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# HISTOLOGICAL AND HISTOCHEMICAL STUDY OF THE LIVER AND GALL BLADDER OF ADULT MALE COMMON CARP *CYPRINUS CARPIO*

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## Abstract

The purpose of this study was to describe, some the histological structures and histochemical features of the liver and gall bladder of common carp. The present study was conducted on fifteen healthy adult male common carp were catching alive from the AL Forat river, mean their standard length was (50.4 ± 3.1 cm), immediately after death, the liver and gall bladder were removed through a longitudinal incision in the abdominal wall and dissected out, 5 specimens from different regions each of the liver and gall bladder were taken and fixed by 10% formalin 24 hours at room temperature, and then treated by routine histological processing. The stains were used, Harries Hematoxylin and Eosin, Masson's trichrome and Periodic acid-Schiff. Histological examination revealed that the liver was covered by a thin capsule with simple squamous epithelium and thin connective tissue as fibroconnective tissues, no triad arrangement of the hepatiocytes is observed, these cells were large, polyhedral cells and centrally nuclei. The liver parenchyma also contains numerous collapsed sinusoids that separated the hepatocytes and found central vein, while there were no Kupffer cells, the hepatic arteries and portal veins were often not associated with one another, were scattered through the liver parenchyma without a well defined arrangement, and they were sometimes accompanied by the bile duct, pancreatic tissue consists of numerous serous acini dispersed down the liver parenchyma forming the hepatopancreas, and not observed the islets of Langerhans. The wall of gall bladder was composed of only three layers; mucosa, circular muscularis and serosa, no villi and goblet cells in wall of gall bladder. The mucosa composed of a only two layers; simple columnar epithelium and lamina propria, has no muscularis mucosae. The histochemical study, The collagen fibers of capsule of the liver by Masson Trichrome stain were pink in color, the hepatic cells were positive to periodic acid Schiff stain, the hepatocytic cytoplasm had granules.

***Keywords*** **:** Common carp, Liver, Gall bladder, Histological, Histochemical

## Introduction

The common carp is a widespread freshwater fish in lakes and large rivers in Europe and Asia, its mostly omnivores (Vilizzi, *et al*., 2015 and Al-Taai and Hussin, 2016). Liver as an associated digestive gland, in garfish fish is compact, unilobular organ and lies in the first third of the abdominal cavity (Monsefi *et al*., 2010; Shahafve, *et al*., 2017), the liver of *Trichomycterus brasiliensis*, lies in the anterior third of the abdominal cavity and has an irregular shape, and has no lobes (Oliveira-Ribeiro and Fanta, 2000), in puffer fish, the liver well developed, with the presence of the gall bladder and the bile duct opening into the first intestinal part (Fagundes, *et al*., 2016). The liver is considered as a good indicator of nutritional pathology due to its function in metabolizing products coming from the digestive tract (Bocina *et al*., 2017). Fish liver histology is characterized by the absence of liver lobules and portal triads that are the basic morphological unit of liver structure in mammals. Although the hepatocytes, blood vessels, and bile ducts are founds (Rocha *et al*., 1994). Fish hepatocytes lack organization in Remack cords, exhibit a radial organization in branching tubules that form liver parenchyma (Gonzalez *et al*., 1993; Rocha *et al*., 1997; Gladys *et al*., 2006; Naguib *et al*., 2009; Sales *et al*., 2017; Taheri *et al*., 2017). The Kupffer cells are present in the hepatic sinusoids, the liver parenchyma of the European hake consists of three lobules, it continuous, with no distinct boundaries, and the hepatocytes are arranged in narrow irregular plates which radiate from the central vein and alternate with numerous sinusoids and contain numerous lipid droplets in their cytoplasm (Bocina *et al*., 2016). Liver parenchyma in garfish consists of the anastomosing plates of hepatocytes separated by sinusoidal capillaries, the sinusoidal capillaries open into the central vein. Hepatocytes are round in shape, with usually one nucleus and lipid droplets in the cytoplasm (Bocina *et al*., 2017), the hepatocytes in black scorpionfish, surrounded by adipocytes, and contain large, spherical nucleus with multiple nucleoli, the nucleus is usually centrally positioned and surrounded by a narrow layer of cytoplasm, the arrays of hepatocytes are separated by system of sinusoidal capillaries (Nazlic *et al*., 2014). The hepatocytes in *Trichomycterus brasiliensis* are polyhedral, with granular cytoplasm, their nuclei are spherical and centrally, the tissues have many blood vessels, some portal areas, and dispersed bile ducts (Malarkey *et al*., 2005). The gall bladder of carp is a large sac situated on the right side of cranial part of the intestinal bulb (Lakshmaiah, 2016). The aim of this study was to describe the histological and histochemical features of the liver and gall bladder of common carp to become available basis data for further sciences.

