Histological Studies on the Liver of Some Plectognthid Fishes (Order: Tetraodontiformes), Red Sea, Egypt

Ahmad M. Azab
Zoology Department, Faculty of Science, Al-Azhar University, Egypt
E-mail: amazab2000@yahoo.com

ABSTRACT

This work was carried out on some plectognathid fishes to study the normal histological structure of the liver. They contained puffer fishes, *Arothron hispidus* and *A. stellatus*; porcupine fish, *Diodon hystrix*; and blue trigger fish, *Pseudobalistes fuscus*. Specimens of these fish species were collected from the Red Sea.

Histological results showed that the liver was composed of hepatic parenchyma which externally covered by a loose connective tissue and a layer of squamous epithelium. Hepatocytes are radially arranged around a central vein in interconnecting laminae of two cells thickness, separated by blood sinusoids. They are big and rounded or oval in shape with transparent cytoplasm, because of the presence of a large quantity of lipids. It contained a large, rounded, and peripherally nucleus with a prominent dark nucleolus.

The pancreatic tissue is distributed in the liver as hepatopancreas. Thin septa of connective tissue separated pancreatic cells from hepatocytes. The pancreatic cells are tall and columnar, with spherical and basal nuclei, having prominent dark nucleoli. Zymogen granules are located in the apical ends of these cells. The results showed the presence of melano-macrophage aggregations in hepatic parenchyma of all studied fish species of tetraodontiformes.

Keywords: Fishes, Plectognathids, Tetraodontiformes, Histology, Liver

INTRODUCTION

The Tetraodontiformes are represented by ten families and approximately 360 species overall; most are marine and dwell in and around tropical coral reefs. The tetraodontiform strategy seems to be defense at the expense of speed, with all species fortified with scales modified into strong plates or spines or with tough, leathery skin (the filefishes and ocean sunfish). Another striking defensive attribute found in the puffer fishes and porcupine fishes is the ability to inflate the body to greatly increase its normal diameter: this is accomplished by sucking water into a diverticulum of the stomach. Many species of the Tetraodontidae, Triodontidae and Diodontidae are further protected from predation by tetrodotoxin, a powerful neurotoxin concentrated in the animals' internal organs. Puffer fishes are generally believed to be the second–most poisonous vertebrates in the world, after the golden poison frog. Liver of pufferfish is highly toxic to most animals when eaten (Keiichi & Tyler, 1998).

Three patterns of organization of vertebrate hepatic parenchyma have been described (Eurell & Haensly, 1982). The first pattern of hepatic parenchyma arrangement consists of hepatocytes, which are radially arranged around a central vein in interconnecting laminae of two cells thickness, sinusoids separating each lamina. Bile canaliculi are located between adjacent hepatocytes. This arrangement occurring in the largemouth bass, *Micropterous salmoides*, the bike, *Esox lucius* and rainbow trout, *Salmo gairdneri*. These laminae, which look like a "muralium", are separated

from each other by sinusoids, formed by reticuloendothelial cells (Bucke, 1971; Hinton *et al*., 1972; Anderson & Mitchum, 1974; Kendall & Hawkins, 1975).

In the second arrangement, which occurring in the hagfish *Myxine glutinosa*; the hepatocytes lie in the form of tubes or tubules with a bile canaliculus running through the center of this structure, sinusoids form an extended network around the tubule (Mugnaini & Harboe, 1967).

In the third arrangement, which occurring in some freshwater and marine fishes, the hepatocytes lie in anastomosing laminae around a central vein, with the bile canaliculi situated intercellularly. This pattern has been established in teleost fish following the classic tubular pattern (Chapman, 1981).

The liver is the largest internal organ. It plays a central role in metabolism of nutrients absorbed in the digestive tract but also in metabolism and detoxification of many toxicants accompanying the foodstuff. The liver receives blood via the hepatic portal vein (70–80%) and the hepatic artery. Nutrients and toxicants absorbed in the digestive tract spread throughout the liver from the hepatic portal vein on the distal part of the organ (Ostrander, 2000).

