

Histological, histochemical and ultrastructural characterization of the pancreas of the grass carp (*Ctenopharyngodon idella*)

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SUMMARY

The purpose of this study is to describe the histological, histochemical and ultrastructural features of pancreas of the grass carp (*Ctenopharyngodon idella*), one of the common herbivorous freshwater fish of Egypt. The pancreas is divided into exocrine and endocrine portions. Exocrine pancreatic tissues consists of scattered serous acini, and is observed in two forms: 1) disseminated in the spleen tissue, in mesentery around intestine and intestinal bulb, and 2) intrahepatically, around the branches of the portal vein. Two alveolar cell types are present in pancreatic acini; centroacinar cells and typical pyramidal acinar cells. Pancreatic stellate cells (PSC) are demonstrated in the perivascular and the periacinar space of the pancreas. The pancreatic acini gave positive reaction to PAS, Best's carmine, and osmium tetroxide, and negative to alcian blue. The acini also show high lipase and alkaline phosphatase activity, and moderate activity for acid phosphatase. Scanning electron microscopy show apical microvilli of the acinar cells, and branched PSC extend their processes between the pancreatic cells. Ultrastructure of pancreatic acini reveals well-developed rER, membrane-bound zymogen granules, and abundant lipid droplets. The duct system is composed of intralobular duct, interlobular pancreatic duct and main duct opened in the intestinal bulb. The endocrine parts of the pancreas are organized as lightly staining Langerhan's islets between exocrine acinar cells found in

the liver, in mesenteries around the intestinal bulb and the intestine, and consisted of three cell types. Alpha cells were the most dominant cells, and were ovoid in shape. Beta cells were polyhedral in shape, and they grouped in small clusters. Delta cells were small fusiform, argyrophilic cells. In conclusion, the present study revealed that the exocrine portion of the pancreas of the grass carp had two forms, disseminated and intrahepatic, with characteristic cellular and histochemical components, and an endocrine portion that consisted of Alpha, Beta and Delta cells.

Key words: Hepatopancreas – Grass carp – Histochemistry – Ultrastructure – PSC

INTRODUCTION

The pancreas of fish is a lobulated compound acinar gland, divided into two compartments with exocrine and endocrine functions. The pancreas plays an important role in the regulation of energy balance and nutrition through the synthesis and release of protein digestive enzymes and hormones (Geyer et al., 1996)

The pancreas of teleosts fish was classified by Rizkalla (1967) into disseminated, compact and intrahepatic types. The disseminated type occurs around gall bladder and in mesentery; the compact pancreas is located in the splenic mesentery, and acts as a discrete organ, while the intrahepatic pancreas is located inside the liver. Exocrine pancreatic tissues that are found in association with the liver around portal vessels are collectively

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called hepatopancreas (Ferguson, 1989). During ontogenesis, the exocrine pancreatic tissue develops around the portal vein, and then penetrates the parenchyma of the liver, or remains extra-hepatic depending on the species (Gonzalez et al., 1993).

The grass carp or white amur (*Ctenopharyngodon idella*) is a large cyprinid fish that is native to large Asian rivers such as the Amur River Basin in Russia and the West River in China (Gulliard and Gasaway, 1978). It is a fast-growing herbivorous fish that usually feeds on grass or other aquatic vegetation, and acts as a good species for aquaculture in Egypt (Earl et al., 1992).

The aim of the present study was to describe the pancreas by light, scanning and transmission electron microscope, focusing on histochemistry and enzyme activity of pancreatic acini with attention to the distribution of islets of Langerhans.

MATERIALS AND METHODS

Sample collection

The materials employed in this study consisted of 16 randomly obtained adult specimens of grass carp, *Ctenopharyngodon idella*, collected from fish a farm in El-Minea during the year. The mean standard length was 35.40 ± 3.01 cm, and the mean standard body mass was 406.40 ± 9.60 g. The fish were deeply anaesthetized with benzocaine (4 mg/L) and decapitated. The specimens were dissected as soon as possible, and the pancreas (with associated organs; intestinal bulb, liver, spleen and intestine) was removed through a longitudinal incision in the abdominal wall.

Histological examination

Samples for histological technique were taken from pancreas associated with liver, spleen, intestinal bulb and intestine, then were dissected at $1 \times 1 \times 0.5$ cm and were immediately fixed in Bouin's fluid for 22 hours. The fixed materials were dehydrated in an ascending series of ethanol, cleared in methyl benzoate and then embedded in paraffin wax. Six paraffin blocks from each above-mentioned samples at 5-8 μ m in thickness were cut transversely and longitudinally and stained with Harris haematoxylin and Eosin (Harris, 1900), Crossmon's trichrome (Crossmon, 1937) for collagenous fibers, Gomori's method (Gomori, 1937) for reticular fibers, Verhoeff's method for elastic fibers (Verhoeff, 1908), Long Ziehl-Neelsen for lipofuscins (Pearse, 1953), improved Kupffer's gold chloride method for demonstration of stellate cells (Wake et al., 1986), Grimelius's silver method for demonstration of argyrophilic cells (Grimelius, 1968) and Maldonado's stain for demonstration of endocrine portion of pancreas (Maldonado and Jose, 1967).

