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The pancreas of the schilbeid catfish, *Clupisoma garua* (Hamilton, 1822): A histological and histochemical study

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Abstract

The objective of this study was to elucidate the cellular architecture of the pancreas in *Clupisoma garua*, a carnivorous river catfish, using light microscopy techniques. The pancreas was diffused in nature, structurally organized into two main components: exocrine serous acini, which produce digestive enzymes and the endocrine islets of Langerhans, which secrete peptide hormones. The pancreatic islets were scattered throughout the organ, each bounded by a thick connective tissue capsule and surrounded by blood capillaries, demonstrating the rich vascularity of the islets. The alpha, beta and delta cells of the islets were identified by their distinct architecture, positions and varying staining intensities. Zymogenic granules of the exocrine acinar cells exhibited an intense tryptophan reaction, reflecting the precursor of numerous pancreatic enzymes related to the fish's feeding habits. The pancreas played a dual physiological role, functioning both as an exocrine organ, with acinar cells responsible for digestion and as an endocrine organ, with islet tissues regulating glucose homeostasis and other vital physiological processes.

Keywords: Garuabachcha, function, morphohistology, pancreas, tryptophan

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Introduction

The pancreas is an endodermal organ composed of both exocrine and endocrine glandular tissue, which collectively produce digestive enzymes and hormones. Within teleosts, the pancreas exists as diffuse tissue, dispersed throughout the peritoneal cavity, intricately intertwined with mesenteries, blood vessels and various organs such as the liver, spleen, intestine and pyloric caeca. In the European eel (*Anguilla anguilla*), the pancreas assumes a compact structure, distinctly discernible as a separate organ (Buddington and Kuźmina, 2000). The exocrine pancreas is a compound gland comprising secretory acini, which synthesize digestive enzymes and store them within zymogen granules (Chakrabati and Ghosh, 2015). The endocrine pancreas is characterized by diffusely scattered pancreatic islets and isolated patches of cells, predominantly small in size, vascularized, interspersed among and within the exocrine acini (Youson *et al*., 2001). In teleost fishes, the cellular organization of the islets is highly variable and their association with the exocrine acinar cells is not always apparent. In jawed fishes, the islet cells evolved as cell masses, which in many bony fishes are bounded with exocrine pancreatic tissue in structures known as Brockmann bodies (Epple and Brinn, 1987). Numerous authors have described the immunocytochemical characterization of the endocrine pancreas in various fish species (Abad *et al*., 1987; Tagliafierro *et al*., 1996; Youson *et al*., 2006; Fortin *et al*., 2015; Hussein *et al*., 2023). Among fish species, four types of endocrine cells have been identified in the islets of Langerhans: A cells produce glucagon, B cells produce insulin, D

cells produce somatostatin and F cells produce pancreatic peptides. However, the population and localization of these cells vary considerably.

The distribution of glandular tissue, along with variations in the arrangement of secretory cells, in the pancreas of fish presents a captivating area of investigation. This exploration not only sheds light on the structural attributes of the gland but also offers valuable insights into the correlation between pancreatic architecture and its functional activity. In the present investigation, the microscopic anatomy of the pancreas in butter catfish, *Clupisoma garua* (Siluriformes; Ailiidae) is studied with a particular focus on delineating its specific location and detailed cytology of both islets of Langerhans and exocrine epithelial units. Inhabiting freshwater rivers, streams, canals and reservoirs, this bottom-dwelling fish species sustains itself by consuming a varied diet consisting of molluscs, crustaceans, shrimps, insects, algae and decaying organic matter. (Talwar and Jhingran, 1991; Akter *et al*., 2019).

Materials and methods

Collection of specimens

Adult specimens of *C. garua* (ranging from 18 ± 6.38 cm in total length; n = 16) were procured from the Bhagirathi-Hooghly river at Kalyani and its surrounding areas in West Bengal, utilizing traditional fishing gear throughout the year 2023. Following collection, the specimens underwent deep anesthesia with Benzocaine (4 mg/L), after which a mid-ventral incision was made to open the body cavity. Pancreatic tissues were then carefully excised from the stomach wall and subsequently prepared for the respective studies.

