Research Article

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New Surgical Technique to Induce Reversible Liver Fibrosis by Surgical Closure of Major Duodenal Orifice in Dogs

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Abstract

This study was conducted to induce and evaluate reversible liver fibrosis in dogs by surgical closure of the major duodenal orifice. The study was performed on six healthy local adult dogs. Reversible liver fibrosis was surgically induced in all animals by surgical closure of major duodenal papilla using absorbable suture material for 60 days. Induced liver fibrosis was assessed by clinical, ultrasonographical examination, laboratory and histological methods. The clinical manifestation of the jaundiced dogs showed reduced food intake, pale-yellowish mucus membrane, inflammatory signs of the wound site and severe postoperative pain. Biochemically, there was significantly increased values of the aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase, indirect bilirubin, direct bilirubin and total bilirubin especially during the first two days after surgery followed by a gradual decrease of these values until the end of the but still higher than normal values. Ultrasonographic examinations showed abnormal changes in the liver tissue such as an increase in both size and wall thickness of the gall bladder and mottled heterogeneous appearance of the liver during the first two weeks following the surgical induction of the hepatic fibrosis. Histological evaluation of liver samples revealed showed necrosis of hepatocytes and deposition of eosinophilic material, infiltration of inflammatory cells, recent thrombus in the hepatic vein, fatty change. Slight clinical, biochemical, ultrasonographic improvement was observed at 30th post-operative day. In conclusion, surgical induction of reversible liver fibrosis in dogs was an easy technique by surgical closure of major duodenal papilla and the results were confirmed by the clinical, ultrasonographical, laboratory and histological examination.

Keywords: liver fibrosis, obstructive jaundice, Ultrasonography, dogs

تقنية جراحية جديدة لإحداث تليف الكبد الإنعكاسي عبرغلق فتحة قناة الصفراء داخل الاثني عشر في الكلاب

الخلاصة

تم اجراء هذه الدراسة لغرض إحداث تليف الكبد الإنعكاسي جراحيا" في الكلاب. أجريت التجربة على ستة من الكلاب المحلية البالغة من كلا الجنسين و السليمة من الأمراض. تم استحداث تليف الكبد الأنعكاسي عن طريق غلق فتحة قناة الصفراء الرئيسية داخل الاثني عشر باستخدام خيط جراحي ممتص نوع بولي كالاكتين. تمت متابعة حيوانات التجربة سريريا" مع قياس مستوى أنزيمات الكبد بالدم و الفحص بالأمواج فوق الصوتية أسبوعيا"و لمدة ستون يوما. في نهاية التجربة تم أخذ عينات من مناطق مختلفة من الكبد لغرض اجراء الفحص النسجي. اظهرت نتائج الدراسة حدوث تليف الكبد الإنعكاسي تدريجيا تمثلت بحصول اصفرار في الغشاء المخاطي للعين و الفم مع فقدان الشهية مع ملاحظة حدوث ألم شديد. لوحظ حدوث تأقلم مع تحسن تدريجي في الحالة الصحية للحيوان. بينت 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. كشفت نتائج الفحص بالامواج فوق الصوتية حدوث تليف الكبد تدريجيا" و الذي تمثل بوجود مناطق عالية الصدى خصوصا في الأسابيع الأولى بعد إجراء العملية الجراحية و حدوث تحسن تدريجي بعد الشهر الاول من احداث التليف. أظهرت نتائج الفحص النسجي تكون نسيج ليفي في نسيج 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. نستنتج مما سبق إمكان احداث تليف الكبد الانعكاسي جراحيا عن طرق غلق فتحة قناة الصفراء الرئيسية و الذي تم تاكيده من خلال نتائج الفحص السريري و المختبري و الفحص بالأمواج فوق الصوتية و الفحص المرضي النسجي.

