

Hepatoprotective effect of water soluble extract of *Coleus barbatus* on cholestasis on young rats¹

Efeito hepatoprotetor do extrato aquoso de *Coleus barbatus* na colestase em ratos jovens

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ABSTRACT

Purpose: To test the effects of water extract of *Coleus barbatus* (WEB) on liver damage in biliary obstruction in young rats.

Methods: Forty 21 day-old male *Wistar* rats were divided into four groups of ten 21 day old (P21) submitted to sham or actual operation (S or L) combined with WEB or Water (B or A). At P48 pentobarbital sleeping time (ST) was measured. At P49 they were submitted to euthanasia to determine of serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), liver wet weight (PFF) and, on hepatic histological slides, the frequency of mitoses (FM), the number of necrotic areas (NN), intensity of fibrosis (IF) and intensity of ductal proliferation (IPD). Two Way ANOVA, the S.N.K. test and the Wilcoxon test for paired multiple comparisons were employed to study the effects of cholestasis and those of EAB and their interactions. The Pearson's coefficient of linear correlation of between paired histological variables separately for the groups LA and LD was determined. The test results were considered statistically significant when the p of α error <0.05 . **Results:** Cholestasis increased the TS, ALT, AST, PFF, MI, NN, IF and IPD. The EAB decreased the TS and IM in the animals without cholestasis (sham operated animals). The EAB decreased the TS, ALT, AST, PFF, MI, NN and IF of the cholestatic animals. In the LA group there was a positive correlation between the IPD and the IF, a negative correlation between the IPD and the FM and a negative correlation between the IF and the FM. In the LD group there was a negative correlation between the NN and the IPD. **Conclusions:** In the absence of cholestasis the EAB reduces the pentobarbital sleeping time and decreases the frequency of mitoses. The EAB has a hepatoprotective effect on the biliary cirrhosis secondary to extra-hepatic biliary obstruction.

Key words: Cholestasis, Extrahepatic. Liver Cirrhosis, Biliary. Liver Cirrhosis, Experimental. Rats.

RESUMO

Objetivo: Testar os efeitos do extrato aquoso de *Coleus barbatus* (EAB) na cirrose biliar secundária por obstrução das vias biliares extra-hepáticas em ratos jovens. **Métodos:** Quarenta ratos *Wistar* machos com 21 dias de vida (P21), foram distribuídos em quatro grupos de 10 animais, submetidos a operação simulada ou dupla ligadura e ressecção do ducto biliar (S ou L) combinados EAB e a Água (B ou A). No P48, foi medido o tempo de sono com o pentobarbital (TS). No P49, foram submetidos a eutanásia para a determinação das atividades séricas do aspartato aminotransferase (AST) e da alanina aminotransferases (ALT); após a eutanásia foram avaliados o peso fresco do fígado (PFF) e, em cortes histológicos do fígado, a frequência de mitoses (FM), o número de áreas de necrose (NN), a intensidade da fibrose (IF) e da proliferação ductal (IPD). Os efeitos da colestase, os do EAB e suas interações foram testados pela ANOVA com dois fatores, e as comparações múltiplas pareadas foram realizadas pelo teste de S.N.K ou teste de Wilcoxon. Também foi determinada a correlação linear de Pearson entre as variáveis histológicas duas a duas separadamente para os grupos LA e LD. O nível de significância estatística para os vários testes foi de p do erro $\alpha <0,05$. **Resultados:** A colestase aumentou significativamente o TS, a ALT, a AST, o PFF, a MI, o NN, a IF e a IPD. O EAB diminuiu o TS e a IM nos animais sem colestase (operação simulada). O EAB diminuiu o TS, a ALT, a AST, o PFF, a MI, o NN e IF na colestase. No grupo LA houve correlação positiva entre a IPD e a IF, correlação negativa entre a IPD e a FM e correlação negativa entre a IF e a FM. No grupo LD houve correlação negativa entre o NN e a IPD. **Conclusões:** Na ausência de colestase o EAB encurta o tempo de sono e diminui a frequência de mitoses. O EAB apresenta efeito hepatoprotetor no modelo de cirrose biliar secundária a obstrução biliar extra-hepática.

Descritores: Colestase Extra-Hepática. Cirrose Hepática Biliar. Cirrose Hepática Experimental. Ratos.

1. Research performed at the Infant Hepatology Division, Department of Pediatrics, School of Medicine of Botucatu, São Paulo State University (UNESP), Botucatu - SP, Brazil.

Introduction

The main indication for liver transplantation in infants and children is biliary atresia, a severe cause of hepatobiliary disease which presents a mortality rate of 100% when untreated^{1,2}.

Ligature and resection of the common bile duct in young rats has been used as a model for obstructive cholestasis and the consequent secondary biliary cirrhosis in infants³.