Thus, the present work was aimed to give a really detailed description of normal liver histology in different species of these plectognathid fishes (Order: Tetraodontiformes), from the Egyptian Red Sea coasts.

MATERIALS AND METHODS

The present work was carried out on live adult specimens of puffer fishes, *Arothron hispidus* and *A. stellatus* (Family: Tetraodontidae); porcupine fish, *Diodon hystrix* (Family: Diodontidae); and blue trigger fish, *Pseudobalistes fuscus* (Family: Balistidae).These specimens were collected from the Red Sea costs at Sharm El-Sheikh and Hurghada by the local fishermen.

After dissection, the liver of each specimen was taken out and immediately fixed in 10% buffered formalin solution for 72 hours. Fixated tissues were dehydrated in aggraded series of alcohols, cleared in xylene, embedded in paraffin wax and cut with microtome at 6 to 7 µm. Sections were mounted on glass slides, deparaffinized and stained by Haematoxylin and Eosin (H&E). Sections were observed under light microscope and photographed by digital camera.

RESULTS

The liver histology of studied plectognathid fishes showed that the liver was composed of a parenchyma covered by a delicate and thin capsule; which composed of a layer of simple squamous epithelial cells and a thin layer of loose connective tissue containing blood vessels, fibrocytes and fibers. Branches of the connective tissue were dispatched into the parenchyma and divided it into irregular hepatic lobules (Figure 1).

Also, liver histology of studied fishes revealed that parenchymal hepatocytes are radially arranged around a central vein in interconnecting laminae or cords of two cells thickness, narrow straight blood sinusoids arising from the central vein separated each lamina. Blood sinusoids are covered by typical elongated endothelial cells with flattened nuclei (Figure 2).

Hepatocytes in tetraodontiform fishes are varied in shape from oval, polyhedral to round. The H&E staining method showed the appearance of vacuolar structures in the hepatic cells in puffer fishes (*A. hispidus* and *A. stellatus*) probably due to the

presence of many lipid droplets, separated by little amounts of cytoplasm. While, hepatocytes of porcupine fish (*D. Hystrix*) and trigger fish (*P. fuscus*) are appeared with transparent cytoplasm. Its cytoplasm is packed by lipids in the form of a large lipid droplet. In all studied fishes, the large amount of lipids displaced cytoplasm and nucleus to the periphery of the cell. Each hepatocyte contained a large, round, and peripherally located nucleus with euchromatin and a prominent dark nucleolus (Figure 3).

The pancreatic exocrine tissue is distributed in the liver of the studied tetraodontiform fishes as hepatopancreas (Figure 1). It has an acinar arrangement, separated from hepatic parenchyma by a thin layer of connective tissue. The pancreatic cells are arranged around branches of the portal vein, separated by a basal membrane and reticular fibers. The hepatopancreatic cells have a particular arrangement, in which the basal region of the inner row of cells is in contact with the basal membrane of the vein, while the basal region of the outer row contacts with the outer layer of connective tissue. The exocrine cells are tall and columnar, and spherical nucleus is basally located, with a prominent dark nucleolus. Zymogen granules are located in the apical ends of these cells. Microscopical observations showed that the pancreatic cells are differentiated from hepatic tissue by their basophilic basal pole and eosinophilic apical cytoplasm (Figures 4 & 5).

Histological observations showed the presence of blood congestion in blood sinusoids in most of studied plectognathid fishes. The presence of hemosiderin pigments was observed on hepatocytes and besides blood sinusoids of tetraodontid species (Figures 2 & 3). Also, the results showed the presence of melano-macrophage aggregations in the hepatic parenchyma of all studied fish species of tetraodontiformes. They are located in the vicinity of the hepatic blood vessels, bile ducts and beside the hepatopancreas. Melano-macrophage cells were big and contained a large amount of cytoplasm and small central nucleus (Figures 4 & 5).

The epithelium of bile ducts is simple. Its epithelial cells are cuboidal (in porcupine fish and trigger fish) or cylindrical (in puffer fish species), and the cells were surrounded by dense connective tissue and some smooth muscle fibers. The epithelial cells have large spherical nucleus and thick brush border (Figure 6).

