

hgMorphology ,Histology and Histochemical comparative study of the Liver in adult female domestic fowl(*Gallus Gallus*), common moorhen(*Gallinula chloropus*)in South Iraq

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Abstract

The aim of this study was determined compared between morphological features and histological structures in the liver of two species of birds that differ in their classification and food habitat by using light microscope. This birds were domestic fowl (*Gallus Gallus*), common moorhen (*Gallinula chloropus*). Twenty birds were used to conduct the current study. These birds were collected from common markets at Basrah province. The morphological result showed the liver in both species was large and bilobed organ. It was consist of two parts (right lobe was larger than left lobe). The left lobe is subdivided into two parts. The histological structure of the organ consist of several lobules separated from each other by tiny trabeculae of connective tissue and was covered by connective tissue capsule. The liver tissue composed of hepatic cells and blood sinusoids in two species of birds. The basic unit of liver is hepatocyte which arranged in plates that radiated around the central vein and between these cords there is sinusoids lined by a layer of fenestrated endothelial cells and Kupffer's cells. The histochemical result revealed that the hepatocyte in the two species of birds were weakly positive to periodic acid Schiff (PAS) while they were positive to bromophenol blue reaction (MBB).

Key words: liver, histology, histochemical, , *Gallus Gallus*, *Gallinula chloropus*

دراسة مظهرية, نسيجية وكيمياء نسيجية مقارنة للكبد لنوعين من الدجاج البالغ دجاج اللحم
المحلي ودجاج الماء في جنوب العراق .

عادل جبار حسين ديار محمد حسين كاظم

فرع التشريخ والانسجة كلية الطب البيطري جامعة البصرة البصرة العراق
كلية الطب البيطري جامعة المتنى المتنى العراق

المخلص

هدفت الدراسة الحالية للمقارنة بين الصفات المظهرية والتراكيب النسيجية لنوعين من الطيور التي تختلف في التصنيف وتغذيتها بواسطة المجهر الضوئي. وهذه الطيور هي دجاج اللحم المحلي ودجاج الماي. واستخدمت في هذه الدراسة عشرون طيرا . هذه الطيور جمعت من السوق المحلي في محافظة البصرة . اظهرت النتائج المظهرية ان الكبد في كلا النوعين هو عضو كبير وتثنائي الفص يتكون من جزئين هما (الفص الايمن والذي يكون اكبر من الفص الايسر) والفص الايسر يكون مقسم الى جزئين . بينما اظهرت نتائج التركيب النسيجي ان

الكبد يتألف من عدة فصيصات مفصولة عن بعضها بواسطة حواجز صغيرة جدا من النسيج الضام ويكون محاط بمحفظة من النسيج الضام. كما بينت الدراسة ان نسيج الكبد متكون من خلايا الكبدية والجيبانيات الدموية في كلا النوعين من الطيور. الوحدة الأساسية في الكبد هي الخلايا الكبدية والتي تكون مرتبة بشكل صفائح شعاعية حول الوريد المركزي وبين هذه الحبال توجد الجيبانيات التي تبطن بطبقة مثقبة من الخلايا الاندوثيلية. في حين اظهرت نتائج الدراسة الكيمياء النسجية وجود تفاعلا موجبا ضعيفا مع تقنية حامض البريوديك- كاشف شيف بينما اظهرت تفاعلا موجبا مع ازرق البروموفيلول للخلايا الكبدية في كلا النوعين.

Introduction

The poultry production has full a main role between agricultural industries in several parts of the world, Chicken meat production has been increase in all regions with the principal in Asia and South America, The Asia is important the world in poultry meat production, followed by North and Central America which had the lead until 1990 (Daghir, 2008). Poultry meat is the then maximum extensively eaten meat in the world, accounting for about 30% of meat production worldwide (Raloff, 2003).

The liver is the large organ of the body and the largest gland tissue. Its roles include absorption of nutrients, production of bile, detoxification and keep of the body metabolic homeostasis which contains the processing of carbohydrates, proteins, lipids and vitamins. The liver also role in the synthesis of plasma proteins, like albumin, fibrinogen, and complement factors (Genten *et al.*, 2009).

