, characterized by their flattened shape and contain a large flattened nucleus that occupies the centre of the cell. The cytoplasm of these cells appeared clear and gave a weak or moderate positive reaction to alcian blue stain (Fig. 2-D). Kupffer cells, were also seen within the cavity and wall of the liver sinusoids with endothelial cells. These cells are characterized by their large size and irregular surface due to the presence of filopodia and lamellipodia (Fig. 2-C). In addition, the cytoplasm of Kupffer cells had large phagosomes containing a mixture of pink ceroid pigments, black melanin and brown hemosiderin pigments (Fig. 2-C). Ito cells or fat-storing cells in which small fat droplets may be found were also shown within the disse space (Fig. 2-C). Mast cells were noticed within the lumina of sinusoids by applying alcian blue technique. These cells had oval or spindle shaped with a pink nucleus surrounded by cytoplasm containing metachromatic granules (Fig. 2-D). Microscopic examination of the liver of the male dhub lizard during hibernation revealed the presence of large quantities of special cellular aggregations of different sizes (small, medium and large) called melanomacrophages that contain dark brown cytoplasmic phagocytic bodies. In the cross section, they are distributed in the form of oval, polygonal or irregularly shaped clumps of cells under the liver capsule and around the central vein, as well as between the central vein and the portal area. However, they are slightly far from the hepatic portal area (Fig. 3-A).

