International Journal of Zoology Studies
ISSN: 2455-7269; Impact Factor: RJIF 5.14
Received: 12-01-2019; Accepted: 26-02-2019
www.zoologyjournals.com
Volume 4; Issue 2; March 2019; Page No. 15-19

Histological and histochemical study of liver and pancreas of sea snake, *Hydrophis cyanocinctus* in Minab Coast of Oman Sea

Mojtaba Ghazizadeh[1], Rahim Abdi[1*], Solmaz Shirali[1], Abdolali Movahedinia[2], Narges Amrollahi[3]
[1] Department of Marine Biology, Faculty of Marine Science, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran
[2] Department of Marine Biology, Faculty of Marine Science, University of Mazandaran, Mazandaran, Iran
[3] Department of Marine Biology, Faculty of Marine Science, University of Hormozgan, Hormozgan, Iran

Abstract
In this research 6 adult sea snake, *H. cyanocinctus* were collected in November from Minab beaches, located in the Sea of Oman. After reviewing and macroscopic examination and confirmation of animal health were euthanized by Chloroform. Then to access of liver and pancreas animals were dissected. Standard procedure of tissue preparation followed and finally sections stained whit Hematoxylin-Eosin, (H&E) and Periodic Acid Schiff's, (PAS) methods, and were observed with a light microscope equipped with a Dino-Lite lens. Microscopic results showed that liver was covered by a very thin capsules. In histological structure of parenchyma was not observed lobular structures and hepatic cords. In some hepatocytes, the nucleus were displaced toward to the periphery. In the sinusoids areas, there were the community of granular brown to dark colors that clearly showed melanomacrophage centers. Bile ducts along with cuboidal structure viewed in portal tracts. In microscopic structure of pancreas was observed that it was composed of two parts, endocrine and exocrine portion. In endocrine portion were observed alpha, beta and delta cells while exocrine portion was includes of communities of compact serous acini.

Keywords: histological, *Hydrophis cyanocinctus*, liver, pancreas, sea snake

1. Introduction
Reptiles are important group of animals that included about 7500 different species [1]. They are very important for maintenance of aquatic and terrestrial ecosystems [2]. Sea snakes are the largest and most complete group of marine reptiles. They constitute 86 percent of total marine reptiles [3]. Sea snake *H. cyanocinctus* has dark transverse lines with olive green colors [4,5]. These lines are vast in the neck and on the under side body are annular [6]. For this reason this species, also called annular sea snake [7]. Maximum body length, 150 cm have been reported for these snakes. Liver is one of the most important in this animals because of the intervention in metabolism, storage, synthesis and elimination of substances unabsorbed. It also produces bile which plays a key role in digestion and absorption of fats [8]. In reptiles, the liver is the largest extrinsic digestive gland and is the site of initial processing of materials absorbed by intestinal capillaries. In snakes it is thin, large, singular and long which expanded along the digestive tract. Some researchers reported that liver plays an important role in the production and storage of glycogen that serves energy for use in various situations, such as sexual activity and save energy especially in cold season [9,10, 11]. Pancreas is another attached organs of digestive system in snakes that is associated with the spleen and gall bladder. Histological data of reptiles liver and pancreas are a valuable source for detecting to physiological situation, [12] ecological position, [13] dietary habits[14] and disease diagnosis [15]. However, there are a little information about the histological aspects of liver and pancreas of sea snakes, especially in this species. For this purpose the present study examined the structure of the liver and pancreas of sea snake *H. cyanocinctus* in coast Minab of Oman sea.

