

Anatomical and Histomorphological Study of Spleen and Pancreas in Berzem (*Barbus pectoralis*)

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Abstract: Berzem; *Barbus pectoralis* (Cyprinidae) inhabiting Karoon River (Southwest Iran). There wasn't any available report on anatomical and histological analysis of *B. pectoralis*. So, in this study, the morphologic and microscopic features of spleen as the important lymphatic organ on immune system of *B. pectoralis* were recognized. A total number of 10 young fish freshly prepared from Karoon River in Khuzestan. After removing the spleen, it was immediately fixed in Bouin's solution and transport to the laboratory. The 5-6 µm sections were made using paraffin embedding techniques and stained by Haematoxylin and Eosin (HandE). The spleen as an elongated organ located on the middle part of digestive canal and microscopically was covered by a capsule of connective tissue and an epithelial layer. Spleen included white and red pulps. There are some similarities and some differences between spleen of this species and mammals. Spleen as the biggest and the most important lymphatic organ and microscopically very similar to spleen of mammals has many functions such as lymphatic cell production. Histomorphology of spleen and pancreas show that in *B. pectoralis*, exocrine part of pancreas is organized inside spleen and like some fishes, splenopancreas structure is present.

Key words: *Barbus pectoralis* % Anatomy % Histomorphology % Splenopancreas

INTRODUCTION

The spleen is usually a solitary, dark red organ in the peritoneal cavity adjacent to the gut wall. The same basic elements as in higher vertebrates are typically present: blood vessels, red and white pulps and ellipsoids [1].

In teleost fish, the immune organs consist of the spleen, pronephros and thymus. With regard to the tissues sampled, the spleen represents suitable choice for examining pathological changes in vivo because it is relatively easy tissue to sample as well as important lymphoid organ in fishes [2].

Since teleost fish have no modularly cavity in their bones, the spleen and kidney serve as the primary haemopoietic organs [3]. As fish have no lymph nodes, the

spleen alone plays an essential role in antigen trapping [4]. The spleen is the major peripheral lymphoid organ [2].

The spleen is covered by a thin fibrous capsule with little evidence of contractile ability [1]. Red pulp is an extensive, interconnecting system of splenic cords and sinusoid capillaries. White pulp, consisting mainly of lymphoid cells typically surrounds arterial vessels, melanomacrophage centers [1].

The exocrine pancreas is a diffuse organ in teleosts. In some species, the exocrine pancreas surrounding the portal vessels extends into the liver and occasionally the spleen. The islets of Langerhans are usually situated within the bounds of the exocrine pancreas, but not within the hepatopancreas. In some species, e.g. dab, *Limanda*

limanda, cod, *Gadus morhua*, wolffish *Anarkichas lupus*, islets are very large and can be seen macroscopically in adult fish [5].

Berzem; *Barbus pectoralis* (Cyprinidae) inhabiting Karoon River (Southwest Iran). The biology of *Barbus* has been widely investigated worldwide [6, 7]. There wasn't any available report on anatomy and histology analysis of *B. pectoralis*. Therefore an attempt has been made to study of macroscopic and microscopic of spleen of *B. pectoralis* and is the first to present complete of spleen based on observation by macroscopic and light microscopic. Conclusively this fish is a good species for aquaculture. Therefore, the present study was performed to investigate the anatomy and histology differences of the spleen in the *B. pectoralis* and to compare it to other fishes.

MATERIALS AND METHODS

A total number of 10 young fish freshly (mean std. total length \pm SD = 31 ± 2.87 cm, mean std. standard length 26.75 ± 2.64 cm, mean body mass 258.5 ± 75.83 g) prepared from Karoon River. Total and standard length of fishes was measured accuracy of (1 mm) and their weight was recorded in accuracy of (0.01 g) [8]. Fishes were anesthetized with overdose of MS222 and then by an incision on the abdominal wall the spleen was removed and the samples were collected with maximum 0.5cm thickness and were fixed in Bouin's solution. Fixed samples were dehydrated in aggraded series of alcohols, cleared in xylene, embedded in paraffin and cut with microtome at 5-6 μ m. Sections were mounted on glass slides, deparaffinized and stained by Haematoxylin and Eosin (H and E) [9]. The studies were observed under light microscope.

