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Intrahepatic- Vascular- Biliary Tract in Hepatopancreas and Liver of Some Teleost Fish in Tigris River (Baghdad, Iraq)

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ABSTRACT

Variations have been detected in the liver of *Barbus luteus*. The liver is surrounded by a thin capsule consisting of connective tissue, with no distinction of lobules or interlobular septa. The hepatocyte–sinusoidal structure appears in a tubular form, and the parenchymal tissue consists of polyhedral hepatocytes arranged in layers. The sinusoids are relatively short, narrow, and tortuous. The exocrine pancreatic tissue is disseminated within the liver parenchyma, forming the hepatopancreas, and is also distributed around the digestive tract. The intrahepatic vascular–biliary tract contains pancreatic tissue, forming several distinct patterns: P-VBAT (pancreatic–venous–biliary–arteriolar tract), P-VAT (pancreatic–venous–arteriolar tract), P-VBT (pancreatic–venous–biliary tract), and P-VT (pancreatic–venous tract). In contrast, the hepatocyte–sinusoidal structure of *Chondrostoma regium* is solid in form, with hepatocytes appearing spherical in shape. Unlike *Barbus luteus*, the presence of pancreatic tissue has not been observed in this species. Consequently, the intrahepatic vascular–biliary tract forms VBAT (venous–biliary–arteriolar tract), VAT (venous–arteriolar tract), VBT (venous–biliary tract), and VT (venous tract). In conclusion, the histological study of the liver and pancreas of the red barb and king barb in Baghdad revealed distinct structural characteristics of these organs in the two fish species. This research provides valuable insights not only into their economic importance in Iraq but also into their potential use in hepatotoxicity studies.

INTRODUCTION

The red barb (*Barbus luteus*) belongs to the class Osteichthyes, family Cyprinidae **(Ghazwan, 2023)**. It is an omnivorous fish **(Maktoof, 2013; Al-Faisal *et al.*, 2023)**. On the other hand, the king barb (*Chondrostoma regium*) also belongs to the family Cyprinidae and has a planktivorous diet **(Ölmez, 2023)**.

The hepatopancreas has various metabolic roles, such as the synthesis of digestive enzymes (alkaline-protease, cellulose, and trypsin enzymes), absorption of digested nutrients, storage of minerals, production of organic materials, and metabolism of carbohydrates and fats **(Kibenge & Strange, 2021)**. The liver is one of the largest internal organs in the body and plays a crucial role in processing and storing nutrients absorbed from the digestive tract. It also has metabolic functions, including protein synthesis, storage of metabolic substances, secretion of bile, and detoxification. The liver is surrounded by a thin connective tissue capsule, called Glisson's capsule, and is supplied with blood by the hepatic artery, which provides oxygenated blood, and the portal vein, which carries blood from the gastrointestinal tract, spleen, and pancreas **(Weber *et al.*, 2020)**.

The liver of teleost fish, including the red barb and king barb, is located in the anterior part of the abdominal cavity and can be either unilobed or multilobed **(Kadhim *et al.*, 2020; Al-Faisal *et al.*, 2023)**. Unlike mammals, fish do not have a separate pancreas but instead possess scattered pancreatic tissue along the gastrointestinal tract **(Luchini *et al.*, 2015)**.

The pancreatic tissue in fish extends along the mesenteric blood vessels, near the bile ducts, around the gallbladder, or between the caeca. The fish pancreas consists of an exocrine part, which secretes digestive enzymes, and an endocrine part, which secretes insulin and glucagon. The pancreas containing pancreatic tissue outside the liver is called the hepatopancreas, while the pancreas located within the spleen is referred to as the spleenopancreas **(Kadhim *et al.*, 2020)**.

In fish, the digestive system shows diverse anatomical structures and functions, which correlate with taxonomy, feeding behaviors, and body shape **(Okuthe & Bhomela, 2020)**. The morphological features of the digestive system provide insights into species activity within their ecosystems and serve as bioindicators of environmental change **(Akoul, 2019)**.

The importance of this research lies in the economic value of *Barbus luteus* and *Chondrostoma regium* and the lack of histological studies on the liver of these fish species in Baghdad, Iraq, where geographical variations may influence the characteristics of the same species. Therefore, this study was conducted to highlight the economic importance of these two fish.

