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Induction of Irreversible Liver Fibrosis by Laparoscopic Closure of Common Bile Duct in Dogs

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Abstract

THIS study was aimed to assess the laparoscopic ligation of common bile duct using a titanium clip in eighteen healthy adult domestic dogs from both sexes, the age ranged from (24±6) months and weight ranged from (20±5) kg. After ligation of common bile duct, the animals were left for 21 days. All animals were subjected to evaluated progression of hepatic fibrosis by clinical, ultrasonography, radiography and laboratory examination. Clinical results such as severe abdominal pain, anorexia, emaciation, jaundice and paleness mucus membrane were observed. Ultrasonographical examination of the liver revealed dilatation of the gallbladder and common bile duct as well as an increased in thickness and echogenicity of liver texture, while the biochemical parameters at the 21 days of closure the common bile duct revealed significant elevation in liver serum enzymes and total, direct and indirect bilirubin at $p \leq 0.05$. In conclusion, the laparoscopic technique of inducing incurable liver fibrosis in dogs by surgical closure of the common bile duct with titanium clips is an unconventional, effective, less complication and straightforward technique to induce fibrosis.

Keywords: Laparoscopic technique, Cholestatic jaundice, Ultrasonographic Examination, Dogs**.**

Introduction

The liver is considered the largest body gland with both endocrine and exocrine functions such as production, collection and concentration of bile in the gall bladder before being drained into the duodenum [1]. Liver fibrosis is defined as the gradual buildup of extracellular matrix (ECM) that diminishes the anatomical organization and composition of the liver that allows it to carry out its essential functions in the body. Pathogenetically, toxic, metabolic, or viral illnesses result in the destruction of hepatocytes and the infiltration of immune cells, which trigger the conversion of hepatic stellate cells (HSCs) into myofibroblasts that make collagen [2]. Liver fibrosis has been proven to be reversible following the elimination of the etiology, particularly in the early stages, so early diagnosis of liver fibrosis is of grat importance for clinical treatment [3]. Cholestasis is the stagnation or a marked decrease in bile drainage and passage. Cholestasis may result from functional impairment of the hepatocytes in the secretion of bile and/or an obstruction at any level of the excretory pathway of bile from the hepatic parenchymal cells level at the basolateral (sinusoidal) membrane of the hepatocytes to the ampulla of Vater, with present in the duodenum[4]. Experimentally, different surgical methods were used for the induction of liver fibrosis by complete or partial bile duct ligation (BDL) with traditional or laparoscopic surgery [5]. The actual favorable non-invasive methods for the diagnosis of liver fibrosis are ultrasonography and serum biomarkers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), as well as total, direct, and indirect bilirubin [6]. The current study's aimed to assess the effect and efficacy of laparoscopy as a novel method for induction of liver fibrosis.

Material and Methods

Experimental Animals

Eighteen healthy local adult dogs from both sexes, aged between (24±6) months and weights was between (20±5) kgs were used to perform this study. These animals were treated with ivermectin at a dose 0.2 mg / kg [7]. The animals were examined

physically and clinically to ensure that they are free of diseases. The animals were kept for 14 days before surgery to ensure adaptation.Vaccin agenst rabies using DHPPIL+R (Dyntec spol. Terezin ,Czech republic).

Preparation of animals for surgery

This study was carried out using a premedication and general anesthesia protocol that included atropine sulfate 1% at a dose of 0.04 mg/Kg B.W.[8], followed by ketamine (10%) and xylazine (2%) at a dose of 15 mg/kg and 5 mg/kg of body weight[9,10], respectively, administered as a mixture intramuscularly. The surgical site was according to the basic traditional principles of surgery.

