Jurnal Kedokteran Hewan March 2023, 17(1):30-34
P-ISSN : 1978-225X; E-ISSN : 2502-5600 DOI: https://doi.org/10.21157/j.ked.hewan.v17i1.26884

HISTOMORPHOLOGY OF PANCREATIC TISSUE IN MEDAKA FISH (*Oryzias javanicus*): STUDY OF DIABETIC ANIMAL MODEL DEVELOPMENT

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ABSTRACT

This study aimed to explore the histomorphology of the pancreatic tissue of medaka fish to develop medaka fish as a diabetic animal model. Adult medaka fish were obtained with a body length of 2.53±0.22 cm and a weight of 265.8±25 g. Medaka fish were fixed using Boiun's fixative solution. Macro anatomical observations of the pancreas were conducted using a stereomicroscope. Histological preparations were made according to the Hematoxylin-eosin staining standard procedure. Pancreatic histomorphology of medaka fish was carried out using a light microscope with 100x and 400x magnification. The macro anatomy observations of the pancreas showed the interconnection with several tissues, such as spleen, gallbladder, liver, fat, and intestines. The pancreas was located toward the liver, oversees the intestines, and covered with fat. Pancreatic histomorphological observations showed exocrine cells composed of serous pyramidal acinar cells and endocrine cells (islets of Langerhans). Intralobular and intercalated ducts (ducts of Boll) were composed of simple cuboidal epithelium. This study concluded that the pancreas organ in medaka fish is a single organ, and histomorphologically has both of exocrine and endocrine cells.

Key words: histomorphology, Langerhans, medaka fish, oryzias, pancreas

ABSTRAK

Penelitian ini bertujuan mengeksplorasi histomorfologi pankreas ikan medaka dalam upaya pengembangan ikan medaka sebagai hewan model diabetes. Ikan medaka dewasa diperoleh dengan ukuran panjang tubuh 2,53±0,22 cm dan berat 265,8±25 g. Ikan medaka yang telah dinarkose dimasukkan ke dalam larutan fiksatif Boiun. Pengamatan makroanatomi pankreas menggunakan mikroskop stereo. Preparat histologi dibuat sesuai dengan prosedur dengan pewarnaan Hematoksilin-Eosin. Pengamatan histomorfologi pankreas ikan medaka dilakukan menggunakan mikroskop cahaya dengan pembesaran 100 dan 400. Hasil pengamatan makroanatomi diperoleh pankreas yang saling berasosiasi dengan beberapa jaringan yaitu limpa, kantung empedu, hati, lemak, dan usus. Pankreas terletak jauh kearah dalam hati, terikat pada bagian usus, dan tertutupi oleh lemak. Hasil pengamatan histomorfologi pankreas menunjukkan bahwa terdapat sel eksokrin yang tersusun atas sel asinar berbentuk piramidal serous dan sel endokrin (pulau Langerhans). Duktus intralobular dan duktus interkalar (Duktus Boll) terdiri atas epitel simpel kuboid. Studi ini menyimpulkan bahwa pankreas pada ikan medaka merupakan organ tunggal dan histomorfologi pankreas memiliki sel eksokrin dan sel endokrin.

Kata kunci: histomorfologi, Langerhans, ikan medaka, Langerhans, oryzias, pankreas

INTRODUCTION

The health problems that are still being faced in Indonesia and other parts of the world today are the high prevalence of infectious diseases and the increasing cases of diabetes mellitus, which is the fourth-ranked degenerative disease in the world that causes death (Handajani *et al.* 2012). Recently, the number of people with diabetes mellitus has increased. It is estimated that 537 million adults aged 20-79 years worldwide (10.5% of the world's population) suffer from diabetes. In 2021 in Indonesia, it was reported that 19.5 million people had diabetes and ranked fifth worldwide (International Diabetes Federation 2021).