MATERIALS AND METHODS

Samples were collected from the Tigris River in Baghdad between April and June 2023, with fish of varying weights and lengths. The live specimens were transported to the laboratory, where they were dissected, and sections of the liver and pancreas were taken. The samples were fixed in Bouin's solution for 24 hours, washed with 70% ethanol to remove the fixative, and prepared for histological sectioning.

Dehydration was carried out using a graded ethanol series (70, 80, 90, 95, and 100%), with each step lasting 45 minutes. The samples were then cleared with xylene for 45 minutes to 1 hour, infiltrated with paraffin, and sectioned using a microtome. The sections were stained with hematoxylin–eosin and examined under a microscope **(Mohsin & Hameed, 2016)**. Images were captured using an Olympus camera attached to an Olympus BH2 microscope in the imaging laboratory of the Department of Life Sciences, College of Science for Women, University of Baghdad.

RESULTS

The results showed that the liver of the red barb was surrounded by a capsule composed of connective tissue, with no evidence of lobules or interlobular septa within the hepatic parenchyma (Fig. 1). The hepatocyte–sinusoidal structure was tubular in form, with hepatocytes arranged in multiple layers surrounded by narrow and tortuous sinusoids. The bile duct, hepatic artery, and portal vein were clearly observed in the portal area (Fig. 2). Exocrine pancreatic tissue was found along the main portal veins and within the adipose tissue surrounding the digestive tract. The intrahepatic vascular–biliary tract contained pancreatic tissue, forming distinct structural patterns: P-VBAT (pancreatic–venous–biliary–arteriolar tract), P-VAT (pancreatic–venous–arteriolar tract), P-VBT (pancreatic–venous–biliary tract), and P-VT (pancreatic–venous tract). The pancreatic tissue consisted of acini, each composed of multiple pancreatic cells containing zymogen granules (Figs. 3–5).

In contrast, the liver of the king barb was surrounded by a very thin capsule composed of connective tissue, and lobules or interlobular septa were absent within the hepatic parenchyma (Fig. 6). The hepatocyte–sinusoidal structure was solid in form, with hepatocytes arranged in a spherical shape containing fat droplets (Fig. 7). Pancreatic tissue was not observed in this species. Consequently, the intrahepatic vascular–biliary tract exhibited four main structural forms: VBAT (venous–biliary–arteriolar tract), VAT (venous–arteriolar tract), VBT (venous–biliary tract), and VT (venous tract) (Figs. 8–10).

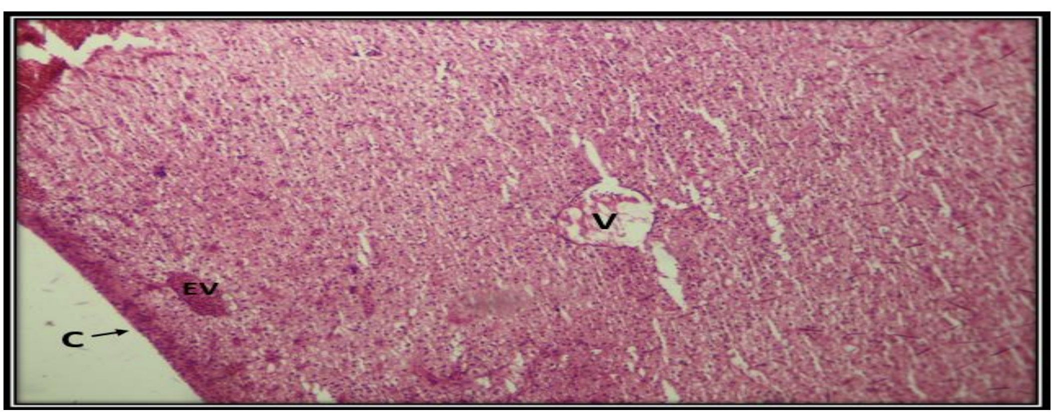

Fig. 1. Liver of *Barbus luteus*: C (capsue), EV (efferent vein), V (afferent vein). X10. (H&E)

