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New surgical model to induce irreversible liver fibrosis by surgical closure of major duodenal orifice in dogs

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Abstract

This study was conducted to induce and evaluate irreversible hepatic fibrosis in dogs by surgical closure of the major duodenal orifice. The study was performed on six healthy local adult dogs. Irreversible hepatic fibrosis was surgically induced in all animals by surgical closure of major duodenal papilla using non-absorbable suture material for 60 days. Induced hepatic fibrosis was evaluated by clinical, ultrasonographical examination, laboratory and histopathological methods. The clinical manifestation of the jaundiced dogs showed reduced food intake, pale-yellowish mucus membrane, inflammatory signs of wound site and severe post-operative pain. Biochemically, there was significant increased values of the aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase, indirect bilirubin, direct bilirubin and total bilirubin especially during 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 change in the liver tissue such as 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 and lasted until the end of the study. Histopathological evaluation of liver samples revealed necrosis of hepatocytes and deposition of eosinophilic material, infiltration of inflammatory cells, recent thrombus in the hepatic vein, fatty change. In conclusion, surgical induction of irreversible hepatic fibrosis in dogs was feasible technique by surgical closure of major duodenal papilla and the results were confirmed by the clinical, ultrasonographical, laboratory and histopathological examination.

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Introduction

Liver fibrosis can be defined as an excessive and progressive accumulation of extracellular matrix which impairs the physiological activities of the liver. Pathogenetically, many diseases (toxic, metabolic, or viral diseases) cause damage of the hepatocytes and immune cells infiltration which stimulate the trans-differentiation of hepatic stellate cells into myofibroblast that produce collagen (1). The common models used to induce hepatic inductions 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 was induced by ligation of common bile duct that can be compared to the change noticed in human biliary cirrhosis (2). 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 (3).

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 (4). Treatment of the underlying cause of 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 attenuates the progression of both liver fibrosis and cirrhosis (5). If fibrosis is created and chronic liver diseases has 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 (6). These structural and functional changes lead to major complication of liver cirrhosis (portal hypertension) which in turn results 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 (4). There are few reports of congenital hepatic fibrosis in different animal species including cats, monkey, calves, foals and dogs (7). This study was aimed to surgically induce irreversible hepatic fibrosis by surgical closure of major duodenal papilla using non-absorbable suture material which is used for first time and to evaluate this induced liver fibrosis by clinical, ultrasonographical, laboratory and histopathological examination.

Materials and methods

Six healthy local dogs of both sexes whose ages ranged between 12 – 36 months and their weights ranged between 15-25 kg were used to conduct this study. The dogs were left for two weeks prior to the surgery to be adapted. Irreversible 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 non- absorbable suture material silk 4/0. Induced hepatic fibrosis was evaluated by clinical, laboratory, sonography and histopathological methods. Prior to surgery, ultrasonography of each animal and collection of blood samples and liver biopsies were performed to compare the normal results with those caused by 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 determined. 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. Ultrasonography of the liver was performed before surgical induction of hepatic fibrosis to ensure the integrity of the hepatic 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. Hepatic wedge biopsies were collected from animals of the experiment before fibrosis induction and two months later following animal euthanasia for histopathological examinations (8).

Statistical analysis

Statistical analysis of the data was performed 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 between groups and P values of less than 0.05 were considered as significant.

Results

The animals were severely dull and depressed. In appetite and subsequent gradual loss of body weight were observed in all animals following surgical induction of hepatic fibrosis. Jaundice was the main clinical feature of the animals with blocked common bile duct which was manifested by pale yellowish mucus membrane of the eye and the oral cavity. 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 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. Macroscopic investigation 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 1).

Biochemical parameters

In general, the results showed significant increase in the level of some evaluated serum enzymes. There were significant increase in the values of serum AST, ALP, ALT, indirect bilirubin, direct bilirubin and total bilirubin especially during the first weeks of the study until the end of the study (Figures 2 and 3).

