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EXPERIMENTAL COMMON BILE DUCT LIGATION IN DOGS (With 2 Tables & 4 Figs.)

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الربط التجريبي للقناة المرارية الرئيسية في الكلاب

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تم دراسة تأثير ربط القناة المرارية في عدد عشرة من الكلاب يتراوح أعمارهم ما بين ٢ - ٥ سنوات وتم التأكد من حالتها الصحية بواسطة الكشف الإكلينيكي والمعملي . وقد ظهرت أعراض الصفراء واضحة نتيجة لزيادة البيلروبين في الدم . وكانت ممثلة في ظهور اصفرار واضح علي الأغشية المخاطية وخاصة علي ملتحمة العين . وقد أوضحت الصورة الدموية عن حدوث إنخفاض معنوي في عدد كرات الدم الحمراء والهيموجلوبين والخلايا المصتة بعد العملية بالمقارنة بقبل العملية . وأوضحت التجربة حدوث ارتفاع معنوي في عدد كرات الدم البيضاء بعد العملية . وبالنسبة للتحليل البيوكيميائية فقد أوضحت الدراسة عن حدوث ارتفاع معنوي في معدل الكلورستيرول والبيلروبين (الكلي والمتحد) عن معدلهم الطبيعي . أما بالنسبة للبروتين الكلي فقد حدث إنخفاض معنوي يتناسب مع طول فترة التجربة . كما أوضحت الدراسة أيضا ارتفاعا معنويا لمعدل أنزيمي الترانس امينيز بعد العملية بمقارنتها بقبل العملية . وأما بالنسبة لعدلات الإليكتروليتات في مصل الدم وكذلك الجلوكوز فقد كانت الفروق غير معنوية .

SUMMARY

Ten clinically healthy dogs their ages varied from 3-5 years constituted the material of this investigation. All animals were put under clinical observation pre and post-operation. Under the effect of general anaesthesia the common bile duct was ligated using catgut. Haematological, clinical, biochemical changes, post-mortem and histopathological examination were carried out following the obstruction at interval time up to three weeks.

Icterus was evident due to hyper haemobilirubinaemia and clinically manifested by yellowish discoloration of visible mucous membranes especially conjunctival mucosa.

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Haemogram picture revealed a significant ($P/0.05$) decrease in total erythrocytic count, hemoglobin concentration and packed cell volume, while total leucocytic count revealed a significant elevation post operation.

Biochemical finding of blood sera declared an increase in blood serum cholesterol, total and conjugated bilirubin than normal. There are also, a decrease in total protein value which varied with the duration of experiment. A highly significant ($P/0.01$) elevation in both Alanine aminotrans-ferase (GPT) and Aspartate aminotransferase (GOT) post-ligation when compared with pre-operation than normal by three time were evident.

INTRODUCTION

In dog, the gall bladder is located on the right side of the visceral surface of the liver at approximately the eighth to the tenth intercostal spaces. Regions of the gall bladder include the neck, body, and fundus with the neck leading directly into the cystic duct. The cystic duct joins with the four hepatic ducts to form the common bile duct.

This duct enters the duodenum approximately 4 cm caudal to the pylorus (NICHEL, et al. 1973; STAFUSS, 1976 and SCHMID, 1978).

Extrahepatic cholestasis is encountered with stenosis or with obstruction of the right or left hepatic ducts or common bile duct. Possible causes included gallstones, cholangitis, neoplasia of the portal hepatic lymph nodes, necrotizing inflammation, and cancer at the head of the pancreas. In all forms of extrahepatic cholestasis there is a massive increase in the level of direct reading (conjugated) bilirubin. (SUTER and OLSSON, 1970; SCHALL, 1973; MITCHELL and JOLLAWS, 1975 and SCHMID, 1978).