Histochemical analysis and enzyme histochemistry

For carbohydrate histochemistry, representative sections from the hepatopancreas and the pancreas around the intestinal bulb were stained by Periodic Acid-Schiff (PAS) technique (McManus, 1946) and Alcian blue (pH 2.5) (Mowry, 1956). Representative sections were stained with Best's Carmine for detection of glycogen (Best, 1906). In addition, some sections were placed in osmium tetroxide (Di Scipio et al., 2008) and then cutting for detection of lipid. Lipase activity was demonstrated by Tween method (Gomori, 1952), acid phosphatase by the Gomori lead nitrate method (Gomori, 1950) and alkaline phosphatase activity by the Gomori calcium method (Gomori, 1952).

Semithin sections and transmission electron microscopic (TEM) preparations

Small specimens of hepatopancreas and pancreas found around the intestinal bulb were fixed in 2.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1M Na-cacodylate buffer, pH 7.3 for 4 hours at 4 °C. They were washed in the same buffer and then post-fixed in 1% osmic acid in 0.1M Na-cacodylate buffer for further 2 hours at room temperature. The samples were then dehydrated in ethanol and embedded in an Araldite-Epon mixture. Semithin sections (1 μ m in thickness) were cut and stained with Toluidine blue. Ultrathin sections, obtained by a Reichert ultramicrotome, were stained with uranyl acetate and lead citrate and examined with a Philips EM 400 electron microscope.

Scanning electron microscopic (SEM) preparation

The body cavity of the fish was opened and the pancreatic tissues in the liver and around the intestinal bulb were immediately washed by 0.1M Na-cacodylate buffer. Then they were fixed in 2.5% paraformaldehyde and 2.5% glutaraldehyde in M Na-cacodylate buffer, pH 7.3 for 4 hours at 4 °C. Thereafter, they were washed in the same buffer and post-fixed in 1% osmic acid in 0.1M Na-cacodylate buffer for further 2 hours at room temperature. The samples were then dehydrated in acetone followed by isoamyl acetate and then subjected to critical point drying method with a polaron apparatus. Finally, they were coated with gold and observed with a JEOL scanning electron microscope (JSM - 5400 LV) at 15 KV.

RESULTS

Gross morphology

The adult grass carp *Ctenopharyngodon idella* is a large fish with a yellowish green colored body and large scales (Fig. 1A). The pancreas appeared as groups of yellowish masses that distributed in mesenteries around the intestine, spleen and intestinal bulb (Fig. 1 B, C). It also was diffusely

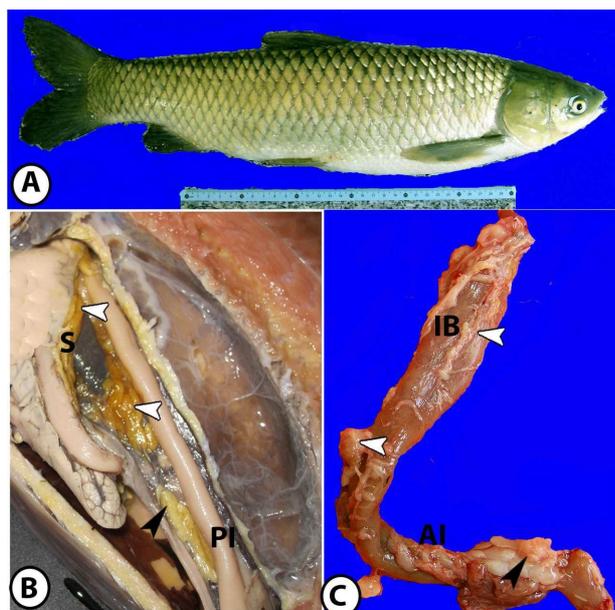


Fig. 1. Gross morphology of grass carp and pattern of distribution of pancreatic tissues. **(A)** adult grass carp *Ctenopharyngodon idella*. **(B)** Pancreatic tissues (white arrowheads) distributed around the spleen (S), and some pancreatic tissues (black arrowhead) found around the posterior portion of the intestine (PI). **(C)** Pancreatic tissues (white arrowheads) diffused around the intestinal bulb (IB), and some pancreatic tissues (black arrowhead) found around the anterior portion of the intestine (AI).

spread within the hepatic tissue to form hepatopancreas, but the pancreas could not be seen macroscopically within the liver.

Histological analysis of the pancreas

The pancreas of the grass carp was a highly lobulated gland, each lobule was composed of closely packed secretory pancreatic acini separated by thin connective tissue septa (Fig. 2A). The pancreas was divided into exocrine and endocrine portions. Exocrine pancreatic tissues of the grass carp were observed in two forms: the first one was disseminated in mesenteries around the intestinal bulb (Fig. 2B) and the anterior portion of the intestine (Fig. 2C), as well as embedded in splenic tissue (Fig. 2D); the second form was intrahepatic, around the branches of the portal vein (Fig. 2E) separated from the hepatic parenchyma by a thin layer of connective tissue mainly of collagenous fibers (Fig. 2F). Abundant amounts of reticular fibers were determined in the pancreatic tissue that appeared as a black colored meshwork encircling the pancreatic acinar cells and concentrated around the blood vessels (Fig. 2G). Fine and few elastic fibers were also demonstrated in the pancreatic tissues (Fig. 2H).

