



A Macroscopic and Microscopic Study of Liver in Female Iraqi Green Freshwater Turtle (*Chelonia mydas*) Linnaeus, 1758 during the Active Period

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Article History	Abstract
Received: 14 April 2022 Revised: 18 October 2022 Accepted: 30 August 2022	<p>The study aims to provide anatomical and histological information about the liver in female Iraqi green freshwater turtles. Ten female green freshwater turtles (<i>Chelonia mydas</i>) were collected from Shatt Al-Hilla and used in this study. They were anesthetized by chloroform in closed chambers. The anatomical information was recorded and the histological sections of the liver were stained by using hematoxylin and Eosin stains. The result showed that the liver of a female green freshwater turtle (<i>Chelonia mydas</i>) is a large elongated organ. The mean weight of turtles is 735 ± 0.04 gm, and the mean weight of the liver is 28 ± 0.02 gm. The ratio between the weight of the liver to the weight of the body was 3.809 %. The liver of (<i>Chelonia mydas</i>) is formed from three lobes right, left and middle (central) lobes. The right lobe is the large one with an average weight of 13 ± 0.022 gm. It looks like a square and has two surfaces ventral and dorsal (visceral) surface. The left lobe is smaller than the right with an average weight of 9 ± 0.05 gm, and its shape is rectangular. The middle lobe is rounded and small. Its mean weight is 7 ± 0.01 gm. Histologically, the liver is covered by mesothelium under its connective tissue layer as a hepatic capsule which divided the liver into lobules in the shape of hexagons with portal spaces, from the central to the walls of the hepatocyte.</p>
CC License CC-BY-NC-SA 4.0	Keywords: Turtle, Testudines, liver, (<i>Chelonia mydas</i>)

1. Introduction

Reptiles are represented by turtles, crocodylians, lizards, and sphenodons as various groups of vertebrates, This group is considered one of the complicated groups in which Testudines are categorized as carapace dorsally and plastron ventrally linked by bone bridges (Kellner & Schwanke, 2001). Approximately 278 species of testudines are distributed in the world (Souza, 2004). The percentage of protein in the feeding of these animals varies between 28 to 30%, relying on 48% of fruits, false and plant stems, 10% of insects, 5% of crustaceans, and 30% of fish. The type of food affects the shape of the liver

(Teran et al., 1995). Glycogen is stored in the liver for all vital activities such as sexual activity and metabolic alterations, especially in climates (Gregory, 1982; Schaffner, 1998). Machado et al., (2005) and Marycz et al., (2007) studied the liver in (*Testudo hermanni*) and other turtles.

In *Phrynops geoffroanus*, *Kinosternon scorpioides*, *Testudo horsfieldi* and *Testudo hermanni*, the location of the liver is in the frontal part of the coelomic chamber, and it is wrapped by the duodenum, stomach, and pancreas (Marycz & Rogowska, 2007; Moura et al., 2017). In *Podocnemis expansa*, Silva et al., (2011) noted that the liver fills the middle space of the coelomic chamber, possessing a right, left, and middle (focal) lobes. The right one is situated in the space between the lateral part of the plastron and the internal rim of the carapace and is attached by a serous membrane. The gall bladder is found in the caudal portion of the right lobe and empties its production in the duodenum. The left lobe is located on the opposite side, and it is similar to the right lobe. The focal lobe is tall, tight and joined between the right and left lobes. In *Trachemys scripta elegans*, Kukental & Matthes (1969) found the liver across the medium third of the coelomic chamber.

