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College of Veterinary Medicine

Induction of reversible and irreversible hepatic fibrosis by surgical closure of major duodenal orifice in dogs models

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Supervised by

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Induction of reversible and irreversible hepatic fibrosis by surgical closure of major duodenal orifice in dogs models

A Thesis Submitted

By

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To

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In

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ABSTRACT

This study was conducted to surgical induce reversible and irreversible hepatic fibrosis in dogs. The study was performed on twelve healthy local adult dogs of both sexes. Animals were divided into two groups of 6 animals in each. The duodenum was opened in both groups and irreversible fibrosis was induced in first group by closing the opening of the main bile duct in the duodenum (major duodenal papilla) with a non-absorbable silk suture. In the second group, reversible fibrosis was induced by using absorbable suture material Polygalactin 910. The experimental animals were followed up clinically for a period of 60 days with measurement of the level of liver enzymes in the blood, ultrasound examination on a weekly basis, in addition to conducting a macropathological and histopathological examination for the period 60 days after the operation. Clinical results showed a gradual occurrence of fibrosis of the liver in both groups, which was represented by pale-yellowish of the mucous membrane of the eyes and mouth with loss of appetite, noting severe pain, especially in the first days after the surgery with a gradual improvement in the health status of the animals of the second group after 30 days of operation While the results of the laboratory test for liver enzymes showed a gradual elevation in the level of these enzymes, especially during the first days after the operation, and this rise continued until the end of the study in both groups. While the results of the ultrasound examination revealed a gradual occurrence of liver fibrosis, which was represented by the presence of high echo areas, especially in the first weeks after the surgery in the animals of first group, while it was noted that liver fibrosis stopped after the fourth week of the operation in the animals of the second group. While the results of the histological examination in the animals of the first group showed the formation of fibrous tissue in the liver with infiltration of inflammatory cells, especially around the bile ducts, in addition to necrosis and degeneration

of hepatocytes with hemorrhage in the liver and sub capsular tissue, while the same pathological changes were observed, but with less severity In the animals of the second group .

We conclude from the above the possibility of surgically causing irreversible and reversible hepatic fibrosis by closing the opening of the main bile duct, which was confirmed by the results of clinical, laboratory, ultrasound, histological and gross pathological examination.

Contents

List of Abbreviations

Abbreviations	Full name
ECM	Extracellular matrix
HSCs	Hepatic stellate cells
NKT	Natural kill T
GB	Gallbladder
KC	Kupffer cell
SECs	Sinusoidal Endothelial Cells
ALT	Alanine aminotransferase
AST	aspartate aminotransferase
ALP	Alkaline phosphatase
TSB	Total Serum Bilirubin
BUN	Blood urea nitrogen
GGT	Gamma-glutamyl transferase
CV	Central vein
PV	Portal vein
TIMP-1	Tissue inhibitors of metalloproteinase – 1
MMPs	Matrix metalloproteinases

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Chapter One

Introduction

Experimentally, different techniques aimed to induce hepatic fibrosis, some of these by surgical methods and others by medicals. The common models used to induce hepatic fibrosis include the administration of hepatotoxins, the ligation of the common bile duct, induction of immune-mediated liver injury and targeted introduction of gene defects. Ligation of the common bile duct in rodents and dogs is a procedure used experimentally in research for many years. In this protocol, a high yield of cirrhosis with morphological changes is induced by ligation of common bile duct that can be compared to the change noticed in human biliary cirrhosis (Tag *et al.,* 2015). Hepatic fibrosis can be defined as an excessive synthesis and deposition of connective tissue proteins, particularly interstitial collagens in the extracellular matrix (ECM) of liver tissue (Bataller & Brenner, 2005a; Roehlen *et al*., 2020). It is an outcome of an abnormally healed wound as a response to chronic liver injury caused by various agents such as ethanol, viruses, toxins, drugs, or cholestasis. The chronic stimuli which initiate fibrosis cause an oxidative stress which acts as a mediators of molecular events that participate in the pathogenesis of hepatic fibrosis (Elpek, 2014). Liver fibrosis is related to major changes in both the quantity and composition of extracellular matrix. In advanced stages of liver fibrosis, extracellular matrix is approximately 6 times more than normal (Bataller & Brenner, 2005a). Although hepatic fibrosis has historically been regarded as one disease, it is clear that the pathophysiology of liver cirrhosis differs according to the underlying etiology, which does not change the perception of liver cirrhosis, but also creates new challenges in the cirrhosis treatment (Böttcher & Pinzani, 2017) . Treatment of the underlying cause of a disease may prevent the progression of normal

liver tissue to cirrhosis and even attempt a regression of the fibrogenic process removal of the harmful agent or stimulus can attenuate the progression of both liver fibrosis and cirrhosis (Arndtz & Hirschfield, 2016). If fibrosis is created and chronic liver diseases have been changed from fibrosis to cirrhosis, major structural changes such as extensive capillarization of sinusoids and development of intrahepatic vascular shunts, as well as endothelial dysfunction can be observed. Endothelial dysfunction is caused by reduced endothelial synthesis of vasodilators, such as nitric oxide, as well as increased vasoconstrictors secretion such as thromboxane A2 and endothelin (García-Pagán *et al*., 2012). These structural and functional changes lead to major complications of liver cirrhosis (portal hypertension) which in turn result in other complications of cirrhosis such as ascites, variceal bleeding, hepatic encephalopathy and failure of kidney. Moreover, liver cirrhosis can be considered as a major risk factor for the development of hepatocellular carcinoma (Böttcher & Pinzani, 2017). The present study was conducted for the following aims :

1- To surgically induce irreversible hepatic fibrosis by closure of major duodenal papilla using non-absorbable suture material which used for first time.

2- To surgically induce reversible hepatic fibrosis by closure of major duodenal papilla using absorbable suture material which also used for the first time.

3- Evaluation the surgically induced hepatic fibrosis through the clinical, ultrasonographical, laboratory, macropathological and histopathological examination.

Chapter Two

Literature review

2-1: Liver anatomy in dogs

Liver is considered as the largest important internal organ in the body where it is located in the front of the abdominal cavity deep to the ribs 7 – 11 on the right side and partially crosses the midline towards the left side (Ozougwu, 2017) .The liver is the biggest gland in the body and performs both endocrine and exocrine functions, including bile generation, collection, and concentration in the gall bladder before it is emptied into the duodenum (Dawood, 2019) .

The upper surface of liver relates entirely to the diaphragm while the visceral surface rests against the abdominal parts such as abdominal esophagus, upper duodenum, hepatic flexure of the colon, stomach, right kidney and suprarenal gland (Ellis, 2011). The ligaments of the liver are primarily responsible for connecting the liver to the diaphragm, abdominal wall and nearby organs, and include the following:

1-The right and left triangular ligaments

2- The coronary ligament

3- The caudate process and the right kidney are connected by the hepatorenal ligament.

4- The falciform ligament is located at the distal end of the ventral mesogastrium. The umbilical vein of the liver runs from the umbilicus to the liver in the fetus along the free border of the falciform ligament. The umbilical vein is lost in mature dogs.

5- The liver's round ligament does not persist, and the falciform ligament is only present as a short fold ventral to the caudal vena cava as it leaves the liver cranially and a much larger fat-filled fold caudally at the level of the umbilicus.

6- The liver's area nuda is a zone of adhesion where the liver is joined to the diaphragm by connective tissue; this area is free of peritoneum and lies to the right and left of the caudal vena cava as it passes through the liver.

7- The lesser omentum is split into the hepatoduodenal and hepatogastric ligaments and converge on the liver's porta (Budras *et al*., 2007; Evans & De Lahunta, 2013).

In general, Deep fissures split the liver into four lobes and four sub lobes, as well as two processes (Figure 2-1).

1- The left hepatic lobe: it is the hepatic part that is located almost entirely to the left of the median plane. This lobe forms about 30-50 % of the total liver and is usually divided into left lateral and medial hepatic sub lobes.

2- The right hepatic lobe: it is smaller than the left hepatic lobe and is located completely to the right of the median plane. This lobe can be accessed between transverse planes that are passed through the dorsal portions of the 6-10 intercostal spaces. It is divided into medial and lateral sub lobes.

3- The quadrate lobe: it is a deep thick lobe of the hepatic tissue that lies in the median plane just between the right medial and the left hepatic lobe. The diaphragmatic surface of this lobe has a fusiform shape. The middle part of its right surface is smoothly excavated by the left half of the fossa carrying the gallbladder.

4- The caudate lobe : is made up of caudate, papillary processes, and an isthmus, which is made up of a collection of hepatic tissues that create a bridge between the caudate and papillary processes.

a- The papillary process is pyramidal in form and generally split into one or two fissures. This process is found in the lesser curvature of the stomach and is loosely surrounded by the minor omentum.

b- The caudate process, which continues to the final rib on the right side, represents the caudal portion of the liver. Its caudolateral portion is positioned deep within the right kidney and thus contains the deep renal impression (Evans & De Lahunta, 2013).

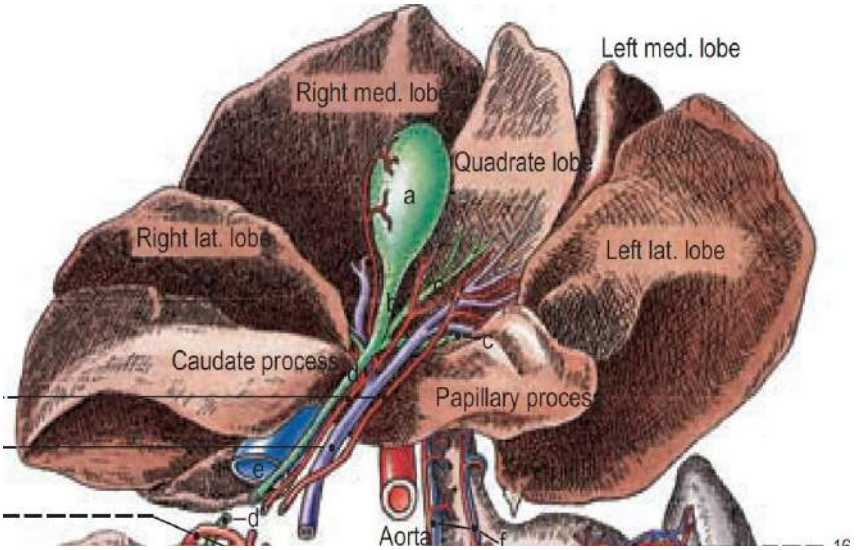

Figure (2-1) : Shows gross anatomy of liver in dogs . (Budras *et al.* 2007)

2- 2: The biliary system

The gall bladder, cystic duct, bile duct, and hepatic ducts, as well as their intrahepatic ramifications, make up the biliary system (Center, 2009).The gallbladder (GB): is part of the digestive system as a tiny sac that is pear-shaped and connected to the liver to the right of the midline within a fossa between the right medial and quadrate liver lobes. Its wall is thin and varies in

size. (Grace *et al.*, 1988). The GB wall, like the intestinal system, has a stratified structure. Simple columnar epithelium lines the luminal mucosal surface. A vascular-rich lamina propria lies underneath the epithelium. The muscularis externa, which is made up of randomly aligned smooth muscle bundles, is located outside of the lamina propria. Diverticula or mucosal invaginations can sometimes extend into the muscularis externa. The adventitia is located outside of the muscularis externa (Jenning and Premanandan 2017) . Bile is produced by hepatocytes and actively released into the bile canaliculi. From there, bile flows through the intrahepatic bile duct system (bile ductules - intralobular ducts and interlobular ducts), exiting the liver through the left and right hepatic ducts, which eventually combine with cystic duct that come from GB to form bile duct which enters and opens as ampulla in the small intestine (duodenum) 5 mm distally to the pyloric region of the stomach (Figure 2-2) (Kook, 2013) .

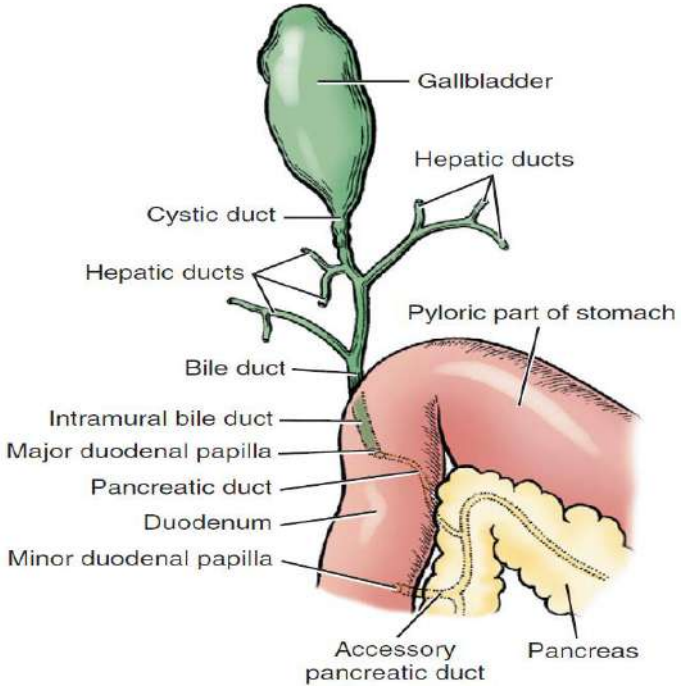

Figure (2-2) shows biliary ducts (Hepatic and cystic ducts), intestine , pyloric part of stomach , major duodenal papilla , minor duodenal papilla in dogs (Evans& De Lahunta, 2013) .

2-3: Blood supply of the liver

The liver receives oxygen and nutrients from blood that comes from two sources: the portal vein, which delivers 80% percent of the blood, and the hepatic artery, which delivers the remaining 20% of the oxygenated blood (Schulze *et al*., Radu-Ionita *et al*., 2020). The portal vein drains the splanchnic viscera (stomach, intestine, pancreas and spleen). The portal vein is formed by the confluence of cranial and caudal mesenteric veins and receives the splenic and gastroduodenal vein before it enters the liver at the porta hepatis. Here the portal vein divided into a short right and a large left branch, which supply the right, left and central divisions of the liver respectively and then subdivide into successively smaller branches . The terminal branches present in the smallest portal tracts, and finally give rise to the inlet venules which penetrate the periportal limiting plate and open into the sinusoids (Van den *et al*., 1995). The hepatic artery originates from one branches of the celiac artery that comes from the aorta and divided into two branches left and right hepatic artery which enter to liver together with the hepatic nerves. The cystic artery originates from the right hepatic branch to supply the gallbladder (Sibulesky, 2013). Blood leaves the liver through the hepatic veins formed by left, right and central hepatic veins which drain into the inferior vena cava and later to the (Evans& De Lahunta, 2013) .

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2-4: Histology of liver in dogs.

The liver is a heterogeneous gland covered with a thin connective tissue capsule called the Glisson capsule that divides the parenchyma into lobules and lobuli (Petcoff *et al.*, 2006). A single lobule is hexagonal in form, and the major structures present in a liver lobule are Hepatocyte plates (make up the majority of the lobule), portal triads (at each hexagonal corner), central vein, liver sinusoids (run between the portal triads and the central vein), hepatic macrophages, bile canaliculi (generated between neighboring hepatocytes' walls), space of Disse : a small space between the sinusoids and the hepatocytes (Figure 2-3) (Ozougwu, 2017).

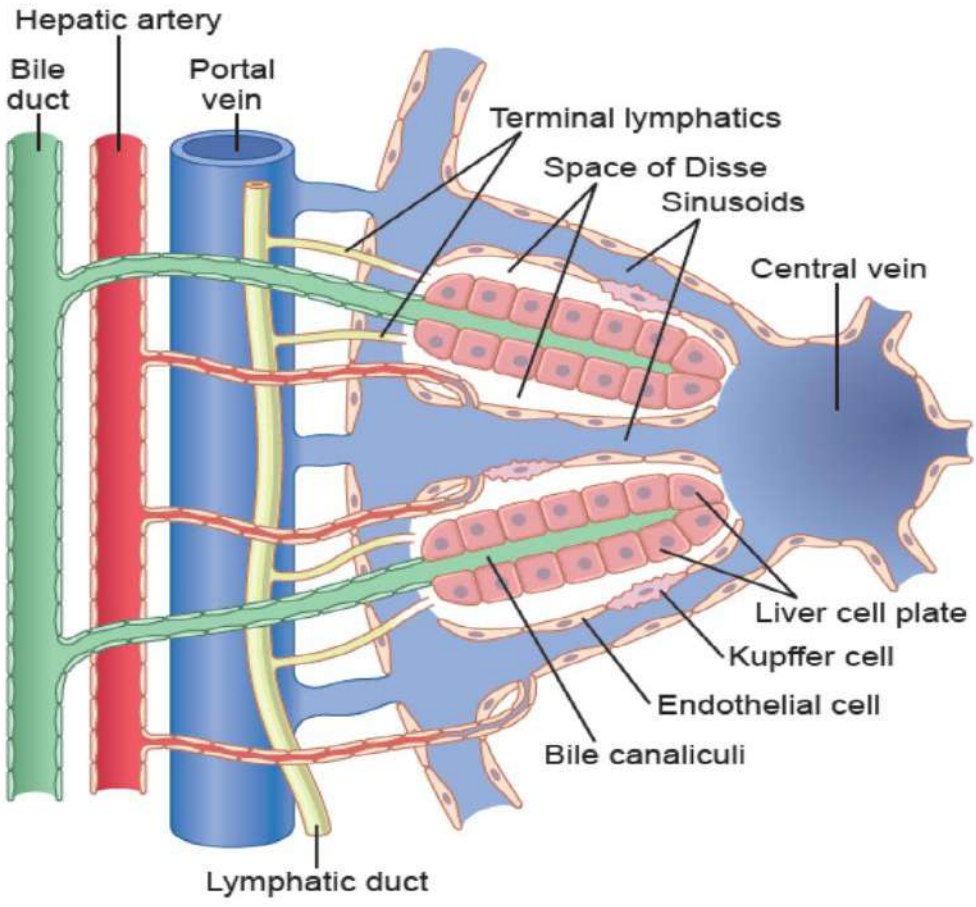

Figure (23) shows liver histology in dogs (Hall, 2016)

There are three vessels which make up the portal triads. (hepatic arteriole, portal venule and bile ductule). Both the arteriole and the venule send blood through the sinusoids in the same direction, to the central vein, which goes to the hepatic vein and the inferior vena cava while the secretion of bile travels in the opposite direction from the central vein, through the bile canaliculi, toward the portal triad, and out to the bile duct (Ozougwu, 2017) (Figure 2-4)

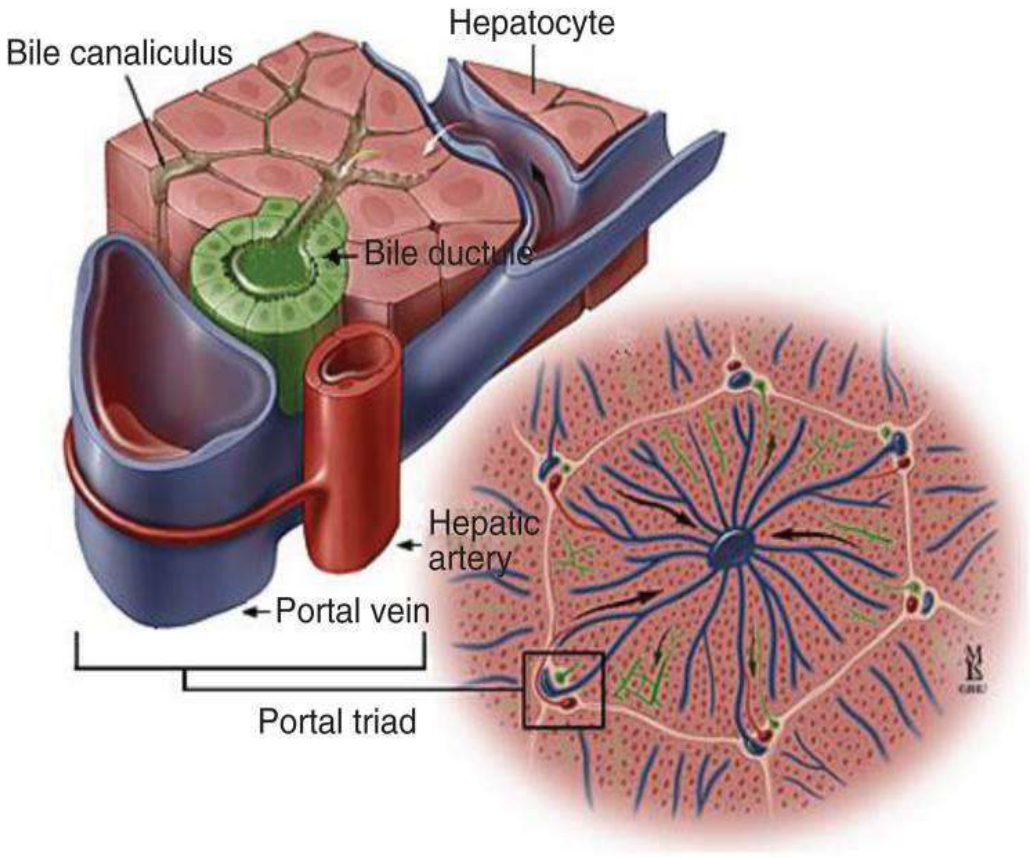

Figure (2 - 4) : shows bile canaliculus, hepatic artery and portal vein (Tabibian *et al.* 2013)

2-5: Cells of the Liver

There are many different cell types which can be found in normal liver such as:

1- Hepatocyte: Hepatocytes are the main cells which represent about 80% of the liver tissue . hepatocyte contains eosinophilic cytoplasm with many mitochondria, as well as basophilic stippling generated by a significant number of rough endoplasmic reticulum and free ribosomes. The nuclei of hepatocytes are spherical, with scattered chromatin and conspicuous nucleoli (Washabau, 2013) .Hepatocytes are the liver's principal functional cells, performing metabolic, detoxifying, and endocrine function and it is targeted mainly by biological and chemical damaging agents of the liver tissue that lead to acute or chronic liver disease. (Kmieć, 2001; Sun *et al*., 2019).

2- Kupffer cells: Kupffer cells have an irregular form with many cytoplasmic extensions and a significant number of lysosomes and phagosomes, which are related with their endocytic activity. They also have a well-developed endoplasmic reticulum, Golgi complex, and secretory vesicles, it can be located in the sinusoidal lumen on top of or between endothelial cells, as well as in the Disse space. They (Smedsrød *et al.*, 1994; Zhang & Bansal, 2020). Kupffer cells begin their formation in the bone marrow with the differentiation of promonocytes and monoblasts into monocytes, and then progress to peripheral blood monocytes before finishing differentiation into Kupffer cells within the liver (Washabau, 2013)

3- Hepatic stellate cells (HSCs): HSCs were discovered in 1876 as black areas stained with gold chloride by German scientist Carl von Kupffer, who called them stellate cells (Cho *et al*., 2020). They are also known as perisinusoidal cells, Ito cells, fat-storing cells or lipocytes . Hepatic stellate

cells (HSC) are the most critical cells in the development of liver damage , located in the midst of the sinusoids epithelium and the liver cell (Ramadori *et al*., 2008). Any process that activates the inflammatory mechanism also activates the HSC. The most significant action of HSC is in the fibrosis process (fibrogenesis) which contribute to hepatic inflammation by their ability to secrete and respond to a wide range of growth factors, in addition to rapid and pleiotropic changes in the function of stellate cells such as proliferation, migration, contractility and increased matrix production (Balabaud *et al*., 2004; Yoshida & Matsuzaki, 2012; Alvani , 2015) .