Laparoscopic closure of common bile duct

The closure of the common bile duct was made laparoscopically. Initially, all animals were positioned in the tilted anti-Trendelenburg with the head elevated higher than the hindlimbs to push the abdominal viscera caudally. pneumoperitoneum was created through the insertion of the veress needle at umbilicus[11], an insufflation tube connected to the electronic CO2 insufflator at 12 mm Hg [12], and a flow rate of 8 L/min [13] was maintained for better visualization and manipulation during laparoscopy. ensure that the versus needle is inside the abdominal cavity so that extra-abdominal insulation is prevented. When proper intra-abdominal pressure was achieved, the veress needle was removed, and a 10 mm cannula (pyramidal trocar and sheath) for a laparoscope (a thin, lighted tube with a camera) was inserted through the umbilical incision to visualize the surgical area inside the abdomen on a monitor. Following abdominal exploration, the liver and gallbladder were revealed. Insertion of another 3 ports was performed under vision. The convergent locations of these ports were, first ±10 cm on right side caudally to the umbilicus, second was ±7 cm parallel to umbilicus on left side and third about ±12 cm on left side caudal to umbilicus. Insertion of 10 mm port on the right side and a 5 mm ports on the left side was done. A babcock forcep was inserted into the left port for gallbladder holding, the other left port was used to insert the hemostatic forceps used for dissection between common bile duct, portal vein and hepatic artery. Once dissection was completed, 10-mm clip applicator introduced through a 10 mm port on the right side, the common bile duct was ligated using 4 extra-large titanium metallic clips (Figure 1).

Post-operative care

After operation, all animals were administered dipyrone by intramuscular injection at a dose of 1 ml per 5 kg of body weight, and the antibiotic (penicillin-streptomycin) was injected intramuscularly for five consecutive days at a dose of 10,000 IU per 15 kg of body weight [14].

Postoperative examinations

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

Clinical examination

The health condition of the animals was evaluated after 21 days after laparoscopic induction of hepatic fibrosis to confirm the main clinical features of common bile duct obstruction.

Macro-pathological examination

All animals were sacrificed for macro-pathological examination 21 days after ligation of the common bile duct to inspect the liver texture and gallbladder.

Ultrasonographic examination

Ultrasound examination of the liver in all animals was done beneath the diaphragm exactly in the right hypochondriac and epigastric regions using the Kaixin Kx5100vet 3,5 microconvex probe (Keebromed USA) prior to the surgical induction of liver fibrosis to ensure the structural safety of the liver tissue. After surgical ligation of common bile duct, ultrasound examination for animals was made for three consecutive weeks in order to assess fibrosis.

Laboratory examination

To evaluate the degree of hepatocyte damage after laparoscopic ligation of the common bile duct, liver function was examined by evaluating the activities of AST, ALT, ALP, and total, direct, and indirect bilirubin in the blood. A blood sample of 10 ml was withdrawn from all animals in the study before the ligation of the common bile duct and after 21 days of the experiment.

Statistics of the study

The liver serum enzymes were statistically analysed via SPSS program v.23 software (SPSS In. Chicago, IL., USA), T test was used to measure the differences in the mean stenosis percentages in different days at $P<0.05$[15].

Results

Clinical investigation

In the first two days after laparoscopic ligation of the common bile duct, the dogs became bored and despondent. Anorexia and progressive emaciation were observed in all animals. Body weight loss was associated with the duration of corresponding cholestasis, and icterus was the main clinical manifestation of dogs suffering from common bile duct obstruction, as evidenced by yellowish skin and pale yellowish mucosa of the eyes and oral cavity, as well as dark yellow urine and clay feces (Fig. 2).

Macro pathological investigation

Macro pathological investigation at 21 days post closure of the common bile duct showed that there was a yellowish discoloration of the liver tissue which was mild to moderate and there was change in size and shape of the gall bladder. The quantities of bile substance reached upto 115 ml (Fig.3).

Radiographical examination:

Radiographical examination revealed the titanium clips used to close the common bile duct became loose and detached, allowing bile to enter the duodenum 21 days after the duct was blocked. (Fig. 4).

Ultrasonographic examinations.

At 21 days post-ligation of the common bile duct, Ultrasonography revealed unusual alterations in hepatic tissue after ligation of the common bile duct, including increased gallbladder size and thickness, enhanced echoes, and a mottled heterogeneous appearance of the liver (Fig. 5).

Laboratory tests

A-Liver enzymes

Alkaline phosphatase (ALP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) revealed a significant elevation in their values at the 21 days of induced liver fibrosis with respect to zero time at p value 0.05 (Fig. 6).

