# Histological study of the liver and biochemistry of the blood of Wistar rats following ligature of right hepatic duct[1]

## Estudo histológico do fígado e bioquímico do sangue de ratos após ligadura do ducto hepático direito

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**ABSTRACT**

**Purpose**: To observe the histological alterations in the liver and biochemistry in the blood that can happen in Wistar rat, after the ligature of right hepatic duct. **Methods**: In this study were used rats (n=46) of Wistar pedigree. The animal groups (n=46) were distributed in 6 experimented sub-groups (n=6). It was held a ligature surgery of the right hepatic duct and euthanasia in 7, 14, 21, 28, 60 and 90 days and the biochemistry control group (n=10), that animals had 2ml of their blood taken by cardiac puncture for biochemistry study with value analyses of bilirubins, transaminasis, lactic desidrogenasis, alkaline phophatase and gamma-glutamil-transferase. Given the expected time of each group, the animals were submitted to anesthesia procedure and cavity re-opening, being held intra-cardiac puncture and with 2ml blood collected for biochemistry analyses. It was proceeded the liver resection, being the liver putted in formol solution to 10% for a period of 24 hours and taken to the histology. **Results**: It was not possible to identify results that express significant differences as the existence of alterations histological and biochemistrily between the different groups. **Conclusion**: At the end of the study, it was not possible to identify histological and biochemistrily alterations that express significant differences between livers of the animals from the right linked hepatic duct and the animals of the control group.

**Key words**: Liver duct. Liver. Histology. Biochemistry. Rat.

**RESUMO**

**Introdução**: a colestase é uma situação grave e geralmente letal. Habitualmente a obstrução do fluxo da secreção biliar ocorre por lesão iatrogênica. Cerca de 80% das lesões das vias **Objetivo**: Observar as alterações histológicas que possam ocorrer no fígado e bioquímicas no sangue de ratos Wistar, após ligadura do ducto hepático direito. **Métodos**: Neste estudo foram utilizados ratos (n=46) da linhagem Wistar. O grupo de animais (n=46) foi dividido em 2 grupos: A experimento (n=36) e B controle bioquímico (n=10), sendo o grupo A subdividido em 6 subgrupos experimento (n=6). Foi realizada cirurgia para a ligadura do ducto hepático direito e eutanásia em 7, 14, 21, 28, 60 e 90 dias. No grupo B controle bioquímico (n=10), os animais tiveram 2ml de seu sangue retirado por punção cardíaca para estudo bioquímico com análise dos valores de bilirrubinas, transaminases, desidrogenase láctica, fosfatase alcalina e gama-glutamil-transferase. Dado o prazo de espera de cada grupo, os animais foram submetidos a procedimento anestésico e reabertura da cavidade, sendo realizada punção intracardíaca, com coleta de 2ml de sangue para análise bioquímica. Foi realizada a retirada do fígado, sendo o fígado fixado em solução de formol a 10 % por um período de 24 horas e encaminhado ao laboratório de histologia. **Resultados**: Não foram encontrados resultados estatisticamente significantes quanto a existência de alterações histológicas e bioquímicas entre os diversos grupos. **Conclusão**: Ao final do estudo não se conseguiu identificar histológica e bioquimicamente, alterações que expressassem diferenças significativas entre os animais do grupo com o ducto hepático direito ligado e os animais do grupo controle.

**Descritores**: Ducto hepático. Fígado. Histologia. Bioquímica. Rato.

## Introduction

Suppression of the bile flow (cholestasis) is a severe and often lethal condition.[1,2,3,4,5,6] Usually the obstruction of biliary secretion is the result of iatrogenic injury.[7] Most of the injuries to the extrahepatic bile duct system stem from surgical procedures on the gallbladder or on the choledochus. About 80% of the injuries to extrahepatic bile duct are diagnosed during and after a cholecystectomy, the most performed surgery on the planet for the treatment of gallbladder calculosis. In approximately 15% of the cases, injuries are identified and treated during the surgery. The remaining 85% become noticeable either through obstructive jaundice or by a profuse and persistent release of bile through the drainage tube — which characterizes a biliary fistula. Generally, jaundice becomes noticeable in two to three days, but in some cases this sign may present itself only a few weeks after the operation.[7] In a study carried out at the Lahey Clinic, 501 patients with biliary tract stenosis following surgical treatment were investigated. It was found that 70% of the patients had been subjected to cholecystectomy with no noticeable pre-op intercurrence. For the following 30% of the patients the following factors were associated with their condition: Operating on contracted gallbladders (scleroatrophic); close association between the gallbladder infundibulum and the common hepatic duct; massive hemorrhage during surgery with a blind attempt to perform the ligature leading to duct trauma or occlusion; ligature of the cystic duct under tension leading to angulation of hepatic duct and consequent double ligature ( cystic and common hepatic).[7] In cholestasis, bilirubins reach their highest levels, contrasting with a mild elevation of the transaminases. Alkaline phosphatase (AF) and gamma-glutamyl-transferase (GGT) also increase considerably under this condition.[8] Cholestasis and regurgitation of biliary pigments into the circulatory system are among the signs and symptoms of biliary obstruction. The mechanism that intervenes secondarily to the obstruction modifies the permeability of biliary canaliculus and exerts a continuous flow of pigment allowing its detection in the plasma.[6,9,10] In many cases, alterations following different episodes of cholestasis by obstruction of main biliary duct are noticed. Functional impairment may or may not be present, and these alterations are perceived by the proportional volume of hepatocytes and on the epithelium of the biliary duct. In a study with 80 Wistar rats, Marinelli, Sanchez, Izquierdo, Burgos, Arce, Del Castilho-Olivares (1987), demonstrated that 30 days after common hepatic duct ligature, significant histological alterations could be seen on the hepatic lobes. In a biochemical assessment, they noticed an increase in bilirubins, alkaline phosphatase, and transaminases and a decrease in the levels of seric albumin.[11] In a histological study with young Wistar rats undergoing common hepatic duct ligature, Medeiros, Freitas and Andrade (1988), confirmed the alterations found by Marinelli et al and suggest that age may be involved in the pathogenesis of injuries resulting from biliary obstruction.[12] Silva Junior, Coelho, Souza, Picinato, Franco, Vanni, Ceneviva (1993), testing the seric levels sensibility of alkaline phosphatase (AF), bilirubins, and gamma-glutamyl-transferase (GGT) as indicators of extrahepatic cholestasis in rats with common hepatic duct ligature, demonstrated that alkaline phosphatase is the ideal marker for induced extrahepatic cholestasis in rats.[13,14] The purpose of the present work is to observe histological alterations that may occur in the liver and biochemical alterations in the blood of Wistar rats following ligature of right hepatic duct.