Coleus barbatus from the Lamiaceae family⁴, commonly known as National Boldo, Brazilian Boldo or False Boldo is used in popular Brazilian medicine in the treatment of liver problems^{5,6}. However, there are no reports in the literature of experimental or clinical studies on the effects of *Coleus barbatus* on cholestasis or other hepatic diseases. A few plants extracts have been tested in bile duct ligated adults rats: *Salvia miltiorrhiza*⁷; *Camellia*⁸ and *Stephania tetrandra*⁹.

Our aim was to test possible hepatoprotective effects of a water extract of *Coleus barbatus* in the model of obstructive cholestasis. To that effect we studied in four groups of young rats (bile duct ligated plus WEB or water and sham operated plus WEB or water) the following variables to ascertain the extent of liver disfunction and damage.

Pentobarbital sleeping time is a easily performed liver function test in rats¹⁰ which evaluates the oxidative metabolism of drugs, which is impaired in chronic cholestatic disease that reduces the activity of enzyme cytochrome P450¹¹. Pentobarbital sleeping time was used in several studies to demonstrate hepatoprotective effects of plant extracts (*Calotropis procera*¹², *Artemisia scoparia*¹³, *Cyperus scariosus*¹⁴ and *Bougainvillea spectabilis*¹⁵).

The serum aminotransferase activities (alanine aminotransferase – AST and aspartate aminotransferase – ALT) are biochemical markers of the rate of hepatocellular lesions¹⁶. In experimental cholestasis, through ligature and common bile resection, a significant increase in these cellular enzymes occurs¹⁶. Fujii, 1997¹⁷ summarized the toxicological correlation between serum biochemical tests and the hepatic histopathology found in his own experiments and those published by other authors, with rats and dogs, and concluded the increase in ALT and AST presents a correlation with the intensity of necrosis.

Hepatic histopathology in laminas of the liver with cholestasis showed the intensity of the following lesions^{18,19}: a) hepatocyte coagulation necrosis²⁰; b) fibrosis²¹; c) ductal proliferation²². The regeneration of liver cells can be observed by the presence of mitosis²³.

Methods

Collection and preparation of the water-soluble extract of Boldo (WEB)

The plant (*Coleus barbatus*) was collected fresh, in its natural habitat, in the district of Rubião Jr., Botucatu, during February 2000. It was identified and registered under the number BTU24169, in the Herbarium of the Botanical Department

of the Biosciences Institute, UNESP – São Paulo State University, Botucatu, São Paulo. The leaves were oven-dried at 60°C for three days and then grounded with a pestle until they constituted a fine powder. This was stored in hermetically sealed plastic flasks and labeled. The water-soluble extract was prepared by passing one liter of water at room temperature through a funnel with 60g of the powder placed in filter paper. The filtrate was stored in 10ml amber colored glass flasks, conserved in a freezer at -20°C for up to eight weeks, while thawed aliquots were maintained in a refrigerator at -4°C for use in a maximum of a two day period.

Forty male Wistar rats were studied, born in the Pediatrics Experimental Laboratory of the Botucatu Faculty of Medicine – UNESP, of nulliparous females, weighing 180 to 220 g at the onset of gestation, provided by the Biotério Central of the Botucatu Campus – UNESP. From birth they were suckled in groups of six animals per dam until the 21st day of life (P21). During this period and for the whole experimental period (P21 to P49), the animals were maintained under controlled environmental conditions – temperature between 22 to 23°C and a 12 hour light/dark cycle.

Experimental procedure

The experimental procedure was approved by the Ethics Commission in Animal Experimentation of the Botucatu Faculty of Medicine / UNESP – São Paulo State University, protocol number 64, 25/10/1999, in accordance with the Principles of Ethics in Animal Experimentation of the Brazilian College of Animal Experimentation (COBEA)²⁴. The maintenance of the animals and anesthetic, surgical and euthanasia procedures were performed according to the recommendations of the Canadian Council of Animal Care (1984)²⁵.

On P21, each animal was randomly assigned to one of the four experimental groups of 10 animals each:

SA Group: sham operation with daily administration, by gavage, of 5µl of water per gram of animal weight, **SB Group:** sham operation with daily administration, by gavage, of 5µl of water-soluble extract of Boldo (WEB) per gram of animal weight, **LA Group:** double ligature and resection of the common bile duct with daily administration, by gavage, of 5µl of water per gram of animal weight, **LB Group:** double ligature and resection of the common bile duct with daily administration, by gavage, of 5µl of WEB per gram of animal weight.

All animals had *ad libitum* access to water and standard feed with 18g of protein per 100g of feed prepared by the Pediatrics Experimental Laboratory of the Botucatu School of Medicine of the São Paulo State University-UNESP (Table 1).

