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Comparative Histological, Histochemical and Ultrastructural Studies on the Liver of Flathead Grey Mullet (*Mugil cephalus*) and Sea Bream (*Sparus aurata*)

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Abstract: The liver is one of the digestive glands that composed of parenchymal cells and lattice fibers. It plays a prominent role in metabolism and acts as storage center for many substances. The histological and histochemical distribution of mucosubstances as well as the ultrastructural characteristics of liver in *Mugil cephalus* (flathead grey mullet) and *Sparus aurata* (sea bream) liver were investigated to compare between the histological characteristics of the livers of two teleosts fishes with different feeding habits. Samples of liver from the investigated species were removed and processed for light and transmission electron microscopy. Histologically, the hepatic parenchyma of flathead grey mullet is consisted of hepatocytes which are radially arranged around the central vein interconnecting laminae of two cell thickness, hepatic triad at the portal area and narrow straight sinusoids separating each lamina. The liver tissue of *Sparus aurata* is consisted of hepatocytes which lie in anastomosing laminae around the central vein with the bile canaliculi intercellularly situated. The hepatopancreas in *Sparus aurata* has an acinar arrangement separated from hepatic parenchyma by a thin layer of connective tissue. Histochemically, the hepatic cells in *Mugil cephalus* are moderately positive to periodic acid schiff and alcian blue reactions, while the hepatocyte of *Sparus aurata* are weakly positive to these stains. The histological structural arrangement of hepatic parenchyma of *Mugil cephalus* resembles that described in mammalian liver. *Mugil cephalus* appeared to be higher phylogenetic status than that of *Sparus aurata.*

Key words: *Mugil cephalus* · *Sparus aurata* · Liver · Histology · Histochemistry · Ultrastructure

INTRODUCTION

Fish liver appears as does the liver of other vertebrates as a key organ which controls many life function and plays a prominent role in fish physiology, both in anabolism (proteins, lipids and carbohydrates) and catabolism (nitrogen, glycogenolysis, detoxification) and it acts as storage center for many substances, mainly glycogen [1-3].

Fish liver is a very interesting model for the study of interactions between environmental factors and hepatic structures and functions [4,5].

Thus research on fish liver is important, especially in the field of problems induced by aquaculture conditions and aquatic pollution [6,7].

A histological investigation may therefore, prove to be a cost-effective tool to determine the health of fish populations, hence reflecting the health of an entire aquatic ecosystem [5,8].

In vertebrates, the liver has a primary array based on hepatocytes, bile canaliculi and sinusoids and structural differences occur among species in stroma and parenchyma three-dimensional organization [9]. The piscine liver is very variable and the few livers viewed in the other classes cannot indicate which, if any, scheme is predominant and , moreover, reveal a notable intra-specific diversity [10].

Three patterns of organization of vertebrate hepatic parenchyma have been described [11]. 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 large-mouth bass, *Micropterus salmoides*, the pike, *Esox lucius* and rainbow trout, *Salmo gairdneri*, *Oligosarcus jenynsii* [12-14]. These laminae,

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which look like a "muralium," are separated from each other by sinusoids, formed by reticular and stellate reticuloendothelial cells [12,14].

Reticular fibers form a network around the laminae [15]. In this arrangement of liver parenchymal cells, the bile canaliculi lie between adjacent hepatocytes [13]. The second arrangement, 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 [16]. Sinusoids form an extended network around the tubule. In the third arrangement, occurring in some freshwater and marine fishes [17,18], 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.

Previous studies have indicated that in teleost fish, the pancreatic exocrine tissue develops around the portal vein during ontogenesis. It remains extra hepatic or penetrates somewhat deeply into the liver parenchyma depending on the species, as *Ictalurus punctatus* [15,19], *Pimelodus maculatus* [20], *Micropogon undulatus* [11], *Serranus cabrilla* [21]. Pancreatic tissue can be differentiated from hepatic tissue by its acinar arrangement. In addition, a thin septa of connective tissue separates the hepatocytes from the exocrine pancreatic cells [4].

Grey mullet, *Mugil cephalus*, is a catadromous fish with world-wide distribution [22]. Grey mullet feed on detritus, diatoms, algae and microscopic invertebrates desmids annelids, crustaceans, bivalves and fish parts, which they filter from mud and sand through their mouth and gills [23,24]. Gilthead sea bream, *Sparus aurata* is one of the most important marine fish for Mediterranean aquaculture [25]. Wild sea bream primarily feeds on molluscs and crustaceans, though occasionally it consumes algae [26].