4- Sinusoidal Endothelial Cells (SECs): Liver Sinusoidal Endothelial Cells are highly specialized endothelial cells representing the interface between blood cells on the one side and hepatocytes and hepatic stellate cells on the other side (Poisson *et al*., 2017) . The liver SECs are flattened, elongated cells which form the sinusoidal wall and only their dilated portion that contains the nucleus protrude into the lumen. The sinusoidal wall is not continuous because of the presence of numerous pores and fenestrations of different size and position and these pores system facilitate transfer of substrates between the blood and the liver parenchyma, and regulates lipoprotein traffic to and from the hepatocytes (Sørensen *et al*., 2015). Liver sinusoidal endothelial cells are thought to play significant roles in the migration of inflammatory cells into hepatocytes and the increased expression of adhesion molecules during liver inflammation and capture degraded agents circulating within the blood (endocytosis), They also produce nitric oxide, endothelin, prostaglandins and cytokines (Tano*i et al*., 2016). The SECs represent the primary barrier between blood and hepatocytes where fluids, solutes, and particles between the blood and space of Disse are filtered (Kmieć, 2001).

5- Pit cells.: Hepatic natural killer cells (NK), and large granular lymphocytes (LGL) are all names for pit cells that are related to various features of their appearance or function and these cells were described for the first by wisse E. in 1976 (Vanderkerken *et al*., 1993). Pit cells are found in the liver sinusoids and frequently attached to endothelial cells, however they can come into touch with Kupffer cells . They are in close contact with the blood. Pit cell pseudopodia can reach the Disse space and directly contact the microvilli of hepatocytes after penetrating the endothelium cells fenestrae (Oz *et al*., 2000) .

NK cells granules are known to contain previously synthesized lytic mediators, which play an important role in the cytotoxic function of these cells which are released to kill tumor- or virus-infected cells (Vanderkerken *et al*., 1993).

6- Oval cells: Are liver stem cells that play akey role in liver regeneration when the hepatocyte response to injury is insufficient (Cohen, 2015). Farber first identified oval cells as having a mildly basophilic cytoplasm and pale blue staining nuclei using hematoxylin and eosin staining (Abdellatif, 2018). These cells are activated by direct signaling from damaged hepatocytes or indirect signaling from HSCs and other inflammatory cells to produce regereration in the liver by proliferating and differentiating into hepatocytes and cholangiocytes (Rehman *et al*., 2013).

2-6: Liver functions

As one of the most important organs, liver has important and main functions in the body due to its ideal location in abdominal cavity to receive and detoxify absorb substances transported via portal blood from the small

and large intestines, stomach, spleen, and pancreas. The main functions of the liver include:

1. Bile Formation and secretion of bile acids: Bile is a complicated aqueous secretion that starts from hepatocytes and is changed distally by absorptive and secretory transport mechanisms in the bile duct epithelium. Bile subsequently reaches the gallbladder, where it is concentrated, or is given straight to the intestinal lumen, in which a variety of endogenous solids are dissolved. Bile is made up of 95% water and contains bile salts, bilirubin phospholipid, cholesterol, amino acids, steroids, enzymes, porphyrins, vitamins, and heavy metals (Boyer, 2013; Baiocchi *et al*., 2019) .

2. Bilirubin production and excretion:Bilirubin results from destruction of senescent red blood cells which is performed by reticuloendothelial system. Hemoglobin is degraded by the reticuloendothelial system and separated into two parts: heme and globin. (Adin, 2021). Globin is hydrolyzed into to amino acids and recycled to form new protein whereas heme is converted enzymatically to green-colored biliverdin which is then converted to yellow-colored bilirubin. Bilirubin is bound to circulating albumin in the blood to form unconjugated bilirubin which carried to the liver and taken up. Hepatocyte joins with glucuronic acid to produce the conjugated form of bilirubin (water soluble) which can be excreted in the bile. Conjugated bilirubin flows within bile into the distal ileum and colon and is deconjugated and converted to urobilinogen by bacterial enzymes to be excreted in the urine and feces (Reece, 2009) .

3. Metabolism of the nutrients such as carbohydrates, proteins, and lipids (Hall, 2016).

4. Substances detoxification and excretion of waste products: The body is potentially protected by liver from toxic substances which are absorbed by the gastrointestinal tract. These substances are brought to the liver through the portal circulation and modified in so-called (first pass metabolism) where little or none of these substances return back again into the systemic circulation (Eurell and Frappler, 2006) .

5. The Liver has immunity function: The liver is a key innate immune organ against bacterial infection. Hepatocytes and immune cells (Monocytes, macrophages, granulocytes, natural killer cells, and dendritic cells) cooperatively control systemic and local bacterial infections from blood that entering through the portal vein and carrying nutrients and bacterial endotoxins from the gastrointestinal tract (Hastings *et al*., 2020).

6. Hemostatic activities are also performed by the liver. Prothrombin, fibrinogen, and coagulation factors are all produced by this organ. Vitamin K is a fat-soluble vitamin that aids in the production of other clotting factors (Ozougwu, 2017) .

7. The liver acts as storage of certain vitamins and minerals: Minerals such as iron and copper are stored in the liver which also stores fat-soluble vitamins such as A, D, E, and K and water-soluble vitamin as B12 (Aspinall *et al.*, 2015; Radu-Ionita *et al*., 2020).

8- Creating circulating plasma proteins: Liver cells are capable of generating the majority of circulating plasma proteins, including albumin, lipoproteins and glycoproteins. Energy, molecular transport, and inflammation are all activities of these hepatocyte-produced proteins (Jenning and Premanandan 2017).

2-7: Hepatic fibrosis

Liver fibrosis is a chronic liver condition that develops as a result of a chronic wound healing response following long-standing liver injury. During hepatic fibrogenesis, the liver parenchyma undergoes fundamental remodelling processes result in cellular injury and set inflammatory responses represented by releasing a variety of cytokines and growth factors that activate and stimulate the transformation of resting hepatic stellate cells into myofibroblast like cells, which initiate an excessive synthesis and accumulation of fibrillar ECM (Lo and Kim, 2017). The uncontrolled and extensive formation of fibrosis leads to distortion of lobular architecture of the liver tissue resulting in nodular formation and cirrhosis. The continuous injury and the subsequent regeneration process may also cause genomic aberrations and mutations that result in the development of hepatocellular carcinoma (George, 2019). The content of the total collagen in a fibrotic liver is about 3 to 10 fold higher than normal liver. However, the results of a previous study of 200 dogs undergoing post-mortem examination for different reasons showed that a 12% of dogs had histological changes consistent with chronic hepatitis (CH), a disease ended with hepatic fibrosis as a defining feature. Therefore, hepatic fibrosis can be considered as a common finding in dogs. The fibrotic liver in both human and dogs shows a similar histological appearance and progression in spite of different causes of chronic hepatitis in both species (Seki & Brenner, 2015; Eulenberg & Lidbury, 2018) . Cholestatic injury which is associated with reduced or obstructed bile flowing within the liver, is the result of primary and secondary diseases such as primary biliary cholangitis, primary sclerosing cholangitis as well as biliary atresia consedared the most common cause of fibrosis. (Bataller & Brenner, 2005a; Berumen *et al*., 2020).

2-8: Pathogenesis of liver fibrosis

Hepatic fibrosis is the liver wound-healing response to the repeated injury. Following an acute liver injury such as viral hepatitis, the necrotic or apoptotic cells are replaced by the regeneration of parenchymal cells. This process is related to an inflammatory response with limited deposition of ECM (Pellicoro *et al*., 2014 Tacke & Trautwein, 2015) . In case of persisting hepatic injury, failure of liver regeneration and substitution of hepatocytes with abundant ECM involving fibrillar collagen are the eventual outcome (Eulenberg & Lidbury, 2018) . The distribution of the deposited fibrous material differs according to the origin of the liver injury. The initial location of the fibrotic tissue in case of both chronic viral hepatitis and chronic cholestatic disorders is around portal tracts, whereas pericentral and perisinusoidal areas are the site of alcohol-induced liver disease(Bataller & Brenner, 2005b) . In advanced fibrotic liver diseases, there is a progression from collagen bands to bridging fibrosis to frank cirrhosis (Bataller &Brenner , 2005b; Fallowfield & Hayes , 2011).

Recent evidence indicates that the fibrosis can be reversible process in spite of the traditional view which emphasizes the irreversible state of hepatic cirrhosis. In hepatic fibrosis which is experimentally induced, the cessation of liver injury leads to fibrosis regression (Eulenberg &Lidbury , 2018; Sun *et al*., 2020). In humans, liver fibrosis may associate with spontaneous resolution following successful treatment of the underlying disease such as iron and copper overload, liver injury caused by alcohol, chronic hepatitis (type C, B, and D), hemochromatosis, secondary biliary cirrhosis and autoimmune hepatitis. The significant regression of hepatic fibrosis may take years to be achieved with variable time relying on the underlying cause of the hepatic disease and its severity. The major mechanism of fibrosis resolution is

represented by an increased collagenolytic activity by Matrix metalloproteinases (MMPs) which responsible for the turnover of matrix proteins ,and considered as anti-fibrotic effect due to their ability to degrade ECM proteins (Naim *et al*., 2017) . which is preceded by the removal of the activated HSCs by apoptosis via stimulation of both death receptors in activated HSCs and a decreased survival factors such as The tissue inhibitors of metalloproteinases (TIMP-1) which can precipitate HSC apoptosis (Bataller & Brenner, 2005b ; Ebrahimi *et al.,* 2018).

On the other hand More recent research has focused on the biochemical changes affecting fibrosis irreversibility. An important advance has been the identification of lysyl oxidase 2 (LOXL2) in catalyzing the cross-linking of extracellular ollagens . LOXL2 stabilizes the ECM, and in more advanced stages reduces fibrosis reversibility (Trautwein *et al*., 2015).

2-9: Diagnosis and assessment of liver fibrosis

1. Liver biopsy: Despite its invasive character, liver biopsy can be considered as the most common reference standard which is highly recommended for diagnosing and staging the hepatic fibrosis (Toosi, 2015). The histopathological examination of the liver biopsy is usually necessary to confirm hepatic diseases. On the other hand, certain disorders like coagulopathies, pleural effusion, peritonitis, suspected liver abscesses and vascular tumors are considered as the main obstacles to pursue a transthoracic liver biopsy (Nyland & Gillett, 1982).

2. Biochemical markers: Biochemical markers of hepatic fibrosis in the serum can be tested and compared with biopsy results (Toosi, 2015). The minimum serum biochemical database includes many agent to be tested such as ALT, AST, ALP, GGT, BUN, creatinine, total bilirubin. Furthermore,

biochemical examination of total protein, glucose, albumin, globulin, and cholesterol is performed in case of suspected hepatic affection (Hall & German, 2016).

3. Ultrasonography: Ultrasonography is an noninvasive, safe method used to differentiate between extrahepatic biliary obstruction and other causes of jaundice (Nyland & Gillett, 1982). However, the correlating of ultrasonographic abnormalities with the history, clinical examination findings, biochemical results, radiographic and laparoscopic observations is necessary for tentative diagnosis of primary hepatic disease. Ultrasonography is considered as the choicest technology which is used to identify various forms of hepatic disease in canines (Kumar *et al*., 2012). During the last two decades, elastography has been used as a quantitative imaging approach to noninvasively evaluate liver fibrosis (William *et al*., 2017). In addition, the non-invasive transient elastography is used for the assessment of the state of hepatic fibrosis (Toosi, 2015). Transient elastography could measure a volume which is 100 times larger than a typical needle biopsy (Eulenberg & Lidbury, 2018). Radiography and abdominal ultrasound are the most frequently imaging techniques used to assess the hepatobiliary system in both dogs and cats (Kozat & Sepehrizadeh, 2017) . Ultrasonography is a frequently used tool to diagnose liver diseases in dogs, but the tentative diagnosis could not be confirmed depending on ultrasonographic imaging alone due to marked overlap in appearance of different hepatic diseases. Histological evaluation remains necessary for the diagnosis of canine hepatic disease (Warren-Smith *et al*., 2012).

2-10: Experimental induction of hepatic fibrosis.

There are common models to induce liver fibrosis such as administration of hepatotoxic agents, surgical ligation of the common bile duct, liver injury induced by immunological stimulation and introduced gene defects or overexpression of transgenes which influence the critical signaling pathways that are involved in the pathogenesis of hepatic fibrosis (Weiler-Normann *et al* ., 2007) . The oral administration of carbon tetrachloride (CCl4) and ligation of the common bile duct (BDL) are considered as the most common methods to induce experimental hepatic fibrosis and/or cirrhosis (Ishibashi *et al*., 2009). Ligation of the common bile duct is a safer method in comparison with carbon tetrachloride. Bile duct ligation causes an acute obstructive jaundice within two weeks which progresses to cirrhosis in 4 or 6 weeks later whereas four weeks are enough to cause a moderate cell necrosis and fatty infiltration due to continuous administration of carbon tetrachloride (Marques *et al*., 2012).

2-11: Treatment Strategies for Hepatic Fibrosis

The therapeutic strategies to treat hepatic fibrosis can be divided into those that reduce myofibroblast activation and those that stimulate both apoptosis of activated myofibroblasts and extracellular matrix degradation. Antioxidants are also commonly used for treatment of hepatobiliary disease in dogs such as S- adenosylmethionine, vitamin E, and silymarin (milk thistle extract), Colchicine (Favier, 2009) (Fallowfield & Hayes, 2011) . Furthermore, treatment by immunomodulatory therapy (Glucocorticoids) and antiviral agents are used to treat hepatic fibrosis. Pirfenidone is an antifibrotic agent which acts via inhibiting nuclear factor-kappa B and its downstream profibrogenic mediators which include PDGF, TGFb-1, and interferon alpha,

leading to reduced HSC activation and deposition of the extracellular matrix. It acts to reduce hepatic fibrosis and inflammation in humans with chronic hepatitis C when given for 2 years (Alejandra *et al*., 2017 ; Seniutkin, 2018) . Hepatoxicity has been reported to be caused as a result of using this drug which is thought to restrict its use in patients suffering from preexisting liver disease (Eulenberg & Lidbury, 2018). Nanoparticles with stimuli sensitive polymers and liposomes have been involved in the treatment of liver fibrosis as well as other hepatic diseases such as cancer (Surendran *et al*. 2017) .

Chapter Three

Material and Methods

3-1: Animals

Twelve healthy local dogs of both sexes whose ages ranged between (6 - 36) months and their weights ranged between (11-25) kgs were used to conduct this study. The dogs were kept in a place designated to house dogs belonging to the department of surgery and theriogenology, College of Veterinary Medicine, University of Mosul , these animals were treated with ivermectin at dose 0.2 mg / kg .

The animals were examined physically and clinically to ensure that they are free of diseases. The dogs were kept for two weeks prior to the surgery to be adapted.

3-2: materials and methods:

3-2-1: Device and tools used in the study

The used tools and devices were listed with their names and origins in Table (3-1) :

Table 3 – 1: shows the name of the equipment we used and where it was made

number	The name of the device or tool	Origin
1	Autoclave	United Kingdom
2	Centrifuge	Germany
3	Rotary microtome	Italy
4	Sonar	U.S.A.
5	Spectrophotometer	China
6	An integrated surgical kit to perform the surgery	multi-origin
7	10ml test tubes	China

8	Disposable syringe	China
9	Digital camera	Japan

3-2-2: Medicines and chemicals used

The names and addresses of manufacture of the chemicals and medicines used in the study were shown in Table (3-2) :

Table 3 – 2: shows the chemicals and drugs used in the experiment, as well as the nation in which they were made

number	chemicals and drugs	origin
1	Xylazine 2%	Interchemie (Netherlands).
2	Ketamine hydrochloride 5%,	(Germany).
3	Penicillin streptomycin blend	kanavet company (Canada).
4	Metalgen Vitafor	Belgium)
5	Spray wounds OTC	Jordan
6	Formaline 10 %	England
7	glucose solution	Germany
8	Absolute alcohol (ethanol)	Sweden
9	Povidine iodine	France
10	Silk 4/0	Romania
11	Silk 2 metric	China
12	polyglactin 910 4/0	China
13	polyglactin 910 2 metric	China
14	Hemotoxylin and eosin dye solutions	Switzerland
15	Masson's trichrome	Italy
16	Xylol	India

3-3: Experiment design

This study was conducted on twelve healthy local adult dogs. Liver fibrosis was surgically induced in all animals by surgical closure of major duodenal papilla. Animal were divided randomally into two groups of 6 animal per each group:

1- First group

Hepatic fibrosis was induced by surgical closure of major duodenal papilla inside the duodenum with 4/0 non- absorbable suture material (silk).

2- Second group

Hepatic fibrosis was induced by surgical closure of major duodenal papilla inside the duodenum with 4/0 absorbable suture material (polyglactin 910).

Induced hepatic fibrosis was evaluated by clinical, laboratory, sonography, macropathologically and histopathological methods (Figure 3-1)

Prior to surgery, all animals in both groups represented the control state where ultrasonography of each animal and collection of blood samples and liver biopsies was performed.

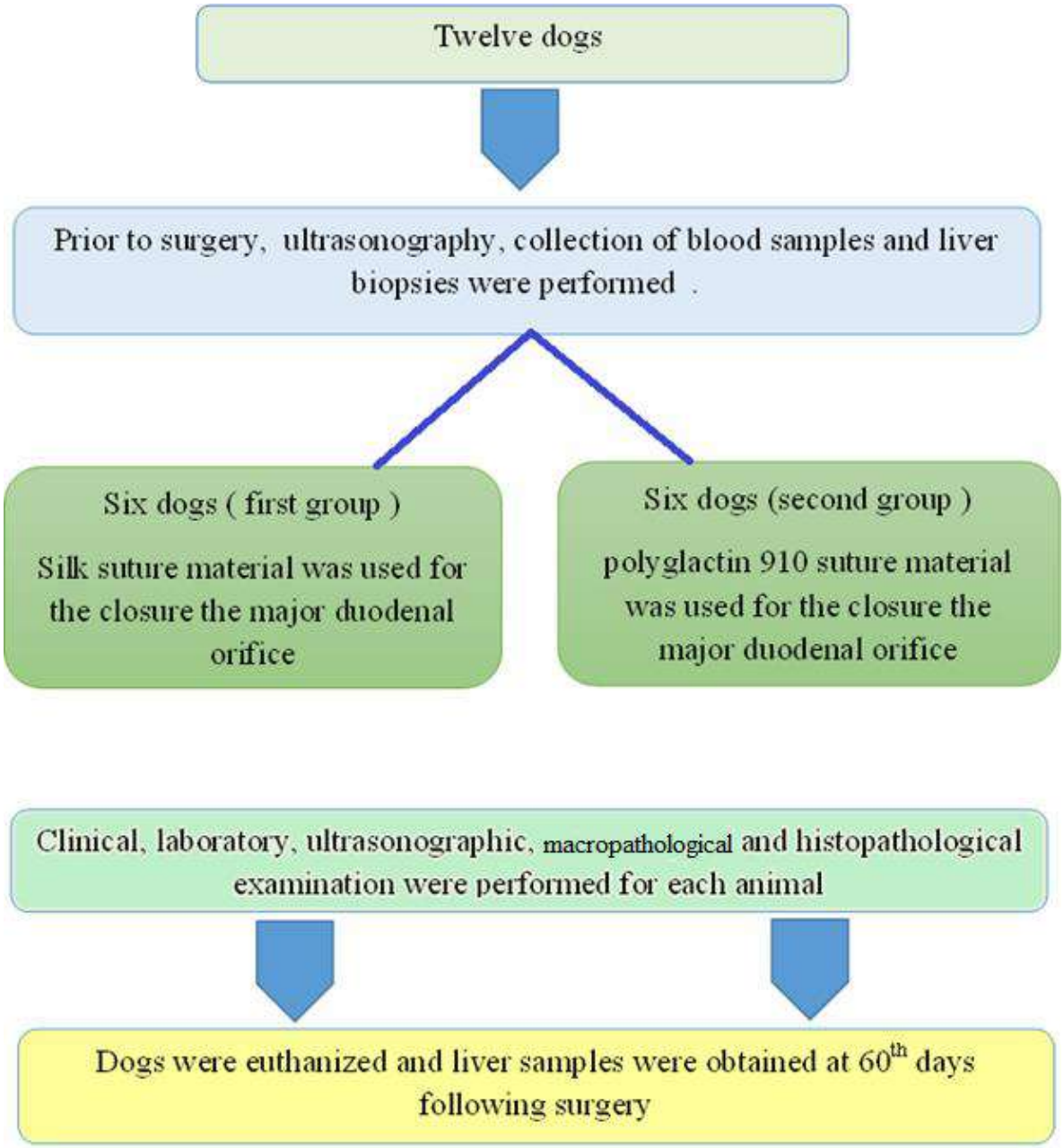

Figure (3 – 1): Experiment design shows animal grouping of irreversible and reversible liver fibrosis in dogs

3-4: Preparation of animals for surgery

The dogs were fasted from food for 24 hours prior to surgery. The animal was anesthetized with mixture of ketamine (5%) and xylazine (2%) at a dose of 10 mg / kg and 2 mg / kg of body weight respectively. The abdomen was prepared from the xyphoid to the umblical region according to the basic traditional principles of surgery.

3-5: Surgical Procedures

The animal was placed on the dorsal recumbency.Laparotomy was performed through a midline abdominal incision of approximately 10 cm leng After that a biopsy of liver tissue was collected and the sample was placed in a container containing buffer formalin concentration (10%) as in (Figure 3-2).