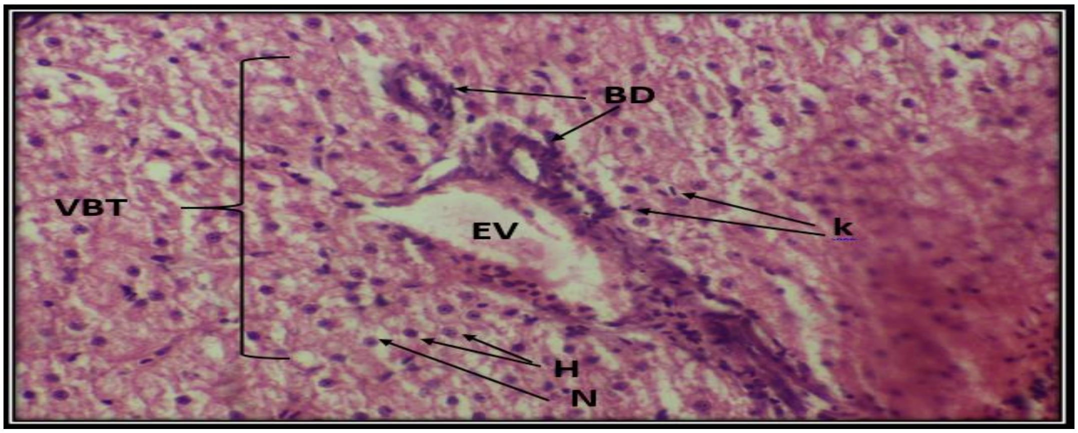

Fig. 2. Liver of *Barbus luteus* illustrating venous-biliary tract ; BD (bile duct), EV (efferent vein), H (hepatocyte), K(kupffer cell), N (nucleus), V (afferent vein), VBT (venous-biliary tract) X40 (H&E)

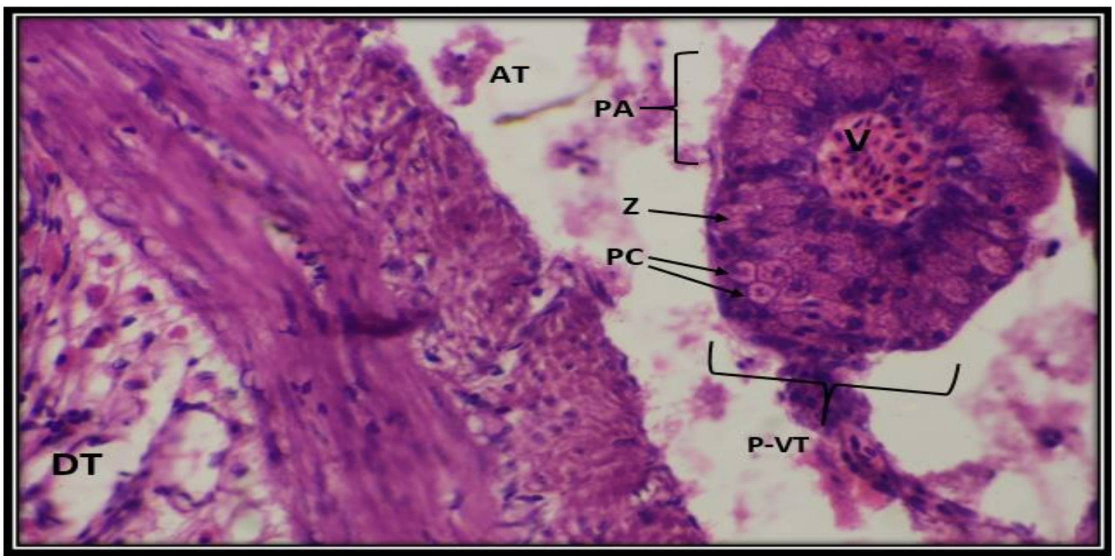

Fig. 3. The pancreatic tissue around digestive tract of *Barbus luteus*: AT (adipose tissue), DT (digestive tract), PA (pancreatic acinus), PC (pancreatic cell), V (afferent vein), P-VT(pancreatic-venous tract) X40 (H&E)