DISCUSSION

The liver of studied plectognathid fishes was externally covered by a loose connective tissue and one-layer squamous cells capsule. In this case, it was similar to that of *Cetonopharingodon idella* (Alboghobeish & Khaksar- Mahabady, 2005), *Oligosarcus jenynsii* (Petcoff *et al*, 2006) *Pangasius sanitwongsei* (Sayrafi *et al*., 2011), and differed from that of *Acipenser stellatus* (Sheybani & Adibmoradi, 2002) whose capsule was composed of dense connective tissue and a layer of cuboidal cells (Monsefi *et al.* 2010).

In the present study, liver of the studied tetraodontiform fishes showed parenchymal lobular arrangement. This histological structure of the liver resembles that described for *Micropogon undullatus* (Eurell & Haensly, 1982), *Mugil cephalus* (El-Bakary & El- Gamal, 2010) and *Aphanius persicus* (Monsefi *et al*., 2010); and differed from acinar arrangement described in the liver of in *A. stellatus* (Sheybani & Adibmoradi, 2002) and *P. sanitwongsei* (Sayrafi *et al*, 2011).

In contrast to that reported for many fish species (Petcoff *et al*., 2006; El-Bakary & El- Gamal, 2010; Monsefi *et al*., 2010; Sayrafi *et al*, 2011), hepatocyte nuclei of

studied tetraodontiform fishes are peripherally located, because cytoplasm is packed by large quantities of lipids.

The results showed that the structure of hepatopancreas in this species was similar to that reported for the other fishes (Alboghobeish & Khaksar-Mahabady, 2005; Sheybani & Adibmoradi, 2002) and even mammals (Eurell & Frappier, 2006); however, there were also considerable structural differences.

In the liver of the studied tetraodontiform fishes, like in many other species (including *C. idella*, *Oligosarcus jenynsii* and *Pangasius sanitwongsei*), exocrine pancreatic tissue or hepatopancreas occurs around the major portal vessels (Alboghobeish & Khaksar-Mahabady, 2005; Petcoff *et al*., 2006; Sayrafi *et al*, 2011).

Kupffer cells, which are typical of the mammalian liver, have not been described in many teleostei. These polymorphous cells are scarce and hardly ever observed in fish liver. When present, they are characterized by phagocytic activity (Munshi & Dutta, 1996). The present study showed the presence of melano-macrophage aggregations in the hepatic parenchyma of all studied fish species of tetraodontiformes. Cells of these melano-macrophage aggregations resemble the form of Kupffer cells with phagocytic activity (Agius, 1980; Munshi & Dutta, 1996; Agius & Roberts, 2003).

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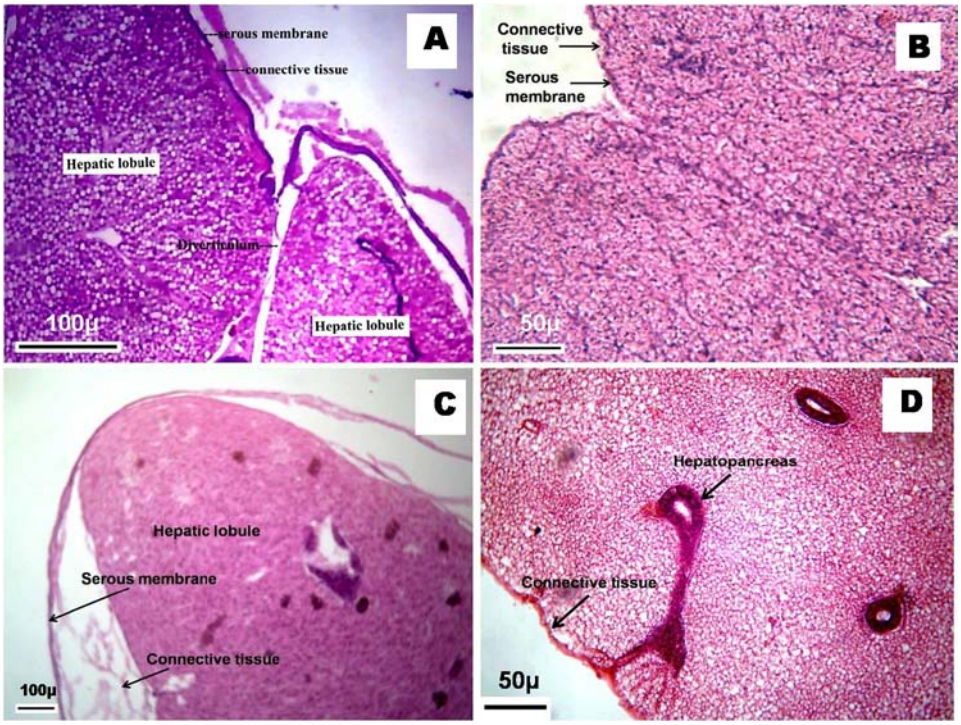

