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Gross Anatomy And Morphology Of Egyptian Water Buffalo's Liver (*Bubalus Bubalis*) With Reference To Some Histochemical And Immunohistochemical Evaluation.

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ABSTRACT

Quite recently, considerable attention has been paid to water buffalo species (*Bubalus bubalis*) and its economic importance. Liver is complex organ and susceptible to many diseases. So, this paper concentrated on the buffalo liver in terms of their anatomy, histology, histochemistry and immunohistochemical characteristics of its cells, with regard to the functional importance. 20 samples of fresh buffalo liver selected, 5 injected by latex for anatomical studies, 5 injected by urographine for radiograph pictures and the last 10 directly fixed in neutral buffered formalin 10% and processed for histological, histochemical and immunohistochemical examination. The result revealed that Portal vein divided into three main branches right, left and caudal omental ones. The hepatic vein branches classified as right, middle and left branch. The common bile duct formed by union of right hepatic duct, left hepatic duct and cystic duct. The liver of buffalo showed a well fibrous capsule of Glisson's with high amount of collagen fibers by Masson's Trichrome stain. Reticular fibers found to have dispersed characteristic shape with Gomori's method. PAS and Best's carmine stains gave positive results. Perl's Prussian demonstrated the presence of hemosiderin and ferric salts in von kupffer cells. These cells stained positive to CD68. Anti-insulin and anti-glucagon showed positive reaction. CK7 and CD10 were immune positive to biliary epithelium.CD34 gave positive to portal endothelial cells. Alfa smooth muscle actin showed unique normal positivity along the hepatic tissue. The results obtained, not only contribute to the knowledge of buffalo species; but also define a normal structure reference for the diagnosis and treatment of liver diseases.

Keywords: *Bubalus Bubalis,* Water Buffalo's Liver, Histochemical

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INTRODUCTION

Buffaloes' values increase day by day as they grow. They need no costly fuel, never rust, and they reproduce. In Egypt, buffaloes play an important role in domestic economy and trade through their effective and powerful participation in the largest proportion of dairy production especially since it is distinguished from other animals by low cholesterol and higher total solids content (protein, fat, minerals). Not only milk production is the only reason for their value, but also have a high proportion of the production of cheap and high quality meat (tasty, lean, low fat content) (Ibrahim, 2012).

Buffalo liver is one of the vital organs of the body (Kelly, 1993) as it is a rich and economic source of essential nutrients especially vitamin A, foliate, riboflavin, zinc, iron and etc. (Devatkal, 2003).

The functional venous vasculature of the ruminant liver is coming from hepatic portal vein and its drainage is accomplished by the help of convergent sinusoidal system to the central vein and then to the hepatic veins (Bank, 2007). The portal vein is formed by the union of tributaries draining the digestive tract, pancreas and spleen in buffalo liver (Ranjbar and Ghadiri, 2011) while the hepatic veins returned functional and nutritional blood of the liver and opened into the caudal vena cava in ruminant (Klages, 1962; Arnautovic and Kremar, 1964; Abdalla et al., 1971; Habel, 1975; Anis, 1977; Mobarak, et al., 1979; Brikas and Tismatas, 1980; Hagras and Osman, 1986; Hagras and Swielium, 1990; Farag, 1990; Santos et al., 1991 and Awad, 2000). Liver biliary system consisted of bile canliculi, intra hepatic bile ducts and extra hepatic bile duct (Abd El-Hady, 2002).

The mammals liver parenchyma is made up of a complex network of epithelial cells, supported by connective tissue and supplied by portal vein and hepatic artery. The hepatic lobule is the structural unit of liver. A roughly hexagonal arrangement of plates of hepatocytes separated by intervening sinusoids which radiate outward from a central vein, with portal triads at vertices of each hexagon (Standring, 2008).

The mammalian liver is a morphologically and functionally complex organ, made up not only of the largely predominant parenchymal cells; hepatocytes, but also non-parenchymal cells, including Ito, Kupffer and sinusoidal endothelial cells as well as other cell types that reside in the sinusoidal compartment (Kmieć, 2001; Malarkey et al., 2005 and Uetsuka et al., 2006).

The perusal of literature revealed that a very limited work has been done on the normal buffalo structure. With regard to this aspect, the present study has been done to elucidate the anatomical, histological, histochemical and immunohistochemical characteristics of the Egyptian water buffaloes filling the knowledge gap and supporting veterinarians in Egypt and all world with basic information to clarify the pathogenesis of the hepatic diseases with its sequential changes on the animal liver preventing tragic loss of livestock as well as their impact on human health.

MATERIALS AND METHODS

Apparently 20 healthy and fresh livers of adult water buffaloes of both sexes were selected from Munib automated slaughter house in Giza during October 2015 to March 2016. The age of the animals and the weight of their livers was between 3 -5 years and 4 -9 Kg respectively. The numbers of samples taken were 20 ones; 10 for anatomical studies and another 10 for histology, histochemistry and immunohistochemical studies.

Gross Anatomical Study

Corrosion casting

10 samples were selected, kept for about 48- 72 h in a refrigerator and casts of portal and hepatic veins were made by injection with latex. The specimens were left in a mixture of 10% formalin and 1% glycerin for seven days before the routine dissection.

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Radiography

By use a cannula into the gall bladder of the liver specimen, the radio opaque suspension (urographin 25%) was injected and the livers were radiographed.

General Histological Study

Ten adult sexually mature apparently healthy buffaloes were used in this study. After slaughtering, the livers were immediately dissected out and sectioned into small pieces. Some of these specimens were fixed in neutral buffer formalin 10% and others fixed in Bouin's fluid. The blocks dehydrated in ethanol, cleared by xylene and embedded in paraffin wax. Serial and step serial sections of 5-6 µm were obtained by rotatory-microtome and stained with Hematoxylin and Eosin (H&E), Masson's trichrome stain for collagen fibers and smooth muscles, Gomori's reticulin stain for reticular fibers, Aldhyde fuchsin stain for elastic fibers (Drury and Wallington, 1980 and Bancroft and Gamble, 2008).

Histochemical Study

Previous paraffin blocks sectioned and stained with periodic acid Schiff (PAS) for detection of neutral mucopolysaccharides, Perl's Prussian blue stain for ferrous and ferric iron, Best's carmine stain for glycogen and Hall's iodine stain for bile (Drury and Wallington, 1980 and Bancroft and Gamble, 2008). The stained sections were observed by light microscopy to record the histological and histochemical features of buffalo liver cells.

Immunohistochemical Study

Deparaffinized sections were autoclaved or incubated in 1% trypsin solution for antigen retrieval. After that, sections were immersed in 0.3% hydrogen peroxide to block internal peroxidase, and in skimmed milk to block non-specific antibody binding. Primary antibodies were PCNA for normal cell mitosis, CD68 for von kupffer cell, CD34 for endothelial cell, CK7 and CK10 for biliary system, alpha smooth muscle actin (α-SMA) for smooth muscle, Anti-glucagon and Anti-insulin for endocrine cells. Biotinylated anti mouse or rabbit immunoglobulin G was applied as secondary antibody. The sections were incubated with peroxidase-labeled streptavidin and visualized in diaminobenzidine-tetrahydrochloride solution then counterstaining was done (Uetsuka et al., 2007).

RESULTS

Anatomical results

Portal vein

After latex injection, the results show that portal vein (Pv) divided immediately up on entering the liver into three main branches right, left and caudal omental ones.

1. The right interlobar branch gave dorsal, ventral and intermediate interlobular veins. Dorsal interlobular vein supplied dorsal part of right lobe, papillary lobe and at the caudate processes of caudate lobe.

- The right dorsal branch (Rd) proceed for about 0.5 -1 cm then it divided into parietal and visceral branches which along its course it gives 8 to 10 smaller branches which arborized in the parenchyma of the process supplying it.
- Ventral interlobular vein (Rv) gives in the middle section of the right lobe the dorsal quadrate branches which supplied the caudodorsal portion of the quadrate lobe and then divided into caudal branches supplying the quadrate lobe and ventral branches supplying the right lobe.
- The intermediate interlobular vein (Ri) had horizontal course and distributed in the diaphragmatic parts of right lobe and papillary process.

2. The left interlobar branch could be considered as the direct continuation of the portal vein which runs at first in the long axis of the liver from porta toward the left lobe and at the boundary between the quadrate and left lobes it bends nearly sharply 80 degree toward the notch for round ligament. So, it showed transverse and umbilical parts.

   - The transverse part that extends from the porta to flexure was nearly 11-12 centimeters and gives off several branches called the omental branches which supplying papillary process, the dorsal border of the liver as well as the caudodorsal part of the left lobe and other quadrate branches to supply quadrate lobe.
   - The umbilical part of the left interlobar branch gave off 3 branches; the dorsal (Ld), intermediate (Li) and ventral (Lv) interlobular branches that radiated into the left and quadrate lobes.