Parasitic cholangitis and cholecystitis occur due to fascioliasis and infestation with *dicrocoelium dentriculum* (BLOOD, et al. 1983). Complete bile duct ligation induced significant liver dysfunction with marked increase in serum bilirubin (SHERLOCK, 1968). Many authors encountered the clinical signs of obstructive jaundice in animals and man. Most of them have constantly shown an increase in serum and urine bilirubin (CHRISTOPH, 1975 and AREVSON, et al. 1978 in dog, MADANI and ADAMS, 1976 in donkey).

Hyperbilirubinaemia cannot be readily differentiated from regurgitation of bilirubin in case of intra hepatic obstruction or from its retention with reduced hepatic uptake (DUNCAN and PRASSE, 1986).

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The objective of this work was planned to investigate the influence of bile duct obstruction upon changes occurred in clinical signs, pathological lesions and the changes in the blood constituents and their use as an aid for accurate diagnosis.

MATERIAL and METHODS

The study was conducted on 10 dogs were proved to be clinically healthy by both clinical and laboratory methods of examinations with different ages, sex and body weight. The animals were put under clinical observation before and after operations.

Surgical technique:

The animals were fasted 24 hours before surgical interference. The dogs were injected intramuscularly with chlorpromazin hydrochloride (Neurazine) in a dose of 1 mg/kg bwt. General anaesthesia was induced intravenously by injection of pentothal sodium until complete main reflexes disappear. The animals were placed in dorsal recumbency with the front part of the body raised to produce a caudal retraction of the intestine and thus facilitate an abdominal approach to the common bile duct. Laboratory mid-line incision was performed for about 10 cm starting from xyphoid cartilage. The common bile duct was exposed and ligated. The abdominal wound was closed in the usual manner. The skin sutures were removed after 7 days from operation.

Haemogram picture and biochemical determination:

Blood samples were collected pre and post-operation by 15 and 21 days. Anticoagulated blood were used for haemogram picture including total erythrocytic and leucocytic count and hemoglobin concentration using automatic cell counter while packed cell volume was determined according to the standard method of hematology as described by COLES (1986).

The clear non haemolysed sera were analyzed biochemically using testkits supplied from Biomerieux (Bains/France) for the determination:

- 1 - Total protein (gm%) after the methods described by WEICHSELBAUM (1946).
- 2 - Blood serum albumin (gm%) after the method of DRUP (1974). Blood serum globulin and albumin/globulin ratio were determined mathematically.
- 3 - Blood serum alanine Aminotransferase and Aspartate amino-transferase (μ /ml) after the method of REITMAN and FRANKEL (1957).
- 4 - Blood serum total bilirubin and conjugated (mg%) after the methods of JENDRASSIK and GROF (1938).
- 5 - Blood serum glucose (mg%) after the method of HULTMAN (1959).
- 6 - Blood serum cholesterol after the method of ZONDER (1977).
- 7 - Blood serum sodium and potassium level were determined by flame-photometer (Corning 400) while blood serum chloride level was estimated using chloride analyzer (model 925).

Histopathological examinations:

Specimens from the liver were taken 15, and 21 days post-operation after scarification of animals. The materials were fixed in 10% neutral buffered formalin and processed by conventional techniques, sections were cut at 5 micron thick, stained with haematoxylin and eosin (H & E).

Statistical analysis of data were performed according to the methods of KALTON (1967).

RESULTS

Clinical findings:

All dogs remained alert, active and without physical signs of illness during 5-days post legation, and after 7-days post-operation appeared as reduced appetite, emaciation, icterus and yellowish staining of the conjunctival mucous membrane. Hepatic palpation revealed pain, and faeces have clay coloration.

Biochemical analysis:

Mean values of haemogram picture and biochemical parameters were illustrated in Table (1 & 2) and Fig. (1 & 2).

Pathological findings:

On necropsy, the gross lesions were restricted to the serous and mucous membrane, and the liver. The membranes were distinctly stained with the yellow colour of bilirubin especially at the late stage. 15 days post-operation the liver was markedly congested, the gall bladder was greatly distended with yellow viscid bile. 21 days post-operation the liver showed pale yellowish descolouration and on cutting the tecture was friable.

