Al-Mukhtar Journal of Basic Sciences 22 (1): 75-84, 2024
Doi: https://doi.org/10.54172/d09k0c70

**Research Article** **Open Access**

# Morphological and Histological Description of Spiny Dogfish shark Liver, *Squalusacanthais*(Linnaeus,1758), Elasmobranchii, Squaliformes

**Nagla A. Elfagi**

*__Corresponding author:__ elfaghi.n.a@sci.misuratau.edu.ly
Department of Zoology, Faculty of Science, Misurata University, Libya.

Received:
03 October 2023

Accepted:
24 February 2024

Publish online:
30 April 2024

**Abstract**

The current study describes the morphological and histological appearance of the liver of the spiny dogfish *Squalusacanthais*, which is classified as Class: Chondrichthyes, Subclass: Elasmobranchi. Morphologically, the liver was observed as large, and consists of two symmetrical lobes connected from the upper side, and the gallbladder was observed in the right lobe. Histologically, liver sections were prepared with H&E stain, and examined with a light microscope. Hepatic parenchyma was surrounded by a capsule of connective tissue and primarily composed of hepatocytes. The nuclei of these cells were observed at the terminal site, with lipid droplets in the cytoplasm. Central veins were observed, surrounded by connective tissue and lined with a squamous epithelial layer. Hepatocytes were, separated by numerous blood sinusoids with connective tissue. Blood arteries were observed, surrounded by thick muscular fiber walls and narrow lumens compared to veins. The portal vein was observed, along with the artery and bile duct, surrounded by a thick connective tissue. The bile duct was surrounded by a layer of muscular fibers, lined with a simple columnar epithelial layer with clear nuclei, and a connective tissue layer. Melanin-containing cells were observed, but no hepatic lobules or connective tissue were seen between them. Additionally, Hepato-pancreatic tissue was not identified.

**Keywords:** Histological Structure; Liver; Hepatocytes; *Squalusacanthais*; Chondrichthys; Elasmobranchs.

## INTRODUCTION

Fish form the largest and most diverse group of vertebrates, teleosts representing more than 25,000 of all known fish species, Chondrichthyes, on the other hand, do not exceed 800 species (Bone & Moore, 2008). The spiny dogfish shark, *Squalusacanthias*, belongs to the class Chondrichthyes, subclass Elasmobranchii, and order Squaliformes. It is a carnivorous fish (Heckmann, 2001) that lives in saltwater in the northeast Atlantic Ocean and the Mediterranean Sea (Marine Biology Blog in Libya).

Morphological, anatomical, and histological studies of the digestive system of fish and its associated glands play an important role in understanding the mechanisms of food digestion and absorption (De Melo Germano et al., 2014). The liver is one of the most important glands associated with the digestive system. It is a relatively large vital organ and has a similar basic function and structure in both Chondrichthyes and teleosts. The liver has many functions similar to those found in mammals, such as the production of vital chemicals for the process of digestion. The liver is highly sensitive to pollution with organic and inorganic compounds, as they can accumulate over time and cause life-threatening conditions due to the liver's ability to remove toxins and store harmful components. It is often used as a biological marker for environmental pollutants (Eppler et al., 2007). Additionally, the liver's functions include storing and producing energy, hormonal balance, and clotting, and it is the center of metabolism (De Melo et al., 2019). In fish, the liver produces bile and maintains metabolic balance, which includes processing and storing carbohydrates, proteins, fats, and vitamins. The liver also plays a major role in synthesizing plasma proteins such as albumin and fibrinogen, and liver tissues vary among species, but there are general features present in most species (Genten et al., 2009). Typically, it is reddish-brown in carnivorous animals, depending on the diet. The liver may be located in the anterior part of the abdominal cavity and adapts its size and shape to the available space between other visceral organs (Sales et al., 2017), or in some species, it may be elongated along the abdomen or attached to other intestines, or it may be a composite organ in the form of a hepato-pancreatic organ.