## Methods

### *Sample*

Male albino rats (Rattus norvegicus albinus) (n=46) of the Wistar strain were used in this experiment. The animals, with weights between 250 and 350g and of the same age (180), were kept in individual plastic cages until the time determined for surgical procedures and euthanasia. Animals were kept under natural light conditions, respecting day and night light cycles, at appropriate temperatures, noise and humidity conditions, receiving proper food with free access to food and water throughout the experiment. Animals were numbered, by simple drawing, and weighed before the first surgical procedures. The animals (n=46) were distributed in two groups. Group A experiment (n=36) and Group B biochemical control (n=10). Group A was further divided into 6 experimental subgroups (n=6). Each is described below:

**Group A** – (n=36) Animals in this group were subjected to surgery with right hepatic duct ligature and euthanasia at the end of the waiting time of each sub-group with the later histological assessment of the liver and biochemical assessment of the blood.

Subgroup A1 - (n=6) In this subgroup, animals were subjected to surgery with the ligature of the right hepatic duct and euthanasia after 7 days for the histological assessment of the liver and biochemical assessment of the blood.

Subgroup A2 - (n=6) In this subgroup, animals were subjected to surgery with the ligature of the right hepatic duct and euthanasia after 14 days for the histological assessment of the liver and biochemical assessment of the blood.

Subgroup A3 - (n=6) In this subgroup, animals were subjected to surgery with the ligature of the right hepatic duct and euthanasia after 21 days for the histological assessment of the liver and biochemical assessment of the blood.

Subgroup A4 - (n=6) In this subgroup, animals were subjected to surgery with the ligature of the right hepatic duct and euthanasia after 28 days for the histological assessment of the liver and biochemical assessment of the blood.

Subgroup A5 - (n=6) In this subgroup, animals were subjected to surgery with the ligature of the right hepatic duct and euthanasia after 60 days for the histological assessment of the liver and biochemical assessment of the blood.

Subgroup A6 - (n=6) In this subgroup, animals were subjected to surgery with the ligature of the right hepatic

duct and euthanasia after 90 days for the histological assessment of the liver and biochemical assessment of the blood.

**Grupo B** - (n=10) In this subgroup, animals underwent anesthesia and had 2ml of blood drawn by cardiac puncture for biochemical analysis of levels of bilirubins (total, direct and indirect), transaminases (TGO and TGP), lactic dehydrogenase (LDH), alkaline phosphatase (AP), and gama-glutamyl-transpherase (GGT).

*Procedures*

Before surgery, animals underwent general anesthesia induced by inhalation of ethylic ether and maintained with a solution of Ketamine hydrochloride (Ketalarâ) and hydrochloride 2-(2.6 **xylidine**)-5.6-dyhidro-4H-1.3-thiazine (Rompumâ), in the doses of 100mg/kg and 10 mg/kg, respectively, with intramuscular application on the inner side of the left thigh.[15,16,17] After reaching plane of anesthesia, with no response to pain stimulus applied on the adipose pad of the animal's paws and absence of corneal reflex, animals were weighed and positioned, horizontally, in dorsal decubitus with all paws held by sticky tape. Next, rats were shaved (by hair plucking) on the anterior wall of the abdomen and antisepsis was carried out with a solution of alcohol and 2% iodine (Figure 1).

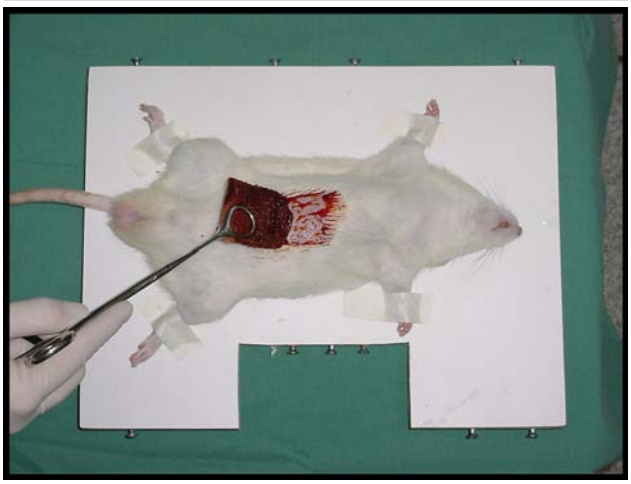

**FIGURE 1** - Picture of rat after antisepsis procedure and delimitation of surgical field.

With a scalpel, an approximately 4cm median incision was performed. Using a Metzembaum scissors, laparotomy was completed with the opening of the linea alba and peritoneum. Next an Adson utostatic retractor was placed to expose peritoneal cavity; an inventory of the cavity was carried out by observing all abdominal viscera (Figure 2). A dissection of the right hepatic duct was performed with the help of a stereoscopic microscope (DF Vasconcelos®) with wide-angle lens and 12,5X magnification. Next, the small intestine and the colon were eviscerated and wrapped in gauze dampened in a saline solution (Figure 3). Exposition of the biliary tract was achieved with the help of small flexible cotton buds (Cotonetes®) dampened in sodium chloride 0.9% (saline).