2. Material and Methods
All stages of this research were conducted under the direct supervision of the vice president of research at the Khorramshahr University of Marine Science and Technology and based on the animal rights protection organization. For this purposes six animals, *Hydrophis cyanocinctus* from family Elapidae (Subfamily hydrophiinae) in November in Minab beaches, located in the Sea of Oman was used. The snakes were euthanized by chloroform. At first samples examined for abnormality signs such as infection, bleeding and skin parasite. After dissection liver and pancreas removed and then fixed in bouin fixative for next histological examination [16]. Rutine procedure of tissue preparation was done while were taken them into alcohol series for dehydration, clearing by xylene and then embedded in paraffin. 6µm thick serial sections of liver and pancreas were cut off with LEICA microtome (RM2245, Germany) [16]. Sections were stained with Hematoxylin-Eosin, (H&E) and Periodic Acid Schiff's, (PAS) methods and were observed with a light microscope equipped with a Dino-Lite lens for histological examination [17, 18].

3. Results
Based on our observations, liver was the largest organ in abdominal cavity that attached to the digestive tract. It was covered with a thin capsule of connective tissue. Pattern of liver that divided into lobules system not found in histological structur in this snake. Hepatocytes were along each other but hepatic cords not found while hepatic sinusoid were present in acinar units. There were some nucleated red blood cells in parenchyma of liver tissue. Portal tracts contained vein branch, gall duct and a number of hepatic artery. Epithelium

of bile duct was laid with cuboidal cells. Cytoplasm of hepatocytes was observed clear, foamy and spherical nucleus where displaced toward the periphery which seems due to be of accumulation of carbohydrates. This nucleus were taken blue color by hematoxylin and eosin. Hepatocytes were stained strongly with PAS staining. In hepatic parenchyma of *H. cyanocinctus* were present numbers of melano-macrophage centers (MMCs), which were observed further in the sinusoidal space with H&E staining. This centers were consists of the community of granular brown to dark colors (Fig. 1C). Pancreas was another accessory glands that attached to the digestive tract (Fig. 1A-1E).

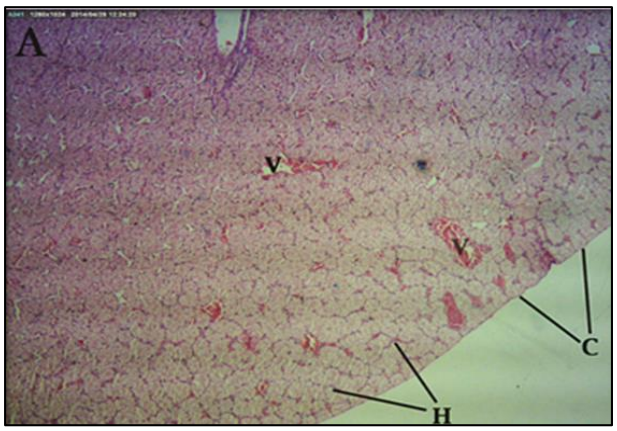

Fig 1A: Histology of the liver in *Hydrophis cyanocinctus.* The liver parenchyma (hematoxylin-eosin stain); C, Capsule; V, Vein; H, Hepatocytes.

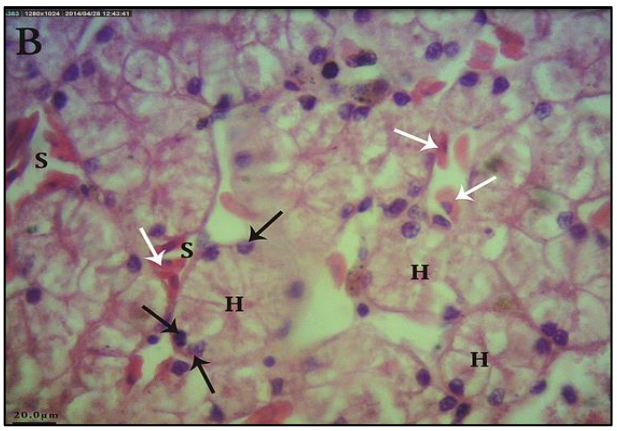

Fig 1B: Hepatocyte structure (hematoxylin-eosin stain); H, Hepatocytes; S, Sinusoidal; black Arrow, Hepatocyte nucleus; white Arrow, Red blood cells.