B-Total, direct and indirect bilirubin:

At 21 days of induction liver fibrosis, the levels of total, direct, and indirect bilirubin were significantly higher than those measured at zero days in animals, at a p value of 0.05. (Fig. 7).

Discussion

This study demonstrated the efficiency of laparoscopic ligation of common bile duct with titanium clips. The induction of irreversible hepatic fibrosis by this technique was linked to lesser complications as compared to traditional surgery in terms of the occurrence of adhesions and infections, as well as the size of the incisional wound [16]. The blockage of common bile duct led in an impaired flow of bile from gallbladder to the duodenal cavity (cholestasis), resulting in elevated ductal mucin, which in turn caused ductal distension. The biliary system is invaded by the bacteria resulting into cholangitis and ascending hepatitis [17].

The obtained clinical findings of icterus occurred as a significant symptom of dogs suffering from common bile duct obstruction are consistent with Andrade et.al.[18] who stated the yellowish pigmentation of tissue and mucous membrane was due to bilirubin deposition. Clay feces was clearly observed as a result of cholestasis due to block of common bile duct. This result was a proven by Vagholkar [19] who reported the conjugated bilirubin failed to enter the intestine and was transformed by bacteria into urobilinogen, which underwent oxidation to stercobilinogen, which was excreted in the feces in the form of stercobilin which imparted a yellow color to the stool. The dark color of urine is caused by the excretion of conjugated bilirubin, which is another symptom of cholestasis [20]. Animals suffered from severe pain for two days after the surgical induction of liver fibrosis due to possible liver enlargement, which compresses the Glisson's capsule, a layer of tissue that surrounds the liver and contains nerves that produce pain perception. Any condition that causes the liver to grow might induce what seems like liver pain when the larger liver presses against its outer capsule this signs has been proven by Hamilton [21].

Macro-pathological features were noticed in the animals of the experiment. The liver texture changed from smooth surface to irregular surface with increased rigidity. These changes occurred due to chronic inflammation which led to excessive production and accumulated extracellular matrix that indicating presence of hepatic fibrosis. These results were similar to those observed by George *et.al.* and Berumen *et.al.* [22,23]. It was found that there was a mild to moderate change in the color of different areas of the liver from the normal color, in addition to the presence of subcapsular hemorrhagic spots and expansion of the common bile duct, these results were proven by Sonne *et.al.* and Milosavljevic *et.al.* [24,25]. Increase in the thickness of the wall of the gallbladder and bile ducts was a hallmark of acute cholecystitis, and may also be due to secondary inflammatory process spreaded from other structures such as the pancreas or the liver this result was in agreement with results reported by Wheeler *et.al.* [26].

Twenty-one days after the duct was closed, bile began to leak into the duodenum due to loose titanium clips that had been utilized to shut the common bile duct. This was discovered by radiographic inspection. These were brought on by the massive buildup of bile, which in turn caused the gallbladder to experience extreme pressure, allowing bile to freely flow from the liver to the duodenum [27].

Ultrasonography showed abnormal morphology-ical changes in liver texture as well as increase in size and wall thickness of the gallbladder. These changes were observed intensively at 21 days of surgery using laparoscopy and fixation with titanium clips, where the liver parenchyma revealed increased echogenicity (hyperechoic) and mottled heterogeneous appearance of the liver instead of the normal appearance, these changes occurred as a result of the closure of the main bile duct, this result was confirmed by Vardar *et.al.* [28].

Laboratory results are frequently unspecific, and while they may refer to hepatic disease, these results may not identify the real cause of illness which may be chronic hepatitis or other diseases that affect the liver [29]. The outcomes showed a significant increase in ALP which indicated the extent of impaired bile flow or cholestasis while, elevation in ALT and AST reflected the extent of hepatocellular injury at 21 days after common bile duct ligation with titanium clips when compared with 0 day. These resulst were in agreement with Iluz-Freundlich *et.al.*[30]. The significant elevation in serum bilirubin often signified problems with bile passage from the liver due to the ductal obstruction. These finding has been proven by Pollock and Minuk [31] .