Introduction

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 (1). 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 act as a mediators of molecular events that participate in the pathogenesis of hepatic fibrosis (2). These 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 of connective tissue proteins such as collagens (3). Cholestatic injury which is associated with reduced or obstructed bile flow within the liver, is the result of primary and secondary diseases such as primary biliary cholangitis, primary sclerosing cholangitis as well as biliary atresia. In advanced fibrotic liver diseases, there is a progression from collagen bands to bridging fibrosis to frank cirrhosis (1). There is recent evidence indicating that even advanced fibrosis is a reversible process in spite of the traditional view which emphasizes on the irreversible state of hepatic cirrhosis. In hepatic fibrosis which is experimentally induced, the cessation of liver injury leads to fibrosis regression (4). The major mechanism of fibrosis resolution is represented by an increased collagenolytic activity which is preceded by the removal of the activated Hepatic stellate cells HSCs by apoptosis via stimulation of both death receptors in activated HSCs and a decreased survival factors such as TIMP-1 which can precipitate HSC apoptosis (5). There are common model 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 (6). The aim of this study was to surgically induce reversible liver fibrosis by surgical closure of major duodenal papilla using absorbable suture material (polyglactine 910) which was used for first time and to evaluate this induced liver fibrosis by clinical, ultrasonographical, laboratory and histopathological examination.

Material and Methods

Six healthy local dogs of both sexes whose ages ranged between (12 – 36) months and their weights ranged between (11-25) kg were used to conduct this study. The dogs were kept for two weeks prior to the surgery for adaptation. Reversible liver fibrosis was surgically induced in all animals by surgical closure of opening of the common bile duct (major duodenal papilla) inside the duodenum with 4/0 absorbable suture material (polyglactine 910) (china). Induced liver

fibrosis was assessed by clinical, laboratory, sonography and histopathological methods. Prior to fibrosis induction, ultrasonography of each animal and collection of blood samples and liver biopsies were performed in order to compare normal results with those resulted following induction of liver fibrosis. 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 examined for each animal of the study prior to operation, then 2 days and weekly until the end of the study to demonstrate the effect of blockage of the common bile duct inside the duodenum. Spectrophotometer (china) was used for enzymatic assays which depends on the signal at a particular wavelength. Ultrasonography of the liver was performed before surgical induction of liver fibrosis to ensure the integrity of the liver tissue. Following fibrosis induction of liver, ultrasonography was done weekly in order to compare results with initial examinations before the surgical operation for a period of two months. Liver tissue biopsies were collected from animals of the experiment before surgery and two month later following animal euthanasia for histopathological examinations.

Surgical Procedures

Under general anaesthesia, animal is clipped, shaved and prepared for aseptic surgery. Animal is placed on the dorsal recumbency. Laparotomy was performed through a midline abdominal incision of approximately 10 cm length. The duodenum was exposed, which is adjacent to the pancreas, we milk the substances that are found in the intestine and then we fix the duodenum with intestinal clamps from both sides. Enterotomy was performed through longitudinal incision (10 cm) in the anti-mesenteric border to close the major duodenal orifice which is located 2-6 cm from the pyloric region. A catheter was inserted to make sure that the bile substance comes out through it. Major duodenal orifice was closed with simple continuous using absorbable suture material (polyglactin 910) (china). The intestine was sutured by Connell and Cushing suture technique using (polyglactin 910) 3/0 (china). The abdominal muscles and peritoneum were sutured by interlocking suture technique. Subcuticular suture technique was used to close skin incision.

Statistical analysis

Statistical analysis of the data were evaluation 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 was used to evaluate the significant results and P values of less than 0.05 were considered as significant.

Results and Discussion

The animals were severely dull and depressed. Inappetite and gradual decrease of body weight were observed in all animals following surgical

induction of liver 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 was observed. All the animals survived until the end of the experiment. Post-operative pain was severe especially during the first two days following the surgical induction of liver fibrosis. There was increase in food uptake due to gradual reduction in post-operative pain but still at lower level leading to gradual decrease in the weight body resulting in emaciation of the animals. Macroscopic investigation revealed apparent swollen liver, engorgement of the bile duct and expanded gall bladder leading to changed shape of the gall bladder. White lines and spots were observed in different areas of the liver indicating the presence of the liver fibrosis. There was discoloration of different areas of the liver manifested by yellowish staining. Evidence of bleeding on the wall of the gall bladder with thickened walls of the both gall bladder and bile ducts were noticed.