The liver in *Phrynops geoffroanus* has four lobes. Silva et al. (2011) noted that the right lateral lobe is the largest lobes and is situated in space between the lateral part of the plastron and the internal rim of the carapace. It is also attacked by a dense serous membrane. The left lateral lobe is found in the field among the rim of the carapace and the left lateral part of the plastron, It is also tied by a dense serous membrane. The numbers of liver lobes differ between the different species of turtles. According to the findings of Machado et al. (2005), in *kinosternon scorpioides* four lobes are named lateral and medial on the right side of the body. Also, there are lateral and medial on the left side in freshwater turtles. In *Phrynops geoffroanus*, Moura et al. (2017) found different numbers of lobes even in *Podocnemis expansa*. They also noted three lobes; right, left, and central lobes. or two lobes in *Lacerta agilis* turtle (Koca et al., 2004; Kukental & Matthes, 2012). Marycz & Rogowska (2007) also found two lobes in *Gheyra mutilate* turtles. For the tortoises (*testudo horsfieldi*) and (*testudo hermanni*), Storer et al., (2000) reported two lobes. For other species of reptiles, there are also two lobes in the turtles (*Trachemys scripta elegans*). Silva et al., (2011) and Marycz & Rogowska (2007) observed the right lobe is greater than the left of the reptiles (*Testudo horsfieldi*) and (*Testudo hermanni*). This difference is also presented in *Phrynops geoffroanus* (Moura et al., 2017).

The size and shape of the liver are varied. For example, in *Phrynops geoffroanus*, it is broad and scraggy with its shape approximately rectangular as well as the right lobe is greater than the left (Moura et al., 2017). Nonetheless, in *Podocnemis expansa*, the liver is enormous and made up approx. 3.096% of the total body weight (Moura et al., 2017). The color of the liver in (*Podocnemis expansa*) changes from dark to light shades of brown (Moura et al., 2017). In (*Phrynops geoffroanus*), Moura et al., (2017) explain that because of melanin deposits, there are black spots in the liver. Their color is pale brown with greater accumulation of glycogen in hepatocytes and the liver varies in color between dark brown to black and paler in some cases (Faria, 2003). In turtles (*Trachemys scripta elegans*), Silva et al., (2011) observed that there are differences in liver color between females and males, becoming reddish-brown or slightly pale yellowish-brown respectively. The gallbladder is present on the caudal face of the organ.

About the fixation of the liver in (*K. scorpioides*), Machado et al., (2005) noted that the lobes are fixed with part of the jejunum, duodenum, ileum, and parts of the large intestine, in addition to the pressure exerted by these viscera and ligaments. Histologically, the liver cover by mesothelium under its connective tissue layer as a hepatic capsule. The parenchyma is divided by this capsule into lobules. The capsule is mainly formed by connective tissue inclosing hepatocytes and sinusoids (Koca et al., 2004). In (*Triturus vulgaris*), the structure of the liver is made of hepatic lobes. In (*P. geoffroanus*) the liver has many lobes, leading to numerous sinusoids and hepatocytes (Moura et al., 2017).

In (*P. geoffroanus*) and (*Testudo graeca*), Ferrer et al., (1987) and Moura et al., (2017) found the liver containing hexagons in shapes with the spaces between them forming a central hexagon and hepatocytes appearing in strings (walls). Gardner & Oberdorster (2016) propose a tubular order of the

liver cells. In (*P. geoffroanus*), Moura et al, (2017) noted the cylindrical order of cells in longitudinal appearance which show two strings enclosed by involuted sinusoidal or as acini formed from central canaliculus surrounded by 2-5 cells in cross appearance. In addition, the hepatocytes of (*P. geoffroanus*) are polyhedral and multi in their sizes with centric and acentric locations nuclei with vacuolated cytoplasm and show large numbers of melanomacrophages in the parenchyma of the liver. In (*Osteolaemus tetraspis*), Storch et al., (1989) noted the cells are polyhedral in shape and the locations of the nucleus are in the middle of the cells.

2. Materials And Methods

Ten female green freshwater turtles (*Chelonia mydas*) were collected from Shatt Al-Hilla in Iraq. The turtles were transferred to the anatomy and histology laboratory at the College of Veterinary Medicine at Al-Qasim Green University. The turtles were anesthetized by chloroform in closed chambers and the weights of each were recorded. Then a chapter between the carapace dorsally and plastron ventrally happened to get the internal organs.

The requirements of the anatomical pedestal of pictures, shape, location and weight of the organ required in this study were recorded. Then all the histological procedures were done after fixation by using formalin (10%). The thickness of the histological sections was 7 micrometers, and then the histological sections of the liver were stained by using hematoxylin and Eosin stains (Luna, 1986).

