HISTOLOGICAL STUDY OF THE LIVER IN TWO FRESHWATER TURTLES IN SOUTHERN IRAQ, EUPHRATES SOFT SHELL TURTLE (*RAFETUS EUPHRATICUS*) AND CASPIAN TURTLE, *MAUREMYS CASPICA* (TESTUDINES)

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ABSTRACT

This report represents the morphohistologically characterization of the liver in two species of turtles in southern Iraq, by using four specimens from each one which captured from the Basra marshes. Morphologically, this organ showed a considerable variation between them, the liver of *R. euphraticus* was cylindrical, irregular and very huge, thickened and consisted of several lobes with clear fissure, while in *M. caspica* was large and wide with an approximately rectangular shape and consists of three lobes, the right one was larger than other, Its colors is variable from red to dark brown. Microscopically, After the histological preparation, the slides were stained with Hematoxylin and Eosin (HE), Periodic Acid-Schiff (PAS), Van Gieson’s and Reticulin for reticular fibers, Microscopic analysis showed the liver covered by a thin layer of connective tissue forming the hepatic capsule, this capsule contributed to the division of the parenchyma into structural units. In all staining techniques showed large quantities of melanomacrophages in the hepatic parenchyma in both turtles. liver of *M. Caspica* probably appeared in longitudinal section as hepatic tubules and likely were arranged as cylinders, In cross section, they resemble acini containing approximately two to five hepatocytes, each hepatocyte are polyhedral with varied size and mostly contain one nuclei , liver in *Rafetus euphraticus*, in longitudinal section, probably arranged as pyramidal shape in histological slices, Whilst in cross section resemble acini containing approximately two to six hepatocytes. Most hepatocytes are polyhedral in shape and contain one nuclei. The cytoplasm in both species appeared as high eosinophilic with more vacuolated found in liver cell of *Rafetus euphraticus* when analyzed by the hematoxylin-eosin staining technique.


INTRODUCTION

Reptiles are considered as complex with great diversity groups, which are represented by many vertebrates, including turtles, crocodiles, lizards and sphenodons. Testudines are characterized by the presence of a shell (carapace and plastron) joined by bone bridges and
are divided according to their habit in terrestrial ones, which are called tortoises, while those living in marine and freshwater are called turtles (Kellner and Schwanke, 2001). *Mauremys caspica* belongs to the family geoemydidae, also called Caspian turtle. The genus Mauremys contains ten species spread in fresh water, such as swamps, marshes, ponds, lakes and rivers, with a small-medium size. They are widely distributed in the Middle East, Turkey, Iran, Syria, Iraq and some areas of Saudi Arabia and Bahrain. (Fritz et al., 2007; Rhodin et al., 2010; Yadollahvand & Kami, 2014). The Euphrates soft shell turtle (*Rafetus euphraticus*) is one of the least known species of the Trionychidae family, The name of this species comes from the fact that the carapace does not have horny plates like many turtles, and is instead made up of a tough, leathery skin which is covered with numerous hard tubercle (Ghaffari et al., 2008; Firouz, 2005). They are found in the Tigris and Euphrates rivers and marshes, southeast Turkey, Syria and Iran, It is a one of the common species in the Iraqi marshlands (Fritz et al., 2007; Rhodin et al., 2010; Bircik & Turga, 2011).