3. The caudal omental branch (Co) supplied the caudodorsal part of papillary process as well as the dorsal portion of the right lobe (Figure 1).

Hepatic vein

They extended from the caudal vena cava (Cv) towards the borders of the liver, generally run perpendicular to the branches of the portal vein, hepatic artery and bile duct system and were embedded deeply in the hepatic parenchyma nearer to the diaphragmatic surface than the visceral surface. In the buffalo, hepatic veins (Hv) divided into; left and middle and right hepatic veins.

A- The left vein (L) collected blood from the left lobe by two radicles; lateral hepatic venule which drained the narrow zone extending along the proximal two thirds of the left lateral border of the liver and medial hepatic venule which along its course give off 4 to7 dorsal and 6 to 9 ventral branches that ramified into the remaining dorsal portion of the left lobe.

B- The middle hepatic vein (M) give in its termination ventral quadrate branch which drained the quadrate lobe and caudal right branch which drained the ventral part of the right lobe furthermore, along its course, it release numerous collateral tributaries which drained the cranial portion of the papillary process and the dorsal part of the quadrate lobe.

C- The right hepatic veins (R) which are, first right hepatic vein that divide into dorsal and ventral branch draining the dorsal partion of the right lobe as well as the caudodorsal angle and the second right hepatic vein that divide into caudate process vein and the caudal continuation of the right hepatic vein draining from caudate process and the right lateral border of the liver respectively. Also buffalo liver has small hepatic veins which receive blood from the dorsal part of the caudate lobe, papillary process and right lobe (Figure 2).

Biliary system

Main bile duct (common hepatic duct) (CBD) is formed by the union of the right and left hepatic ducts and then joins together with cystic duct which comes from gall bladder to form common bile duct that open in the duodenum.

A- The right hepatic duct (RHD) characterized by a shot trunk of about 2 cm long and formed by union of right dorsal branch which drain bile from caudal portion of papillary process and the caudate process, and cystic duct which receive bile from ventral part of right lobe and caudal portion of quadrate lobe.

B- Left hepatic duct (LHD) received along its course omental branches which collect bile from papillary process and dorsal branch of quadrate lobe which collect bile from the dorsal part of caudal portion of the quadrate lobe. Left hepatic duct has transverse part and umbilical part. The transverse part of the left hepatic duct considered as direct continuation of the parent duct and divided into dorsal and intermediate branch. The dorsal branch collects bile from dorsal portion of left lobe and cranial part of papillary process while the intermediate branch receives bile from middle portion of left lobe. The umbilical part of the left hepatic duct has 2 branches; the left ventral branch which collect bile from ventral part of the left lobe and cranial quadrate branch which collect bile from ventrocranial part of quadrate lobe.

C- The cystic duct (CD) showed as direct continuation of the neck of the gall bladder which received right ventral branch and caudal quadrate branch. (Figure 3)

Histological Results

Buffalo liver is composed of polygonal structures called hepatic lobules in which hepatocytes form hundreds of irregular plates arranged radially in cord like pattern around a small central vein (Fig. 4) and between hepatocytes, sinusoids are lined with their characteristic of fenestrated endothelium and their content of von kupffer and Ito cells. Hepatocytes are large cuboidal or polyhedral epithelial cells with large round central nuclei and esinophilic cytoplasm. At the periphery of each lobule; there are three to six portal areas which contain branches of hepatic artery, portal vein and bile duct that comprise portal triad and also contain lymphatic and nerve fibers (Fig.5). Binucleated hepatocytes were observed near the portal areas. It is so difficult to distinguish between lobule boundaries because of scanty connective tissue that connect lobules with each other. The results thus obtained are showing a moderate amount of collagen in the wall of the central veins and dense connective tissue that formed mainly of collagen fibers in the portal areas of buffalo liver than any other area in liver tissue, additionally the buffalo liver surrounded by well-developed collagenous capsule. (Figs. 6&7). It is covered externally by visceral membrane from the peritoneum formed by mesothelial flat cells, capsula serosa. Additionally in sub capsular zone, there were more collagen fibers. Also, in these zones, the nuclei of the hepatocytes were hyper chromatic. The distribution of elastic fibers present more in portal areas while its less amount in the other parts of the buffalo liver's tissue. Elastic fibers are interspersed among collagen fibers (Fig. 8). These investigation provided that the reticular fibers of buffalo liver have its own characteristic in its distribution between hepatocytes which arranged in two forms; one network and the other appeared as black lines which are dispersed and not connected to each other. In addition their spread is in high quantity between hepatocytes unlike their presence in the portal areas (Fig. 9).

Histochemical Results

The Liver of the water buffalo showed highly positive reaction with best's carmine stain which represents the amount of the glycogen is huge (Fig. 10) and also to PAS (Fig. 11). The stellate macrophage (von kupffer) cells of the buffalo liver showed positive reaction to the Perl's Prussian stain ((Fig. 12). Hall's method demonstrated the staining of the bile in the buffalo liver cells by using the oxidizing action of fouchet's reagent to convert bile pigment into an easily identifiable green biliverdin (Fig. 13) Which located in the bile canaliculi, solitary bile duct which dispersed among hepatocytes and portal bile duct.

Immunohistochemistry Results

In the water buffalo liver, von kupffer cells were demonstrated by CD68 antibodies, and showed immunopositive cells characterized by more abundant in periportal areas. Unlike centrilobular areas, in which low reaction were detected (Fig.14). Sinusoidal endothelial cell (EC) continuously expressed CD34 in the periportal area only. On the other hand, the centrilobular sinusoids being mostly negative (Fig. 15). Normal mitosis of the hepatic cells exhibited by using PCNA (proliferating cell nuclear antigen) (Fig. 16). Sections of water buffalo liver tissue expressed CD10 positivity along the bile canaliculi and the luminal borders of bile ducts. Also the buffalo liver bile ducts gave positive reaction with CK7 (cytokeratin 7) antibodies to demonstrate biliary system (Figs. 17&18). Around the terminal and sublobular hepatic veins positive staining of pericytes and smooth muscle cells was observed by alpha smooth muscle actin antibodies while, endothelial cells were consistently negative. Portal areas showed strong positivity around the bile ducts and in the arterial tunica media and moderate positivity in the wall of the portal veins. Slightly irregular moderate staining to Alfa smooth muscle observed in the perisinusoidal spaces throughout the hepatic parenchyma, additional positive reaction observed bounding the hepatic stellate cells (HSC) 'von kupffer' and Ito cells ' Vitamin A-storing cells' (Figs. 19 &20). Positive reaction to insulin and glucagon anti bodies were detected which characterized by their distribution in the hepatic parenchyma (Figs. 21&22).

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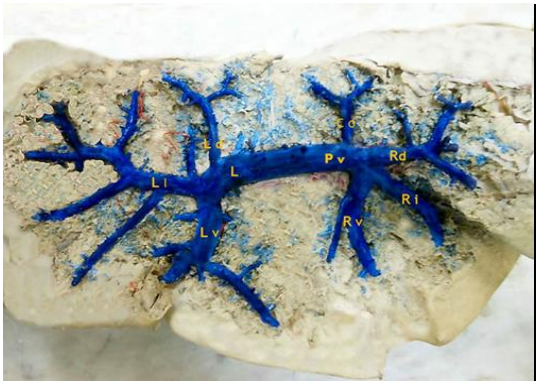

Fig.1 Photomicrograph of water buffalo liver showed branches of portal vein (Pv) ; right ,left and caudal omental ones (Co). Right branch divided into; right dorsal (Rd), right intermediate (Ri) and right ventral (Rv) while the left branch divided into left dorsal (Ld), left intermediate (Li) and left ventral (Lv).

Fig.2 photomicrograph of water buffalo liver demonstrated the main branches of the hepatic vein (H v) which classified as; right branch (R), middle branch (M) and left one (L).

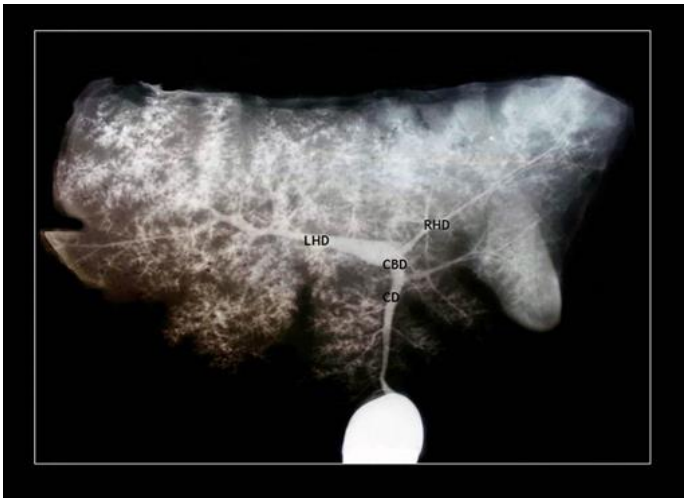

Fig.3 photomicrograph of a radiograph of the liver of water buffalo showing biliary system branches; right hepatic duct (RHD), left hepatic duct (LHD), cystic duct (CD) which are connected to each other forming the common bile duct (CBD).