The liver in avian provides exocrine secretions to the digestive tract and its exocrine secretion is called bile, which mix fats and raise the pH of the duodenal digest, Bile is created in the hepatocytes and secreted into bile canaliculi situated on the lateral surfaces of connecting liver cells, These canaliculi drain into interlobular ducts, which unite to form the right and left hepatic ducts, which in turn drain into the gall bladder (Hoppe, 1999).

The adult chicken usually has a dark red to brown red colored liver (Clark, 2005). The liver is separated into right and left portions which are fused

cranially at the midline, the right lobe is larger in the domestic fowl and turkey the left lobe is divided into the dorsal and ventral parts (Whitlow, 2000). The parenchyma of liver in birds resemble the liver of mammalian but there is some unlike in histological features such as lacking of lobules and interlobular trabeculae, the principal cell of liver is the hepatocyte. (Caceci, 2006).

The liver of coot bird (*fulica atra*) is resample to the liver of fowl that it lies in the ventral part of the body cavity and appears red-brown in color and separated into two lobes, right and left, Histologically the liver of coot bird surrounded by thin capsule of connective tissue that continue to subdivided the liver into lobules, The hepatocyte which arranged radially around the central vein, These cells are polygonal in shape and have rounded nucleus and there is present of sinusoids between hepatocyte which lined by flattened endothelial cells. (Hanan, 2013).

The liver of Mallard showed result histochemical study formed differ size of glycogen granules in cytoplasm of hepatocytes and in some specimen the glycogen large granules arranged around the central vein because the bird in starvation period. (Maha, 2015).

The aim of the study was determined compared between domestic fowl (*Gallus Gallus*) and common moorhen (*Gallinula chloropus*) morphological, histological, histochemical finding and

measurement of some structures in the liver .

Materials And Methods

A total twenty birds consist of two group ,each group contain ten domestic fowl (*Gallus Gallus*),ten common moorhen (*Gallinula chloropus*) were used in this study. These birds were collected from common markets at Basrah province, this study carried out in college of veterinary medicine/University of Basrah. The birds anesthesia by the drug Diazepam at a dose of (5 mg / kg) of body weight (Shindala,1999).. Liver were isolated from the celomic cavity the organ cut into pieces (5cm). The specimens were immersed in 10% neutral buffered formalin solution for 2 days, after well fixation the specimens was dehydrated by (70%, 80%, 90%, 95% and 100%) ethanol Alcohol each for two hours and then specimens was cleared in xylene for one hour after that embedded in paraffin wax and then the blocks were sectioned serially at 6µm thickness and stained with following stains. Mayer's Hematoxylin and Eosin routine stain for general features identification, Lillies Allochrome stain for connective tissue and Masson's Trichrome stain for the staining of the collagenous and smooth muscle fibers (Bancroft and Stevens, 2010). Periodic Acid schiff (PAS) stain It is especial stain used for carbohydrate, muco-proteint, glycoprotein and basement membrane (Luna,1968), Mercury Bromophenol blue (MBB) stain for protein detection (Widhi Dubey and Trivedi ,2012). The measurement of each structure by ocular micrometer to histometrical analysis.

Result

A gross examination of the liver in the two species of birds is largest gland in

domestic fowl (*Gallus Gallus*), common moorhen (*Gallinula chloropus*). It located caudally and ventrally to the heart and was associated with the gizzard that lied in the mid-coelomic cavity of the body. The liver have two surface. This surface character smooth convex ,concave and the gland subdivided two parts right and left lobe. The lobe left declines represent proventriculus sites, part of the gizzard and spleen as there are declines in the right lobe also represent sites both part of the gizzard and both ends of the duodenum upward and downward. (fig.1)

The liver of the domestic fowl (*Gallus Gallus*) was larger than the common moorhen (*Gallinula chloropus*) and it was dark red in colour and consisted of left and right lobes which were joined cranially at the midline by an interlobar portion. The left lobe has concavity in its top where the heart stabilized, and it was subdivided two secondary lobes (dorsal and ventral) the dorsal lobe was wider and shorter than the ventral lobe, while the right lobe has concavity from its ventral side where the gizzard was stabilized, and it didn't contain the incision that divided it into secondary lobes as it is in the left lobe. (fig. 2,A)