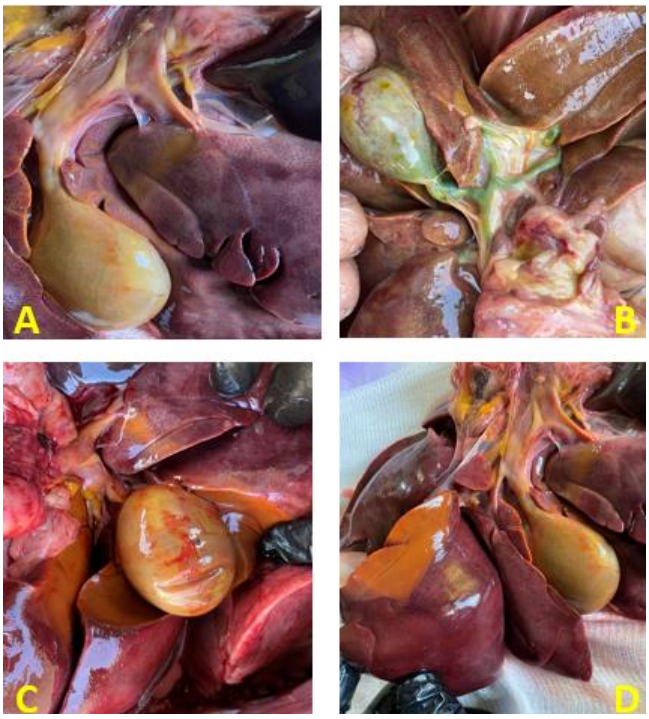

Figure 1: shows swollen gallbladder (A), engorgement of bile duct (B) hemorrhage in the wall of gallbladder (arrow) (C), liver lobe pigmentation with bile (D).

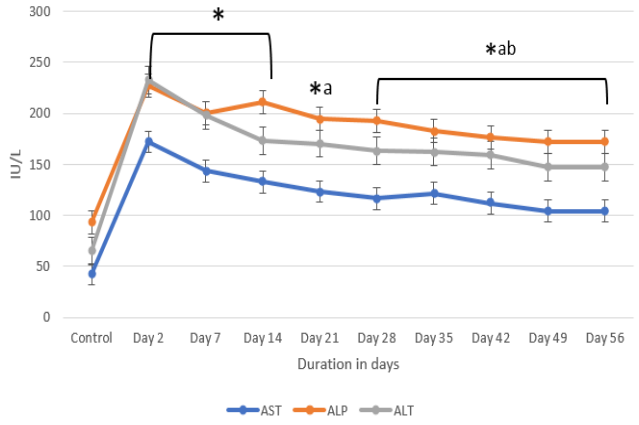

Figure 2: shows changes in serum liver levels of ALT, ALP and AST following surgical induction of irreversible liver fibrosis in animals of the first group compared to the normal levels prior to fibrosis induction. * Significant difference between control and experimented groups. Small letters mean significant within experimented groups.

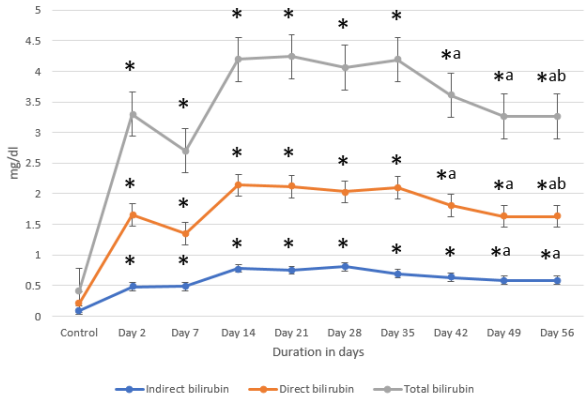

Figure 3: shows changes in serum liver levels of indirect bilirubin, direct bilirubin and Total serum bilirubin following surgical induction of irreversible liver fibrosis in animals of the first group compared to the normal levels prior to fibrosis induction. * Significant difference between control and experimented groups. Small letters mean significant within experimented groups.

Ultrasonographic examinations

Results of the ultrasonographic examinations revealed 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 4-7).