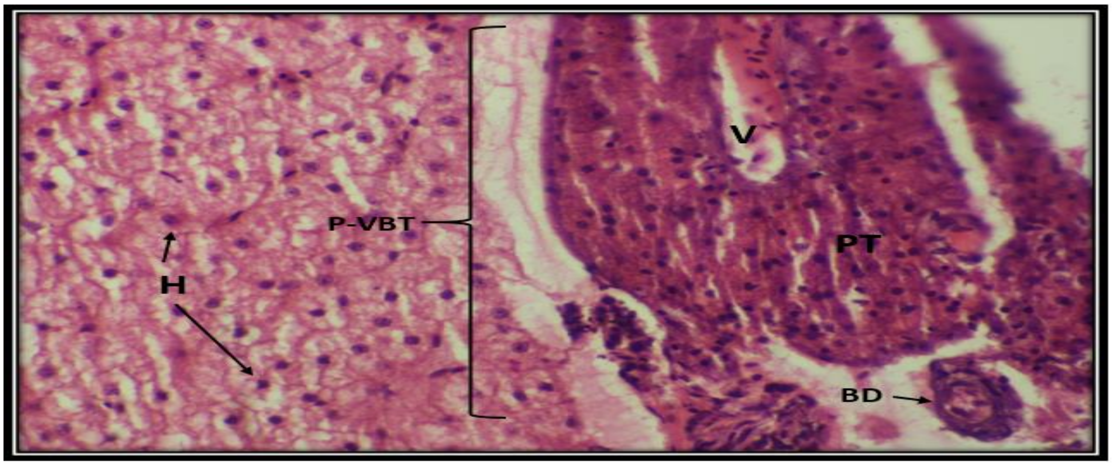

Fig. 4. Liver of *Barbus luteus* illustrating the pancreatic tissue: BD (bile duct), H (hepatocyte), PT (pancreatic tissue), P-VBT(pancreatic-venous-biliary tract) ,V (afferent vein). X40 (H&E)

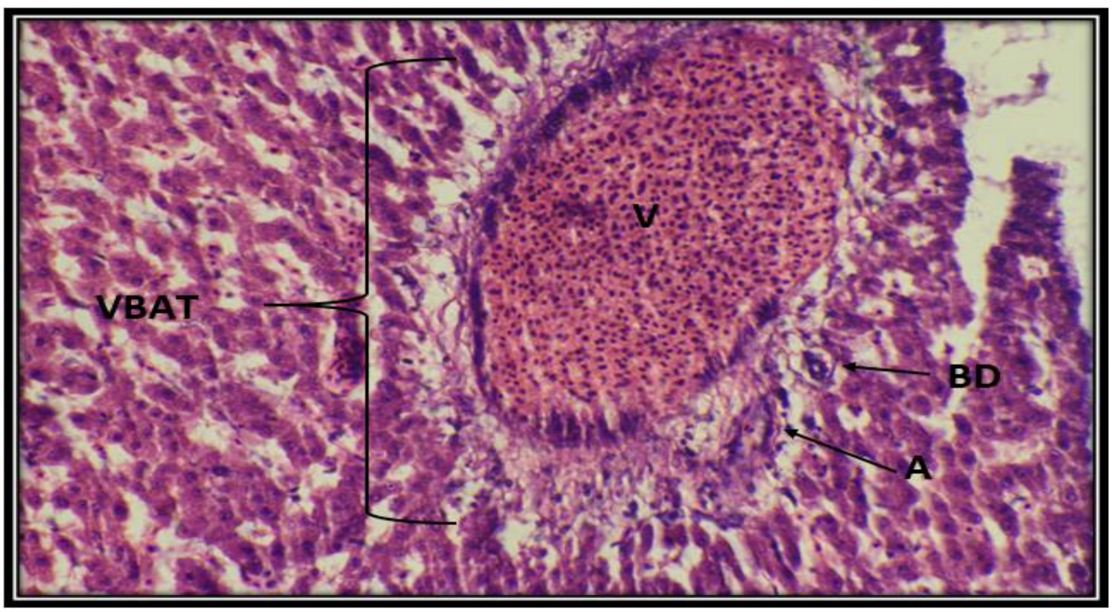

Fig. 5. Liver of *Barbus luteus* illustrating the venous-biliary-arteriolar tract: A (artery), BD (bile duct), VBAT(venous-biliary-arteriolar tract) ,V (afferent vein). X40 (H&E)