The liver of the common moorhen (*Gallinula chloropus*) was appears red-brown in color and divided into parts this of left and right lobes like the liver of (*Gallus gallus*) but it is smaller in size. The left lobe was subdivided into the dorsal and ventral parts smaller in size from the right lobe and it has concavity in its top where is the gizzard was stabilized .The right lobe is greater and longer than the left lobe. (fig. 2,B)

The histological our results of this study revealed that the liver in the two species of birds were covered of capsule which is composed of irregular

dense connective tissue and collagen with some elastic fibers with Mayer's Hematoxylin and Eosin (fig.3,A) and showed with the Masson's trichrome stain (fig.3,B).

This capsule appears thicker in the common moorhen (*Gallinula chloropus*) was $(165 \mu\text{m} \pm 12.8452)$, whereas, in the (*Gallus gallus*) was $(72.5 \mu\text{m} \pm 17.5000)$. The capsule called Glisson's capsule and that separated the gland into basic units that hepatic lobules. The lobulation that not clear in the birds because the ill distinct hepatic septa and found between the liver lobules the portal area which consist of branch of portal vein and branch of the hepatic artery and bile duct that lining by one layer cuboidal cells resting on basal lamina (fig.3,D). (fig.4,D)

The mean diameter of center vein in domestic fowl (*Gallus Gallus*), common moorhen (*Gallinula chloropus*) were $(735 \mu\text{m} \pm 42.9535)$ and $(445 \mu\text{m} \pm 42.0119)$ respectively.

The mean diameter of portal vein, hepatic artery and hepatic duct in domestic fowl (*Gallus Gallus*) were $(320.79 \mu\text{m} \pm 202.284311)$, $(250 \mu\text{m} \pm 26.4575)$ and $(315 \mu\text{m} \pm 22.9129)$ respectively, whereas in common moorhen (*Gallinula chloropus*) were $(610.19 \mu\text{m} \pm 193.056338)$, $(235 \mu\text{m} \pm 28.3725)$ and $(395 \mu\text{m} \pm 32.3265)$ respectively.

The parenchymal of liver consist of hepatocytes constitute parallel cords to the capsule whereas it was arranged radially inward composing small lobules and acini. This hepatocytes it is polyhedral in shape, and have rounded nucleus and this plates are separated by blood sinusoids and arranged in radiation form around central vein. (fig.3,A) (fig.4,A)

Also observed the presence of blood sinusoids in the liver and the board that

are lined with two types of cells, the first type cells are flattened endothelial cells which are nuclei elongated, dark-colored, linked to this type cells with each other loosely. The second type of cells is Kupffer cells, which are drenched in cavities of blood sinusoids as phagocyte cells and nuclei which are spherical in shape dark, cytoplasm are projections and extends this as larger than the previous type cells.

It showed the branches of portal triads that lined by endothelial cells and surrounded by some smooth muscle fiber (fig.5,B) so that consisted hepatic vein which features thin wall and large lumen while hepatic artery that features thick wall and small, winding cavity and contain internal elastic membrane as for bile duct were detected at the periphery of parenchymal lobules which lined by simple cuboidal epithelium and surrounded by a loose connective tissue. There are smooth muscles around larger ducts.

Histochemically, The hepatocytes were weakly positive to periodic acid Schiff (PAS) reaction in the two species of birds. (Fig.5,A) (fig.6,A) The hepatocytes in the study of birds showed a positive reaction with bromophenol blue reaction (MBB) stain for the proteins which showed the collections of protein inside the hepatocytes. (Fig.5,C) (fig.6,B)

Discussion

The morphological results in this study in the liver of the two species of birds was a red bilobed and large organ that lied in the mid-coelomic cavity, these agreed with (Whittow, 1998; Schmidt *et al.*, 2003) such as captive bustards (Bailey *et al.*, 1997), ostrich (Illanes *et al.*, 2006; Stornelli *et al.*, 2006) and other vertebrates (Petcoff *et al.*, 2006; Seyrafi *et al.*, 2009; Monsefi *et al.*, 2010; El-Bakary and El-Gammal, 2010; Alshamarry *et al.*, 2010).