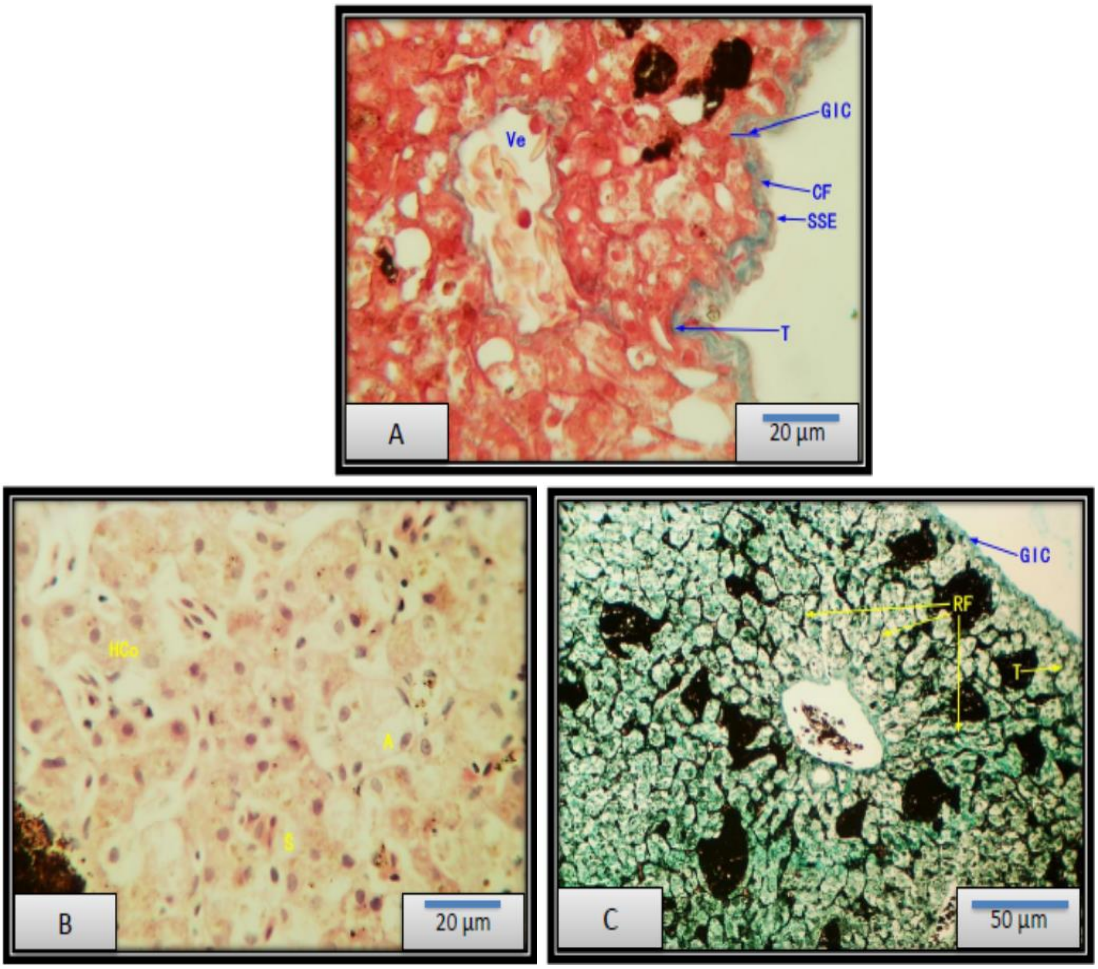
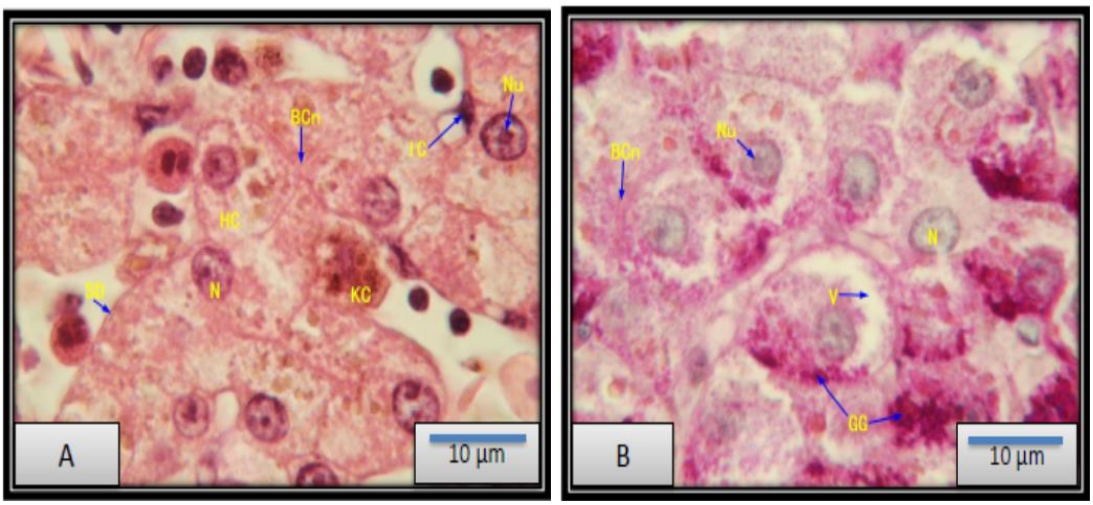


Fig. 1. Cross section in the liver showing (A) Glisson's capsule with (Masson trichrome satin, 400 x); (B) Hepatic cords with (Haematoxylin Harris and eosin stain, 100 x); (C) Reticular fibres with (Gomori's methenamine-silver nitrate, 100 x): Collagen fibres (CF), Central Vein (CV), Glisson's Capsule (GlC), Hepatic acinus (A), Hepatic Cords (HCo), Large portal area (LPA), Reticular fibres (RF), Simple squamous epithelium (SSE), Sinusoid (S), Trabeculae (T), Vein (Ve).