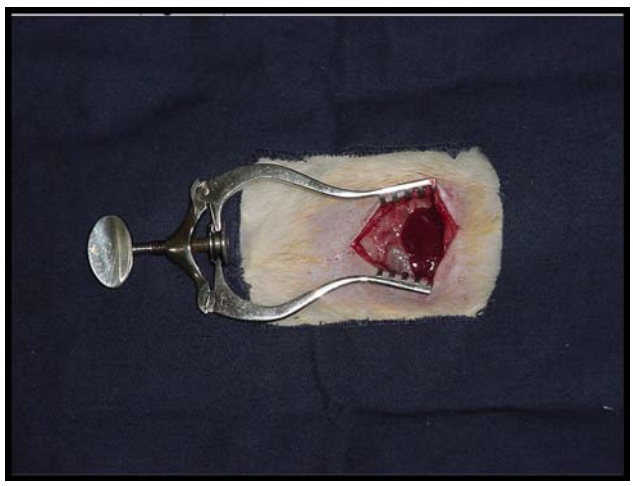

**FIGURE 2 -** Picture of the open cavity

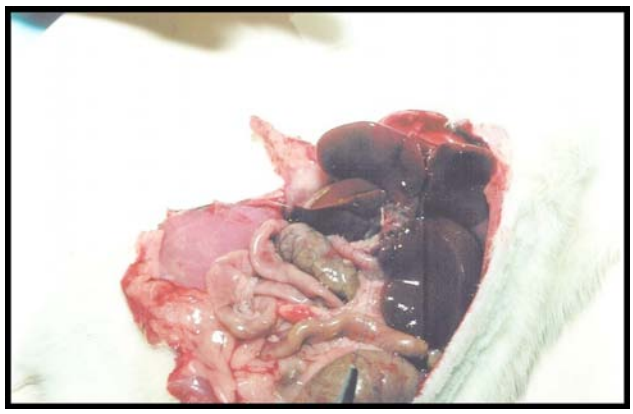

**FIGURE 3** - Picture of animal in the experiment group with exposed viscera during the procedure.

Biliary tract was identified between pancreatic tissue and the hepatic hilum; right hepatic duct was identified and double ligated with polypropylene suture (Prolene®, Ethicon) 7-0, at 1cm of its exposition, outside the hepatic parenchyma, and sectioned between ligatures. The animal's viscera were returned to the cavity. The closure of the recto-abdominal sheath was performed with 4-0 absorbing thread (Catgut®, Ethicon) and continuous anchored suture. The skin and the subcutaneous mesh were sutured with nylon monofilament thread (Mononylon®, Ethicon) 4-0, with one single plane and continuous anchored suture. After each groups' respective waiting time, animals underwent anesthesia and re-opening of the cavity through thoraco-phreno-laparotomy for cardiac puncture and liver removal (Figure 4). Initially an intracardiac puncture was performed for the collection of 2ml of blood with a 3ml syringe (Plascalp®) and insulin needle (Rymco®). Blood was stored in a specific vial for blood collection and sent immediately for biochemical analyses in the Clinical Analysis Lab at the University Hospital (UH) of the Federal University of Santa Catarina (Figure 5). Next the whole liver was removed, including the right hepatic duct. Liver was removed by section with scissors—cutting of ligaments, at splenic and hepatic angles. All other remaining adhesions and/or bands were removed in blocs, along with the hepatic ducts and diaphragm muscle (Figure 6).