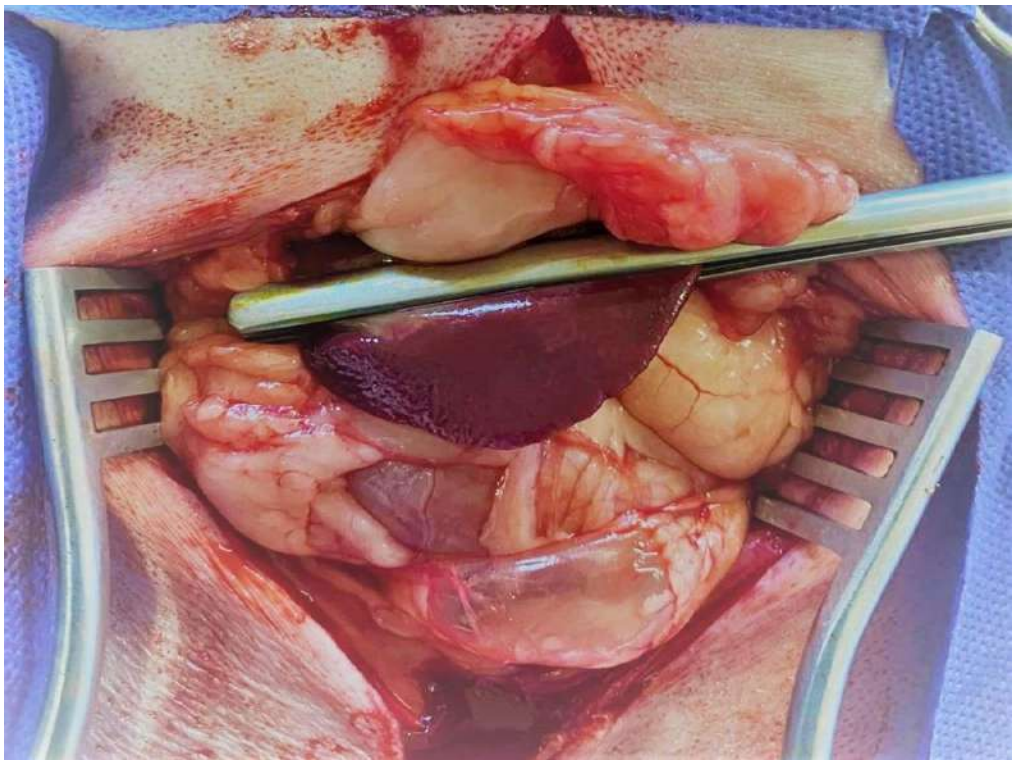

Figure (3 –2): Surgical collection of liver biopsy prior to the induction of liver fibrosis

The duodenum was exposed and milked from contents that were found in it (Approximatelly 15 cm from the pyloric orifice) which is adjacent to , and then the duodenum was clamed by doyen intestinal clamps (Figure 3-3). Enterotomy was performed through longitudinal incision (10 cm) in the anti-mesenteric border to close the major duodenal orifice which was located 2-6 cm from the pyloric region (Figure 3-4). A catheter was inserted in to the major duodenal orifice to make sure that the bile substance has come out through it (Figure 3-5).

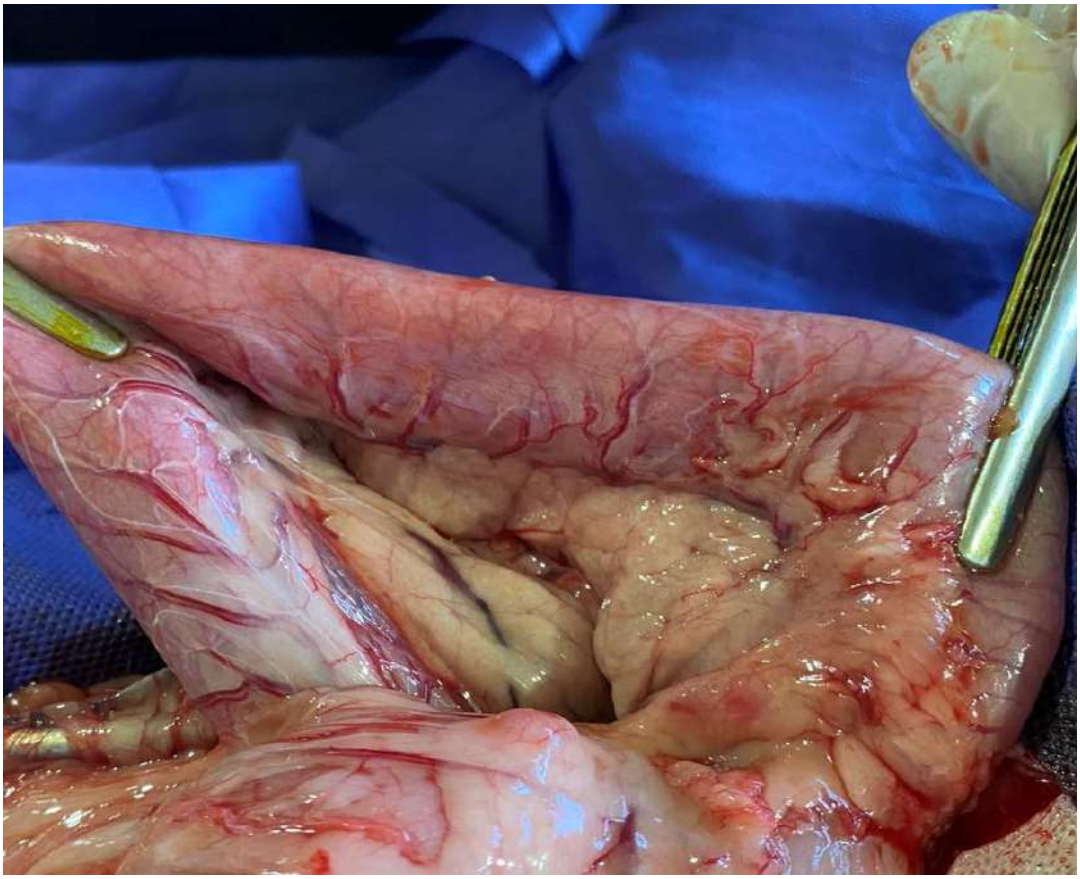

Figure (3 - 3): Shows the loop of duodenum which were entertomy was made

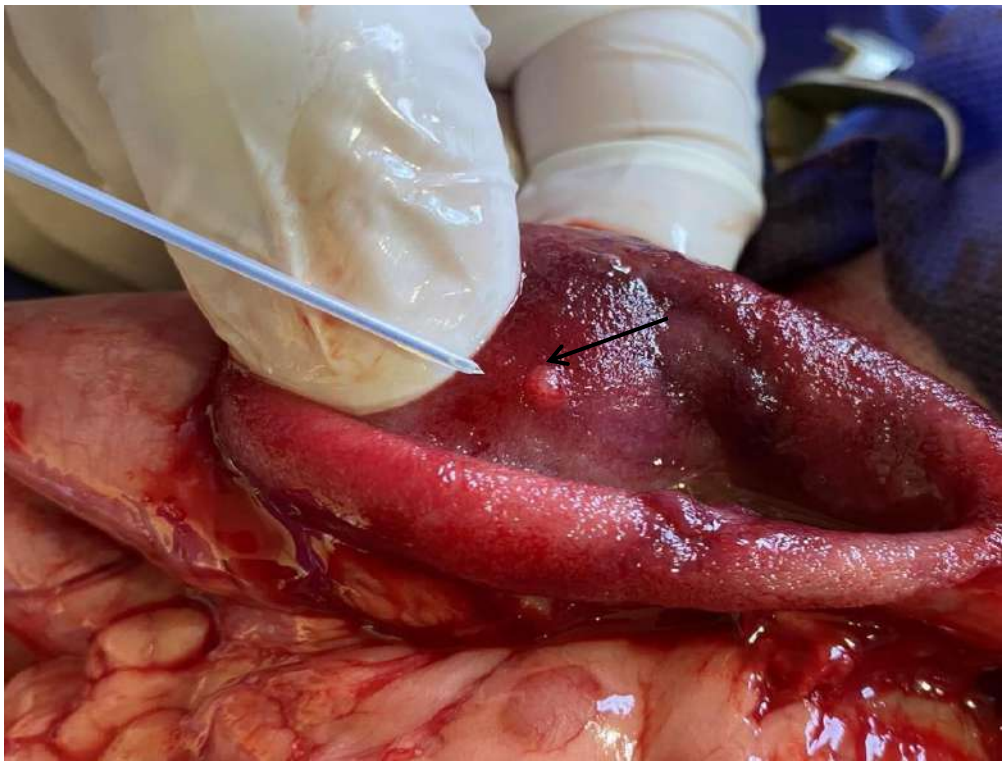

Figure (3 - 4): Major duodenum papilla (arrow) which was surgically closed to induce irreversible and reversible liver fibrosis .

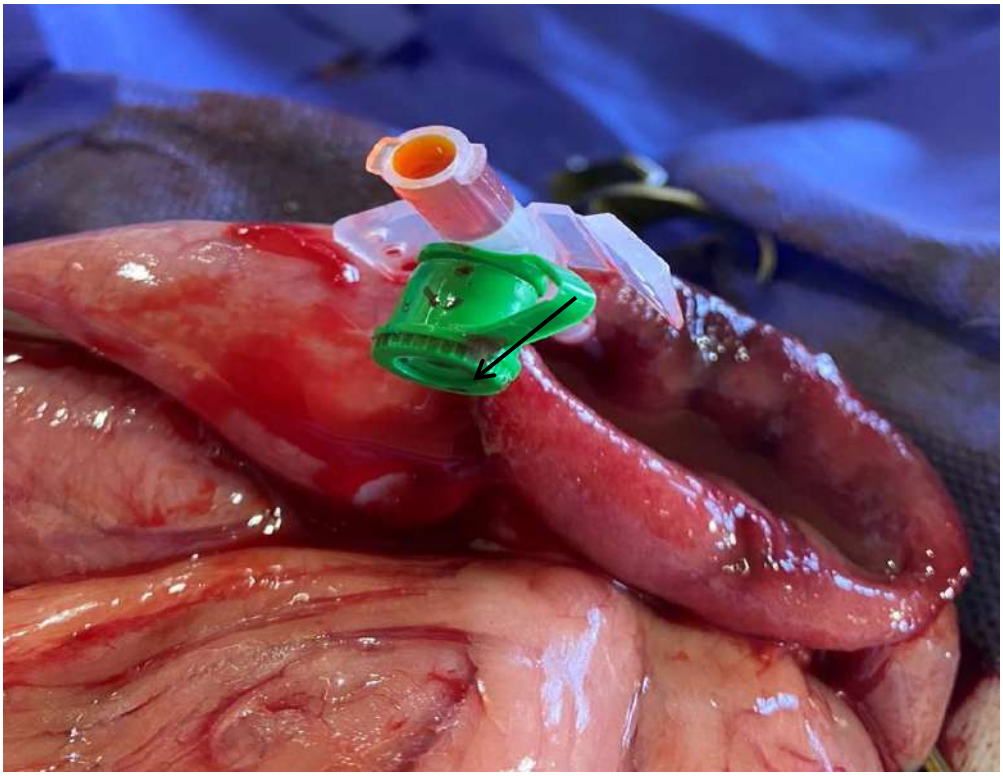

Figure (3 - 5) :Flow of bile fluid (arrow) throw catheter which was inserted into the major duodenal papilla .

Major duodenal orifice was closed with simple continuous by non-absorbable suture material (silk) in group one (Figure 3-6), whereas in the group two absorbable suture material was used (polyglactin 910) .

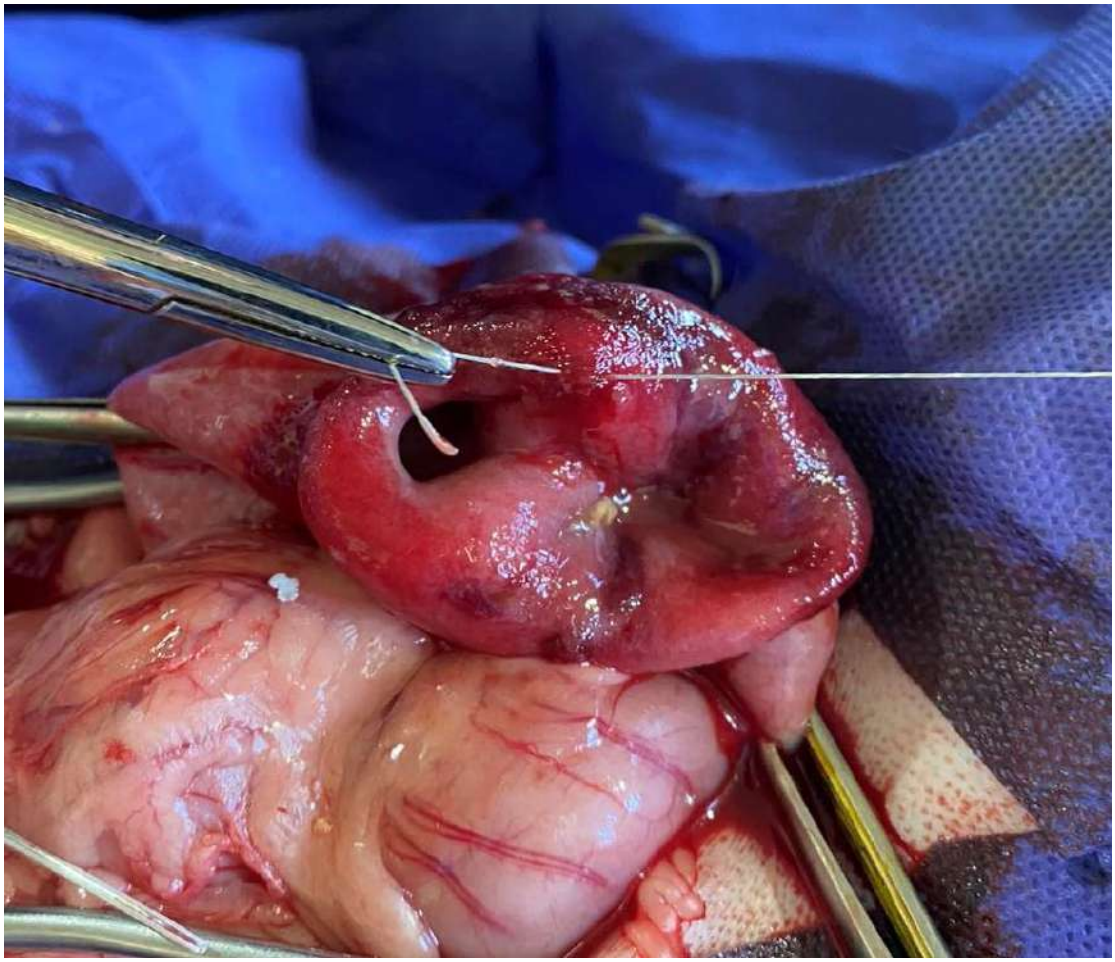

Figure (3 -6) surgical closure of major duodenal papilla to induce liver fibrosis in group one .

Enterotomy incision was sutured by Connell and Cushing suture technique using polygactine suture material No. 3-0. The abdominal muscles and peritoneum were sutured by interlocking suture technique using polygactine suture material No. 2. Subcuticular suture technique using polygactine suture material No. 1 was used to close skin incision.

3-6: Post-operative care

After operation the all animals was administered dipyrone by intramuscular injection at a dose of 1 ml / 5 kg of body weight, and the antibiotic (penicillin-streptomycin) was injected intramuscularly for five consecutive days at a dose of 10,000 IU and 15 mg / kg of body weight.

3-7: Postoperative examinations

The following tests were carried out for each of the experimental animal as follows:

3-7-1: Clinical examination

The health status of the animals in two groups was monitored after surgical induction of the hepatic fibrosis for 60 days after surgery by observing the animal's movement, activity and appetite for food.

3-7-2: Laboratory tests

To investigate the course of hepatocellular injury following surgical induction of hepatic fibrosis, serum levels of aspartate aminotransferase (AST) , alkaline phosphatase (ALP) , alanine aminotransferase (Alt) , indirect bilirubin and direct bilirubin were determined. A blood sample of 5 ml was withdrawn from the jugular vein for each animal of the study prior to operation, two days following surgery and then weekly until the end of the study to demonstrate the effect of surgical blockage of the common bile duct inside the duodenum on fibrosis progress .

3-7-3: Ultrasound test

Ultrasound test of liver for all animals was performed before surgical induction of hepatic fibrosis to ensure the integrity of the hepatic tissue. Following fibrosis induction of liver, ultrasound test for dogs was made weekly in order to compare results with initial examinations before the surgical operation for a period of two months.

3-7-4: Macropathological examination.

Macropathological examination was performed to all animals at 6o days after operation to inspected examined the abdominal cavity, liver and gallbladder.

3-7-5: Histological evaluation

Hepatic biopsies were collected from animals of the experiment before surgery and two month later following animal euthanasia for histopathological examinations .Also gall bladder biopsies were collected from animals of the experiment at two month after operation Fixation of liver and gallbladder samples was performed by using neutral buffered formaline solution 10% and skin specimens were embedded in paraffin, stained with hematoxylin-eosin (Luna, 1968) and Masson's trichrome and examined under a light microscope (Amin & Mahmoud, 2011).

3-8: Statistical analysis

Statistical analysis of the data was made by using the statistical SPSS program v.23 software (SPSS In. Chicago, IL., USA). All results were expressed as mean ± standard error (mean ± S.E.). One way ANOVA and LSD test were used to evaluate the significance between groups and P values of less than 0.05 were considered as significant.

Chapter four

Results

4-1: Clinical findings

All the animals survived until the end of the experiment. In the first group, the animals were severely dull and depressed. In appetite and gradual loss of body weight were observed in all animals following surgical induction of hepatic fibrosis. Bodyweight were correlated with corresponding cholestatic duration and Jaundice was the main clinical feature of the animals with blocked common bile duct where pale yellowish mucus membrane of the eye and the oral cavity was clearly noticed. Post-operative pain was severe especially during the first two days following the surgical induction of hepatic fibrosis. Food uptake increased due to gradual decrease in post-operative pain but still at lower level leading to gradual decrease in the weight body resulting in cachexia of the animals (Figure 4-1).

The clinical findings in the second group were similar to that of the first group with the exception that there was gradual clinical improvement which started at the 30^{th} post-operative day. The improvement was manifested by gradual increase of body weight and disappearance of jaundice signs.

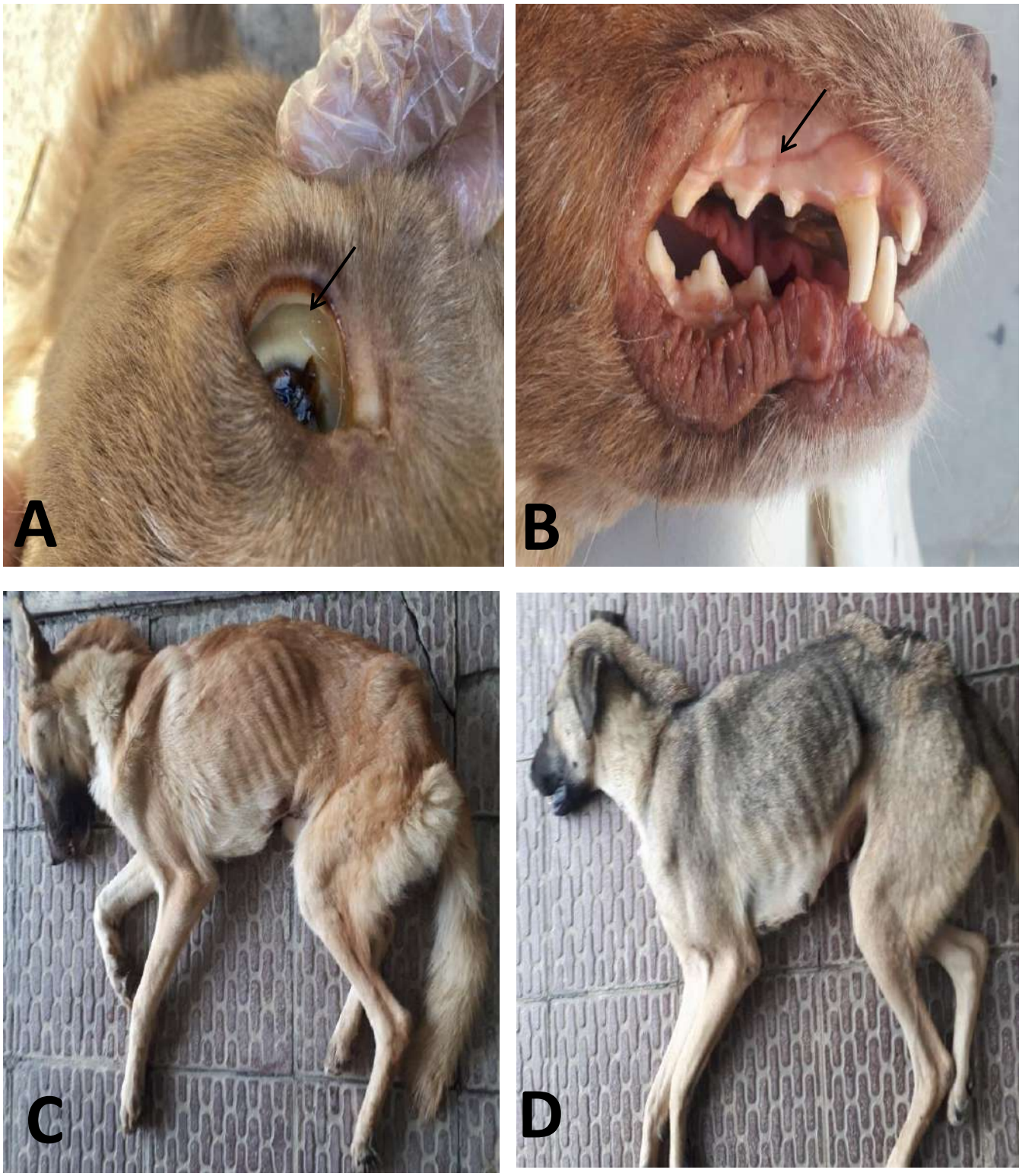

Figure (4 – 1) :Show the clinical signs in first group. .A: yellowish mucus membrane of eye (arrow) .B: yellow oral mucus membrane (arrow) . C and D: dogs suffering from cachexia .

Macropathological investigation at 60 days post operation showed apparent swollen liver and engorgement of the bile duct and expansion of the gall bladder resulting in changed shape of the gall bladder. White lines and spots were noticed in different areas of the liver indicating the presence of the hepatic fibrosis. There was discoloration of different region of the liver manifested by yellowish staining. Evidence of bleeding on the wall of the gall bladder with increased wall thickness of the both gall bladder and bile ducts were observed (Figure 4-2)

Similar but less intensive macroscopic features were noticed in the animals of the second group. The duodenal orifice of the common bile duct was found to be opened due to absorption of the polyglactin 910 suture material which was used to block the orifice resulting in the free passage of the bile secretion into the duodenum when the gall bladder was squeezed by hand. There was mild to moderate yellowish discoloration of the liver tissue and the white lines and spots were lesser in size and intensity. There was normal size and shape of the gall bladder due to free passage of the bile and lesser engorgement of the bile duct.