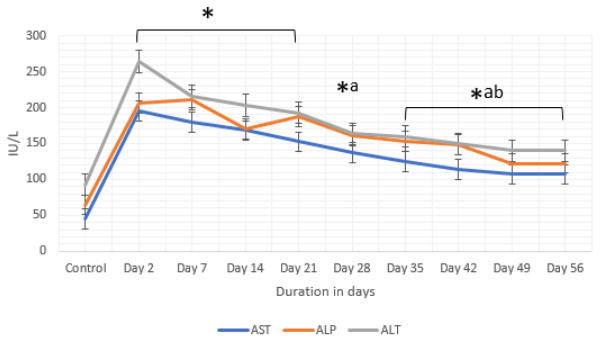

Figure 1 : shows changes in serum liver levels of AST , ALP and ALT following surgical induction of reversible liver fibrosis compared to the normal levels prior to fibrosis induction . * Significant difference between control and experimented values. Small letters means significant within experimented values.

Biochemical parameters

The results biochemical examination showed significant increase in the level of evaluated serum enzyme. There were significant elevated values of serum aspartate aminotransferase AST, alkaline phosphatase ALP, alanine aminotransferase ALT, indirect bilirubin, direct bilirubin and total bilirubin especially during the first weeks of the study and lasted until the end of the study figure (1 and 2).

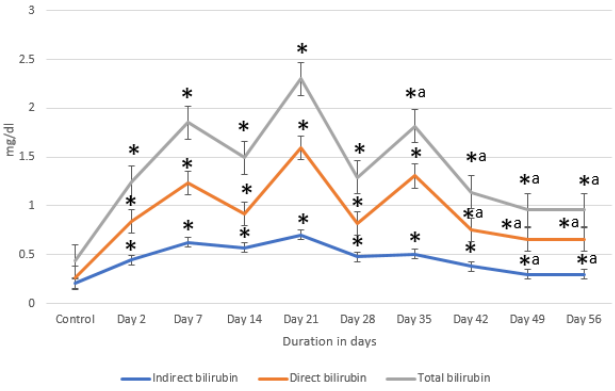

Figure 2 : shows changes in serum liver levels of Total serum bilirubin , direct bilirubin and indirect bilirubin following surgical induction of reversible liver fibrosis compared to the normal levels prior to fibrosis induction . * Significant difference between control and experimented values. Small letters means significant within experimented values.

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Ultrasonographic examinations

Results of the ultrasonographic examinations sowed abnormal change in the liver tissue 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 following surgical induction of the liver fibrosis with slight improvement observed at 30th post-operative day and lasted until the end of the study (figures 3, 4, 5 and 6)

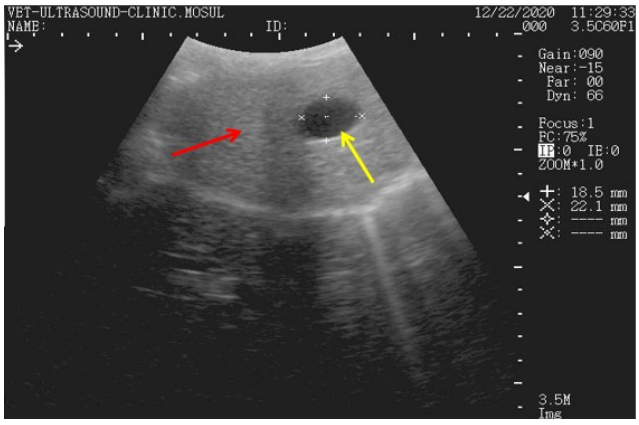

Figure 3: Ultrasonography shows normal liver tissue (red arrow) and normal gallbladder (yellow arrow).