Studies of fish liver structure and function can indeed be considered as a starting point of comparative and phylogenetic studies among vertebrates [4].

The aim of the present investigations was to perform a histological analysis and histochemical distribution of carbohydrates in liver tissue of *Mugil cephalus* and *Sparus aurata* and to compare between the histological characteristics of the livers of two teleosts fishes with different feeding habits.

MATERIALS AND METHODS

Sample Collection: Five live adult flathead grey mullet, *Mugil cephalus* (total body length 21-29 cm) and six adult sea bream, *Sparus aurata* (total body length 19-26.5 cm) were collected from Mediterranean Sea at Damietta region, Egypt.

Fishes were used without sexual distinctions, after their identification, the body cavity were opened through a midventral incision and the liver was immediately fixed in Bouin's solution.

Light Microscopy: After fixation, samples of liver were dehydrated in an ethanol series, cleared in xylene and embedded in paraffin wax and sectioned at 5μm. After dewaxing with xylene and hydration in ethanol series of descending concentration, sections were stained for general histological purposes with haematoxylin and eosin stain.

For carbohydrate histochemistry, sections were combined stained with periodic acid-Schiff (PAS) for identification of glycogen; Alcian blue (pH 2.5) for carboxylated and some sulfated glycoconjugates [27,28].

Transmission Electron Microscopy: Small fragments of liver were placed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer at pH 7.2 for 3h at room temperature. After rinsing in phosphate buffer the specimens were postfixed in 1% buffered osmium tetroxide at pH 7.2 for 3h at 4C. They were then dehydrated and embedded in araldite. Thin sections were stained with uranyl acetate and lead citrate and examined with JEOL electron microscope and photographed.

RESULTS

Histologically, the liver of *Mugil cephalus* shows hepatic parenchymal arrangement consists of hepatocytes, which are radially arranged around a central vein in interconnecting laminae of two cells thickness, narrow straight sinusoids separating each lamina (Fig.1a,b). Hepatocytes vary in shape from round to oval. Each hepatocyte contains a large, round, centrally situated nucleus with a prominent dark nucleolus. Hepatocytes show an appearance of some small vacuolar structures in the hepatic cells, probably due to the presence of lipids and the biliary tract was detected.

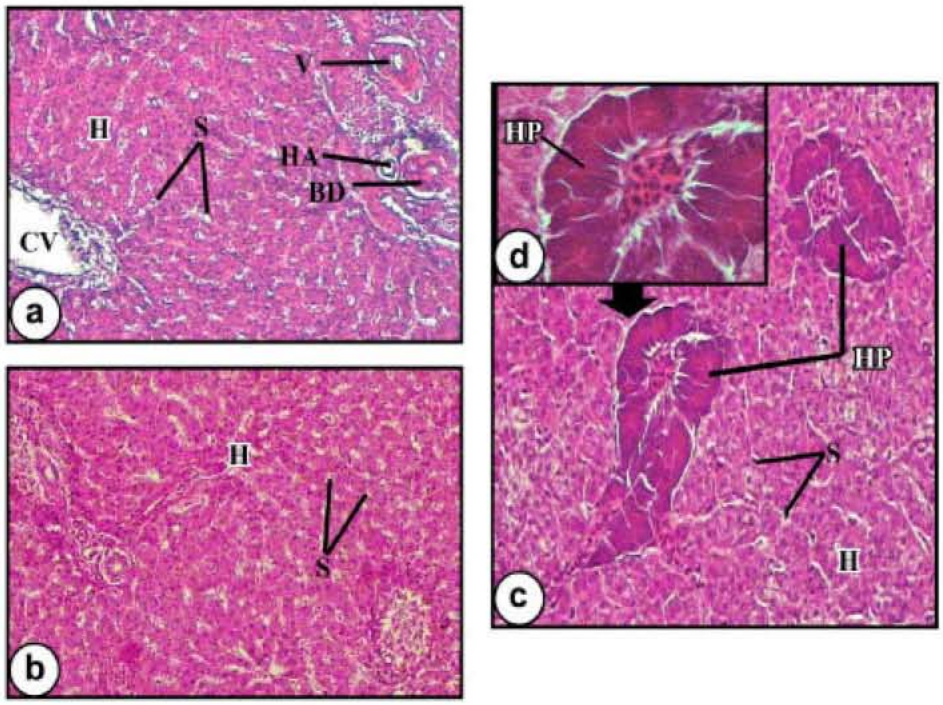

Fig. 1: a), b) Histology of hepatic parenchyma of *Mugil cephalus* Showing hepatocytes (H), sinusoid (S), central vein (CV), portal vein (V), hepatic artery (HA) and bile duct (BD). (HE X100, X400).
c), d) Histology of hepatic parenchyma of *Sparus aurata* showing hepatocytes (H), sinusoid (S) and intra hepatic exocrine pancreatic tissue (HP). (HE X100, X400).