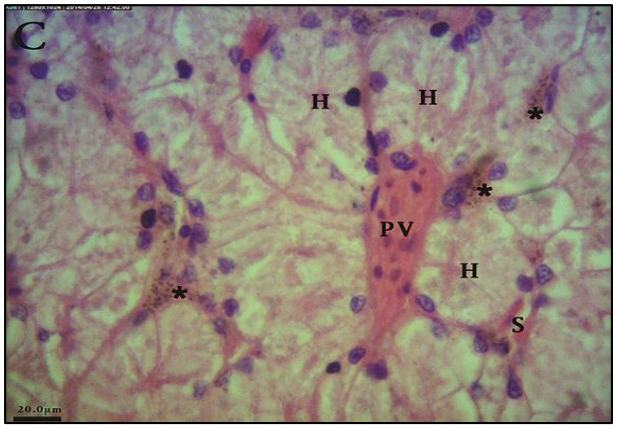

Fig 1C: The melano-macrophage centers in the liver (hematoxylin-eosin stain), *; H, Hepatocytes; S, Sinusoidal; PV, branches of portal vein.

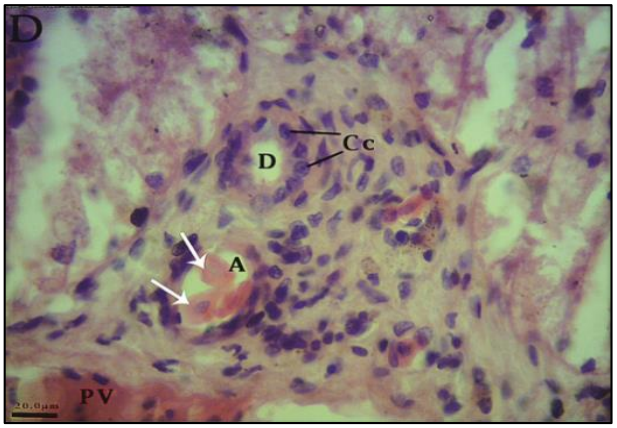

Fig 1D: The portal tracts in the liver (hematoxylin-eosin stain); D, Bile duct; A, Hepatic artery; Cc, Cuboidal cells.

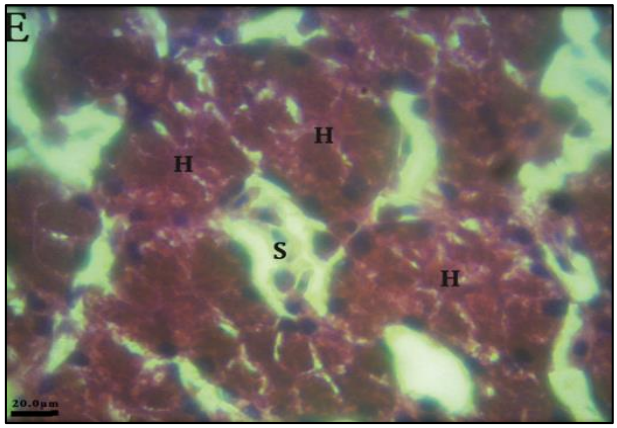

Fig IE: Reaction of Hepatocytes with PAS staining (periodic acid–Schiff's stain); H, Hepatocytes; S, Sinusoidal.

Pancreas, from outside was lined by a thin capsule of connective tissue. In Parenchymal study of pancreas is composed of two parts, endocrine and exocrine portion. Compared to mammals, exocrine structure immediately is located on the under of capsule. In this study, the endocrine cells were included alpha, beta and delta cells. Alpha cells had round, large and euchromatin nucleus but beta cells were further in number and had small, round heterochromatin nucleus. Also delta cells were the lowest in number and had larger, elliptical and euchromatin nucleus. A portion of

exocrine was located close to the center of pancreas and had contains of communities of compact serosal acini and each of them composed of several serosal cells. The number of cells in each acini was variable between 4-10 and had basophilic cytoplasm Containing zymogen granules. Their nucleus were observed basophilic and with bright nucleolus. In center of each acini were observed centroacinar cells and they were surrounded by a thin connective tissue along with fibroblasts (Figs. 2A-2D).