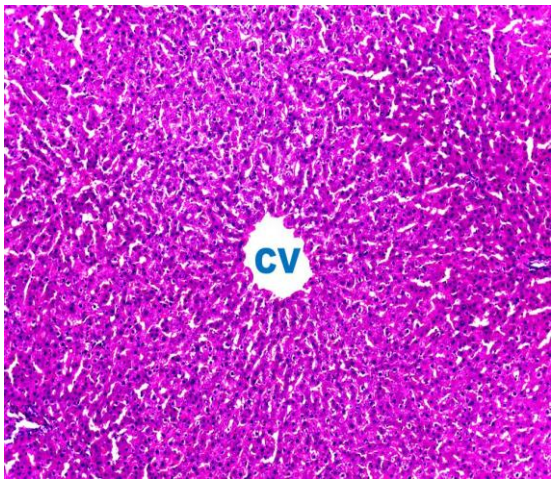

Fig. 4: photomicrograph of water buffalo liver showing the central vein (cv) arising from it sheets of hepatocytes and sinusoids inbetween. (H&E X100)

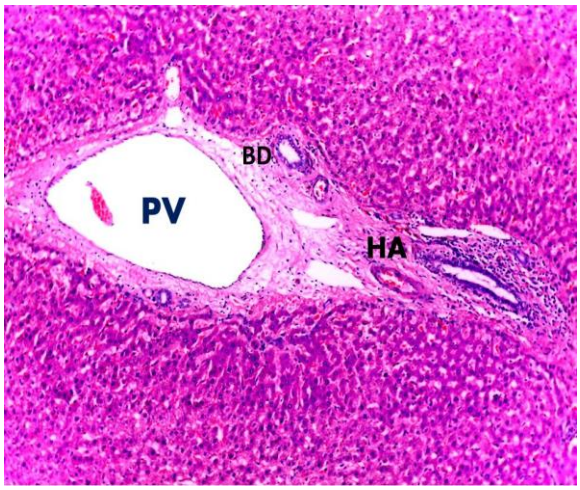

Fig. 5: photomicrograph showing portal area of water buffalo liver in which branch of portal vein (PV), hepatic artery (HA) and bile duct (BD) are present and surrounded by interlobular connective tissue in the periphery of the lobule. (H&E X100)

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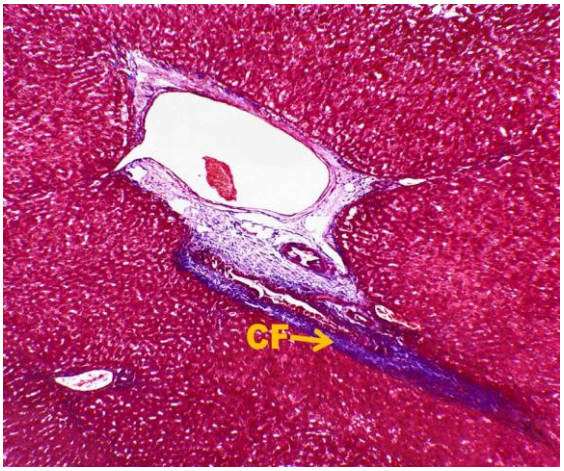

Fig. 6: photomicrograph of the portal area of the water buffalo liver to clarify the collagen fibers which stained blue (CF) moreover, the cytoplasm of hepatocytes are stained red. (Masson's Trichrome X40)

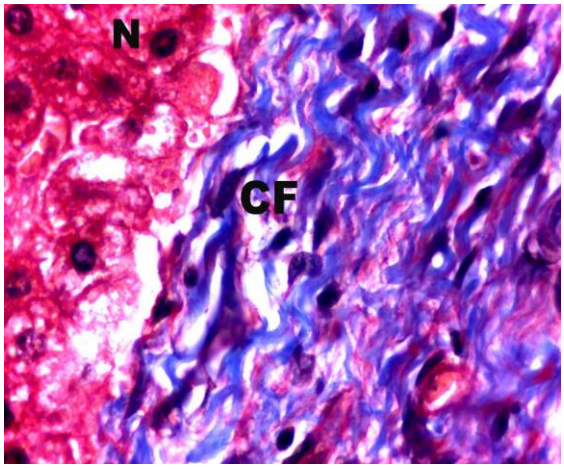

Fig. 7: photomicrograph of the Glisson's capsule of the water buffalo liver to demonstrate collagen fibers (CF) with its blue staining. Notice hyper chromatic nucleus of hepatocyte (N) (Masson's Trichrome X1000)

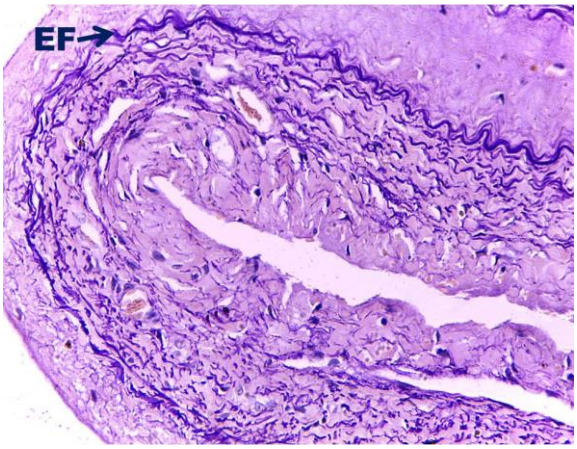

Fig. 8: photomicrograph of a portal vein in the water buffalo liver, showing elastic fibers stain deep violet (EF). (Aldehyde Fuchsin x400)

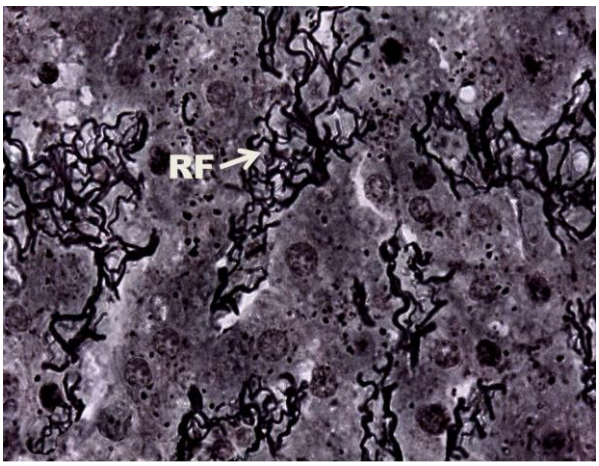

Fig. 9: photomicrograph of liver water buffalo tissue showing the reticular fibers (RF) in a dispersion manner of black lines between hepatic cells. (Gomori's Reticulin X1000)

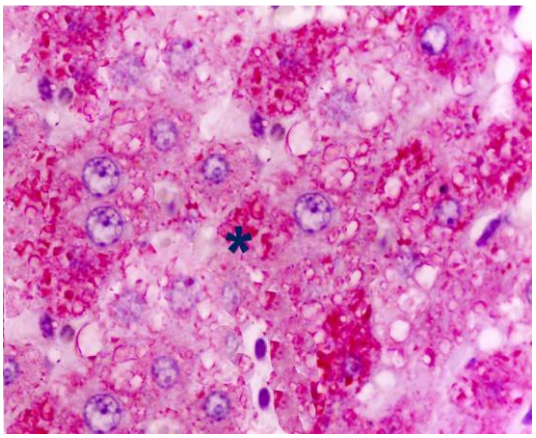

Fig. 10: photomicrograph of water buffalo liver demonstrated the bright red granules (*) in the cytoplasm of the hepatic cells are glycogen deposits. (Best's carmine X1000)

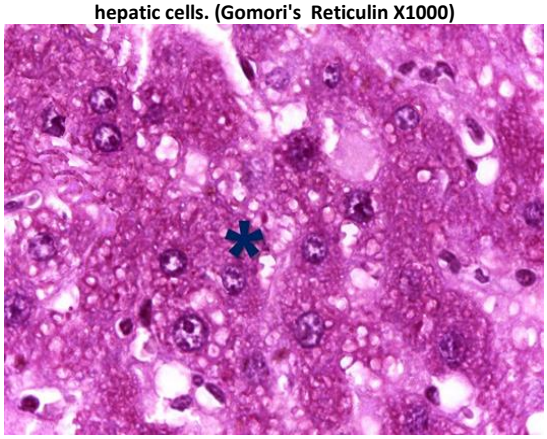

Fig.11: photomicrographs of water buffalo hepatic cells showed intense reactivity to PAS (*). (PASX1000)