Fig.1: Sections in the liver of tetraodontiform fishes showing general structure of hepatic parenchyma covered by a delicate capsule of connective tissues and a serous epithelium. (A) Porcupine fish, *Diodon hystrix*; (B) Puffer fish, *Arothron hispidus*; (C) Puffer fish, *A. stellatus*; and (D) Trigger fish, *Pseudobalistes fuscus*.

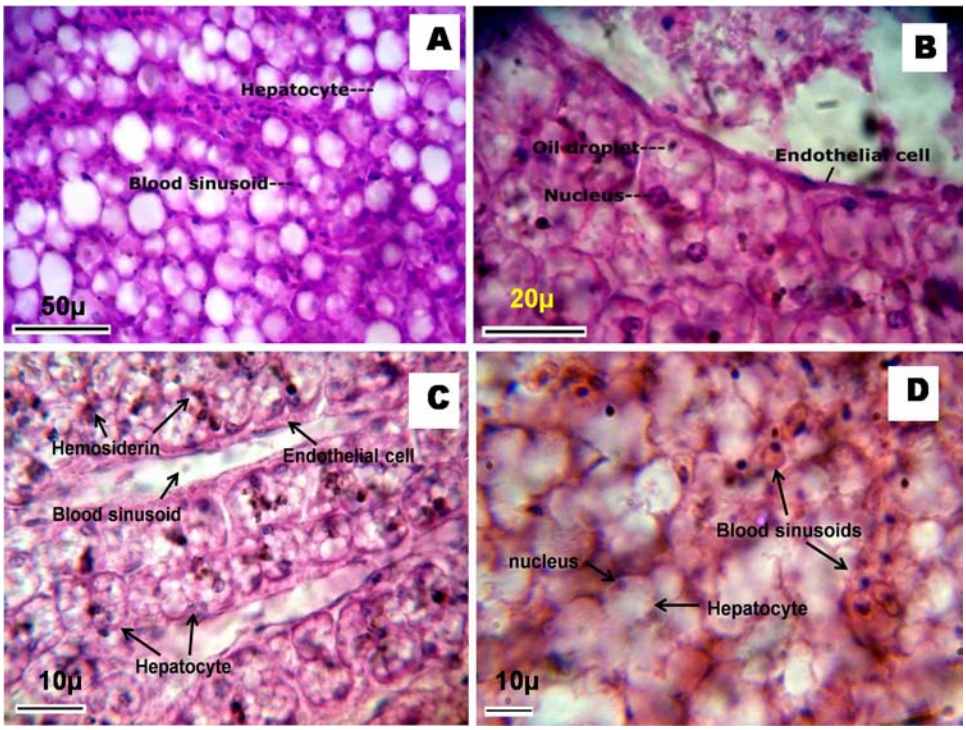

Fig. 2: Sections in the liver of tetraodontiform fishes showing the arrangement of hepatic laminae and Blood sinusoids. (A) P. fish, *D. hystrix*; (B) Puffer fish, *A. hispidus*; (C) Puffer fish, *A. stellatus*; and (D) Trigger fish, *P. fuscus*.