The liver shows in this study the presence of two secondary lobes in the end of the left lobe of the liver. The later result also disagrees in the ostrich where the left lobe of the liver was subdivided into three secondary lobes while the right lobe was undivided (Stornelli *et al.*, 2006) while there were no further lobular subdivisions in the liver of Houbara Bustards (Bailey *et al.*, 1997) and in contrast with (Nicke *et al.*, 1977) who described that the left lobe of Passenger Pigeon liver consists of three parts, distal processes a small median one, and one upon either side of double its size, the result agreed with (Shaymaa, 2015) that described the liver in *Coturnix coturnix* was the left lobe divided into two portions, dorsal and ventral lobe.

Histologically, our result of the liver in study birds is large lobed lined by capsule which composed of irregular dense connective tissue that continues to subdivide the liver into lobes and appears thicker in the common Moorhen (*Gallinula chloropus*) these agreed which appears thicker in the *Larus canus* (Hani *et al.*, 2013). This capsule also reported in other birds and vertebrates (Sheybani and Adibmoradi, 2002; Schmidt *et al.*, 2003; Illanes *et al.*, 2006; Firmiano *et al.*, 2011 and Xie *et al.*, 2011).

The parenchyma of liver showed an arrangement consisting of hepatocytes, which were arranged in either cords one cell or two cells thickness around sinusoids. While it resembled the results of the studies that have

described for many vertebrates that have hepatocyte cords consisted of one - two cell thickness (Stornelli *et al.*, 2006; Bertolucci *et al.*, 2008; El-Bakary; El-Gammal, 2010; Sayrafi *et al.*, 2011 and Xie *et al.*, 2011 and Eyhab *et al.*, 2016). The hepatocytes vary in shape among the two species, the reason of that may be due to the difference of each animal species (Alshamary *et al.*, 2010).

Lumen of sinusoids contained erythrocytes and macrophages also the wall of sinusoids contained Kupffer cells which are important for the maintenance of liver functions under physiological and pathological circumstances (Wardle, 1987). They play an important role in the modulation of immune responses via antigen presentation and suppression of cell activation and proliferation of T-cells (Sun *et al.*, 2003)

Histochemically, showed that the hepatocytes were weakly reactive to PAS and positive reaction with MBB in the two species of birds. These results check the importance of the liver in the metabolism of fats, carbohydrates and other nutrients and this also agreed with the results of previous studies on the liver in different vertebrates (Akiyoshi and Inoue, 2004; Petcoff *et al.*, 2006; Seyrafi *et al.*, 2009; El-Bakary and El-Gammal, 2010; Immanuel and Palavesam, 2010; Firmiano *et al.*, 2011; Sayrafi *et al.*, 2011 and Xie *et al.*, 2011).