The lumen of the sinusoids contains erythrocytes and macrophages. Large cells resting on the luminal surface of the sinusoid endothelium are present. These cells are known as Kupffer cells. Sinusoids are covered by typical endothelial cells with flatten nucleus (Fig. 1a).

Veins are scattered through the liver parenchyma without a well defined arrangement and they are surrounded by hepatic parenchyma or pancreatic tissue, sometimes accompanied by an artery or bile duct (Fig.1a). Triads consists of hepatic vein and artery and bile duct were detected at the periphery of parenchymal lobules.

Histological sections of liver from *Sparus aurata* revealed parenchymal arrangement consists of hepatocytes, which lie in anastomosing laminae or cords around a central vein, with the bile canaliculi situated intercellularly. Irregular shaped sinusoids which contain erythrocytes appeared throughout the interstice between the hepatic plates (Fig.1 c,d). A homogenous-sized hepatocytes with large spherical centrally located nuclei with a prominent nucleolus. The cytoplasm of hepatocytes appeared pale stained, probably due to the presence of lipids which did not significantly affect the size of the cells. A well-developed biliary tract was detected.

In *Sparus aurata*, the exocrine pancreas or hepatopancreas has an acinar arrangement, separated from hepatic parenchyma by a thin layer of connective tissue. The pancreatic cells are arranged around a branch of the portal vein, separated by a basal membrane and reticular fibers (Fig.1 c, d).

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, spherical nucleus is basally located, with prominent dark nucleolus. Zymogen granules are located in the apical ends of these cells. Microscopical observation showed that the pancreatic cells are differentiated from hepatic tissue by their basophilic basal pole and eosinophilic apical cytoplasm (Fig. 1 c, d).

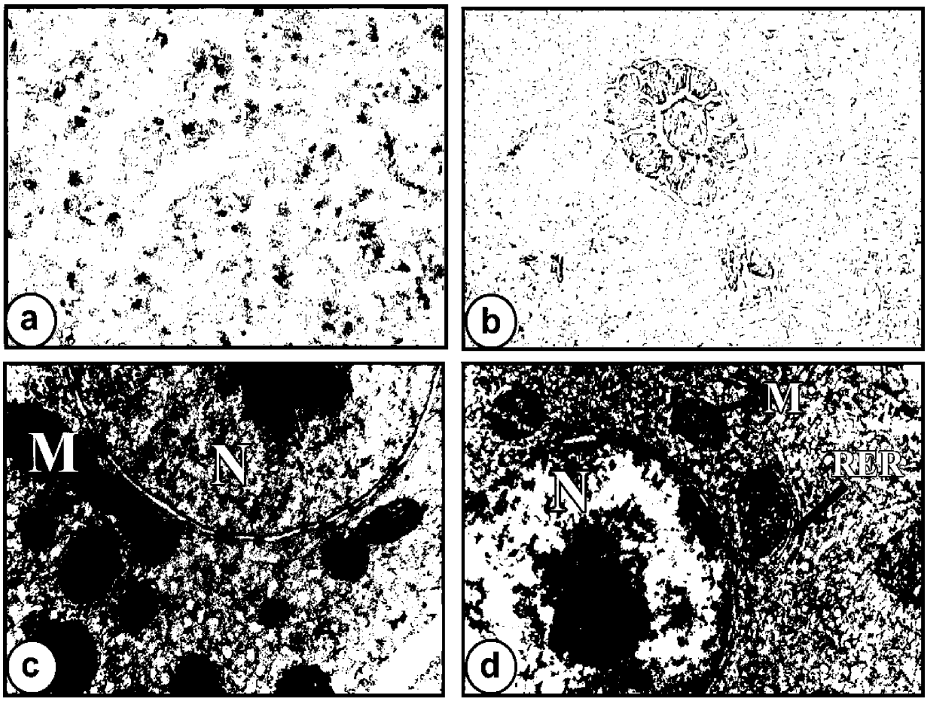

Fig. 2: a) Histochemistry of hepatic parenchyma of *Mugil cephalus* showing glycogen granules positively reacted with PAS. (AB-PAS X100).
b) Histochemistry of hepatic parenchyma of *Sparus aurata* showing hepatopancreas positively reacted with AB. (AB-PAS X100).
c) Hepatocyte ultrastructure of *Mugil cephalus* showing granulated nucleus (N) and few rough and large amount of smooth endoplasmic reticulum and mitochondria (M). (X13000).
d) Hepatocyte ultrastructure of *Sparus aurata* showing heterochromatic nucleus (N), rough (RER) and vesiculated smooth endoplasmic reticulum and mitochondria (M). (X13000).

