Morphology and histology of the liver of Oblada melanura (Linnaeus, 1758) (Teleostei: sparidae) in tripoli coast, Western Libya

MORPHOLOGY AND HISTOLOGY OF THE LIVER OF *OBLADA MELANURA* (LINNAEUS, 1758) (TELEOSTEI: SPARIDAE) IN TRIPOLI COAST, WESTERN LIBYA

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ملخص

وصف التركيب التشريحي والنسيجي الطبيعي لكبد الكحلة : تختلف الخصائص المورفولوجية والنسيجية لكبد الأسماك باختلاف الأنواع، لذا يصعب اعتماد كبد معين كنموذج ينطبق علي كل الأسماك العظمية . سمكة الكحلة *Oblada melanura* هي أكثر أنواع الأسماك شيوعًا في الأسواق الليبية والتابعة لعائلة سباريدي ولها قيمة إقتصادية مهمة. تهدف هذه الدراسة إلى وصف التركيب التشريحي والنسيجي الطبيعي لكبد الكحلة لبناء قاعدة بيانات محلية ودعم زراعة الأحياء البحرية. تم الحصول على 215 عينة من ساحل طرابلس حيث تراوح الطول الكلي بين 127 إلى 261 ملم ووزن الكبد بين 0.10 إلى 4.53 جم. تم تثبيت الكبد في فورمالين 10% ، ثم قطعت شرائح سمكها حوالي 7 ميكرون وصبغت بالهيماتوكسيلين والإيوسين و PAS. الكبد مثلث الشكل وله فصين: الأمامي كبير والخلفي أصغر ، و سطحين: ظهري أملس و بطني مفصص. مثلت أنسجة الكبد أكبر نسبة بينما كانت النسبة الأقل لأنسجة البنكرياس ، إلى جانب وجود الأوعية الدموية والقنوات الصفراوية. كانت خلايا الكبد متعددة الأضلاع ، مع نواة كروية ونوية بارزة مظلمة. لوحظت وجود أنسجة البنكرياس في الجزء الحشوي من الكبد ، و يتكون من حويصلات بنكرياسية والتي تمثل الجزء الخاص بالإفراز القنوي، في حين يوجد الجزء المختص بالإفراز اللاقنوي خارج نسيج الكبد. خلصت هذه الدراسة إلى أن غدة الإفراز القنوي للبنكرياس في الكحلة موجودة داخل أنسجة الكبد.

الكلمات المفاتيح: أوبلادا ميلانورا، كبد، أنسجة، الشكل الظاهري، سباريدي، ليبيا.

RESUME

Morphologie et histologie du foie d'*Oblada melanura* (Linnaeus, 1758) (Teleostei : Sparidés) de la côte de Tripoli, à l'ouest de la Libye : Les caractéristiques morphologiques et histologiques du foie des poissons varient selon les espèces. Aucun modèle spécifique n'a décrit le foie de Téléostéen « *Oblada melanura* », poisson sparidé très commune sur les marchés libyens et à importance économique. Le but de cette étude vise à décrire la structure anatomique et histologique du foie d'O. melanura. L'étude de 215 spécimens de la côte de Tripoli, d'une longueur totale allant de 127 à 261 mm et de poids du foie de 0,10 à 4,53 g, a été effectuée. Les foies ont été fixés dans du formol à 10 %, puis sectionnés à 7 µm et colorés à l'hématoxyline et à l'éosine et au PAS. Morphologiquement, le foie est en forme de triangle avec deux lobes, un antérieur plus grand et un postérieur plus petit et il a deux surfaces : dorsale lisse et ventrale lobulaire. Histologiquement, la plus grande proportion est constituée d'un tissu hépatique, tandis que la plus petite est d'un tissu pancréatique, ainsi que des vaisseaux sanguins et des voies biliaires. Les hépatocytes sont polygonaux, avec un noyau sphérique et un nucléole proéminent foncé. Du tissu pancréatique a été observé dans la partie viscérale du foie, formée par des acinis exocrines pancréatiques, alors qu'aucun tissu endocrinien n'a été trouvé dans le tissu hépatique. Cette étude a permis de conclure que la glande exocrine du pancréas d'O. melanura se trouvait à l'intérieur du tissu hépatique.

Mots-clés : **:** *Oblada melanura*, Hépatopancréas, Histologie, Morphologie, Sparidés, Libye

ABSTRACT

The morphological and histological characteristics of the fish liver varies among species. No specific model described the liver of Teleostei. '*Oblada melanura*' which is the most common sparid fish species in the Libyan markets with significant economic effects. ~~The aim of~~ This study aim to describe anatomical and histological structure of the liver of *O. melanura*. 215 specimens, with a total length ranged from 127 to 261 mm and the liver weight from 0.10 to 4.53 g, were obtained from Tripoli coast. The livers were fixed in 10% formalin, then sectioned at 7 µm and stained with hematoxylin and eosin and PAS. Morphologically, the liver is a triangle shaped with two lobes, bigger anterior and smaller posterior and has two surfaces: smooth dorsal and lobular ventral. Histologically, the largest proportion is the liver tissue, while the smallest is the pancreatic tissue, as well, blood vessels and bile ducts. The hepatocytes are polygonal, with spherical nucleus and a dark prominent nucleolus. Pancreatic tissue was observed in visceral portion of liver, formed by pancreatic exocrine acini, whilst no endocrine tissue found in the liver tissue. This study concluded that the exocrine gland of the pancreas of *O. melanura* found inside the liver tissue.

Keywords: *Oblada melanura*, Hepatopancreas, Histology, Morphology, Sparidae, Libya

INTRODUCTION

Oblada melanura is the most common sparid fish species in the Libyan markets with important economies (Shtewi *et al*., 2018). As other vertebrates, the digestive system of the fish consists of pancreas, liver, stomach and intestinal tract (Kjorsvik and Rhersen, 1992). Size, shape and volume of the liver fish are depended on the available space between other visceral organs of common cavity (Bertolucci *et al*., 2008). The liver is divided into lobes located in the anterior part of the common cavity, cranially and ventrally, with a reddish-brown color in most teleost fish and the numbers of lobes are different in some fishes (Bruslé and Anadon, 1996). However, there is one lobe in salmonidae or three lobes in scomberidae and erythrinidae (Demir, 1992), while a few species, such as *Pimephales promelas* and *Hypostomus francisci*, have multi-lobes (Rizkalla and Emsheri, 1978; Ferguson, 1989; Sales *et al*., 2017). However, Gingerich (1982) mentioned that most fish have a single-lobed liver. |In general, the liver of teleost has three lobes (Bruslé and Anadon, 1996).