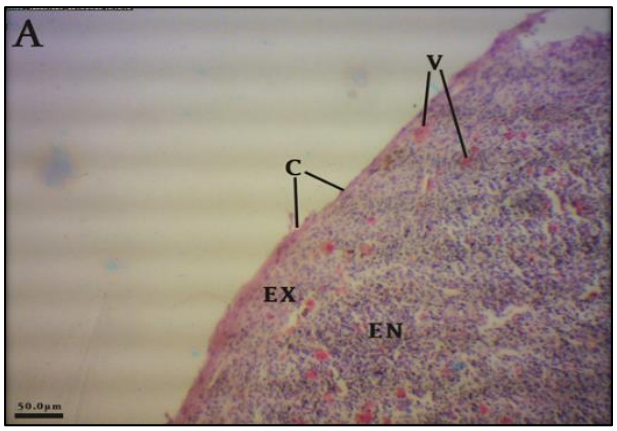

Fig 2A: Histology of the pancreas in *Hydrophis cyanocinctus.* The pancreas parenchyma (hematoxylin-eosin stain); C, Capsule; V, Vein; EN, Endocrine part; EX, exocrine part.

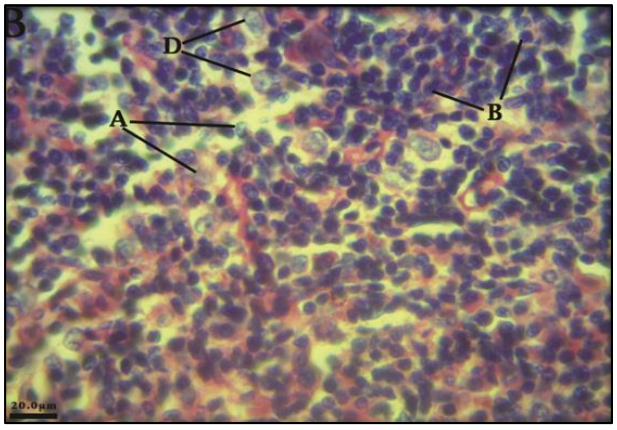

Fig 2B: The Endocrinepart of pancreas (hematoxylin-eosin stain). A, Alpha cells; B, Beta cells; D, Delta cells

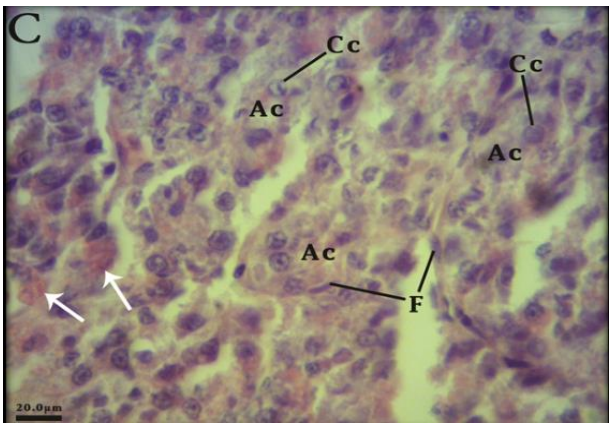

Fig 2C: The exocrinepart of pancreas (hematoxylin-eosin stain); Ac, Acini; F, fibroblasts; Cc, Centroacinar Cells; white Arrow, zymogen granules.