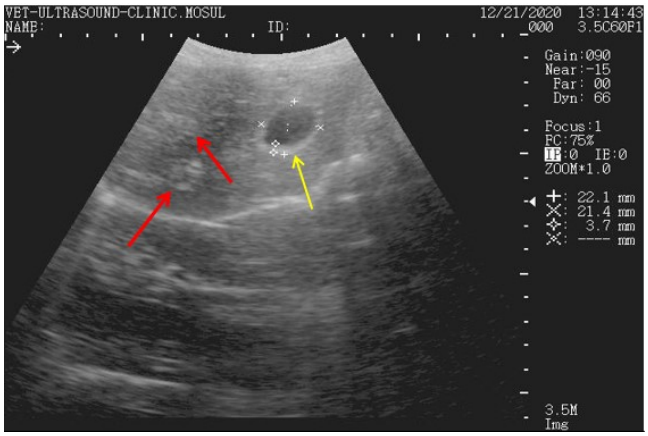

Figure 4: Ultrasonography at the 2nd post-operative week shows thickness in the wall of gallbladder (yellow arrow), heterogeneous mottled appearance of the liver (red arrows).

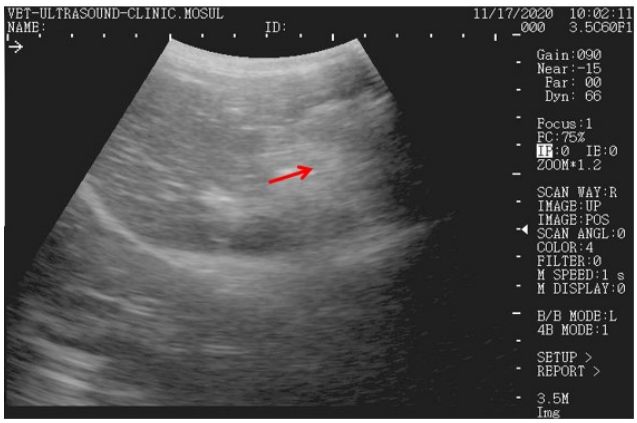

Figure 5: Ultrasonography at the 4th post-operative week show mottled appearance of the liver (red arrows).

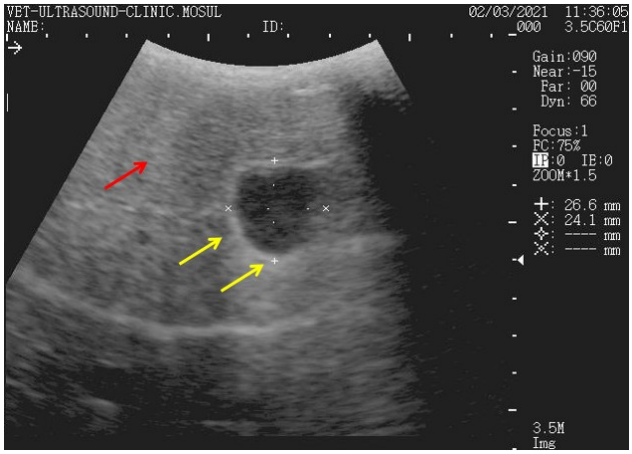

Figure 6: Ultrasonography at the 8th post-operative week shows less sever changes (red arrow) and increase the thickness of the gallbladder wall was observed (yellow arrows).

Histological evaluation

The histopathological examination of normal liver tissue revealed normal tissue architectures

without any pathological lesions (Figure 7). Microscopic examination showed 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) was observed. Some hepatocytes appeared as a polygonal cells with a granular eosinophilic cytoplasm with prominent nuclear (figure 8).Other sections showed recent thrombus in the hepatic vein, and focal infiltration of inflammatory cells (Figure 9). Sever subcapsular and parenchymatous hemorrhage with coagulation necrosis of the hepatocytes (Figure 10) were noticed. Other sections showed sever deposition of eosinophilic material (fibrosis) around portal vein, hepatic artery and bile duct. The epithelial cells lining the bile duct suffered from hyperplasia (Figure 11). Histological examination showed fatty change or steatosis which was manifested by intra cytoplasmic accumulation of fat droplets in the form of vacuoles and fibrosis was observed by Masson's trichrome staining (Figure 12).