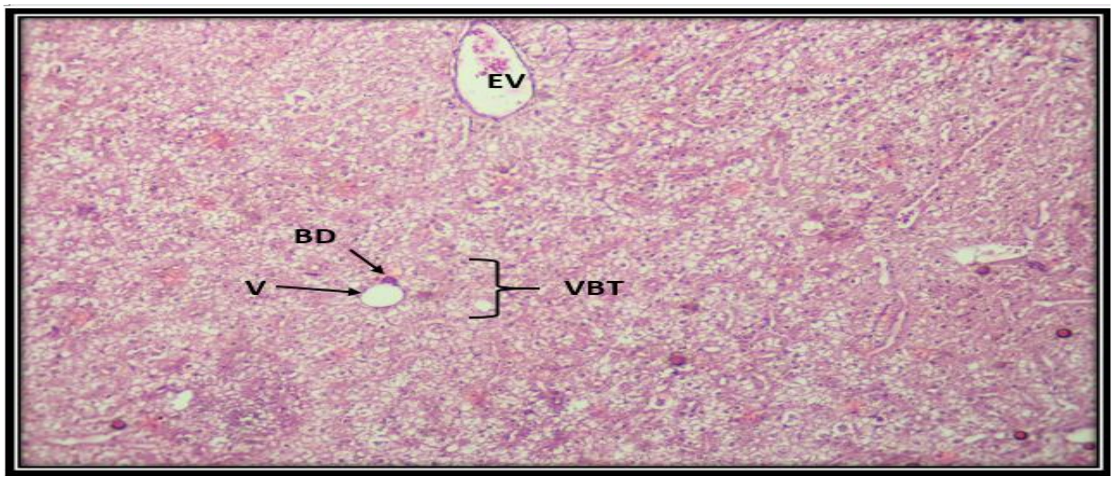

Fig. 6. Liver of *Chondrostoma regium* illustrating the venous-biliary tract: BD (bile duct), EV (efferent vein), VBT (venous-biliary tract) ,V (afferent vein) X10. (H&E stain)

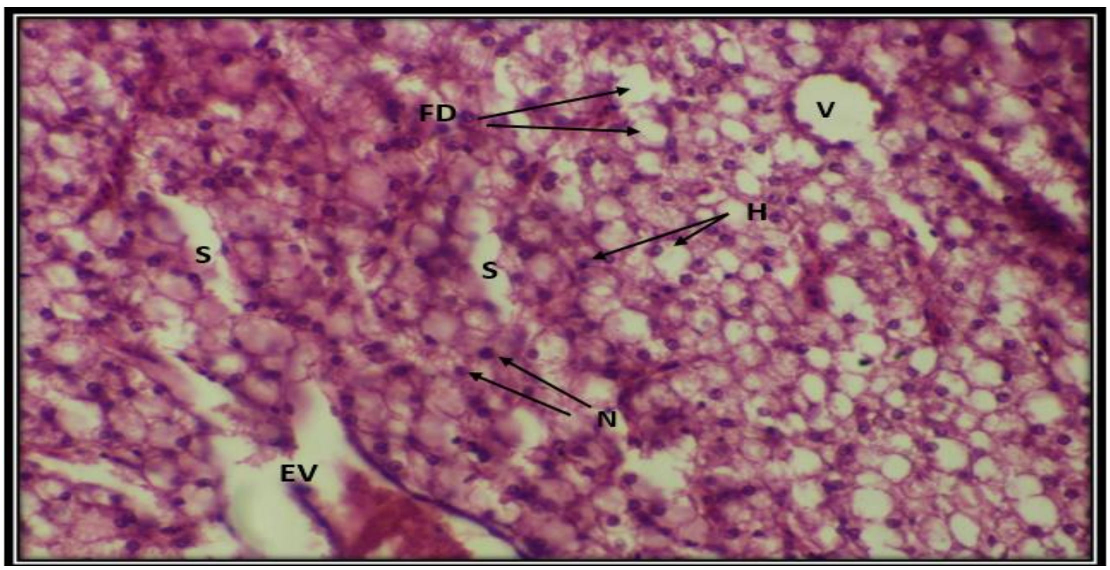

Fig. 7. Liver of *Chondrostoma regium*: EV (efferent vein), FD (fat droplet), H(hepatocyte), N (nucleus), S (sinusoids) ,V (afferent vein). X40 (H&E)