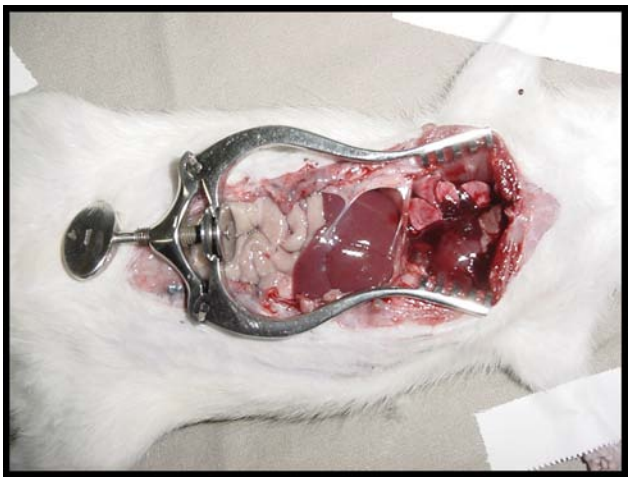

**FIGURE 4** - Picture of an animal in the experimental group after relaparotomy (Thoraco-phreno-laparotomy).

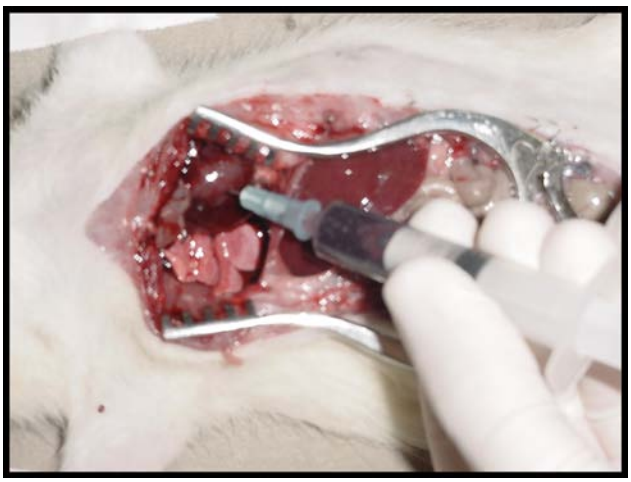

**FIGURE 5 -** Intracardiac puncture on anesthetized animal.

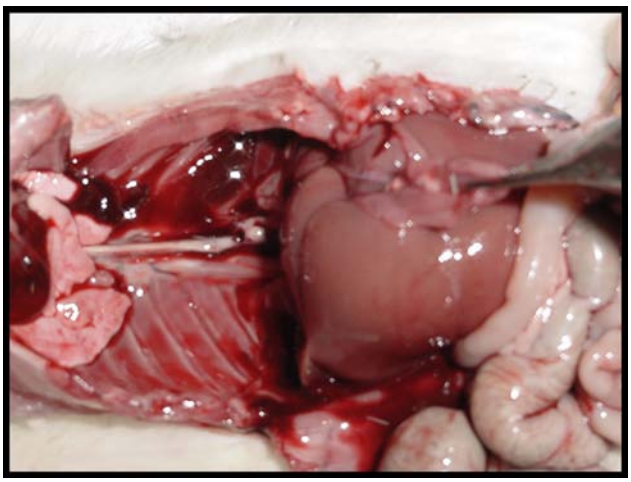

**FIGURE 6 -** Picture of hepatectomy on animal in the experiment group.

Later, with the animal under anesthesia, euthanasia by exsanguination was performed. The carcass was place inside a specific plastic bag and properly disposed of in the experimental surgery hospital garbage. Liver, after being removed, had its lobes recognized in its right, left, causal and median divisions. The left and right lobes were separated and sectioned transversally in its biggest diameter with the help of a 23 steel blade, and remained in 10% formaldehyde solution for 24 hours (at 12 hours interval the whole liquid would be replaced by a volume 10x bigger than the volume of the specimen (Figure 7).

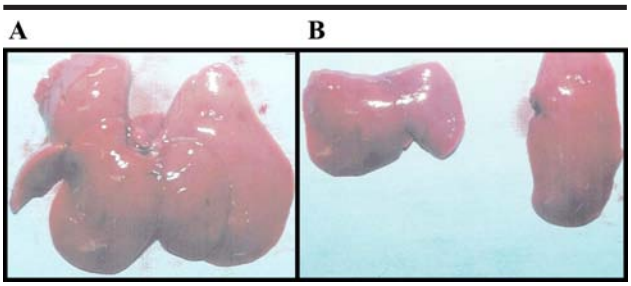


**FIGURE 7** - A - Picture of removed liver. B - Picture of the right lobe transversally sectioned.

After fixation, the specimens were rinsed with water and immersed in a 70% alcohol solution. Specimens were then code-numbered and sent to the histology lab to undergo routine histological procedure and obtain 7 to 10 mm semi-serial paraffin-embedded, hematoxylin and eosin (H.E) stained cuts. Ten histologic cuts were obtained for each of the hepatic lobes; slides were assessed by two trained observers who did not know the specimen's codification, which prevented the identification of which group of animals the analyzed cuts belonged to. The following criteria were taken into consideration in the analysis:

a) Presence or absence of morphologic alterations in the hepatic lobes.

b) Mean size of 2 hepatic lobes in each cut of the slice, measured with an optic ruler previously calibrated according to the method proposed by Mandarim de Lacerda for linear measurements[18,19,20].

c) Presence or absence of granulation tissue and/or an increase in the number of polymorphonuclears and/or mononuclears in the examined cuts.

d) Histological alteration of the connective tissue present in the portal-space and in the centrilobular area of each analyzed lobe.

For the biochemical study, the following analyses were carried out: Alkaline phosphatase (AF), gamma-glutamyl-transferase (GGT), total bilirubin (TB), direct bilirubin (DB), indirect bilirubin (IB), transaminases, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic dehydrogenase (LDH). The reagent kit used was the *Dimension® clinical chemistry system*, existing in the clinical lab of the UFSC. In the histologic study, right and left lobes of a same liver were analyzed — the left serving as control for the right one — through 10 semiserial cuts apiece, 7 to 10 ìm in thickness and 200 mm clearance between cuts, covering an area of 2cm of the analyzed hepatic lobe. The analysis of the slides was carried out on a conventional optic microscope using 10X and 40X magnification. The following parameters were assessed in this analysis:

1 - With a 10X magnification lens:

**a -** Presence or absence of fibrosis on the hepatic lobe, lymphocytic infiltration in any area of the cut lobe and histologic alterations around biliary ducts and portal-spaces.

**b** - Size, in micrometer, of at least two hepatic lobes transversally cut within each histologic cut, using an optic ruler previously calibrated according to the method proposed by Mandarim Lacerda[20] for linear measurements.

2 - With a 40X magnification lens the following procedures were carried out:

Focusing on one hepatic lobe, transversally sectioned within the histologic cut, a wide-angle lens (with 100 divisions previously calibrated in accordance to the method employed by Tramonte, Carvalho, Ortellado, Serafim, Dambrós, d'Acampora[32]) was placed on the border of the connective tissue existing in one of the portal-spaces of the said lobe, delimiting a counting area towards the central vein of the chosen lobe. Next, a counting was carried out for the number of polymorphonuclears, mononuclears, or macrophagic cells existing within the area delimited by the wide-angle. After the histologic analyses, the codes for each slide were revealed and tables were put together following the protocol for histological analysis. (attach 7) After these procedures, microphotographies (using Nikon- Labophot 2) were taken to illustrate each of the found alterations, allowing the documentation of the observed histologic alterations in the groups of analyzed animals. The results thus obtained were subjected to statistic analyses to assess quantitatively the possible morphological differences observed among the groups of animals analyzed in this study. All biochemical and histological results were tabulated on MS Excel software and statistically analyzed using the SPSS 8.0 program. Since biochemical measurements did not present a normal distribution, non-parametric tests were applied. Mann-Whitney U Test[33,34], which compares measurements of two independent samples, was applied in the comparison between of each of the groups in the experiment and the control group and the differences were tested in a 95% significance level ($p < 0.05$).

**Results**

The table below indicates the mean values of all sub-groups used in the present study. No significant difference ($p<0,05$) was found, except for the DB values in the A6 sub-group (ligature and euthanasia at 90$^{th}$ day). Values of the control group were adopted as normal. The table below demonstrates that no parenchyma necrosis and no color alteration on the hepatic surface of any liver in the experiment was found. A single nodule in an animal of sub-group A4 (ligature and euthanasia at 28$^{th}$ day) was found. The table below indicates the presence of hilum adhesions in all operated animals and hilum fibrosis in 9 cases, three of which in sub-group A5 (ligature and euthanasia at 60$^{th}$ day). The table below indicates that it was not possible to observe any significant histological alteration in the size of the hepatic lobes analyzed through H.E. stained cuts. The sequence of microphotography below indicates that it was not possible to find any histological alteration worth of closer observation among the several groups of analyzed animals. The first sequence shows 10x magnified, H.E. stained slides of 4 groups (Figure 8). The second sequence shows 40x magnified, H.E. stained slides of 4 groups (Figure 9).