TABLE 1 – Feed composition (18g/100g of proteins) prepared by the Pediatrics Department Experimental Laboratory

DIETARY COMPONENTS	g/kg
Protein (casein)	1.8
Saline Mixture ^{26,27}	0.4
Fiber (corn cob)	0.4
Vitamin Mixture ¹⁸	0.1
Sodium Benzoate	0.01
Corn Oil	0.8
Sucrose	1.0
Cornstarch (Maizena [®])	5.49

The ligation and resection of the common bile duct was performed according to the technique presented by Cameron and Oakley²⁸ with modifications²⁹.

The sham operation followed exactly the same technique described above with exception of the ligatures and common bile duct resection.

Sleeping time by pentobarbital

On P48, the sleeping time test was performed using pentobarbital. The animals received pentobarbital (concentration of 3g/100ml of solution) through intraperitoneal injection at a dosage of 50 mg/kg of rat body weight (or 0.0017 ml/g of animal weight) and time was measured in minutes, from the loss of position reflex to its recuperation.

Euthanasia was performed on P49, beginning with an intraperitoneal injection of sodium pentobarbital, followed by posterior bleeding. The blood and liver were collected for biochemical tests:

- aminotransferase dosage: ALT (alanine aminotransferase) and AST (aspartate aminotransferase) in IU/L by the automated colorimetric enzymatic method using Technicon[®] equipment, model RAXT;

- Liver wet weight in g using Mettler Toledo digital scales - model PB 3002, maximum weight = 3.100 g, minimum weight = 0.5 g, sensibility 0.1 g.

Histological lesions

A fragment of the right lobe of the liver, 0.3 to 0.35 cm thick, was removed from each animal. Histological sections of a around 4 µm thick were stained with hematoxylin and eosin stains. A semi-quantitative analysis of a slide from each animal was performed and scores from zero to three for the intensity of fibrosis and ductal proliferation were attributed to each animal. Areas of necrosis were counted in the whole slide, and the number of mitosis in ten fields at high magnification were counted. Three independent measurements of each of the histopathological variables mentioned above were performed.

Statistical procedures

The mean and standard deviation or median values of each variable for each group were calculated. The results of each variable for each experimental group were compared according to the following steps:

1) testing for normality and equality of variances of the values obtained for each group;

2) if the data passed these tests, a two way analysis of variance was applied, where one factor was the administration or non-administration of WEB and the other the presence (double ligation and common bile duct resection) or absence of cholestasis (sham operation). The effects of each factor were tested independently of the effect of the other, as well as the interaction between both factors;

3) when a significant interaction between factors was found ($p \leq 0.05$), multiple paired comparisons were performed using the Student-Newman-Keuls (S.N.K.) method, identifying pairs of groups with significant differences among them (p of $\alpha \leq 0.05$);

4) if the measurements did not satisfy the criteria of item 1, mathematical transformations were performed and the transformed values that passed the tests were employed in the calculations as in item 2 above. Pentobarbital sleeping time and aminotransferase were substituted by their rank number.

5) histological variables (frequency of mitosis, number of areas of necrosis and intensity of ductal proliferation) were analysed by means of a factorial delineation using a generalized linear model, presuming a Poisson's distribution and a canonic (logarithmic) connection. A 1/6 value was added to the observations for a better fit to the model. For this analysis the means of the 30 readings of each group were used, $n=30$ (3 readings per animal from each experimental group). However this model did not result in an adequate fit: in the case of the number of mitosis excessive dispersion occurred and in the case of the variables number of areas of necrosis, intensity of fibrosis and the intensity of ductal proliferation a sub-dispersion occurred. Even when the interaction was not significant, we opted to perform the decomposition of the effects of the drug and ligation factors in the nestling effect which were tested using the Wilcoxon test ($p < 0.05$).

Pearson's analysis of linear correlation was applied to paired histological variables (p of $\alpha < 0,05$) separately for groups LA and LB. For this analysis the means of the 3 measurements per animal from each experimental group ($n=10$) were used.

Table 2 shows the mean and standard deviation values of the variables for which no mathematical transformations were necessary and the median of those values for which transformations were necessary. The graphs in figures 1 and 2 show the means (solid points), the standard deviations above and below the mean (lines) and the individual values (hollow points) of the measurements, or their transformations, performed on each experimental group.

Calculations were performed by the Sigma Stat program for Windows[®] 2.0, Jandel Corporation or the SAS program.