Liver is an important gland, which is accessory to the digestive system. It is a large organ and has a multiple and complex functions and considered as the center for metabolic processes and playing a key role in absorption and digestion of fats, carbohydrates storage, as well as the production of most plasma proteins (Akat and Gocmen, 2014; Firmiano et al., 2011). The liver produces red blood cells during embryonic stages, and removes toxins from the blood in addition to the role in bile secretion. (Kardong, 2006; Ross and Pawlina, 2006). The liver plays a role in the production and storage of glycogen, the stored energy that can be used in several situations, for example, during sexual reproduction efficiency and metabolic changes during periods of the year and particularly in species affected by the cold climatic changes (Schaffner, 1998; Marycz, et al., 2009). The liver is a mixed gland surrounded by a thin capsule of connective tissue, the Glisson capsule, dividing the parenchyma into lobules and lobuli. The histological unity of the liver is composed of the liver lobuli, with classic, portal and acini conceptions. The liver has two types of irrigation: one constituted by the hepatic artery, which provides arterial blood to the gland, and the other derived from the portal vein, carrying blood from the esophageal and gastrointestinal tracts, from spleen and pancreas, Liver parenchyma consist of a group of cells which arranged around a central vein, cells are polygonal with a spherical nucleus shape. (Kent and Carr, 2001, Ross et al., 2003). Few authors have described the liver of reptiles macro- and microscopically, Schaffner (1998) analyzed the liver of reptiles in general, while Moura et al. (2009) studied the microscopic aspects of the organ of the freshwater turtle *phrynops*
geoffroanus and Machado Júnior et al. (2005) described the macroscopic aspects of this gland in the scorpion mud turtle *Kinosternon scorpioides*, Moura et al. (2012) studied the liver of *podoenemis expansa*. However, the few morphological studies that have been made of the digestive system of hard and soft shell turtles do not include a morphological description of the liver of this species. Little is known about the morphological aspects of most turtles in habitating South Iraq. Aiming to contribute to this line of research, the objective of the present work was to investigate the liver of two species of turtles macro- and microscopically and to describe its anatomical relationships.

**Material and methods**

The animals used in this experiment were four from both species of turtles that captured from the Basra marshes. The animals coelomic cavity was opened, completely exposing the viscera (Wyneken, 2001), a description was made of the anatomical relationships, and fragments were collected and fixed in 10% formalin. The histological procedures were carried out in the laboratory of scientific marine center. The specimens were processed according to the routine histological technique (Bancrotand and Stevens, 1982). Six slides, each containing at least two slices, were prepared from each piece and were subjected to the staining techniques by routine Hematoxylin-eosin stain, Van Gieson’s for collagen, Periodic acid-Schiff (PAS) for glycogen and glycoproteins (GPs) and Gomori argentie impregnation for reticular fibers, mounted by use a D.P.X, Examined the microscopic sections by using an optical compound microscope type (Olympus, japan) and picked up images of tissue sections by optical imaging microscope type Kruss (German) with a digital camera type HDCE-50B.

**Results**

Morphological description of liver *Refetus euphraticus* was cylindrical, irregular shape and thickened that consist of a several lobe Figure 1 (A) was filling almost the entire cranial portion of the coelonic cavity and surrounding the pancreas, duodenum and stomach Figure 1 (C-D). While the liver of *Mauremys caspica* was wide and thin with an approximately rectangular shape that consist of three lobes, Figure 1 (D-F), was filling almost the entire cranial portion of the coelonic cavity. The color of the liver in *Mauremys caspica* was reddish brown however the liver of *Refetus euphraticus* was dark brown to almost black with a light spots represented a melanocyte, showed a deep fissure that separated a liver in to
several lobes, two lobes in liver of *Mauremys caspica*, the right lobe was the elliptic shape and larger than the rest, while the medium was very thin, very small and which Splited partially into caudoventral part and caudodorsal parts. Liver of *Refetus Euphraticus* had several lobes was described, the medium lobe was a heart like shape and larger than as it other. Gall bladder in *Refetus euphraticus* was a cylindrical, greenish color and located down the right lobe, while in *Mauremys caspica* is appeared a wide sac, a pale greenish color is located under the median lobe of liver.

Microscopic analysis showed that the liver in both types was covered by a thin layer of connective tissue forming the hepatic capsule and it was Surrounded from outside by mesothelium, this capsule contributed to the division of the parenchyma into structural units, called hepatic lobules, Hepatic capsule of *Rafetus euphraticus* appeared thicker than the liver capsule of *Mauremys caspica* Figure2 in all staining techniques showed large quantities of melanomacrophages in the hepatic parenchyma in both types of turtles. Figure2(A-C).