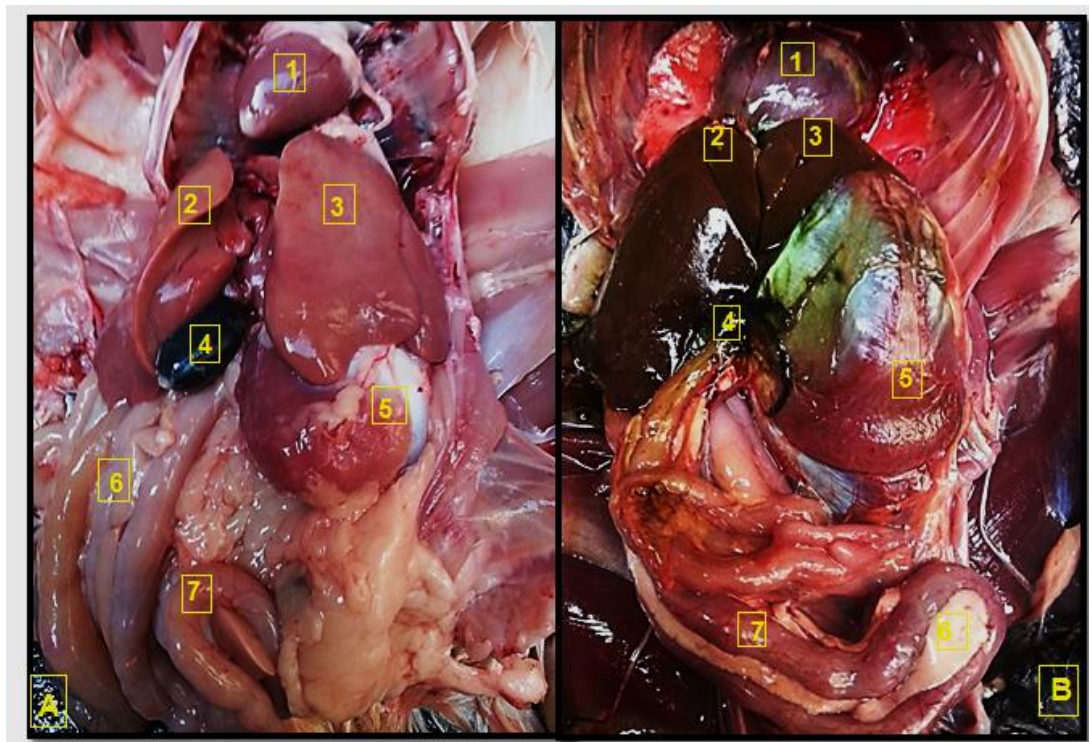


Fig.(1):Dissected of abdominal cavity show the position and relation of liver : (A)-domestic fowl (*Gallus Gallus*) , (B)common moorhen(*Gallinula chloropus*):-1-Heart, 2- right lobe of Liver, 3- left lobe of Liver, 4-gall bladder, 5- Ventricular ,6- Pancreas and 7-Duodenum.

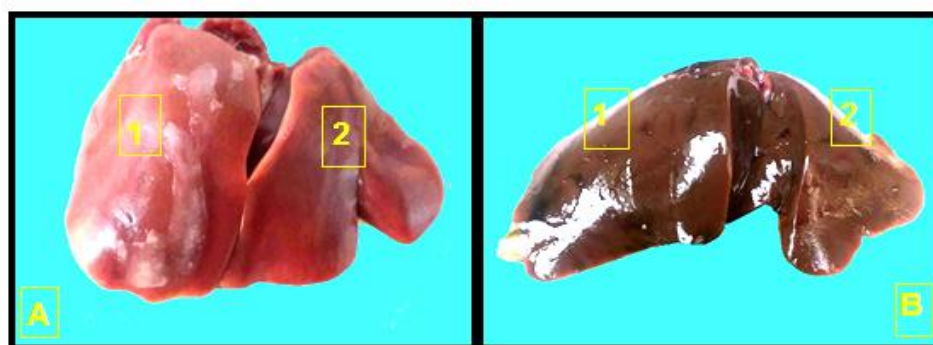


Fig. (2) The liver showing: (A) domestic fowl (*Gallus Gallus*) .(B) common moorhen (*Gallinulachloropus*). 1- Right lobe 2- left lobe.