The experimental use of animals, which developed widely in the 18th and 19th centuries, continues with certain rules and scientific grounds. According to one study, nearly 100 million animals, from fish to primates, were used for experimental purposes in 2010 (Greek and Greek 2003). In European countries, 93% use vertebrate animals as experimental animals (Taylor and Alvarez 2019). Rodents are one of the most common vertebrates used as experimental animals. Mice are one of the rodents used in this study because of their size, ease of use, and fast reproduction, and they have 95% gene similarities with humans (Pan *et al.* 2018). Other vertebrate groups were also used for some of the studies. For example, in 2016, about 500 thousand fish and 9,000 amphibians were used in research (Sadat and Nurunnabi 2012). Zebrafish (*Danio rerio*) and medaka (Oryzias) are both important model organisms in biomedical research (Lawrence *et al.* 2012).

Medaka fish (*Oryzias javanicus*) is one of Indonesia's endemic fish that has the potential and can be developed into animal models such as zebrafish (Sari *et al.* 2018). *O. javanicus* has the same characteristics as zebrafish, such as short growth cycles, transparent embryos, and rapid development (Zhu *et al.* 2018), and has potential as experimental animals with physiological and genetic qualifications as animal models (Furutani-Seiki and Wittbrodt 2004). Zebrafish and medaka fish have been used effectively for screening drugs, active substances, and natural ingredients (Khazaee and Ng 2018). Genes that regulate the same carbohydrates in mammals have been detected in zebrafish and medaka. In addition, the pancreas of medaka fish has a function similar to that

 Revised 07-03-2023, Accepted 13-03-2023

of mammals in terms of glucose homeostasis, including producing and secreting insulin, glucagon, somatostatin, and digestive enzymes such as amylase (Biemar *et al.* 2001). Despite its advantages, however, working with zebrafish requires handling skills to extract organs for histological purposes (Benchoula *et al.* 2019). Medaka fish have been selected as alternative animal models for various diseases, including diabetes. However, inducing type 1 diabetes in adult medaka fish varies depending on unpredictable factors. Thus, an optimized protocol for establishing type 1 diabetic medaka fish should be developed before testing the anti-diabetic activity of a potential treatment.

MATERIALS AND METHODS

The sample used in this study was ten adult *O. javanicus* sorted based on body length and weight criteria. *O. javanicus* was weighed using a digital scale, and body length was measured using a calliper. *O. javanicus* was reared based on the guidelines of the Institutional Animal Care and Use Committee (IACUC, 2018) and has ethical approval 029/KEH/SKE/IX/2022 from the animal ethics committee of the Schools of Veterinary and Biomedical, IPB University. The water circulation system in each pond was flowed continuously to the two aquariums according to the fish habitat with a 14-hour light and 10-hour dark cycle. Air quality was maintained at a conductivity of 800 µS, temperature 27-28° C, and pH 7.6-7.8.

Prior to anatomical and histological observation, medaka fish were anesthetized using cold water with a temperature of 0-5° C until signs of anesthesia were observed. Then the fish are transferred to the surgical board. Surgery was performed on the caudal fin and the abdominal organs were dissected with the stereomicroscope assistance. The abdominal cavity was presented in its entirety. The fish was then transferred to a solution of Bouin's fixative. The samples were stained using Hematoxylin-Eosin (HE) staining refers to the Kiernan (2008) method. Observation was carried out microscopically using an Olympus BX31 binocular microscope at 100x and 400x magnification to observe the pancreatic tissues of medaka fish. Data from anatomical and histological morphological observations of medaka fish pancreas were analyzed descriptively and qualitatively.

RESULTS AND DISCUSSION

Anatomy of Medaka Fish (*Oryzias javanicus*)

Ten adults medaka fish which was were observed in this study showed a relatively straight body profile from the head to the base of the dorsal fin and a convex belly from the head to the base of the anal fin. The morphology of *O. javanicus* in this study differed from other medaka species, which has a yellow stripe on the dorsal to the caudal fin. The anal fin in males has a copulatory organ characterized by a protrusion, transparent body with black spots, large eyes, seven fins consisting of pectoral fins, pelvic fins, anal fins, dorsal fins, and caudal fins (Figure 1A). The body length of the medaka fish was 28.12±0.95 mm, and the body weight was 0.18±0.02 g. Research conducted by Magtoon and Termvidchakorn (2009) described in detail the anatomy and morphology of *O. javanicus with* standard length is 22.2-30.8 (26.6±2.2) mm.