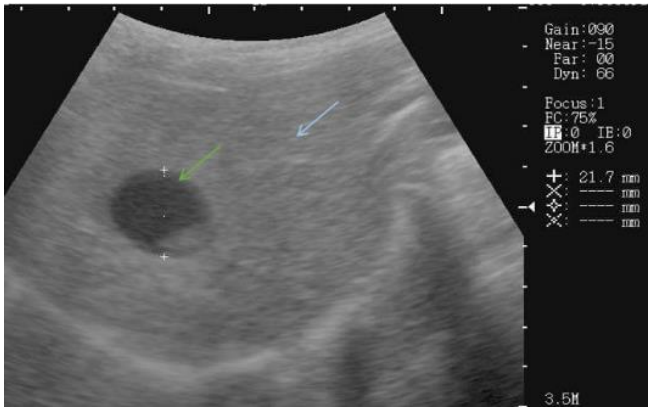

Figure 4: Ultrasonographic image shows normal liver tissue (blue arrow) and normal gallbladder (green arrow).

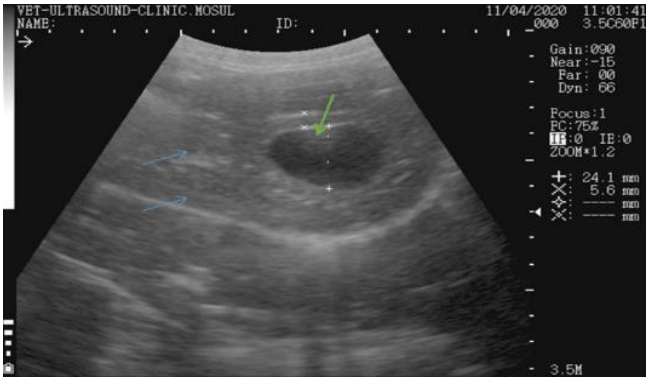

Figure 5: Ultrasonographic image at the 2nd post-operative week shows thickness in the wall of gall bladder (green arrow) heterogeneous and mottled appearance of the liver (blue arrows).

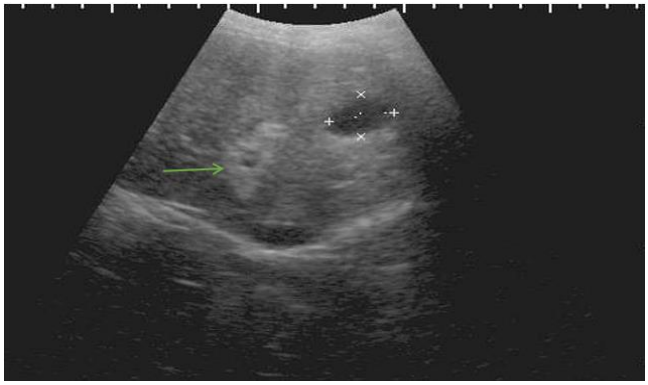

Figure 6: Ultrasonographic image at the 4th post-operative week shows heterogeneous and mottled appearance of the liver fibrosis (green arrow).

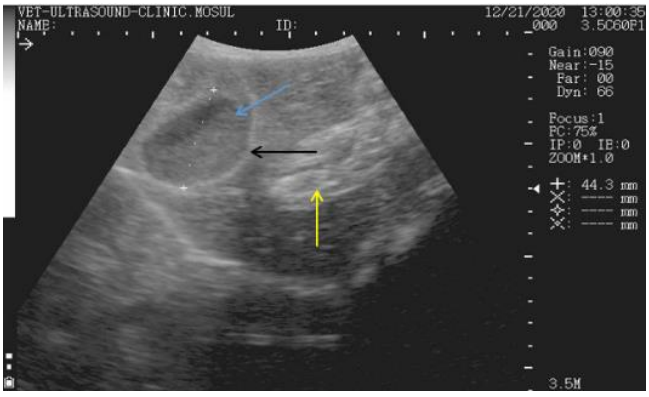

Figure 7: Ultrasonographic image at the 8th post-operative week shows increased size of gallbladder (blue arrow), increased thickness in the wall of gall bladder (black arrow) and heterogeneous and mottled of the liver appearance (yellow arrow).