Conclusion

As a new technique, it is possible to induce laparoscopic irreversible cirrhosis in dogs by surgical closure of the common bile duct with titanium clips. The technique was simple and straightforward, and the results were validated by radiographic, ultrasonography, laboratory, and clinical examinations that confirmed the effectiveness of laparoscopic ligation in inducing liver fibrosis.

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Authors Contribution

The authors contributed equally

Conflicts of interest

The authors state that they have no conflicts of interest.

Ethical considerations

The present study was approved by the Institutional Animal Care and Use committee of the College of Veterinary Medicine, University of Mosul (UM.VET.2021.026).

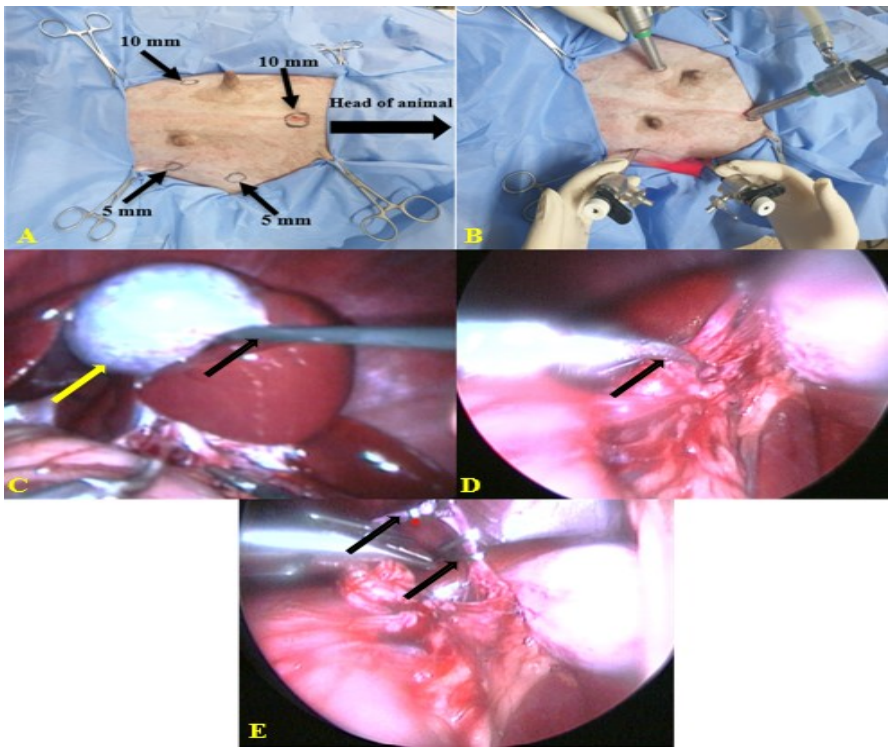

Fig. 1. Shows (A) the site and size of ports entry on the animal body (B) the insertion of different size of cannulas in ports of entry on the animal body(C) the Babcock forceps (Black arrow) used to hold the gallbladder (Yellow arrow) (D) the hemostatic forceps (Black arrow) used for dissection between common bile duct, portal vein and hepatic artery (E) the common bile duct was ligated using 4 extra-large titanium metallic clips (Black arrow).