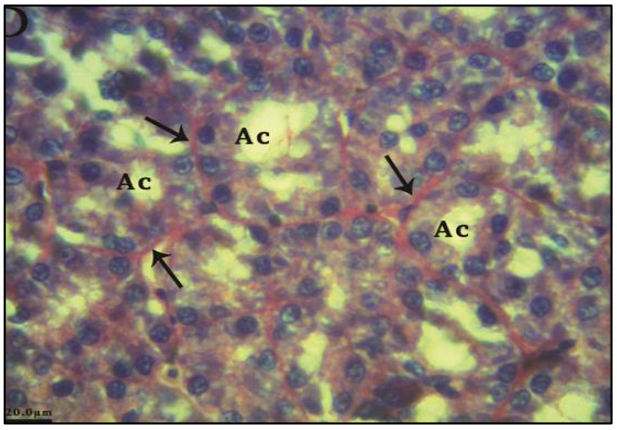

Fig 2D: Reaction of exocrine part of pancreas with PAS staining (periodic acid–Schiff's stain); Ac, Acini; black Arrow, The Connective tissue at around of acini.

4. Discussion

The histological results, from liver microscopic observation of *H. cyanocinctus* showed this organ covered by mesothelium, a very thin layer of connective tissue as hepatic capsule that reported by Schaffner [19]. It involved in maintenance of mesothelium and divided the parenchyma into lobules, and created lobular structures [20, 21, 22]. On the other hand connective tissue of capsule supports of hepatic cell, sinusoidal capillaries and the port triad structure, in many reptiles, such as lizard, *Tropidurus troquatus* newt, *Triturus vulgaris*, turtle, *Podocenomis xpansa* and freshwater turtles, *Phrynops geoffroanus* [23]. According to Gardner and Oberdorster (2006), the lobular structur pattern of liver not common in all reptile, or may even be completely absent in some species [24]. For example, there is not lobulation in the lizards, *Hemidactylus frenatus*, salamander, *Notophthalms viridescens*, *Caiman*, *latirostris* and even in some fish species, such as, *Micropogon undulates* [25]. In current study, as previous research the lobular structures of the liver was not observed and parenchyma had a unified state, only hepatocytes were observed in form of small polygonal lobules. The histological structure of liver of sea snake *H.cyanocinctus* was different with histological structures of some vertebrates. Because, hepatocytes are classified in strings or cords in the most vertebrates which constitute the hexagonal or portal spaces in the around them, and begins to makeup them from a central vein [26]. While hepatocytes in the present snake were similar to the acini containing about 2-5 hepatocellular, which are located around a central canaliculi. In a study on hepatocytes of turtle, *Phrynops geoffroanus* and in longitudinal section, they reported that they were cylindrical or tubular form with double strings which surrounded by twisted sinusoidal capillaries [27]. But in cross section they reported resemble acini containing approximately two to five hepatocytes surrounding a probable central biliary canaliculus. Tubular structure of hepatocytes in some vertebrates, including reptiles is reported [28]. Hepatocytes of *H. cyanocinctus* were polyhedral in shape and their size was different also the nucleus were shifted toward to the corner which was different with research results of [29, 30]. Cytoplasm with H&E staining was clear and eosinophilic but reacted strongly with PAS staining this indicated presence high levels of glycogene in it that is very common in reptiles [31]. The among of hepatocytes observed

sinusoids that have red blood cells. Adjacent Sinusoids with hepatocytes can lead to flow of material in this area, maintaining the integrity of the liver parenchyma. In sinusoids areas was found a large quantity of melano-macrophage centers. They observed in hepatic parenchyma of amphibians, reptiles and some fish. The MMs are components of an internal, pigmented cell system in the liver and spleen tissues of reptiles which have closely related to lymphoid cells. These cells have various functions among them synthesis of melanin, fagocytosis and neutralization of free radicals [32]. These cells are plentiful in the amphibians and reptiles, but in the snakes are less plentiful. The portal tracts in *H. cyanocinctus* contained one vein branch, a gall duct with cuboidal epithelial and number arteries that was cuboidal the epithelial of gall duct. The Histological findings of pancreas in sea snake, *H. cyanocinctus* revealed rarely interstitial connective tissue exist, in the parenchyma. As a result, lobular structure not found in the pancreas. But there was an external connective tissue which making the capsule. Similar of our finding obtained have been reported in many reptiles. The pancreas in this snake such as other reptiles included exocrine and endocrine parts. But unlike published research island structure was not seen in the endocrine region. While island structures is reported in amphibians, even reptiles and snakes [33]. In this study exocrine portion was community of the serosal units, similar to other snakes. With PAS staining, cells in the endocrine portion were detectable. They were alpha, beta and delta that secrete insulin, glucagon and somatostatin. The presence of these cells have been reported in all common snakes, but they are different in number and size [33].