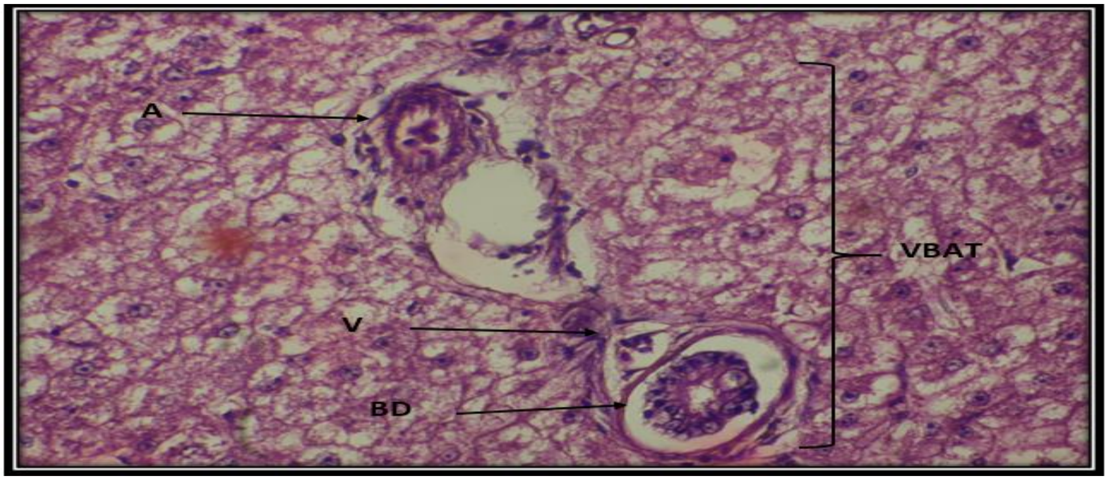

Fig. 8. Liver of *Chondrostoma regium* illustrating the venous-biliary-arteriolar tract: A (artery), BD (bile duct), VBAT(venous-biliary-arteriolar tract) ,V (afferent vein). X40 (H&E)

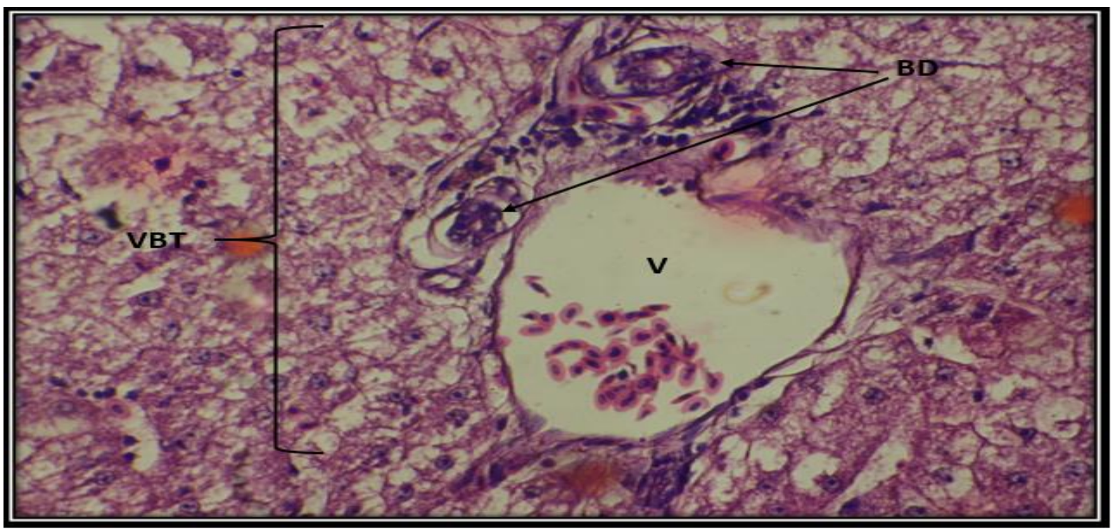

Fig. 9. Liver of *Chondrostoma regium* illustrating the venous-biliary tract: BD (bile duct), VBT (venous-biliary-tract),V (afferent vein) X40. (H&E)