On microscopic examination, 15 days post-operation, the vasculature were congested, the bile canaliculi showed bile casts and the hepatocytes revealed slight degenerative changes reached to fatty change in two cases (Fig. 3). 21 days post-operation the dystrophic changes in the hepatic parenchyma reached to diffuse necrobiotic changes associated with moderate activation of the reticuloendothelial cells, bile thromboi was seen within the dilated bile canaliculi (Fig. 4).

DISCUSSION

The gall bladder functions as a reservoir for the bile excreted by the liver, probably concentrate the bile and regulates its discharge through the common bile duct into

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the duodenum. (SCHMID, 1978 and STAFUSS, 1976). It was important to emphasize that the similar clinical signs of jaundice which appeared on the animals following common bile duct ligation, have extensively been recorded by many authors (SCHIFF, 1969; CHRISTOPH, 1975 and BLOOD, et al. 1983). Such an observation may indicate that signs can appear in natural affection after seven days post bile obstruction.

Icterus was also evident due to hyper bilirubinaemia either total or conjugated and clinically manifested by yellowish coloration of visible mucous membrane especially conjunctival mucosa.

Haemogram picture revealed a significant ($P/0.05$) decrease in TRBCs., Hb concentration and PCV value post operation if compared with pre-operation values. Meanwhile a slight significant elevation in leucocytic count post-operation was evident. The obtained values for haemogram picture coincided within the normal values which previously recorded in dogs by COLES (1986); DUNCAN and PRASSE (1986) and SCHALM (1986).

Blood serum electrolytes showed a non significant variations between pre and post operative values and the recorded values are with the normal level in dogs as described by COLES (1986).

Blood serum glucose levels showed a non significant variations. The obtained values coincided with the normal recorded levels of dogs COLES (1986).

Total protein values showed a marked decrease and their fractionation either in albumin or globulin values. Decreased values were accounted by increasing time (post-operation). This decrease can be attributed to the disturbance in the metabolism of the animals. The obtained data coincided with that previously obtained by SHERLOCK (1968).

Blood serum transaminasis either (GPT) and (GOT) showed highly elevation in their values during obstruction if compared with preoperation. This elevation can be attributed on the basis that obstruction leads to hepatic cell necrosis consequently leads to elevation in transaminasis level. The obtained data coincided with those previously obtained by COLES (1986) and DUNCAN and PRESSE (1986). Hepato cellular permeability alteration was evident by high activity of the leakage enzyme (GPT).

Blood serum bilirubin and cholesterol level showed a highly significant elevation than normal values and this can be attributed to their accumulation in the blood as a result of obstruction. Jaundice appear always due to increasing of blood serum bilirubin either conjugated or total by 2 mg%. This observation was clearly evident in our investigation and coincided with those previously obtained by SCHIFF (1969); SHERLOCK (1968) and BLOOD, et al. (1983).

The histopathological examination of the liver revealed the picture of obstructive jaundice which similarly described by CHORS (1962) and JUBB, et al. (1985).

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Finally, it can be concluded that blood serum transaminases, serum bilirubin and cholesterol levels can be used as confirmatory biochemical tests used for diagnosis of bile duct obstruction.

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Table (1): Mean values of biochemical parameters in examined dogs.