The liver tissues of fish differ from those of mammals in that hepatic cells rarely tend to form cords or distinctive lobules, and the typical portal triads are not clear (Mumford et al., 2007). The structural tissue of the liver is enclosed in a thin capsule of fibrous connective tissue and consists primarily of multi-surfaced hepatocytes with a visible cellular membrane usually with central nuclei. Glycogen deposits are often dissolved and fats are stored, resulting in a wide variety of liver tissues (Genten et al., 2009). The central veins can be randomly observed throughout the hepatic parenchyma, and structures with small gaps are apparent in hepatocytes due to the presence of fats. The presence of glycogen may indicate the fish's ability to synthesize or break down glycogen according to metabolic needs. The hepatic sinusoids are distributed among the hepatocytes that form a wide network. These sinuses are lined with blanket cells with dark nuclei and Kupffer cells that are star-shaped with large dark oval nuclei. In addition, a large amount of connective tissue is identified around blood vessels, and a network of blood vessels and bile ducts is found within the tissue components of the liver (Mokhtar, 2017).

There are limited studies on the liver tissues of elasmobranchs. A study conducted by (De Melo et al., 2019) indicated that the liver in the blue shark *Prionaceglauca* is the largest gland in the body, located in the abdominal cavity and is shaped like two elongated lobes, with the tip of the lower lobe tapered in a semicircular shape, while the right and left lobes of the liver are symmetrical. Elasmobranchs do not have a swim bladder, which requires them to constantly move to avoid drowning, which is why they have a high-fat content to float, as the liver acts as a hydrostatic organ.

Oguri (1978) in a study on dogfish, (Adams et al., 2015) in their study on the longfin mako shark (*Isuruspaucus*), and (Gajié et al., 2020) in their study on the catshark (*Scyliorhinuscanicula*) indicated that hepatocytes contain a large number of fat droplets and contain brown pigment granules called melano-macrophage centers MMC.

(Seyrafi et al., 2009) in their study on *Pangasiushypophthalmus*, as well as (Hibiya, 1982; Mumford *et al*., 2007; Coetzee, 2018) described the liver as being covered with a thin capsule consisting of a serous layer and connective tissue, part of which extends into the liver parenchyma. The liver lobules were not visible, and the liver parenchyma mainly consisted of multiple surface hepatocytes with central nuclei, with many hepatic sinusoids and cavities for fat storage. The hepatocytes of the liver (spherical shaped) are irregularly distributed with sinusoids in between (Hibiya, 1982; Mumford et al., 2007).

The hepatic ducts that collect yellow bile from the liver were observed by (Borucinska et al., 2009) in their study on the liver of three species of fish. The portal triad, consisting of the hepatic vein, hepatic artery, and bile duct, has been described in teleost, but studies are limited to elasmobranchs (Akiyoshi& Inoue, 2004; Coetzee, 2018).

Due to the importance of comparative studies of fish in interpreting some phenomena and evolutionary relationships imposed by environmental, genetic, and life conditions on this vertebrata in general, this research was designed to study the morphological and histological structure of the liver in a type of fish belonging to the class of chondrichthyes.

## MATERIALS AND METHODS

The current study was conducted on a type of chondrichthyes, specifically the spiny dogfish *S. acanthais* (Figure 1). The samples were collected from the fishing port in the area of Qasr Ahmed, and the area of Abu Qareen in the east of Misuratah, with a total of 4 samples. Morphometric measurements were conducted on the samples; the average lengths were (67.2 cm), and the average weights were (2.5 kg). The samples were then transferred to the animal science department laboratory at the Faculty of Science, Misrata University, in plastic polythene bags with ice to preserve them until the study was conducted. The fish were identified visually, and external morphometric measurements were taken. A longitudinal incision was made in the abdominal cavity using a scalpel from the beginning of the anus to the gill chamber, and then the liver was removed and placed in a dissecting dish to identify the main parts. Afterward, it was washed with a saline solution, cut into small pieces, and immediately transferred to 10% neutral formalin fixative to preserve it until the next step.

For histological studies, parts of the liver were taken and cut into small pieces, and placed in vials containing formalin w (10%). Each vial was labeled with the name of the fish used in the study and the specific part of the sample. The study samples were then sent to the tissue unit in the Misrata laboratory. The targeted study samples were processed in an ascending series of ethanol (70-100%), then soaked in xylene, embedded in paraffin wax, and then cut into tissue sections using the rotary microtome model Cut 5062 (an Argentic company SLEE Medical) with a thickness of 5 micrometers. The tissue sections were stained with hematoxylin and eosin (H&E) dyes, washed with water, dehydrated in an ascending series of ethanol, soaked in xylene, and mounted with DBX glue for examination under a light microscope (Bancroft & Stevens, 1977). The tissue sections were then photographed using a Motic BA 310 Digital microscope.