**TABLE 1** - Rats' blood biochemical findings – mean of biochemical measurements of the hepatic profile of each subgroup.

| GROUPS | TGO | TGP | GGT | AF | TB | DB | IB | LDH |
|---|---|---|---|---|---|---|---|---|
| A1 | 137.33 | 94.66 | 3.16 | 130.66 | 0.161 | 0.063 | 0.098 | 853.8 |
| A2 | 201.50 | 119.33 | 4.66 | 159.00 | 0.201 | 0.080 | 0.121 | 973.1 |
| A3 | 115.50 | 108.50 | 4.00 | 133.00 | 0.113 | 0.071 | 0.041 | 882.1 |
| A4 | 127.66 | 110.16 | 4.00 | 122.16 | 0.081 | 0.035 | 0.046 | 815.8 |
| A5 | 193.33 | 86.83 | 2.50 | 130.50 | 0.158 | 0.063 | 0.081 | 971.83 |
| A6 | 115.00 | 114.66 | 3.16 | 95.66 | 0.165 | 0.083* | 0.081 | 849.8 |
| CONTROL | 130.90 | 105.20 | 4.00 | 127.00 | 0.148 | 0.052 | 0.096 | 714.9 |

** Observed value was p= 0.02.*

**TABLE 2** - Microscopic findings on hepatic surface – presence of necrosis, color alteration and nodules on the hepatic surface.

| SUBGROUP | NECROSIS | COLOR ALTERATION | NODULES | # OF ANIMALS |
|---|---|---|---|---|
| A1 | 0.00 | 0.00 | 0.00 | 6 |
| A2 | 0.00 | 0.00 | 0.00 | 6 |
| A3 | 0.00 | 0.00 | 0.00 | 6 |
| A4 | 0.00 | 0.00 | (1) 16.66% | 6 |
| A5 | 0.00 | 0.00 | 0.00 | 6 |
| A6 | 0,00 | 0.00 | 0.00 | 6 |
| TOTAL | 0.00 | 0.00 % | 2.27% | 36 |

**TABLE 3** - Microscopic finding on the hepatic hilum – presence of adhesions and fibrosis

| SUBGRUPO | ADHESIONS | FIBROSIS | # OF ANIMALS |
|---|---|---|---|
| A1 | (6) 100.00 % | (0) 0.00% | 6 |
| A2 | (6) 100.00 % | (0) 0.00% | 6 |
| A3 | (6) 100.00 % | (0) 0.00% | 6 |
| A4 | (6) 100.00 % | (0) 0.00% | 6 |
| A5 | (6) 100.00 % | (3) 50.00% | 6 |
| A6 | (6) 100.00 % | (6) 100.00% | 6 |
| TOTAL | (36) 100.00 % | (9) 25.00% | 36 |

**TABLE 4** - Histologic findings on rats's livers – mean size of the hepatic lobes in each sub-group

| SUBGRUPO | RIGHT LOBE (in micrometers) | LEFT LOBE (in micrometers) | # OF ANIMALS |
|---|---|---|---|
| A1 | 53.08 | 50.58 | 6 |
| A2 | 45.50 | 45.75 | 6 |
| A3 | 47.75 | 47.91 | 6 |
| A4 | 49.16 | 50.83 | 6 |
| A5 | 50.33 | 53.33 | 6 |
| A6 | 47.16 | 48.16 | 6 |

**TABLE 5 -** Number and cause of death of animals in the control group.

| LIKELY CAUSE | # OF ANIMALS | POST-OP DAYS |
|---|---|---|
| Hypothermia | 2 | Immediate |
| Hypovolemic shock | 1 | Immediate |
| Choleperitoneum | 1 | 2 PO |

**TABLE 6 -** Observed sings in experiment animals

| SUBGROUP | JAUNDICE | CHOLURIA | ACHOLIA | TOTAL |
|---|---|---|---|---|
| A1 | 0.00 | 0.00 | 0.00 | 6 |
| A2 | 0.00 | 0.00 | 0.00 | 6 |
| A4 | 0.00 | 0.00 | 0.00 | 6 |
| A5 | 0.00 | 0.00 | 0.00 | 6 |
| A6 | 0.00 | 0.00 | 0.00 | 6 |

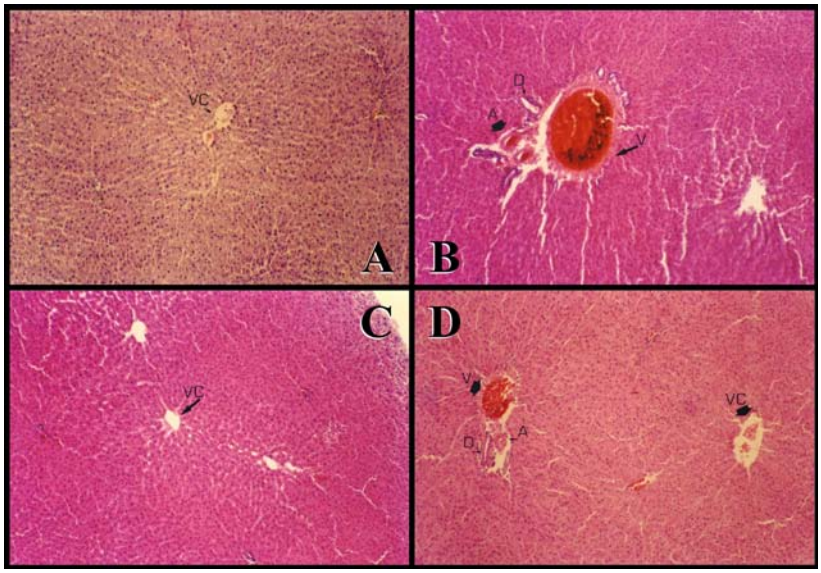


**FIGURE 8** - Right lobe (procedure) microphotography of groups (A) A2 (14 days), (B) A3 (21 days), (C) A4 (28 days) and (D) A5 (60 days). (10X HE). It shows centrilobular vein (VC); portal-space (EP); portal-space vein (V); Biliary Duct (D); Artery (A).