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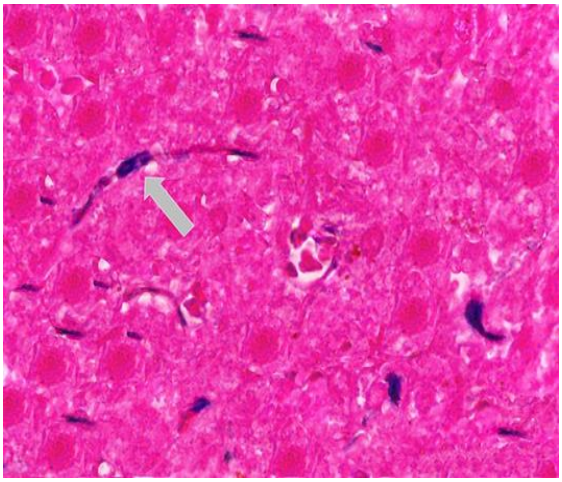

Fig. 12: photomicrograph of water buffalo liver demonstrated the dark blue coarse granules (arrow) of hemosiderin and ferric salts inside the von kupffer cells between hepatic cells. (Perl's Prussian X1000)

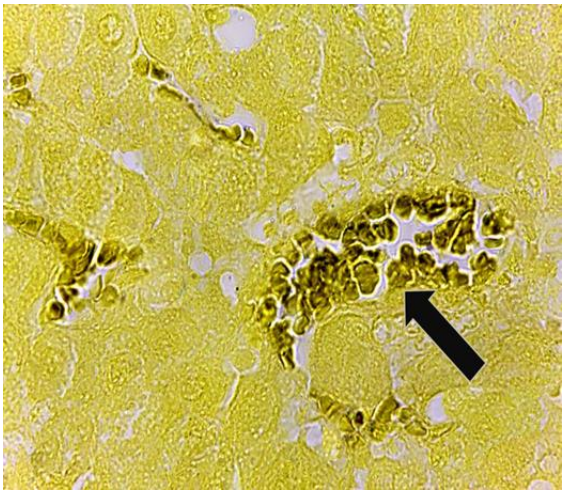

Fig.13: photomicrograph of the water buffalo liver in which, the bile pigments appear green (arrow) against yellow background. (Hall's Iodine X1000)

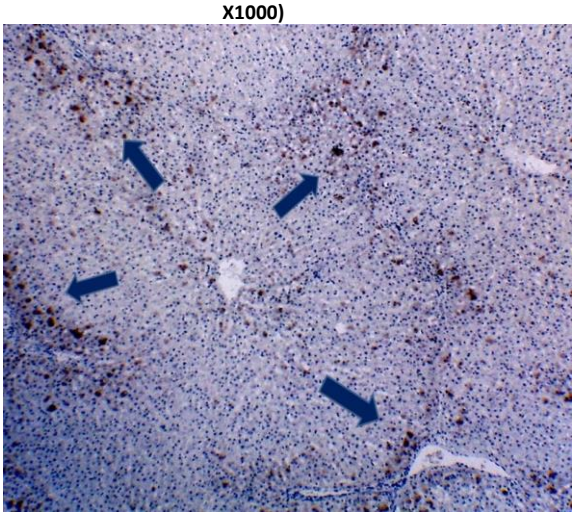

Fig.14: photomicrograph of water buffalo liver showed the positive reaction of von kupffer cells to the CD68 antibodies and clarified the spreading of the cells more in portal areas (arrows) in the form of specific lobe shape unlike, its fewer amounts in centrilobular areas. (CD68 X40)

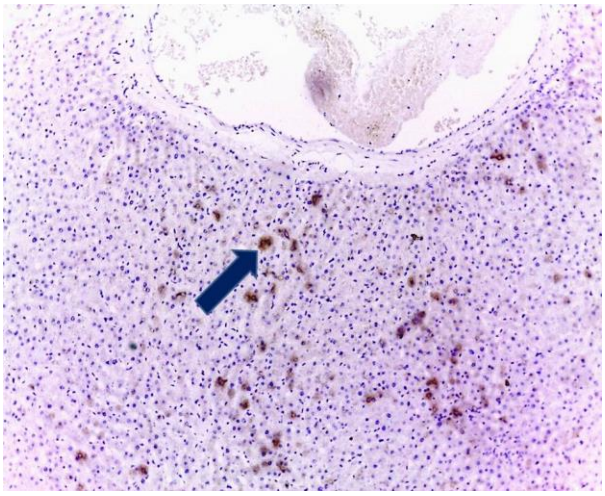

Fig.15: photomicrograph of the water buffalo liver in which CD34 expression showing focal positivity of the sinusoids, restricted to the periportal area (arrow). (CD34 X100)

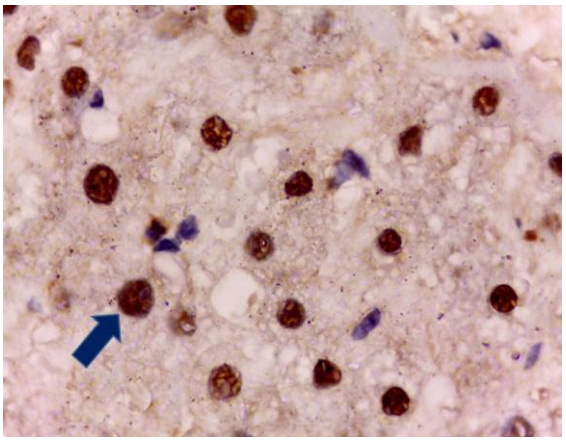

Fig. 16: photomicrograph of normal liver of water buffalo showed expression of the PCNA gene (arrow) to the hepatic cells in a normal case. (PCNA X1000)

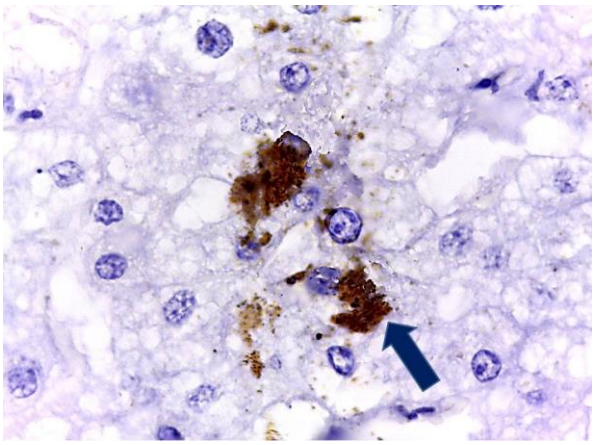

Fig. 17: photomicrograph of water buffalo liver showed positive reaction along the biliary system (CD 10 X1000)

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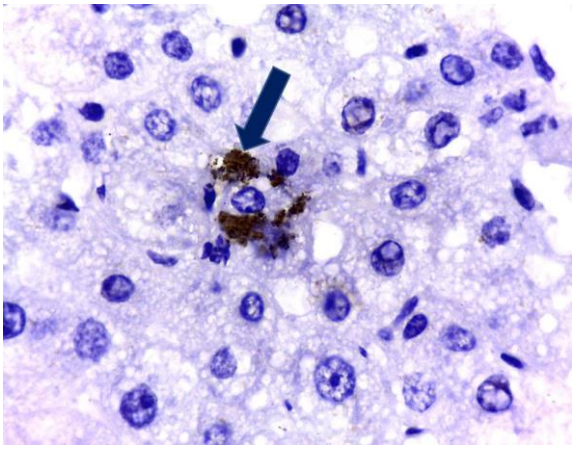

Fig. 18: photomicrograph of water buffalo liver clarified positive reaction of bile ducts to the Cytokeratin 7 antibodies. (CK 7 X1000)

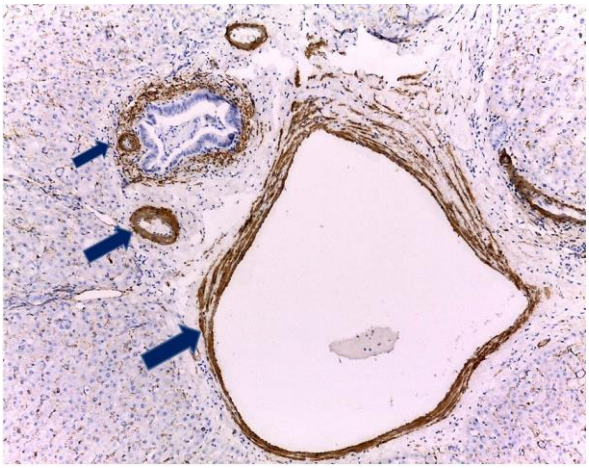

Fig.19: photomicrograph of Portal areas of water buffalo liver stained showing positive reaction to α-SMA around the bile ducts, in the arterial tunica media, and in the wall of the portal veins. There is slightly irregular moderate staining in per sinusoidal spaces throughout the parenchyma. (α-SMA X100)