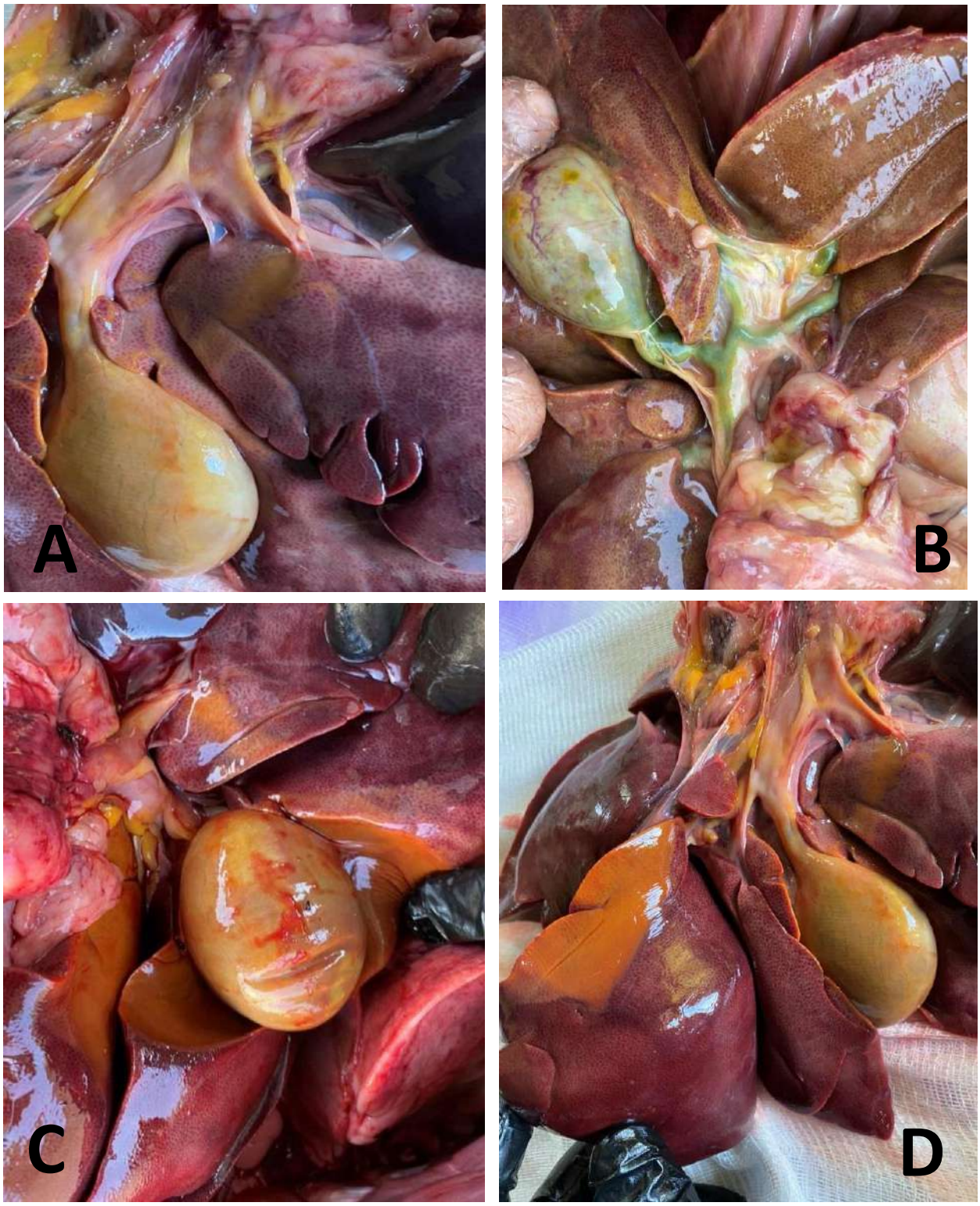

Figure (4 – 2) Shows macropathological investigation at 60 days post operation in first group . A: Swollen gallbladder, B: Engorgement of bile duct. C: Hemorrhage in the wall of gallbladder (arrow) , D: Liver lobe pigmentation with bile (D) .

4-2: Biochemical parameters

1- Aspartate aminotransferase (AST):

In the first group serum AST levels showed significant elevation during all the post-operative days with respect to day zero. In addition, the results revealed a significant decline from 21days to last week when compared to the 2nd, 7th and 14th days which showed no significant differences between them.. The values of the 5th, 6th, 7th and 8th weeks revealed a significant decline with respect to 3rd and 4th weeks. Where as in second group serum AST levels showed significant elevation during all the postoperative days with respect to day zero. Also AST levels showed a significant elevation during 3rd and 4th weeks with respect to 5th, 6th, 7th and 8th weeks (Figure 4-3).

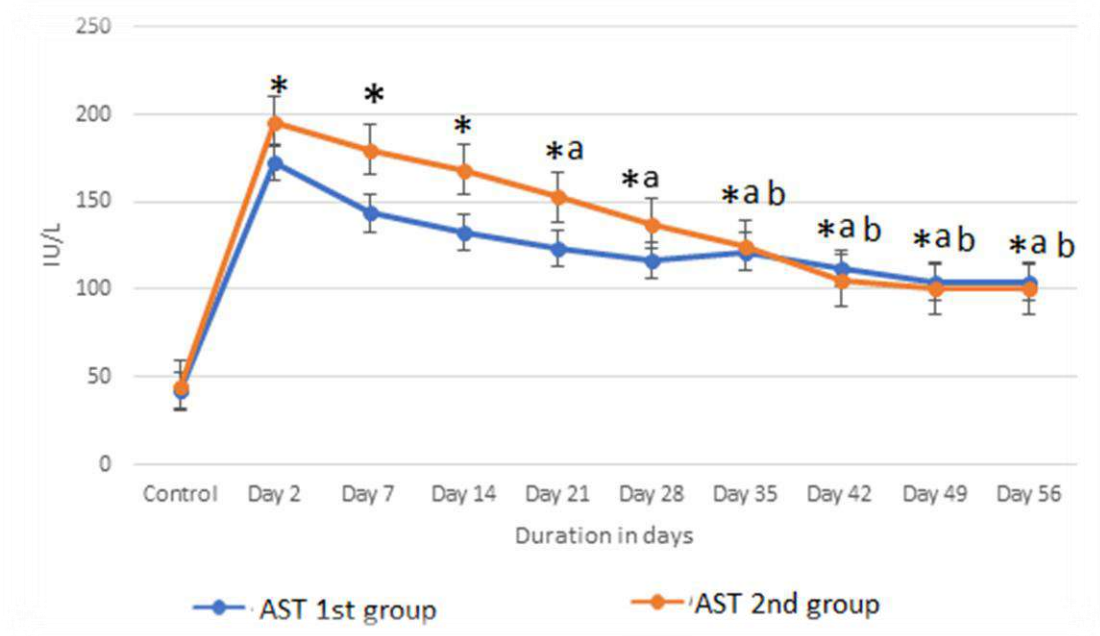

Figure (4 –3) : Shows changes inliver level of AST in animals of the first and second groups compared to the normal levels prior to fibrosis induction. *: Significant difference between control and experimented groups. Small letters: Means significant within weeks in the same group.

2- Alkaline phosphatase (ALP):

In the first group serum ALP levels showed a significant elevation during all the postoperative days with respect to day zero. In addition to that, during the first 4th weeks revealed significant elevation compared to last 4th weeks. While the last two week exhibit significant decline in ALP values compared to other weeks. Where as in second group serum ALP levels showed significant elevation during all the postoperative days with respect to day zero. In addition to that, the 2nd, 7th 14th and 21th days revealed a significant elevation compared to last four weeks (Figure 4-4).

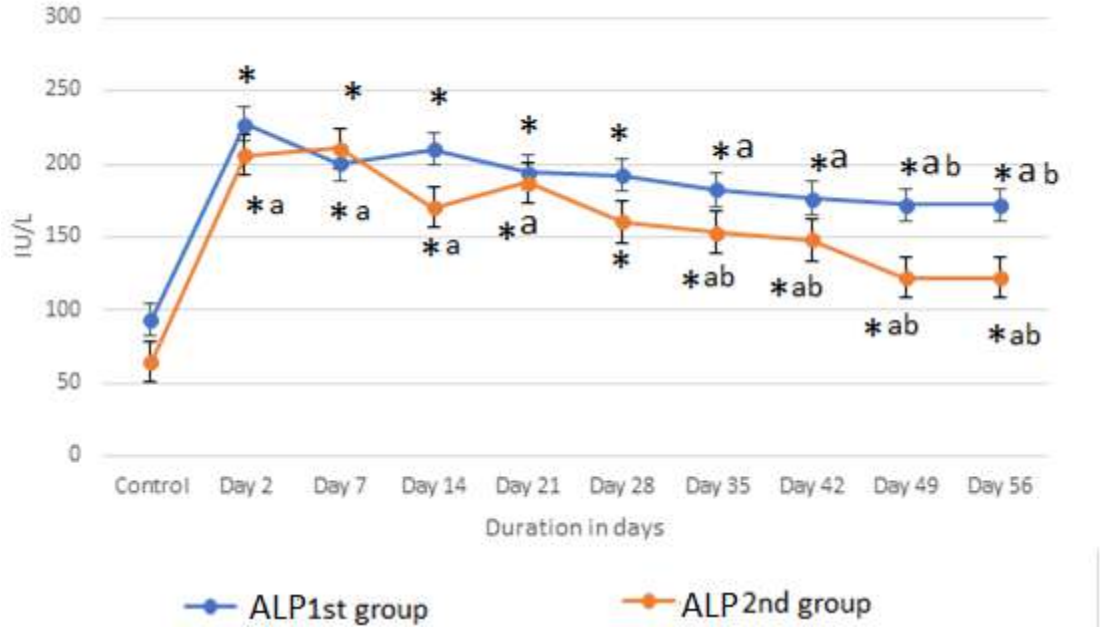

Figure (4 –4): Shows changes in liver level of ALP in animals of the first and second groups compared to the normal levels prior to fibrosis induction. *: Significant difference between control and experimented group. Small letters: Means significant within weeks in the same group.

3- Alanine aminotransferase (ALT):

In the first group a sharp significant increase in ALT levels in the 2nd day with respect to control values day zero as well as the ALT values remains high with consequence of the study day until the last day of treatment with respect to the day zero. A significant decline of serum ALT starting from the 3nd week until the last day of study with respect to 1st and 2nd week despite of its elevation to time zero. Whereas in second group sharp significant increase in ALT levels in the 2nd day the study with respect to control values day zero was formed. During the 1st week of study the ALT level showed significant decrease in relation to progress of time until last day of the study despite of the higher values compared to last four weeks (Figure 4-5).

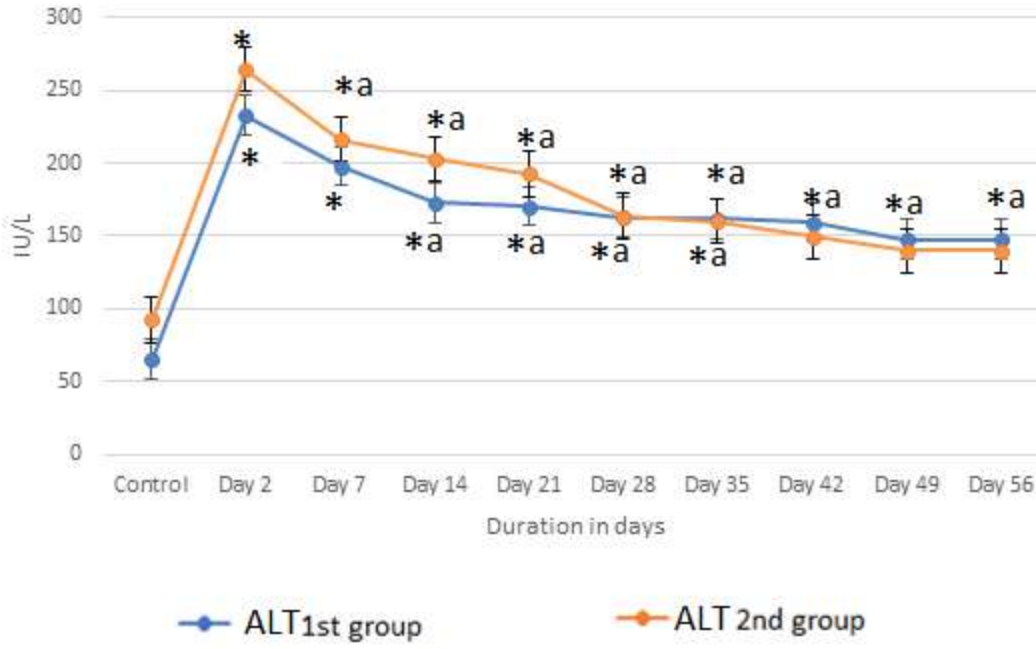

Figure (4 –5): Shows changes in liver level of ALT in animals of the first and second groups compared to the normal levels prior to fibrosis induction . *: Significant difference between control and experimented group. Small letters: Means significant within weeks in the same group.

4- Indirect bilirubin

In the first and second groups indirect bilirubin levels showed a significant increase from the 1st to the last weeks of the study compared to day 0 while the last two weeks revealed significant decline of indirect bilirubin levels compared to previous weeks of the study (Figure 4-6) .

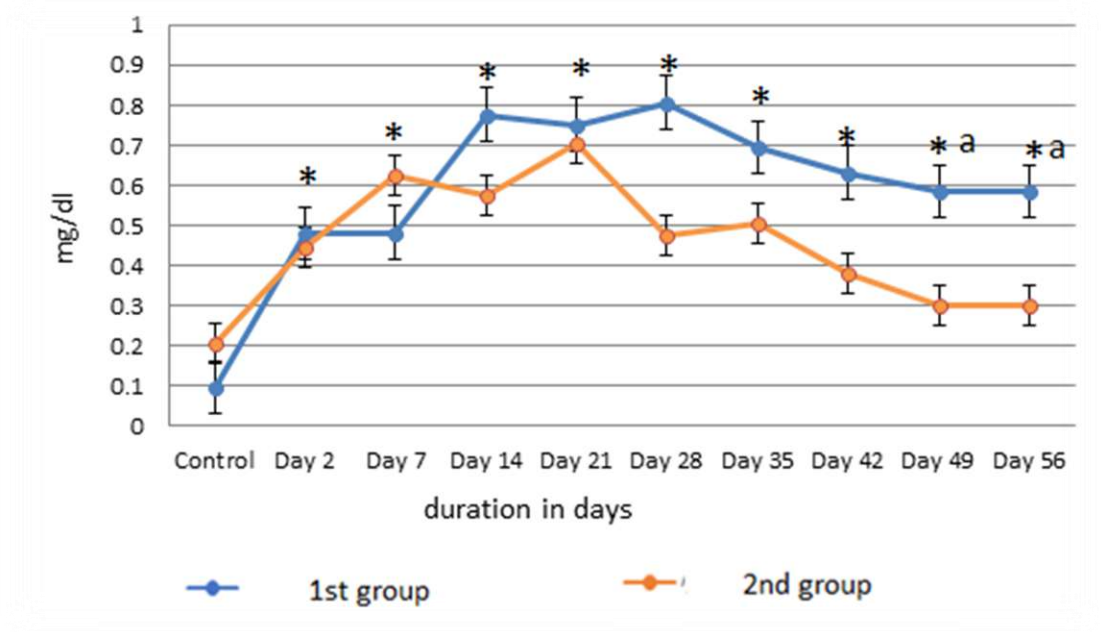

Figure (4 –6): Shows changes in liver levels of indirect bilirubin in animals of the first and second groups compared to the normal levels prior to fibrosis induction. *: Significant difference between control and experimented group. Small letters: Means significant within weeks in the same group.

5- Direct bilirubin

In first and second group despite the significant elevation of direct bilirubin levels starting from second days to the last week compared to time zero, a significant decline level starting from the 4th to last week of the study (Figure 4-7).

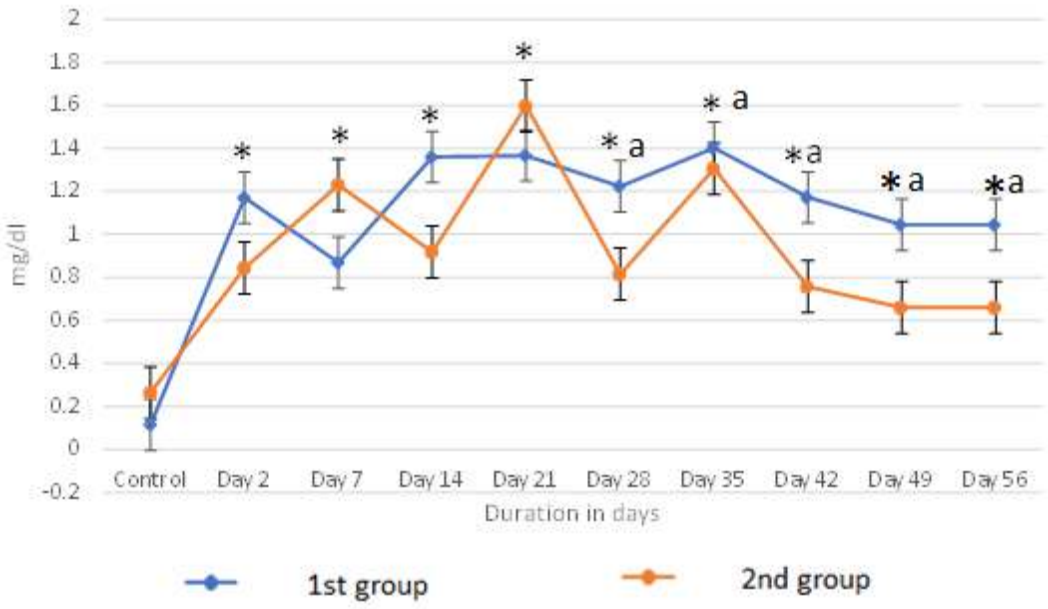

Figure (4 –7): Shows changes in liver levels of direct bilirubin in animals of the first and second groups compared to the normal levels prior to fibrosis induction. *: Significant difference between control and experimented group. Small letters: Means significant within weeks in the same group.

6- Total serum bilirubin (TSB):

In both groups TSB levels exhibits significant variations as same as direct bilirubin (Figure 4-8).

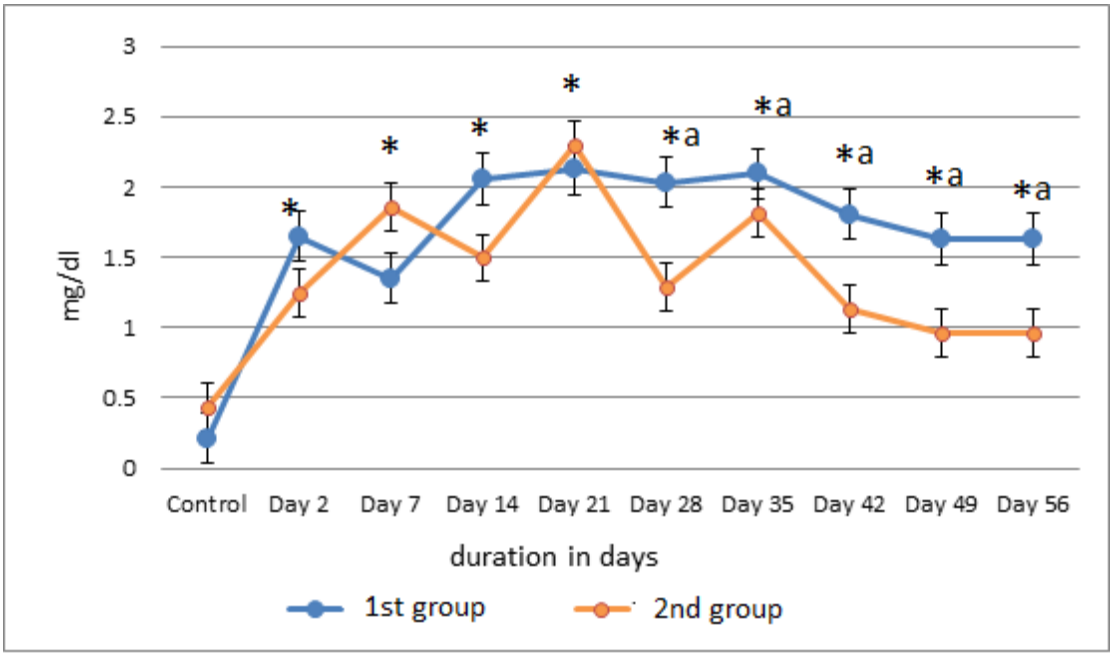

Figure (4 –8): Shows changes in serum liver levels of TSB following surgical induction of irreversible and reversible liver fibrosis in animals of the first and second groups compared to the normal levels prior to fibrosis induction . *: Significant difference between control and experimented group. Small letters: Means significant within weeks in the sam group.

4-3: Ultrasonographic examinations.

Results of the ultrasonographic examinations showed abnormal change in the liver tissue in both groups such as increased size and wall thickness of the gall bladder and mottled heterogeneous appearance of the liver instead of the normal appearance (weak homogenous appearance). These changes were observed two weeks after the surgical induction of the hepatic fibrosis and lasted until the end of the study in first group (Figures 4 – 9 - 4 – 12), while in the second group, the ultrasonography examinations showed a gradual decrease in the severity of these change from the 4th post-operative week until the end of the study (Figures 4 – 13 - 4 – 16) .

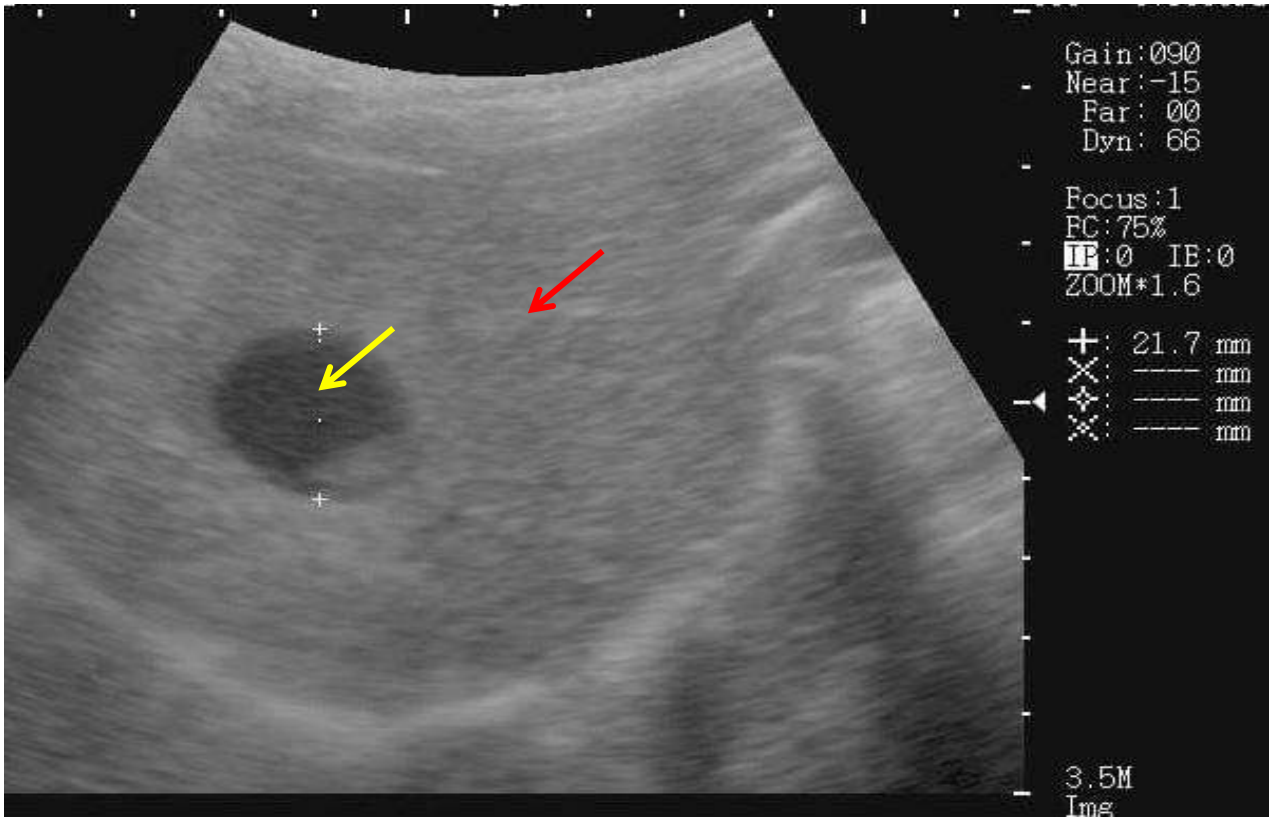

Figure (4 – 9): Ultrasonography of the first group shows normal liver tissue (red arrow) and normal gallbladder (yellow arrow) .