3. Results and Discussion

The liver of female green freshwater turtles (*Chelonia mydas*) is a large elongated organ extending along the right and the left cranial part of the coelomic cavity. Its location is limited between the lungs dorsally and pancreas, stomach and duodenum ventro-cranially (fig,1). This result is well-matched with Moura et al. (2017). In (*Phrynops geoffroanus*), (*Kinosternon scorpioides*), (*Testudo horsfieldi*) and (*Testudo Hermanni*) Machado et al.(2005) and Storch et al., (1989) reported that the liver fills the entire frontal space of the coelomic chamber (cavity), and it is enveloped by the pancreas, duodenum and stomach. Meanwhile, Moura et al., (2017) pointed out that in (*Podocnemis expansa*) the liver is located in the middle space of the coelomic chamber.

The color of the liver in female green freshwater turtles is pale brown with black spots. The mean weight of turtles is 735 ± 0.04 gm, and the mean weight of the liver is 28 ± 0.02 gm. The ratio between the weight of the liver to the weight of the body was 3.809 % (tab,1). These results are close to the findings reported by Marycz & Rogowska (2007) which showed that the liver size in *Podocnemis expansa* is large, wide, and it represents 3.096% of all body mass. The increase in the percentage may be because our study relied on female turtles that need a larger stock in the liver to lay eggs.

The liver of (*Chelonia mydas*) consists of three lobes; the right, left, and middle (central) lobes which connect between them (fig,2). This is in agreement with the results reported by Marycz & Rogowska (2007) which show that the liver of (*Podocnemis expansa*) has three lobes; right, left, and middle lobes. This, however, differs from what Schaffner 1998) reported; where there are five lobes in (*kinosternon scorpioides*) two left and three right, or four lobes named the right lobe, the lateral one, the medial one, the left lobe lateral one, and the medial one in freshwater turtles (*Phrynops geoffroanus*) by Machado et al., (2005) or two lobes in (*Lacerta agilis*) by Moura et al.,(2017). In (*Gheyra mutilate*), (*testudo Hermanni*), and (*testudo horsfieldi*) two lobes were identified (Gregory, 1982; Moura et al., 2017; Kukental & Matthes, 1969).

The right lobe in female green freshwater turtles is large (fig,2). Gregory (1982) also observed the right lobe is greater than the left in the tortoises (*Testudo horsfieldi*) and (*Testudo hermanni*). This difference was also supported by Machado et al.(2005) in (*Phrynops geoffroanus*). The average weight of the right lobe of the liver of female green turtle (*Chelonia mydas*) was 13 ± 0.022 gm, placing it amongst the inner carapace border and outer plastrons part (Tab,1). The result is similar to what Marycz Rogowska (2007) reported. In the liver of (*Podocnemis expansa*), the right lobe of a female green turtle connects with the middle lobe by a serous fold in the free edge. It looks like a

square having two surfaces; ventral and dorsal (visceral) surfaces and four borders cranial, caudal, lateral, and medial border (fig,2).

The ventral surface of the right lobe in female green freshwater turtles is characterized by a smooth appearance that is in contact with the plastron, while the visceral surface is irregular due to the presence of deep fissures. This gives a lobed view of the liver (secondary lobes) which resemble a pyramid in shape and is not equal in size. The apexes of lobes are directed dorsally and the base of the pyramid is directed ventrally.

The gall bladder is a small sac located on the dorsal surface of the right lobe in female green turtles. Its contents are emptied by a canal that opens in the duodenum. In (*Podocnemis expansa*), the cranial border of this lobe is thick and attached to the duodenum by connective tissue. The caudal border is thin and directed caudally, and the lateral border is rounded, long and thick surrounded by the lateral portion of the plastron. The medial border is a sharp concave border tied with the middle lobe by a serous membrane. The left lobe of the female green freshwater turtle was smaller than the right with an average weight of 9 ± 0.05 gm (tab,1). It is located in the left confined space between the carapace and the plastron (fig,1). These results were compatible with those reported by Machado et al.(2005): In (*Podocnemis expansa*), the shape of the left lobe is rectangular and has two surfaces and four borders. The ventral surface is also characterized by smoothness while the dorsal surface is more regular than in the right lobe. The cranial border is thick and attached to the anterior part of the stomach. The caudal border is thin and directed caudally, and the lateral border has a thick straight edge adjacent to the left side of the plastron. The medial border communicates with the middle lobe.

