

Morphology and Histochemistry of the Liver of Carnivorous Fish *Hemisorubim platyrhynchos*

Morfología e Histoquímica del Hígado del Pez Carnívoro *Hemisorubim Platyrhynchos*

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SUMMARY: The aim of this study was to characterize the normal structure of the liver of *Hemisorubim platyrhynchos*, a carnivorous freshwater catfish found in Neotropical region, using gross anatomy, histology and histochemistry. Anatomically, the liver presents C-shaped and only two lobes: smaller right and bigger left. The gallbladder is located in right lobe and shows elongated shaped. Histological analysis demonstrated that the hepatic parenchyma is made of two hepatocytes plates surrounded by sinusoids. The hepatocytes are polygonal-shaped cells, with spherical nucleus and a dark prominent nucleolus. The cytoplasm presents large amount of lipids and glycogen deposits PAS positives. There are no hepatic lobules or portal triads. Bile ducts are lined by columnar epithelial cells with apical mucosubstances PAS and AB positive. Furthermore, the liver presents melano-macrophages centers, distributed next to the blood vessels and bile ducts, constituted by cells accumulating pigments, whose presence may be related to the nutritional status of the fish. Moreover pancreatic tissue was observed in visceral portion of liver, formed by exocrine pancreatic tissue and islet organ, constituting an extrahepatic pancreas.

KEY WORDS: Anatomy; Histology; Hepatocytes; Extrahepatic Pancreas; Teleost.

INTRODUCTION

The fish liver appears as a key organ, which controls many functions and plays an important role in fish physiology, both in anabolism as in catabolism (Bruslé & Anadon, 1996). The size, shape and volume of liver are adapted to the available space between other visceral organs of general cavity (Bertolucci *et al.*, 2008). According to Bruslé & Anadon, although there are variations between the species, the liver of teleosts in general presents three lobes. The main cellular type of the liver is the hepatocytes which are arranged as cords forming cellular plates, each of which separates several lacunae: the vascular (sinusoids) and biliary (canaliculi) network (Eurell & Haensly, 1982; Bruslé & Anadon; Bombonato *et al.*, 2007).

The fish liver also presents the melano-macrophages, which are distinctive groupings of pigment-containing cells that are usually organized in melano-macrophage centers (Hartley *et al.*, 1996). Melano-macrophage centers increase in size or frequency in conditions of environmental stress and have been suggested as reliable biomarkers for water quality in terms of both deoxygenation and iatrogenic chemical pollution (Agius & Roberts, 2003).

Fishes are very susceptible to environmental variations and respond significantly to pollution. The fish liver is a very interesting model. It is considered a target organ for the study of interactions among environmental factors and structure and/or hepatic functions (Bruslé & Anadon). In this sense, the morphological characteristics of the liver have been associated with physiological status in fish (Caballero *et al.*, 1999) and used as an indicator of environmental quality (Rocha *et al.*, 1994; Al-Youseif *et al.*, 2000; Gochfeld, 2003).

Hemisorubim platyrhynchos belongs to the family Pimelodidae and Siluriformes order. This is a migratory species without parental care that is widely distributed in the Neotropical region. Reports indicate its presence in the Orinoco, Amazon, Paraguay, Uruguay and Paraná River basins. According to Bressan *et al.* (2009), these are nocturnal carnivorous fish that has been a reduction in the population size due to habitat destruction as a result of the building of hydroelectric dams that interrupt the flow of migration required for reproduction. This species is valuable for aquaculture because of the quality and flavor of its meat

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and the absence of intramuscular bones. Thus, the aim of this study was to describe the morphological and histochemical characteristics of the liver of *H. platyrhynchos*, with the goal of increasing available knowledge of the morphofunctional aspects in carnivorous Neotropical fish, especially to improve the aquaculture.