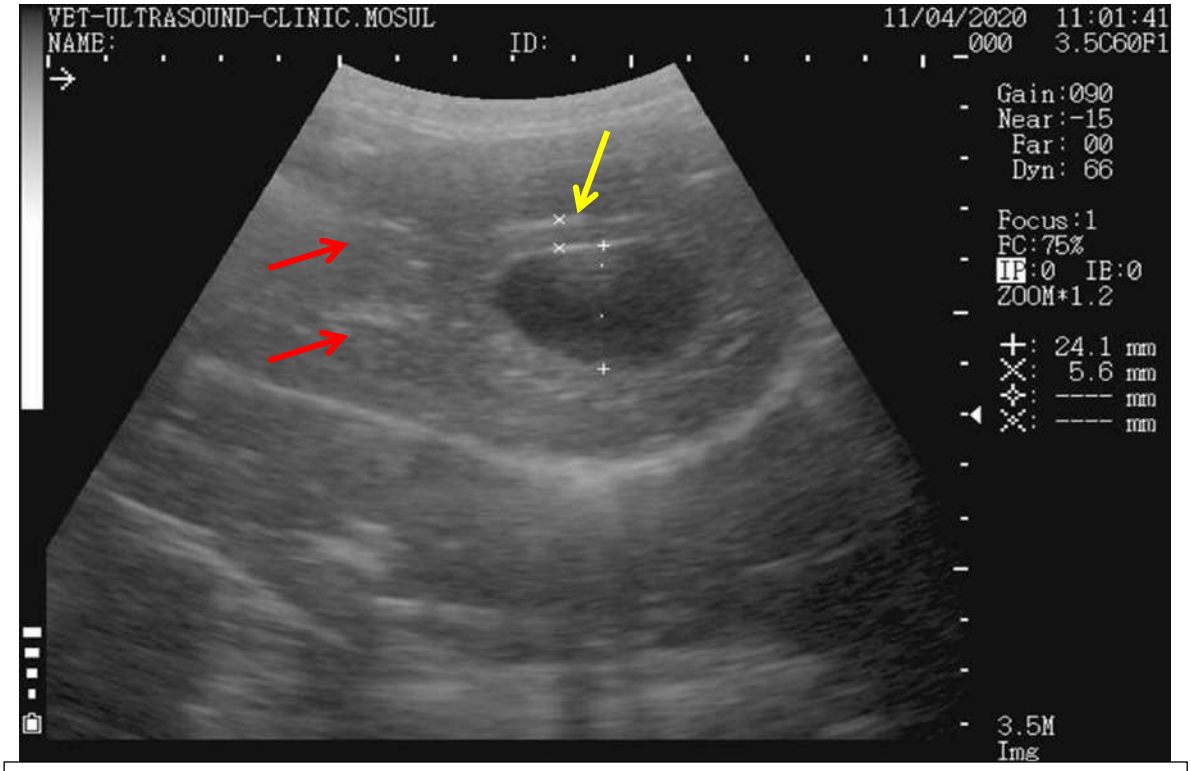

Figure (4 – 10): Ultrasonography of first group at 2nd post – operative week shows thickness in the wall of gall bladder (yellow arrow) and heterogeneous mottled appearance of liver tissue (red arrows).

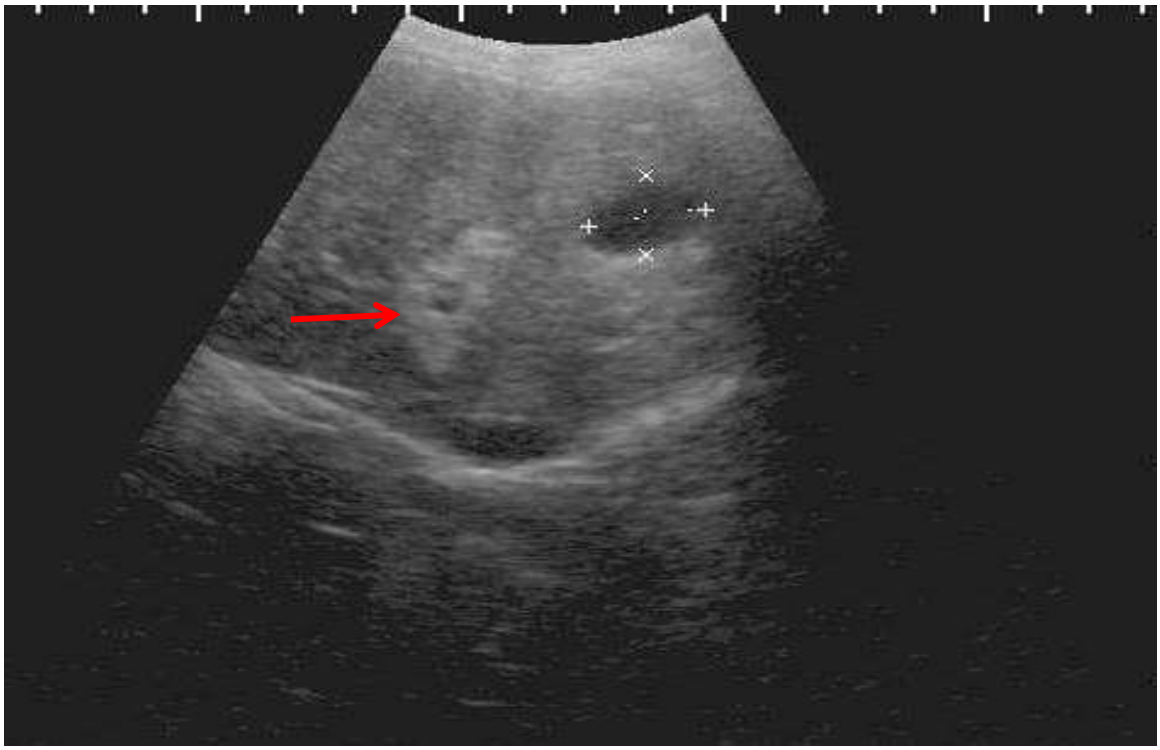

Figure (4 – 11): Ultrasonography of the first at 4th post – operative week shows heterogeneous and mottled appearance of the liver (red arrow)

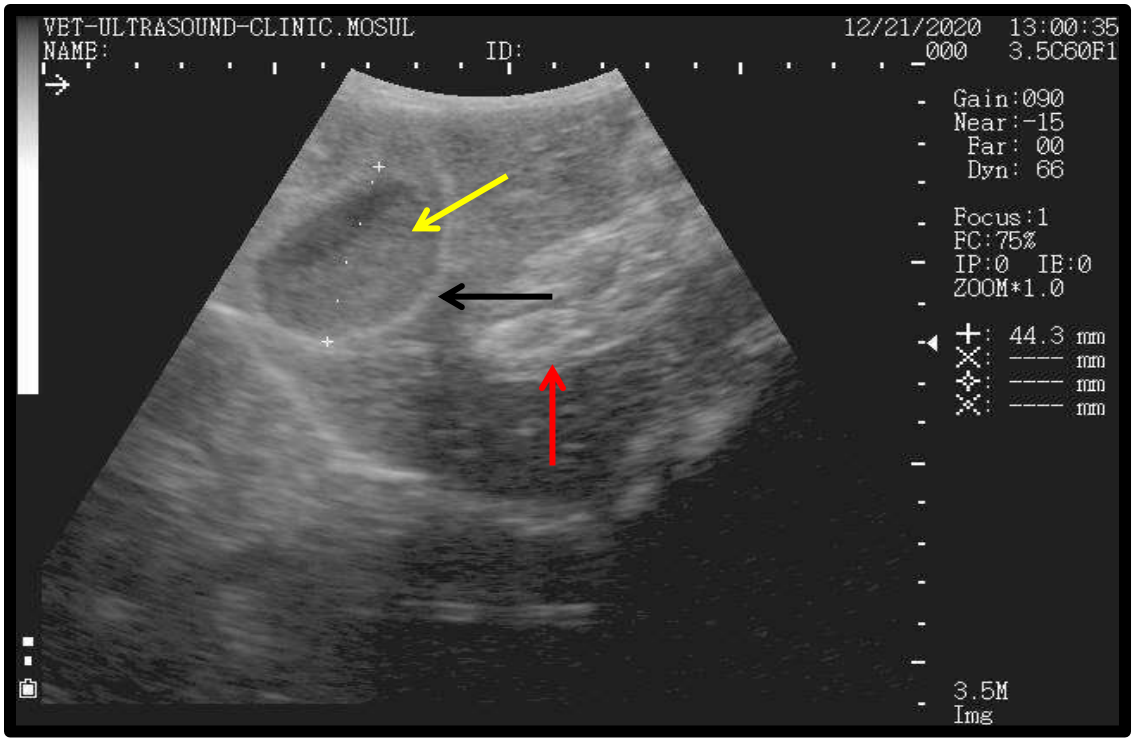

Figure (4 – 12): Ultrasonography of the first at 8th week shows increase in size of gallbladder (yellow arrow) increase thickness of the wall of gallbladder in (black arrow) and heterogeneous mottled appearance of the liver (red arrow) .

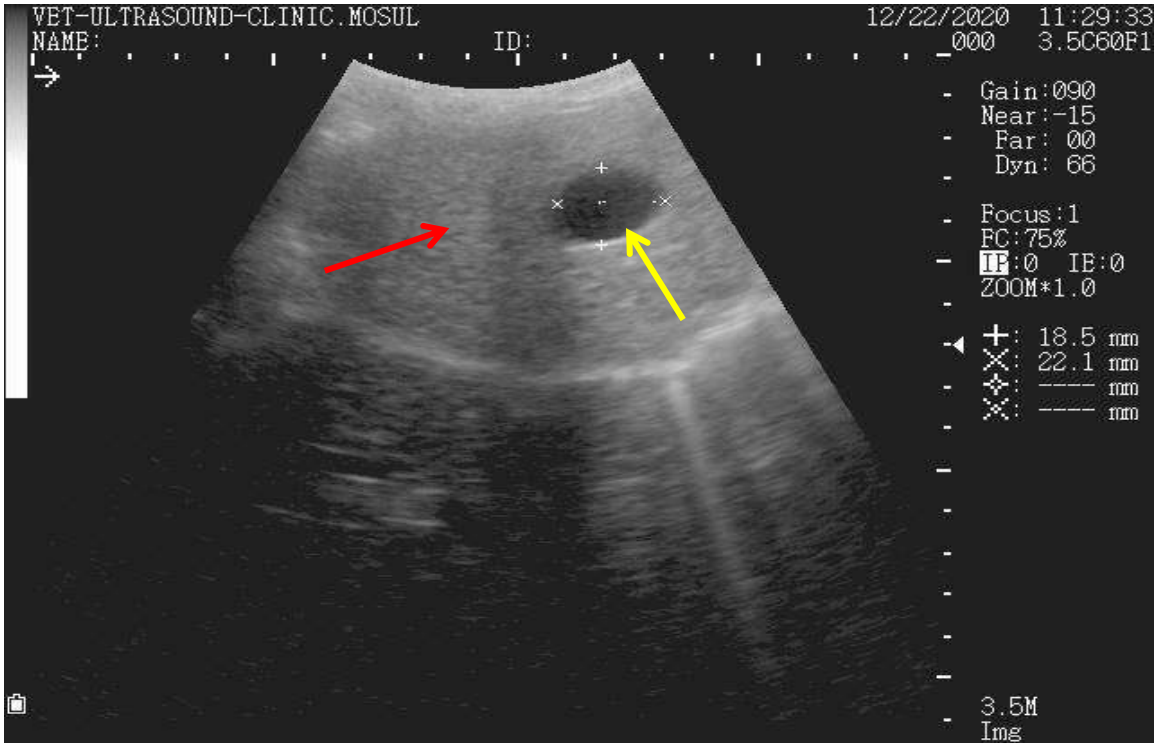

Figure (4 – 13): Ultrasonography in second group shows normal liver tissue (red arrow) and normal gallbladder (yellow arrow) .

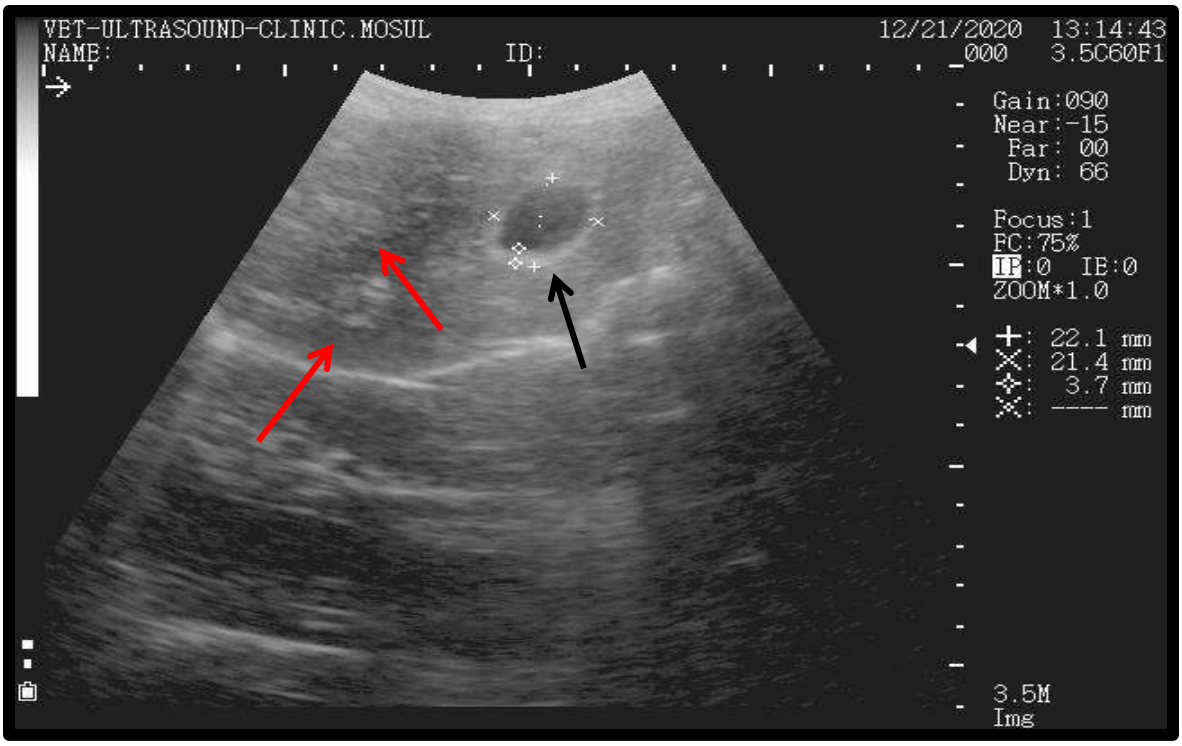

Figure (4 – 14): Ultrasonography of the second group at the 2nd week) shows thickness in the wall of gallbladder (black arrow) , heterogeneous mottled appearance of the liver (red arrows).

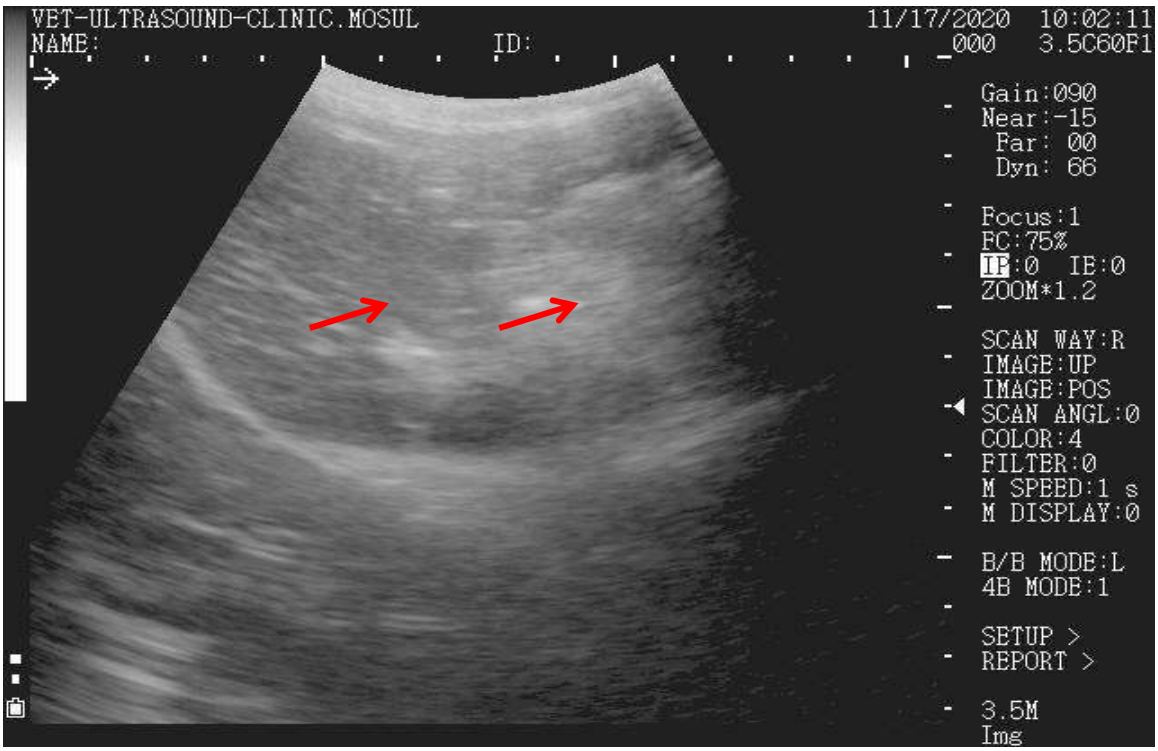

Figure (4 – 15) : Ultrasonography of the second group at the 4th week shows mottled appearance of the liver (red arrows) .

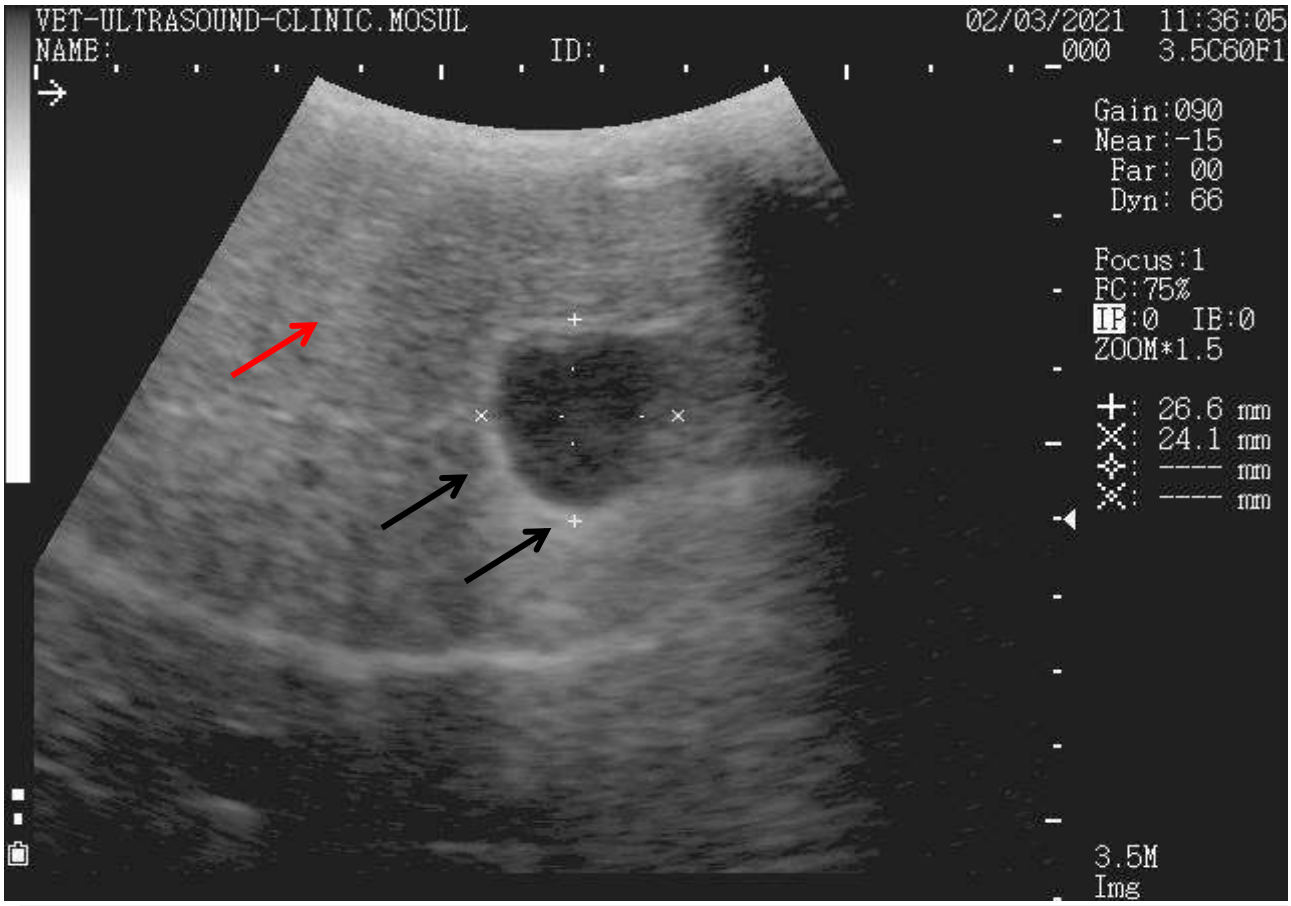

Figure (4 – 16): Ultrasonography of the second group at the 8th week shows less sever changes in liver tissue (red arrow) and increase the thickness of the gallbladder wall is present (yellow arrows) .

4-4: Histological evaluation

The microscopic examination of normal liver tissue in both groups showed normal tissue architectures without any pathological lesions (Figure 4-17).