Liver is described as a multifunctional organ acting in digestion and production of biochemical necessary for digestion, detoxification, storage, production of vitellogenin and the synthesis of serum proteins and it plays an important role in the body's metabolic balance as well as the deposition and metabolism of carbohydrates and fat (Bruslé and Anadon, 1996; Roberts and Ellis, 2001; Menke *et al*., 2011). Furthermore, the most important function of the liver in the digestion is to secrete bile by hepatic cells into the hepatic duct then passing to duodenum, while on stomachless fish, the bile duct opens into the anterior chamber of the intestines (Anderson and Mitchum, 1974).

The liver of some teleosts has aggregates of macrophages cells called melanomacrophage centres (MMC) and found in the other organs such as kidney and spleen (Sales *et al*., 2017). Histologically, MMC are distinctive groups of pigment-containing cells such as melanin, hemosiderin and lipofuscins (Gomes *et al*., 2015). MMC are present in the hepatic parenchyma, usually seen in the vicinity of hepatic arteries, portal veins, or bile ducts (Manju *et al*., 2012). Size and number of MMCs depending on the species, age and health status (Agius, 1980). As well as, the presences, numbers and area of MMCs used as biological indicator in environmental impact studies (Ribeiro *et al*., 2011; Gomes *et al*., 2015).

The morphology of the liver is correlated functionally to the physiological state of the fish. Therefore, it is considered as a biomarkers organ and important model (Caballero *et al*., 1999). Subsequently an indicator of environmental quality for exploring environmental changes and contamination (Rodrigues and Fanta 1998). Notably, the histological investigation of the liver is significant to understanding the interactions among the environmental factors, the hepatic structures, and the functions (Au, 2004; Maharajan *et al*., 2016).

The histological structure of vertebrate liver has been studied for a long period of time and classified into main models; the lobular model as in mammalian liver and the tubular model as in other vertebrate liver (Yao *et al*., 2012). Interestingly, a plenty of the previous studies of fish liver, they were exhibited a great variety in the histological structure of the liver organ among different species and within the same family (Schar *et al*., 1985; Robertson & Bradley 1992). Furthermore, many studies mentioned that it would be impossible to describe the liver of teleosts in a general aspect (Rocha and Monteiro, 1999; Flores-Lopes and Malabarba, 2007). Subsequently, the histological investigations of fish liver are indeed for the specific identification among variable species (Figueiredo-Fernandes *et al*., 2007).

Liver of Osteichthyes is a very large, separate, encapsulated and sinusoidal perfused gland (Wolf and Wolfe, 2005). Histologically, hepatocytes are the main cells in the liver; they are arranged around the capillary space sinusoids in fish (Bombonato *et al*., 2007; Nejedli and Gajger, 2013). The pancreas of teleosts is classified into disseminated, compact and intrahepatic types (Mokhtar, 2017). During ontogenesis, the exocrine pancreatic tissue develops around the portal vein and can remain extrahepatic or penetrate more or less deeply and the proportion into the liver parenchyma depends on the species of fish (Brusle and Anadon, 1996; Nejedli and Gajger, 2013). Kjorsvik and Rehersen (1992) stated that pancreatic tissues started in the posterior part of the liver after the hatching. Therefore, some researchers are named it hepatopancreas when the exocrine glands of the pancreatic tissue are present in the liver of some fishes. Meanwhile, others are named liver (Geyer *et al*., 1996; Petcoff *et al*., 2006; El-Bakary and El-Gammal, 2010; Agamy, 2012; Maharajan *et al*., 2016; Bertucci *et al*., 2017). On the other hand, there are some fish that have pancreases organ separate from the liver as in *Lithognathus mormyrus*, *Prochilodus argenteus*, *Astyanax altiparanae* and *Clarias gariepinus* (Rizkalla and Emsheri, 1978; Ribeiro *et al*., 2011; Chehade *et al*., 2014; Sayed and Younes, 2017). Whereas, the gallbladder is present in some fishes and absent in the other species such as cod and some species of teleost fishes (Gingerich, 1982; Hinton *et al*., 2001).

Morphological and histological characteristics of the fish liver and pancreas are obviously needed further investigation. The present study is specifically to describe the anatomical and histological structure of the liver of *O. melanura* in details. To the author's knowledge, this is the first study on the liver of *O.*

melanura, which able be to support the mariculture of this species, as well as to improve the local database.

MATERIALS AND METHODS

The work was conducted at marine biology laboratory, Zoology department, Tripoli university, Libya. 215 specimens of *O.melanura* were caught from artisanal fishers in Tripoli fishing harbor (32° 53' 33" N and 13° 10' 48" E). The total length (cm), total weight and liver weight (g) were measured for all specimens and calculated of hepatosomatic index (HSI) by equation:

$$HSI = \frac{\text{Liver weight } (g)}{\text{Total body weight}(g)} * 100$$

(Bukhari *et al*., 2012)

Liver was rapidly removed and cut into small pieces, then were fixed in buffered 10% formalin (Bancroft and Gamble, 2008). The fixed liver pieces were washed in running water, dehydrated through a graded ethanol solutions series from 70 to 100 %, cleared by xylene and embedded in paraffin. Then, the blocks were sectioned at 7 µm thickness on a rotary microtome. Tissue sections were stained with hematoxylin and eosin (Culling, 1963) and Periodic Acid Schiff (PAS) (Suvarna *et al*., 2012). The stained sections were mounted in DPX and observed under the transmitted light microscope (Axiostar company).