MATERIAL AND METHOD

Animals. Fifteen adult specimens of *H. platyrhynchos*, with a body length of 35.2 ± 2.3 cm, were obtained from Pirai Pisciculture (Terenos, State of Mato Grosso do Sul, Brazil). The animals were anesthetized and euthanized with an overdose of benzocaine and then were dissected with a longitudinal incision along the ventral region. The present study was approved by the Ethical Committee for Research of the Faculty of Sciences at São Paulo State University – UNESP, Bauru, SP, Brazil, under protocol n°. 1144/46/01/10.

Gross anatomy. The features of liver were analyzed and photo-documented in situ with others organs of the digestive tract. Posteriorly, the liver of five specimens was removed and dissected to analysis and documentation using a Leica M50 stereomicroscope (Germany) and stored in 10% formalin.

Histological and histochemical studies. Tissue fragments of the liver of ten specimens were fixed in Bouin's solution immediately after dissection. After fixation, the samples were washed with 70% ethanol, dehydrated in graded ethanol solutions and embedded in historesin. Histological sections (2-3 mm) were stained with hematoxylin-eosin (HE) and 1% toluidine blue (TB). For histochemical studies the samples were embedded in paraplast and sections (5-7 mm) were submitted to reactions with Alcian blue (AB) at pH 1.0 and 2.5 and periodic acid-Schiff (PAS) (Suvarna *et al.*, 2012). The sections were analyzed and photo-documented using Olympus BX50 microscope (Japan).

RESULTS

Gross anatomy. The liver of *H. platyrhynchos* is located in the cranial region of the general cavity, cranially to stomach and anterior intestine (Fig. 1A). The liver presents a reddish-brown color, C-shaped and is divided in two lobes. The right one is smaller than the left lobe, and is near the gallbladder. The left lobe presents gastric imprint (Figs. 1A-1C). Gallbladder possesses elongated shape and blue-green color. The visceral face of the liver presents a tissue

of light color, where the portal vein and bile duct are located, surrounded mainly by pancreatic tissue (Fig. 1C).

Histological and histochemical studies. Histological sections of the liver show a parenchyma covered by a thin capsule of connective tissue lined with a simple squamous epithelium (Fig. 1F). The hepatic parenchyma is made of two cellular plates surrounded by sinusoids. Each plate shows polarized hepatocytes with a sinusoidal face that can be considered the basal region of hepatocyte, and a biliary face that can be the apical region of hepatocyte. Between two neighboring sinusoids, the hepatocytes are arranged as cords that can have two cells thick or can present branches and/or anastomoses of cords (Figs. 1F and 1G). The liver shows no triads or lobules division, but presents an organization of the sinusoids converging to the center-lobular vein (Figs. 1F and 1G). The hepatocytes are polygonal-shaped cells, with spherical nucleus usually centrally located and a prominent dark central nucleolus. The cytoplasm is eosinophilic and presents large amount of lipids. With histochemical analysis with PAS reaction, glycogen deposits were identified in the cytoplasm of the hepatocytes.

The pancreatic tissue in *H. platyrhynchos* was observed in the visceral face of the liver and gradually invades the liver along the branches of the portal vein (Figs. 1D and 1E). However, pancreatic tissue was not observed in hepatic parenchyma. The exocrine pancreatic tissue consists of clusters of pyramidal cells mostly organized in acini. The acinar cells have a dark basophilic cytoplasm, distinct basal nucleus and many eosinophilic zymogen granules. Moreover the pancreas also presents the endocrine tissue as islet organ (Fig. 1D).

The center-lobular veins present simple squamous epithelium, while the arterioles possess a simple cubic epithelium. The bile system consists of bile canaliculi and ducts. The bile canaliculi were observed in the apical border of the neighboring hepatocytes, toward the bile ducts. The bile ducts consist of a simple colunar epithelium (Fig. 1H). In the cytoplasm of the epithelial cells of the bile ducts it was observed a strong reaction of PAS and a moderate reaction of AB pH 1.0 and 2.5 (Figs. 1I and 1J).