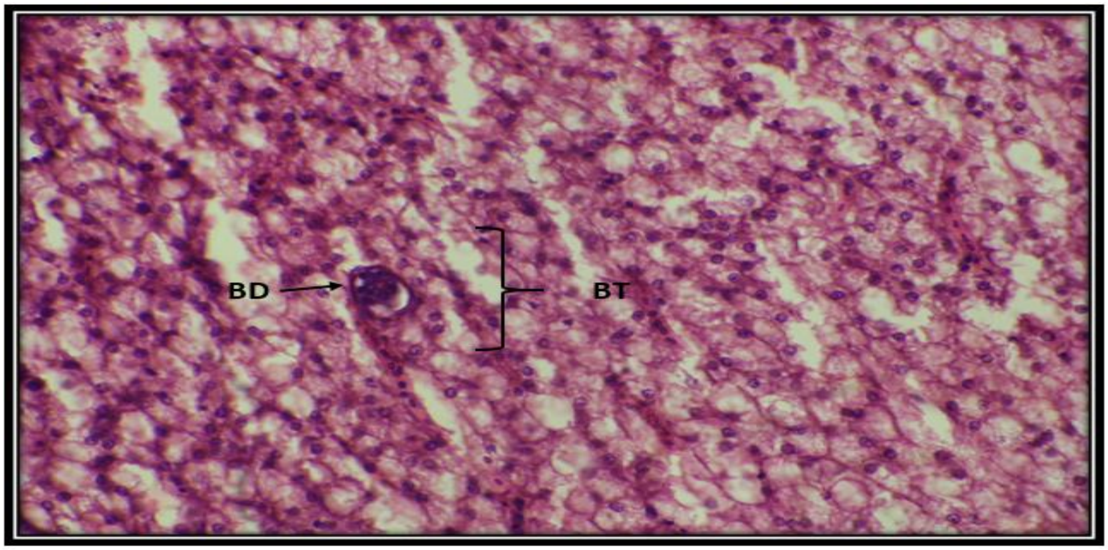

Fig. 10. Liver of *Chondrostoma regium* illustrating the isolated biliary tract not associated to blood vessel: BD (bile duct), BT(biliary tract). X40 (H&E)

DISCUSSION

In higher vertebrates, the presence of lobes and central veins within the liver is considered a characteristic feature, whereas in fish, it is generally not well defined and varies depending on the species **(Akoul, 2019)**. The results of the current study revealed structural differences between the livers of *Barbus luteus* (red barb) and *Chondrostoma regium* (king barb), despite both belonging to the family Cyprinidae. Although there were similarities in the overall basic structure, the liver of both species was surrounded by a thin layer of dense connective tissue, with no distinct lobes or partitions observed within the hepatic parenchyma. These findings are consistent with previous studies on the zebrafish, which also belongs to the Cyprinidae family **(Ota & Shiojiri, 2022)**. However, in *Oligosarcus jenynsii*, a member of the Caracidae family, the liver is divided into irregular lobes by exocrine pancreatic tissue, accompanied by connective tissue **(Petcoff *et al.*, 2006; Elsayed *et al.*, 2022)**.

Furthermore, the current study showed that the hepatocyte system in the red barb is tubular in form, while in the king barb it is solid. The hepatocyte–sinusoidal structure in fish livers has been classified into three types: cord-like, tubular, and solid **(Hussein *et al.*, 2023)**. In the cord-like form, hepatocytes are arranged in simple layers with dilated sinusoids and straight capillaries. The tubular form is characterized by bilayers with irregular sinusoids, while the solid form consists of multiple hepatocyte layers with short and narrow sinusoids. Primitive fish species typically exhibit tubular or solid hepatocytes, and the order Cypriniformes is considered primitive **(Sales *et al.*, 2017)**.

The present results also showed that hepatocytes in the red barb are polyhedral with a centrally located spherical nucleus containing up to three nucleoli, whereas in the king barb the hepatocytes are spherical, with cytoplasm filled with lipid droplets and a single centrally located nucleus, which becomes peripheral in the presence of large lipid droplets. These differences may be attributed to dietary variation between the two species and are consistent with the hepatocyte systems observed, where polyhedral hepatocytes are associated with the tubular form and spherical hepatocytes with the solid form. Such differences may also arise within the same species due to gender, age, water temperature, or hormonal changes during the life cycle **(Kibenge & Strange, 2021)**.

The results of this study confirmed that the hepatocyte–sinusoidal structure of the red barb liver was tubular. Odokuma and Omokaro also classified hepatocyte–sinusoidal structures in fish livers into cord-like, tubular, and solid forms **(Odokuma & Omokaro, 2015)**. The morphology and distribution of intrahepatic vascular and biliary systems showed considerable variation among different fish species. Bile is synthesized by hepatocytes and flows through intrahepatic bile canaliculi, bile ductules, and bile ducts **(Hussein *et al.*, 2023)**. The bile duct structure has been classified into four types: (a) isolated, (b) biliary–arteriolar tract (BAT), (c) biliary–venous tract (BVT), and (d) portal-tract type. In the present study, the red barb exhibited four tract types: P-VBAT (pancreatic–venous–biliary–arteriolar tract), P-VAT (pancreatic–venous–arteriolar tract), P-VBT (pancreatic–venous–biliary tract), and P-VT (pancreatic–venous tract). In contrast, the king barb exhibited VBAT (venous–biliary–arteriolar tract), VAT (venous–arteriolar tract), VBT (venous–biliary tract), and VT (venous tract).