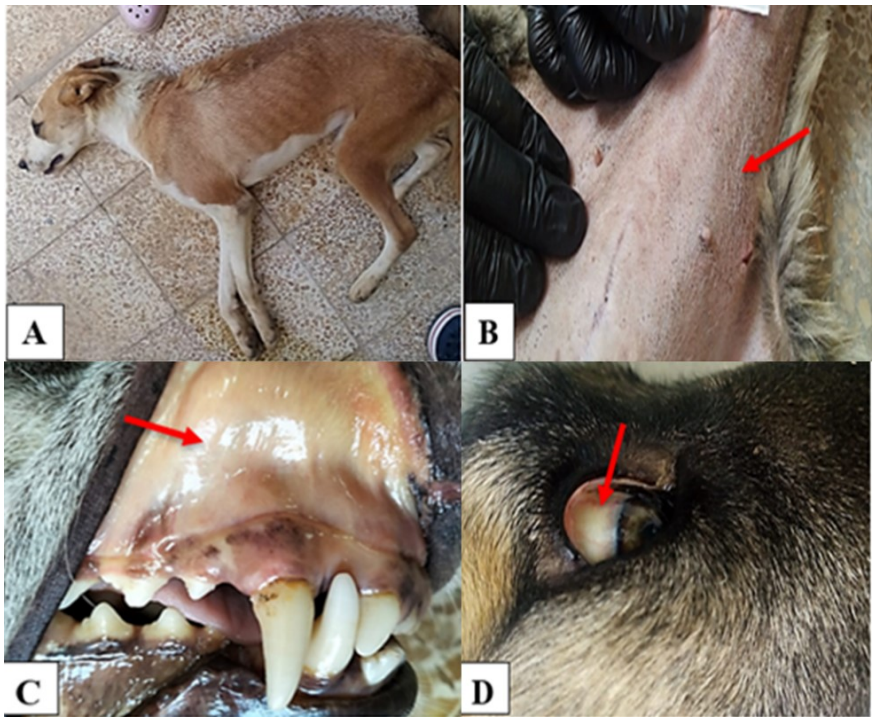

Fig. 2. Shows clinical observations after induction of fibrosis (A) loss of body weight. (B) mild yellowish of skin (Red arrow). (C) mild yellowish of oral mucus membrane (Red arrow). (D) mild yellowish of mucus membrane of eye (Red arrow).

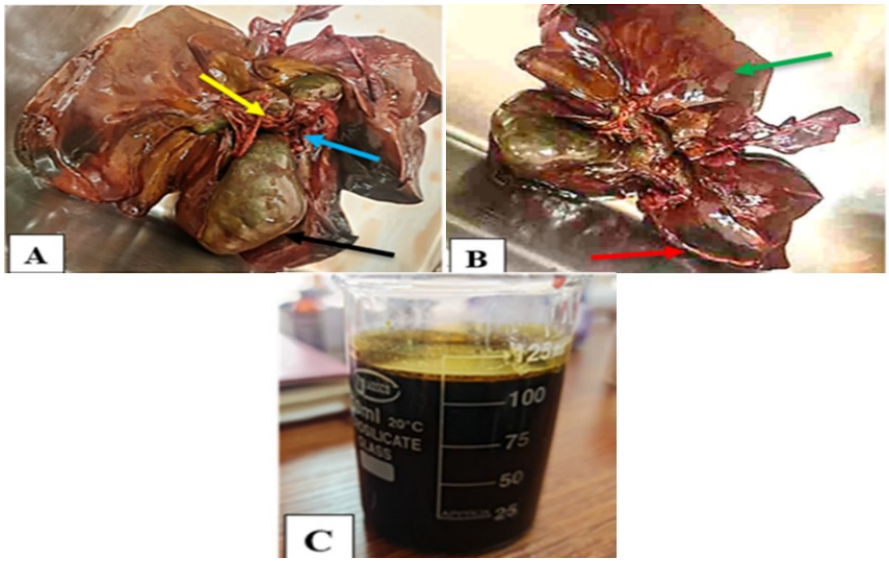

Fig. 3. Shows Macropathological features after closure of common bile duct by laparoscopic surgery using titanium clips. (A) Titanium clips (Blue arrow), expansion of the gall bladder (Black arrow), expansion of common bile duct (Yellow arrow). (B) deformity in shape and size with discoloration in different region of liver (Green arrow), hemorrhage under the liver capsule (Red arrow). (C) quantities of bile more than normal reaching to (115 ml) at the 21 days