The hepatic parenchyma also presents the melano-macrophage centers, located around the blood vessels and bile ducts (Figs. 1H-1J). Melano-macrophage centers are usually nodular and are lined by a thin connective tissue that enters the organ as septa. The macrophages of melano-macrophage centers present a small peripheral nucleus. It is observed many dark pigments in the cytoplasm of these cells, mainly in the periphery of the melano-macrophage centers. With PAS technique, the macrophages present weak intensity of reaction (Fig. 1I).

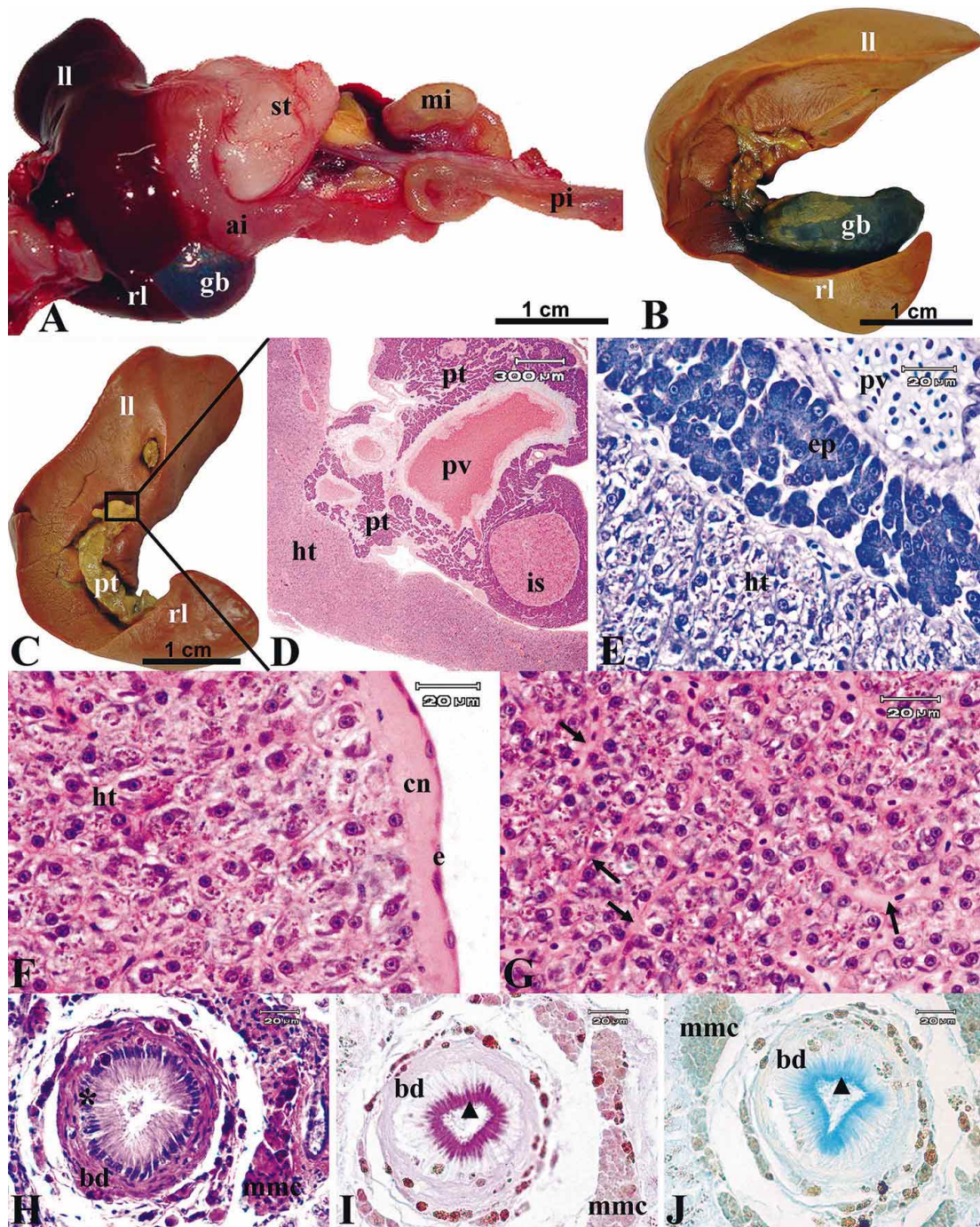


Fig. 1. A: Digestive system of *Hemisorubim platyrhynchos*. B: Ventral view of the liver, showing the right lobe (rl), left lobe (ll) and elongated gallbladder (gb). C: Visceral face of the liver, note the pancreatic tissue (pt). D: Histological section of the transition of the hepatic tissue (ht) to the pancreatic tissue (pt) (HE). E: Histological section of the hepatic tissue (ht) and the exocrine pancreatic tissue (ep) (AT). F: Transversal section of the liver evidencing the squamous epithelium (e), connective tissue (cn) and hepatocytes (HE). G: Hepatic parenchyma with hepatocytes cords, evidencing the sinusoids (arrows) (HE). H: Transversal section of the liver, evidencing the bile duct (bd) with simple columnar epithelium with basal nucleus (asterisk) and melano-macrophage centers (mmc) (HE). I: Bile duct (bd) showing apical region of the epithelial cells with PAS-positives mucosubstances (arrowhead). J: AB-positives mucosubstances (arrowhead) in apical region of the epithelial cells of bile duct (bd). Legend: ll-left lobe; rl-right lobe; gb-gallbladder; st-stomach; ai-anterior intestine; mi-middle intestine; pi-posterior intestine; is-islet organ; mmc-melano-macrophage centers; ht-hepatic tissue, pt-pancreatic tissue, pv-portal vein.

DISCUSSION