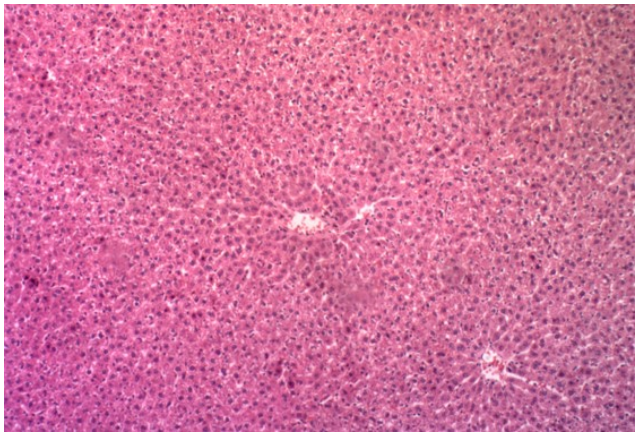

Figure 7: Photomicrograph shows normal tissue of liver. H&E. X10 stain

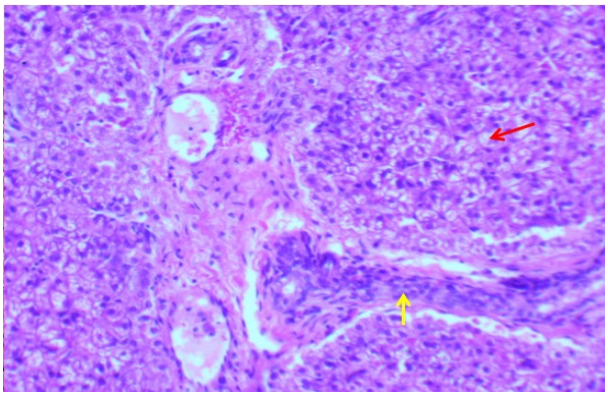

Figure 8: Photomicrograph shows vacuolar degeneration (red arrows) and interlobular infiltration of inflammatory cells in portal area (yellow arrow) H&E stain X10

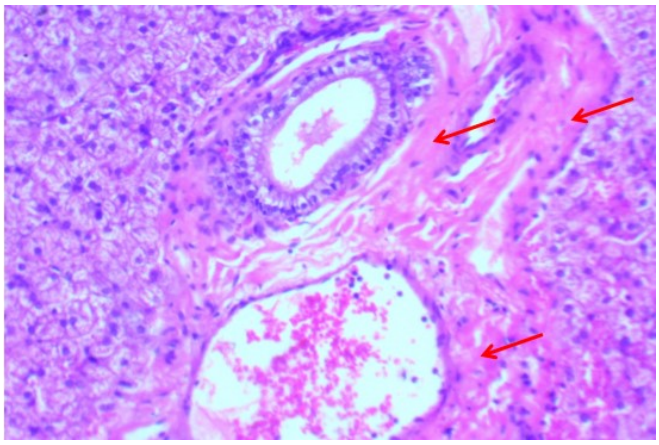

Figure 9: Photomicrograph shows severe hemorrhage (red arrows) H&E. X10 stain.

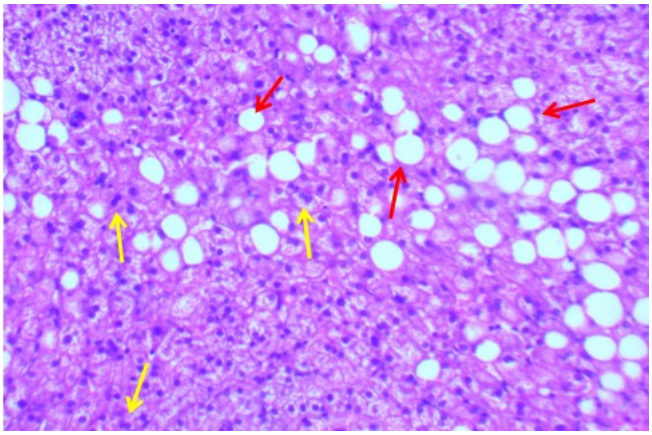

Figure 10: Photomicrograph shows steatosis (fatty change) (red arrow) and double nuclei (regeneration) (yellow arrow) H&E stain X10.