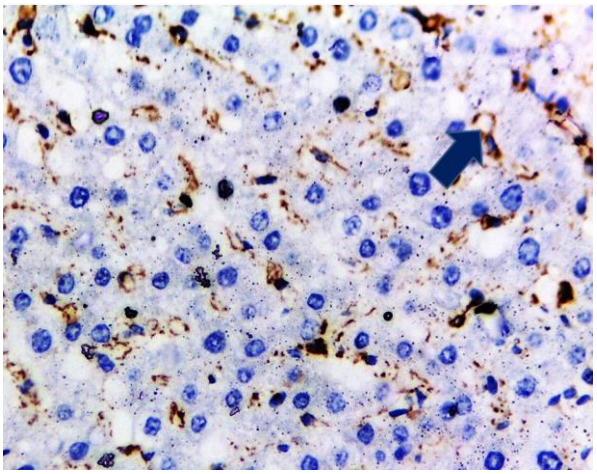

Fig. 20: photomicrograph of water buffalo hepatic cells in which, HSC stain positive. A positive cell containing one large vacuole (arrow-head) and a dislocated nucleus seen. (α- SMA X400)

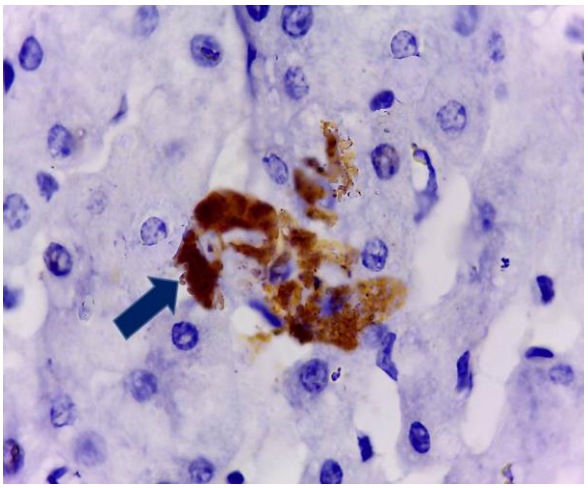

Fig. 21: photomicrograph of water buffalo liver showed immune positive reaction to the insulin antibodies as demonstrated in a brown color (Anti Insulin X1000)

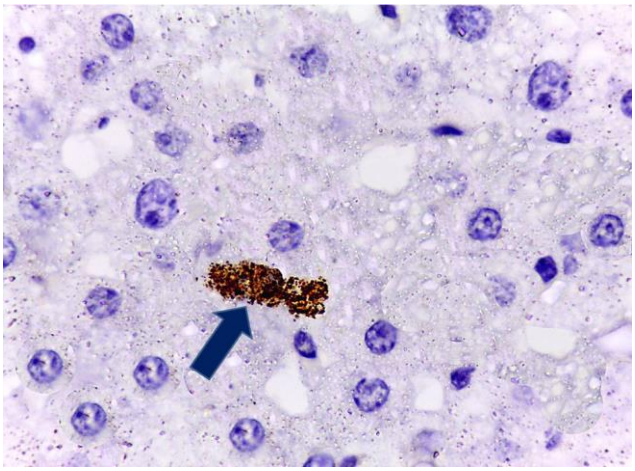

Fig. 22: photomicrograph of water buffalo liver clarified positive reaction of the extra hepatic bile ducts to the glucagon antibodies. (Anti Glucagon X1000)

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DISCUSSION

The present study of buffalo liver, recorded that portal vein divided immediately up on entering the liver into three main branches right, left and caudal omental ones in contrast to Anis (1977), El-Gaafary et al. (1979) and Abd-Elhady (2000) who demonstrated that in the buffalo portal vein was divided into three branches; left, right and right dorsal while, Dyce et al. (2002) stated that the portal vein after entering the liver divided into right and left branches that supply the liver chiefly. Anuradha & Singah (2004) agreed that in Indian water buffaloes the portal vein divided into right (dorsal) and left (ventral) interlobar veins. In cow, the portal vein in cow divides into a very short right branch and a long left branch (Zanco et al., 1994). Nickel et al. (1973) and Habel (1975) in the ox, recorded that portal vein gives two branches; left and right. However, Klages (1962) demonstrated three branches of the portal vein and Barone (1976) described four intra hepatic branches in the ox and mentioned that portal vein when reaching the porta hepatis, gave off the right dorsal branch, the vein of the caudate process and then divided into a right and a left branch. Happich (1961) and Klages (1962) in sheep, reported three intra hepatic branches of the portal vein, and designated them as; left, right and right dorsal. However, Heath (1968) and Hagras & swielim (1990) mentioned that in the same animal, the portal vein enters the liver porta and immediately receives a small vein from the gallbladder and the cystic duct and then divides into the left and right branches. Horowitz & Venzke (1966) in the goat recorded two intra hepatic branches of the portal vein, a short right and a long left branch. However, Hagras & Osman (1986) in the same animal, demonstated three branches; left, right ventral and right dorsal. In contrast to Awad (2000) who described four main branches R.sinister, R. dexter, R. dorsalis dexter as well as R. omentalis caudalis. Abdalla et al. (1971) in the camel, observed the division of the portal vein into three main branches, cranial, ventral and caudo dorsal. Hagras & Osman (1986), Tadjalli & Akhavan (2003) and Farag (1990) recorded nearly similar observations.

Our investigation revealed that in buffalo liver, the right interlobar branch of portal vein gave dorsal, ventral and intermediate interlobular veins. Dorsal interlobular vein supplied dorsal part of right lobe, papillary lobe and at the caudate processes of caudate lobe, the right dorsal branch proceed for about 0.5 -1 cm then it divided into parietal and visceral branches which along its course it gives 8 to 10 smaller branches which arborized in the parenchyma of the process supplying it. Ventral interlobular vein gives the dorsal quadrate branches which supplied the caudodorsal portion of the quadrate lobe and then divided into caudal branches supplying the quadrate lobe and ventral branches supplying the right lobe. The intermediate interlobular vein had horizontal course and distributed in the diaphragmatic parts of right lobe and papillary process. Nearly similar results clarified by Anis, (1977), El-Gaafary et al. (1979), Abd-Elhady (2000) and Ranjbar & Ghadiri (2011) in the same animal. Similar results in sheep recorded by Happich (1961), Klages (1962), Heath (1968) and Hagras & swielim (1990). Heath (1968) added that the right branch proceeds ventrally and gives rise to three or four major branches and a variable number of minor branches which supply a wedge shaped segment of the liver tissue. The same result in camel discussed by Hagras & Osman (1986), Farag (1990) and Tadjalli & Akhavan (2003). The right branch in cow gives off four or five secondary branches that supply the right and caudate lobes (Zanco et al; 1994).

The left interlobar branch of the portal vein runs at first in the long axis of the liver from porta toward the left lobe and at the boundary between the quadrate and left lobes. So, it showed transverse and umbilical parts. The transverse part gives off several branches called the omental branches which supplying papillary process, the dorsal border of the liver as well as the caudodorsal part of the left lobe and other quadrate branches to supply quadrate lobe. The umbilical part of the left interlobar branch gave off 3 branches; the dorsal, intermediate and ventral interlobular branches that radiated into the left and quadrate lobes. The caudal omental branch supplied the caudodorsal part of papillary process as well as the dorsal portion of the right lobe. Similar results are shown by Anis, (1977), El-Gaafary et al. (1979), Abd-Elhady (2000) and Ranjbar & Ghadiri (2011) in buffalo; Hagras & Osman (1986) and Awad (2000) in goat; Hagras & Osman (1986), Farag (1990) and Tadjalli & Akhavan (2003) in camel.

This study of buffalo liver's hepatic vein showed that hepatic veins divided into; left and middle and right hepatic veins. Similar results in the same animal resulted by Anis (1977), Mobarak et al. (1979), Abd-Elhady (2000) and Shirai et al. (2005); in ox by Habel (1975); in sheep by Hagras & swielium (1990); in the goat by Hagras &Osman (1986) and Awad (2000) while in the cattle, recorded two large hepatic veins; right and intermediate, in addition to 12 to 35 minor veins (Passafaro et al., 1997). In the camel, Fahmy et al. (1971), Osman & Ragab (1986) and Farag (1990) reported that presence of two large hepatic veins; left and middle.

The medium sized veins, were represented by the V.processus caudati, the V. omentalis caudalis as well as 2 to 4 Vv. Dorsales lobi dextri. In addition to a large number of small sized veins.