## Materials and Method

The study was performed using fifteen of healthy male adult common carp *Cyprinus carpio* during March and April 2019. Were catching alive from the AL Forat river, with age about (7- 12) months, mean their standard length was (50.4 ± 3.1) cm, immediately after death (Kadhim *et al*., 2019), liver and gall bladder were removed through a longitudinal incision in the abdominal wall and dissected out, 5 specimens from different regions of the liver and gall bladder were taken and fixed by 10% formalin 24 hours at room temperature, and then treated by routine histological processing, embedding with paraffin wax (58-60 ℃) and

sectioning to 5-7µm. The stains were used, Harries Hematoxylin and Eosin for demonstrating the general histological components, Masson's trichrome stain was used for nuclei, collagen, muscles and Periodic acid-Schiff (PAS) for glycoproteins (Luna, 1968), The slides were then dipped in xylene and mounted with cover slip using DPX mounting medium. The slides were examined under light microscope to study the general histology and histochemistry features of liver and gall bladder. The mean thickness of capsule, diameter each of central vein, hepatic artery and hepatic duct, the mean and the standard error were calculated for 5 slides for each region of liver (Al-Rawi and Kalaf-Allah, 1980).

## Results

The structure of the liver of the common carp was consists of continuous mass of cells called hepatocytes. The hepatic cells were large, polyhedral and densely granulated with abundant glycogen and centrally located distinct nuclei, the liver cells mainly composed of a continuous compact field of hepatocytes, and scattered with connective tissue enclosing the bile duct and vessels (Fig.1-7). The liver parenchyma of the carp contains hepatocytes, numerous sinusoids that separated the hepatocytes and found central vein, and not observed of Kupffer cells (Fig. 2,3). The central veins are so irregularly dispersed that a of the portal triads. Sinusoids were collapsed being filled with glycogen, hepatocytes appear very vacuolated and pale in hematoxylin and eosin section (Fig. 1-3). Veins are scattered through the liver parenchyma without a well defined arrangement, and they are surrounded by hepatic parenchyma or pancreatic tissue, sometimes accompanied by an artery or a bile duct (Fig. 2). the liver was covered by a thin capsule with simple squamous epithelium and thin connective tissue as fibroconnective tissues (Fig.5,6,7). The main stored substances in liver cells are glycogen and lipids, the appearance of vacuolar structures in the hepatic cells, probably due to the presence of lipids (Fig.5,6). The mean thickness of capsule, diameter each of central vein, hepatic artery and hepatic duct of cranial, middle and caudal lobes of liver were (187.2 ± 1.5), (627.9 ± 1.9), (167.7 ± 2.1) and (329.6 ± 2.5); (152.3 ± 1.2), (587.3 ± 2.5), (153.9 ± 2.9) and (311.2 ± 2.7) and (76.2 ± 0.5), (317.2 ± 0.9), (87.1 ± 2) and (119.6 ± 1.5) µm respectively (Table 1). The wall of gallbladder of common carp in this study was composed of only three layers; mucosa, muscularis and serosa. The narrow mucosal layer, was inner layer lined the lumen, it composed of a only two layered structure containing simple columnar epithelium and lamina propria, no has muscularis mucosae, The mucosal epithelium is simple columnar composed of enterocytes with brush border, there are no goblet cells between them, and there were no mucosal folds in wall of the gall bladder. Lamina propria, is a connective tissue layer adjacent to the epithelium, thus separating the mucosa from the muscularis externa, and composed of layer of the dense, irregular connective tissue, has large blood vessels, lymphatic vessels, adipose tissue, also rich with collagen fibers, muscularis externa, consist of one thin circular layer of the smooth muscle cells (Fig.8). Serosa, appeared as thin layer of loose connective tissue cover by a layer of mesothelial cells of the visceral peritoneum, thin layer of simple squamous epithelium, the serosa was cover the muscularis externa (Fig. 8). Histochemically, The collagen fibers of capsule of the liver by Masson trichrome stain were pink in color (Fig.5), hepatic cells were positive to periodic acid Schiff stain, the hepatocytic cytoplasm had granules (Fig. 4,6,7).

