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Histological study of hepatopancreas in Hi Fin Pangasius (*Pangasius sanitwongsei*)

Reza Sayrafi¹, Gholamreza Najafi¹*, Hooman Rahmati-holasoo², Aref hooshyari¹, Ramin akbari³, Sara shokrpoor³ and Masoomeh Ghadam⁴

¹Department of Anatomy, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. ²Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. ³Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. ⁴Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

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This study was undertaken to determine the histological structures of the hepatopancreas in Hi Fin Pangasius (*Pangasius sanitwongsei*). Microscopic results showed that exocrine pancreatic tissue was located in liver and consists of serous acini that had one or more centroacinar cells each. Thin septa of connective tissue separated parenchyma of liver from exocrine pancreatic cells. Endocrine parts of pancreas were observed in a few number of cell masses in various sizes among exocrine pancreatic cells. Hepatopancrease was covered by a thin capsule and lobulation was not clearly seen in liver. Microscopic structure of hepatopancreas in this species was more similar to other fishes; however, it also had structural differences.

Key words: Hepatopancreas, histology, Pangasius sanitwongsei.

INTRODUCTION

Farming of Pangasius family is industrially performed all over the world for human consumption and as ornamental fishes. In the case of Hi-Fin Pangasius (*Pangasius sanitwongsei*), study of gastrointestinal tract and adnexa are of high importance. Since this fish is a carnivore and has high content of protein in the ration, its digestive system must be very active metabolically and enzymatically. The liver plays a crucial role through secretion of bile salts, metabolism and detoxification of poisons. The pancreas also plays an important role by secreting different kinds of hormones. Hence, in this study, liver and pancreas have been studied histologically.

The interest in knowing how liver structure differs in vertebrates is an old driving force and the piscine liver in particular has always been a matter of debate and renditions. Another point of interest is that fish liver is prone to tumors. Therefore, it is being used as a model in the study of carcinogenesis (Bailey et al., 1996). In the present study, a strong basis on both normal cytology and histology of fish liver is definitely of importance for supporting pathology studies.

In vertebrates, liver has a primary array based on hepatocytes, bile canaliculi and sinusoida. Structural differences occur among species in stromal and parenchymal three-dimensional organizations. Although this structure differs among different species of fishes, it is not often distinctive. As in other vertebrates, the pancreas in fish is an organ that consists of endocrine (islets of Longerhans) and exocrine parts.

In some fish species, the pancreatic tissue gradually invades the liver along the branches of the portal vein. The combined hepatic and pancreatic tissue are collectively called the hepatopancreas (Alboghobeish and Khaksar-Mahabady, 2005; Pousty and Seddig-Marvasti, 2000).

In our previous study (Seyrafi et al., 2009), we showed that liver and pancreas were found to be present as hepatopancreas in *Pangasius hypophthalmus* and there were lots of similarity with the fishes with hepatopancreas. The present study is of high importance regarding Hi-Fin Pangasius (*P. sanitwongsei*) as a carnivore

^{*}Corresponding author. E-mail: g.najafi2006@yahoo.com.

Abbreviations: H&E, Haematoxylin and eosin; PAS, periodic acid-schiff.

and has high demand for metabolic and enzymatic activity of its liver and pancreas, contributing to digestion and absorption of nutrients

MATERIALS AND METHODS

Ten five-month-old Hi Fin Pangasius (*Pangasius sanitwongsei*) of either sexes have been included in this study. The fish were deeply anaesthetized by immersion into 5 ml/L aqueous solution of ethylene glycol monophenyl ether. Then the hepatopancreases were taken out. Tissue samples were fixated in 10% buffered formalin solution for 72 h. Fixated tissue were dehydrated in aggraded series of alcohols, cleared in xylene, embedded in paraffin and cut with microtome at 6 to 7 μ m. Sections were mounted on glass slides, deparaffinized and stained by Haematoxylin and Eosin (H&E), Periodic Acid-Schiff (PAS) and Weigret's Iron Haematoxylin (Humansan, 1997). The Sections were observed under light microscope.