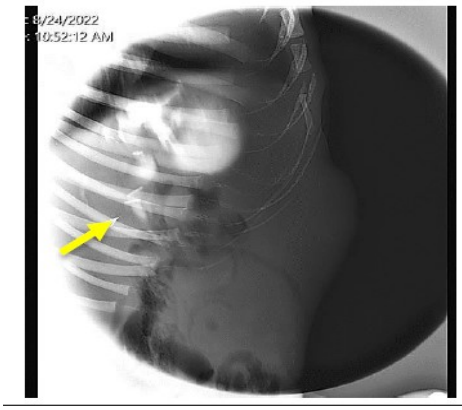

Fig. 4. Radiographic image shows the free passage of bile into the duodenal cavity when used Titanium clips (Yellow-arrow)

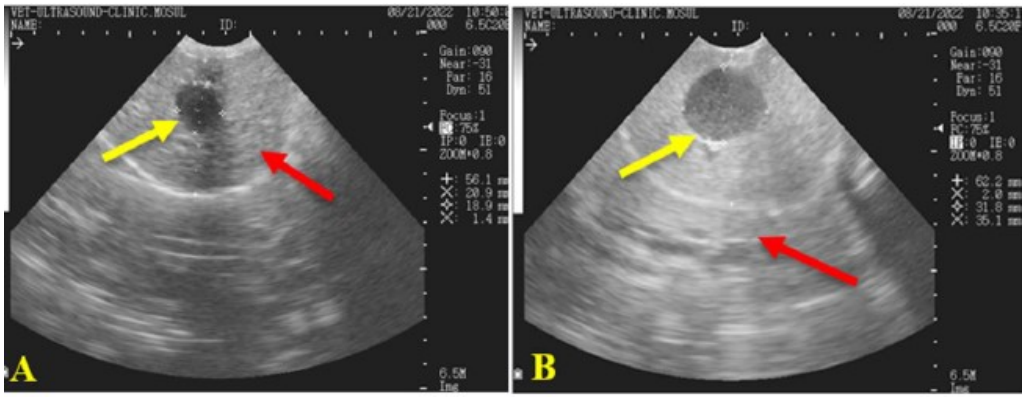

Fig. 5. Ultrasonography of liver (A) at 0 day shows normal liver tissue (red-arrow) and normal gall bladder (yellow-arrow), (B) at 21 days shows increase in the size and thickening of the wall of gall bladder (yellow-arrow) and heterogeneous mottled appearance of liver tissue (red-arrows).

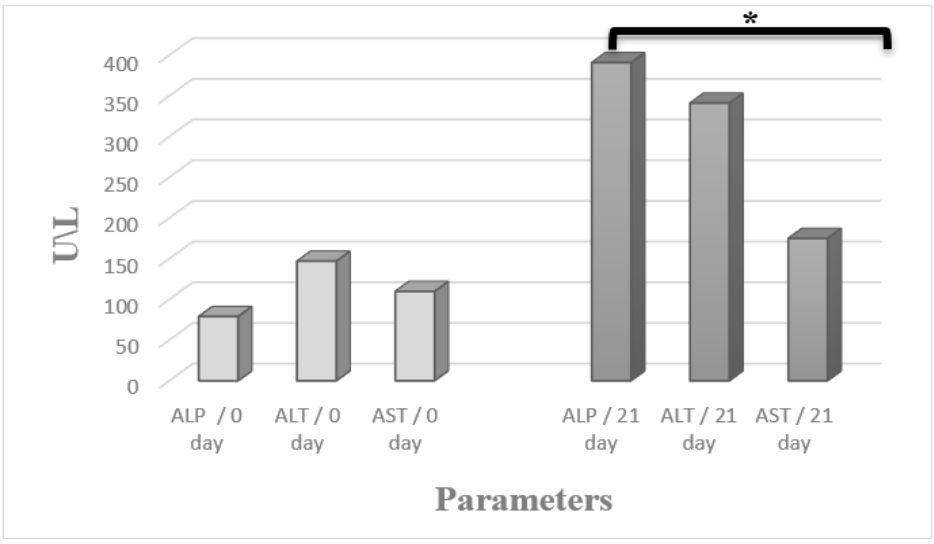

Fig. 6. Shows a significant elevation in the liver levels of ALP, ALT, and AST at day 21 in comparison to day zero of induction of liver fibrosis. (n=9).