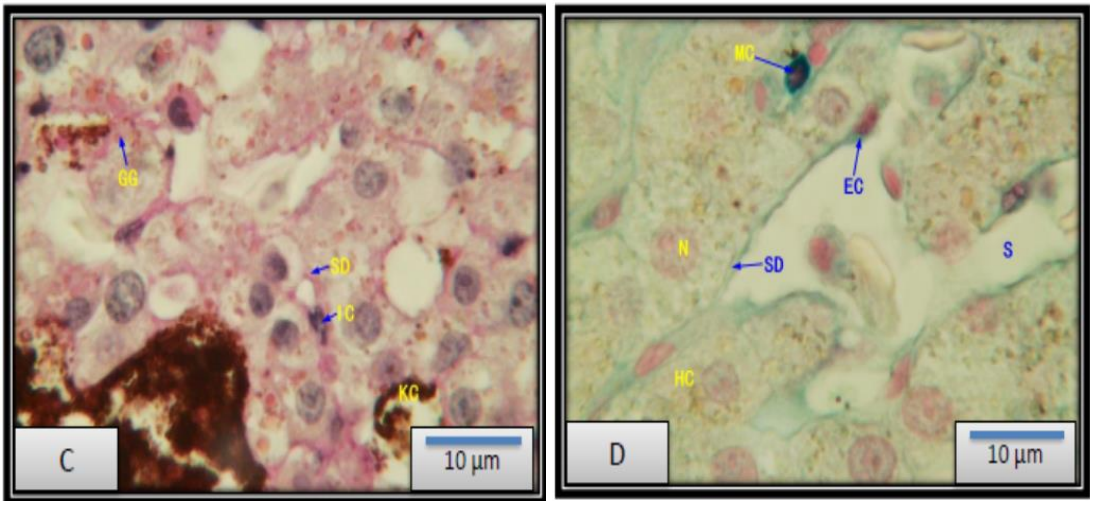
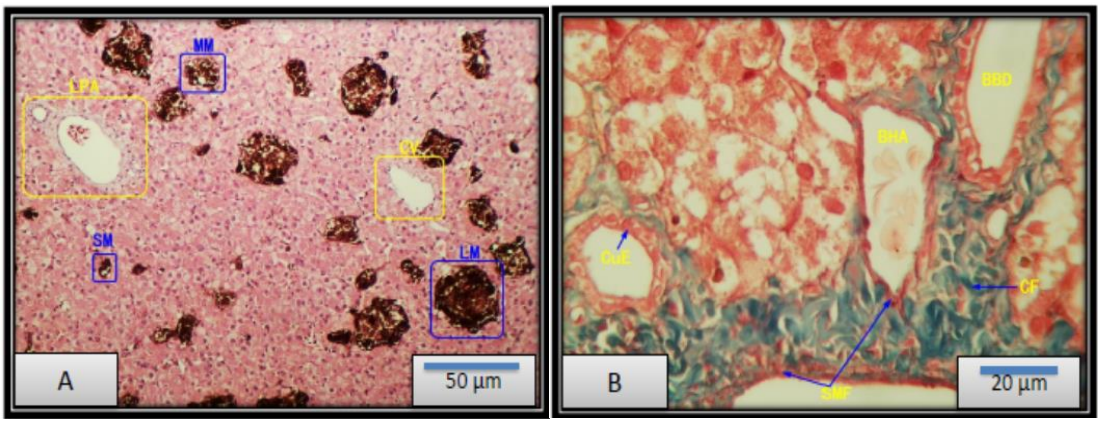


Fig. 2. Cross section in the liver show: (A) Hepatocyte with (Haematoxylin Harris and eosin stain, x 1000), (B) Glycogen granules during the beginning of hibernation with (Periodic acid-Schiff stain, x 1000), (C) Glycogen granules during the end of the hibernation with (Periodic acid-Schiff stain, x 1000), (D) Mast cell with (Alcian blue stain, x 1000) : Bile canaliculi (BCn), Disse space (SD), Endothelial cell (EC), Glycogen granules (GG), Hepatocyte (HC), Ito cell (IC), Kupffer cell (KC), Mast cell (MC), Nucleus (N), Nucleolus (Nu), Sinusoid (S), Vacuole (V).