RESULTS

P. sanitwongsei liver surface was covered by a delicate and thin capsule of a loose connective tissue that was composed of layer of squamous cells. Branches of the connective tissue were dispatched into the parenchyma of the liver and no lobulation was present (Figure 1). It should be noted that in the sections, the connective tissue was thicken around hepatocytes and acini-like aggregation of hepatocytes were present in the parenchyma of the liver (Figure 3). Hepatocyte was fairly big and polygon with transparent cytoplasm, because of the presence of glycogen and lipid droplets during the process of preparation of histologic specimens. The nucli were spherical with euchoromatin and distinct nucleolus. The cytoplasm of hepatic cells showed considerable variation based on functional activity, particularly in glycogen and fat storage. The H&E staining method showed the appearance of vacuolar structures in the hepatic cells, probably due to the presence of lipids (Figures 1 and 2). With PAS reactions, large glycogen deposits were identified throughout the cellular parenchyma (Figure 3).

Some of the hepatocytes surface lines the sinusoidal channel (sinusoidal surface), while other surfaces are in contact with adjacent hepatocyte (intercellular surfaces), and some of the adjacent hepatocyte surfaces had bile canaliculi running between them. Sinusoids were covered by endothelial cells with flattened nucleus. Kupffer cells were found among the sinusoidal endothelium. These cells were small and few in number. There were no epithelial cells in bile canaliculi except for hepatic cell walls that were connected to interlobular bile ducts with simple cuboidal cells (Figure 4). These ducts were distally connected to extrahepatic ducts.

The pancreatic exocrine tissue consisted of acini with centroacinar cells and differentiated from hepatic tissue by this arrangement and its characteristic stain. Acinar cells had a euchoromatic nucleus in base and their cytoplasm was basophilic, but the apex of each cell contained eosinophilic zymogen granules. Centroacinar cells were the first cells of exocrine part ducts of pancreas. These cells were smaller than pyramidal acinar cells with euchoromatic nucleus. Intralobular ducts were simple cuboidal.

Thin septa of connective tissue separated exocrine pancreatic cells from the hepatocytes. The exocrine pancreas was arranged around a branch of the portal vein and separated by basal membrane and reticular fibers (Figure 3). Endocrine part of pancreas was observed among exocrine acinar cells in the form of few cell masses in various sizes. Longerhan's islets were surrounded by a delicate connective tissue. In H&E staining of Longerhan's islets, only α and β cells were differentiated. α and β cells were big euchoromatic with elliptical neuclous and small spherical with heterochoromatic neuclous, respectively.

DISCUSSION

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

External surface of liver in *P. sanitwongsei* was covered by a loose connective tissue and one-layer squamous cells capsule. In this case, it was similar to that of *Cetonopharingodon idella* (Alboghobeish and Khaksar-Mahabady, 2005) and differed from that of *Acipenser stellatus* (Sheybani and Adibmoradi, 2002) whose capsule was composed of dense connective tissue and a layer of cuboidal cells.

Like most fish species, lobular organization of hepatic tissue was not conspicuous in the liver of *P. sanitwongsei* (Marina and Claudia, 2007; Rocha et al., 2008; Sheybani, 2006). The hepatic parenchyma of fish is very homogeneous and the hepatocytes are polygonal-shaped cells, appearing hexagonal (Petcoff et al., 2006; Rocha et al., 1998), often weakly basophilic when compared to those of mammals. The nucleus of fish hepatocytes is generally a single, centrally located and spherical nucleus with a clear, dark nucleus.

Hepatocytes in *P. sanitwongsei* were arranged in acinar form. Such arrangement is reported by Sheybani and Adibmoradi (2002) in *A. stellatus*. However, in most species of fishes like *Cetnopharyngodon idella* (Alboghobeish and Khaksar-Mahabady, 2005; Geyer et al, 1999), hepatocytes are arranged in columns forming hepatic cords. Like other fish species, it is not possible to distinguish the portal lobules and the triads (Gonzalez et al., 1993). The organization of the biliary tree in this species was not different from that of other fishes and higher vertebrates.

In Pangasius sanitwongsei, like in many other species



Figure 1. Histological picture of the hepatopancreas of *P. sanitwongsei.* 1, Capsule; 2, hepatocyte; 3, exocrine pancreas; 4, centroacinar cell (H&E ×100).