**Table 1 :** Measurement of thickness of capsule, diameter of central vein and hepatic artery of the liver of common carp (µm) (X± S.E).

| Part / Measure | Cranial lobe of liver | Middle lobe of liver | Caudal lobe of liver |
|---|---|---|---|
| Thickness of capsule | 187.2 ± 1.5 | 152.3 ± 1.2 | 76.2 ± 0.5 |
| Diameter of central vein | 627.9 ± 1.9 | 587.3 ± 2.5 | 317.2 ± 0.9 |
| Diameter of hepatic artery | 167.7 ± 2.1 | 153.9 ± 2.9 | 87.1 ± 2 |
| Diameter of hepatic duct | 329.6 ± 2.5 | 311.2 ± 2.7 | 119.6 ± 1.5 |

## Discussion

Similar to the mammals liver, the Teleost liver plays an important role in the metabolic homeostasis of the body, this role includes the processing of carbohydrates, proteins, lipids, and vitamins. In addition, it also plays a key role in the synthesis of serum proteins such as albumin, fibrinogen, complement factors, and acute-phase proteins, and the liver play important functions in lipid storage, also for the production of bile for intestinal lipid breakdown, as well as for the breakdown and excretion of metabolic products, and detoxification processes (Speilberg and Nafstad, 1994; Akiyoshi and Inoue, 2004; Hoehne-Reitan and Kjorsvik, 2004; Vicentini *et al*., 2005; Nejedli and Gajger, 2013; Pronina *et al*., 2014; Al-Taai *et al*., 2016). The fish liver differs from the mammals liver in that the hepatocytes are not clearly organized in cords or lobules and the typical portal triads are not apparent (Faccioli *et al*., 2014). Liver parenchyma in garfish consists of the anastomosing plates of hepatocytes separated by sinusoidal capillaries. The sinusoidal capillaries open into the central vein. Hepatocytes are round in shape, with usually one nucleus and lipid droplets in the cytoplasm (Bocina *et al*., 2017). The biliary system also differs from that of mammals in that intracellular canaliculi occur, which eventually anastomose to form bile ducts. The bile ducts fuse and ultimately form the gall bladder, which contains greenish bile that is conducted to the intestine via the common bile duct (Karachle and Stergiou, 2010). The liver of carp is composed of a parenchyma covered by a thin capsule of connective tissue, same as in *Oligosarcus jenynsii* (Gladys *et al*., 2006). In the present study, absence of division into hepatic lobules and the lack of portal triads as also recorded in many teleosts (Faccioli *et al*., 2014; Sales *et al*., 2017). The hepatocytes are large in size, polygonal in shape with homogenous granular cytoplasm and centrally located distinct nuclei, similar to (Geyer *et al*., 1996; Tripathi *et al*., 2012; Kasoni *et al*., 2017; Mustafa *et al*., 2017), no Kupffer cells were observed in liver of common carp, that agree with (Nazlic *et al*., 2014) in *Scorpaena porcus*, but not agree with (Bocina *et al*., 2016), who mention the Kupffer cells found in liver of hake. The liver parenchyma of common carp contained tubular acinar glands which constituted the exocrine pancreas forming the

hepatopancreas. This arrangement also in garfish (Bocina *et al*., 2017). However, the hepatopancreas is not observed in higher vertebrates (Kasoni *et al*., 2017).

The wall of gall bladder, consist of mucosa, muscularis and serosa, similar that in European hake (Bocina *et al*., 2016) and in *Scorpaena porcus* (Nazlic *et al*., 2014), but in *Catla caltla*, consist of mucosa, submucosa and muscularis (Tripathi *et al*., 2012). The mucosa is lined by simple columnar epithelium, muscularis, is made of one circular layer of smooth muscle cells, serosa is made of mesothelial layer, similar to (Tripathi *et al*., 2012) in *Catla catla*; in black scorpionfish (Nazlic *et al*., 2014), but (Bocina *et al*., 2016) mention the muscularis externa in hake consists of two muscular layers; the inner circularly and outer longitudinally arranged muscles. The capsule of the liver is positive with Masson Trichrome stain, and the hepatic cells, take positive reaction with periodic acid-Schiff, that similar with (Oliveira-Ribeiro and Fanta, 2000) in *Trichomycterus brasiliensis*, Some regions of the hepatic cells are weakly positive to the periodic acid-Schiff due to some glycogen stored in their cytoplasm (Malarkey *et al*., 2005).