RESULTS

The spleen of Berzem; *B. pectoralis* is a reddish-brown and as an elongated organ located on the middle part of digestive canal. The spleen was located caudally to the liver, ventrally to the swim bladder, dorsally to the medium caudal portion biliary, dorsoventrally flattened and it presented irregular surface (Fig. 1). The results showed that the spleen has a capsule and a small trabeculae extent into the parenchyma which can be divided into a red and white pulp as in other vertebrates. The capsule composed of one layer including an epithelium of squamous to cuboidal cells with some small

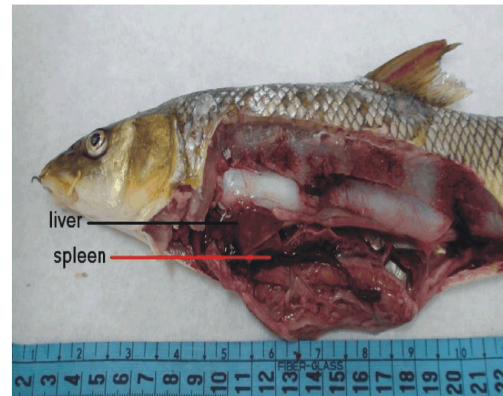


Fig. 1: *B. pectoralis*. Abdominal cavity opened and organs such as liver and spleen were observed.



Fig. 2: Histological picture of spleen in *B. pectoralis* (H and E, X10): Sp: spleen; capsule (arrowhead).

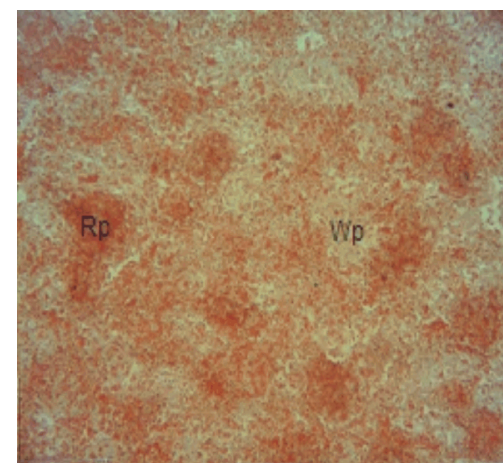


Fig. 3: Histological picture of spleen in *B. pectoralis* (H and E, X10) showing the nodular arrangement of the germinal nodes (white pulp) and stroma of connective tissue with red blood cells (red pulp). Wp: white pulp; Rp: red pulp.



Fig. 4: Histological picture of spleen in *B. pectoralis* (H and E, X40) showing the nodular arrangement of the germinal nodes (white pulp) and stroma of connective tissue with red blood cells (red pulp). Wp: white pulp; Rp: red pulp; capsule (arrowhead).

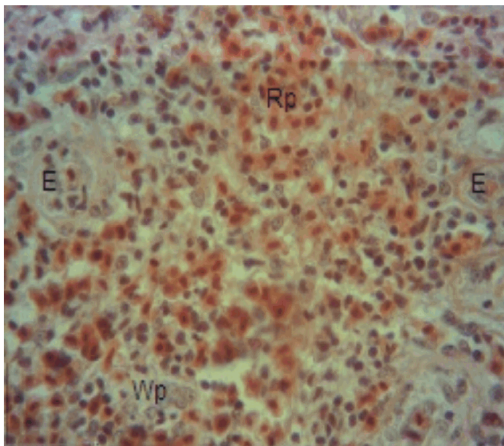


Fig. 5: Histological picture of spleen in *B. pectoralis* (H and E, X40) showing the nodular arrangement of the germinal nodes (white pulp) and stroma of connective tissue with red blood cells (red pulp): Wp: white pulp; Rp: red pulp; E: ellipsoid.

round secretory cells (Figs. 2, 4). Under which a waving layer of elastic fibers and then a condensed connective tissue were located. White pulps consisted of a lymphatic nodule, like that of mammals, but also an ellipsoid capillary branch, unlike mammals that differ from this aspect (Figs. 3,4,5). The ellipsoids are terminations of arterioles with a narrow lumen that run through a sheath of reticular fibers, reticular cells and macrophages (Figs. 5, 6). The red pulp which may occupy the majority of the organ is containing too many sinusoids filled with