Parameters	units	Before operation		
		Before operation	15 days	21 days
Sodium	mmol/L	135.50 ± 1.50	134.40 ± 3.30	126.00 ± 4.60
Potassium	mmol/L	4.90 ± 0.90	4.30 ± 0.60	5.90 ± 1.20
Chloride	mmol/L	120.00 ± 3.50	125.00 ± 4.40	104.00 ± 5.60
Glucose	mg/dl	88.00 ± 3.40	92.30 ± 4.50	99.10 ± 4.40
Cholesterol	mg/dl	189.50 ± 10.4	275.30 ± 16.3 **	335.50 ± 14.5 **
Total protein	gm/dl	7.50 ± 0.90	6.90 ± 0.75	5.50 ± 1.20
Albumin	gm/dl	4.80 ± 1.10	3.50 ± 0.90	2.50 ± 0.80
Globulin	gm/dl	3.70 ± 0.50	3.40 ± 1.20	3.00 ± 1.50
GPT	mu/ml	65.00 ± 3.10	132.00 ± 4.50 **	198.00 ± 5.90 **
GOT	mu/ml	35.00 ± 4.10	80.00 ± 5.50 **	120.00 ± 6.30 **
Total bilirubin	mg/dl	0.55 ± 0.01	3.60 ± 0.90 **	3.90 ± 1.10 **
Cong. bilirubin	mg/dl	0.09 ± 0.01	2.10 ± 0.90 **	2.50 ± 1.20 **

** = Highly significant (p<0.01).

* = Significant (p<0.05).

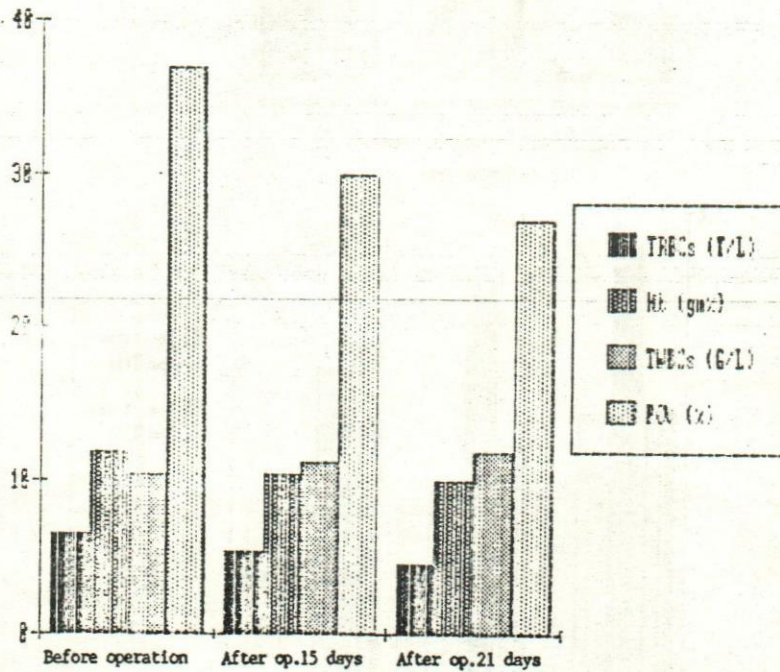
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Table (2): Haemogram picture in examined dogs.

Parameters	units	Before operation	After operation	
			15 days	21 days
TRBCs	T/L	6.60 ± 0.50	5.40 ± 0.40 **	4.60 ± 0.90 **
Hb	gm%	11.90 ± 0.80	10.50 ± 0.60	10.00 ± 0.50 **
TWBCs	G/L	10.50 ± 1.50	11.30 ± 1.10	11.90 ± 0.40
PCV	%	37.00 ± 2.50	30.00 ± 1.50 **	27.00 ± 1.50 **

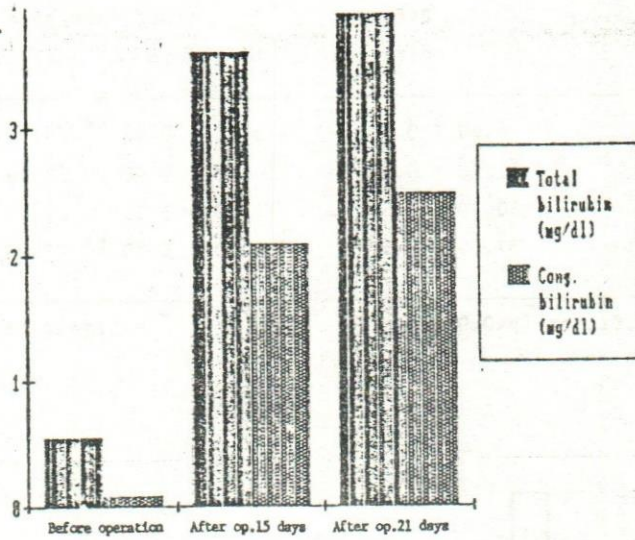
** = Highly significant (p<0.01).