The left vein of buffalo liver collected blood from the left lobe by; lateral hepatic venule and medial hepatic venule which along its course give off 4 to7 dorsal and 6 to 9 ventral branches that ramified into the remaining dorsal portion of the left lobe. The middle hepatic vein give ventral quadrate branch and caudal right branch furthermore, along its course, it release numerous collateral tributaries, while the right hepatic veins which are first right hepatic vein that divide into dorsal and ventral branch and the second right hepatic vein that divide into caudate process vein and the caudal continuation of the right hepatic vein. Also buffalo liver has small hepatic veins which receive blood from the dorsal part of the caudate lobe, papillary process and right lobe. The similar results was discussed by Habel (1975), Anis (1977), Mobarak, et al. (1979) and Abd-Elhady (2000) in buffalo; Hagras and Osman (1986), Hagras &Swielium (1990) and Awad (2000) in the small ruminant and Farag (1990) in the camel. In bovine liver, the same result and also, the hepatic veins drained three major areas, such as the left lobe, right lobe, and between the two lobes, as the left hepatic vein, right hepatic vein and intermediate hepatic vein, respectively (Shirai et al., 2005) . Dyce et al. (2002) added that, the venous blood from the liver sinusoids reaches the center of the liver hepatic veins, which at the dorsal border of the liver; open into the embedded caudal vena cava.

This study clarified that in buffalo liver, the main bile duct (common hepatic duct) is formed by the union of the right and left hepatic ducts and then joins together with cystic duct which comes from gall bladder to form common bile duct that open in the duodenum. The same results are described by Anis (1977) and Abd-Elhady (2000) in the buffalo, Derrico (1976), Hagras and Osman (1986), Hagras &Swielium (1990) and Awad (2000) in the small ruminant and added that, in such cases the cystic duct opens into the right duct. On the other hand, in ox the common hepatic duct was formed by the union of the right and left hepatic duct, while the cystic duct either opened directly into the common hepatic duct to form the common bile duct as demonstrated by Raghavan & Kachroo (1964), Bevandic et al. (1967), Nickel et al. (1973), Habel (1975) and Dyce (1996) as well as reported in sheep by May (1964). According to Bevandic et al. (1967) and Derrico (1976) in sheep as well as Hagras and Osman (1986) in some cases of the goat, demonstrated that the formation of the bile duct by the triple convergence of the right, left and cystic ducts. While in the camel, the common hepatic duct was formed by the union of three main trunks; cranial, ventral and caudodorsal as reported by Arnautovic et al. (1964).on the other hand Osman &Ragab (1986) and Farag (1990) recorded similar observations and named these branches as left, rightdorsal and right ventral.

The right hepatic duct characterized by a short trunk and formed by union of right dorsal branch (R. dorsalis dexter) and cystic duct (ductus cysticus). Similar results are observed by Anis (1977) and Abd-Elhady (2000) in the buffalo, Hagras and Osman (1986) in the goat, Hagras &Swielium (1990) in sheep and Bevandic et al. (1967) in the ox. Shirai et al. (2005) added that in the bovine liver, the right branch of the hepatic duct was short and very complex.

The cystic duct showed as direct continuation of the neck of the gall bladder which received right ventral branch and caudal quadrate branch. Same observations were given by Anis (1977) and Abd-Elhady (2000) in the buffalo, Hagras & Osman (1986) and Awad (2000) in the goat, Hagras &Swielium (1990) in sheep and Bevandic et al. (1967) in the ox.

The left hepatic duct has transverse part and umbilical part. The transverse part of the left hepatic duct considered as direct continuation of the parent duct and divided into dorsal and intermediate branch. The umbilical part of the left hepatic duct has 2 branches; the left ventral branch and cranial quadrate branch. similar results were discussed by Anis (1977) and Abd-Elhady (2000) in the buffalo, Hagras and Osman (1986) in the goat, Hagras &Swielium (1990) in sheep. However Awad (2000) in the goat, reported that the left dorsal branch arose independently, but the intermediate and ventral branches originated by a common trunk. While, Shirai et al. (2005) reported that in bovine liver, the bile ducts of the left lobe generally consisted of four ducts, of which the left intermediate superior bile duct was the best developed.

In this observations the connective tissue which cover the liver of water buffalo is well fibrous Glisson's capsule consisting of great quantity of the collagen fibers and connective tissue cells and it covered externally by visceral membrane from the peritoneum formed by mesothelial flat cells, capsula serosa and this approved

with Ekataksin (2000) in ox and Prunescu (2002) in buffalo, suborders Suiformes and Ruminantia. So, the capsule of Glisson contributed high degree of arterial vascularization and resistance structure for supporting the great amount of hepatic parenchyma. While this capsule is thinner and less vascularized in domestic animals and man (Trautmann and Fiebiger, 1957) and in Carnivora (Prunescu, 2002)

In this investigation intra lobular connective tissue consisted of reticular fibers while in the portal areas were dense collagenous tissue rich in elastic fibers and no demarcation of the lobule this in line with Banks (2007) and disagreement with Ham (1969) and Barone, (1976) whose considered that the connective septa separating more or less completely the hepatic lobules began from the capsule of Glisson and met the perivascular connective tissue marking the limits of the numerous hepatic lobule. Such a connective superstructure of the main hepatic vessels in the portal area formed an inner resistant and elastic skeleton capable of maintaining the funiction of the great mass of hepatic parenchyma Prunescu (2002) in ox and bison.

This result describe that inbetween hepatocytes, reticular connective tissue appeared in a unique form; dispersed and not connected to each other and this distribution may contribute in continuous supply between hepatic sinusoids. The connective tissue around portal triad was well and had; prominent thickness of the collagen sheath around large and medium sized vein, hepatic artery and portal bile ducts. This observation was demonstrated by Prunescu (2002) in buffalo, Banks (2007) in man and domesticated animals, Carollo et al. (2012) in giraffe, scimitar oryx and Mrs Gray's lechwe livers and Madhan and Raju (2014) in cow, sheep and goat; while Carollo et al; (2012) discussed that in the cattle, goat and reindeer livers, the connective tissue was represent by few quantities in the capsule, portal areas, and delineating the lobular septa.

Similar to Paunch (2002) result, Other independent vessels which travel long lines through the buffalo hepatic lobules were the hepatic arterioles or capillaries. They were protected towards the neighbouring hepatocytes and sinusoids by thick positive coats of collagen fibers. The central veins presented a moderate amount of collagen in its wall.

Large polyhedral hepatocytes with round and centrally located nuclei were observed in the liver of water buffalo as recorded by Madhan and Raju (2014) in cow, goat and human and Singh et al. (2014) in goat. While the former author demonstrated that hepatocytes were larger in sheep. Binucleated hepatocytes were observed near the portal areas, while the nuclei of the hepatocytes were hyperchromatic in the subcapsular zone. These differences may be due to the phylogenical or evolutional and/or developmental. The same results confirmed by paunch 2012 in the same animal, Hall (1996) in rat, Endo et al. (2000) in camel, Galletti and Jauregui (2000) in human and Eroschenko (2008) in mammalian liver.

Liver buffalo showed high reactivity to the amount of glycogen by best's carmine method and PAS which also stained the neutral mucopolysaccharide and this referred to the normal function of the liver in synthesis of glycogen from glucose and storing it in the hepatocytes upon somatic demand. These results were discussed by Lillie (1947) and Banks (2007). Doley et al; (2006) added that strong reaction was observed in buffalo foetii.

The buffalo liver is a morphologically and functionally complex organ, made up not only of the most predominant parenchymal cells (hepatocytes), but also non-parenchymal cells (N-PCs), including Ito, Kupffer and sinusoidal endothelial cells (SECs), as well as other cell types that reside in the sinusoidal compartment and this confirmed by Neyrinck et al., (2000) and Kmieć (2001). These non-parenchymal cells play an important role in the regulation of many hepatocyte functions (Kmieć, 2001and Parker & Picut 2005) as well as in the immune biology of the liver, in both normal and pathological conditions (Carollo et al., 2012).

In buffalo, the perisinusoidal stellate cells (Kupffer) presented great vacuoles distorting the nucleus. Perls-positive secondary lysosomes containing ferric ion accumulations demonstrated. This reaction was positive for small groups of hepatocytes scattered in the liver parenchyma. Same results in the same animal observed by paunch (2002).

In this investigation the sinusoids arranged between cords like hepatocytes. This hepatic sinusoidal arrangement has been largely attributed to evolutionary trends of vertebrates for metabolic functions (such as synthesis of plasma proteins, fibrinogen and prothrombin and the regulation of blood glucose and lipids) (Beresford and Henninger, 1986 and Barbara et al; 2014).