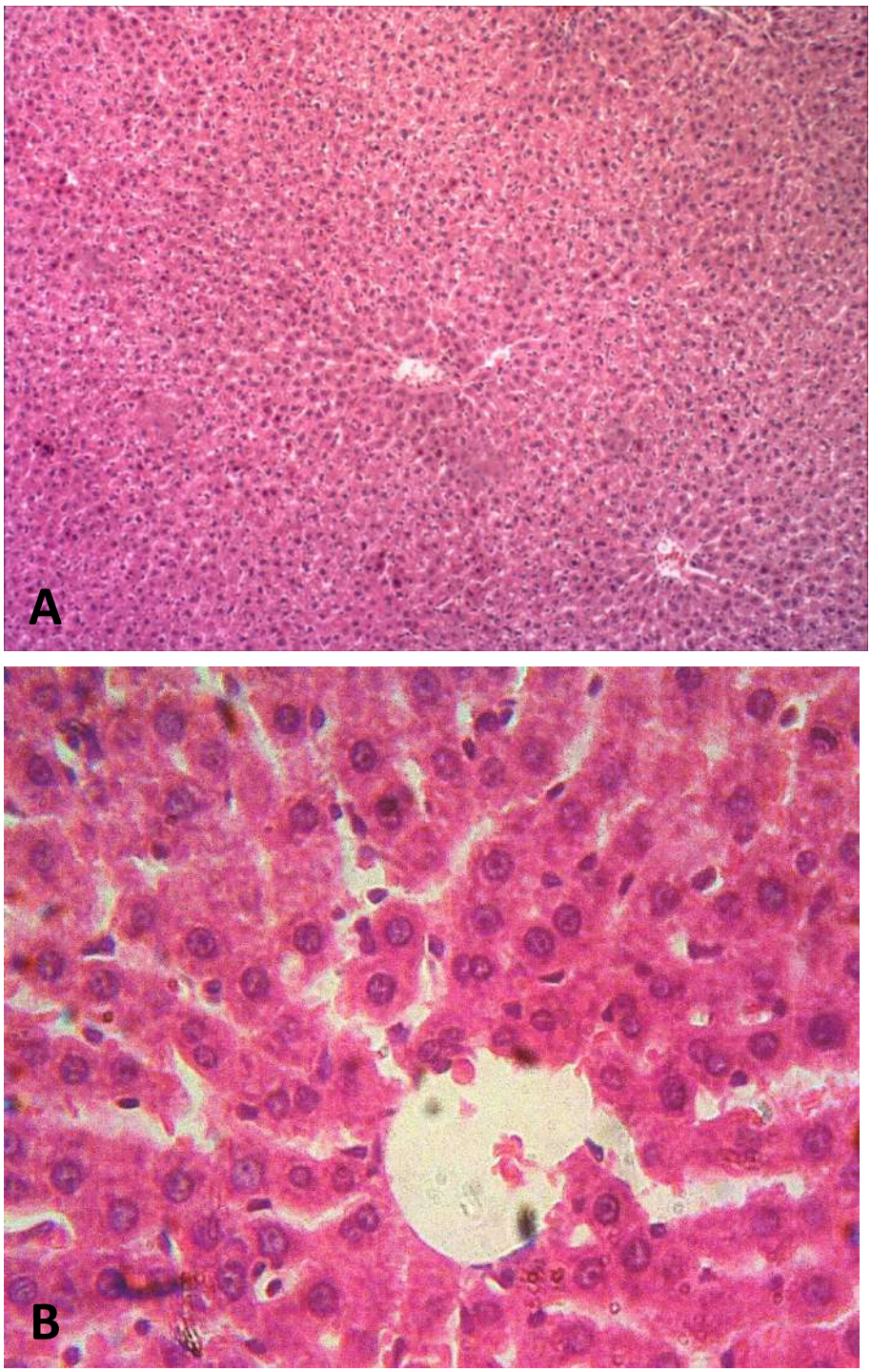

Figure (4 – 17) : Photomicrograph shows normal tissue of liver. H&E stain X10 (A) and normal tissue H&E stain X40 (B)

First group :

Microscopic examination showed coagulative necrosis of hepatocytes and deposition of eosinophilic material in the portal area, around the hepatic artery and branch of bile duct in the portal area with infiltration of inflammatory cells in the interlobular space, central vein and around the blood vessels in the portal area. Hemorrhage in the hepatic tissue was observed with congestion of blood vessels (central vein, portal vein), thickening of hepatic artery wall. Some hepatocytes appeared in the form of polygonal cells with a granular eosinophilic cytoplasm and prominent nucleus (Figure 4-18).

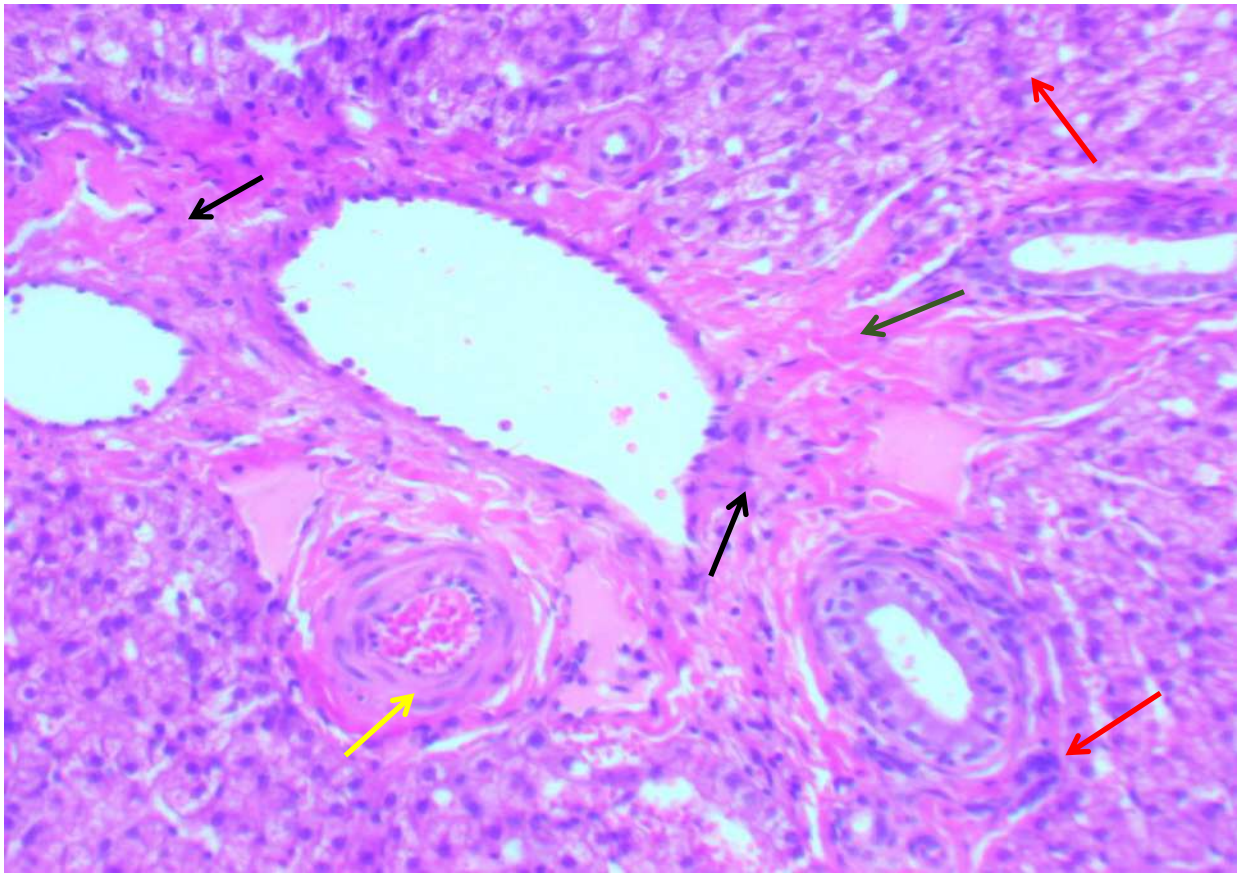

Figure (4 – 18): Photomicrograph for the liver in the first groupat 60 days post operation shows deposition of eosinophilic material in the portal area (black arrows) hemorrhage in hepatic tissue (green arrow), thickening , congestion in blood vessels (yellow arrow) and necrosis of hepatocytes (red arrows) H&E stain X40 .

Other section showed recent thrombus in the hepatic vein, and focal infiltration of inflammatory cells (Figure 4-19)..

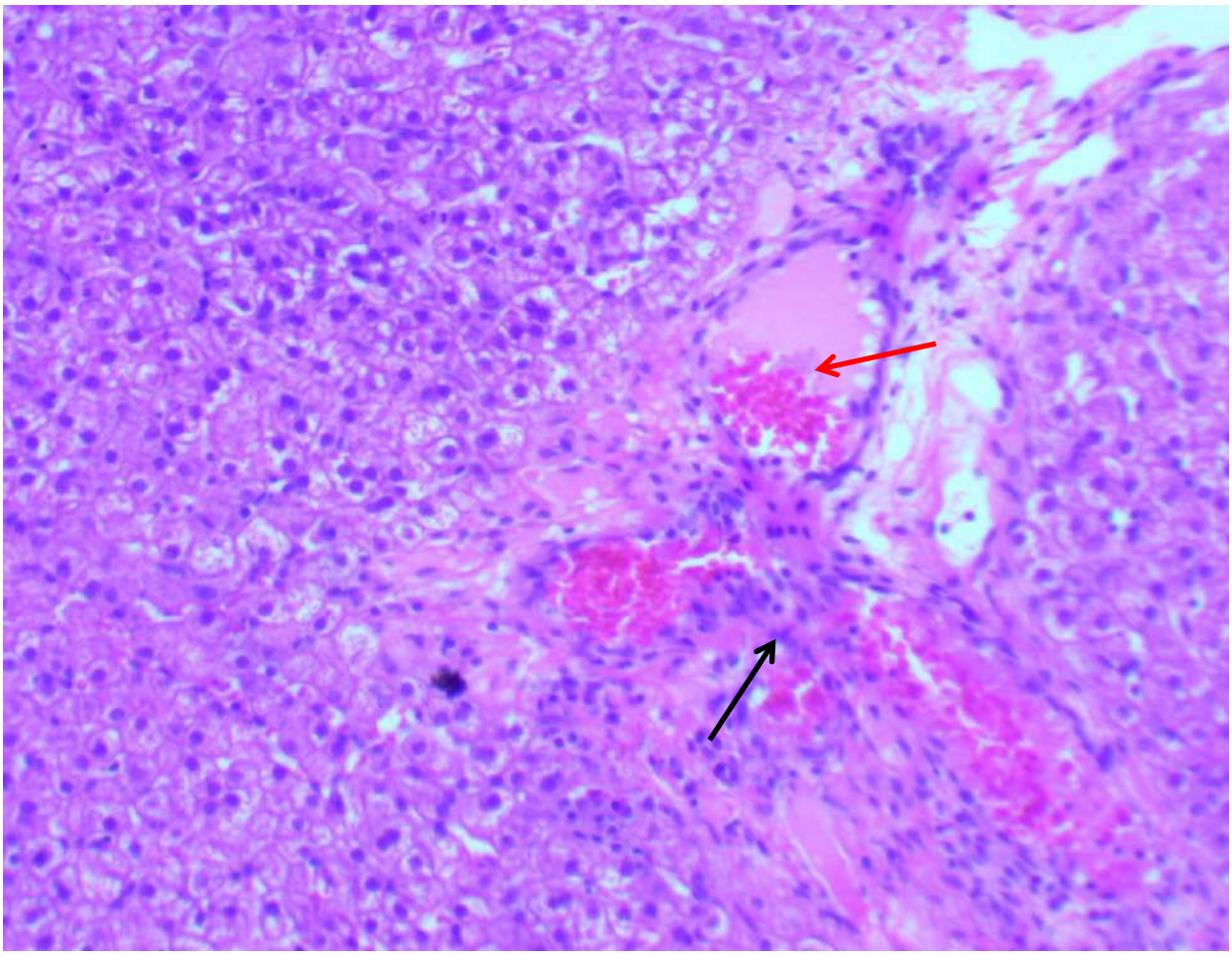

Figure (4 – 19): Photomicrograph o the liver in the first group at 60 days post operation shows recent thrombus in hepatic vein (red arrow) , and inflammatory cells (black arrow) at the end of the study in the first group following induction of irreversible liver fibrosis . H&E stain X40 .

Severe hemorrhage beneath the capsule and paranchymatous tissue of liver with coagulative necrosis of the hepatocytes were observed (Figure 4-20)..

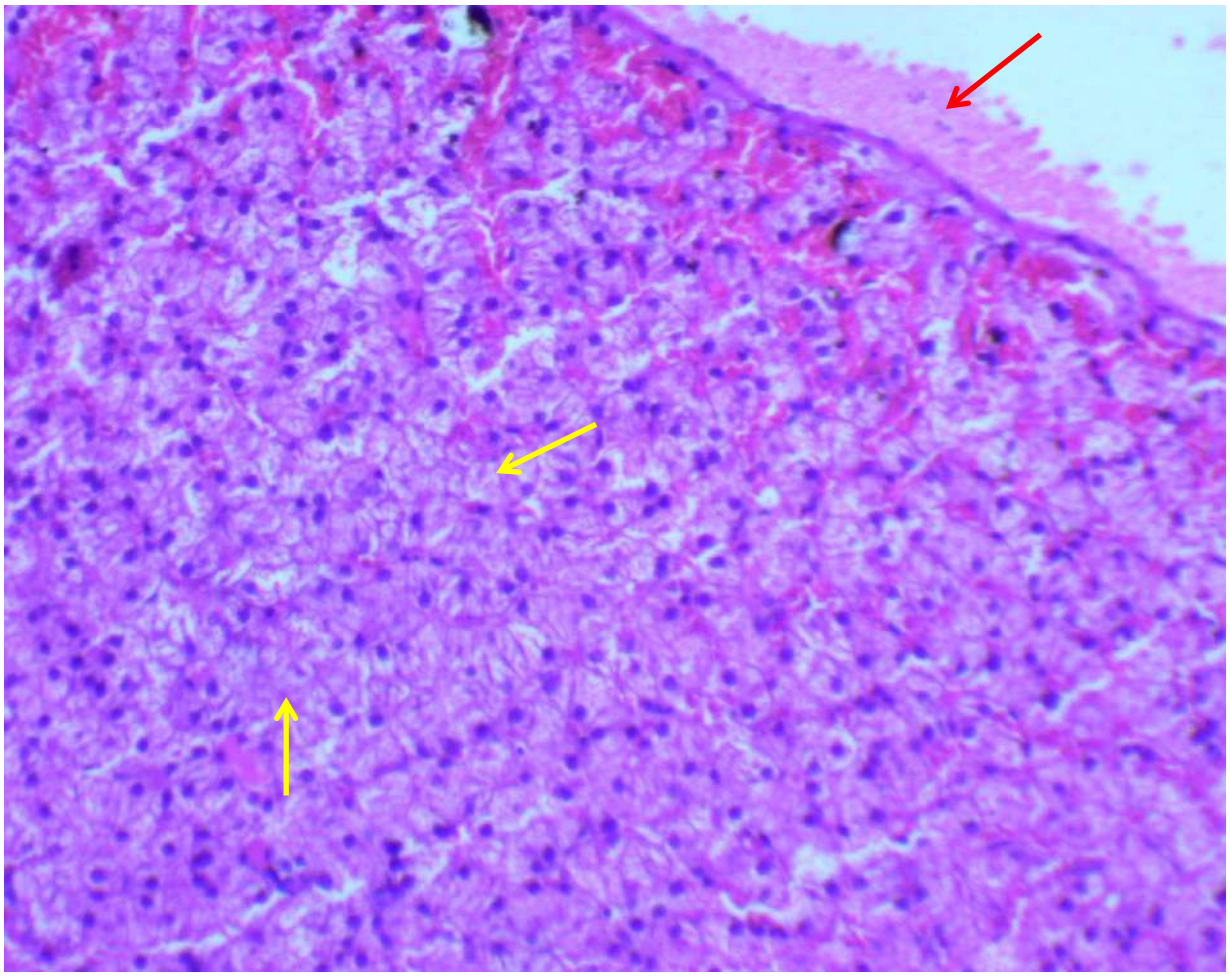

Figure (4 – 20): Photomicrograph of the liver in first group at 60 days post operation shows thickening of hepatic capsule (red arrow) and coagulative necrosis (yellow arrows) . H&E stain X40 .

Other sections showed severe deposition of eosinophilic material (fibrosis) around portal vein hepatic artery and bile duct. The epithelial cells lining the bile duct suffered from hyperplasia (Figure 4-21).

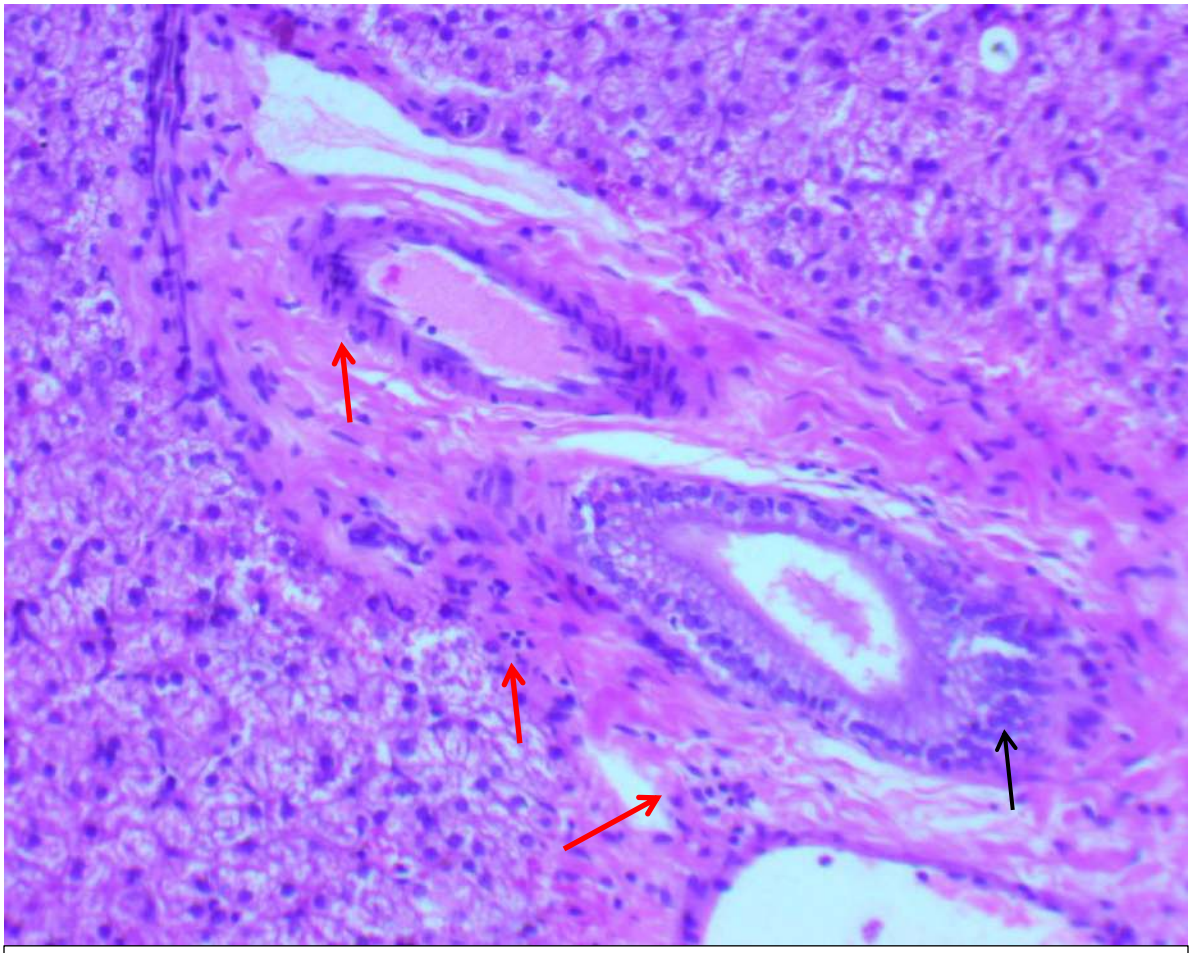

Figure (4 – 21): Photomicrograph of th liver in e first group at 60 shows sever deposition of esinophilic material (fibrosis) around portal vein , hepatic artery and bile duct (red arrows) . The epithelial cells lining the bile duct suffered from hyperplasia (black arrow) . H&E stain X40 .

Vacuolar degeneration of hepatocytes , Deposition of eosinophilic material in the portal area and increased thickening the wall of bile duct, hepatic artery with congestion of blood vessels (Figure 4 - 22) .

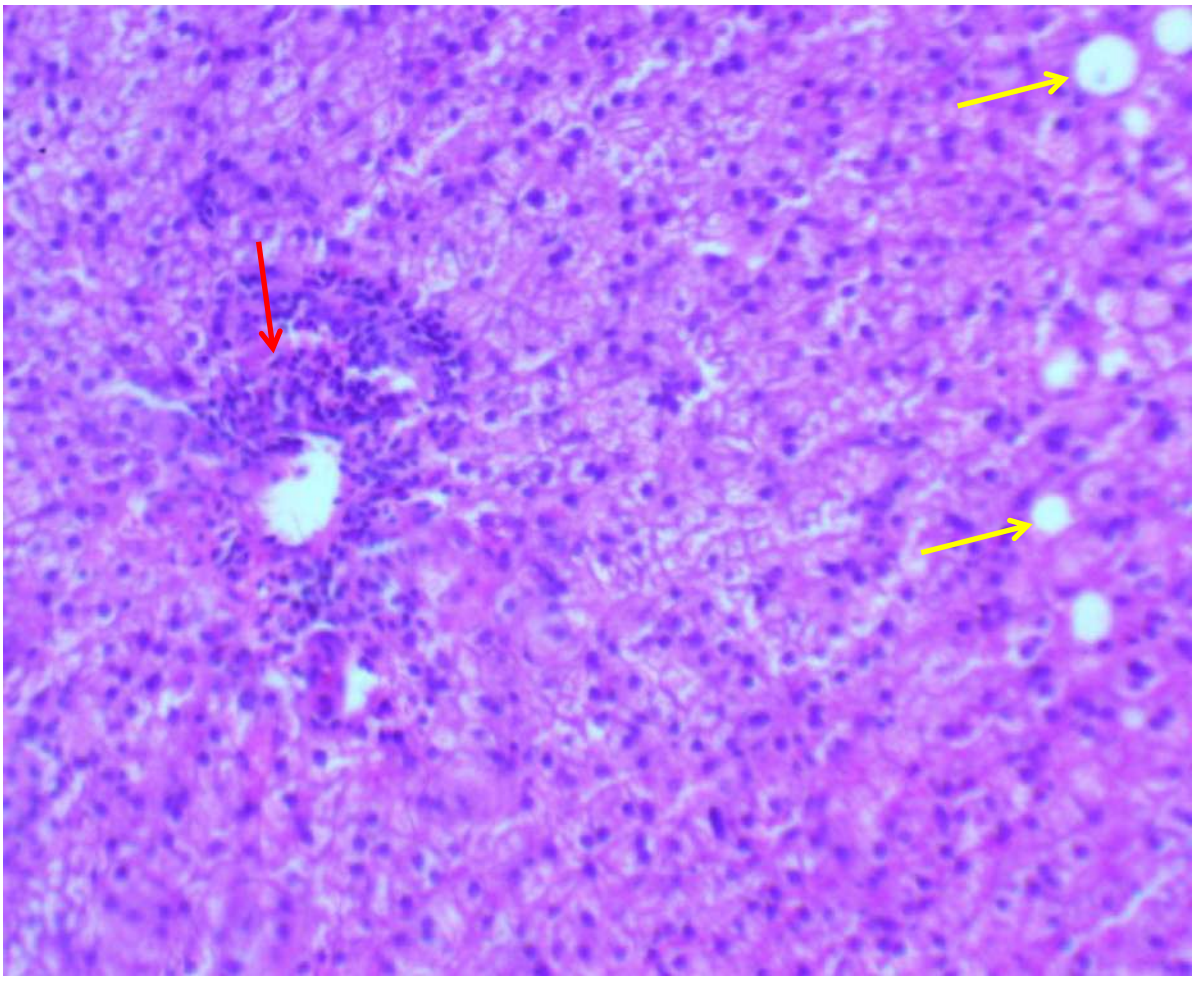

Figure (4 – 22) : Photomicrograph of the liver in first group at 60 shows vacular degeneration (yellow arrows) and infiltration of inflammatory cells around central vein (red arrow) . H&E stain X40.