The portal area supported by connective tissue, composed mainly of collagen fibres and contained fibroblasts. Triad structures were embedded in this connective tissue and included one or more than one branch of the bile ductule that lined with simple cuboidal epithelium, a branch of the hepatic portal vein, and usually one branch of the hepatic artery. The branch of the hepatic artery is characterized by its small lumen compared to the portal vein, and both are lined with a layer of endothelial cells (Fig. 3-B). The central vein, was round or oval in shape and lined with a row of endothelial cells were penetrated by the sinusoids that drained into the central vein lumina. A thin layer of loose connective tissue that supported the lining of the central vein, contained collagen fibres, that appeared in a pale blue colour with Masson's trichrome stain (Fig. 3-C). Aggregation of hematopoietic tissue was also observed in the hepatic parenchyma represented by different stages of blood cells (Fig. 3-D).



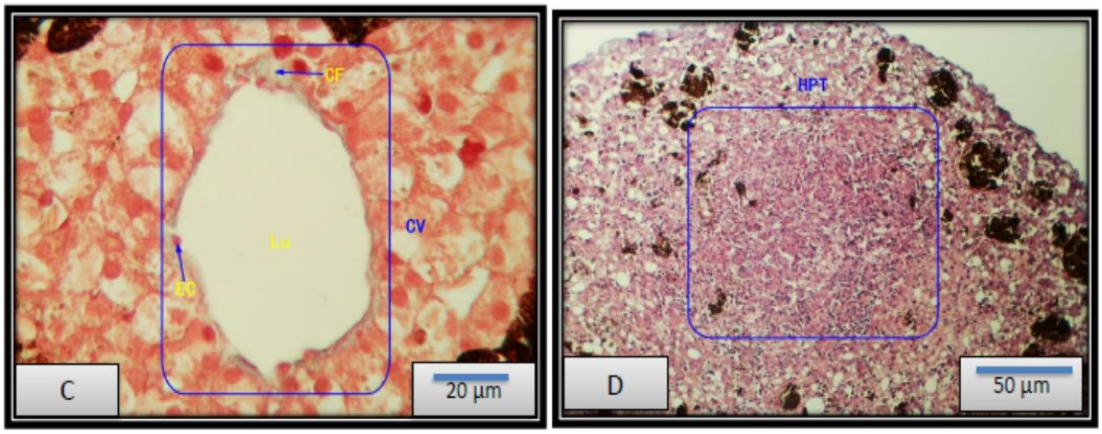


Fig. 3. Cross section in the liver show: (A) Melanomacrophage with (Hematoxylin Harris and eosin stain, x 100), (B) Collagen fibres in the portal area with (Masson trichrome satin, x 400), (C) Collagen fibres in central vein with (Masson trichrome satin, x 400), (D) The hematopoietic tissue with (Haematoxylin Harris and eosin stain, x 100) : Branch of Bile Duct (BBD), Branch of hepatic artery (BHA), Collagen fibres (CF), Simple cuboidal epithelium (CuE), Central Vein (CV), Endothelial cell (EC), Hematopoietic tissue (HPT), Large melano-macrophages (LM), Large portal area (LPA), Lumen (Lu), Medium melano-macrophage (MM), Small melano-macrophages (SM), Smooth muscle fibres (SMF).