RESULTS

Hepato somatic index (HSI)

The total length of 215 specimens of *O.melanura* was ranged from 127 to 261 mm (211±26.2 SD). The liver weight of 215 specimens was measured and ranged from 0.1 to 4.53 g (0.87± 0.62). While HSI value was calculated from 0.29 to 3.46g (0.82± 0.42).

Anatomy of the liver

The liver of *O.melanura* is located in the anterior region of abdominal cavity near the cranial cavity, direct after heart. The liver is a triangle shaped and consist of two lobes: bigger anterior and smaller posterior. It has two surfaces: smooth dorsal and lobular ventral (Figure 1). The ventral or visceral face is concave, surrounded by the esophagus and parts of stomach and intestine. The liver of *O.melanura* is a reddish-brown in color.

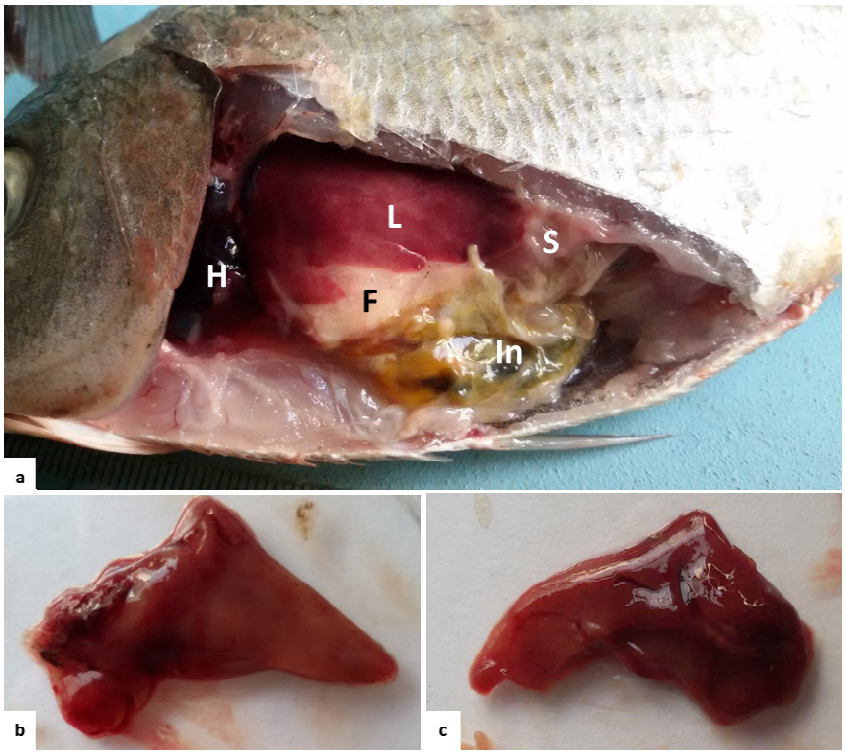

Fig 1. Gross section shows the anatomy of *O.melanura*, (a) ventral view of the visceral organs, liver (L), heart (H), fat tissue (F), stomach (S), intestine (In). (b) dorsal surface, (c) visceral surface of the liver.

Histology of the liver

The liver of *O.melanura* was covered with a thin capsule of dense irregular connective tissue, mostly collagen fibers and simple squamous epithelium. The largest proportion of the liver is hepatic tissue, while The smaller proportion is pancreatic tissue, as well, blood vessels and bile ducts (Figure 2a).

Overall, the sections of the liver, the lobe is not divided into lobules and the typical portal triads are not obvious, but the liver tissue appeared as cords converging around the sinusoids to the center vein (Figure 2b). The central veins are varied in size, shape and lined with endothelial cells (Figure 2b). The hepatic parenchyma was organized in hepatocyte

plates surrounded sinusoids. The hepatocytes are polygonal in shape with spherical nucleus usually centrally, dark prominent nucleolus and homogeneous cytoplasm. The sections of the liver tissue consisted of cords of hepatocytes arranged between neighboring sinusoids. The hepatocytes are array generally in plates with two hepatocytes thick. The sinusoidal capillaries are narrow and branched also connecting to central vein. In the sections which stained with PAS-positive glycogen stain showed that the hepatocytes were rich in glycogen (Figure 2b), furthermore some hepatocytes contained vacuoles (Figure 2c). The blood sinusoids are lined with distinct endothelial cells interspersed among the hepatocytes. The sinusoids are irregularly distributed between the polygonal hepatocytes with very prominent nuclei as showed in Figure 2b. In the addition, Küpffer cells are small cells with a dark and elongated nucleus; they located amongst the hepatocytes, around some sinusoids and sometimes appeared at groups (Figure 2b).

The exocrine pancreatic tissue is a characteristic feature in the liver of *O.melanura* and it consists of a large number of acini. Exocrine pancreatic tissue composed of secretory cells organized in acinus, and the portal vein located in the center region of the acinus (Figure 2c). Exocrine pancreatic tissue was appeared as scattered tubular acinar glands, so it was observed behind the capsule of liver tissue and separated from the hepatic parenchyma by a thin septa of connective tissue (Figure 2d). The secretory cells of the acinus were pyramidal, darkly basophilic cytoplasm stained and near the bases of the cells, in contrast the apical parts of the exocrine pancreatic acinus cells rich eosinophilic zymogen granules, as well as, the nucleus has eccentric and prominent nucleolus (Figures 2 c & e). The portal vein connected directly to the sinusoids in the liver tissue for the distribution of the secretions by one canal or more (Figure 2e). The bile system consists of bile canaliculi and ducts. Bile ducts appear as round channel in structures (Figure 2f).