The middle lobe of (*Chelonia mydas*) is a bridge rounded small one. Machado et al.(2005) noted the same result in (*Podocnemis expansa*) turtle. The mean weight of the middle lobe was 7 ± 0.01 gm, and the lobe is stretched between the right and left lobes. Both ventral and dorsal surfaces are smooth, and the pancreas extends from the cranial part of the dorsal surface along the front (cranial) edge and holds tight between the cranial border from one side and the duodenum and the last part of the stomach. Our result is similar to what Machado et al.(2005) reported. Regarding the liver of (*Phrynops geoffroanus*), it is enveloped by the pancreas, and this is also confirmed with scorpion mud turtle (*Kinosternon scorpioides*) (Schaffner, 1998) and tortoises (*Testudo horsfieldi*), (*Testudo hermanni*) (Gregory, 1982).

The thickness of the middle lobe in freshwater turtles decreases gradually from the cranial border toward the caudal border. There are many triangular shapes as a result of the presence of several cracks in the caudal border. The fixation of the liver in a green freshwater turtle (*Chelonia mydas*) throws off the neighboring organs as well as their connecting ligaments to the right and the left end with a carapace (Schaffner, 1998). Histologically, the liver of a green freshwater turtle is covered by mesothelium. Under its connective tissue, there is a layer as a hepatic capsule which divided the pulp of the liver into lobules. It takes hexagons in appearance with portal spaces enclosing them beginning from the central vein with hepatocytes grouped into walls (fig,3). The result concurred with what was reported by (Silva et al. 1989; Storer et al., 2000) regarding (*Triturus vulgaris*) and (*Testudo graeca*) respectively.

The hepatocytes in green freshwater turtles are arranged in cylinder shapes. When examined under a microscope, strings 2-3 in number appear in a longitudinal section. The strings are surrounded by coiled sinusoidal capillaries, or acini with a central biliary canaliculus that is surrounded by 7-8 hepatocytes in cross-section (fig,4). These results are similar to those reported by Gardner & Oberdorster (2016) concerning the tubular arrangement of hepatocytes. However, our results are different from Schaffner (1998) in (*Phrynops geoffroanus*) about the number of strings which are double in longitudinal sections or 2-5 in cross section. In addition, the hepatocytes in the liver of (*Chelonia mydas*) are tacks oval, rounded an hexagonal in shape with large sizes. The central location of nuclei or modicum is shifted toward the edge, viewing cytoplasm vacuolated with large amount of melanomacrophages in liver tissue.

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Table 1 : The mean weight of turtles, liver, right lobe, left lobe and meddle lobe (gram), (n=10, M±S.E)

Anatomical parameters	Mean ± S.E
Mean weight of turtle (<i>Chelonia mydas</i>)	735±0.04
Mean weight of liver	28±0.02
Mean weight of right lobe	13±0.022
Mean weight of left lobe	9±0.05
Mean weight of middle lobe	7±0.01