Exocrine pancreatic tissue consisted of scattered serous acini. Two alveolar cell types were present in the acini; centroacinar and typical acinar cells (Fig. 3A). The centroacinar cells were polyhedral cells attached by their basal ends to the portal

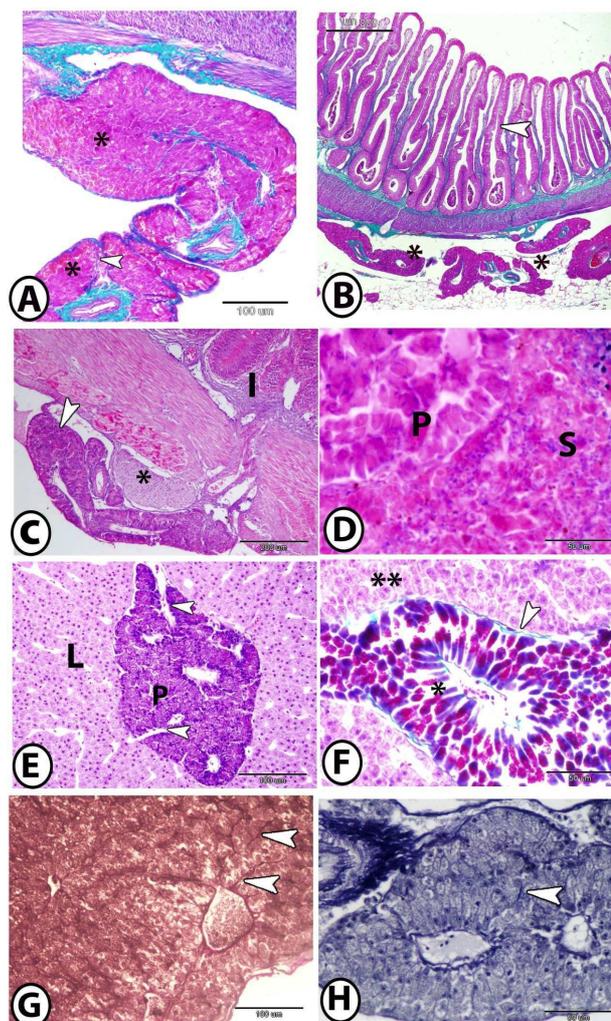


Fig. 2. Distribution of exocrine portion of the pancreas. **(A)** The pancreas is formed of many lobules (asterisks) separated by connective tissue septa (arrowhead) (Crossmon's Trichrome). **(B)** Disseminated form of exocrine pancreatic tissues (asterisks) distributed in the mesenteries around intestinal bulb (arrowhead indicate folds of intestinal bulb) (Crossmon's Trichrome). **(C)** Disseminated form of exocrine pancreatic tissues (arrowhead) distributed in the mesenteries around the intestine (I). Note the presence of pale staining islets of Langerhans (asterisk) scattered between the exocrine portion of pancreas (H&E). **(D)** Disseminated form of exocrine pancreatic tissues (P) distributed in the splenic tissue (S) (H&E). **(E)** Intrahepatic form of exocrine portion of pancreas or hepatopancreas formed of clusters of pancreatic acini (P) distributed around branches of the portal vein (arrowheads) of the liver (L) (H&E). **(F)** Exocrine pancreatic tissue (*) separated from hepatic parenchyma (**) by a thin layer of connective tissue capsule (arrowhead) (Crossmon's Trichrome). **(G)** Abundant amount of reticular fibers (arrowheads) distributed around pancreatic acini (Gomori's staining). **(H)** Fine elastic fibers (arrowhead) distributed around pancreatic acinar cells. (Verhoeff's staining).

veins and their apices toward the central lumen, and represent the terminal end of the pancreatic duct system (Fig. 3A). The typical acinar cells were polyhedral in shape, arranged in groups to form