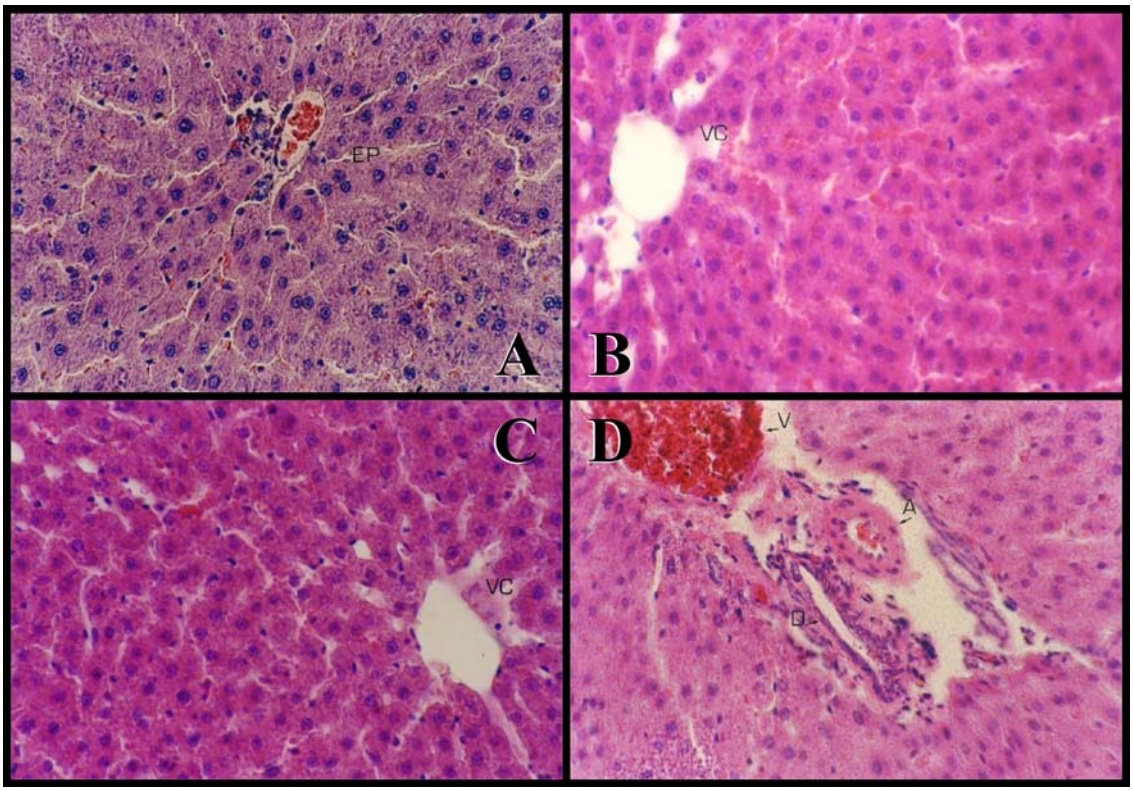


**FIGURE 9** - Right lobe (procedure) microphotography of groups (A) A2 (14 days), (B) A3 (21 days), (C) A4 (28 days) e (D) A5 (60 days). It shows centrilobular vein (VC); portalspace (EP); artery (A); and duct (D). The presence of hepatocytes with one and two nucleus can be observed (40X HE).

**Discussion**

The choice of this particular animal as experimental model was considered because of the following factors: a rat is defined as an animal with standardized characteristics; its small dimension allow an adequate keeping of several animals in a small spaces; lower cost; relative facility of handling; great resistance to infection; short life span; and high scale reproduction. In addition, the existing knowledge on the anatomy, physiology, behavior, and genetics of its several strains, allow the results obtained in experimental research to contribute for a better understanding of medical problems.[15,35,36,37,38] A rat's liver is a dark-brown organ, located below the diaphragm, predominantly on the right side. It comprises 4 lobes: A median lobe, the biggest of all, right lateral lobe, left lateral lobe, and a small caudal lobe.[39] The common hepatic duct is formed by the junction of the hepatic ducts coming from each lobe. It runs towards the duodenum where it opens at the papilla, at approximately 25mm in a caudal direction to the pylorus. At this point, it is surrounded by a muscle structure similar to humans Oddi's sphincter. Its diameter is uniform, with about 0.5 to 1.0mm, and has neither a cystic duct nor a gallbladder.[17,36,37,38,40] As there is no evidence in the literature of a communication between the hepatic lobes of rats, this experiment was developed in an attempt to simulate what happens in humans, albeit accidentally, which can even result in death. During the experiment, 4 deaths occurred, 2 of which immediately after anesthesia and probably due to hypothermia. The 2 other deaths happened in consequence of (1) hypovolemic shock, and (1) choleperitoneum. Waynforth, HB; Flecknell PA[37] report that hypothermia is the major cause of deaths during anesthesia in rats. All anesthetics (including Xylasine + Ketamine[21]) alter the thermo-regulatory center and decrease body temperature in consequence. This fact is more often noticed in small animals; given their big exchange surface in relation to their body mass, implications are more profound[17]. The anesthesia technique used in this experiment proved efficient, despite the two deaths. In some animals, however, at the end of the surgery, it was necessary to apply anesthetic supplementation, which was done by inhalation of a small quantity of ether through a mask. One must be careful with the respiratory depression caused by this anesthetic agent and apply only small doses while observing the animal's respiratory and heart rates. In order to avoid hypothermia, a 40w bulb light was used to keep animals warmed during immediate post-op until full recovery from anesthesia. It is worth stressing that during the surgery, the presence of an assistant to retract the hepatic lobes is fundamental for a perfect exposition of the biliary tract and adequate sectioning. As retractor, cotton buds dampened in a saline solution were used and proved efficient in exposing the lobes without injuring the tissues. Another area of concern was the dissection of the right hepatic lobe, intimately in contact with the portal vein. Serious injury to the latter may be caused by any slight mistake. This