In this study α-SMA observed in perisinusoidal, Kupffer and Ito cell. This was recorded in normal manner by IJzer et al., (2006) in canine and Uetsuka's (2007) in ruminants as they excluded fibrosis. While the former author in human and rats recorded this expression as pathological feature to increase the contractility of the kupffer cells. On the other hand, Uetsuka's (2007) and Carollo et al., (2012) not observed this expression in Ito cells as absence of its activation and not transformed into myofibroblast like cells. It may be due to species-specific expression pattern.

In water buffalo, kupffer cells show positive to perl's Prussian, the indicator for haemosiderin pigment. This is considered as haemopoietic function (Doley et al., 2006 and Singh et al., 2014). Also Kupffer cells showed positive reaction to CD68 and more distribution in portal areas than in central areas in accordance with Parker & Picut, (2005) and Carollo et al. (2012) in ruminant. This apparent immuno- histochemical heterogeneity was expected, differently tissue localizations and species. Also due to intensity of staining for these markers decreases with decreasing cell size, suggesting that these cells display a more immature phenotype. As large Kupffer cells located in periportal regions appear to have more active metabolically and more scavenging functions (Hall, 1996 and Naito et al., 2004) and phagocyte the great majority of bacterial products coming from the gut, and consequently are responsible for the onset of the acute phase response (Motta, 1984, Prunescu et al., 2002, Lopez et al., 2011, Carollo et al., 2012) in other mammals and (Carollo et al., 2012) in ruminant.

In this study immuno- reactive cytokeratins 7 and CD10 are expressed in normal bile duct epithelium of water buffalo while Hall (1996) recorded cytokeratins 7 and 19 in rats and humans and referred these cytokeratins provide part of the skeletal structure of the biliary cell responsible for transport of hepatocyte-secreted materials across the canalicular cell to its lumen (Hall, 1996). Interesting structure was the solitary bile duct encountered sometimes in the liver parenchyma. These solitary bile ducts were formed by typical biliary cells and making the connection between the bile canaliculi with the portal bile ducts Prunescu (2002) in buffalo.

Buffalo liver expressed positive reaction to anti-insulin and anti-glucagon antibodies which referred to the presence of pancreatic endocrine cells in liver. This explained the presence of genes which can express endocrine cells of the pancreas. This result come in touch with what discussed about; it is possible to transdifferentiate the liver to pancreas (Rao et al; 1986). Recently showed that cells derived from a population of human fetal liver cells could be induced to acquire certain cell properties after the expression of the transcription factor pancreatic duodenal homeobox 1 (PDX-1) (Zalzman et al; 2005). In mice Insulin mRNA and Protein-Producing Cells are present in the liver of diabetic mice (Kojima et al; 2004).

PCNA (proliferating cell nuclear antigen) demonstrated in liver of water buffalo which is gene encodes an essential DNA replication accessory protein hence; it has been used to express the cell regeneration condition (Dervan et al., 2000) to increase metabolic demands (Singh et al; 2014).

CONCLUSION

Inspection at abattoirs, for hygienic quality, involves both ante and postmortem examination which include gross and microbiological investigation. Liver is one of the most important organs as it is involved in many metabolic disorders and parasitic diseases. It may harbor pathogens which are dangerous for human consumption when passed with localized or mild infection. In addition the aim was to identify the fundamentals of the normal liver structure in buffaloes that to date have not been fully investigated, in order to improve the present structural framework, to which one can refer for describing possible hepatic diseases. This might then lead to a better quality of care and management of these animals.

REFERENCES

[1] Abd El-Hady, E.F.M. (2002): Gross anatomical studies on the liver of buffalo calves. Thesis, M. V. SC. Cairo Univ.

[2] Abdalla, O., Aranautovic, I. and Fahmy, M.F.A. (1971): Anatomical study of the liver of the camel (Camelus dromedarius).1.Topography and morphology. Acta Morphol. Neer I .Scand; 9: 85-100.

[3] Anis, H. (1977): Some anatomical studies on the liver of the buffalo in Egypt (Bos bubalis L.). Thesis, M. V. SC. Zagazic Univ.

[4] Anuradha, S. and Singah, O. (2004): Venography of intrahepatic portal veins of the liver in buffalo (Bubalus bubalis). Indian J. Anim. Sci., 74: 1024-1026.
[5] Arnautovic, I. and Kremar, I. (1964): A contribution to the knowledge of the intrahepatic blood vessels and gall ducts in sheep. Veterinaria, sarajeva, 13: 15-28.
[6] Awad, A.S. (2000): Gross anatomical study on the liver of the goat. Thesis, M. V. Sc. Cairo Univ.
[7] Bancroft, J.D. and Gamble, M. (2008): Theory and practice of histological techniques.6th ed. Churchill Livingston Edinburgh, London and New York.
[8] Banks, W. J (2007): Text Book of Applied Veterinary Histology. 4th ed. Baltimore: Williams & Wilkins, PP- 362-72.
[9] Barbara, Y., Geraldine, O. and Phillip, T. (2014): Histology of the Digestive Tract: Wheater's Functional Histology. 11th ed. Philadelphia: Elsevier, Churchill Living Stone; p. 274-80.
[10] Barone, R. (1976): Anatomie compare des mammiferes domestiaues laboratoire d'anatomie. Ecole nationale veterinaire, Lyon, pp. 506-651.
[11] Beresford, W.A. and Henninger, J. M. (1986): A tabular comparative Histology of the liver. Arch Histol Jap; 49:267-81.
[12] Bevandic, M., Arnautovic, I., Kremar, I. and Lorger, J. (1967): Comparative survey of the bile ducts of domestic animals.Veterinaria, Sarajevo, 16, 301-314.
[13] Brikas, P. and Tismatas, C. (1980): Anatomic arrangement of the hepatic veins in the goat. Amer. J. Vet. Res., 41, 5, 796-799.
[14] Carollo, V., Giancamillo, A. D., Vitari, F., Schneider, R. and Domeneghini, c. (2012): Immunohistochemical Aspects of Ito and Kupffer Cells in the Liver of Domesticated and Wild Ruminants. Open Journal of Veterinary Medicine, 2, 129-136.
[15] Derrico, A.A. (1976): Contribution on the study of the intra and extra hepatic bile duct in ovine. I. Behavior of ductus choledocus. II. Systematization of R. principalis dexter. Rev. Fac. Med. Vet. Zootec. Vinv. S. paule, France, 13, 1. 85-117.
[16] Dervan, P. A., Magee, H.M., Buckley, C. and Carney, D. N. (2000): Proliferating cell nuclear antigen counts in formalin-fixed paraffin-tissue correlate with Ki-67 in fresh tissue. Am J Clin Pathol; 97(suppl):S21-S28.
[17] Devatkal, S., Mendiratta, S.K., Kondaiah, N. (2004): Quality characteristics of loaves from buffalo meat, liver and vegetables. Meat Science 67 (2004) 377–383.
[18] Doley, P.J., Roy, K.S. and Kumar, A. (2006): Histochemical development of prenatal liver in non-descript Indian buffalo. Indian Journal of Animal Sciences 76: 787-789.
[19] Drury R.A.B. and Wallington, E.A. (1980): Carleton's Histological techniques.5th ed. Oxford, New york Toronto, Oxford Univ. Press.
[20] Dyce, K.M., Sack, W.O., and Wensing, C.J.G. (2002): Textbook of Veterinary Anatomy. W.B.Saunders Company. Philadelphia. London.Toronto. Toronto, Montreal, Sydney and Tokyo.
[21] Ekataksin, W. (2000): The isolated artery: an intrahepatic arterial pathway that can bypass the lobular parenchyma in mammalian liver. Hepatology, 31: 269–279.
[22] EL-Gaafary, M.A, EL-Gindy, E.M and Anis, H. (1979): Some morphological features on the intrahepatic part of the portal vein in buf falo in Egypt. Vet. Med. J., 27, 61- 69.
[23] Endo, H., Gui-fang, C., Dugarsuren, B., Erdemtu, B., Manglai, D. and Hayashi, Y. (2000): On the Morphology of the Liver in the Two!humped Camel "Camelus bactrianus". Anat. Histol. Embryol. 29, 243-246.
[24] Eroschenko, V.P. (2008): Difiore's Atlas of histology with functional correlations. 11th Ed. Philadelphia: Lippincott Williams & Wilkins, pp- 277-81.
[25] Fahmy, M.F.A, Abdalla,O. and Arnautovic, j. (1971): Anatomical studies of the liver of the camel (Camelus dromedaries). Iii. The hepatic veins. Acta. Morpho L. – Neer I. Scand, 9, 221-228.
[26] Farag, F.M.M. (1990): Some gross anatomical studies on the liver of the one humped camel (Camelus drome darius).Thesis hp. O. Vet, Med. Cairo, Univ.
[27] Galletti, P. M. and Jauregui, H. O. (2000): The Biomedical Engineering HandBook, Second Edition. Ed. Joseph D. Bronzino Boca Raton: CRC Press LLC, 2291:2305.
[28] Habel, R.E (1975): Ruminant digestive system. In Sisson and Grossman's anatomy of the domestic animals 5th ed Revised by Getty, R. (1975). W.B. Saunders. Philadeliphia, London, Toronto pp.908-913.
[29] Hagras, S.M. and Osman, F.A. (1986): Parenchymal distribution of the hepatic artery of goat. Egypt. J. Histol. 9. 1, 93-96.
[30] Hagras, S.M. and Swielim, G.E.A. (1990): Gross anatomy on the blood vessels and bile duct in the liver of sheep. The new Egyptian. J. of Med. Vol., 1, No. 2, pp. 1066-1082.