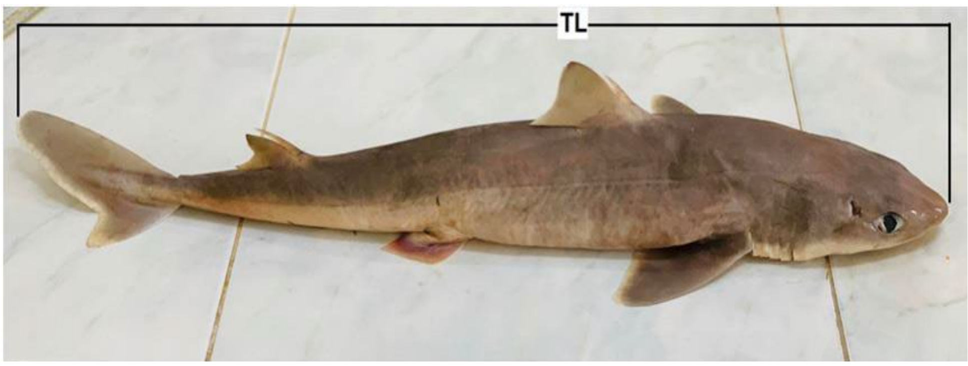


**Figure: (1).**The general appearance of the fish studied: *S acanthais*, Total length(TL).

## RESULTS ANDDISCUSSION

The morphological examination showed that the liver of the *S. acanthais* fish occupies most of the abdominal cavity. It is large, takes a ventral position relative to the stomach and intestines, has a pinkish-yellowish color, and consists of two symmetrical lobes connected by an isthmus. Each lobe is elongated, broad at the top, tapered at the end. Additionally, the gallbladder was observed in the right lobe as shown in (Figure 2: A, B). These results are consistent with the findings reported by (De Melo et al., 2019) in their study of the liver of the *Prionaceglauca* shark, describing it as the largest gland in the body, located in the abdominal cavity, consisting of two symmetrical elongated lobes with a tapered lower end in a semi-circular shape, and with the gallbladder extended in the right lobe. In his study on the liver of the European Spotted Dogfish, (Oguri, 1978) described it as relatively small in size, which contradicts the current consensus that elasmobranchs typically have large livers. This was confirmed by (Genten et al., 2009; De Melo et al., 2019), who stated that the liver occupies more than 80% of the abdominal cavity. Like other chondrichthys, sharks do not have a swim bladder and must constantly move to avoid sinking. The average density of the liver is related to the fat content, and the high oil content of these fish allows them to float more easily in the water column, acting as a hydrostatic organ to help them maintain their position. The liver represents 20% of the total body weight (de Melo, et al. 2019), and its size and weight vary depending on the species, age, and season. According to (Brusle & Anadon, 1996), up to 90% of the liver can be made up of oil. There are also differences in liver structure between males and females, as well as between mature and immature fish. It has been observed that fat storage begins at the embryonic stage, where hepatocytes accumulate fat in the cytoplasm according to the growth of the embryo, as demonstrated by (De Melo et al., 2019) in their study on sharks.

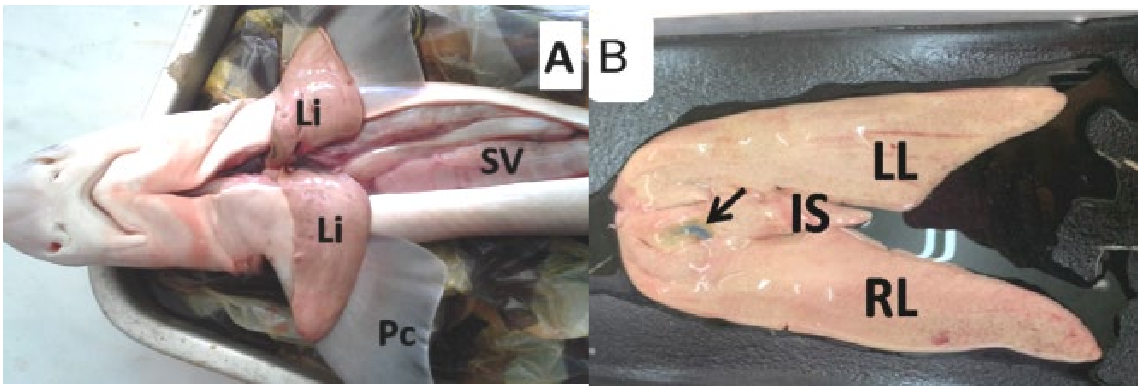


**Figure: (2).**The phenotype and location of the liver in Dogfish shows : A **-** liver lobes(Li), spiral valve (SV), pectoral fin (pc), B **-** right lobe of the liver (RL), left lobe of the liver(LL), gallbladder (arrow), isthmus (Is).