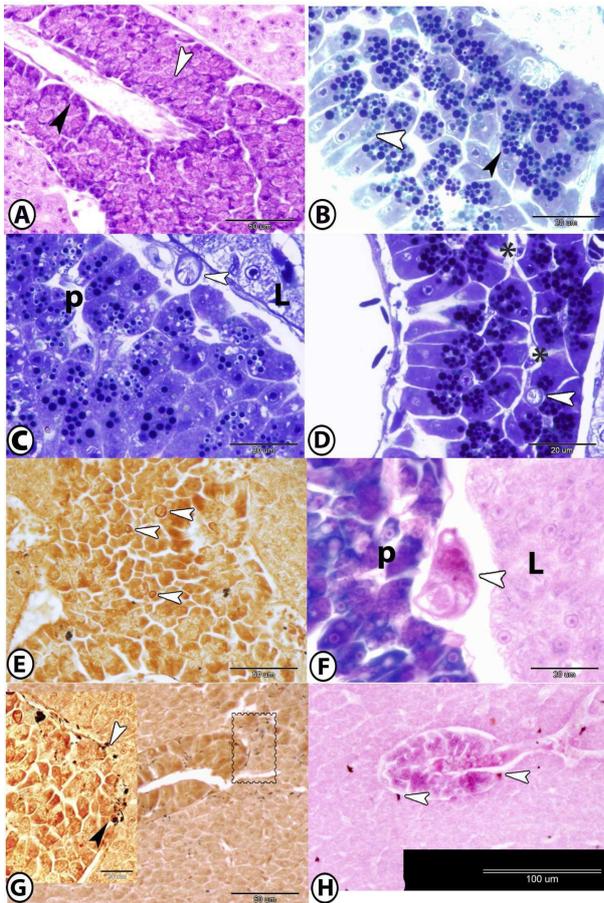


Fig. 3. Structure of the pancreatic acini. **(A)** The pancreatic acini formed from two types of cells; centroacinar (black arrowhead) and typical acinar cells (white arrowhead) (H&E). **(B)** Semithin sections of pancreatic acinar cells showing dark zymogen granules (black arrowhead) and light ones (white arrowhead) (Toluidine blue). **(C)** Semithin sections of exocrine pancreatic tissue showing rodlet cells (arrowhead) between pancreatic acini (P) and liver (L) (Toluidine blue). **(D)** Semithin sections of pancreatic acini showing rodlet cells (arrowhead) interspersed between pancreatic acinar cells. Many small (pancreatic stellate cells) PSC (asterisks) extended between the acinar cells are noticed (Toluidine blue). **(E)** Argyrophilic rodlet cells (arrowheads) distributed between pancreatic tissue (Grimelius silver staining). **(F)** Macrophage center (arrowhead) organized between liver (L) and pancreas (P) (Kupffer's Gold Chloride method). **(G)** PSC (square, arrowheads) gave positive reaction to Grimelius silver stain. **(H)** PSC (arrowheads) gave positive reaction to Kupffer's Gold Chloride staining.

the pancreatic acini with central narrow lumen, where the products of these exocrine cells are secreted. The basal cytoplasm was basophilic, and the apical cytoplasm contained eosinophilic zymogen granules, which varied in density (Fig. 3A). The nucleus was spherical and basally located with a prominent central nucleolus. Semithin sections revealed the presence of rounded zymogen granules of variable size and some of them appeared dark in colour with toluidine blue and others were observed in light colour (Fig. 3B). Rodlet cells were observed in the external surface of

hepatopancreas, as well as between the acinar cells, and were characterized by rounded to oval shape and intracytoplasmic granules or rodlets surrounded by a thick capsule (Fig. 3 C, D). These cells also gave positive reaction with Grimelius silver stain (Fig. 3E). Melanomacrophage centers with heterogenic contents of mainly lipofuscins were observed in hepatopancreas in association with blood vessels (Fig. 3F). Pancreatic stellate cells (PSC) were demonstrated by Improved Kupffer's Gold Chloride and Grimelius silver stain to be small branched cells with many cytoplasmic processes in perivascular and periacinar space (Fig. 3 G, H).

Histochemistry and enzyme activity of pancreatic acini

Exocrine pancreatic cells revealed PAS- positive reaction (Fig. 4A). Abundant amounts of glycogen were demonstrated in the pancreatic parenchyma by Best's Carmine stain that also revealed a stronger reaction in centroacinar cells (Fig. 4B). Negative reaction of the pancreatic acini to Alcian Blue was recorded (Fig. 4C). The lipid droplets in the cytoplasm of pancreatic acinar cells stained brown to black by osmium tetroxide (Fig. 4D).

The acini showed high activity for alkaline phosphatase in the form of blackish granules scattered in the cytoplasm and zymogen granules (Fig. 5 A, B). Moderate activity for acid phosphatase was determined in form of homogenous dark granules in the pancreatic acinar cells (Fig. 5 C, D). The acini also showed high lipase activity in the form of numerous black granules scattered throughout the acinar cells (Fig. 5 E, F). All cuts of the different specimens of the hepatopan-

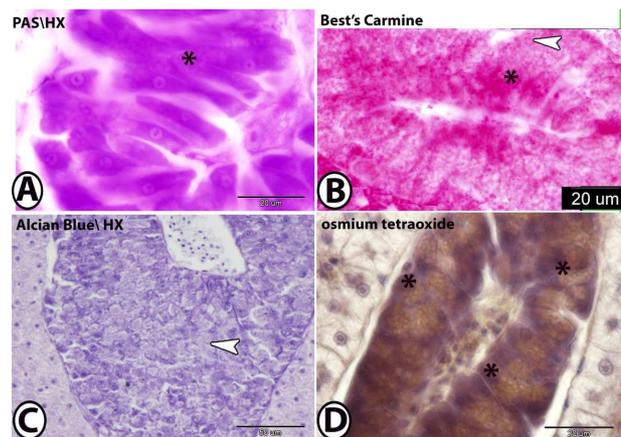


Fig. 4. Histochemistry of pancreas. **(A)** Pancreatic cells showing PAS-positive reaction (asterisk). **(B)** Pancreatic acini showing strong Best's Carmine reaction (arrowhead), however stronger reaction is demonstrated in centroacinar cells (asterisk). **(C)** Negative Alcian blue reaction of acinar cells (arrowhead) is noted. **(D)** Exocrine part of pancreas showing positive reaction to osmium tetroxide (asterisks).