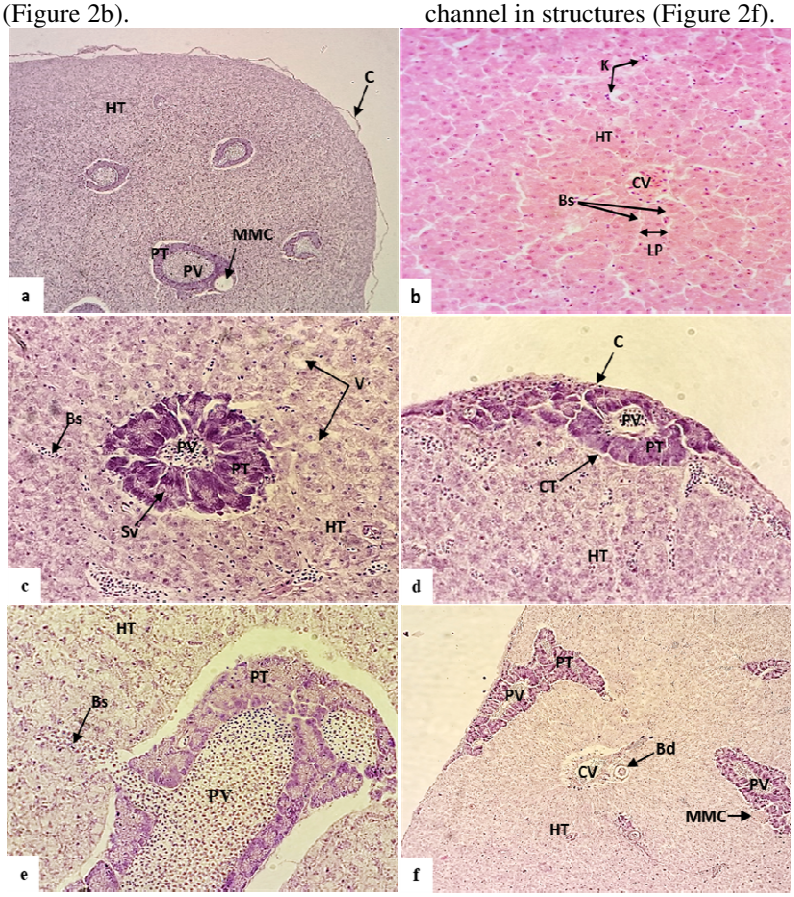

Fig 2. Histological sections of liver of *O.melanura* showing (a) Hepatic tissue, exocrine pancreatic tissue and capsule (PAS. 200X); (b) Hepatic parenchyma with hepatocytes cords (H&E. 400X); (c) & (d) Hepatic tissue and exocrine pancreatic tissue (PAS. 400X); (e) Exocrine pancreatic tissue connected with blood sinusoid (PAS. 400X); (f) Hepatic tissue, exocrine pancreatic tissue and MMCs with diads (central canal and bile duct) (PAS. 200X). blood sinusoid (Bs), bile duct (Bd), capsule (C), central vein (CV), endothelial cells (arrowhead), hepatic tissue (HT), Küpffer cell (K), liver plate (LP), melano-macrophage center (MMC), pancreatic tissue (PT), portal vein (PV), secretory vesicles (Sv), vacuole (V).

The bile ducts consist of a simple cubic or columnar epithelium and surrounded by a concentric layer of collagen and smooth muscle fibers. Bile ducts located mostly near to central vein in the liver tissue and closed to exocrine pancreatic tissue behind the blood supply (Figures 2f, 3a & b). The epithelial cells of the

bile duct cytoplasm stained strongly by PAS, bile canaliculi originate between adjacent hepatocytes, but it does not explicit (Figures 3 b & c).

Melano-Macrophage center (MMC) is a special category of macrophage which contains a variety of pigments and appears as pigment cluster and capsuled by simple sequamous epithelium. MMC present usually between the exocrine pancreatic tissue and hepatic parenchyma; hence, the MMCs usually found in between the pancreatic tissue and hepatic tissue, however the surface of the MMCs that faces the pancreatic tissue is larger than the hepatic tissue (Figure 3d). Moreover, some of the MMCs were observed surrounded by the exocrine pancreatic tissue in the interstitial tissue (Figure 3e), while no MMCs were noticed in the hepatic parenchyma. MMCs were covered by one layer of the epithelial cells or more (Figures 3e & f). The macrophages presented a small peripheral nucleus with different pigments inside the cytoplasm such as black melanin pigments (Figure 3e).