Source: Authors

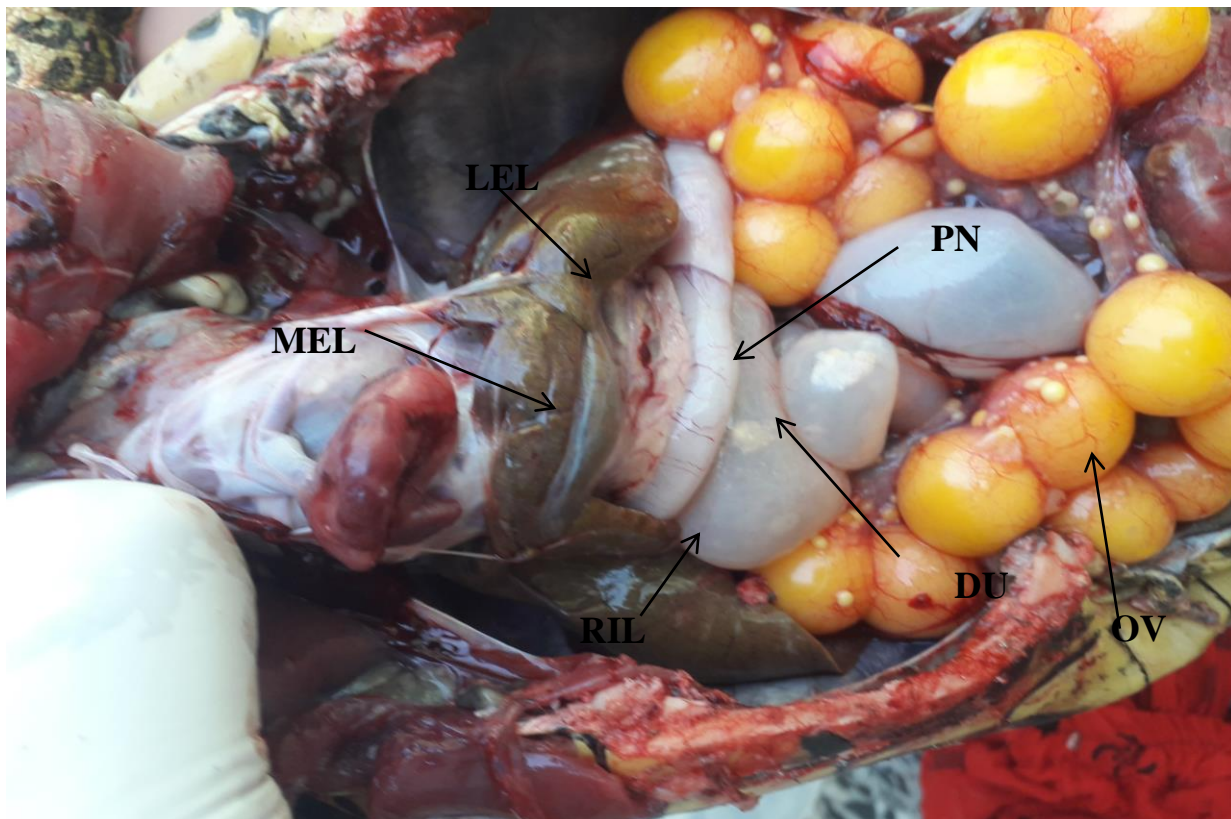


Figure 1. Photographs (Sony camera) of the liver turtle (*Chelonia mydas*), coelomic cavity. RIL right lobe, LEL left lobe, MEL meddle lobe, DU duodenum, PN pancreas, OV ovary.