The histological appearance: The histological examination results in (Figure 3:A, B) show a section passing through the liver of Spiny dogfish *S.acanthais* composed of typical functional units associated with fish liver, including the basic structural tissue of the liver (parenchyma), blood vessels, and the biliary canal system, surrounded by a thin capsule of connective tissue. The hepatic parenchyma appears primarily as hepatocytes with a clear cellular membrane, rich in lipid droplets. Due to the large amount of fat in the liver, these hepatocytes vary in size and shape and do not have visible cytoplasm in H&E preparation, but they have an unstained appearance (white) (Figure 3). It seems that the organization of the liver parenchyma in elasmobranchs are less organized than in teleost. Additionally, some functional units such as biliary canals were less prominent. The high content of fat in the liver may have led to an evolutionary change in elasmobranch hepatic parenchyma, where hepatocytes were observed to be organized in separate clusters, separated by a thin layer of connective tissue that likely provides support to the parenchyma. The high fat content may lead to a less stable organ structure without supportive connective tissue (Coetzee, 2022). The blood vessels consist of spherical arteries, large veins, and small blood sinuses. The arteries have a significantly thicker muscular layer and connective tissue layer surrounding the vessel compared to the larger veins. It is also difficult to distinguish the central veins and portal veins. The section shows the presence of the central vein surrounded by connective tissue and containing blood cells, as observed in (Figure 3& 4), and the numerous blood sinuses that drain into the central veins are visible due to the single file of red blood cells visible in these capillaries. The figure (Figure 3: A, B) also indicates the leakage of blood cells from blood vessels that appear as blood clots, although the liver does not provide the histological structure of the typical hexagonal liver of mammals, and liver lobules or the connective tissue separating them were not observed. Also, Hepato-pancreatic tissue, often identified in some fish species, was not identified.

The results of this study are consistent with the study conducted by (Seyrafi et al., 2009) in their study of *P.hypophthalmus* and Coetzee (2022) in their study on three species of Elasmobranches, *Sphyrnalewini, Carcharhinubrevipinna,* and *Aetobatus narinari,* where they described the liver as covered with a thin capsule composed of the serous layer, and liver lobules were not clearly seen, And the hepatic parenchyma is primarily composed of multi-surfaced hepatocytes usually with central nuclei, and there were many liver sinusoids and gaps for storing fat, and macrophages cells, but (Seyrafi et al., 2009) pointed out the presence of pancreatic-secreting tissues in the liver, which was not consistent with the current study and Coetzee's study. (2022) where they did not observe the presence of pancreatic tissues within the hepatic parenchyma.

In (Figures 3: C, D), the external capsule appears composed of connective tissue lined with squamous epithelial cells that surround the parenchyma of the liver. Also, the different hepatocytes appeared to differ in shape and size, surrounded by a visible cellular membrane, and the nuclei of these cells were observed at the peripheral site of the cytoplasm and not central because they contained droplets of fat. This is consistent with what (De Melo et al., 2019) pointed out in their study of the liver in the shark *P. glauca* and (Coetzee, 2022) in his study of three species of elasmobranches fish *S. Lewin, C. brevipinna,* and *A. narinari,* where they mentioned that hepatocytes appear in different sizes of fat gaps that are introduced into the cells, leading to a shift in their nuclei from the center to the periphery. Given that the liver contains a tremendous amount of fat in all the sections examined, the liver tissue appears like adipose tissue.

Also, the results showed the presence of macrophages that play a role in engulfing foreign bodies and removing bacterial causes of inflammation and disease that enter the bloodstream. This is consistent with what (Seyrafi, et al., 2009); Coetzee, 2022) have pointed out, and may be present due to environmental hepatic toxins from human pollution (Gajić et al., 2020). The presence of these cells in hepatic parenchyma is consistent with what was found in the study by (Adams et al., 2015; Gajić et al., 2020) on different species of elasmobranches. Additionally, histological examination revealed the presence of melanomacrophage centers, which contain melanin granules, and these structures appeared mostly spherical and stained dark brown in H&E.