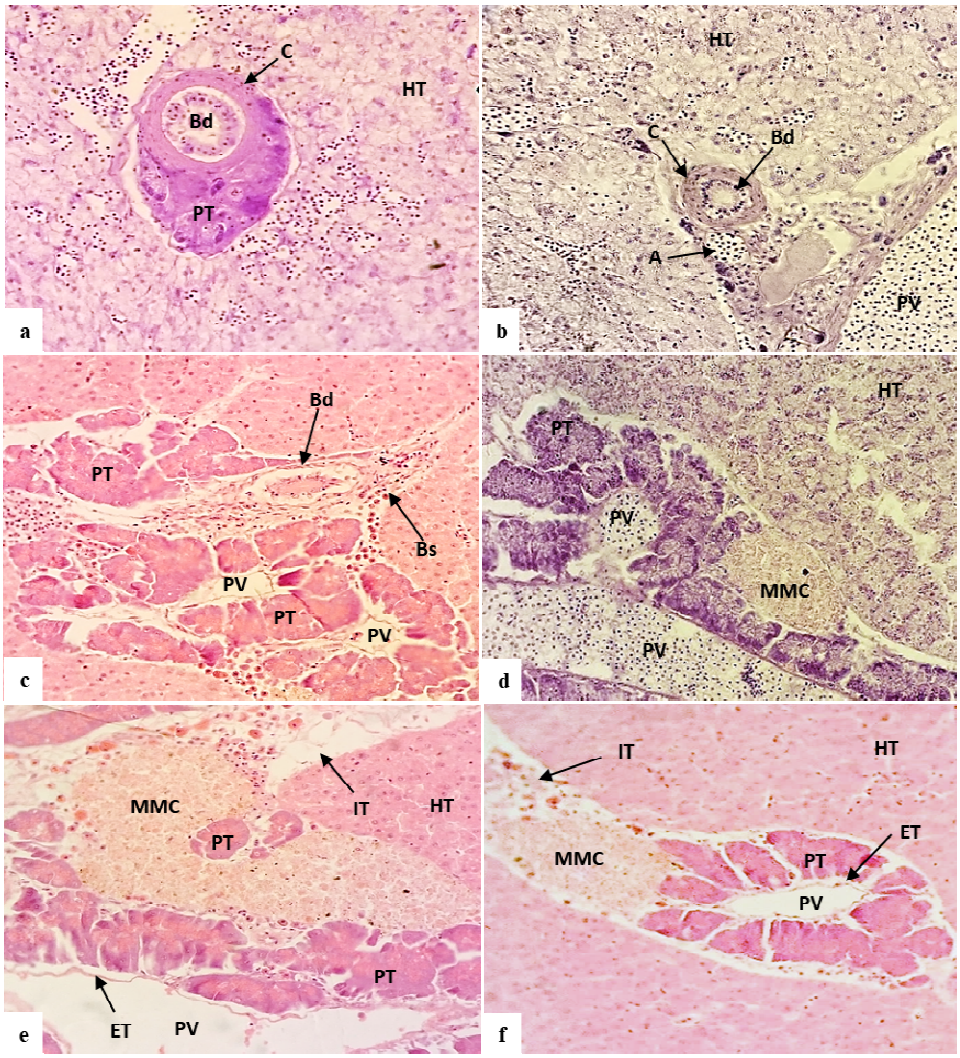

Fig 3. Histological sections of liver of *O.melanura* showing (a) Bile duct with exocrine pancreatic tissue (PAS. 400X); (b) Bile duct beside to blood supply and portal vein (PAS. 400X); (c) Large bile duct connected to blood sinsoid (H&E. 400X); (d) Melano-macrophage center between hepatic tissue and exocrine pancreatic tissue (PAS. 400X); (e) & (f) Melano-macrophage center surrounded by the exocrine pancreatic tissue in the interstitial tissue (H&E. 400X). arteriole (A), blood sinusoid (Bs), bile duct (Bd), capsule (C), Epithelial tissue (ET), hepatic tissue (HT), interstitial tissue (IT), melano-macrophage center (MMC), pancreatic tissue (PT), portal vein (PV).

DISCUSSION

The HSI of *O.melanura* specimens in this study appeared relatively at normal average, which is reflect the good health of the liver condition. Many factors might be play a role to decrease of HSI such as trophy or necrosis of hepatocyte, exposed to metal, sexual maturation, season and photoperiod or quantity and quality of food (Haug and Gulliksen, 1988; Larsson *et al*., 1984; Busackers *et al*., 1990;

Hung *et al*., 1990). furthermore, liver is sensitive organ to a great variety of environmental factors and the morphological characteristics associated with physiological status in fish (Caballero *et al*., 1999; Gochfeld, 2003). The position of the liver of *O.melanura* was in the anterior region direct after heart as other teleost species, triangle in shape and it consist of two lobes; anterior and posterior, while some teleosts have no lobulation like *Serranus cabrilla* or two lobes, right and left such as *H. platyrhynchos* (Bruslé and Anadon, 1996; González *et al*., 1993; Faccioli *et al*., 2014). The size, shape and volume of liver are adapted to the available space between other visceral organs of general cavity (Bruslé and Anadon, 1996). The color of the liver of *O.melanura* was reddish-brown similar to *Hemisorubim platyrhynchos* and *Hoplias* aff. *Malabaricus*, while *Geophagus brasiliensis* was yellow-brown, *Hypostomus francisci* was dark-yellow color and *Dicentrarchus labrax* was light brown color. There are many factors associated with liver color such as food habits and health conditions (Bertolucci *et al*., 2008; Faccioli *et al*., 2014; Sales *et al*., 2017; El-Fergani *et al*., 2012).

In the liver parenchyma of *O.melanura*, the hepatocytes are arranged in cords, there is no division in the hepatic lobules and lack of portal triads was observed as recorded in most teleosts (Hampton *et al*., 1985; Gonzalez *et al*., 1993; Bertolucci *et al*., 2008; Bombonato *et al*., 2007; Faccioli *et al*., 2014; Sales *et al*., 2017). On the other hand, few species of fish had triads such as *Caranx spp* and *Lutjanus bohar* (Gonzales, 1992). The hepatocytes are polygonal with a single spherical nucleus similar to other teleosts; such as *Limanda limanda, Oligosarcus jenynsii*, *Sparus aurata* and *H. platyrhyncho* (Petcoff *et al*., 2006; El-Bakary and El-Gammal, 2010; Bucke *et al*., 1984; Faccioli *et al*., 2014). The liver of *O.melanura* was rich in glycogen and contained few lipid vacuoles, while El-Bakary and El-Gammal (2010) and Faccioli *et al*. (2014) were noticed few glycogen granules and large amount of lipids in other species. This differences are might be effect by many factors mainly nutrition and the histochemical contents of the hepatocytes, which describe the two groups of fish; glycogen-rich or lipid-rich cytoplasm (Akiyoshi *et al*., 2001) or both types like *H. francisci* (Sales *et al*., 2017). However, the previous variations are associated with distinct swimming behavior, therefore active fish is rich in glycogen, while occupier fish had large lipid reserves (Akiyoshi *et al*., 2001). Moreover, Santos and Vinagre (1991) mentioned that the amount of glycogen increased or decreased after the development, while Hyvaeriner *et al.* (1985) suggested that liver glycogen varied in amount according to the age and seasonal changes. The sinusoidal structures of *O.melanura* was cord-like form, while *G. brasiliensis* and *H.* aff. *malabaricus* showed tubular-like form (Sales *et al*., 2017). The sinusoids are lined with endothelial cells with elongated dark nuclei and Küpffer cells of *O.melanura*. Interestingly, Menke *et al*. (2011) observed that the zebrafish liver does not have Kuppfer cells.