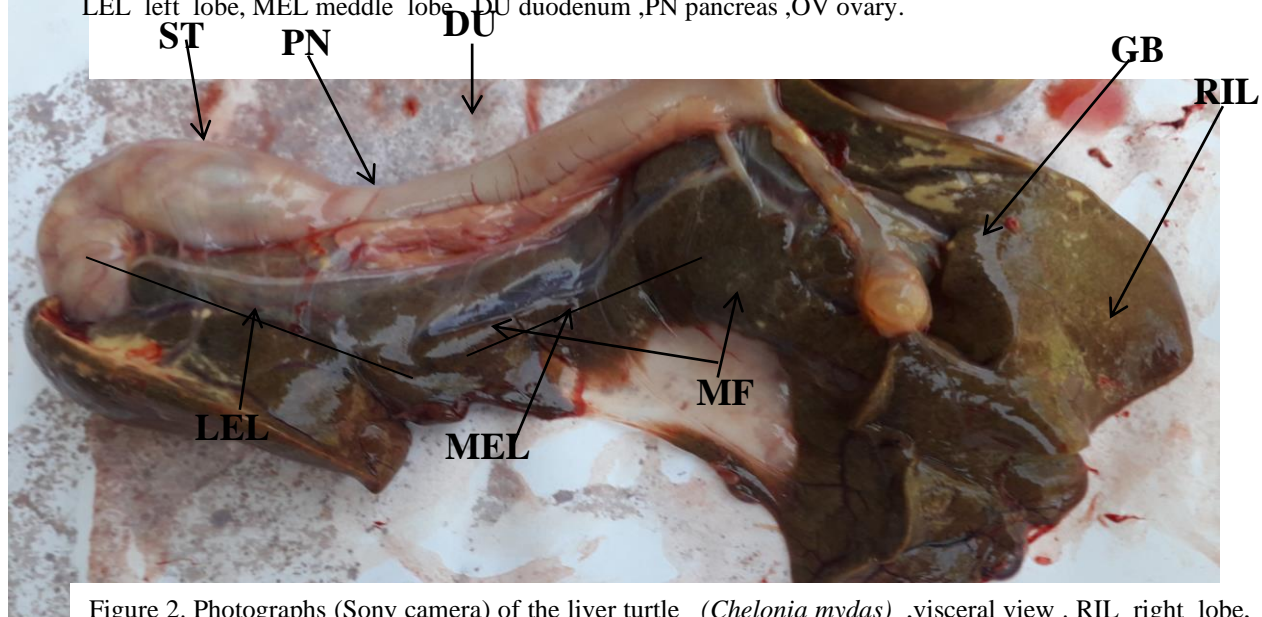


Figure 2. Photographs (Sony camera) of the liver turtle (*Chelonia mydas*), visceral view. RIL right lobe, LEL left lobe, MEL meddle lobe, DU duodenum, PN pancreas, ST stomach, GB gallbladder, MF mesenteric folds.

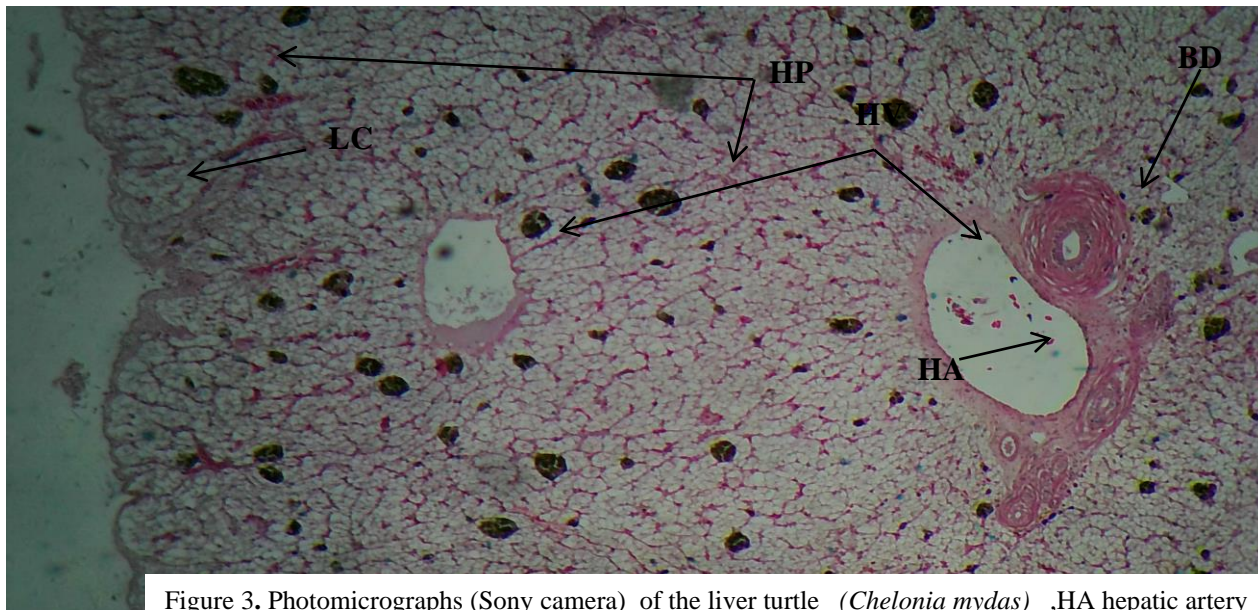


Figure 3. Photomicrographs (Sony camera) of the liver turtle (*Chelonia mydas*) ,HA hepatic artery ,HV hepatic vein ,BD bile duct ,HP hepatic pigments ,HC hepatic capsule . H & E stain 10 X.