Histological evaluation

The microscopic examination of normal liver tissue in both groups revealed normal tissue architectures without any pathological lesions (Figure 8). 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 and portal vein) and thickening of hepatic artery wall. Some hepatocytes appeared polygonal cells with a granular eosinophilic cytoplasm with prominent nuclear (Figure 9). Other section showed recent thrombus in the hepatic vein, and focal infiltration of inflammatory cells (Figure 10). Sever hemorrhage beneath the capsule and parenchymatous tissue of liver with coagulation necrosis of the hepatocytes (Figure 11). Other section 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 12). Vacuolar degeneration of hepatocytes and deposition of eosinophilic material in the portal area lead to thickening the wall of bile duct and hepatic artery with congestion of blood vessels (Figure 13). Histological examination showed fatty change or steatosis which was characterized by intra cytoplasmic accumulation fat droplets which appeared as vacuoles and fibrosis when stain the slide with Masson's trichrome (Figure 14).

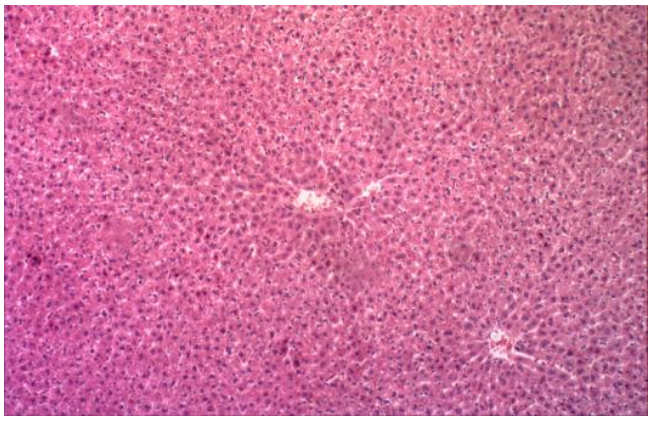

Figure 8: Photomicrograph shows normal tissue of liver. H&E stain. X10.

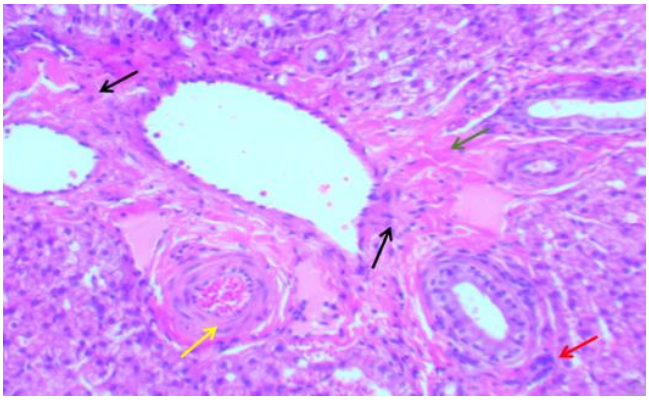

Figure 9: Photomicrograph shows deposition of eosinophilic material in the portal area (black arrows), hemorrhage in hepatic tissue (green arrow), thickening and congestion in blood vessels (yellow arrow) and necrosis of hepatocytes (red arrow) H&E stain. X10.

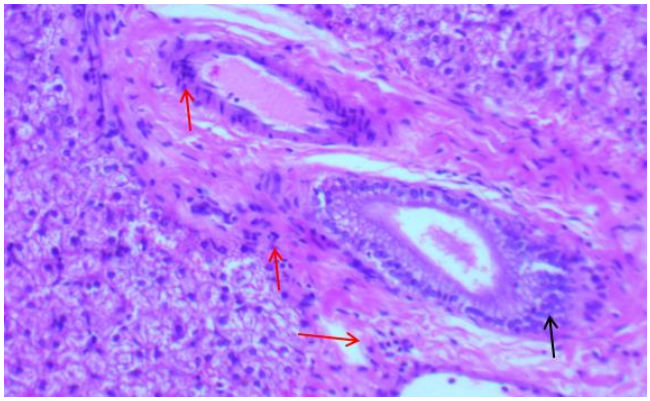

Figure 12: Photomicrograph shows sever deposition of esinophilic material (fibrosis) around portal vein, hepatic artery and bile duct (red arrows) and the epithelial cells lining the bile duct suffered from hyperplasia (black arrow). H&E stain. X10.