5. References

1. Firmiano EM, Cardoso NN, Vieira DA, Sales A, Nascimento AA. Histological study of the liver of the lizard Tropidurus torquatus Wied 1820, Squamata: Tropiduridae. Journal of Morphological Sciences. 2011; 28(3):165-170.
2. Ahmed YA, El, Hafez AAE, Zayed AE. Histological and Histochemical Studies on the Esophagus, Stomach and Small Intestines of *Varanus niloticus*. Journal of Veterinary Anatomy. 2009; 2(1):35-48.
3. Voris HK. The role of sea snakes (Hydrophiidae) in the trophic structure of coastal ocean commumities. Journal of the Marine Biological Association of India. 1972; 14(2):429-442.
4. Rasmussen AR. Systematics of sea snakes: a critical review. Symp. Zool. Soc. Lond. 1997; 70:15-30.
5. Rasmussen AR, Murphy JC, Ompi M, Gibbons JW. Uetz P.Marine reptiles. PLo SONE. 2011; 6(11):e27373.
6. Rezaie, Atagholipour R, Riyahi, Bakhtiari A, Sajj adi M, Kong Yap C. Metal concentrations in selected tissues and main prey species of the annulated sea snake *Hydrophis cyanocinctus* in the Hara Protected Area, northeastern coast of the Persian Gulf, Iran. Marine Pollution Bulletin. 2012; 64(2):416-421.
7. Accordi F, Bracci M A, Ventola F. Pancreatic endocrine cells in *Bufo bufo*: immunocytochemistry and ultrastructure. Journal of Anatomy. 1998; 192(2):195-202.
8. Christiansen JL, Grzybowski JM, Kodama RM. Melanomacrophage aggregations and their age relationships in the yellow mud turtle, Kinosternon flavescens (Kinosternidae). Pigment Cell Research. 1996; 9(4):185-190.
9. Agius C, Roberts RJ. Melano–macrophage centres and their role in fish pathology. Journal of fish Diseases. 2003; 26(9):499-509.
10. Moura LR, Santos ALQ, Beletti ME, Vieira LG, Orpinelli S R, De Simone SBS. Morphological aspects of the liver of the freshwater turtle *Phrynops geoffroanus* Schweigger, 1812 Testudines, Chelidae. Brazilian Journal Morphological Sciences. 2009; 26(3-4):129-134.
11. Zieri R. Taboga SR, Oliveira C. Melanocytes in the testes of *Eupemphix nattereri* (Anura, Leiuperidae): histological, stereological and ultrastructural aspects. The Anatomical Record. 2007; 290(7):795-800.
12. Guida G, Gallone A, Maida I, Boffoli V, Cicero R. Tyrosinase gene expression in the Kupffer cells of Rana esculenta L. Pigment Cell Research. 2000; 13(6):431-435.
13. Moura LR, Santos ALQ, Beletti ME, Vieira LG, Orpinelli S R, Alves Júnior J R. Morphological aspects of the liver of the *Podocnemis expansa* (Testudines, Podocnemididae). Journal of Morphological Sciences. 2012; 29(3):159-166.
14. Storch V, Braunbeck T, Waitkuwait WE. The liver of the West African crocodile *Osteolaemus tetraspis*: an ultrastructural study. Journal Of Submicroscopic Cytology And Pathology. 1989; 21:317-327.
15. Micale V, Perdichizzi F. A quantitative and histochemical study on melano-macrophage centres in the spleen of the teleost fish *Diplodus annularis* L. Journal of Fish Biology. 1990; 37(2):191-197.
16. Syed Ali S, Syed Ali MM, Hafeez MA, Ahmad MM. Microangioarchitecture of the islets of Langerhans in the snakes, *Naja naja*, Vipera russelli, and Echis carinatus. Cell and Tissue Research. 1991; 266(1):83-88.
17. Cicero R, Scuito S, Chilemi R, Sichel G.. Melanosynthesis in the Kupffer cells of amphibian. Comparative Biochemistry Physiology. 1982; 73:477-479.
18. Manera M, Serra R, Isani G, Carpené E.. Macrophage aggregates in gilthead sea bream fed copper, iron and zinc enriched diets. Journal of Fish Biology. 2000; 57(3): 457-465.
19. Schaffner F, The Liver. In: Gans C. and Gaunt A.S.(eds), Biology of the Reptilia, (Morphology G), Society for Study Amphibians and Reptiles, Ithaca, New York. 1998; 19:513-514.
20. Koca YB, Gurcu B, Balcan E.. The Histological Investigation of Liver Tissues in Tritures Karelinii and Tritures Vulgaris (*Salamandridae Urodela*). Russian Journal of Herpetology. 2004; 11(3):223-229.
21. Edwards MA, Frazer TK, Jacoby CA. Age and growth of invasive lion fish (*Pterois* spp.) in the Caribbean Sea, with implications for management. Bulltine Marine Science. 2013; 90:953-966.
22. Buchan AM, An immunocytochemical study of endocrine pancreas of snakes. Cell and Tissue Research. 1984; 235(3):657-661.
23. Moura LR, Santos ALQ, Beletti ME, Vieira LG, Orpinelli S R, De Simone SBS. X Morphological aspects of the liver of the freshwater turtle *Phrynops geoffroanus* Schweigger, 1812 Testudines, Chelidae. Brazilian Journal Morphological Sciences. 1984; 26(3-4):129-134.