These results were consistent with the findings of (Oguri, 1978) in his study of the liver of European spotted dogfish, where he noted that hepatocytes contain a large number of fat droplets, as well as brown pigment granules called melanin granules, which are usually irregularly distributed in liver parenchyma and serve as evidence of environmental stress. They are also an indicator of water quality and oxygen content, as well as a sign of chemical pollution in the environment where fish live (Agius and Roberts, 2003). Some studies on elasmobranch species have also reported the presence of MMCs, such as the study conducted by (Gajić et al., 2020) on the shark (*Scyliorhinuscanicula*). Also, in a study conducted by (Borucinska et al., 2009) on three species of elasmobranchs, the mako shark, the thresher shark, and the blue shark, the authors reported the presence of MMC in the liver, testes, kidneys, and spleen. According to (Adams et al., 2015), MMC was found in the liver of the shark *Isuruspaucus*, it has detoxification and recycling functions in the liver, as it breaks down endogenous and exogenous materials and then becomes metabolic dumps (Mooney, 2012).

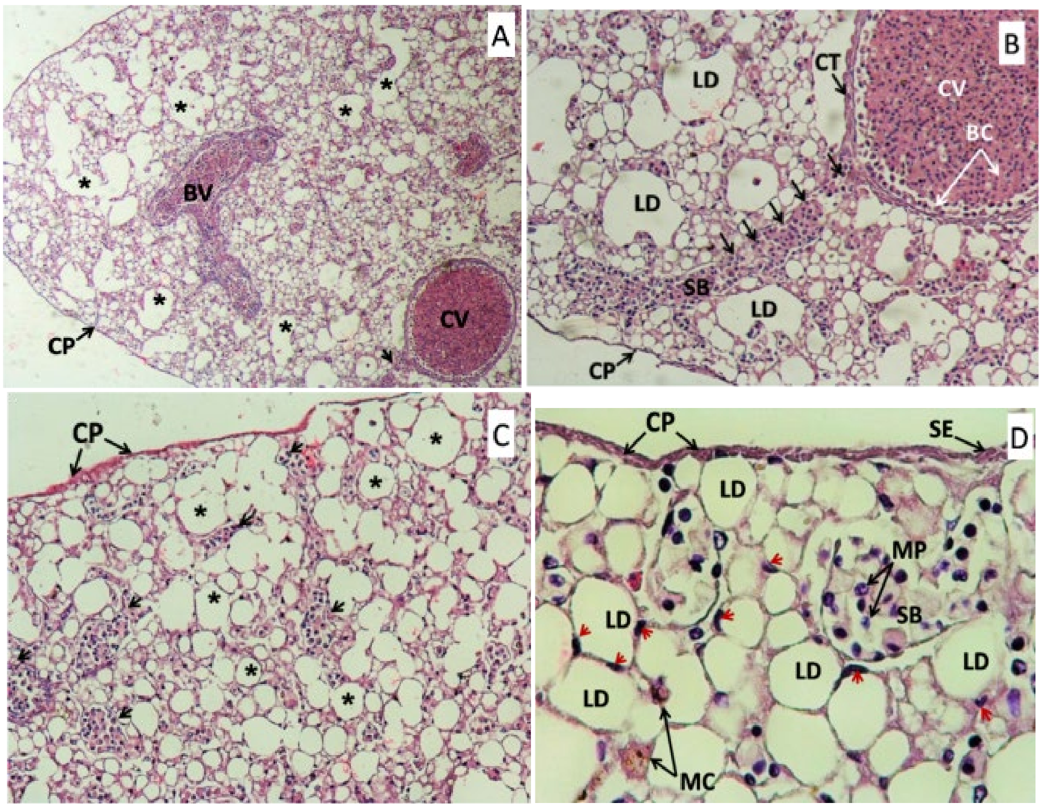


**Figure: (3).** Histological sections through the liver in a dogfish (*S.acanthias*)
**A-**Capsule (CP), central vein (CV), blood vessel (BV), hepatocytes containing fat droplets (*), blood leak (Arrowhead in black) 40X, H&E.
**B-** Capsule (CP), central vein (CV), connective tissue surrounding the central vein (CT), blood cells (BC), hepatocytes containing fat droplets (LD), blood sinusoid (SB), blood leak (arrows) 100X, H&E.
**C-**Capsule (CP), blood sinusoids (Arrowhead), hepatocytes containing fat droplets (*) 100X, H&E.
**D-**Capsule (CP), squamous epithelial cells (SE), blood sinusoids (SB), hepatocytes containing fat droplets (LD), hepatocyte nuclei (Arrowhead in red), macrophage cells (MP), melanocyte centers (MC) 400X, H&E.