Anatomy of *O. javanicus* Pancreas

The macro anatomical observations showed that the pancreas of *O. javanicus* is a single organ (Figure 2B), while in other several types of fish, the pancreatic organ was found in association with liver tissue called hepatopancreas (Mokhtar 2015). The pancreas of *O. javanicus* located along the intestine, which has four lobes: the gallbladder lobe, spleen lobe, middle lobe, and ventral lobe. The gallbladder-spleen lobes wer scattered around the gallbladder and covered a small part of the spleen. The middle lobe was the most significant part of the pancreas, which was concentrated in the intestine. The ventral lobe was part of the gallbladder-spleen lobe that extends into the intestinal slits in thin ropes (Figure 2A).

The *O. javanius* pancreas has similarities with the pancreas of zebrafish (*Denio rerio*) (Chen *et al.* 2007; Kinkel and Prince 2009), humans and other mamals such as rats and mice (Dolenšek *et al.* 2015; Jennings *et al.* 2015). The pancreas of *O. javanicus* was well distributed in the intestinal mesentery, and surrounded by several tissues such as the intestine, spleen, gallbladder, and liver. The pancreas of *O. javanicus* was covered by fat, located deep into the clear and cloudy liver (Figure 2A, Figure 2B).

Histology of *O. javanicus* Pancreas

Histological analysis of the pancreas of *O. javanicus* was performed using hematoxylin and eosin staining. The results of histomorphological observations of the *O. javanicus* pancreas were presented in the form of photomicrographs. Observations showed that *O. javanicus* consisted of exocrine cells, endocrine cells, and several supporting components in the pancreas such as ducts, connective tissue, fat, and blood vessels.

Exocrine cells in the pancreas of *O. javanicus* consisted of serous pyramidal acinar cells with a grape-like structure. These cells are the constituent structures of the pancreas and other organs that produce certain proteins in mammals and some other animals (Figure 3C, Figure 3E). Exocrine cells in the pancreas of medaka fish are very similar to those of mammals (Wang *et al.* 2020) and consist of basophilic cytoplasmic cells (Menke *et al.* 2011). Serous pyramidal secretory cells with grape-like structures were known as acini. Pancreatic acini are tissues that produce digestive enzymes in the small intestine (Hall and Hall 2021). Endocrine cells of *O. javanicus* consisted of clusters of cells known as the islets of Langerhans, which consist of oval-shaped cells with a rounded nucleus. These cells were arranged in irregular cords or clumps around capillaries and separated from acinar tissue by a thin layer of connective tissue (Figure 3C). Islets of Langerhans on hematoxylin-eosin

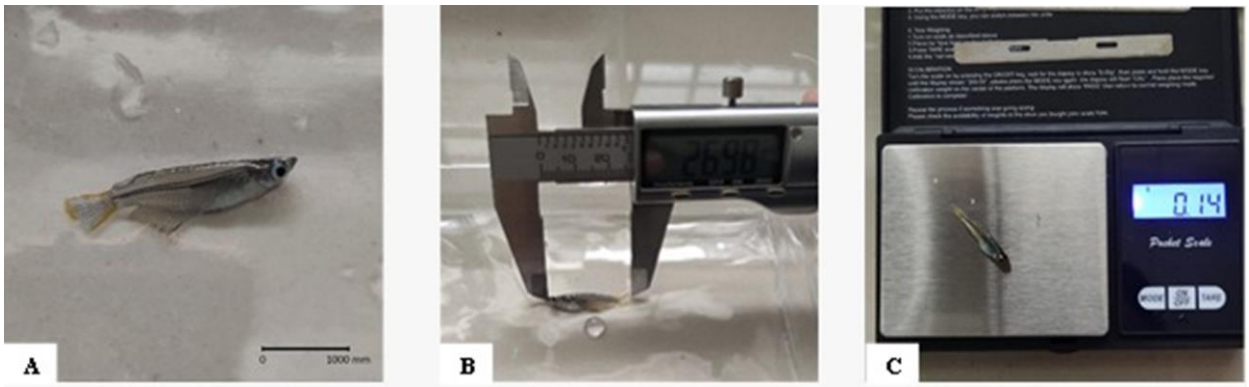

Figure 1. Anatomy and morphology observation of *O. javanicus*. A= *Oryzias javanicus* (bar: 1000 mm), B= Body length measurement *Oryzias javanicus*, C= Weighing *Oryzias javanicus*