complication, observed in one case, lead to hypovolemic shock with hemoperitoneum and death of the animal due to uncontrollable hemorrhage caused by the iatrogenic rupture of the vein. The right hepatic lobe itself must be handled with extreme care. Being a small, sensible structure, sudden maneuvers must be avoided – as for any other tissue. This was also observed in one animal whose rupture of hepatic duct lead to choleperitoneum and chemical peritonitis and ultimately death at the second day of post-op. These two deaths from surgery complication took place in the immediate postoperative, a period constantly under observation. All these complications took place with the first operated animals and were attributed to the learning curve of the surgical procedure. All animals whose outcome was death underwent necropsy and were replaced. Although jaundice was already expected sometime during the study, no sclerotic color alteration was observed in any animal throughout the experiment, and the same goes for choluria. According to Popper[41], cholestasis consists of a blockage of the cananicular bile flow. There is a failure in the secretory capacity of the hepatocyte in consequence of the accumulation of blood in the products normally excreted by the bile. Diminished biliary flow may be inferred through the increase of biliary acids in the plasma. The pigment bilirubin is made mainly of hemoglobin and, to a minor extent, of the degradation of mioglobulin and hepatic synthesis. Bilirubin binds to albumin forming a relatively stable protein-pigment complex to be transported to hepatic parenchyma. This complex, called indirect reacting bilirubin, only takes part in the Van der Berg's diazoreaction after treatment with alcohol or other substances that break the protein binding. Once in the hepatocyte, albumin is removed and bilirubin is conjugated to glycuronic acid, forming a diglycuronic, which is hydrosoluble and is excreted by the biliary canaliculus. This substance causes immediate diazoreaction and is thus called direct reacting. Its analysis is useful in the assessment of jaundices, which may be related to the increase in the offer, alteration of transportation, alteration in uptake, alteration in conjugation, excretion deficiency and other mechanisms. TGO is present in the liver, myocardium, bone muscles, kidneys and pancreas. Cell damage to any of the said tissues results in TGO serum level elevation. As for the liver, more marked increases accompany acute cell damage, regardless of the cause; extremely high levels in patients with hepatitis can be observed. TGO is only mildly elevated in cirrhosis and biliary obstruction. Along with other enzymes, this test is useful in the diagnosis of myocardial infarction and hepatic diseases. Patients with acute kidney disease, bone muscle disease, pancreatitis or trauma may present transitorily elevated levels. TGP is particularly more applicable to assessing liver disease, since hepatic content exceeds greatly myocardial concentration. These elevations accompany acute hepatocellular damage. Elevated levels are found in infectious and toxic hepatitis, pancreatitis, cirrhosis, obstructive jaundice, biliary obstruction and hepatic carcinoma. Lactic dehydrogenese (LDH) levels may also rise in hepatic disease, proliferation of neoplasic cells, myocardial and pulmonary infarction, acute leukemia, hemolytic anemia, megaloblastic anemia, bone muscle necrosis, intense shock and hypoxia. Alkaline phosphatase is mainly present in the liver, bones, intestinal epithelium, and placenta.[8] This enzyme supplies an assessment of the power of intra and extra-hepatic biliary tree. The elevation of this enzyme is demonstrated in 94% of patients with extra-hepatic biliary tract obstruction due to neoplasia, and in 76% of those where the obstruction is caused by calculus. Intra-hepatic biliary obstructions and cholestasis also give rise to an increase of the enzymatic level.[7] Determining its levels is important in the assessment of hepatic and bone disturbances in the extra-hepatic biliary obstruction. Especially, in PAGET disease and cirrhosis. Gamma-glutamyl-transferase (gamma-glutamul-transpeptidase or GGT) is present in many tissues, increases in the serum not only in hepatobiliary diseases, but also after myocardial infarction, neuromuscular disease, pancreatic disease (even in the absence of biliary obstruction), pulmonary disease, diabetes, and during the ingestion of ethanol and other microsomial enzyme inducers. This enzyme takes part in the transfer of aminoacids and peptides through the cellular membrane e possibly involved in glutathione metabolism. The measurement of GGT was proposed as a sensible screening test for hepatobiliary disease and for monitoring ethanol abstinence, however, because of its high sensibility, many individuals who tested positive were cleared of any identifiable hepatic disease when further tests were performed. False-positive results are found in approximately 10% of tests of hospital controls.[42] In this experiment several tests of hepatic function were carried out for all groups (TB, DB, IB, AF, GGT, LDH). Only in the 90-day group (A6) a significant increase of direct bilirubin was found. The fact that only in one group of animals (A) one single alteration in direct bilirubin was observed, cannot be used to infer that significant biochemical alterations of the several analyzed parameters take place, since the number of metabolic factors associated with the many existing biochemical pathways in the hepatocytes of these animals may cause significant biochemical alterations without necessarily compromising liver functions. Another factor that adds up is the work by Silva Junior et al (1993) which demonstrates alkaline phosphatase to be the ideal marker for induced extra-hepatic cholestasis in rats[13]. In all procedure groups non-significant values of alkaline phosphatase were found. In regards to macroscopic alterations, upon sacrificing the animals adhesions in the hepatic hilum, stemming from the handling necessary to perform the ligature, were found in all animals. Hilum fibrosis was evident in 50% of the animals of group A5 (60 days waiting time) and 100% of animals in group A6 (90 days waiting time), demonstrating a regenerative response following probable post-manipulation inflammatory process. The fact that 100% of animals in subgroup A6 presented hilum fibrosis and underwent cavity reopening may have caused some kind of extra-hepatic canalicular obstruction leading to a significant increase in direct bilirubin. This anedoctal finding,