The BAT type has been reported in nearly all species, forming pathways that connect with either the isolated type or the portal-tract type. However, no clear correspondence between bile duct morphology and phylogenetic advancement has been established, suggesting that fish livers are organized by a biliary system similar to that of other vertebrates **(Nazlić *et al.*, 2014; Mokhtar, 2015)**. Hepatocytes, the chief parenchymal cells of the liver, play essential roles in metabolism, protein synthesis, detoxification **(Menke *et al.*, 2011; Weber *et al.*, 2020)**, and stimulation of innate immunity through the production of immunoregulatory proteins **(Hussein *et al.*, 2023)**.

A study on molly fish illustrated that hepatocyte morphology is similar to mammalian liver cells, with cytoplasm rich in filaments, mitochondria, well-developed smooth and rough endoplasmic reticulum, lysosomes, Golgi apparatus, and numerous glycogen and lipid droplets. Comparable findings have been reported in various fish species such as Teleostei, Salmonidae, grass carp, and Atlantic salmon **(Petcoff *et al.*, 2006; Akoul, 2019; Kibenge & Strange, 2021)**.

The current study also demonstrated that hepatic sinusoids in both red barb and king barb livers were narrow, relatively short, and tortuous. Similar features have been reported in other bony fish, such as common pandora and whiting, where hepatic sinusoids are irregularly shaped and located between hepatic plates. Specialized B, E, F, and R cells were identified in the walls of hepatopancreatic tubules, playing important roles in digestion through enzyme production, absorption of water and nutrients, intracellular digestion, and protein synthesis. These nutrients are absorbed through the hepatopancreatic tubules, while some are absorbed directly through the midgut wall **(McGaw & Curtis, 2024)**.

Regarding the biliary system, the present study found that the red barb has a portal biliary system, consisting of a bile duct accompanied by the hepatic artery and portal vein, while the king barb has an isolated biliary system, with bile ducts scattered individually within the liver parenchyma **(Teles *et al.*, 2017)**. In bony fish, the biliary system is classified into four types: isolated, biliary–arteriolar tract, biliary–venous tract, and portal-tract **(Ali *et al.*, 2023; Hussein *et al.*, 2023)**.

In the current study, external pancreatic tissue extending along the main portal veins and within the adipose tissue surrounding the digestive tract was observed in the red barb but absent in the king barb. This feature is common in bony fish, where external pancreatic tissue is dispersed within the mesentery, forming clusters in the connective tissue surrounding digestive organs or scattered within peritoneal adipose tissue. Fish lack a distinct pancreas, instead possessing dispersed pancreatic islets within the spleen and mesentery around the intestines, as well as in the branches of the portal vein within the liver **(Bruslé & Anadon, 2017)**. Similar findings have been reported in *Ctenopharyngodon idella* (grass carp), a member of the same family as the red barb, and in *Rhamdia quelen* (catfish), where pancreatic clusters are distributed within the adipose tissue along the digestive tract. In zebrafish, another cyprinid, the pancreatic tissue is distributed along the digestive tract but not within the liver **(Tan *et al.*, 2013)**. In the red barb, pancreatic tissue appeared as grape-like clusters composed of multiple pancreatic cells containing numerous zymogen granules, which store enzymes required for protein, carbohydrate, lipid, and nucleotide digestion.

CONCLUSION

The main histological differences between the livers of the two fish species are as follows: in the red barb, dispersed pancreatic tissue is present within the hepatic parenchyma, whereas in the king barb, pancreatic tissue is absent. The hepatocyte system in the red barb is tubular, while in the king barb it is solid. Red barb hepatocytes are polyhedral with a centrally located spherical nucleus, whereas in the king barb the hepatocytes are spherical, with a centrally located nucleus that shifts peripherally in the presence of large lipid droplets. The biliary system of the red barb corresponds to the portal type, while in the king barb it is isolated. These differences may be attributed to dietary variations between the two species, despite both belonging to the same family (Cyprinidae).

Authors contribution

Aveen R. Mohsin and Abeer M. Hussain: Methodology and manuscript writing.

Zainab karim AL-Timimi: Methodology

Ruqaia Ali Duraye: Writing the manuscript.

Duha hameed and Ahmed Flayyih Hasan : Organize manuscript according to journal style, correspondence file, respond to reviewers' comments.

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Conflict of interest

The authors declare that there are no conflicts of interest or personal relationships that could influence the research paper.

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