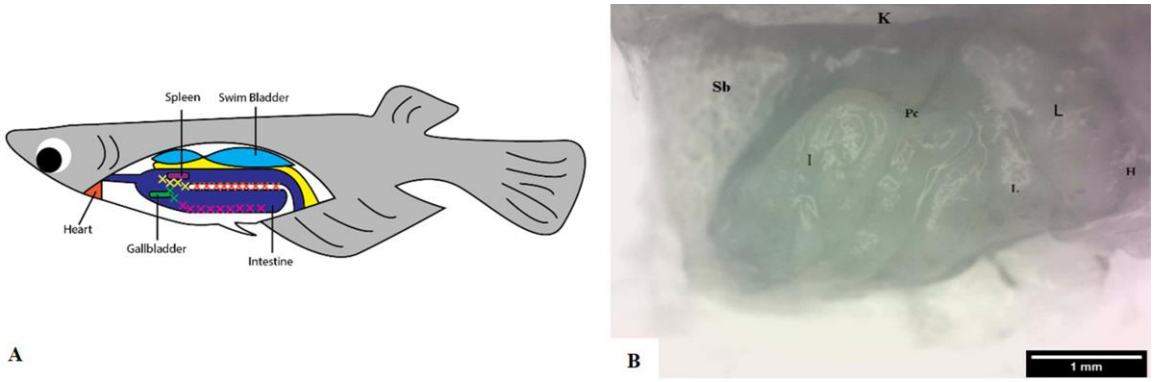

Figure 2. Anatomy of the pancreas. A= Illustration of the location of the pancreas of *Oryzias javanicus* marked with an X symbol, lobes of the gallbladder (green); spleen lobe (yellow); Middle lobe (red); ventral lobe (pink). B= Morphology and anatomy of the abdominal organs in *Oryzias javanicus*, Sb= Swim bladder, K= Kidney, Pc= Pancreas, I= Intestine, L= Liver, H= Heart. Scale bars 1 mm

staining were visible compared to acinar cells (Figure 3F). Qadori (2011) observed that the cytoplasm on the islets of Langerhans was more basophilic so that it looks clearer than acinar cells, which were acidophilic. The observed pancreas of *O. javanicus* consists of two main parts of the pancreas: (1) the islets of Langerhans located in the dorsal part of the abdominal cavity associated with several organs such as the gallbladder, intestines, and fat which are commonly referred to as the Brockman body (Figure 3A, Figure 3B, Figure 3C); (2) numerous small accessory islets, varying in size and shape, located in the gallbladder area and scattered throughout the body cavity among the exocrine tissues within the mesentery connecting the stomach, liver, liver, and gallbladder, and along the common bile duct (choledochal duct) (Figure 4C).