The hepatic cells of *Mugil cephalus* are moderately positive to the periodic acid-Schiff and Alcian blue reactions due to carbohydrate stored in their cytoplasm. Although, large and rosette shaped glycogen deposits were identified in the cytoplasm of parenchymal hepatocytes (Fig.2a).

The hepatocytes of *Sparus aurata* are weakly positive to the periodic acid-Schiff and Alcian blue reactions due to small contents of carbohydrate stored in their cytoplasm. But, the hepatopancreatic cells revealed high positive periodic acid-Schiff and the branch of the portal vein contains cells with positive Alcian blue reaction (Fig. 2b).

At ultrastructural level, the hepatocytes of *Mugil cephalus* have a round spherical nucleus with granulated chromosomes and prominent nucleolus, elongated and spherical mitochondria, rough and large amount of vesiculated smooth endoplasmic reticulum (Fig. 2c).

Ultrastructurally, the hepatocytes of *Sparus aurata* have a spherical nucleus with euchromosomes and heterochromosomes, prominent nucleolus and nuclear membranes and pores. Rough endoplasmic reticulum connecting with nuclear membrane and within the cytoplasm was investigated. Spherical mitochondria and numerous vesiculated smooth endoplasmic were also found in the cytoplasm.

DISCUSSION

In the present study, liver of *Mugil cephalus* showed hepatic parenchymal arrangement consists of hepatocytes, which are radially arranged around a central vein in interconnecting laminae of two cells thickness, narrow straight sinusoids separating each lamina. This histological structure of the liver resembles that described for the Atlantic croaker *Micropogon undulatus* [11], for

Liza species [29], for the stripped weakfish *Cynoscion guatucupa* [30] and for rainbow trout *Oncorhynchus mykiss* [31].

The hepatocyte-sinusoidal structure is physiologically important, not only because hepatocytes takes up large molecules from the sinusoid, but also because a large number of macromolecules (e.g., lipoproteins, albumin and fibrinogen) are secreted into the sinusoid. In the cord-like form, hepatocytes are closely contacted with sinusoidal capillaries that form a dense network as in mammalian livers [32,33].

The livers of the *Mugil cephalus* have some lipid vacuoles within the hepatocytes and the biliary tract was detected. In the livers of *Sparus aurata*, large contents of lipid and well-developed biliary tract were observed. Biliary tract structures were concerned with dietary habits and adapted the hepatic function, including lipid metabolism [2].

The present study revealed that the hepatic parenchymal arrangement in *Sparus aurata* consists of hepatocytes, which are composed of anastomosing two-cell-layered cords. They are similar to those in the channel catfish, *Ictalurus punctatus* [19], the rainbow trout, *Salmo gairdneri* [18], to some extent, the Atlantic croaker, *Micropogon undulatus* [11], the tiger fish, *Hydrocynus forskahlii* [9], *Oreochromis niloticus* [34] and *Astyanax altiparanae* [7].

The exocrine pancreas or hepatopancreas has an acinar arrangement, separated from hepatic parenchyma by a thin layer of connective tissue. The pancreatic columnar cells are arranged around a branch of the portal vein, separated by a basal membrane and reticular fibers.

Exocrine pancreatic cells of *Sparus aurata,* exhibit similar characteristics to those of other teleosts [20,34,35].

The present study revealed differences in hepatic parenchymal arrangement between *Mugil cephalus* and *Sparus aurata*. The histological structural arrangement of the *Mugil cephalus* liver resembles that described for mammalian arrangement.

Fish livers with a higher phylogenic status had structures identical to the mammalian arrangement, which possessed higher metabolic functions [2]. In the present study, difference was seen in the periodic acid-Schiff and Alcian blue reactions, between *Mugil cephalus* and *Sparus aurata,* fishes with different feeding habits. The liver of *Sparus aurata* showed less glycogen and higher lipid.

In conclusion, a possible positive correlation between hepatic parenchymal arrangement, hepatocyte structures and feeding habits may be related to the phylogenic advancement.

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