Figure 3. Histological picture of the hepatopancreas of *P. sanitwongsei.* 1, Acini of exocrine pancreas; 2 and 3, septa of connective tissue; 4, duct; 5, acini-like aggregation of hepatocytes (PAS ×100).



Figure 2. Histological picture of the hepatopancreas of *P. sanitwongsei.* 1, Acini of exocrine pancreas; 2, duct (H&E ×400).

including *C. idella* and *Oligosarcus jenynsii*, pancreatic tissue occurs around the major portal vessels. This is exocrine pancreatic tissue located in the liver (Alboghobeish and Khaksar-Mahabady, 2005; Petcoff et al., 2006). In contrast to *C. idella* whose exocrine pancreas consists of serous cells clusters (Alboghobeish and Khaksar-Mahabady, 2005), in this species, the pancreatic exocrine tissue consists of acini with centroacinar cells that were similar to *Acipenser stellatus* and *Serranus cabrilla* (Sheybani and Adibmoradi, 2002; Gonzalez et al., 1993).

Conclusion

Histomorphology of liver and pancreas showed that in *P. sanitwongsei*, exocrine part of pancreas is organized



Figure 4. Histological picture of the hepatopancreas of *P. sanitwongsei.* Bile canaliculi among adjacent hepatocells are observed in dark lines (Weigret's Iron Haematoxylin ×100).

inside liver and like other fishes, hepatopancreas structure is present.

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REFERENCES

Alboghobeish N, Khaksar-Mahabady M (2005). Histological study of

liver and pancreas in *Cetonopharingodon idella*. J. Sch. Vet. Med. 11: 25-34.

- Bailey GS, Williams DE, Hendricks JD (1996). Fish models for environmental carcinogenesis: the rainbow trout. Environ. Health. Perspect, 104: 5-21.
- Eurell J, Frappier BL (2006). Dellmann's Text book of Veterinary Histology. 6thed. Blackwell Publishing, Iowa, USA, pp: 201-208.
- Geyer HG, Nel MM, Swanepoel JH (1999). Histology and ultrastructure of the hepatopancreas of the tigerfish (*Hydrocynus forskahlii*). J. Mor. 227(1): 93-100.
- Gonzalez 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): 363-373.
- Humansan GL (1997). Animal Tissue Technique. 4 th ed. Sanfracisco, USA, pp. 208-210, 309-311.
- Marina MP, Claudia BR (2007). Histopathology of gill, kidney and liver of Neotropical fish caged in an arban stream, Neotropical Ichthyology. 5(3): 327-336.
- Petcoff GM, Diaz AO, Escalante AH, Goldemberg AL (2006). Histology of the liver *oligosarcus jenynsii* (ostariophsi, characidae) from Los Pades Lake, Argentina. Ser. Zool. 96(2): 205-208.

- Pousty I, Seddigh-Marvasti SAH (2000). An Atlas of Fish Histology. 2nd Ed. University of Tehran press, Tehran, Iran, pp:149-165.
- Rocha E, Monteiro RAF, Pereira CA (1998). Liver of the brown trout (*Salmo trutta*) (Teleostei, Salmonidae): A stereological study at light and electron microscopic levels. Anat. Rec. 3: 317-328.
- Rocha E, Rocha MJ, Galante MH, Silva MW, Monteiro RAF (2008). The hepatocytes of the brown trout (*Salmo trutta fario*): a stereological study of their number and size during the breeding cycle. Ichthyol. Res. 5(5): 415-419.
- Seyrafi R, Najafi Gh, Rahmati-holasoo H, Hajimohammadi B, Athari S.Sh (2009). Histological Study of Hepatopancreas in Iridescent Shark Catfish (*Pangasius hypophthalmus*). J. Anim. Vet. Adv. 8(7): 1305-1307.
- Sheybani MT (2006). Microscopic structures of the liver and pancreas associated with their ducts Persian Sturgeon. Vet. J. Isla. Azad Univ. Garm. Bran. 1(1): 33-38.
- Sheybani MT, Adibmoradi M (2002). Histological study of the liver and pancreas and their ducts in *Acipenser stellatus*. J. Fac. Vet. Med. Univ. Tehran. 57(1): 19-23.