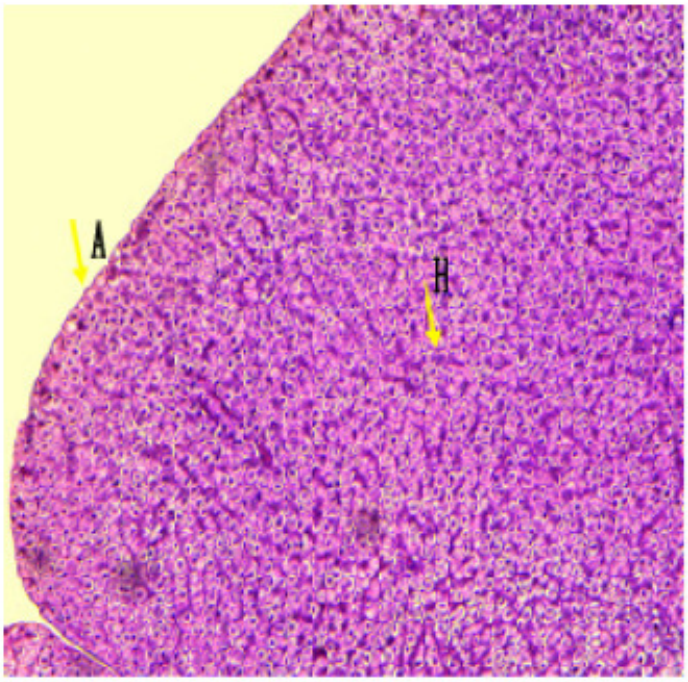


**Fig. 1 :** Cross microscopic section of cranial lobe of liver : A. Capsule, H. hepatocyte, H&E stain (X200).

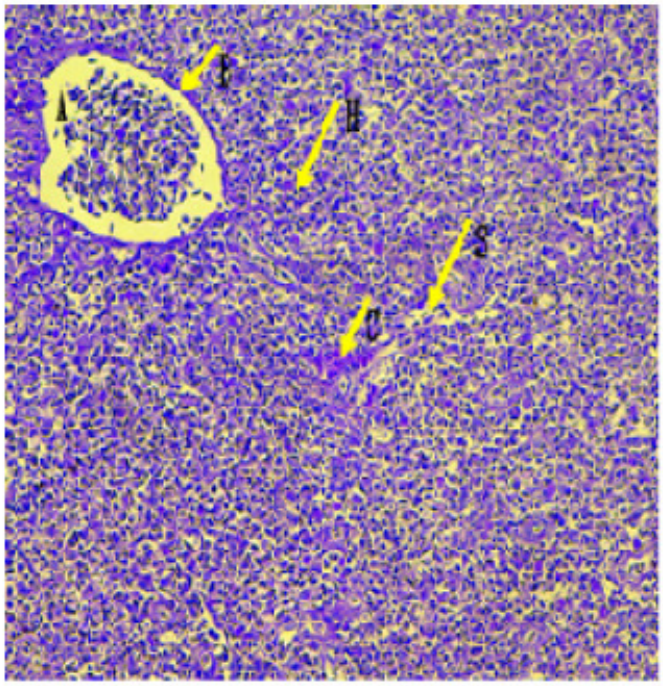


**Fig. 2 :** Cross microscopic section of caudal lobe of liver: (A). central vein, (B). epithelium, (C). glycogen granules, (H). hepatocyte, (S). blood sinusoid, PAS stain (X400).

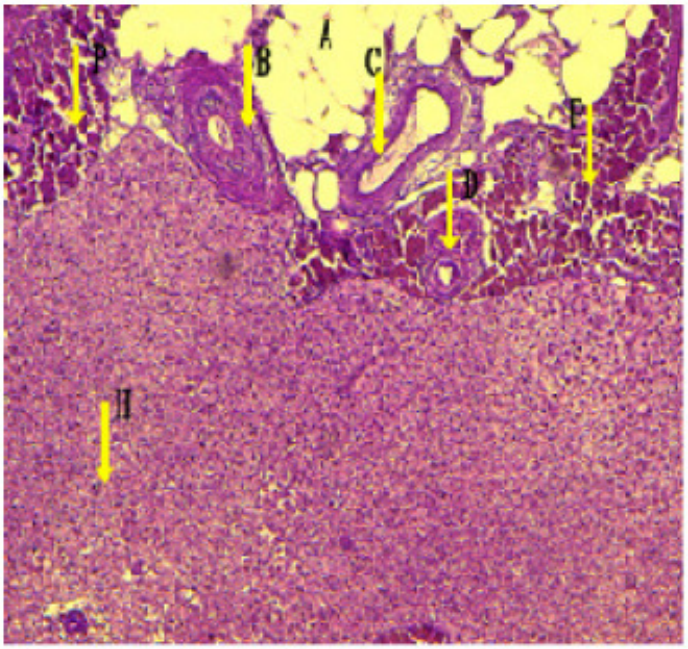


**Fig. 3 :** Cross microscopic section of middle lobe of liver: A. adipose tissue, B. bile duct, C. central vein, D. hepatic artery, H. hepatocyte, P. exocrine acini of pancreas, **H&E stain (X 200).**