Histological examination showed fatty change or steatohepatitis which was characterized by intra cytoplasmic accumulation of fat droplets which appeared as vacuoles and fibrosis was clearly observed by staining with Masson's trichrome (Figure 4- 23).

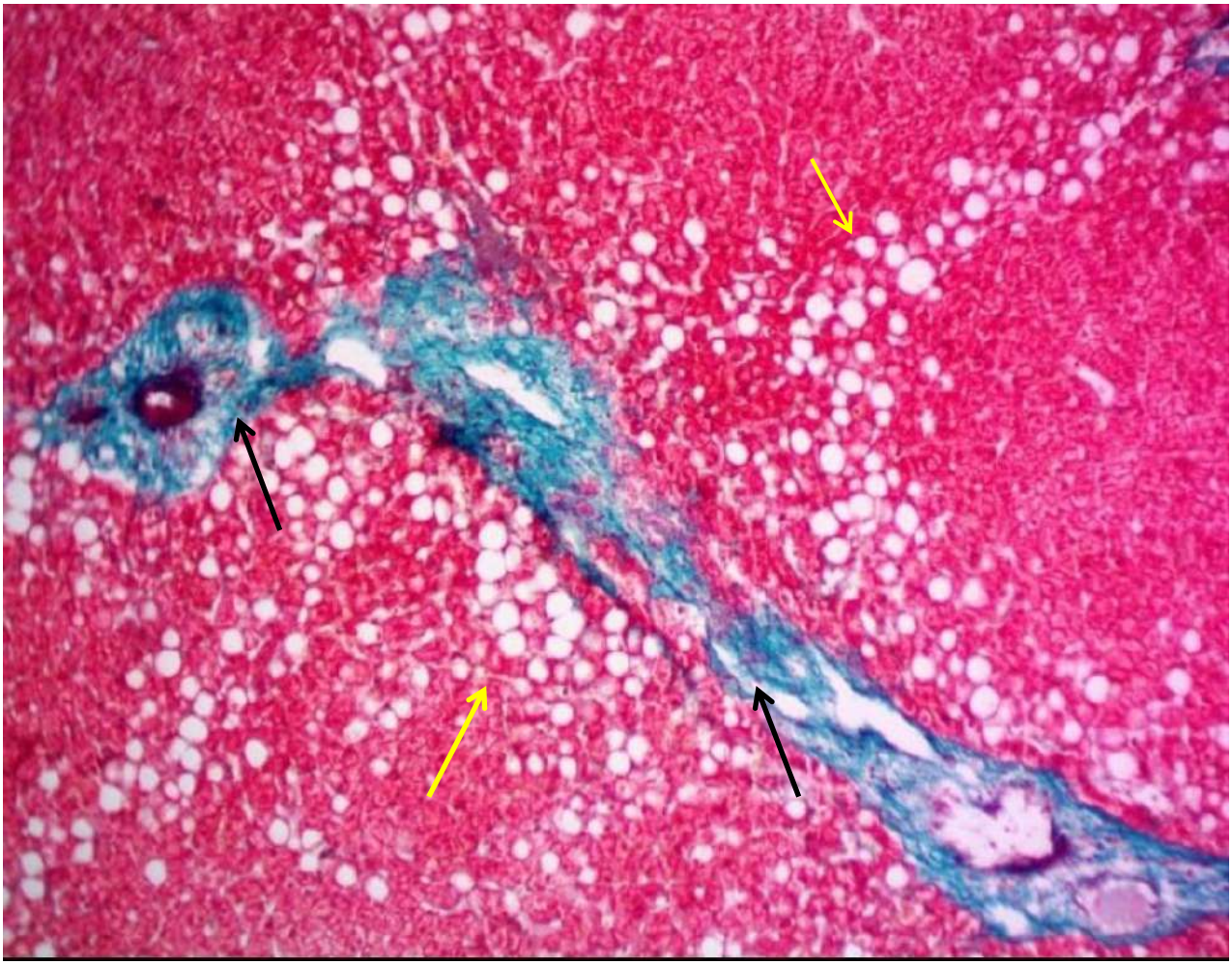

Figure (4 – 23): Photomicrograph of the liver in first group at 60 shows fatty changes (yellow arrows) and fibrosis in the portal area (black arrows) . Masson's trichrome stain X40.

Second group

Microscopic examination shows generalized congestion of blood vessels, Vacuolar degeneration of hepatocytes , deposition of eosinophilic material in the portal area and slight infiltration of inflammatory cells in the portal area were observed (Figure 4 - 24) .

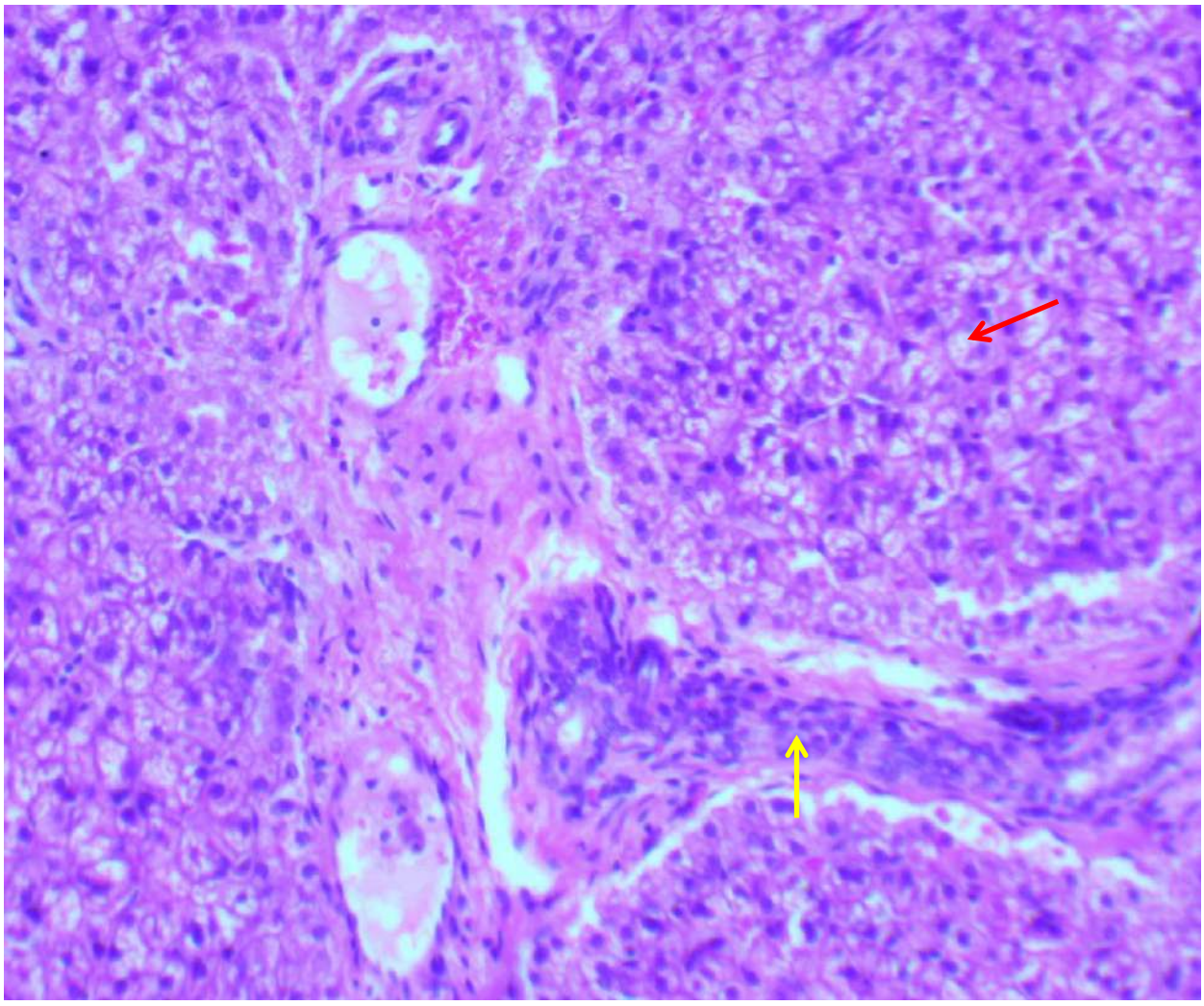

Figure (4 – 24) : Photomicrograph of the liver in second group at 60 shows vacular degeneration (red arrows) and interlobular infiltration of inflammatory cells in portal area (yellow arrow) H&E stain X40.

Severe hemorrhage was observed (Figure 4 - 25) , Histological examination showed fatty change or steatosis which was characterized by intra cytoplasmic accumulation of fat droplets which appeared as vacuoles with double nucleus indicating to regeneration of the liver tissue (Figure 4-26). Atrophy of hepatocytes resulted into dilation of sinusoids, sever deposition of eosinophilic material in the portal area and infiltration of inflammatory cells were observed (Figure 4 - 27)

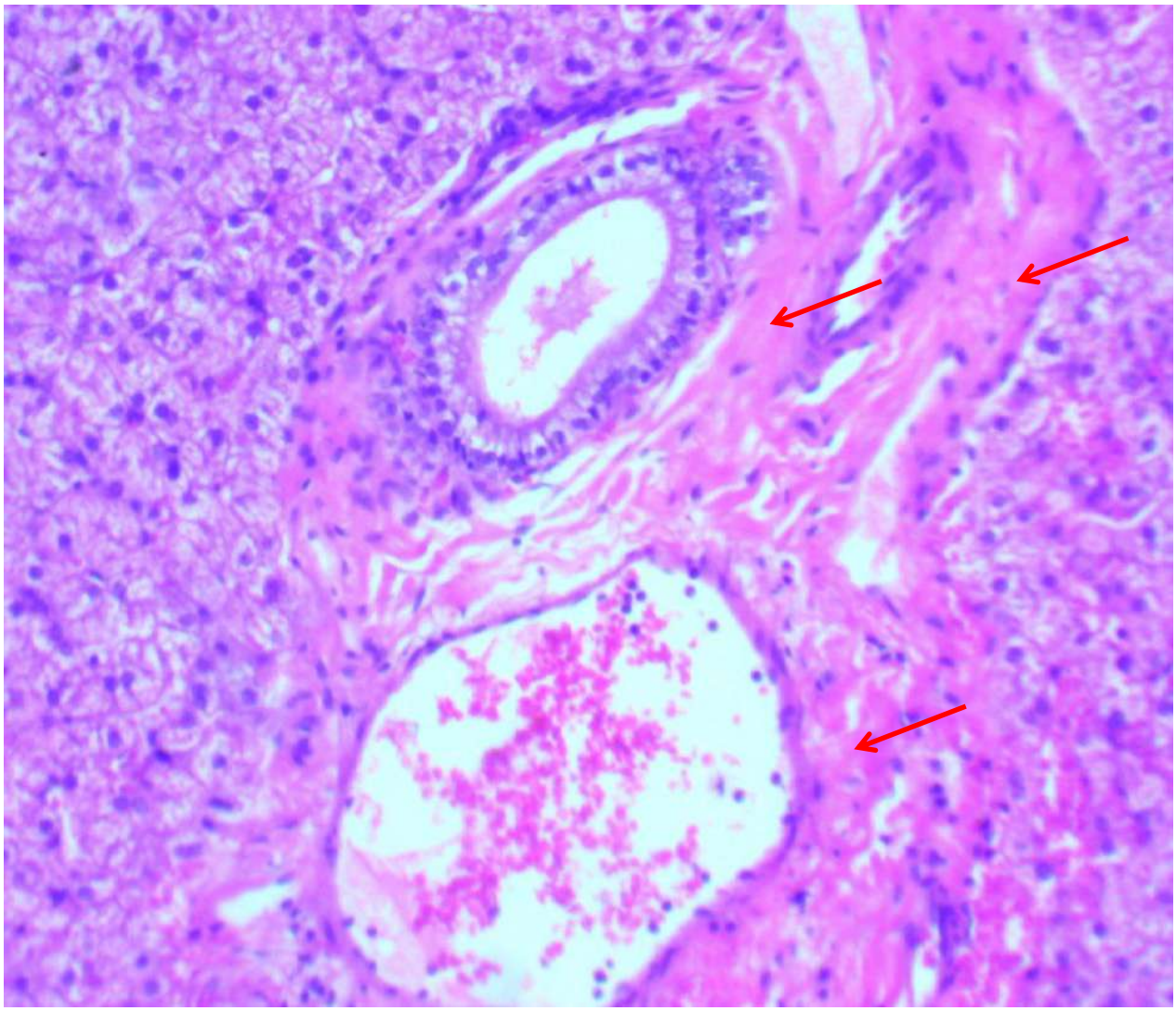

Figure (4 – 25): Photomicrograph of the liver in second group at 60 shows severe hemorrhage (red arrows) H&E stain X40.

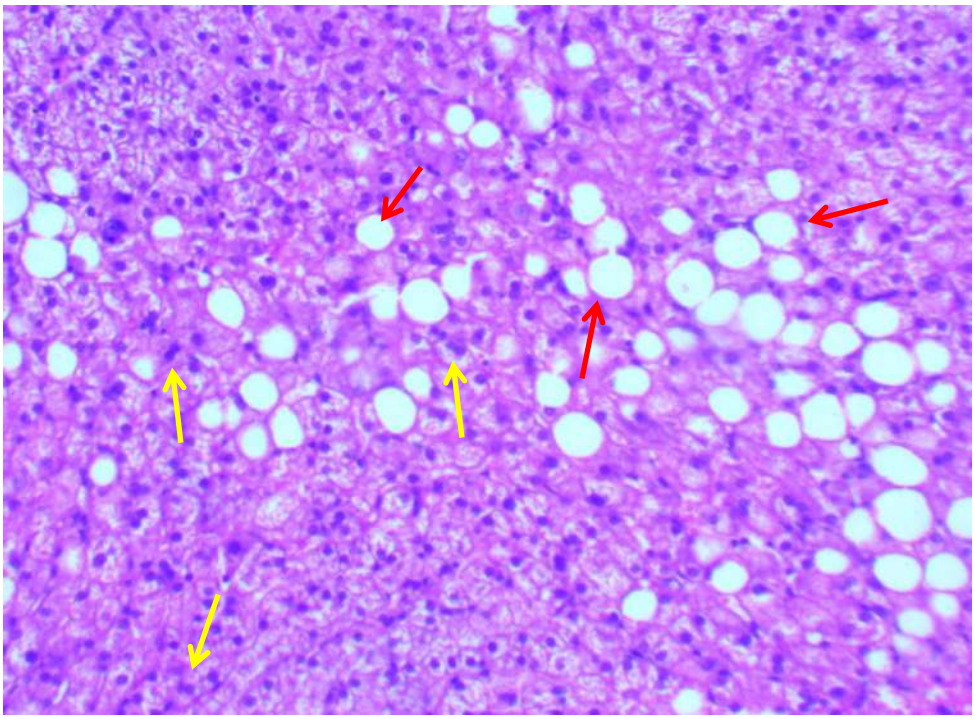

Figure (4 – 26): Photomicrograph of the liver in second group at 60 shows fatty change (red arrow) and double nuclei (regeneration) (yellow arrow) H&E stain X40.

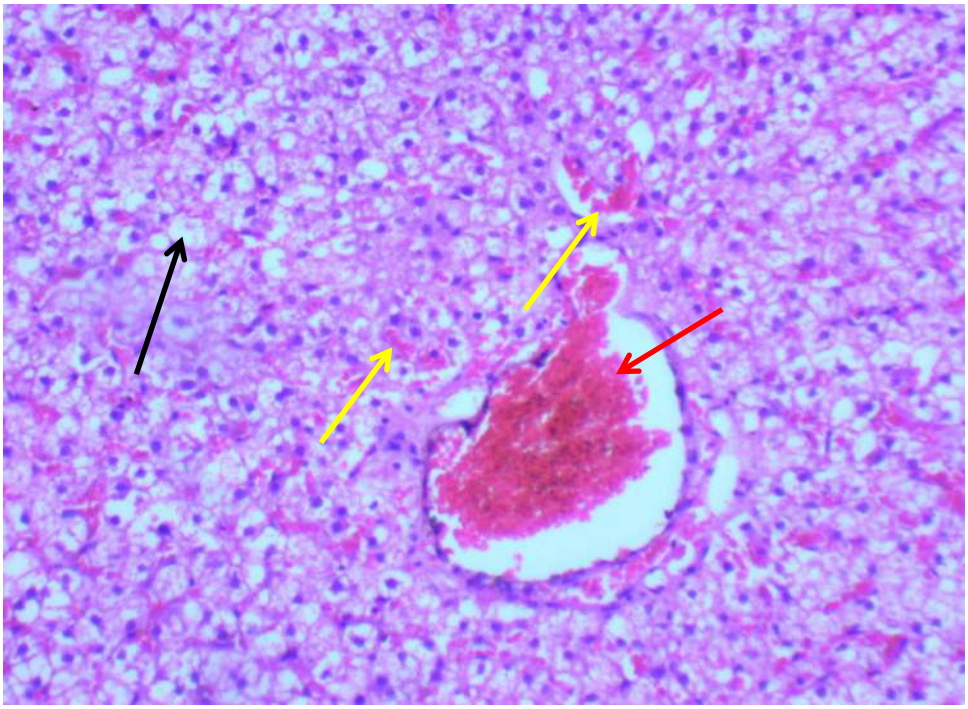

Figure (4–27): Photomicrograph of the liver in the second group at 60 show s vacuolar degeneration of hepatocytes (black arrow), congestion in sinusoids (yellow arrows) and hemorrhage in portal area (red arrow) H&E stain X40.

By staining with special stain (Masson's trichrome) was used a less fibrous tissue was noticed in comparison to first group was observed (Figure 4 – 28) .

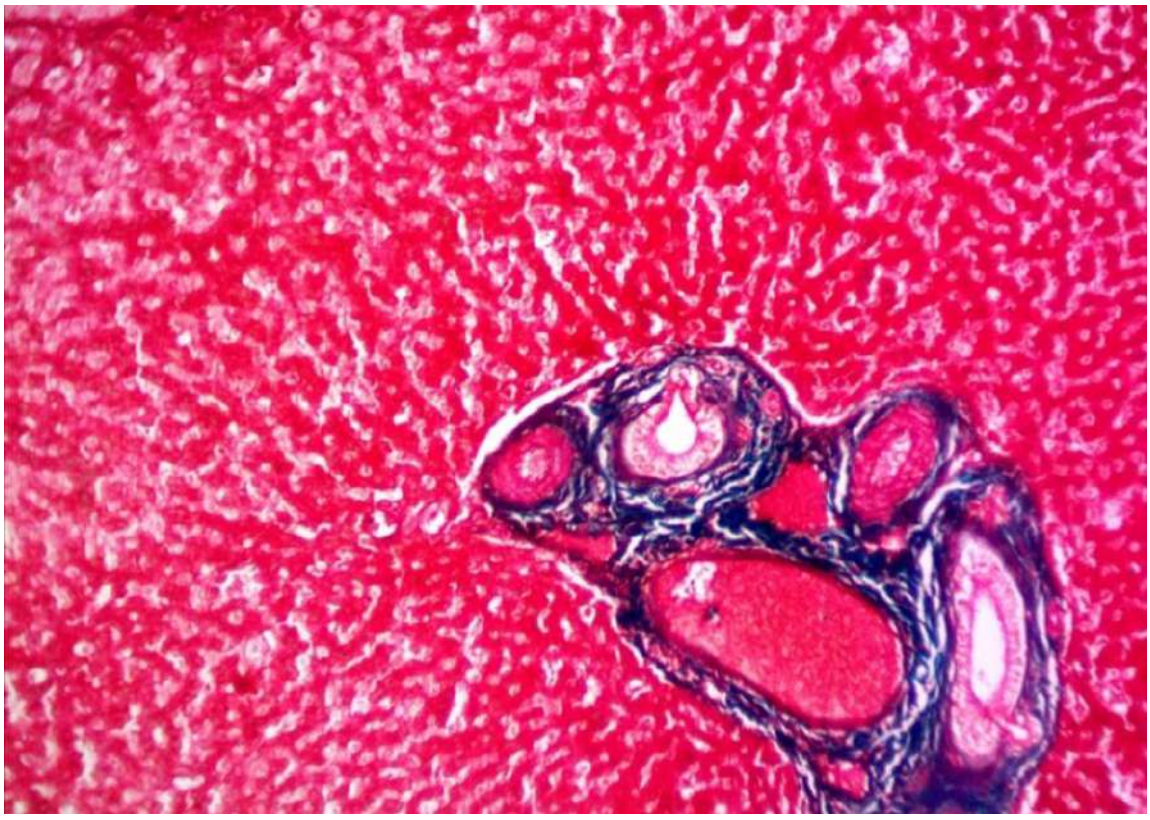

Figure (4 – 28) : Photomicrograph of the liver in second group at 60 shows less fibrosis in the portal area comparison to the first group . Masson's trichrome stain X40

Gall bladder

Histological examination of gall bladder in two group showed inflammation of gall bladder (cholecystitis) which was characterized by infiltration of inflammatory cells in lamina and properia papillary projection of epithelial cells (Figure 4 - 29) . Infiltration of inflammatory cells in the muscular layer and adventitia was observed (Figure 4 - 30)

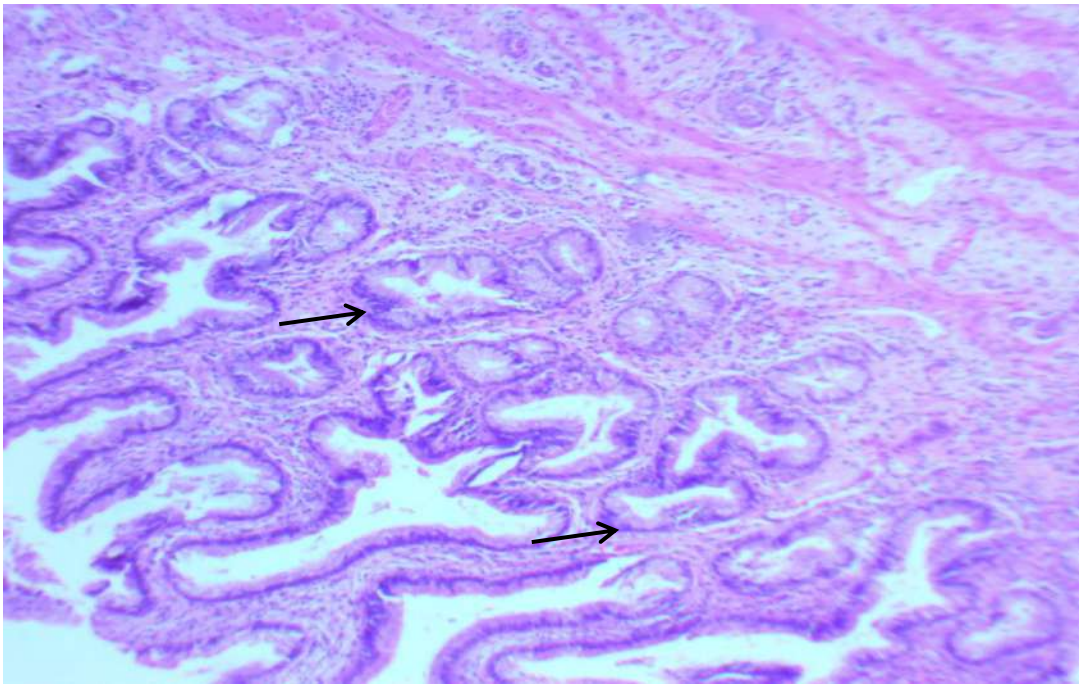

Figure (4 – 29): Photomicrograph of gall bladder at 60 days post operation in first and second groups shows inflammation of gall bladder (cholecystitis) infiltration of inflammatory cells in lamina properia papillary and projection of epithelial cells (black arrow) H&E stain X40 .