* = Significant (p<0.05).

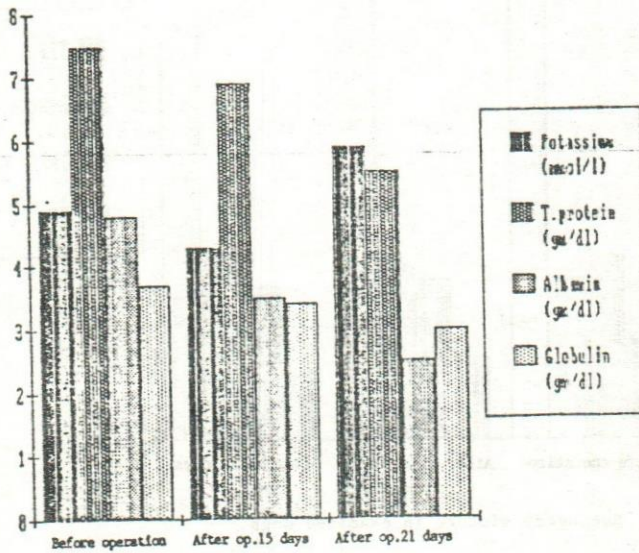


Haemogram picture in examined dogs.

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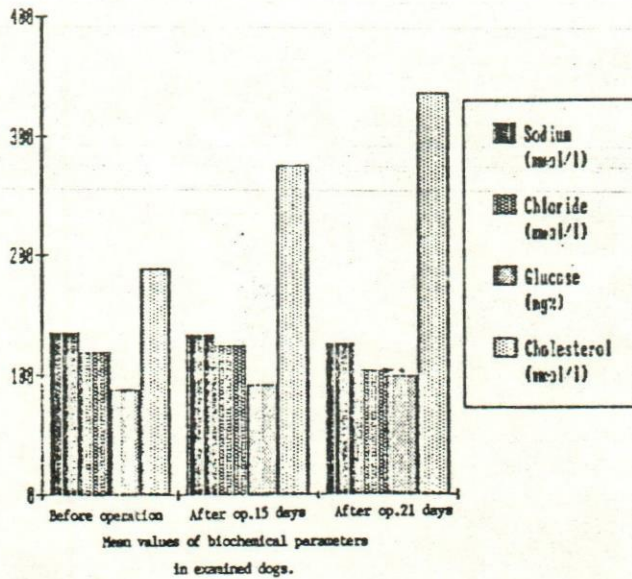
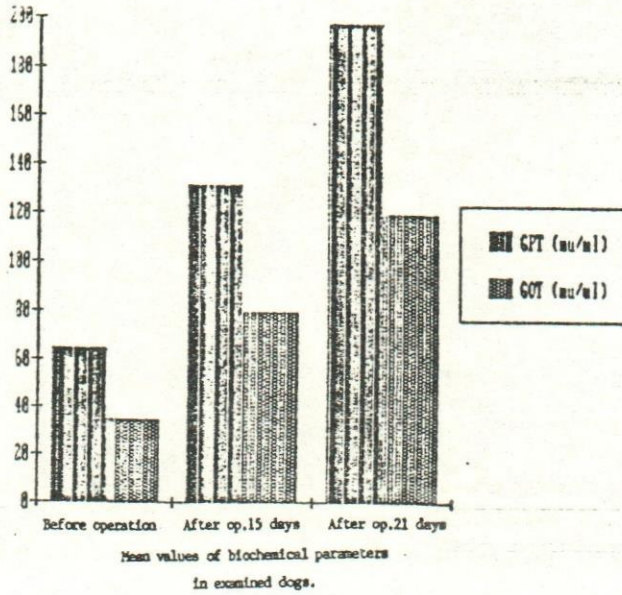