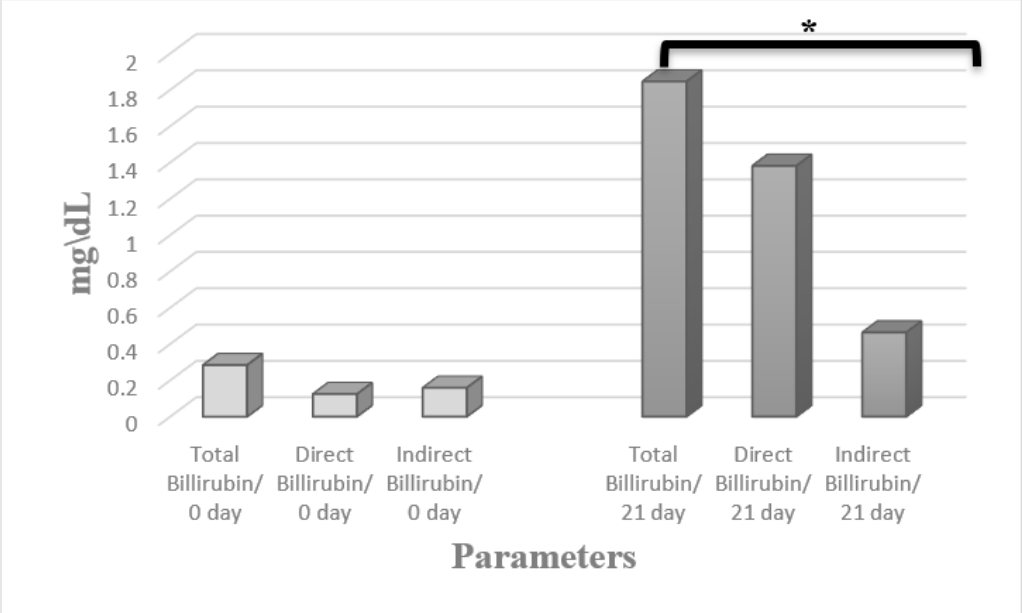

Fig. 7. Shows a significant elevation in the liver levels of total, direct and indirect bilirubin at 21 days in comparison to day zero of inducing liver fibrosis. (n=9)

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إحداث تليف الكبد الذي لا رجعة فيه عن طريق إغلاق القناة الصفراوية المشتركة بالمنظار في الكلاب

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المستخلص

هدفت هذه الدراسة إلى تقييم ربط القناة الصفراوية المشتركة بالمنظار باستخدام مشبك التيتانيوم في ثمانية عشر كلباً منزلياً بالغاً سليماً من كلا الجنسين، تراوحت أعمارها بين (24±6) شهراً وتراوح وزنها بين (20±5) كغم. بعد ربط القناة الصفراوية المشتركة، تُركت الحيوانات لمدة 21 يومًا. تم إخضاع جميع الحيوانات لتقييم تطور التليف الكبدي عن طريق التصوير السريري والموجات فوق الصوتية والتصوير الشعاعي والفحص المختبري. وقد لوحظت النتائج السريرية مثل آلام شديدة في البطن، وفقدان الشهية، والهزال، واليرقان، وشحوب الغشاء المخاطي. كشف الفحص بالموجات فوق الصوتية للكبد عن توسع المرارة والقناة الصفراوية المشتركة بالإضافة إلى زيادة في سماكة وبنية نسيج الكبد، في حين كشفت المعلمات البيو كيميائية في 21 يومًا من إغلاق القناة الصفراوية المشتركة عن ارتفاع كبير في إنزيمات مصل الكبد والإجمالي. ، البيليروبين المباشر وغير المباشر عند $p<0.05$. في الختام، فإن تقنية المنظار لإحداث تليف الكبد غير القابل للشفاء في الكلاب عن طريق الإغلاق الجراحي للقناة الصفراوية المشتركة بمشابك التيتانيوم هي تقنية غير تقليدية وفعالة وأقل تعقيدًا ومباشرة لإحداث التليف.

الكلمات المفتاحية: تقنية المنظار، اليرقان الركودي، الفحص بالموجات فوق الصوتية، إنزيمات مصل الكبد، الكلاب