DISCUSSION

The results of the current study showed a number of important aspects related to the histological composition of the liver gland in the dhub-lizard during the hibernation period. The liver parenchyma in *U. aegyptius microlepis* was coated by a thin layer of loose connective tissue that forms the hepatic capsule, which is overlaid by a mesothelium. A similar description of the hepatic capsule was documented by Firmiano *et al.* (2011) in the lizard *Tropidurus torquatus* and Moura *et al*. (2012) in the giant Amazon river turtle, *Podocnemis expansa*. However, in contrast to our result, Marycz *et al*. (2009) identified a distinct thickening in the dense connective tissue that forms the liver capsule of the red-eared turtles after hibernation. According to Schaffner (1998), the presence of a hepatic capsule is common in all vertebrates. Using silver stain showed that the liver parenchyma was supported by a dense and highly branched reticular network. This finding corresponds to the results of Moura *et al.* (2012) in the liver of *Podocnemis expansa*. The liver parenchyma in *U. aegyptius microlepis* is not divided into lobules as shown in the mammals or in the turtles by Moura *et al.* (2009). The absence of a lobulation pattern in the liver was also confirmed by previous studies on other species of lizards such as Al-Shuaily (2012) and An & Zhang (2019). According to Gardner & Oberdorster (2006), the structural pattern of the liver organized into distinct lobules is not discernible in all reptilian species. In the current study, hepatocytes were usually arranged into cords of one or two layers thick. However, sometimes three to five cells arranged in the form of acini were also present, while Akiyoshi *et al.* (2016) found only the pattern of combined two- and one-cell-thick plate type, in two families belonging to lizards, Gekkonidae and Agamidae. Also in contrast to our study, Ahmed *et al*. (2018) observed in the liver of *Varanus niloticus* that hepatocytes are grouped in alveoli similar to the glands or in tubules, each of them composed of three to eight cells. This difference in the arrangement of hepatocytes is a species dependent and may be due to their biological functions in the body (Odokuma & Omokaro 2015). The hepatocytes appeared in polyhedral, oval, and pyramidal shaped with one or two nuclei which are central or peripheral in location and surrounded by acidophilic cytoplasm containing vacuoles and granules of glycogen. This is in agreement with the study by Nafady & Awadalla (2019) on *Acanthodactylus boskianus*. The present study indicated degeneration changes in some hepatocytes during this season, in line with the study of Marycz *et al*. (2011), who reported that the body mass loss of the red-eared turtle was accompanied by a tendency of hepatocytes to contract during hibernation. Hepatocytes are separated from the sinusoid lining by a narrow (Disse) sub endothelial space, in which Ito cells are located. The sinusoids are in line with endothelial cells and macrophage cells called Kupffer cells. These cells are also found in previous studies on other reptilian species such as results obtained by Al-Shuaily (2012), Zhang (2019) and Thongboon *et al*. (2019). Analysis by PAS reaction revealed the presence of dark pink colour glycogen granules with heterogeneous distribution within the cytoplasm of hepatocytes at the beginning of the hibernation and towards the end of the hibernation period, these granules decreased in quantity and appear in light pink colour. This result indicated the accumulation of glycogen granules in the liver as a source of energy at the onset of hibernation, which was gradually consumed by the lizard to maintain its life during this

unsuitable thermal conditions. Nafady & Awadalla (2019) noticed in *Acanthodactylus boskianus* that hepatocyte cytoplasm contained a strongly pas-reactive glycogen granules of variable sizes, which clumped together as massive patches condensed mainly at one hepatocyte pole. Sections stained with alcian blue showed a negative response of the hepatocyte cytoplasm to this stain, which refers to absence of the acid mucopolysaccharides in these cells during the winter season. All the stains used in our study showed the presence of melanomacrophages. According to the study by Akiyoshi *et al*. (2016), melanomacrophages were observed in sinusoids capillaries in the suborder Lacertilia, but not in the sub-order Serpentes (except the Black-headed sea snake). Melanomacrophages have various functions, including melanin synthesis, maintenance of the phagocytic ability at low temperatures and neutralization of free radicals (Johnson *et al*. 1999; Jacobson, 2007; Franco-Belussi *et al*. 2012; Akiyoshi *et al.* 2016). With respect to the portal area in the liver of our study, its histological structure was similar to that found in liver of *P. expansa* by Moura *et al*. (2012) who reported that most of the portal areas contained a branch of a portal vein and a bile duct, however, few possessed arteries. Hematopoietic tissue was observed in our study also found by Mezyad (2015) in the liver of two species of Iraqi turtles. Jacobson (2007) stated that blood cell formation centres include the bone marrow, liver, and spleen.

CONCLUSION

During the winter hibernation season, the functional activities of the dhub-lizard reaches the lowest levels necessary to maintain life to resist unsuitable thermal conditions. In this period. The liver undergoes changes to maintain life represented by the accumulation of glycogen granules as a source of energy directly before this period and increase of liver pigmentation to maintenance the phagocytic ability.

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