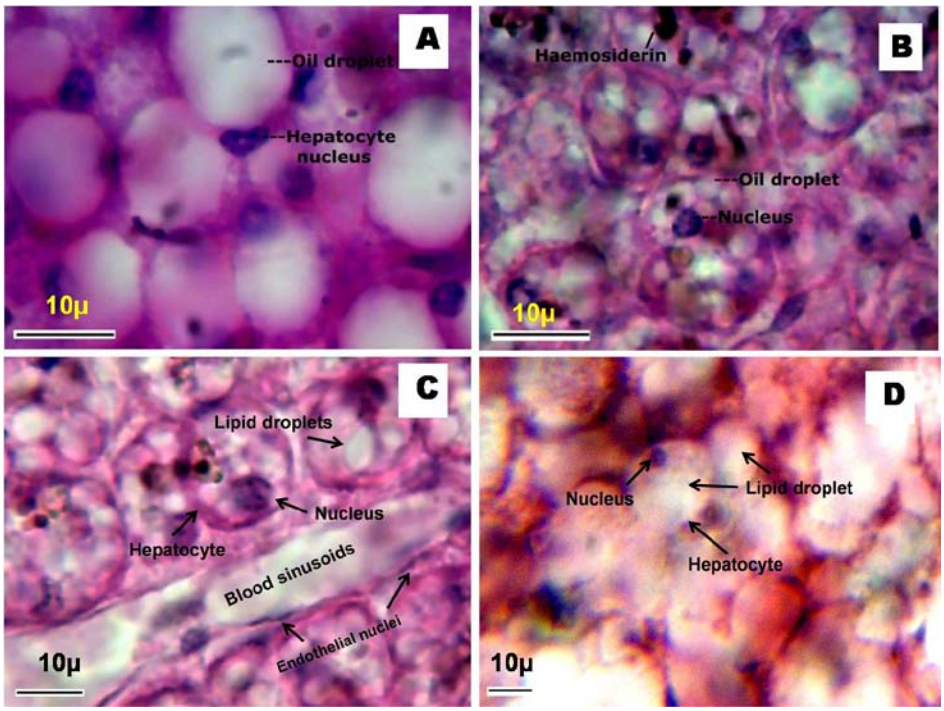

Fig. 3: Sections in the liver of tetraodontiform fishes showing the structure and shape of hepatocytes. (A) P. fish, *D. hystrix*; (B) Puffer fish, *A. hispidus*; (C) Puffer fish, *A. stellatus*; and (D) Trigger fish, *P. fuscus*.