The exocrine pancreatic tissue of *O.melanura* was intrahepatic type like *Ictalurus punctatus*, *Astyanax altiparanae Oreochromis niloticus*, *Oligosarcus jenynsii*, *Leporinus macrocephalus*, zebrafish, *Pagellus erythinus* and *G. brasiliensis* (Kendall and Hawkins, 1975; Bertolucci *et al*., 2008; Shtewi *et al*., 2014; Petcoff *et al*., 2006; Bombonato *et al*., 2007; Menke *et al*., 2011 Nejedli and Gajger, 2013; Sales *et al*., 2017). While the pancreas of *Ctenopharyngodon idella* have a disseminated form, an intrahepatic form, and *Gadus morhua* have an immensely among the pyloric caeca (Mokhtar, 2017; Pedersen and Petersen, 1992). The acinus cells of *O.melanura* rich in zymogen granules of variable size, some of them dark in color and others light color, that indicate the presence of different enzymes within the granules. (González *et al*., 1993; Field *et al*., 2003).

The bile ducts of *O.melanura* lined with simple cuboidal epithelium in small ducts and with simple columnar epithelium in large ducts similar to other teleosts (Faccioli *et al*., 2014; Sales *et al*., 2017). The biliary channels are always near to portal or central veins and exhibit diads and seemed to be randomly dispersed in the parenchyma, this structure is similar to *C. idella* (Mokhtar, 2017).

The MMCs of the liver of the *O.melanura* are located near the blood vessels, bile ducts and exocrine pancreatic tissues similar to some teleosts such as *H. platyrhynchos* and *Cynoscion guatucupa*, while they were observed only near blood vessels in *Hoplias malabaricus* and *Leporinus macrocephalus* (Díaz *et al*., 1999; Lemes and Braccini, 2004; Bombonato *et al*., 2007; Faccioli *et al*., 2014). The MMCs located often near to the blood supply because of the relation between the breakdown product of hemoglobin and the pigment) Sayed and Younes, 2017). Herraez and Zapata (1986) suggested a certain relationship between the need for iron in erythropoiesis and the disappearance of MMCs. Few dark pigments appeared in the MMCs of *O.melanura*, while many dark pigments showed in *H. platyrhynchos* (Faccioli *et al*., 2014). However, some authors were suggested to use the number and size of MMCs as biomarkers in environmental impact assessment like water quality (Agius and Roberts, 2003; Fishelson, 2006).

CONCLUSION

The anatomical and histological characteristics of the fish liver varies among species, however, there is no specific model has been described the liver of Teleostei. Thus, each species of Teleostei needs

further investigation and more extensive studies. Taken all together, study of *O.melanura* can be used to support the mariculture and expansion the locally database, as well as, improve the Libyan markets for all the species of Sparidae family.

BIBLIOGRAPHY

Agamy, E. (2012). Histopathological Changes in the Livers of Rabbit Fish (*Siganus canaliculatus*) Following Exposure to Crude Oil and Dispersed Oil. Toxicol. Pathol., 40:1128-1140.

Agius, C.; Roberts, R. J. (2003). Melano-macrophage centers and their role in fish pathology. J. Fish Dis., 26(9):499-509.

Agius, C.(1980). Phylogenetic development of melano–macrophage centres in fish. J. Zoology, 191(1):11-31.

Akiyoshi, H.; Inoue, A.; Hamana, A. (2001). Comparative histochemical study of the livers of marine fishes in relation to their behavior. Bull. Fac. Life Env. Sci. Shimane Univ., 6:7-16.

Anderson, B.G.; Mitchum, D.L. (1974). Atlas of Trout Histology. Wyoming Game Fish Department Bulletin, USA. No, 13.

Au, D.W.T. (2004). The application of histo-cytopathological biomarkers in marine pollution monitoring: a review, Mar. Pollut. Bull., 48: 817–834.

Bancroft, J.D.; Gamble, M. (2008). Theory and practice of histological techniques. Pp.168-69.

Bertolucci, B.; Vicentini, C. A.; Franceschini-Vicentini, I. B.; Bombonato, M. T. S. (2008). Light microscopy and ultrastructure of the liver of *Astyanax altiparanae* Garutti and Britski, 2000 (Teleostei, Characidae). Acta Sci. Biol. Sci., 30(1):73-6.

Bertucci, J I; Blanco, A M; Canosa, L F; Unniappan, S. (2017). Direct actions of macronutrient components on goldfish hepatopancreas in vitro to modulate the expression of ghr-I, ghr-II, igf-I and igf-II mRNAs. Gen. Comp. Endocr., 250:1–8.

Bombonato, M. T. S.; Rochel, S. S.; Vicentini, C. A.; Franceschini Vicentini, I. B. (2007). Estudo morfológico do tecido hepático de *Leporinus macrocephalus.* Acta Sci. Biol. Sci., 29(1):81-5.

Bruslé, J.; Anadon, G. G. (1996). The structure and function of fish liver. Fish morphol., 76:545-551.

Bucke, D.; Watermann, B.; Feist, S. (1984). Histological variations of hepato-splenic organs from the North Sea dab, *Limanda limanda* (L.). J. Fish Dis., 7(4):255-68.

Bukhari, A.S.; Syed, H.E.; Broos, K.V.; Stalin, A.; Singhal, R.K..; Venubabu, P. (2012). Histological variations in liver of freshwater fish *Oreochromis mossambicus* exposed to 60Co gamma irradiation. J. Environ. Radioact., 113:57-62.

Busacker, G. P.; Adelman, I. R.; Goolish, E. M. (1990). Growth. h. 363–382 in Schreck, C. Methods for Fish Biology. American Fisheries Society. Maryland, USA.

Caballero, M. J.; López-Calero, G.; Socorro, J.; Roo, F. J.; Izquierdo, M. S. & Férnandez, A. J. (1999). Combined effect of lipid level and fish meal quality on liver histology of gilthead seabream (*Sparus aurata*). Aquac., 179(1-4):277-90.

Chehade, C; Cassel, M; Borella, M I; Costa, F.G. (2014). Morphologic study of the liver of lambari (*Astyanax altiparanae*) with emphasis on the distribution of cytokeratin. Fish Physiol. Biochem., 40:571–576.

Culling, C.F.A. (1963). Handbook of Histological Techniques (Including Museum Techniques) 2nd Edition, London, Butterworhts. Pp. 553.