Main islets of Langerhans (Brockman body) *O. javanicus* were seen as white nodules, separated from exocrine tissue and surrounded by exocrine cells. A group of Langerhan was surrounded and penetrated to varying degrees by exocrine cells and connective tissue (Figure 4A, Figure 4B). The islets of Langerhans have a capsule of fine fibrous connective tissue, and each lobe of the pancreas contains an intralobular duct with simple cuboidal epithelial structures connecting the lobes, as well as intercalated ducts (ducts of Boll) with cuboidal epithelial structures that extend from exocrine cells to striated ducts (Figure 4D, Figure 4E, Figure 4F). Pancreatic tissues were stained using HE to observe the histomorphology of *O. javanicus* pancreas. The pancreas of *O. javanicus* consist of exocrine (acinar cells) and endocrine (Langerhans islets) compartments (Figure 3C, Figure 3D, Figure 3E, Figure 3F). It was reported that each compartment has specific function (Otsuka *et al.* 2015). The hematoxylin-eosin staining is an acid-base reaction (Dey 2018). Hematoxylin is a cationic dye which stain the nuclei a bluish-black color. The anionic dye eosin was used as a cytoplasmic dye with a pink color in cytoplasm (Meschel 2012). The β cells, α cells, and δ cells in pancreatic tissues of medaka fish was stained differently using HE staining. Pancreatic β cells was observed with dark blue colour, while α and δ cells were showed paler color in their cytoplasm (Figure 4A). It's mean that cytoplasm of β cells more acidophilic than other cells. The histomorphological pancreatic tissues in *O. javanicus* was similar with the humans (Meschel 2012), and other mamals such as rats (Wresdiyati *et al.* 2016).

Insulin in fish is involved in glucose oxidation, fat synthesis, glucose transportation, and glucose uptake by liver and muscle cells. Glucagon functions in breaking down glycogen and fat in the liver (Rahardjo *et al.* 2011). In a study conducted by Palazón-Fernández *et al.* (2011) were able to identify four islets of Langerhans cells in fish in the form of α cells (glucagon), β cells (insulin), δ cells (somatostatin), and pancreatic polypeptide (PP or F cells) using immunohistochemical methods.

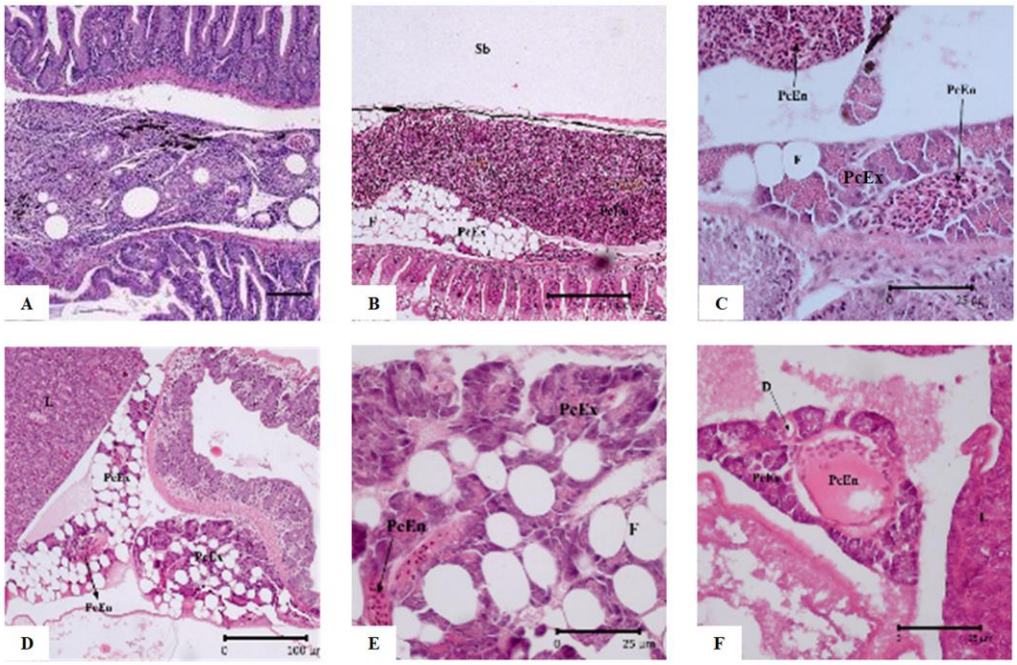

Figure 3. Histomorphology of *Oryzias javanicus* pancreas. A-F= Sagittal section with hematoxylin-eosin staining. Sb= Swim bladder, PcEn= Pancreatic endocrine cells/islet of Langerhans, PcEx= Exocrine cells/acinar cells, I= Intestines, L= Liver, F= Fat (F), D= Intralobular tract. The main A-C islets of Langerhans. D-F islets of Langerhans with smaller and varied sizes are scattered throughout the pancreatic tissue among the exocrine tissues. A scale bar 250 µm, B scale bar 100 µm, C scale bar 25 µm, D scale bar 100 µm, E-F scale bar 25 µm