The portal triads were identified in some tissue areas, where the portal vein and its blood cells, the blood artery, and the bile duct are collected, as the connective tissue appears thick and blue around the bile ducts and veins (Figure 4:A, B, C, D). The bile duct appears surrounded by a layer of muscle fibers and lined with a simple columnar epithelial layer, with clear nuclei that appear dark in color, and a layer of connective tissue near the portal vein, as in (Figure 4: B). However, it cannot be compared to the typical portal triad found in mammalian livers, and this is consistent with what was mentioned by (Genten et al., 2009; Mumford et al., 2007), and Coetzee (2022) in their study on several species of Chondrichthyes.

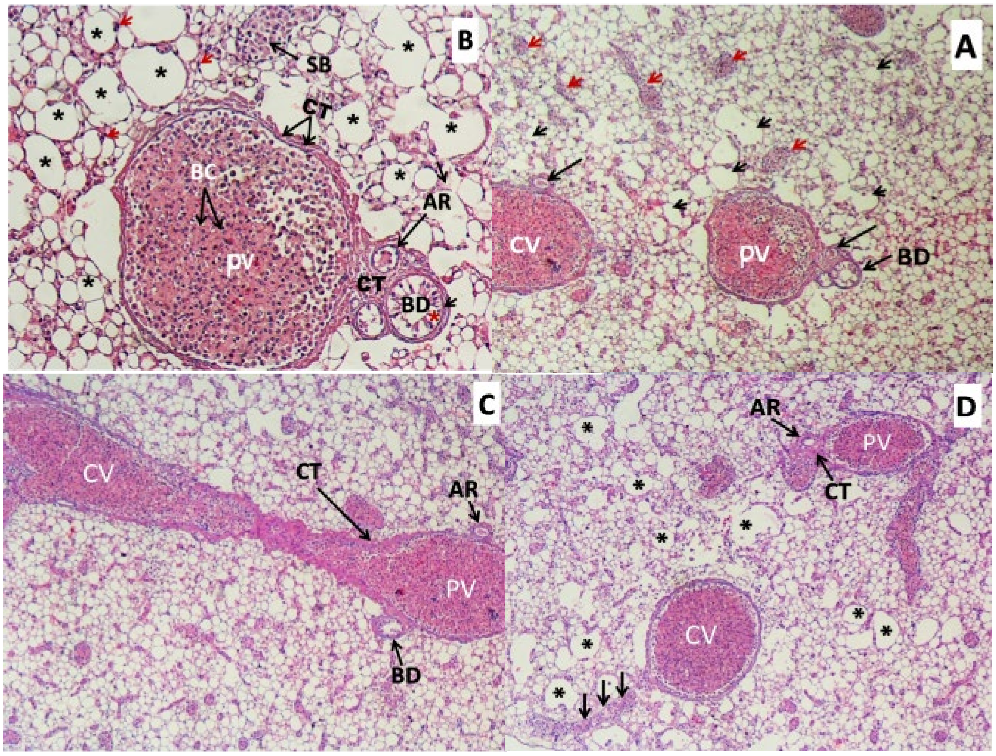


**Figure: (4). A-** Central vein (CV), portal vein (PV), bile duct (BD), blood artery (long arrow), blood sinusoids (Arrowhead in red), hepatocytes with fat droplets (Arrowhead in black) 40X, H&E. **B-**Hepatocytes showing fat (*in black), hepatocyte nuclei (Arrowhead in red), blood sinusoids (SB), portal vein (PV), red blood cells (BC), artery (P), bile duct(BD), connective tissue (CT), columnar epithelial cells (* in red) 100 X, H&E. **C-** Central vein (CV), portal vein (PV), bile duct (BD), blood artery (P), connective tissue (CT) 40X, H&E. **D-** Portal vein (PV), blood artery (P), connective tissue (CT), hepatocytes containing fat droplets (*), central vein (CV), blood leak (Arrowhead in black) 40X, H&E