Preparation for histology

The pancreatic tissues were initially fixed by immersion in aqueous Bouin's fluid for approximately 18-24 hour. Subsequently, they underwent repeated washing in 70% ethanol to eliminate picric acid, followed by dehydration through a graded series of ethanol and clearing with xylene. The tissues were then infiltrated in paraffin wax of 56-58˚C using a thermostat controlled vacuum paraffin embedding bath for a duration of 1½ hour. Serial paraffin sections were cut at a thickness of 4 µm employing a rotary microtome (Weswox MT-1090A). Following standard histological procedures, the deparaffinized sections were stained with Mallory's triple (MT) (Mallory, 1936), Heidenhain's azan (HA) (Kiernan, 2008), Haematoxylin phloxine (HP) (Suvarna *et al*., 2019) and Aldehyde fuchsin (AF) (Halami, 1952) stains.

Preparation for histochemistry

Tissue samples were fixed in 10% neutral formalin for a duration of 16-18 hour. Following proper dehydration in an ascending series of ethanol and subsequent clearing in xylene, the tissues were embedded in paraffin wax of 52-54˚C. Paraffin blocks were then sectioned into slices with a thickness of 8 µm. These sections underwent the p-Dimethyl-amino-benzaldehyde (DMAB) - nitrate method for the detection of tryptophan, as described by Adams in 1957.

The stained slides were mounted with DPX, observed and photographed under a Zeiss Primo Star light microscope, equipped with a Tucsen 5.0 MP camera.

Results

The pancreas in *C. garua* (Fig. 1A) is not a separate, discrete organ. The pancreatic tissue is diffused and located in the mesentery that supports the esophagus, stomach, liver and intestinal loop, mostly confined to the anterior region (Figs. 1B-C). A significant portion of this tissue is attached to the distal portion of the stomach wall.

Histology

The pancreas is enveloped by loose fibrous tissue and consists of compositionally distinct exocrine and endocrine portions (Fig. 2A). The endocrine portions are interspersed among the exocrine acinar cells as small masses of varying sizes. The exocrine pancreas is a diffuse structure consisting of a branching network of ducts and associated acini (Figs. 2B-C). The serous acini contain pyramid-shaped acinar cells, organized in groups around a central lumen and fine blood capillaries (Fig. 2D). Each acinar cell features a basal homogeneous area with a large spherical nucleus and zymogen granules located at the apical ends of these cells, with the number of granules varying (Fig. 2E). The branches of exocrine tissue and their associated ducts form lobules, which are held together by loose connective tissue (Fig. 2D). The secretory products quit the acini via intralobular ducts, which are characterized by small lumina lined with low cuboidal epithelium (Figs. 2B-C). The pancreatic islets are encased in a thick connective tissue capsule, which separates them from the exocrine cells (Figs. 2E-F). Using special staining techniques such as Mallory's triple, Heidenhain's azan and Aldehyde fuchsin, three cell types are identified in each islet of Langerhans: alpha, beta and