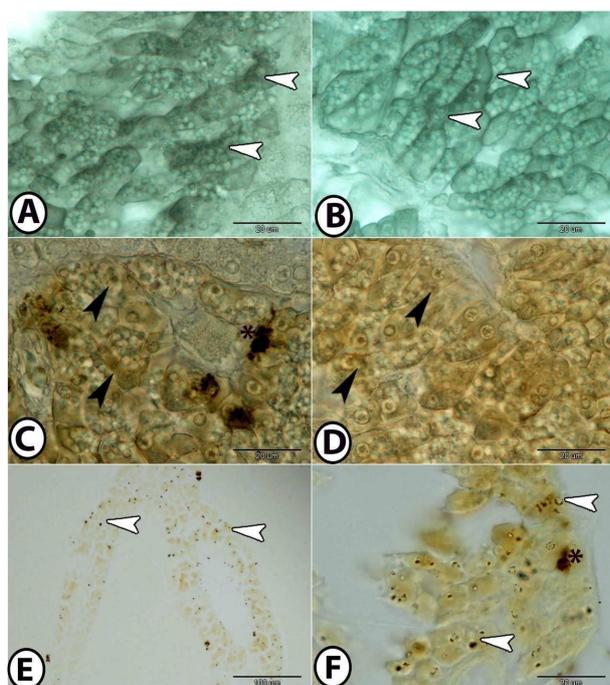


Fig. 5. Enzyme activity of the pancreatic acini. **(A,B)** High alkaline phosphatase activity (arrowheads) of pancreatic acini (Gomori Calcium method). **(C,D)** Moderate acid phosphatase activity (arrowheads) of pancreatic acini. High activity is observed in macrophage centers (asterisk) (Gomori Lead Nitrate method). **(E,F)** High lipase activity (arrowheads) of pancreatic acini. High activity is observed in macrophage centers (asterisk) (Tween method).

creas and pancreas around the intestinal bulb had the same histochemical and enzymatic findings.

Scanning electron microscopy of pancreatic acini

The pancreatic acinus was formed of 12-22 acinar cells. They were rounded to oval in shape and measured 70-100 μm in diameter. Pancreatic serous acinus was surrounded by a connective tissue capsule (Fig. 6A). The acinar cells were covered by minute microvilli (Fig. 6B). The acini are also organized into the form of columnar cords of acinar cells (Fig. 6C). Oval shaped PSC were observed in association with pyramidal pancreatic cells as their dendritic-like processes extended in between them (Fig. 6 B, C, D). The lateral surfaces of the acinar cells exposed a number of rounded elevations, which probably corresponded to zymogen granules (Fig. 6D). The pancreas associated with the intestinal bulb and hepatopancreas had the same SEM features.

Ultrastructure of pancreatic acini

The acinar cells of the grass carp were pyramidal in shape, and their cytoplasm contained many rounded membrane-bounded secretory zymogen granules of variable size that contained electron dense materials with a mean diameter of 1.5 μm (Fig. 7A). The granules were distributed in the cytoplasm, but mainly concentrated in the apical portion of the acinar cells (Fig. 7B). The nuclei of these cells were rounded and basally located with

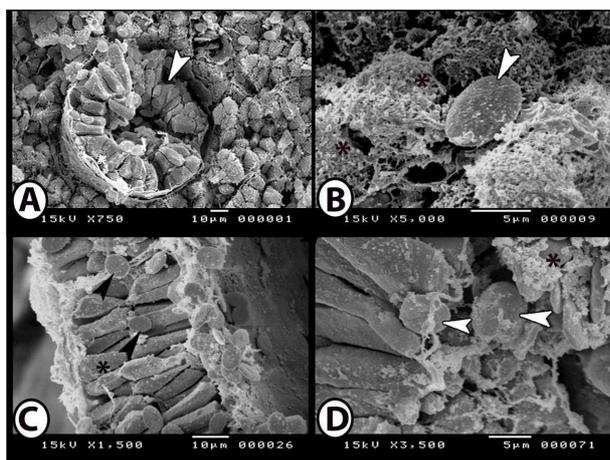


Fig. 6 (middle). Surface architecture of the pancreatic acini. **(A)** Serous pancreatic acini surrounded by connective tissue capsule (arrowhead). **(B)** Pancreatic acinar cells are covered by microvilli (asterisks). PSC (arrowhead) is observed between the acinar cells. **(C)** PSC (arrowheads) extended their processes among columnar cords of pancreatic acinar cells (asterisk). **(D)** PSC (arrowheads) extended their processes among the pancreatic cells. Note presence of elevations of zymogen granules (asterisk).