In mammals, the accumulation of fat within hepatocytes is considered a disorder known as hepatic steatosis. When there is excess fat for a prolonged period, hepatocytes may become damaged and inflamed. In sharks, however, this condition is a healthy and necessary adaptation to help them float, as well as to serve as a glycogen store for conversion to glucose and energy, because they are animals with large energy expenditure. Another function of fat in the liver is its importance during embryonic development, where females use this fat to maintain and nourish their young (De Melo, et al., 2019). Although the accumulation of fat in the shark liver is considered fairly normal, it is also listed here as a change that should be considered in toxicological studies, since the fat content (cytotoxicity) can increase due to exposure to toxic substances since the liver is the main organ of detoxification and is important for the metabolism of fats, proteins, carbohydrates, bile secretion, excretion of toxic substances and a large blood supply, it can play an important role in the extent of exposure to toxic substances (Hibiya, 1982; Van Dyk et al., 2007; Mohamed, 2009; Yancheva et al., 2015; &De Melo et al., 2019). The liver is the main organ for the biological accumulation of substances due to the presence of enzymes that metabolize toxins, which often harm the organ (Mumford et al., 2007; &Borucinska et al., 2009). Elasmobranch species mainly use proteins and fats as part of their energy reserves (Remme et al., 2005). The main component of the liver is squalene, a fatty acid that is also used in the production of cosmetic products (Remme et al., 2005; & Cardeñosa, 2019). Therefore, fat accumulation can be considered somewhat normal, but exposure to toxins can also increase levels above normal, resulting in changes in fat or hepatic steatosis, as suggested in (Adams et al., 2015) study on longfinmako fish.

## CONCLUSION

The liver of spiny dogfish, is large, occupying most of the abdominal cavity, and its tissue structure differs from the typical mammalian liver, with the absence of hepatic lobules and typical portal triads being unclear. Hepatocytes appear with varying sizes and contain a huge amount of fat, resembling adipose tissue. In mammalian livers, fat accumulation is considered hepatic steatosis, while in shark livers, it is a healthy and necessary condition. Since Elasmobranchs do not have a swim bladder, the liver acts as a hydrostatic organ to help them float. Pancreatic tissue is absent.

## ACKNOWLEDGEMENT

The researchers express their thanks and appreciation to sister Ibtisam Mohammed for helping to bring the samples.

## ETHICS

Authors should address any ethical issues that may arise after the publication of this manuscript.

**Duality of interest:** The authors declare that they have no duality of interest associated with this manuscript.

**Author contributions:** Contribution is equal between authors.

**Funding:** No specific funding was received for this work.

## REFERENCES

Adams, D., Borucinska, J., Maillet, K., Whitburn, K. & Sander, T. (2015). Mortality due to a retained circle hook in a longfin mako shark /*surs paucus* (Guitart-Mandav). *Journal of Fish Disease*, 38(7): 621-628.

Agius, C., & Roberts, R. J. (2003). Melano-macrophage centres and their role in fish pathology. *Journal of fish diseases*, *26*(9), 499-509.

Akiyoshi, H., & Inoue, A.(2004). Comparative histological study of teleost livers in relation to phylogeny. Zoological Science, 21(8): 841-850.

Bancroft, J. D., & Stevens, A. (1977). Theory and practice of histochemical techniques. *Churchilli Vingstone*, *New York pi*, *16*, 164-195.

Bone, Q., & Moore, R. (2008). *Biology of Fishes* (3rd ed.). Taylor and Francis. https://doi.org/10.1201/9781134186310

Borucinska, J.D., Kotra, K., Shackett, M.& Barker,T. (2009). Melanomacrophages in three species of free-ranging sharks from the northwestern Atlantic, the blue shark *Prionacae glauca* (L), the shorttih mako, *Isurus oxyrhinchus* Rafinesque, and the thresher, *Alopias vulpinus* (Bonnaterre), *Journal of Fish Diseases*, 32(10): 883-891.

Brusle, J., & Anadon, G. G. (1996). The structure and function of fish liver. Fish Morphology. *Edited by Munshi, JSD and Dutta, HM Science Publishers Inc.*

Cardeñosa, D. (2019). Genetic Identification of threatened shark species in pet food and beauty care. Conservation Genetics, 20: 1383-1387.

Coetzee, H. J. (2018). The effect of organochlorine pesticides aldrin and methoxychlor on the health status of *Clarias gariepinus* by means of a histology-based approach. M.Sc. Dissertation. Department of Zoology. University of Johannesburg. South Africa.