Demir, N. (1992). İhtiyoloji, İ.Ü. Yayın., İstanbul. Sayı:3668.

Díaz, A. O.; González Castro, M.; García, A. M.; Devincenti, C. V.; Goldemberg. A. L. (1999). Morphological and histochemical characterization of liver from stripped weakfish, *Cynoscion guatucupa* (Cuvier, 1830). Biocienc., 7(1):67-78.

El-Bakary, N. E. R.; El-Gammal, H. L. (2010). Comparative Histological, Histochemical and Ultrastructural Studies on the Liver of Flathead Grey Mullet (*Mugil cephalus*) and Sea Bream (*Sparus aurata*). Global Vet., 4(6):548-53.

El-Fergani, E. S; El- Gharbawy, S. M.; Saleh, R. A.; Al-Sarraj, K. R.; El-Mor, M. E. (2012). Histological and histochemical studies on the liver of sea bass fish (*Dicentrarchus labrax* L.) M.Sc Thesis. Omar Al-Mukhtar University, Faculty of Science, Zoology Department. Pp.115.

Faccioli, C K; Chedid, R.A.; Bombonato, M.T.S.; Vicentini, C.A.; Franceschini-Vicentini, I.B. (2014). Morphology and Histochemistry of the Liver of Carnivorous Fish *Hemisorubim platyrhynchos*. Int. J. Morphol., 32(2):715-720.

Ferguson, H.W. (1989). Systemic Pathology of Fish: A text and Atlas of Comparative Tissue Responses in Diseases of Teleosts. Iowa State University Press, Ames, IA. Pp. 263.

Field, H.A.; Dong, P.D.; Beis, D.; Stainier, D.Y. (2003). Formation of the digestive system in zebrafish. II. Pancreas morphogenesis. Dev. Biol., 261(1):197-208.

Figueiredo-Fernandes, A.M, Fontai'nhas-Fernandes, A.A; Monteiro, R.F; Reis-Henriques, M.A.; Rocha, E. (2007). Spatial relationships of the intrahepatic vascular-biliary tracts and associated pancreatic acini of Nile tilapia, Oreochromis niloticus (Teleostei, Cichlidae): a serial section study by light microscopy. Ann. Anat., 189:17–30.

Fishelson, L. (2006). Cytomorphological alterations of the thymus, spleen, head-kidney, and liver in cardinal fish (Apogonidae, Teleostei) as bioindicators of stress. J. Morphol., 267:57-69.

Flores-Lopes, F.; Malabarba, L.R. (2007). Alterações histopatológicas observadas no fígado do lambari Astyanaxn jacuhiensis (Cope, 1894) (Teleostei, Characidae) sob influência de efluentes petroquı´micos. Biociências, 15:166–172.

Geyer, H.J.; Nel, M.N.; Swanepoel, J.H. (1996). Histology and ultrastructure of the hepatopancreas of the tiger fish, *Hydrocynus forskahlii.* J. Morphol., 227:93-100.

Gingerich, W.H. (1982). Hepatic toxicology of fishes. In Aquatic Toxicology (L. J. Weber, ed.). Raven Press, New York. pp. 55–105

Gochfeld, M. (2003). Cases of mercury exposure, bioavailability and absorption. Ecotoxicol. Environ. Saf., 56(1):174-9.

Gomes, J. M.; Ribeiro, H. J.; Procópio, M. S.; Alvarenga, B. M.; Castro, A. C.; Dutra, W. O.; Junior, J. D. C. (2015). What the erythrocytic nuclear alteration frequencies could tell us about genotoxicity and macrophage iron storage?. PloS one, 10(11).

González, G. (1992). Contribution à la connaissance des processus ciguatérigènes. These de Doctorat (specialite Oceanologie), Universite de Perpignan, Pp. 335.

González, G.; Crespo, S.; Brusle, J. (1993). Histo-cytological study of the liver of the cabrilla sea bass, *Serranus cabrilla* (Teleostei, Serranidae), an available model for marine fish experimental studies. J. Fish. Biol., 43(3):363-73.

Hampton, J.A.; Mc Cuskey, P.A.; Mc Cuskey, R.S.; Hinton, D. E. (1985). Functional units in rainbow trout (*Salmo gairdneri*) liver: I. Arrangement and histochemical properties of hepatocytes. Anat. Rec., 213(2):166-75.

Haug, T.; Gulliksen, B. (1988). Variations in liver and body condition during gonad development of Atlantic haliput, *Hippo glossus* (L.). Fisk Dir. Skr. Ser. Hau Unders., 18: 351-363.

Herraez, M.P.; Zapata, A.G. (1986). Structure and function of the melano-macrophage centres of the goldenfish *Carassius auratus*. Vet. Immunol. Immunopathol., 12:117-126.

Hinton, D. E.; Segner, H.; Braunbeck, T. (2001). Toxic responses of the liver. Target organ toxicity in marine and freshwater teleosts, 1:224-268.

Hung, S.S.O.; Groff, J.M.; Lutes, P.B.; Alkins, F.K.F. (1990). Hepatic and intestinal histology of juvenile white sturgean fed different carbonhydrates. Aquaculture, 87:349-360.

Hyvaeriner, H.; Holopainen, I.J.; Piironon, J. (1985). Anaerobic wintering of crucian carp (*Carassius carassius* L.) 1. annual dynamics of glycogen reserves in nature, Comp. Biochem. Physiol., 82:797-803.

Kendall, M.W.; Hawkins, W.E. (1975). Hepatic Morphology and Acid Phosphatase Localization in the Channel Catfish *(Ictalurus punctatus*). J. Fish. Res. Board Can., 32(8):1459-1464.

Kjorsvik, E.; Rehersen, A.L. (1992). Histomorphology of the early yolk-sac larva of the atlantic aalibut (*Hippoglossus hippoglossus* L.) an indication of the timing of functionality. J. Fish Biol., 41:1-19.

Larsson, A.; Haux, C.; Sjobeck, M.L.; Lithner, G. (1984). Physiological effects of an additional stressor on fish exposed to a simulated heavy-metal-containing effluent from a sulfide or smeltery. Ecotoxicol. Environ. Saf., 8:118-128.