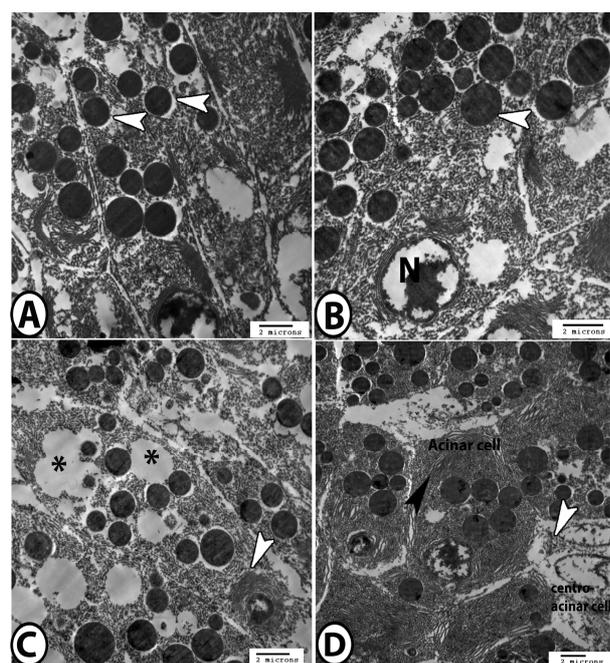


Fig. 7 (bottom). Ultrastructure of pancreatic acini. **(A)** Acinar cells are filling of zymogen granules (arrowheads). **(B)** The rounded nucleus (N) of pancreatic cells located in the basal portion and zymogen granules concentrated apically (arrowhead). **(C)** Concentrically arranged rER (arrowhead) and many lipid droplets (asterisks) are observed. **(D)** Electron lucent centroacinar cells with ribosomes (white arrowhead) adjacent to acinar cells with abundant rER (black arrowhead).

a distinct central nucleolus. The heterochromatin was represented by small dense granular areas attached to the inner side of the nuclear envelope (Fig. 7B). Abundant well-developed rough endoplasmic reticulum with concentrically distributed dilated cisternae filled the most basal region of the cell, with small amounts also occurring in the apical region among zymogen granules (Fig. 7 C, D). Many lipid droplets of variable size were observed in the acinar cells (Fig. 7C). Centroacinar cells were observed adjacent to the typical acinar cells, and were characterized by a more electron lucent cytoplasm with few organelles mainly free ribosomes and oval euchromatic nucleus (Fig. 7D). All cuts of the different specimens of the hepatopancreas and pancreas around the intestinal bulb had the same TEM findings.

The duct system of exocrine pancreatic tissue

A number of blood vessels with erythrocytes and pancreatic ductules were scattered in the pancreatic tissue (Fig. 8A). The duct system of the pancreas consisted of intralobular, interlobular pancre-

atic ducts and main duct. The intralobular pancreatic ducts were small (80-100 μm in diameter), present in the pancreatic lobules and consisted of a simple cuboidal epithelium surrounded by layers of collagenous and elastic fibers (Fig. 8A, B). The interlobular pancreatic ducts were larger (500-750 μm in diameter), present between the pancreatic lobules and the mucosa was thrown into mucosal folds (Fig. 8C, D). The wall of the interlobular duct consisted of simple columnar epithelium with numerous apical lipid droplets, goblet-like cells with basal nucleus, rodlet cells and small dark basal cells, and was surrounded by layers of collagenous fibers (Fig. 8E). The apical brush border of the epithelial cells of the interlobular duct gave strong Alcian blue- positive reaction (Fig. 8F). The main duct was the larger duct (1100-1400 μm in diameter), opened in the middle portion of intestinal bulb and surrounded by collagenous fibers (Fig. 8G). The main pancreatic duct was composed of a simple columnar epithelium with PAS- positive apical portion and some silver- positive argyrophilic cells (Fig. 8H, I).