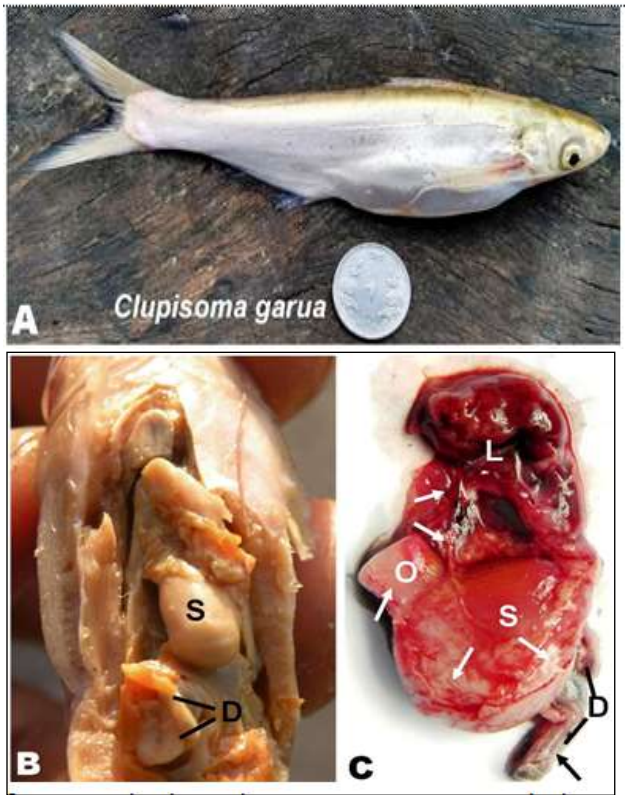

Figure 1. Distribution of pancreatic tissue in *C. garua*. **(A)** Lateral view of *Clupisoma garua*. **(B)** Dissected view shows the alimentary canal and the position of diffuse pancreas. S for stomach and D for duodenum. **(C)** The presence of pancreatic tissue (arrows) on the surface of oesophagous (O), stomach (S), lobes of liver (L) and within duodenal loop (D).

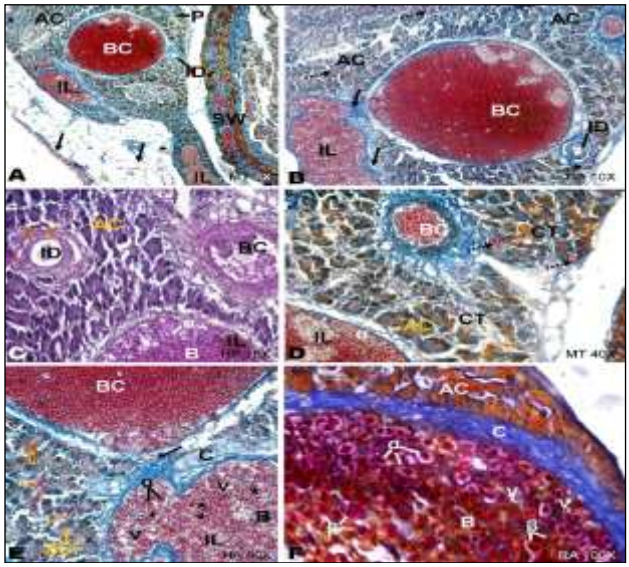

Figure 2. Photomicrographs of pancreatic sections in *C. garua*stained with Mallory's triple (MT), Heidenhain's azan (HA), Haematoxylinphloxine (HP) and Aldehyde fuchsin (AF) stain. (**A)** Pancreatic tissue (P), covered with connective tissue (arrows) and attached to stomach wall (SW) consists of clusters of acinar cells (AC) surrounding the islet of Langerhans (IL). Note the presence of blood capillaries (BC) and intralobular ducts (ID) in the PT. **(B)** IL is separated from exocrine AC by thick capsule (solid arrows). Notably, fine blood vessels (broken arrows) and ID are observed among acini. BC marks blood capillaries. **(C)** The Exocrine pancreas shows clusters of pyramidal AC, ID lined with cuboidal epithelium (arrow heads) and BC. The vascularized endocrine IL shows phloxinophilicα cells adjacent to blood cells (B). **(D)** Acinar cells (AC) are arranged into small lobules around a central lumen (L), surrounded by interlobular connective tissue septa (CT) containing blood vessels (broken arrows). Notably, α cells are present around the periphery of the islets of Langerhans (IL). BC for blood capillaries. (**E)** Acinar cells (AC) are characterized by diffused zymogen granules (ZG) and nuclei (N). IL covered by a connective tissue capsule (C) contain α cells, β cells (asterisks) and δ cells (arrow heads). Clusters of blood cells (B) form capillary network (solid arrow) with BC. Broken arrows indicate scattered blood vessels between the acinar cells. (**F)** A magnifiedview of pancreasshows IL separated from AC by connective tissue septa (C). IL contains α, β and δ cells (broken arrows) adjacent to B.

delta cells. Alpha cells are acidophilic and oval shaped with dark nuclei, primarily located around the islet periphery (Figs. 2C-F). Beta cells are predominant and concentrated more towards the center of the islet. They are polyhedral in shape and have large, rounded central nuclei. The nuclear membrane and nucleolus are distinct and well defined. These cells contain cytoplasmic ground substances and take up aldehyde fuschin stain (Fig. 2F). Delta cells are few in number and scattered throughout the islets (Figs. 2E-F). They exhibit variable shapes and have homogenous cytoplasm. The blood capillaries surrounding the endocrine cells highlight the rich vascularity of the islets.