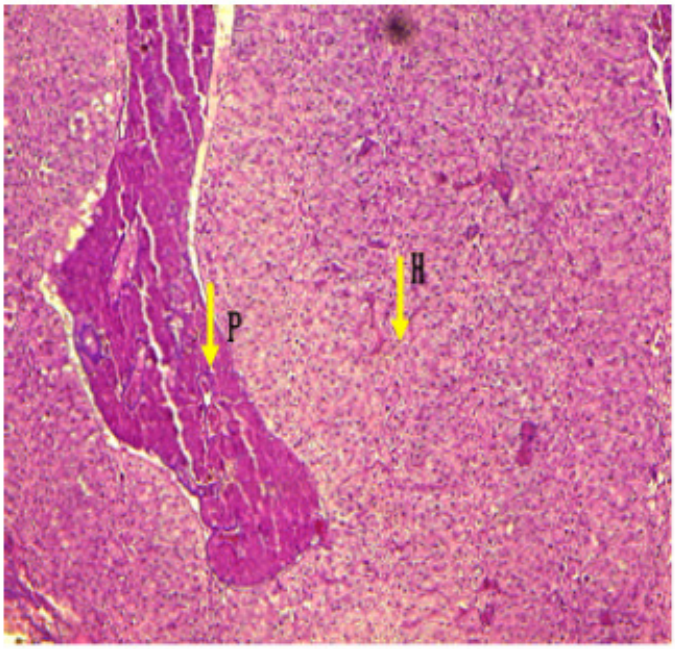


**Fig. 4:** Cross microscopic section of cranial lobe liver: H. hepatocyte, P. part of pancreas, **H&E stain (X 200).**

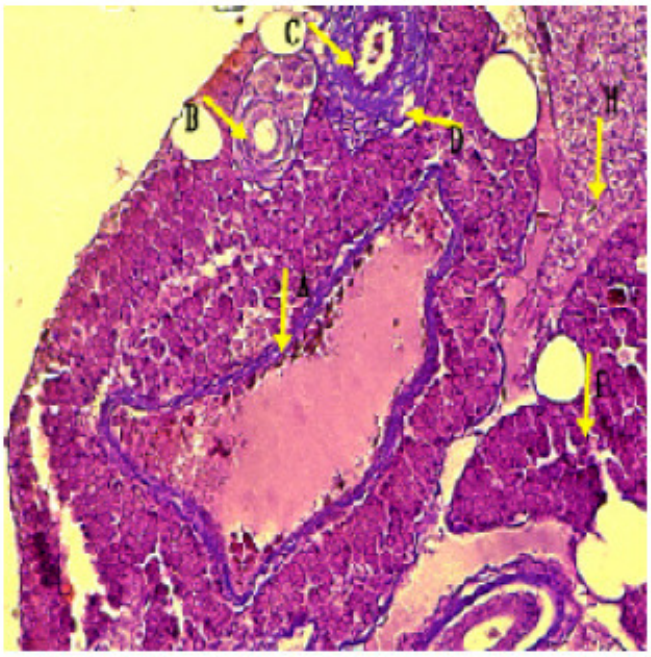


**Fig. 5 :** Cross microscopic section of middle lobe of liver: A. central vein, B. bile duct, C. hepatic artery, D. portal vein, H. hepatocyte, P. exocrine acini of pancreas, **H&E stain (X 400).**