however, requires a deeper investigation to be proven scientifically. From a histological point of view, cholestasis is characterized by retention of biliary pigment, in a decreasing order, in the hepatocyte, canaliculus and Kupffer cells[43]. In the hepatic parenchyma, cholestasis induced histological alterations are primarily found in the portal-space area and may reach as far as the lobe periphery. Throughout this experiment, no microscopic histological alterations were found to indicate biliary pigment retention within the hepatic lobes; neither has any increased in the size of the hepatic parenchyma been observed in the histological cuts of the right lobe. Hepatocytes located around the central vein of the hepatic lobes did not show any morphologic alteration during optical microscopy examiniation; it was not possible to observe sinusoid size increase in this region. Histologically, the presence of hepatic fibrosis in only one animal of subgroup A4 (28 days waiting time) was seen as pericapsular whitish mass. In the literature review, no studies employing the present methodology were found. All the studies found in the literature employed ligature of the common hepatic duct. Among these, authors such as Marinelli et al[11] carried out studies with 80 Wistar rats demonstrating that after 30 days of common hepatic duct ligature, canicular proliferation and portal fibrosis around hepatic lobes took place. These findings were confirmed by Medeiros, Freitas and Andrade[12] in 1988. Biochemical analyses indicated an increase of bilirubins, alkaline phosphatase and transaminases with a decrease in albumin levels. Therefore, this is the first study in the literature in which a specific duct ligature (the right one in this investigation) is extensively analyzed from a biochemical point of view. In the long term, bile duct obstruction will lead to intra and extra fibronodular alterations — the latter being also called mechanic cholestasis. The response to the chronic biliary obstruction is known as secondary biliary cirrhosis. Other causes of secondary biliary cirrhosis include prolonged mechanical obstruction, sclerosing cholangitis, cystic fibrosis, and congenital biliaty cysts.[43] Regardless of the causing agent of the hepatic lesion, the liver will apparently react in five ways: 1. Necrosis. 2. Degeneration. 3. Inflammation. 4. Regeneration. 5. Fibrosis. Necrosis may follow practically any lesion whose changes are significant, taking a toll on hepatocytes. However, before it becomes characteristically necrotic, hepotocytes may become swollen and edematous, with irregularly compact cytoplasm and great clear spaces. Retained biliary material may have a swollen, frothy, and diffuse aspect. These characteristics are related to degeneration. Inflammation is defined by the afflux of acute or chronic inflammatory cells to portal-spaces or parenchyma.[42] Regeneration may take place and is visible by the thickening of hepatocyte cords (or their proliferation) and certain disorganization of the parenchyma structure. Fibrosis occurs through increased collagen deposition, initially around portal-spaces or centrolobular vein, it may also deposit in the space of Disse. Should fibrosis persist, liver will be divided into regenerating hepatocyte nodules surrounded by scarring tissue (cirrhosis). In our optical microscopy observations, no significant increase in fibrosis was detected in any group. Extra-hepatic obstruction causes deep changes in the liver. In a terminal stage, liver surface shows a green-yellowish pigmentation together with jaundiced body liquids and tissues. On the cut surface, the liver is hardened and slightly granular. In microscopy, bile ducts stay distended and frequently contain thickened bile. Portal-spaces are interconnected by fibrous septs and look edematous; there is a narrow zone of edema and ductal proliferation at the junction between parenchyma and septs. Cholestatic characteristics may be intense with cytoplasmatic and canalicular bile accumulation, extense hepatocyte degeneration and formation of biliary lakes. However, once cirrhosis regeneration nodules set in, biliary stasis may become less apparent.[44] The observation of biliary ducts on the portal-space of right hepatic lobes did not indicate a significant increase in their caliber, nor was it possible to confirm the presence of increased edema on this area in all observed group of animals. The observed quantity of polymorphnuclears and mononuclears on the areas stipulated by the employed method did not indicate a significant difference between right and left lobes. Such result strongly indicates that, in the different analyzed groups of animals, physiologic and/or immunological alterations in the cells of hepatic lobes, induced by the obstruction of the right lobe of these animals, were not enough to cause a morphologic alteration likely to be observed microscopically. The results obtained through microscopic analyses show no incidence of necrosis, inflammation or fibrosis in the examined area, in all groups. Morphologic structure remained equally unaltered in all groups (refer to pictures 10 and 11), demonstrating that waiting times between 7 and 90 days are insufficient to cause any alteration. This contradicts post-op findings for ligature of common bile duct, where an intense alteration process takes place in intra-hepatic bile ducts, portal-spaces and hepatocytes. Despite this, it is important to stress that the histological results obtained in this study indicate that the ligature of only one of the lobular branches (the right one) that participate in the formation of the common hepatic duct does not cause alterations likely to be observed microscopically. Total absence of alterations also excludes the possibility of a degenerative or inflammatory process with consequent regeneration or fibrosis, for neither hepatocyte cord thickening nor collagen deposition was observed. In the literature review of studies with humans, Abrantes[45] in seven patients treated with a non-conventional technique for hepatic ducts junction lesion, in a mean 11-year-5-month follow-up, noticed no alteration in hepatic function in a biochemical assessment of patients who underwent ligature of right hepatic duct. The technique consisted of ligature of the right hepatic duct and anastomosis of the left hepatic duct with the retropancreatic segment of the choledochus or with the excluded jejunal loop. Drummont, DAF; Abrantes, WL; Soares, RSQ[46] in 1995 reported that after five years follow-up of patients treated for lesions of the left hepatic duct caused by fire-arms, no hepatic function alterations were

observed. These findings, although observed in humans, are similar to the morphologic data observed in this experiment with rats. Therefore, it suggests a communication between the liver lobes of Wistar rats that offset the obstruction of one of the ducts. It is necessary to study this communication further in order to prove this hypothesis. In the understanding of the authors, the absence of histological and biochemical alterations is owed to the anastomosis between the several extra-hepatic canaliculi, at the hilum level, that drain the bile dammed up by the ligature in question; further studies need to be carried out to prove this hypothesis.

## Conclusion

The present study did not identify histological or biochemical alterations that expressed significant differences between animals that underwent ligature of right hepatic duct and those in the control group in regards to hepatic parenchyma lesion.

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