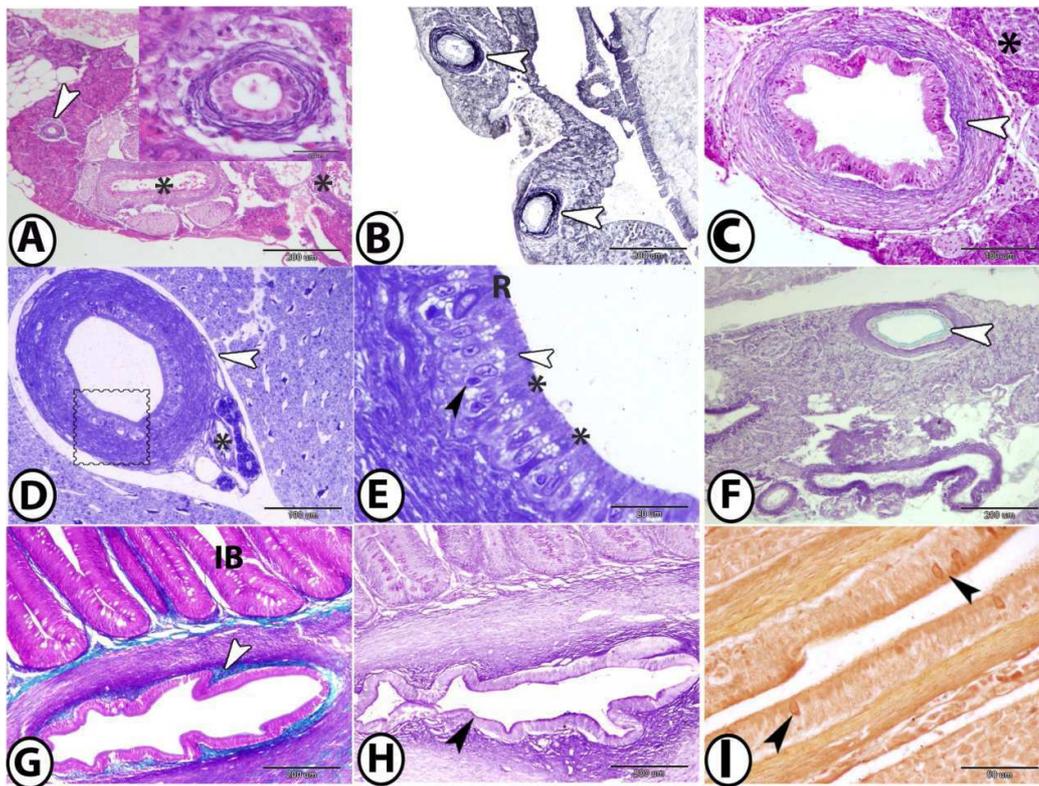


Fig. 8. The duct system of the exocrine pancreatic tissue. (A) Intralobular pancreatic duct (arrowhead & inserted figure) is lined by cuboidal cells surrounded by collagenous fibers. Note presence of many blood vessels (asterisks) in the pancreatic tissue (H&E). (B) Intralobular pancreatic duct surrounded by elastic fibers (arrowheads) stained by Verhoeff's staining. (C) Interlobular pancreatic duct (arrowhead) with folded mucosa located between pancreatic lobules (asterisk) (H&E). (D) Low magnification in semithin section of interlobular pancreatic duct (arrowhead) stained by Toluidine blue showing its organization between the pancreatic tissue (asterisk). (E) Higher magnification to the square in Fig. D showing the wall of interlobular duct stained by Toluidine blue indicates its epithelium that lined by simple columnar cells (white arrowhead), goblet-like cells (asterisks), basal cells (black arrowhead) and rodlet cells (R). (F) Interlobular duct showing Alcian Blue- positive brush border (arrowhead). (G) Main pancreatic duct surrounded by collagenous fibers (arrowhead) opened in intestinal bulb (IB) (Crossmon's trichrome). (H) Main pancreatic duct showing PAS-positive apical portions (arrowhead). (I) Epithelium of the main duct contained argyrophilic cells (arrowheads) stained by Grimelius silver method.

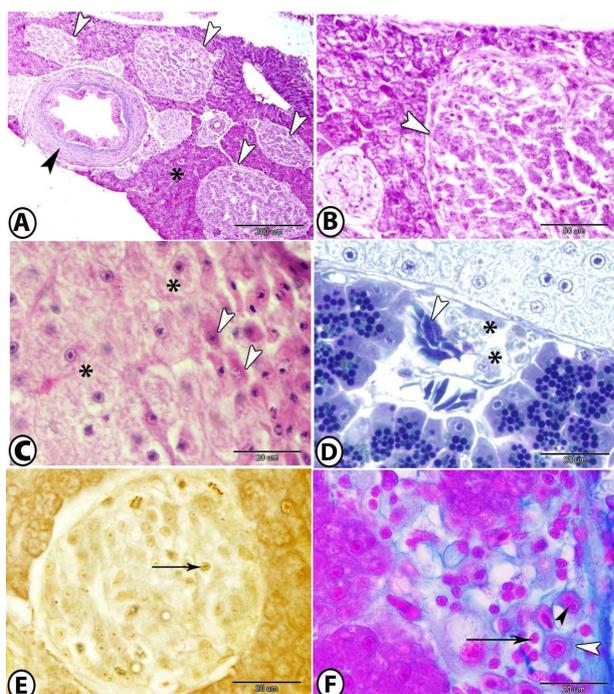


Fig. 9. Distribution of endocrine portion of the pancreas. **(A)** Langerhan's islets (white arrowheads) are organized between exocrine acinar cells (asterisk) and around pancreatic duct (black arrowhead) (H&E). **(B)** Islet is surrounded by thin connective tissue capsule (arrowhead) (H&E). **(C)** Oval shaped Alpha cells (arrowheads) and large polyhedral Beta cells (asterisks) (H&E). **(D)** Semithin sections of islet of Langerhan's showed oval shaped Alpha cells (arrowheads), large polyhedral Beta cells (asterisks) (Toluidine Blue). **(E)** Small argyrophilic Delta cells (arrow) are determined (Grimelius silver stain). **(F)** Cytoplasm of Alpha cells stain purple colour (black arrowhead), Beta cells stain violet blue (white arrowhead) and Delta cells stained light blue (arrow) by Maldonado's staining.

Endocrine parts of the pancreas were organized in the liver, around the intestinal bulb, and in mesentery of anterior part of the intestine, as a number of lightly staining Langerhan's islets of different sizes and shapes between exocrine acinar cells and around the pancreatic duct (Fig. 9A). Each islet was surrounded by a thin capsule of connective tissue, and consisted of three cell types that were arranged in the form of irregular cords and clumps (Fig. 9B). Alpha cells were the most dominant cells and most common on the periphery that appeared ovoid in shape with intense acidophilic cytoplasm. Their nuclei were large oval in shape with distinct nucleoli (Fig. 9C, D). Beta cells were large in size, polyhedral in shape with a granular cytoplasm and rounded eccentric nucleus and were grouped in small clusters (Fig. 9C, D). Delta cells were solitary and few in number (2-3 / islet), small in size, fusiform in shape and argyrophilic with silver stain (Fig. 9E). By Maldonado stain, the cytoplasm of Alpha cells stained purple in colour and the cytoplasm of Beta cells stained violet-blue, while the cytoplasm of Delta cells stained light blue (Fig. 9F).