Histochemical localization of tryptophan

The serous acini display a blue color, indicating positive reactions for tryptophan (Fig. 3A). The zymogenic granules of the acinar cells in the exocrine pancreatic tissue, considered to be secretory granules, show a strong tryptophan reaction (Fig. 3B). In contrast, the islet of Langerhans between the acini exhibit a very weak reaction. The blood cells in the capillaries show moderate tryptophan reaction.

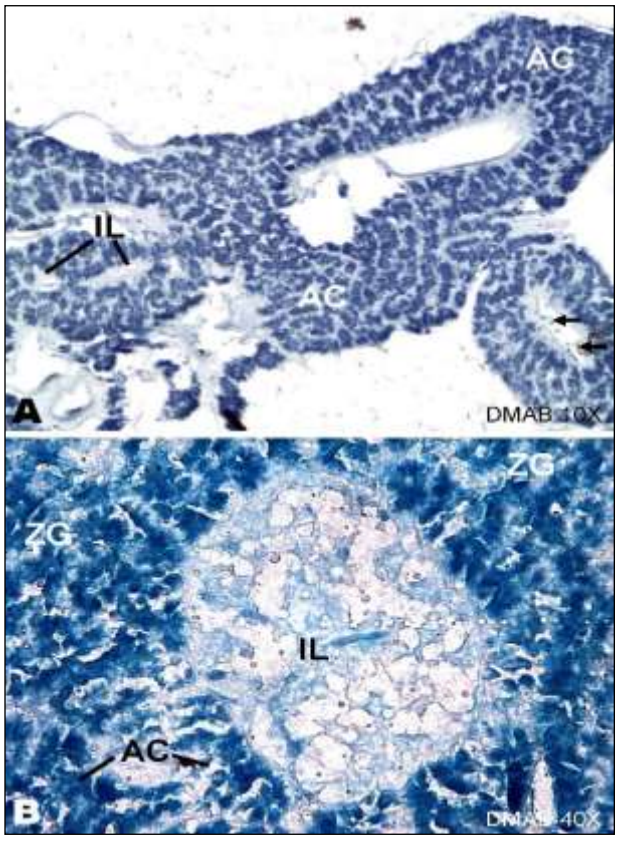

Figure 3. Photomicrographs of pancreatic sections in *C. garua* showing histochemical test for trypotophan (DMAB). **(A)** Displays strong tryptophan localization in the acinar cells (AC) of exocrine pancreatic tissue. Blood cells (arrows) show moderate reaction while the islet of Langerhans (IL) exhibit a weak reaction. **(B)** Shows acute tryptophan reaction in the zymogen granules (ZG) of AC and a weak reaction in IL.

Discussion

Fishes exhibit remarkable diversity in their food and feeding habits, resulting in consequent modifications to their alimentary canal and associated structures. In higher vertebrates, the pancreas assumes a compact and lobulated form whereas in teleosts, it disperses throughout the peritoneal cavity and shows a wide array of structural, morphological and anatomical variations. In the present study, the pancreas is observed as diffuse tissue in *C. garua*, intricately associated with mesenteries and other organs such as the oesophagus, stomach, liver and intestine. Gonzalez *et al*. (1993) documented the existence of a diffuse pancreas in *Serranus cabrilla*, which extends through the mesentery and forms islets within the connective tissue surrounding certain digestive organs. Alternatively, it disperses within the intraperitoneal adipose tissue. Similar findings have also been reported in *Paralichthys olivaceus* (Kurokawa and Suzuki, 1995), *Danio rerio* (Yee *et al*., 2005), as well as *Sperata aor* and *Chitala chitala* (Ghosh *et al*., 2016).

Conclusion

The pancreas in *C. garua* is made up of diffuse tissue that contains both exocrine and endocrine components. Digestion related enzymes are secreted by exocrine acinar cells, filled with zymogen granules, while hormones that control several metabolic processes are released by the endocrine tissue. Transmission electron microscopy and immunocytochemistry are required for further study on the pancreas of *C. garua* in order to identify the cellular components of the islets and ascertain their precise physiological significance.

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