Coetzee, H. J. (2022). *A histological study of selected target organs of three elasmo- branch species from the coast of KwaZu- lu-Natal, South Africa* (Doctoral dissertation, University of Johannesburg

de Melo Germano, R., Stabille, S. R., de Britto Mari, R., Pereira, J. N. B., Faglioni, J. R. S., & de Miranda Neto, M. H. (2014). Morphological charact- eristics of the P terodoras granulosus digestive tube (V alenciennes, 1821) (O steichthyes, D oradidae). *Acta Zoologica*, *95*(2), 166-175.

De Melo, L. F., Cabrera, M. L., Rodrigues, A. C. B., Turquetti, A. D. O. M., Ruivo, L. P., Bruno, C. E. M., & Rici, R. E. G. (2019). Morphological Description of Blue Shark Liver, Prionace glauca (Linnaeus, 1758), Elasmobranchii, Carcharhiniformes. *Int. J. Adv. Eng. Res. Sci*, *6*, 286-290.

Eppler, E., Caelers, A., Shved, N., Hwang, G., Rahman, A. M., Maclean, N., ... & Reinecke, M. (2007). Insulin-like growth factor I (IGF-I) in a growthen- hanced transgenic (GH-overexpressing) bony fish, the tilapia (*Oreochromis niloticus*): indication for a higher impact of autocrine/paracrine than of endocrine IGF-I. *Transgenic research*, *16*(4), 479-489.

Gajic A, AliC A,Kahric A, Bilalovic N, Supié J & Besirovic H. (2020). Melanomacro- phage centeres and diseases occurring in lesser- spotted catsharks, Scyliorhinus canicula (L.), from the southern Adriatic Sea -importance for monitoring. *Acta Adriatica*, 61(2): 175-184.

Genten, F., Terwinghe, E., & Danguy, A. (2009). *Atlas of fish histology*. CRC Press.

Heckmann, R.(2001). Roundworms and their cousins: commen fish invaders. *Journal of Aquacultural of Magaz*. 33-44.

Hibiya, T. (1982). An atlas of fish histology: Normal and pathological features. Kondasha Ltd. Tokyo.

Mohamed, F. A. S. (2009). Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. *World Journal of Fish and Marine Sciences*, 1(1): 29-39.

Mokhtar, D. M. (2017). *Fish histology: from cells to organs*. Apple Academic Press.

Mooney, A.(2012). An assessment of the health status and edibility fish from three impoundments in the North West Province. South Africa. M.Sc. Dissertation. Department of Zoology. University of Johannesburg. South Africa.

Mumford, S., Heidel, J., Smith, C., Morrison, J., MacConnell, B., & Blazer, V. (2007). Fish histology and histopathology. *US Fish and Wildlife National Conservation Training Center, Amerika Serikat*.

Oguri, M. (1978). Histochemical observations on the interrenal gland and liver of european spotted dogfish. *Bull. Jap. Soc. Sci. Fisheries, 44*, 703-9.

Remme, J.F., Stokes, I.S. & Larssen, W.E. (2005). Bioactive lipids in deep-sea sharks. *Research Council of Norway. Report* A0510.

Sales, C. F., Silva, R. F., Amaral, M. G., Domingos, F. F., Ribeiro, R. I., Thomé, R. G., & Santos, H. B. (2017). Comparative histology in the liver and spleen of three species of freshwater teleost. *Neotropical Ichthyology, 15*

Seyrafi, R. E. Z. A., Najafi, G., Rahmati-Holasoo, H., Hajimohammadi, B., & Shamsadin, A. S. (2009). Histological study of hepatopancreas in iridescent shark catfish *Pangasius hypophthalmus . Journal of Animal and Veterinary Advances*, *8*(7), 1305-1307.

Van Dyk, J. C. , Pieterse, G. M. & Van Vuren, J.H.J. (2007). Histological changes in the liver of Oreochromis mossambicus (Cichlidae) after exposure to cadmium and zinc. Ecotoxicology and Environmental Safety, 66(3): 432-440.

Yancheva, V., Velcheva, L., Stoyanova, S. & Georgieva, E. (2015). Histological biomarkers in fish as a tool in ecological risk assessment and monitoring program s: A Review. Applied Ecology and Environmental Research, 14(1): 47-75.

https://libyamarinebiology.wordpress.com