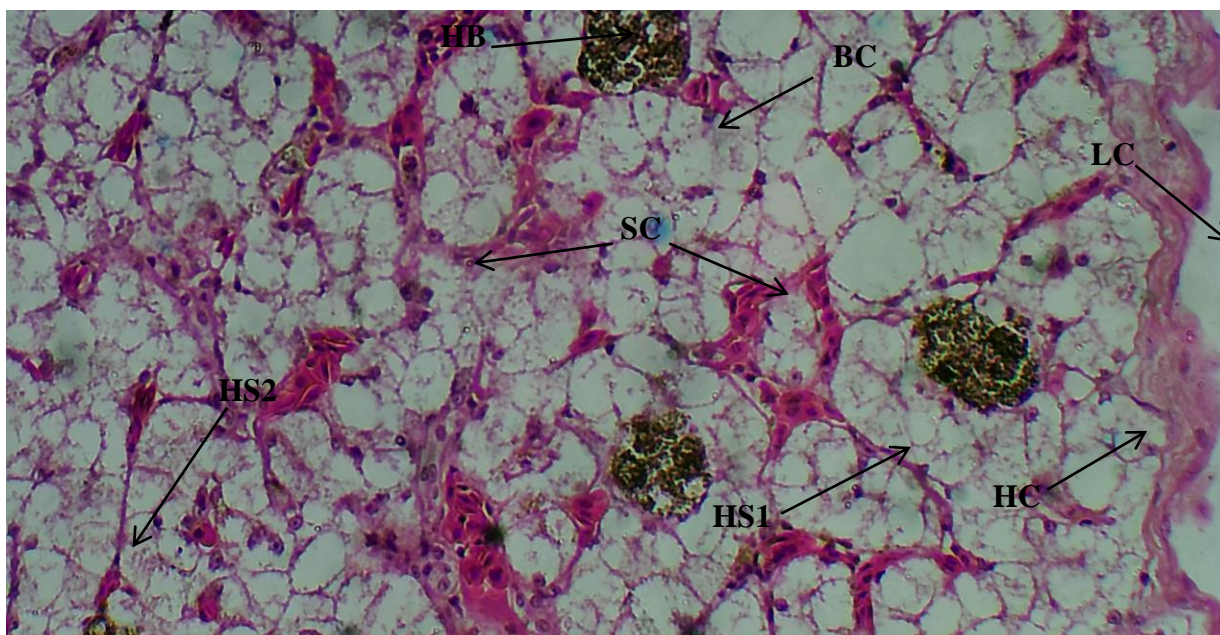


Figure 4. Photomicrographs (Sony camera) of the liver turtle (*Chelonia mydas*) , HS1 hepatocyte (Crosse appearance) ,BC biliary canaliculus ,SC sinusoidal capillaries ,HS2 hepatocyte (longitudinal appearance), HN hepatocyte nucleus ,HC hepatocyte ,LC liver capsule . H & E stain.40X.

4. Conclusion

The liver of green freshwater turtle(*Chelonia mydas*) consists of three lobes with hepatocytes that change in size and shape depending on the season. The hepatocytes in green freshwater turtles are arranged in cylinder shapes. When examined under a microscope, strings 2-3 in number appear in a longitudinal section. The strings are surrounded by coiled sinusoidal capillaries, or acini with a central biliary canaliculus that is surrounded by 7-8 hepatocytes in cross-section.

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Conflict of interest:

The authors declare no conflict of interest.

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