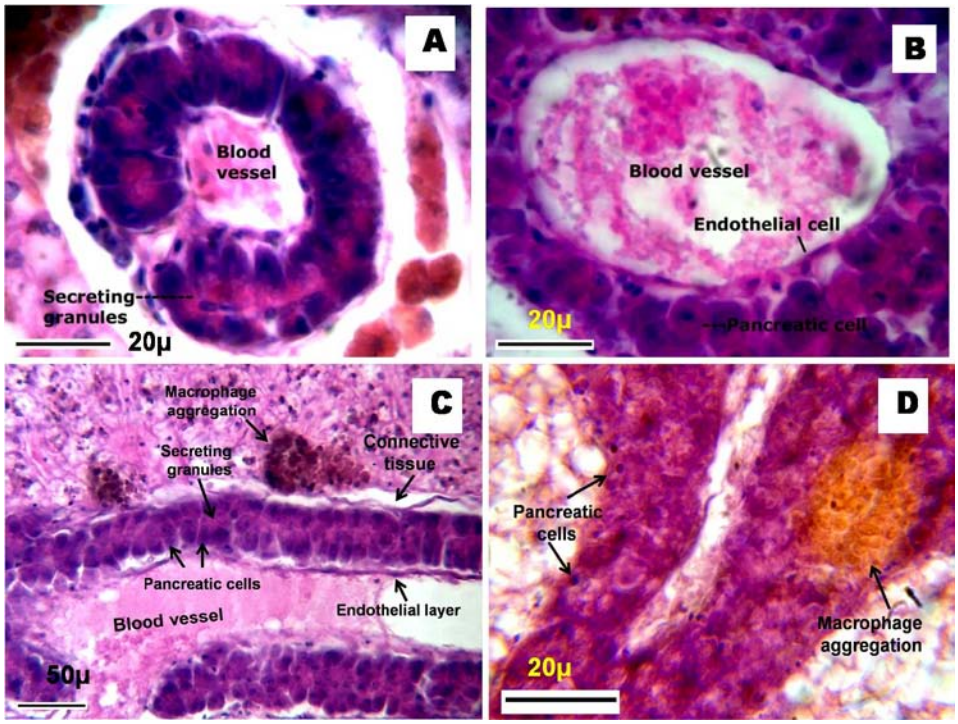

Fig. 4: Sections in the liver of tetraodontiform fishes showing the presence of hepatopancreas. (A) P. fish, *D. hystrix*; (B) Puffer fish, *A. hispidus*; (C) Puffer fish, *A. stellatus*; and (D) Trigger fish, *P. fuscus*.

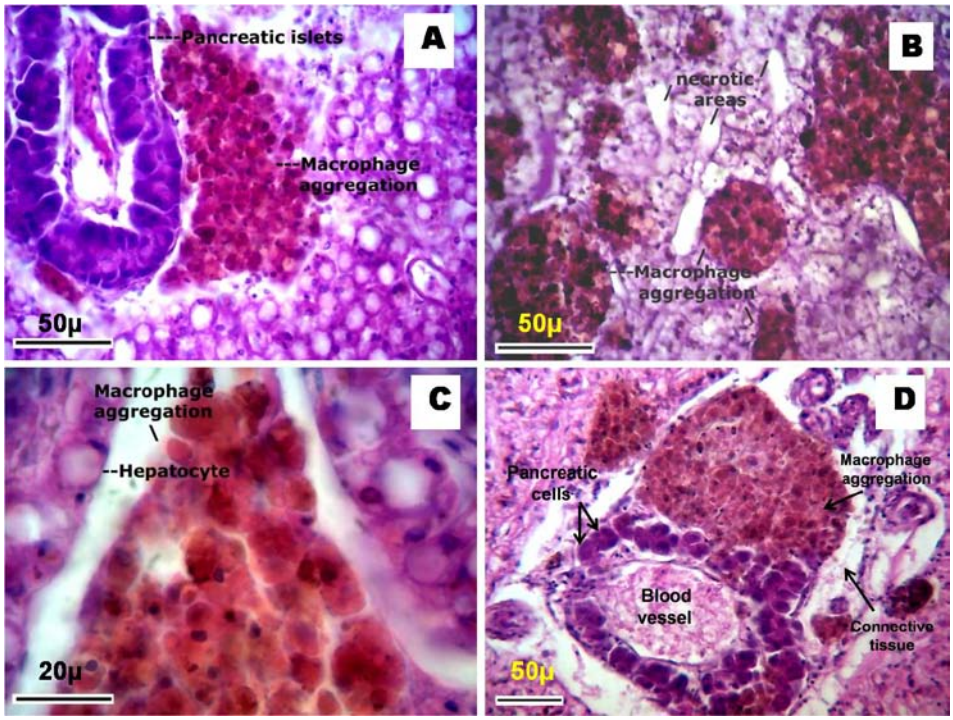

Fig. 5: Sections in the liver of tetraodontiform fishes showing the presence of melano-macrophage aggregations. (A & C) P. fish, *D. hystrix*; and (B & D) Puffer fish, *A. hispidus*.