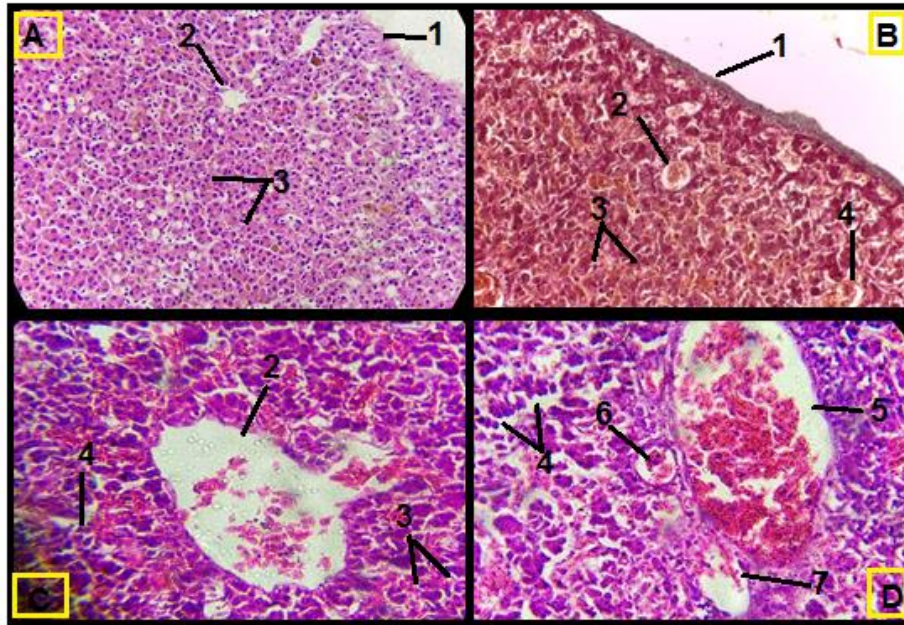


Fig. (3):Cross section of the Liver in (*Gallus Gallus*) showing:
 (A) 1. Capsule , 2. Central vein, 3.Hepatocyte (H & E stain X40).
 (B) 4. Sinus (Masson's Trichrome stain, X100)
 (C) (H & E stain X400)
 (D) 5.portal vein, 6. Hepatic artery and 7.Bile duct (H & E stain X100).

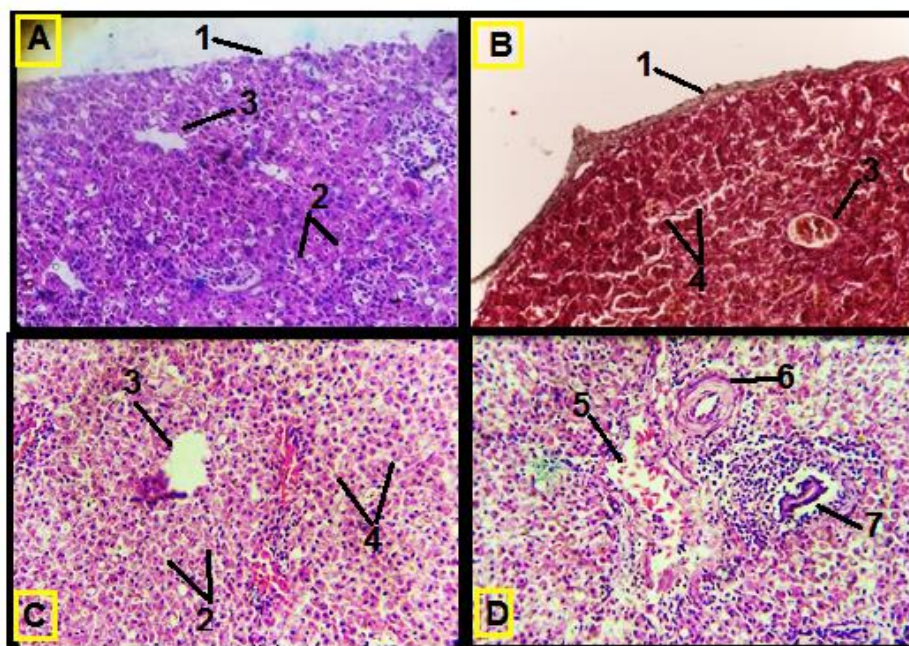


Fig. (4): Cross section of liver in (*Gallinula chloropus*) showing:
 (A) 1- Capsule, 2- Hepatocyte 3- Central vein (H & E stain X40).
 (B)4-liver sinusoid (Masson's Trichrome stain, X100).
 (C) (H & E stain ,X400).
 (D) 5-portal vein 6- Hepatic artery and 7- Bile duct (H & E stain ,X100).