[31] Hall, W. C. and Rojko, J. L. (1996): The Use of Immunohistochemistry for Evaluating the Liver. Toxicologic Pathology 24; 4-12.
[32] Ham, A.W. (1969): Liver. In: Histology, Sixth Edition, JB Lippincott Co., Philadelphia and Toronto, pp. 711–723.
[33] Happich, A. (1961): Blutgefass versorgung der verdaungsorgan in Bauch and Beckenholle, einges chlieslich leber, Milz und Bauchs peichel druse beim schaf. Diss. Vet. Med. Hannover cited by Osman, F.A. and Hagras, S.M. (1986).
[34] Heath, J. (1968): Original distribution of portal blood in the sheep. Amer.J.Anat.122, (1), 95-106.
[35] Horowitz, A. and Venzke, W.G. (1996): Distribution of blood vessels to the post diaphragmatic digestive tract of the goat: coeliac trunk, gastroduodenal and splenic tributaries of the portal vein. Amer. J. Vet. Res. 27, 1293-1315.
[36] Ibrahim, M.A.R (2012): Water buffalo for our next generation in Egypt and in the world. Scientific Papers, Animal Science, Series D, vol. LV.
[37] IJzer, J., Roskams, T., Molenbeek, R. F., Ultee, T., Penning, L. C., Rothuizen, J. and SGAM, T. (2006): Morphological characterisation of portal myofibroblasts and hepatic stellate cells in the normal dog liver. Comparative Hepatology; 5:7. pp. 1-9.
[38] Kelly, W.R. (1993): The liver and biliary system. In: Pathology of Domestic Animals. 4th edn., Jubb KVF, Kennedy PC and Palmer N (eds.), Academic Press, London, UK.
[39] Klages, C.H (1962): Anatomische untersuchungen des Gefassver laufs der leber neugeborner schafe and geburtsreifer Rinder. Diss. Vet. Med. Hannover. Cited by Osman, F.A. and Hagras, S.M. (1986).
[40] Kmieć, Z. (2001): Cooperation of Liver Cells in Health and Disease. Advances in Anatomy, Embryology and Cell Biology, Vol. 161, No. 1, pp. 1-151.
[41] Kojima, H., Fujimiya, M., Matsumura, K., Nakahara, T., Hara, M. and Chan, L. (2004): Extrapancreatic insulin-producing cells in multiple organs in diabetes. PNAS; 101: 8 PP. 2458–2463.
[42] Lillie, R. D. (1947): Studies on the preservation and histologic demonstration of glycogen. Bull. Int. Assoc. Med. Mus. 28:23-61.
[43] Lopez, B. G., Tsai, M. S., Baratta, L. J., Longmui, K. J. and Robertson, R. T. (2011): Characterization of Kupffer cells in livers of developing mice. Comp. Hepatol., 10(1):2.
[44] Madhan, K. E and Raju, S (2014): Comparative Histology of Human and Cow, Goat and Sheep Liver. Journal of Surgical Academia; 4(1):10-13.
[45] Malarkey, D. E., Johnson, K., Ryan, L., Boorman, G. and Maronpot, R. R (2005): New Insights into Functional Aspects of Liver Morphology. Toxicological Pathology, Vol. 33, No. 1, pp. 27-34.
[46] May, N.D.S. (1970): The anatomy of the sheep. 2nd ed. Univ. of the Queensland press.
[47] Mobarak, A.M., EL-Gaafary, M.A. and Anis, H. (1979): Some anatomical studies on the liver of the buffalo in Egypt. IV. Biliary system. Egypt. Vet. Med. J., 27, 259-263.
[48] Motta, P. M. (1984): The three-dimensional microanatomy of the liver. Arch.Histol. Jpn. 47(1):1-30.
[49] Naito, M., Hasegawa, G., Ebe, Y. and Yamamoto, T. (2004): Differentiation and function of Kupffer cells. Med Electron Microsc 37:16–28.
[50] Neyrinck, A. M., De Wispelaere, L. D., Vanhulle, V. P., Taper, H. S. and Delzenne, N. M. (2000): Are Kupffer Cells In- volved in the Metabolic Adaptation of the Liver to Die- tary Carbohydrates Given after Fasting? . Biochimica ET Biophysica Acta—General Subjects, Vol. 1475, No. 3, pp. 238-244.
[51] Nickel, R., Schummer, A., and Seiferle, E. (1973): The Viscera of the Domestic Mammals. Translation and Revision by W.O. Sack. Verlag Paul Parey. Berlin, Hamburg.
[52] Osman, F.A. and Ragab, S.A. (1986): Intrahepatic veins and bile ducts of one humped camel (camelus dromedaries). Egypt. J. Histol. 9, 2, 245-249.
[53] Parker, G.A. and Picut, C. A. (2005): Liver Immunobiology, Toxicologic Pathology, Vol. 33, No. 1, pp. 52-62.
[54] Passafaro, R., Souza, W, M, De; Miglino, M.A. (1997): The caudal vena cava and ostia of the hepatic veins in Nelore cattle. Brazilion J. Vet. Res. and Anim. Scie. 34, 2, 70-72.
[55] Prunescu, C.C., Prunescu, P., Krasińska, M. and Krasiński, Z.A (2002): Liver histological structure in adult European bison. Bison bonasus, Via medica, Vol. 61, No. 3, pp. 137–142.
[56] Raghavan, D. and Kachroo, P. (1964): Anatomy of the ox.Indian council of Agricultural Research, New Delh.
[57] Ranjbar, R. and Ghadiri, A.L. (2011): Observation of Intrahepatic Branching Pattern of the Portal Vein in Water Buffaloes of Iran. Asian Journal of Animal and Veterinary Advances 6 (5): 508-516.
[58] Rao, M. S., Bendayan, M., Kimbrough, R.D. and Reddy, J. K. (1986): Characterization of pancreatic-type tissue in the liver of rat induced by polychlorinated biphenyls. J Histochem Cytochem 34:197–201.

[59] Santos, R.M.B., Parda, I.L.S. and Didio, L.J.A (1991): Hepatic veins and liver segmentation in ovine (ovisaries). Braz. J. Res. Anim. Sci, Vol. 28, No. I, p. 19-30.

[60] Shirai, W., Sato, T., Shibuya, H., Naito, K. and Tsukise, A. (2005): Three-Dimensional Vasculature of the Bovine Liver. Anat. Histol. Embryol. 34, 354–363.

[61] Singh, G., Farooqui, M. M, Prakash, A., Pathak, A. and Kumar, P (2014): Histogenesis of Prenatal Liver of Goat (Capra hircus). Indian Journal of Veterinary Anatomy 25 (2): 61-64.

[62] Standring, S. (2008): Gray's Anatomy - Anatomical basis of clinical practice. 40th ed. London: Churchill Livingstone, pp. 1173-5.

[63] Tadialli, M., Akhavan, R. (2003): Anatomical study on intrahepatic branches of portal vein in one humped camel (Camelus dromedarius). J Camel Pract Res; 10(2):201-6.

[64] Trautmann, A. and Fiebiger, J. (1957): The Histology of Domestic Animals. 8th ed, Ithaca, New York, pp. 221-229.

[65] Uetsuka, K., Nishikawa, S., Isobe, K. and Nakayama, H. (2007): Histopathological characteristics of Kupffer cells and Ito cells in the Porcine and Bovine liver. J. Vet. Med. Sci. 69(7): 767- 770.

[66] Uetsuka, K., Nishikawa, S., Yasoshima, A., Nakayama H. and Doi, K (2006): Histopathological Characteristics of Ito Cells and Kupffer Cells in the Feline Liver. Journal of Veterinary Medical Science, Vol. 68, No. 3, pp. 235-242.

[67] Zalzman, M., Anker-Kitai, L. and Efrat, S. (2005): Differentiation of Human Liver-Derived, Insulin-Producing Cells Toward the Cell Phenotype. DIABETES, VOL. 54, pp. 2568-2575.

[68] Zanco, N., Reginato, A., Miglino.M and Dildo, L. (1994): The structures of the portal vein in bovine fetuses of Nelore breed. Brazil J. Vet. Res. Anim. Sci., 31: 13-18.