Lemes, A. S.; Braccini, M. C. (2004). Descrição e análise histológica das glândulas anexas do trato digestório de *Hoplias malabaricus* (Bloch, 1794), (Teleostei, Erythrinidae). Biodivers. Pampeana, 2(1):33-41.

Maharajan, A.; Rufus Kitto, M.; Paruruckumani, P.S.; Ganapiriya, V. (2016). Histopathology biomarker responses in Asian sea bass, *Lates calcarifer* (Bloch) exposed to copper. J. Basic Appl. Zool., 77: 21–30.

Manju, M.; Akbarsha, M.A.; Oommen, O.V. (2012). In vivo protective effect of dietary curcumin in fish *Anabas testudineus* (Bloch). Fish Physiol. Biochem., 38:309–318.

Menke, A.L; Spitsbergen, J.M; Wolterbeek, A.P.M.; Woutersen, R.A. (2011). Normal Anatomy and Histology of the Adult Zebrafish. Toxicol. Pathol., 39: 759-775.

Mokhtar, D.M. (2017). Fish histology: from cells to organs. Apple Academic Press, New Jersey. Pp. 246.

Nejedli, S.; Gajger, I. T. (2013). Hepatopancreas in some sea fish from different species and the structure of the liver in teleost fish, common pandora, *Pagellus erythinus* (Linnaeus, 1758) and whiting, *Merlangius merlangus euxinus* (Nordmann, 1840). Vet. Arhiv., 83(4):441-452.

Pedersen, T.; Petersen, I.B. (1992). Morphological changes during metamorphosis in rod (*Gadus morhua* L.) with particular reference to the

stomach and pyloric caeca. J. Fish Biol., 41:449-461.

Petcoff, G. M; Díaz, A. O.; Escalante, A.H.; Goldemberg, A.L. (2006). Histology of the liver of *Oligosarcus jenynsii* (Ostariophysi, Characidae) from Los Padres Lake, Argentina. Iheringia, Sér. Zool., 96(2):205-8.

Ribeiro, H.J.; Procópio, M.S.; Gomes, J.M.M.; Vieira, F.O.; Russo, R.C.; Balzuweit, K.; Chiarini-Garcia, H.; Castro, A.C.S.; Rizzo, E.; Jr, J.D.C. (2011). Functional dissimilarity of melanomacrophage centres in the liver and spleen from females of the teleost fish *Prochilodus argenteus*. Cell Tissue Res., 346:417–425.

Rizkalla, W.; Emsheri, O. (1978). The pancreas of the marine teleost fish, *Lithognathus mormyrus* L., with special reference to its islet tissue. The Libyan J. Sci.,8(A):1-20.

Roberts, R.J.; Ellis, A. E. (2001). The anatomy and physiology of teleosts. In: R.J. Roberts (ed.) Fish Pathology, 3rd edition, Bailliere Tindall, London. pp. 12–54.

Robertson, J.C.; Bradley, T. M. (1992). Liver ultrastructure of juvenile Atlantic salmon (*Salmo salar*). J. Morphol., 211:41–54.

Rocha, E.; Monteiro, R. A. F. (1999). Histology and cytology of fish liver: a review. In: Chehade C; Cassel M; Borella M I; Costa F G. (2014). Morphologic study of the liver of lambari (*Astyanax altiparanae*) with emphasis on the distribution of cytokeratin. Fish Physiol. Biochem., 40:571–576.

Rodrigues, E.; de, L.; Fanta, E. (1998). Liver histopathology of fish Brachydanio rerio Hamilton Buchman after acute exposure to sublethal levels of the organophosphate Dimethoate 500. Rev. Bras. Zool., 15(2):441–450.

Sales, C.F.; Silva, R.F.; Amaral, M.G.C.; Domingos, F.F.T.; Ribeiro, R.T.M.A.; Thomé, R.G.; Santo, H.B. (2017). Comparative histology in the liver and spleen of three species of freshwater teleost. Neotrop. Ichthyol., 15(1):1-12.

Santos, E.A.; Vinagre, A.S. (1991). Carbonhydrate metabolizm during embryonic and larval development of *Odonthostes humensis* (De Buer,1953) (Pisces-Atherinidae), J. Fish Biol., 39:239-344.

Sayed, A.H; Younes, H. A. M. (2017). Melanomacrophage centers in *Clarias gariepinus* as an immunological biomarker for toxicity of silver nanoparticles. J. Microsc. Ultrastruct., 5:97–104.

Schar, M.; Maly, I.P.; Sasse, D. (1985). Histochemical studies on metabolic zonation of the liver in the trout (*Salmo gairdneri*). Histochemistry, 83:147–151.

Shtewi, H.H.; Ansher, H.A.; Alaswed, E.R. (2018). Relative Growth, Reproduction and Feeding Habits of The Saddled Bream, *Oblada Melanura* (Linnaeus, 1758) in Tripoli Coast, Western Libya. Libyan Journal of Veterinary and Medical Sciences, 1(2): 6-5.

Shtewi, H.H.; Khalil, M. T.; Goda, A.M.A.; Sleem, S.H.; Habashy, M.M. (2014). Acidifiers substances as growth promoters for the freshwater prawn, *Macrobracium rosenbergii* and the Nile tilapia, *Oreochromis niloticus*. PhD. Thesis. Ain Shams University, Faculty of Science, Zoology Department. Unpublished. Pp.258.

Suvarna, K.S.; Layton, C.; Bancroft, J.D. (2012). Bancroft's Theory and Practice of Histological Techniques. 7th ed., London, Churchill Livingstone. Pp.672.

Wolf, J.C.; Wolfe, M.J. (2005). A brief overview of nonneoplastic hepatic toxicity in fish. Toxicol. Pathol., 33:75–85.

Yao, Y.; Lin, J.; Yang, P.; Chen, Q.; Chu, X.; Cheng, G.; Hu, J. (2012). Fine structure, enzyme histochemistry, and immunohistochemistry of liver in zebrafish. Anat. Rec., 295:567–576.