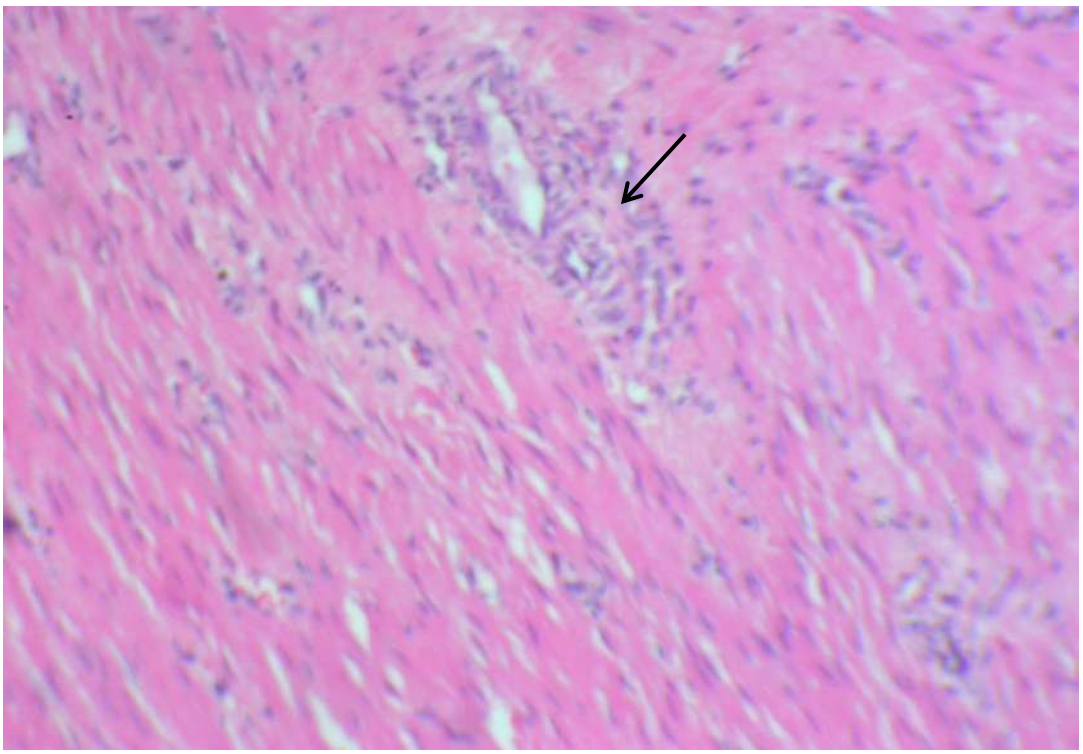

Figure (4 –30): Photomicrograph of gall blader at 60 days post operation in first and second groups shows infiltration of inflammatory cells in muscular layer of gallbladder (black arrow) .H&E stain X40 .

Chapter five

Discussion

This study confirmed the success of induction of hepatic fibrosis in dogs by the ligation of duodenal biliary orifice following enterotomy in the duodenum by means of direct surgical ligature of the major biliary papilla using non absorbable silk and absorbable polygalactin 910, and this technique demonstrated that the induction of irreversible and reversible liver fibrosis through the surgical closure of the major duodenal papilla was associated with less complications in comparison to the results of the previous related studies in which common bile duct ligation was used as a model to induce liver fibrosis which was related to many complications such as bleeding complications due to accidental injury of the accompanying blood vessels during or rapidly following surgery , severe infections (ranging from peritonitis to sepsis), bile leakage into the peritoneal cavity due to inaccurate ligation (Liedtke *et al*., 2013). Pancreatitis can be considered as a common cause of extrahepatic biliary obstruction where fibrosis, edema, and inflammation of the bile duct (cholangitis) may occur as it passes through the inflamed pancreatic parenchyma (Larson, 2016). Chronic liver injury causes a progressive response which leads to eventual liver fibrosis characterized by both quantitative and qualitative alteration of hepatic ECM. This alteration involves an increased proliferation, and accumulation of ECM by collagen deposition. The oxidative stresses have a critical role to activate hepatic stellate cell during hepatic fibrogenesis (Liu *et al*., 2006) .

Silk suture material consist of silk fibroin protein from Bombyx mori (70%) and coating material (30%). These sutures are considered as non-biodegradable sutures because duration of approximately 2 year is required for

complete bio-degradation (Banche *et al*., 2007). Therefore, silk suture as non-absorbable material was chosen to surgically close the major duodenal papillae to induce the irreversible liver fibrosis in the first group of this study.

The absorbable suture material (polyglactin 910) was reported to retain 57% of its initial strength within 2 weeks and 16% after 4 weeks (Puerta *et al*., 2011). In general, if a suture material is knotted, this suture may be weakened by 10–40%, relying on the material of synthesis and if a suture fails it could be always attributed to the break or loosening at the knot (Capperauld & Bucknall, 1984). In the current study, absorbable suture material (polyglactin 910) was used for the surgical closure of the major duodenal papillae to induce the reversible liver fibrosis in the second group of this study where expected spontaneous biliary decompression was ensured by the self-release of this material due to rapid degradation. This result was in agreement with (Kahramansoy *et al*. 2012) who used absorbable suture material (polyglactin 910) for the ligation of the common bile duct to induce reversible obstructive jaundice in rats.

The body weight was slightly but not significantly reduced in complete bile duct ligation in comparison with the control dogs and the biochemical abnormalities were not associated with the presence of ascites (Bosch *et al*., 1983). Clearly, partial bile duct ligation as an internally controlled and reproducible technique was used widely to induce and study liver injury and subsequent repair along with or without biliary physiology and drug metabolism. For studies aimed to assess the progression and systemic influence of chronic cholestasis, complete bile duct ligation was considered the most dependable method (Yokota *et al*., 2018). Unlike humans, there is difficult identification of the underlying etiology of chronic hepatitis in dogs. however, recent experiments reported some similarities between liver reaction

patterns and the pathogenesis of the chronic hepatitis in both men and dogs leading to the common evolution of fibrosis in the two species (Cerquetella *et al* ., 2012).

Clinically, the hepatic disorder is not characterized by specific signs. Signs such as anorexia, vomiting, diarrhea, polydipsia and polyuria were differently noticed. Furthermore, other signs like fatigue, jaundice, hepatic encephalopathy and ascites were also noticed in various association to the disease, relying on its severity. Coagulopathies, cirrhosis and portal hypertension were closely related to liver failure. In dogs suffering from chronic hepatitis, ascites was regarded a negative prognostic sign which is already known for humans (Cerquetella *et al* ., 2012). The current study has supported the clinical, ultrasonographic and histological results of previous studies using various animal models and techniques to induce hepatic fibrosis.

The clinical results of the present study in the first group involved inappetite and gradual loss of body weight following surgical induction of hepatic fibrosis. Jaundice was the main clinical feature of the animals with blocked common bile duct where pale yellowish mucus membrane of the eye and the oral cavity. Post-operative pain was severe especially during the first two days following the surgical induction of hepatic fibrosis. Food uptake increased due to gradual decrease in post-operative pain but still at lower level leading to gradual decrease in the body weight of resulting in cachexia of the animals. The results of our study were in agreement with that of *(Elhiblu et al*., 2015) who reported the signs of affected dogs by hepatic insufficiency based on clinical examination. The clinical findings in the second group were similar to that of the first group with the exception that there was gradual clinical improvement which started at the 30th post-operative day. The improvement was manifested by gradual increase of body weight and

disappearance of jaundice signs. Our findings agreed with (Li & Sydney Chung, 2001) who reported the functional and morphologic improvement of the liver fibrosis in rats.

Macropathological investigation showed engorgement of the bile duct and expansion of the gall bladder resulting in a changed shape of the gall bladder. White lines and spots were noticed in different areas of the liver indicating the presence of the hepatic fibrosis. There was discoloration of different region of the liver manifested by yellowish staining. Evidence of bleeding on the wall of the gall bladder with increased wall thickness of the both gall bladder and bile ducts were observed. Similar findings were also observed by (Milosavljevic *et al*., 2018). Similar but less intensive macropathological features were noticed in the animals of the second group. The duodenal orifice of the common bile duct was found to be opened due to absorption of the polyglactin suture material which was used to block the orifice resulting in the free passage of the bile secretion into the duodenum which was confirmed the gall bladder was squeezed by hand following animal euthanasia There was mild to moderate yellowish discoloration of the liver tissue and the white lines and spots were lesser in size and intensity.. There was a normal size and shape of the gall bladder due to free passage of the bile and lesser engorgement of the bile duct. The icterus usually resulted from increased production of bilirubin, increase of bilirubin enterohepatic circulation and deficiency in both of hepatic uptake and bilirubin conjugation (Sonne *et al.* 2018)

Obstruction of the common bile duct results from a variety of primary conditions such as pancreatitis, duodenitis, duodenal foreign body, cholelithiasis, gallbladder mucocele, cholecystitis, neoplasia, malformations and parasitic infection of the bile duct, extrinsic compression, and fibrosis. Complete bile duct obstruction leads to an impairment of bile flow from the

liver to the duodenum (cholestasis) resulting into increased ductal mucin which contributes to duct distention. In this case, the biliary tree was subjected to be colonized by bacteria causing cholangitis and ascending hepatitis and subsequent hepatic dysfunction as a result of liver cell damage (Negasee, 2021). Cholestasis (reduced excretion of bile) may cause accumulation of bile acids in liver, which enhances the biliary epithelial cells to secrete many agents such as tumor necrosis factor (TNF). The accumulated bile acids in liver tissue have stimulated HSC activation, invasiveness, and fibrogenesis via up-regulating the gene expression of type-I collagen and procollagen ∂-1 during the early phase of hepatic fibrosis (Ahmad & Riaz, 2012).

Laboratory findings were usually unspecific, and even though they might indicate a hepatic problem but they did not show whether the condition was a chronic hepatitis or another problem affecting the liver (Sterczer *et al.*, 2001). The results of the present study showed a significant increase in the serum bilirubin, AST, ALT and ALP levels during the first two post-operative days and continued to be high until the end of the study in both group of the experiment while (Cömert *et al*., 2004) concluded that the mean total protein levels in the serum of the animals subjected to bile duct ligation did not cause statistically significant difference in comparison to the control group. It is well known that cholestasis and liver cell necrosis leads to increased serum bilirubin, AST, ALT and ALP levels. By contrast, because chronic liver disease did not result from bile duct ligation for 10 days, the total serum protein levels were not significantly affected in jaundiced mice.

In addition to the increased total and direct bilirubin, elevated level of alkaline phosphatase , AST and ALT in the affected dogs with complete bile duct ligation was observed by (Bosch *et al* ., 1983). These results were also in an agreement with the results of the both groups of the current study. The

means of alanine aminotransferase (ALT) and aspartate transaminase (AST) which are well-established serum markers of liver injury showed rapid increase and peak during the first 7 and 20 days following bile duct ligation. Thereafter, ALT and AST stead reduction by the day 30 was noticed and remained constant until 60 days following surgery. In a recent experiment it was reported that serum AST and ALT concentrations increased up to 5-10 fold than normal during the first week following BDL surgery and reduced after two weeks (Tag *et al*., 2015). Total serum bilirubin is considered as a measure of the cholestasis degree. It is important to note that mice with failed partial bile duct ligation as a result of technical problems like bile duct or vascular injuries will show serum values as same as complete bile duct ligation mice with significant increase of liver enzymes and total serum bilirubin. Clinically, mice with failed partial bile duct ligation will be more jaundiced and distressed with signs of bile leakage, ascites, peritonitis (Yokota *et al*., 2018).

In the current study, the results of the ultrasonographic examinations showed abnormal change in the liver tissue in both groups such as increased size and wall thickness of the gall bladder and mottled heterogeneous appearance of the liver instead of the normal appearance (weak homogenous appearance). These changes were observed two weeks after the surgical induction of the hepatic fibrosis and lasted until the end of the study in first group. In the second group, the ultrasonography examinations showed a gradual decrease in the severity of these change from the 4th post-operative week until the end of the study. The use of the ultrasonography to diagnose liver fibrosis and cirrhosis was supported by (Bataller & Brenner, 2005a) who reported the importance of ultrasonography to detect and assess the changes in the hepatic

parenchyma (in terms of liver echogenicity and nodularity) in patients suffered from moderate to severe fibrosis or cirrhosis.

Our histopathological results revealed necrosis of hepatocytes and deposition of eosinophilic material in the portal area, around the hepatic artery and branch of bile duct in the portal area with infiltration of inflammatory cells in the interlobular space, central vein and in the portal area around the blood vessels.. Hemorrhage in the hepatic tissue, congestion of blood vessels (central vein , portal vein, thickening of hepatic artery wall. Histological examination showed fatty change or steatosis which was characterized by intra cytoplasmic accumulation fat droplets which appeared as vacuoles. Sever hemorrhage beneath the capsule and paranchymatous tissue of liver. Similar findings were observed by (Tag *et al*., 2015) who reported formation of the persinusoidal fibrosis on day 10 following surgery while periportal fibrosis which was permanently increased until the end of the study was fully developed at 20 days. BDL triggers the proliferation of biliary epithelial cells and oval-shaped hepatocyte progenitors leading to proliferation of the bile ductules with portal inflammation and fibrosis. Cholangiocyte proliferation was initiated following BDL at the edge of the portal tract (Marques *et al*., 2012). Transforming growth factor beta (TGF β) is secreted by perisinusoidal hepatic stellate cells which is directly involved in the fibrogenesis process via stimulated release of collagens, glycosaminoglycans and inhibitors of metalloproteinases (Favier, 2009). Although the underlying mechanisms still undetermined, activated HSCs are believed to play an important role in the development of hepatic fibrosis that occur in the chronic diseases of the liver. Furthermore, in the development of hepatic fibrosis and cirrhosis, many cytokines exerted marked effects by autocrine and paracrine roles(Liu *et al*.,

Conclusions

1. Induction of irreversible and reversible hepatic fibrosis via closure of major duodenal orifice was new successful model, simple and accurate method.
2. Closure of major duodenal orifice to induce hepatic fibrosis was associated with fewer complications as avoidance of the traumatic pancreatitis, biliary leakage and peritonitis was feasible by using this method.
3. The clinical signs, sonographic, laboratory and histopathological changes in the second group were of less severity and intensity after the 60^{th} days post operation in comparison to the first group.

Recommendations

1. We hope that the summary of this protocol will be necessary for successful establishment of liver fibrosis model in other animals and could guarantee reliable and reproducible results.
2. Animals can be left for longer periods than sixty days to determine the maximum period to be tolerated by animals suffering from induced hepatic fibrosis.
3. Thermal occlusion of the major duodenal orifice can be used to induce hepatic fibrosis instead of closure of the orifice by suture material.
4. In general, therapeutic strategies such as antioxidants and antifibrotic agents could be used to treat the reversible hepatic fibrosis after removal of the underlying primary cause of this disease.

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الخلاصة

تم اجراء هذه الدراسة لغرض إحداث تليف الكبد الانعكاسي و غير الانعكاسي جراحيا" في الكلاب. تم اجراء التجربة على اثني عشر حيوانا من الكلاب المحلية البالغة والسليمة من الأمراض ومن كلا الجنسين. فسمت حيوانات الدراسة عشوائيا" الى مجموعتين، شملت كل مجموعة ستة حيوانات. تم فتح الاثني عشر في كلا المجموعتين وتم استحداث تليف الكبد الغير الانعكاسي في المجموعة الأولى عن طريق غلق فتحة قناة الصفراء الرئيسية داخل الاثني عشر باستخدام خيط جراحي غير ممتص نوع حرير، أما في المجموعة الثانية فقد تم استحداث تليف الكبد الانعكاسي باستخدام خيط جراحي ممتص نوع بولي كلا كتين ٩١٠ . تمت متابعة حيوانات التجربة سريريا "لمدة ٦٠ يوما مع قياس مستوى أنزيمات الكبد بالدم و الفحص بالأمواج فوق الصوتية أسبوعيا" اضافة الى اجراء الفحص المرضي الغياني والمرضي النسجي للفترة ٦٠ يوما بعد العملية". اظهرت نتائج السريرية حدوث تليف الكبد تدرجيا" في كلا المجوعتين و التي تمثلت بحصول اصفرار في الغشاء المخاطي للعين والفم مع فقدان الشهية مع ملاحظة حدوث ألم شديد خصوصا" في الايام الأولى التي تلت العملية الجراحية مع حدوث تحسن تدريجي للحالة الصحية لحيوانات المجموعة الثانية بعد ٣٠ يوما من العملية بينما اظهرت نتائج الفحص المختبري لأنزيمات الكبد حصول إرتفاع تدريجي في مستوى هذه الأنزيمات خصوصا خلال الايام الاولى بعد اجراء العملية و استمر هذا الارتفاع الى نهاية التجربة. في كلا المجموعتين. في حين كشفت نتائج الفحص بالأمواج فوق الصوتية حدوث تليف الكبد تدريجيا" و الذي تمثل بوجود مناطق عالية الصدى خصوصا" في الأسابيع الأولى بعد إجراء العملية الجراحية في حيوانات المجموعة الاولى في حين لوحظ توقف حدوث تليف الكبد بعد الاسبوع الرابع من اجراء العملية. في حيوانات المجموعة الثانية. فيما أظهرت نتائج الفحص النسجي المرضي في حيوانات المجموعة الاولى تكون نسيج ليفي في الكبد مع ارتشاح للخلايا الالتهابية خصوصا" حول القنوات الصفراوية اضافة الى تنخر و تنكس الخلايا الكبدية مع حصول نزف في نسيج الكبد و تحت المحفظة في حين لوحظ حدوث نفس التغيرات المرضية ولكن بشدة اقل في حيوانات المجموعة الثانية. نستنتج مما سبق إمكان احداث تليف الكبد الغير انعكاسي و الانعكاسي جراحيا عن طرق غلق فتحة قناة الصفراء الرئيسية و الذي تم تأكيده من خلال نتائج الفحص السريري ، المختبري، الفحص بالأمواج فوق الصوتية ، الفحص المرضي العياني والنسيجي.

شكر وتقدير

الحمد لله رب العالمين والصلاة والسلام على أشرف الأنبياء والمرسلين نبينا محمد وعلى آله وصحبه أجمعين .

أتقدم بالشكر والتقدير إلى عمادة كلية الطب البيطري متمثلة بعميد الكلية ومعاون العميد للشؤون العلمية ومعاون العميد للشؤون الإدارية وفقهم الله وجزاهم كل خير.

كما أوجه الشكر والعرفان بالجميل الى مشرفي واستاذي الفاضل الاستاذ (احمد خلف علي) الذي منحني الكثير من وقته ، وكان لرحابة صدره وسمو خلقه وأسلوبه المميز في متابعة الرسالة أكبر الأثر في المساعدة على اتمام هذا العمل ، وأسأل الله العلي القدير أن يجازيه خير الجزاء وأن يكتب صنيعه في موازين حسناته.

كما أقدم شكري وتقديري لأساتذتي في فرع الطب الباطني لجهودهم المبذولة في فحص الحيوانات بجهاز السونار .

واقدم شكري وتقديري لأساتذتي في فرع التشريح و فرع الأمراض لجهودهما القيمة في قراءة وتصوير المقاطع النسيجية جزاهم الله خير الجزاء .

ولا يفوتني أن أتقدم بجزيل الشكر والامتنان إلى منتسبي فرع الجراحة وعلم تناسل الحيوان من اساتذة وموظفين ، والتمس العذر لكل من قدم لي المساعدة ولم يتم ذكره .

بسم الله الرحمن الرحيم

﴿ لَا يُكَلِّفُ ٱللَّهُ نَفۡسًا إِلَّا وُسۡعَهَاۚ لَهَا مَا كَسَبَتۡ وَعَلَيۡهَا مَا ٱكۡتَسَبَتۡۗ رَبَّنَا لَا تُؤَاخِذۡنَآ إِن نَّسِينَآ أَوۡ أَخۡطَأۡنَاۚ رَبَّنَا وَلَا تَحۡمِلۡ عَلَيۡنَآ إِصۡرٗا كَمَا حَمَلۡتَهُۥ عَلَى ٱلَّذِينَ مِن قَبۡلِنَاۚ رَبَّنَا وَلَا تُحَمِّلۡنَا مَا لَا طَاقَةَ لَنَا بِهِۦۖ وَٱعۡفُ عَنَّا وَٱغۡفِرۡ لَنَا وَٱرۡحَمۡنَآۚ أَنتَ مَوۡلَىٰنَا فَٱنصُرۡنَا عَلَى ٱلۡقَوۡمِ ٱلۡكَٰفِرِينَ ٢٨٦ ﴾

[سورة البقرة : ٢٨٦]

إحداث تليف الكبد الانعكاسي و غير الانعكاسي عن طريق الإغلاق الجراحي لفتحة الاثني عشر الرئيسية في نماذج الكلاب

رسالة تقدم بها

مروان حازم خليل

إلى

مجلس كلية الطب البيطري في جامعة الموصل

و هي جزء من متطلبات نيل شهادة الماجستير

في أختصاص الطب البيطري / الجراحة البيطرية

بإشراف

الأستاذ المساعد الدكتور

أحمد خلف علي الجبوري

١٤٤٣ هـ **٢٠٢١م**

جامعة الموصل
كلية الطب البيطري

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مروان حازم خليل

رسالة ماجستير

الطب البيطري / الجراحة البيطرية

بإشراف

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