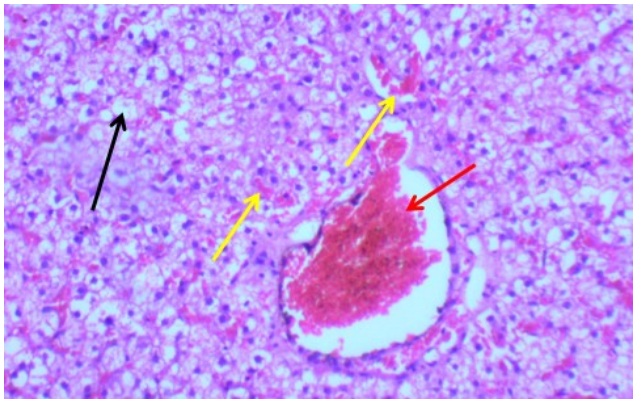

Figure 11: Photomicrograph shows: vacuolar degeneration of hepatocytes (black arrow) and show congestion in sinusoids (yellow arrows) and hemorrhage in (red arrow) H&E stain X10.

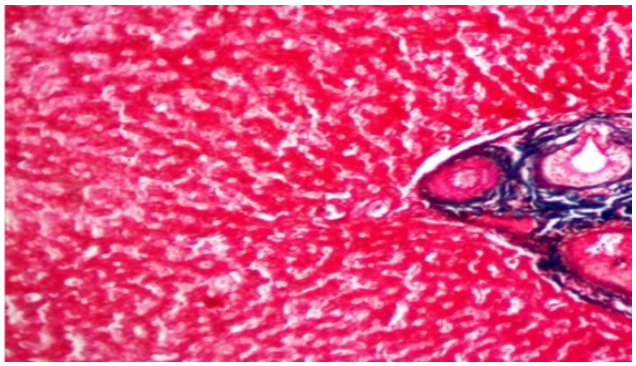

Figure 12: Photomicrograph shows the induced fibrosis in the portal area. Masson's trichrome stain X40

The findings of this study demonstrated that the induction of 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 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 (7). 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 (8). The absorbable suture material polyglactin 910 was reported to retain 57% of its initial strength within 2 weeks and 16% at 4 weeks (9). 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 (10). 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 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 (11) who used absorbable suture material

(polyglactin 910) for the ligation of the common bile duct to induce reversible obstructive jaundice in rats. The clinical results of the present study 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. The results of our study were in agreement with that of Elhiblu et al., 2015 (12) who reported the signs of affected dogs by hepatic insufficiency based on clinical examination. 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 and Sydney Chung 2001 (13) who reported that the functional and morphologic improvement of the liver in rats. Macroscopic investigation showed 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. Similar findings were also observed by Milosavljevic et al., 2018 (14). 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 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. The icterus is usually resulted from increased production of bilirubin, increased of bilirubin enterohepatic circulation and deficiency in both of hepatic uptake and bilirubin conjugation (15). 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 is subjected to be colonized by bacteria causing cholangitis and ascending hepatitis and subsequent hepatic dysfunction as a result of liver cell damage (16). Laboratory findings are usually unspecific, and even though they may indicate a hepatic problem but they do not show whether the condition is a chronic hepatitis or another problem affecting the liver (17). The results of the present study revealed 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 as reported by Cömert et al., 2004 (18) who

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. In the current study, the results of the ultrasonographic examinations showed abnormal change in the liver tissue 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 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 and Brenner 2005 (5) 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. 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 parenchymatous tissue of liver. Similar findings were observed by Tag et al., 2015 (19) who reported formation of the per-sinusoidal 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. Bile duct ligation 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 (20).

Conclusion

The induction of reversible liver fibrosis in dogs by surgical closure of major duodenal papilla was possible and easy technique and the results were confirmed by the clinical, ultrasonographical, laboratory and histological examination. Closure of major duodenal orifice to induce liver fibrosis was related to less complications such as avoidance of the traumatic pancreatitis, biliary leakage and peritonitis.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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