The liver of *Hemisorubim platyrhynchus* is divided into two lobes, different of what was observed in other Neotropical fishes, as *Hoplias malabaricus* (Lemes & Braccini, 2004), *Leporinus macrocephalus* (Bombonato *et al.*), *Astyanax altiparanae* (Bertolucci *et al.*), who possess three lobes. In the specie studied, the liver shows reddish brown color due to the rich vascularity, indicating that the animals were in good nutritional status and in normal health (Bruslé & Anadon).

The basic histological constitution of the liver is highly conserved among species and is similar in many teleosts (Kendall & Hawkins, 1975; Bruslé & Anadon). In the liver parenchyma of *H. platyrhynchus*, the hepatocytes are arranged in cords and it was no division in lobules and portal triads was observed, as evidenced in other teleosts (Kendall & Hawkins; Hampton *et al.*, 1985; Gonzalez *et al.*, 1993; Vicentini *et al.*, 2005; Bombonato *et al.*; Bertolucci *et al.*). The hepatocytes of *H. platyrhynchus* are polygonal and present basal nucleus and apical cytoplasm with few glycogen granules but large amount of lipids, similar to observed in *Sparus aurata* (El-Bakary & El-Gammal, 2010). In *Oligosarcus jenynsii*, Petcoff *et al.* (2006), observed that the hepatocytes are polyhedral cells with spherical nucleus and according to Bucke *et al.* (1984), these cells are specialized in the accumulation of reserve substances, mainly glycogen and lipids.

Alongside the hepatocytes cords of *H. platyrhynchus*, sinusoids containing blood plasma converge on the center-lobular vein. According El-Bakary & El-Gamma, the hepatocytes-sinusoidal structure is physiologically important, not only because hepatocytes takes up large molecules from the sinusoid, but also because a large number of macromolecules are secreted into the sinusoid. The bile ducts of *H. platyrhynchus* present simple columnar epithelium with apical mucosubstances PAS and AB positives. The occurrence of neutral and acid mucosubstances at the apex of the epithelial cells of the bile ducts can be indicative of a protective function, important to prevent possible injuries caused by hepatocytes secretions.