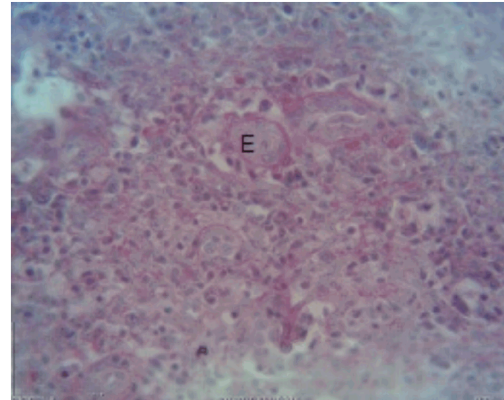


Fig. 6: Histological picture of spleen in *B. pectoralis* (H and E, X 40): E: ellipsoid

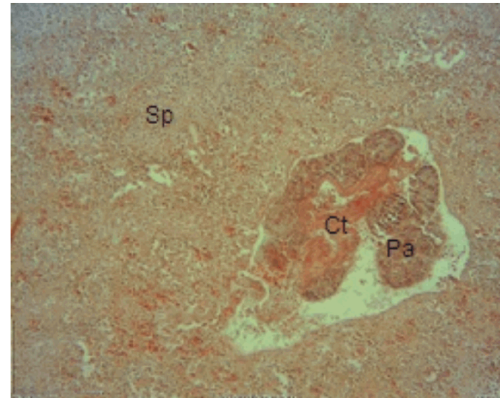


Fig. 7: Histological picture of spleen in *B. pectoralis* (H and E, X40): Sp: spleen; Pa: pancreas; Ct: connective tissue

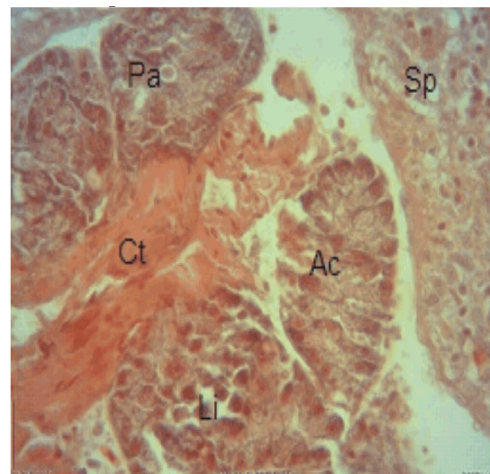


Fig. 8: Histological picture of pancreas in *B. pectoralis* (H and E, X40): Sp: spleen; Pa: pancreas; Ac: acini of pancreas; Li: langerhanse islets; Ct: connective tissue

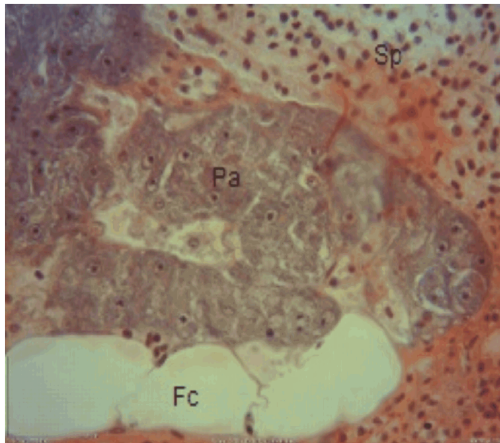


Fig. 9: Histological picture of pancreas in *B. pectoralis* (H and E, X40): Sp: spleen; Pa: pancreas; Fc: Fat cell

red blood cells surrounded by some trabeculae and diffused lymphatic tissues throughout the red pulp of spleen (Figs. 3, 4, 5).

In *B. pectoralis* the exocrine pancreas is a compound acinar gland, with secretory units or acini scattered within spleen. Each acinus consists of a single layer of pyramidal broad based cells that rest on a basal lamina. The nucleus of the acinar cell lies towards the base of the cell and the basophilic cytoplasm towards the lumen of the acinus contains zymogen granules that are acidophilic (Figs. 7, 8, 9). The endocrine components of the pancreas, the islets of Langerhans, are enclosed in a thin capsule and consist of poorly stained cords of cells with large distinct nuclei, interspersed with, blood sinuses (Fig. 8).