Mean values of biochemical parameters
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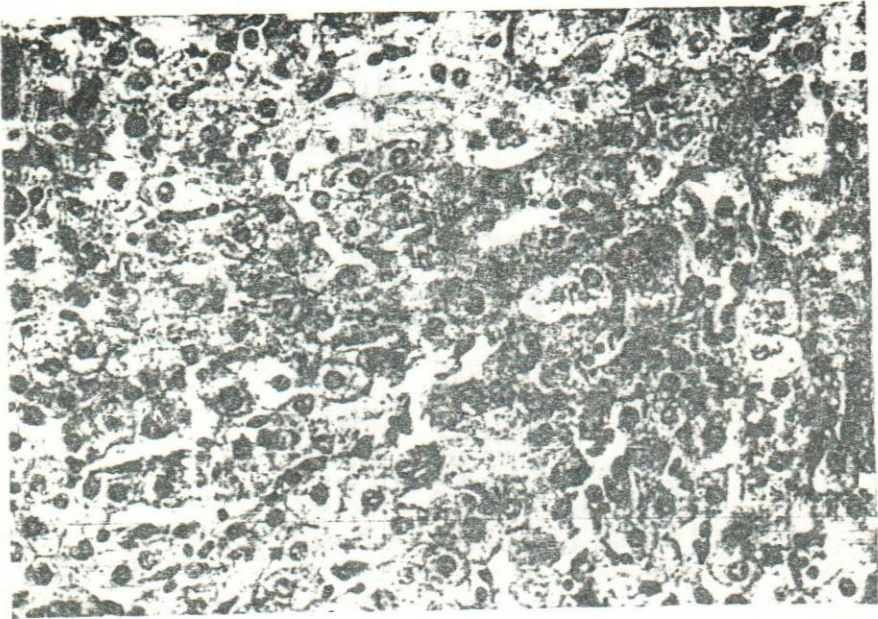


Fig. (3): Liver showing slight degenerative changes (H&E X 400).

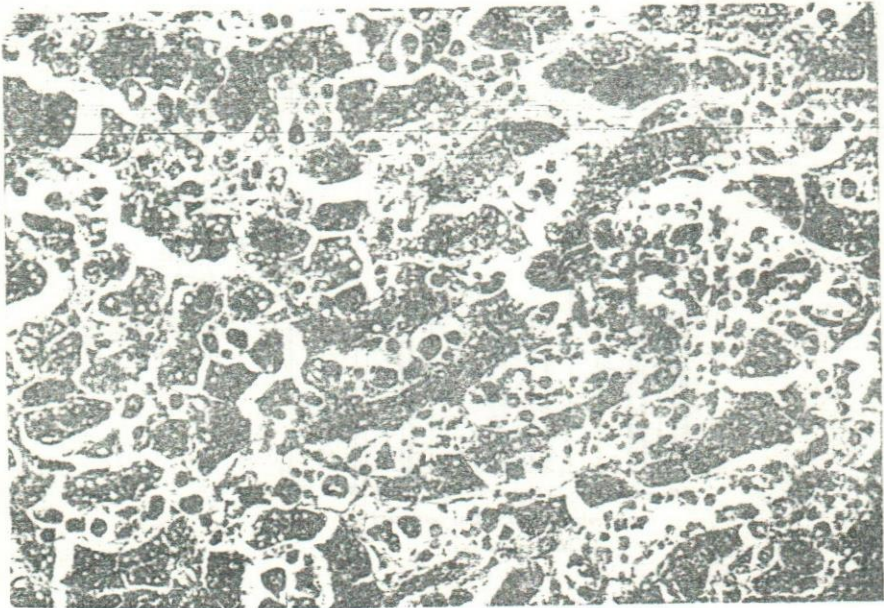


Fig. (4): Liver showing degenerative change and bile thrombosis (H&E X 400).