DISCUSSION

The pancreas of fish is responsible for many vital functions. The present study revealed that the exocrine portion of pancreas of the grass carp had two forms, disseminated and intrahepatic. It consisted of closely packed pancreatic acini that exhibited the characters of protein secreting cells as it formed of pyramidal acinar cells with many zymogen granules and abundant rER. Similar finding were observed by Sheibani and Yali (2006) in *Huso huso* and Naguib et al. (2009) in *Schilbe mystus* and *Labeo niloticus*.

The zymogen granules contain proenzymes responsible for the digestion of protein, carbohydrates, fats and nucleotides (Alboghobeish and Khaksar-Mahabady, 2005). The acinar cells of the grass carp showed differences in staining activity of zymogen granules by toluidine blue, which indicates the presence of different enzymes within the granules. This is supported by enzyme histochemistry of the acini, which revealed different ratios of specific digestive enzymes, as they showed high lipase and alkaline phosphatase activity and moderate activity for acid phosphatase. It is well known that this fish has no stomach and the presence of these different active enzymes may help in food digestion. Also, the histochemical analysis of the pancreatic acini of the grass carp revealed presence of neutral mucopolysaccharides, glycogen and lipid, thus indicated high storage activity of these cells.

PSCs with its dendritic-like processes were observed between the pancreatic acinar cells of grass carp by scanning electron microscopy and by specific stains. PSC comprise 4-7% of the pancreatic cell mass (Apte et al., 1998). They have received several names such as fat storing cells, lipocytes, pericytes and parasinusoidal cells (Wake, 1980). They are detected ultrastructurally by the presence of multiple lipid droplets in their cytoplasm and are found in a wide variety of species, ranging from lampreys (primitive fish) to humans and have been observed in many tissues; including liver, adrenal gland, spleen, ductus efferent and uterus (Wake et al., 1987; Geerts, 2001). These cells expressed specific filamentous proteins such as desmin and vimentin, and myofibroblast marker α -smooth muscle actin (Yokoi et al., 1984; Zhao and Burt, 2007). Kordes et al. (2009) reported that PSC are Vit A storing cells and during injuries are transformed into an activated form that produces large amounts of fibronectin and laminin (extracellular matrix proteins), resulting in fibrosis. Shimizu et al. (2005) added that these cells have an immune function since it engulfs the damaged pancreatic parenchymal cells.

Rodlets cells were observed in the pancreatic acinar cells and the epithelium of pancreatic duct of grass carp. Bielek (2005) considered these cells

as migrating secretory cells. These may also be considered as non-specific immune cells, involved in immunity as their number is increased in parasitic infection (Reite, 2005). Reite and Evensen (2006) considered them a type of eosinophilic granulocyte. These cells observed in many organs of fish include heart, kidney, gills and gut. Mokhtar and Abd-Elhafeez (2014) confirmed the secretory function of these cells ultrastructurally by holocrine mode of secretion.

Melanomacrophage centers were detected in the exocrine portion of the pancreas. The number and contents of melanomacrophage centers differs according to species, health status and age (Agius, 1980). They usually concentrate lipofuscin, melanin, ceroid or hemosiderin pigments (Munshi and Dutta, 1996).

Both centroacinar and duct cells apparently secrete bicarbonate and water since carbonic anhydrase, the enzyme responsible for formation of bicarbonate, has been demonstrated in their epithelia by Sheibani and Yali (2006). The presence of goblet-like cells in the wall of the interlobular duct suggests an adaptation for protection against pathogens as these cells were also observed in common carp by Neuhaus et al. (2007). The strong PAS-positive apical portion of the main pancreatic duct indicated presence of neutral mucopolysaccharides materials and may suggest transport of molecules across the cell membrane. The main duct of the grass carp pancreas opened into the intestinal bulb and its wall contained argyrophilic cells that may be somatostatin or glucagon producing cells as these cells present in the related species *Cyprinus caprio* (Kong et al., 2002).

The endocrine portion of the grass carp pancreas was distributed around exocrine pancreatic tissue, in the liver, and around intestinal bulb and intestine. These descriptions resemble those given for islet aggregates in Salmoniformes (Wang et al., 1986). While in some species, the endocrine portion of the pancreas is found as an independent organ called Brockmann bodies that are located in mesentery adjacent to the spleen (Chiba et al., 1976). The "Brockmann body regions" from species such as tilapia (*Oreochromis niloticus*) are under investigation as a source of islet tissue for human transplant because of the compacted and pure nature of the "region" (Yang and Wright, 1995). The endocrine portion of grass carp was composed of A, B & D cells but in some species an additional Ff cells is present (Kobayashi et al., 1976). Immunohistochemical analysis to endocrine cells of the grass carp is recommended.

In conclusion, this study describes the histological, histochemical and ultrastructural features of the pancreas of grass carps (*Ctenopharyngodon idella*). Exocrine pancreatic cells were observed in two forms; disseminated in the spleen tissue, in mesentery around intestine and intestinal bulb and intrahepatically around branches of the portal vein.

Pancreatic stellate cells (PSC) were demonstrated in the perivascular and periacinar space of pancreas. The pancreatic acini gave positive reaction to PAS, Best's carmine, osmium tetroxide and negative to alcian blue. The acini also showed high lipase and alkaline phosphatase activity, and moderate activity for acid phosphatase. The duct system was composed of intralobular duct, interlobular pancreatic duct and main duct opened in the intestinal bulb. Langerhan's islets were consisted of three cell types: Alpha, Beta and Delta cells.

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