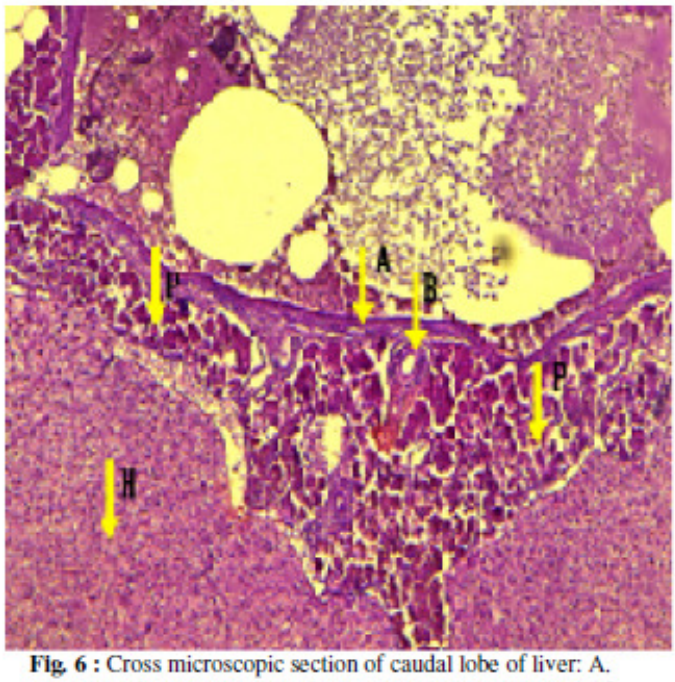


**Fig. 6 :** Cross microscopic section of caudal lobe of liver: A. septum, B. hepatic artery, H. hepatocyte, P. exocrine acini of pancreas, **H&E stain (X 200).**

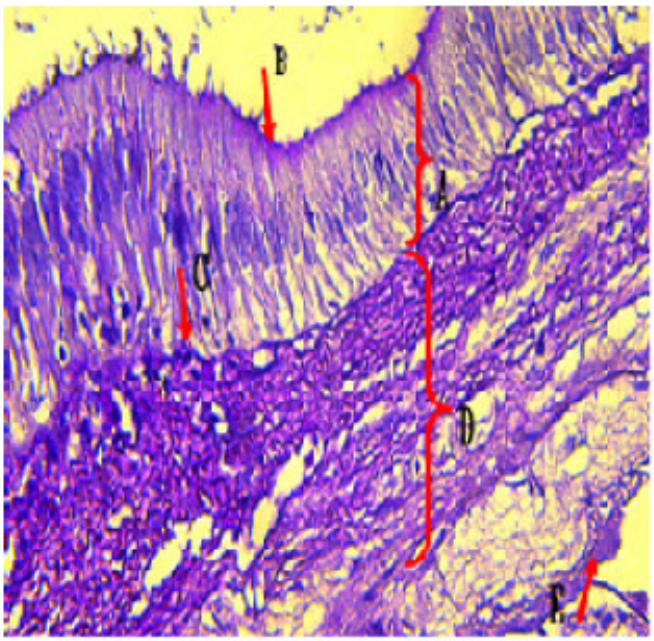


Fig. (7): Cross microscopic section of gall bladder: A. epithelium, B. brush border, C. basement membrane, D. muscularis, E. serosa, **H & E stain (X 200).**

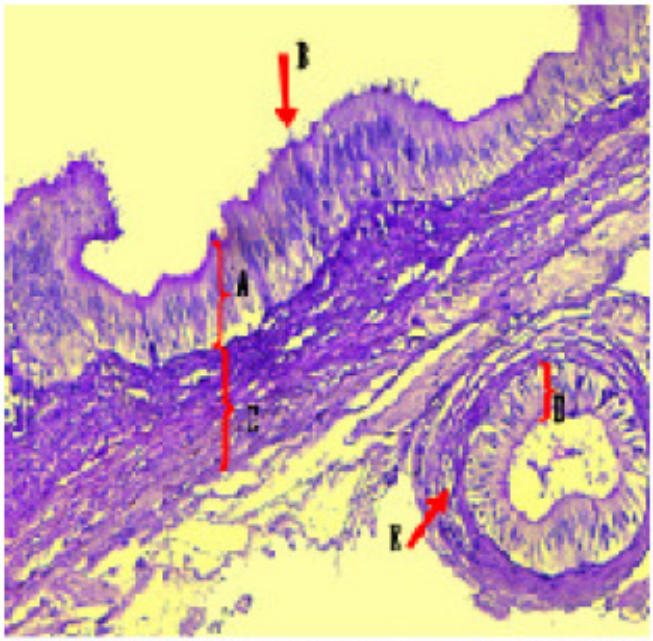


**Fig. 8 :** Cross microscopic section of gall bladder: A. epithelium of bladder, B. brush border, C. muscularis, D. epithelium of duct, E. blood vessel, **H & E stain (X 200).**

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