24. Gardner SC, Oberdorster E. Toxicology of reptiles, Boca Raton: CRC Press, USA, 2006,
25. Eurell JA, Haensly W. The histology and ultrastructure of the liver of Atlantic croaker *Micropogon undulates.* Journal of Fish Biology. 1982; 21(1):113-125.
26. Starck JM, Cruz, Neto AP, Abe AS. Physiological and morphological responses to feeding in broad-nosed caiman (*Caiman latirostris*). Journal of Experimental Biology. 2007; 210(12):2033-2045.
27. Ross M, Kaye G, Pawllna W. Histología textoy atlas color con Biología Celular y Molecular. 4Edition, Panamericana, Argentina, 2003.
28. Brito-Gitirana L, Storch V. Temperature induced alterations in the liver of wall lizard (*Hemidactylus frenatus*): morphological and biochemical parameters. Micron. 2002; 33(7-8):667-672.
29. Balslev, Clausen A, Mc Carthy JM, Carey HV. Hibernation reduces pancreatic amylase levels in ground squirrels. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 2003; 134: 573-578.
30. Goldblatt PJ, Hampton JA, Didio LN, Skeel KA, Klaunig JE. Morphologic and histochemical analysis of the Newt (*Notophthalmus viridescens*) liver. The Anatomical Record. 1987; 217(4):328-338.
31. Scalia M, Geremia E, Corsaro C, Santoro C, Sciuto S, Sichel G. The extracutaneous pigmentary system: evidence for the melanosynthesis in Amphibia and Reptilia liver. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry. 1988; 89(4):715-717.
32. Unver S, Saraydın SU. Macroscopical and Histological Analysis of Gonadal Development of *Squalius cephalus* (L., 1758) in Tödürge Lake, Turkey. pakistan veterinary journal. 2012. 32(1):55-59.
33. Sichel G, Scalia M, Corsaro C. Amphipia Kupffer cells. Microscopy Research and Technique. 2002; 57(6):477-490.