Histologically, hepatocytes in the liver of *M. Caspica* probably appeared in longitudinal section as hepatic tubules and likely were arranged as cylinders Figure3(A-B), Whilst in cross section resembled acini that contain approximately two to five hepatocytes Figure3 (C-D), The hepatocytes of *M. caspica* are polyhedral in shape and their sizes varying. That was observed in each Hepatocytes are contained one or two nuclei, mostly large, rounded and eccentric region with dark oval nucleolus. The cytoplasm appeared slightly vacuolated and more eosinophilic when analyzed by the hematoxylin-eosin staining technique Figure4(A-B).

While in liver of *Rafetus euphraticus* was probably appeared as hepatic tubules in longitudinal section and likely arranged as pyramidal shape in histological slices Figure4(C-D), Whilst in cross section resemble acini containing approximately two to six hepatocytes Figure5(A-B), Most hepatocytes are polyhedral in shape in cross section. Each hepatocytes of *Rafetus euphraticus* was probably contains one or two nuclei, mostly were rounded, eccentric regions with pale rounded nucleolus The cytoplasm appeared highly vacuolated and more eosinophilic when analyzed by the hematoxylin-eosin staining technique. Figure5(C).

Sinusoids in both types of liver turtles are interspersed within hepatocytes, which vary widely in size. The lumen of sinusoids was lined with flattened endothelial cells and contains erythrocytes and macrophages as phagocyte and also called kupffer cell Figure5(D). In both liver of turtles, the hepatic portal area of interlobular artery, vein and bile duct were observed. The lumen of interlobular artery was smaller than vein., the
Branches of portal area were lined by endothelial cells and covered with some smooth muscles fibers, the interlobular bile duct was composed of simple cuboidal epithelium tissue Figure6(A-B). With respect to the portal tracts in both types they were found to be supported by abundant connective tissue Figure6(C-D). moreover, the liver of *M. Caspica* was little reactive to PAS, in comparison with of *Rafetus euphraticus* were regarded more reactive with PAS staining technique Figure7(A-B), by using Reticulin staining Demonstrated the hepatic parenchyma of both types were supported by reticular fibers surrounding hepatocytes, blood vessels and sinusoids. Figure7(C-D) Hematopoietic tissue was observed in subcapsular region and portal triads of *Rafetus euphraticus* but less obvious in the liver of *M. Caspica*. Figure7(E).
Figure 1: A, photograph of soft shell turtle (*Refetus euphraticus*), (B-C), (ventral view) of liver of *R. euphraticus*,. (D). Photograph of Mauremys caspica, (E-F) the coelomic cavity (ventral view) of Mauremys caspica. Vertical view of the liver. RLL - right lateral lobe; LLL - left lateral lobe; ML - median lobe, SI - small intestine; LIG - large intestine right medial lobe; LML - left medial lobe; RML. SI - small intestine; LIG - large intestine; GB - gall bladder. figure 2. 50MM.