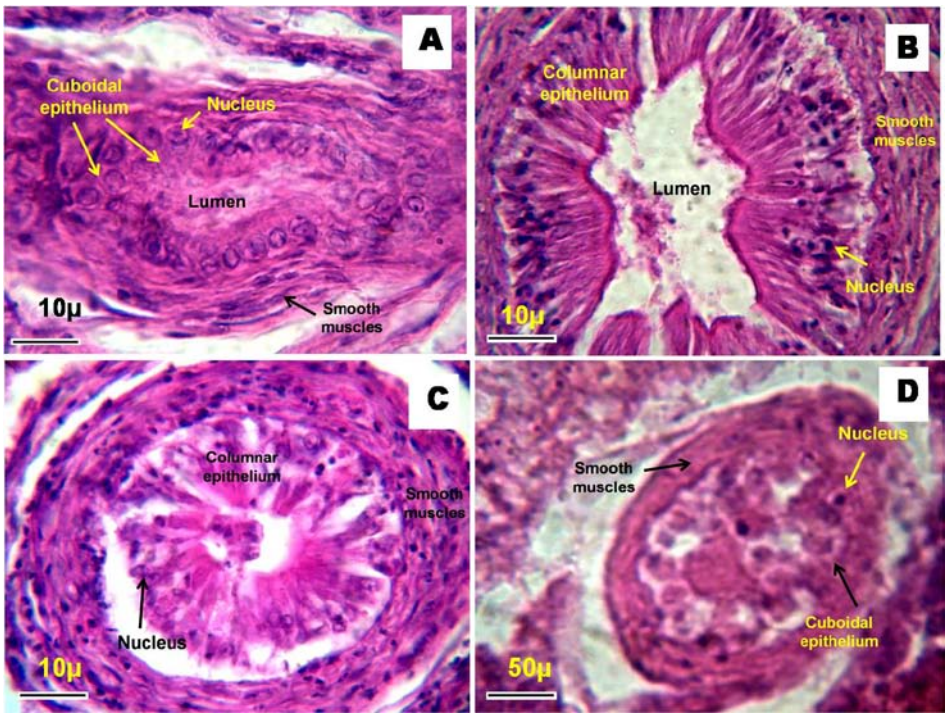

Fig. 6: Sections in the liver of tetraodontiform fishes showing the structure of bile duct. (A) P. fish, *D. hystrix*; (B) Puffer fish, *A. hispidus*; (C) Puffer fish, *A. stellatus*; and (D) Trigger fish, *P. fuscus*.

ARABIC SUMMARY

دراسة نسيجية على كبد بعض الأسماك معقوفة الفكين (رتبة: تتراودونتيفورميس)، البحر الأحمر، مصر

أحمد مسعد عزب
قسم علم الحيوان- كلية العلوم- جامعة الأزهر، مصر
E-mail: amazab2000@yahoo.com

أجريت هذه الدراسة للتعرف على التركيب النسيجي العادي للكبد في بعض الأسماك معقوفة الفكين. وقد اشتملت الدراسة على أربعة أنواع تنتمي إلى ثلاثة فصائل من الأسماك معقوفة الفكين هي: فصيلة أسماك الفهقة (Tetraodontidae)، فصيلة أسماك الشكعة (Diodontidae)، وفصيلة أسماك الخنزيرة (Balistidae). وقد تم تجميع عينات هذه الأسماك من ساحل البحر الأحمر المصري بالغردقة وشرم الشيخ.

أوضحت النتائج أن كبد الأسماك محل الدراسة تتكون من نسيج برنشيمي كبدي مغطى من الخارج بغلالة رقيقة من نسيج ضام وطبقة طلائية حرشفية. تنتظم الخلايا الكبدية بشكل شعاعي حول وريد مركزي على هيئة صفائح كبدية بسمك خليتين او اكثر وتفصلها عن بعضها البعض الجيبيات الدموية. الخلايا الكبدية كبيرة الحجم ومستديرة الشكل أو بيضاوية الشكل ذات سيتوبلازم شفاف بسبب تكدس كميات كبيرة من الدهون. تحتوي هذه الخلايا على أنوية كبيرة حافية الموقع ذات نويات واضحة داكنة اللون.

ينتشر النسيج البنكرياسي في كبد جميع الأسماك معقوفة الفكين محل الدراسة في صورة بنكرياس كبدي، ويفصل هذا النسيج البنكرياسي عن الخلايا الكبدية فواصل رقيقة من نسيج ضام. الخلايا البنكرياسية اخراجية الافراز تكون طويلة أو عامودية ذات أنوية دائرية وقاعدية الموقع ونوياتها داكنة اللون. تظهر الحبيبات الافرازية عند النهايات القمية لهذه الخلايا البنكرياسية. وقد أظهرت النتائج أيضا وجود تجمعات من خلايا الملتقمات الكبيرة الصباغية في كبد جميع أنواع الأسماك معقوفة الفكين محل الدراسة.