The melano-macrophage centers of the liver of the *H. platyrhynchus* are located near the blood vessels and bile ducts. Díaz *et al.* (1999) reported the melano-macrophage centers near hepatic arteries, bile ducts and hepatopancreas in liver of the *Cynoscion guatucupa*. However, in *Hoplias malabaricus* (Lemes & Braccini) and *Leporinus macrocephalus* (Bombonato *et al.*) these centers were observed only near blood vessels. The melano-macrophage

centers of the *H. platyrhynchus* present many dark pigments, mainly in peripheral cells. In *Cynoscion guatucupa*, this tissue presented lipofuscin, ceroid and melanin pigments (Díaz *et al.*). According to Agius & Roberts, melano-macrophage centers usually contain a variety of pigments, including melanin, and these increase in range and volume in older fish or in the presence of diseases. Hartley *et al.*, concluded that these centers are critical elements of the immune system of fish, including immune response against foreign materials such as altered cells or infectious agents.

The pancreatic tissue in *H. platyrhynchus* is observed in the visceral portion of the liver and gradually invades the liver along the branches of the portal vein. The fish pancreas is generally diffusely spread within the fat and mesenteries that connect the intestine, stomach, liver and gallbladder (Youson *et al.*, 2006). The exocrine pancreatic tissue develops around the portal vein during ontogenesis and can remain extrahepatic or penetrate more or less deeply into the liver parenchyma depending on the fish species (Bruslé & Anadon). Although the exocrine pancreatic tissue of *H. platyrhynchus* penetrates in the liver in some regions, acini were not observed in the hepatic parenchyma, constituting an extrahepatic pancreas, unlike what was observed in other teleosts as *Oligosarcus jenynsii* (Petcoff *et al.*), *Ictalurus punctatus* (Kendall & Hawkins), *Oreochromis niloticus* (Vicentini *et al.*), *Leporinus macrocephalus* (Bombonato *et al.*), *Astyanax altiparanae* (Bertolucci *et al.*). The exocrine pancreatic tissue produces digestive enzymes, such as trypsin, amylase and carboxypeptidase A, that are delivered to the digestive tract through a network of ducts (Field *et al.*, 2003). Islet organ also was observed in pancreatic tissue of *H. platyrhynchus* and, according to Beccaria *et al.* (1990), there are four principal cell types in the islet organ of fishes: cells-A producing glucagon-like, cells-B producing insulin, cells-D producing somatostatin, and cells-PP which secrete the pancreatic polypeptide. The presence or absence of these cell types is of interest from the point of view of both the ontogenetic and phylogenetic history of this tissue among fishes (Youson *et al.*).

The results obtained in this study enabled the characterization of the normal structure of the liver of *H. platyrhynchus*, assisting in the analysis of adverse conditions, such as water contamination, nutritional status and health of fishes, which cause changes in the normal structure of the liver (Hartley *et al.*; Ruiz-Picos & López-López, 2012).

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RESUMEN: El objetivo de este estudio fue caracterizar la estructura normal del hígado de *Hemisorubim platyrhynchos*, un bagre carnívoro de agua dulce encontrado en la región neotropical, utilizando anatomía macroscópica, histología e histoquímica de mucosustancias. Anatómicamente, el hígado presenta una forma de C y sólo dos lóbulos de tamaño diferente: menor derecho y mayor izquierdo. La vesícula biliar se encuentra en el lóbulo derecho y presenta forma alargada. El análisis histológico demostró que el parénquima hepático está hecho de dos placas de hepatocitos rodeados por sinusoides. Los hepatocitos son células poligonales con núcleo esférico y un nucléolo oscuro prominente. El citoplasma presenta gran cantidad de lípidos y depósitos de glucógeno PAS positivos. No hay lóbulos hepáticos o triadas portal. Los conductos biliares están revestidos por células columnares epiteliales con mucosustancias apicales PAS y AB positivos. Además, el hígado presenta centros de melanomacrófagos, distribuidos junto a los vasos sanguíneos y conductos biliares, constituidos por células que acumulan pigmentos, cuya presencia puede estar relacionada con el estado nutricional de los peces. Por otra parte, el tejido pancreático se observó en la porción visceral de hígado, formado por tejido pancreático exocrino y órgano islote, que constituye un páncreas extrahepático.

PALABRAS CLAVE: Anatomía; Histología; Hepatocitos; Páncreas Extrahepático; Teleósteos.

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