Figure 2: A-B) Longitudinal section of liver in *Rafetus euphraticus* showing the hepatic capsule (Hca), sinusoids (sc), Melanomagrophage (Mm). (C-D), ) Longitudinal section of liver in *Mauremys caspica*, showing the hepatic capsule (Hca), central vein (cv) and the Melanomagrophage (arrow). A, C stain with H.E stain, B, D stain with Van Gieson.
Figure 3: A-B) Longitudinal section of liver in Mauremys caspica, showing the hepatic cords (Hoc) is arranged as cylindrical tubules around the central vein (cv), branch hepatic portal vein (BPV), branch of the bile duct (BBD), C-D), Cross-section of liver in Mauremys caspica., tubules are arranged as acin, hepatocyte (H), Melanomagrophage appear as cluster (Mm), sinosiod (SC), kuffer cell( kc), and endohtelial cell(EC),A stain with van Gienson, B,C,D, with H-E stain.
Figure 4: A-B) the hepatocyte of liver in Mauremys caspica, appear the nucleus (NH), Kuffer cell (KC), sinusoids (SC), C-D), Longitudinal section of liver in Rafetus euphraticus showing hepatic cords (Hoc) is arranged as pyramidal shape, Melanomagrophage appear as a cluster (Mm). A,B, D stain with H-E stain, B stain with van Gienson.
Figure 5: Cross-section of liver in *Rafetus euphraticus*, tubules are arranged as acini, hepatocyte (H), contain one or two nucleus (N), Melanomagrophage (Mm), appear as cluster. C, the hepatocyte is contain eccentric nucleus and more vaculated cell, D, appear a hepatic capsule (Hca) with sinusoids (sin)
Figure 6: A-B) liver of Mauremys caspica is showing branch of portal vein (BPV), branch of bile duct lined with simple cuboidal epithelium, hepatocyte (H), kuffer cell (kc), Melanomacrophage (Mm) and sinusoids arrow, C-D. liver of Rafetus euphraticus showing portal space, branch of portal vein (BPV), connective tissue (CT), branch of portal artery (BPA), A,B stain with H-E staining, C,D staining with Van Gensein stain.
Figure 7: A, liver of Mauremys caspica stain with PAS, glycogen (G). B, liver of Rafetus euphraticus with PAS stain, (C-D) liver of Rafetus euphraticus showing reticular fibers in tubules and sinusoids, BPV, branch of portal vein, cv, central vein, BBD, branch of bile duct. (F) Hematopoietic subcapsular tissue (arrow), Hca, hepatic capsule, Mm, melnomacrophage. A, B PAS stain, C, D, Reticulin, F, H-E stain.
Discussion:

Reptilian liver is very important model for the study of interactions between environmental factors and Hepatic structures, especially in the field of problems induced by pollution in both aquatic and terrestrial systems (Hopkins et al., 2001; Ganser et al., 2003; Amaral et al., 2012). Testudines can tolerate severe prolonged unfavorable conditions due to their adaptive capacity, specific anatomy, and their specialized biochemistry and physiology, including conditions related to the liver (Moura et al., 2012).

Morphological description of the liver in two species of turtles in Iraq showed considerable variation between them, the liver of Rafetus euphraticus was cylindrical, irregular and very huge and thickened, while in Mauremys caspica was large and wide with an approximately rectangular shape. Its colors is variable from red to dark brown. The livers of reptiles differs in size, shape and appearance among species of this class (Schaffner, 1998; Gardner and Oberdörster, 2006). In both species of turtles studied, liver usually grows and may fill the entire available space in the abdominal area between the heart and the stomach, Schaffner (1998) reported that the liver in Testudines is wide, located ventrally and extended from one edge to another and occupies 2-5% of the total body mass. A similar characteristic was described by Firmiano et al. (2011) when studied the liver of the lizard Tropidurus torquatus, also Marycz et al., 2009 demonstrated that this organ consists of two lobes of equal size when he studied the liver in red-eared turtle (Trachomys scriptaelegans). The liver in M. caspica consists of three lobes, the right one was larger than other, This result agrees with the finding by Moura et al. (2012), but disagree with the results by Firmiano et al. (2011), where this organ in Rafetus euphraticus consisted of several lobes with clear fissure, Marycz and Rogowska, (2007) reported the presence of two lobes of the liver when studied these organs in both type of terrestrial tortoises, This result agrees with the finding by Machado Júnior et al. (2005) for K. scorpioides, who reported the presence of five lobes in the liver of these animals, but Moura et al. (2009) noted the presence of four lobes of the liver in the freshwater turtle Phrynops geoffroanus. This is due to the fact that these reptiles have long bodies Schaffner (1998), According to Hildebrand and Goslow (2006), the liver of vertebrates may contain several lobes arranged in various patterns that have no known functional or systematic purpose.