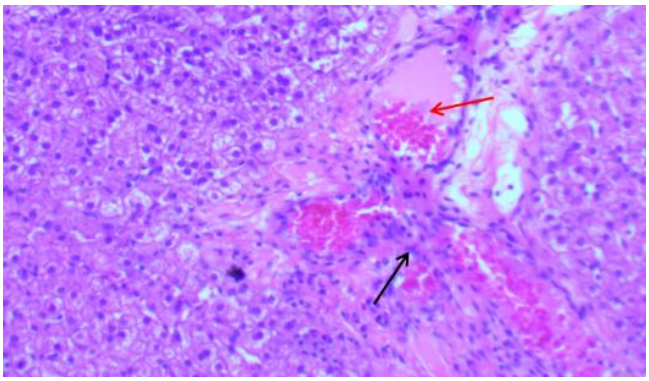

Figure 10: Photomicrograph shows recent thrombus in hepatic vein (red arrow) and inflammatory cells (black arrow). H&E stain. X10.

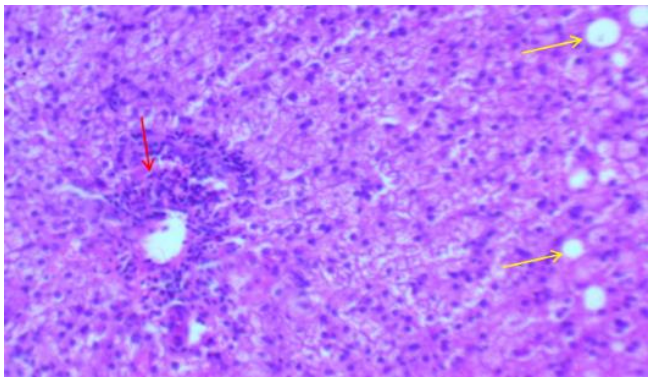

Figure 13: Photomicrograph shows vacuolar degeneration (yellow arrows) and infiltration of inflammatory cells around central vein (red arrow). H&E stain. X10.

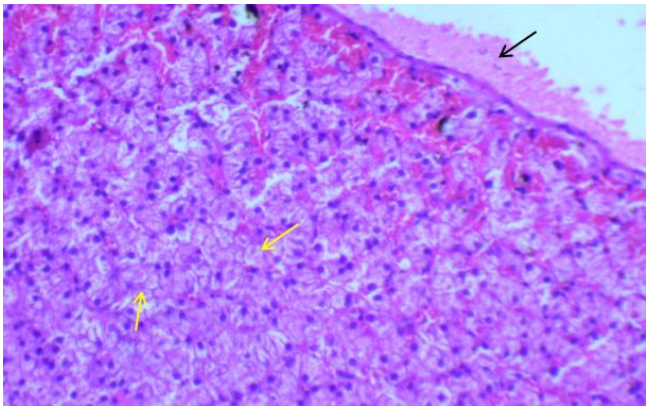

Figure 11: Photomicrograph shows thickening of hepatic capsule (black arrow) and coagulative necrosis (yellow arrows). H&E stain. X10.

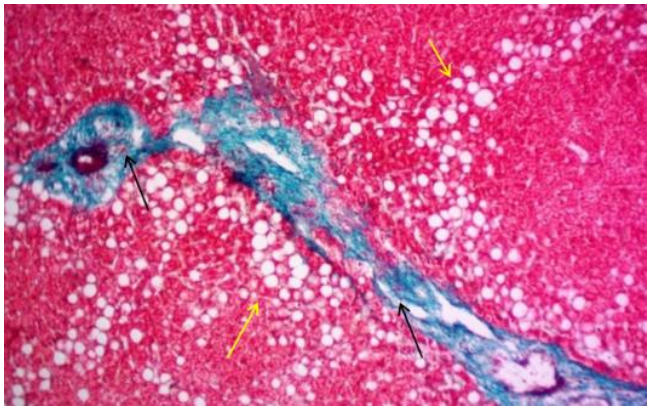

Figure 14: Photomicrograph shows fatty changes (yellow arrows) and fibrosis in the portal area (black arrows). Masson's trichrome stain, X40.