DISCUSSION

The spleen of *B. pectoralis* is a reddish-brown and as an elongated organ located on the middle part of digestive canal as same as other fishes [10]. The spleen was located caudally to the liver, ventrally to the swim bladder, dorsally to the medium caudal portion biliary, dorsoventrally flattened and it presented irregular surface. In lungfish (dipnoans) the spleen is enclosed within the stomach wall and the spiral valve of the intestine. In holocephalans (rabbit fish, *chimaera*) the spleen is associated with the pancreas and is lying free in the peritoneal cavity [11]. In teleosts the spleen is usually smaller than in holocephalans and elasmobranchs and in teleosts the structure of spleen varies very much [11].

In present study, the spleen in *B. pectoralis* has a capsule and a small trabeculae extent into the parenchyma which can be divided into a red and white pulp as in other

vertebrates. The capsule composed of one layer including an epithelium of squamous to cuboidal cells with some small round secretory cells while in the *Acipenser persicus* capsule composed of three layers including an epithelium of cuboidal to columnar cells with some small round secretory cells with eosinophilic granules [10]. Also, in the *Acipenser persicus* under the capsule a dense lymphatic tissue containing some melanomacrophages was observed while in *B. pectoralis* was not observed. Under which a waving layer of elastic fibers and then a condensed connective tissue were located. White pulps consisted of a lymphatic nodule, like that of mammals, but also an ellipsoid capillary, unlike mammals that differ from this aspect.

The red pulp contained too many sinusoids filled with red blood cells surrounded by some trabeculae and diffused lymphatic tissues throughout the red pulp of spleen. In present study, red pulp of *B. pectoralis* similar to *Huso huso* may occupy the majority of the organ [12].

In non salmonid species, aggregations of closely packed melanomacrophages form melanomacrophage centers, which may be bound by a thin argyrophilic capsule, surrounded by white pulp and associated with thin-walled, narrow blood vessels [3, 13]. However in salmonids, the accumulations of melanomacrophages are less well-defined and lack a capsule, but the association with blood vessels and lymphocytes is retained [4]. In present study, melanomacrophage in young *B. pectoralis* was not observed.

The ellipsoids are terminations of arterioles with a narrow lumen that run through a sheath of reticular fibers, reticular cells and macrophages [14]. Ellipsoids appear to have a specialized function for plasma filtration and the trapping of blood-born substances, particularly immune complex [15, 16].

In *B. pectoralis* the exocrine pancreas is a compound acinar gland, with secretory units or acini scattered within spleen. Each acinus consists of a single layer of pyramidal broad based cells that rest on a basal lamina. The nucleus of the acinar cell lies towards the base of the cell and the basophilic cytoplasm towards the lumen of the acinus contains zymogen granules that are acidophilic. The endocrine components of the pancreas, the islets of Langerhans, are enclosed in a thin capsule and consist of poorly stained cords of cells with large distinct nuclei, interspersed with, blood sinuses. All vertebrate islets except those in cyclostome have three functionally independent cell type, alpha cells producing glucagon, beta cells producing insulin and delta cells. The islets are ductless glands [17].

The pancreas of teleost fish, amphibians and reptiles is similar to that found in mammals, but two major differences are observed in some species. Many fish and some amphibians and reptiles, possess a pancreas that has an intimate association and admixture of cells, with the spleen or liver. This combined organ is termed a splenopancreas or hepatopancreas, respectively [17]. In our previous study, we showed that pancreatic tissue in *cetenopharyngodon idella* gradually invades the liver along the branches of the portal vein. The combined hepatic and pancreatic tissue are collectively called hepatopancreas [18].

In conclusion, the important morphologic characteristics of the spleen studied in the *B. pectoralis* for the first time and showed some similarities and some differences between spleen of this species and mammals. Spleen as the biggest and the most important lymphatic organ and microscopically very similar to spleen of mammals has many function such as lymphatic cell production. Histomorphology of spleen and pancreas that in *B. pectoralis* for the first time show that exocrine part of pancreas is organized inside spleen and like some fish, splenopancreas structure is present.

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