The microscopic analysis revealed that the liver in both species studied were covered by a thin layer of connective tissue forming the hepatic capsule, According to Schaffinar (1998) the liver of reptiles is bilobed, dark brown to black in coloration in healthy adult animals and surrounded by a connective tissue capsule, Glisson capsule, this capsule
contributes in the division of the parenchyma into lobules (Ross et al., 2003). This capsule in liver of Rafetus euphraticus is thicker than M.caspica, which may be due to consumption of stored materials during hibernation, Marycz et al., 2009 demonstrates considerable morphological changes in the liver parenchyma and thickness of the liver fibrous capsule that may be visible during hibernation.

Histologically, in both turtle livers hepatic tubules were observed, In M. caspica, the tubules were arranged as cylinders and acini, while in R. euphraicus they were arranged as pyramidal shape in histological slices. This results was different from reported by Moura et al. 2012, who demonstrated the hepatocytes of liver of P. expansa in longitudinal section which resemble double strings of cells surrounded by twisted sinusoidal capillaries, Firmiano et al., 2011 also reported the lobules arranged as polyhedral in shape and separated by a thin layer of interlobular connective tissue, These characteristics have been found by Gardner and Oberdorster (2006) who was suggested a tubular arrangement of the hepatocytes with laminar strings. These tubules have two cells in most vertebrates, including reptiles (Storch et al., 1989). This information agree with our finding in the liver of both turtles, where the hepatocytes are probably arranged as cylinders which, in longitudinal section and, in cross section were appeared as acini surrounded by twisted sinusoidal capillaries. In some fishes, the hepatocytes are arranged as glands surrounded by biliary canaliculi (Hampton et al., 1989). Most hepatocytes of both liver turtle were polyhedral in shape and may be appear as pyramidal form which come in varied size in histological slices, mostly hepatocyte contain one rounded nucleus which was located mostly in eccentric regions. Similar observation was reported by Moura et al. (2009) for Phrynops geoffroanus, and Firmina et al., 2011 for liver of the lizard T. torguatus, While Petcoff et al., 2006. who showed the hepatocyte in fish liver varying from polyhedral to rounded shape, Each hepatocyte contain large, rounded and central nucleus with a prominent dark nucleolus. A finding differs from that reported by Storch et al., (1989) for Osteolaemus tetraspis, who found nuclei located in the center of the cells.

The cytoplasm in both species appeared as high eosinophilic with more vacuolated found in liver cell of Rafetus euphraticus when analyzed by the hematoxylin-eosin staining technique. Moreover, it was reactive to PAS, indicating the presence of glycogen. All the staining techniques used here detected large quantities of melanomacrophages in the hepatic parenchyma in the liver of both turtle, melanomacrophage is probably involved in many functions in organs of heterothermic vertebrate as synthesis of melanin phagocytosis and...
protection against pathogen in addition to neutralization of free radicals. (Johnson et al., 1999; Agius and Roberts 2003 and Gallon et al., 2007). These cells are normally found in the liver of amphibian, reptiles and some fishes (Agius and Roberts 2003; Ribeiro et al., 2011 and Barni et al., 1999). these results are similar to those found by Moura et al. (2009) for liver of the Phrynops geoffroanus. Also Akat & Göçmen (2014) was demonstrated these cells in liver parenchyma of L. arikani. However, Reticulin staining detected the parenchyma was supported with reticular fiber network around the hepatocytes and vessels of the portal space and sinusoidal capillaries, This is in accordance with the results obtained by Koca et al.,(2004) when they studied the liver of the smooth newt (Triturus vulgaris), and Moura et al., (2012) for liver of the Podocnemis expansa. A mesh of reticular fibers between the sinusoids and the trabecules of hepatocytes was observed in liver of Oligosarcus jenynsii (Petcoff et al., 2006). In liver of both turtle (as in mammals 3), the hepatic parenchyma were separated by sinusoids that are lined with kuffer cells and endothelial cells, the kuffer cells serves as a sinusoidal macrophage (Jacobson, 2007).

References