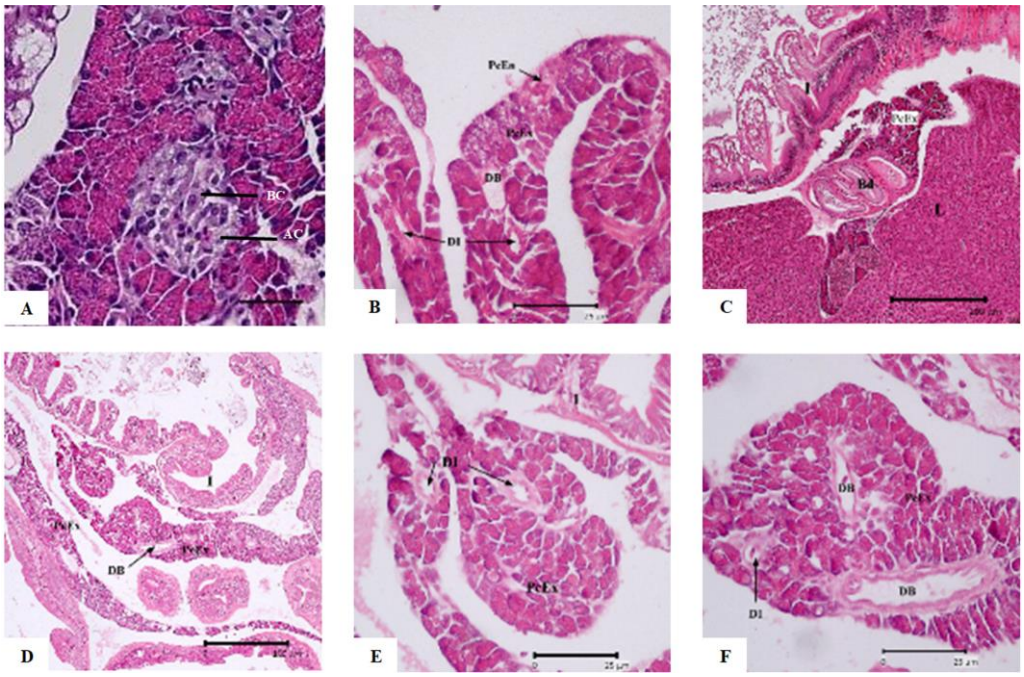

Figure 4. Histomorphology of *Oryzias javanicus* pancreas. A-F= The coronal section with hematoxylin-eosin staining. I= Intestines, PcEx= Exocrine cells/acinar cells, PcEn= Endocrine cells/islet of Langerhans, DI= Intercalary ducts, DB= Intercalary ducts/ ducts of Boll, L= Liver, BD= Bile ducts. C. pancreas associated with bile ducts. D-F intralobular duct with a simple cuboidal epithelial structure. D-F intercalary ducts (B ducts of Boll) with simple cuboidal epithelium extending from exocrine cells leading to striated ducts. A scale bar 250 µm, B scale bar 25 µm, C scale bar 100 µm, D scale bar 100 µm, E-F scale bar 25 µm

CONCLUSION

The Pancreas of *O. javanicus* is a single organ near the duodenum, spleen, gallbladder, and liver. The histomorphology of the pancreas of *O. javanicus* consists of two compartments, exocrine cells (acinar cells) and endocrine cells (islets of Langerhans). The Langerhans islet has β cells, α cells and δ cells. Based on the anatomy, histomorphology, and physiology of the pancreas, *O. javanicus* suggested can be used as an animal model for diabetic mellitus.

ACKNOWLEDGEMENT

The author would like to thank the Directorate General of Higher Education, Ministry of Education

and Culture, Republic of Indonesia who has funded this research through a master thesis research scheme in 2022. Contract Number 082/E5/PG.02.00.PT/2022.

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