Discussion

The findings of this study demonstrated that the induction of irreversible 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 (9). 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 (10). Chronic liver injury causes a progressive response which leads to eventual liver fibrosis characterized by both quantitative and qualitative alteration of hepatic extracellular matrix (ECM). This alteration involves an increased proliferation, accumulation of ECM by collagen deposition. The oxidative stresses have a critical role to activate hepatic stellate cell HSC during hepatic fibrogenesis (11). 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 years is required for complete bio-degradation (12). Therefore, silk suture as non-absorbable material was chosen to surgically close of the major duodenal papillae to induce the irreversible liver fibrosis. Clinically, the hepatic disorder is not characterized by specific signs. Signs such as anorexia, vomiting, diarrhea, polydipsia and polyuria are differently noticed. Furthermore, other signs like fatigue, jaundice, hepatic encephalopathy and ascites are also in variable association to the disease, relying on its severity. Coagulopathies, cirrhosis and portal hypertension are closely related to liver failure. In dogs suffering from chronic hepatitis, ascites is regarded a negative prognostic sign which is already known for humans (13). The current study has reported 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 involved in appetite 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 weight body of resulting in cachexia of the animals. The results of our study were in agreement with that of Elhiblu *et al*. (14) who reported the signs of affected dogs by hepatic insufficiency based on clinical examination.

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*. (15). 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 (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*. (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 addition to the increased total and direct bilirubin, alkaline phosphatase levels and SGOT and SGPT in the affected dogs with CBDL (19). These results were also in an agreement with the results of the current study. 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*. (2) 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.

Conclusion

The induction of irreversible hepatic fibrosis in dogs by surgical closure of major duodenal papilla was feasible and easy technique and the results were confirmed by the clinical, ultrasonographical, laboratory and histological examination.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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طريقة جراحية جديدة لإحداث تليف الكبد الغير الإنعكاسي عبر غلق فتحة قناة الصفراء داخل الاثني عشر في الكلاب

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الخلاصة

تم إجراء هذه الدراسة لغرض إحداث تليف الكبد الغير الانعكاسي جراحيا في الكلاب. أجريت التجربة على ستة من الكلاب المحلية البالغة من كلا الجنسين والسليمة من الأمراض. تم استحداث تليف الكبد الغير الانعكاسي عن طريق غلق فتحة قناة الصفراء الرئيسية داخل الاثني عشر باستخدام خيط جراحي غير ممتص نوع سلك بعد إجراء فتحة بطول ١٠ سم. تمت متابعة حيوانات التجربة سريريا مع قياس مستوى أنزيمات الكبد بالدم والفحص بالأمواج فوق الصوتية أسبوعيا ولمدة ستون يوما. في نهاية التجربة تم أخذ عينات من مناطق مختلفة من الكبد لغرض إجراء الفحص النسجي. أظهرت نتائج الدراسة حدوث تليف الكبد الانعكاسي تدريجيا تمثلت بحصول اصفرار في الغشاء المخاطي للعين والفم 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 ارتفاع تدريجي في مستوى هذه الأنزيمات خصوصا خلال الأيام الأولى بعد إجراء العملية واستمر هذا الارتفاع إلى نهاية التجربة. كشفت نتائج الفحص بالأمواج فوق الصوتية حدوث تليف الكبد تدريجيا والذي تمثل بوجود مناطق عالية الصدى خصوصا في الأسابيع الأولى بعد إجراء العملية الجراحية. أظهرت نتائج الفحص النسجي تكون نسيج ليفي في نسيج الكبد مع ارتشاح للخلايا الالتهابية خصوصا حول القنوات الصفراوية إضافة إلى تنخر وتنكس الخلايا الكبدية مع حصول نزف فنسيج الكبد وتحت المحفظة. نستنتج مما سبق إمكان إحداث تليف الكبد الانعكاسي جراحيا عن طرق غلق فتحة قناة الصفراء الرئيسية والذي تم تأكيده من خلال نتائج الفحص السريري والمختبري والفحص بالأمواج فوق الصوتية والفحص المرضي النسجي.