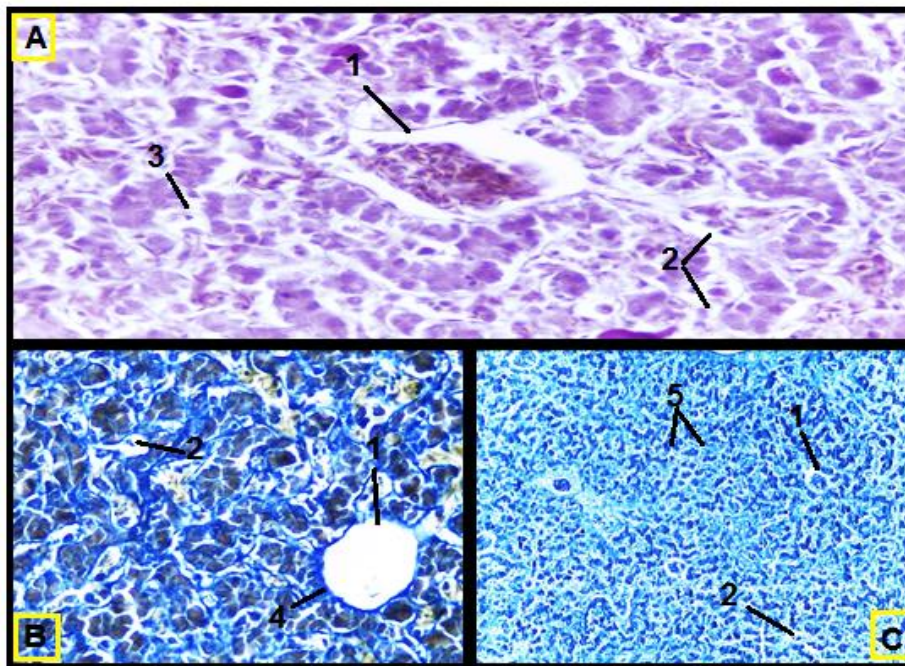


Fig. (5) Cross section of Liver (*Gallus Gallus*) showing that :
 (A) 1-central vein 2- liver sinusoid, 3- Kupffer's cells (PAS stain X400).
 (B) 4- endothelial cell (Lillies Allochrome stain, X100).
 (C) 5- Hepatocyte (MBB stain X40).

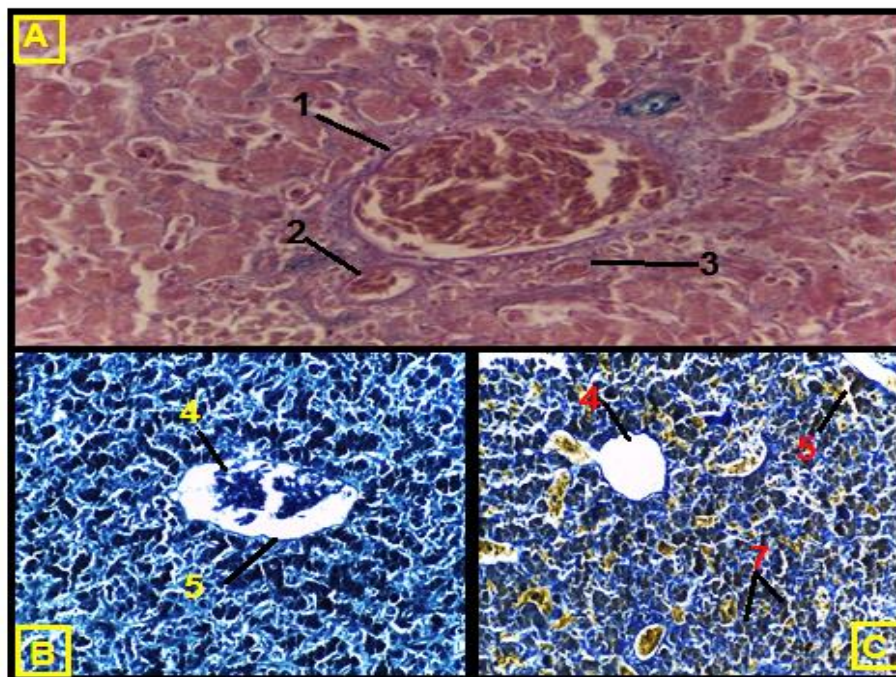


Fig.(6) Cross section of the Liver (*Gallinula chloropus*) showing that :
 (A) 1- Hepatic portal vein, 2- Bile duct and 3- Hepatic portal artery (PAS stain X400).
 (B) 4- central vein and 5- endothelial cell (MBB stain, X400).
 (C) 6- liver sinusoid 7- Hepatocyte (Lillies Allochrome stain, X100).

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