Results

Sleeping time with pentobarbital (ST), aminotransferase serum activities (AST and ALT) and liver wet weight (LWW)

Cholestasis caused a statistically significant prolongation in **ST** in animals which received WEB (medians: (LB=172.80 min.)>(SB=97.30 min.)) and in those which did not receive WEB (medians: (LA=374.30 min.)>(SA=111.30 min.)). The prolongation of the **ST** was decreased by WEB in the animals with cholestasis (Medians: (LB=172.80 min.)<(LA=374.30 min.)). The same effect was seen in animals without cholestasis (medians: (SB=97.30 min.)<(SA=111.30 min.)), Figure 1A and Table 2.

Cholestasis caused a statistically significant increase in **ALT** in animals which received WEB (medians: (LB=105.50 IU/L)>(SB=38.00 IU/L)) and those which did not receive WEB

(medians: (LA=150.00 IU/L)>(SA=37.00 IU/L)). The increase in **ALT** caused by cholestasis was decreased by the administration of WEB (medians: (LB=105.50 IU/L)<(LA=150.00 IU/L)) (Figure 1B and Table 2).

Cholestasis also caused a statistically significant increase in **AST** in the animals which received WEB (medians: (LB=25.50 IU/L)>(SB=12.00 IU/L)) and in those which did not receive WEB (medians: (LA=34.00 IU/L)>(SA=10.50 IU/L)). The increase in **AST** caused by cholestasis was decreased by the administration of WEB (medians: (LB=25.50 IU/L)<(LA=34.00 IU/L)) (Figure 1C and Table 2).

Cholestasis caused a statistically significant increase in **LWW** in animals which received WEB (means: (LB=7.22 g)<(SB=9.71 g)) and in those which did not receive WEB (means: (LA=17.68 g)>(SA=10.74 g)). The increase of **LWW** caused by cholestasis was decreased by WEB ((means: (LB=7.22)<(LA=17.68)) (Figure 1D and Table 2).

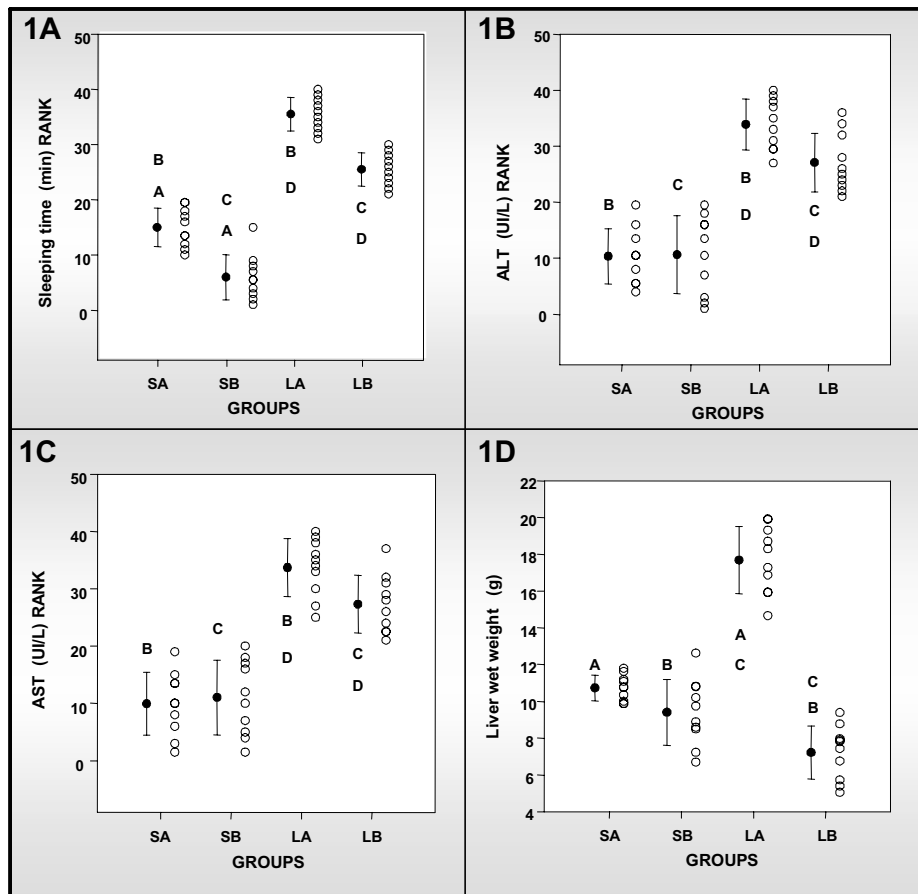


FIGURE 1 – Means, standard deviations and individual values of sleeping time (min.) (**1A**) of aminotransferase serum activity ALT (IU/L) (**1B**), aminotransferase serum activity AST (IU/L) (**1C**), and liver wet weight (g) (**1D**) in rats, in groups of 10, submitted to one of the following treatments: **SA**: sham operation with daily administration of water; **SB**: sham operation with daily administration of WEB; **LA**: double ligature with common bile duct resection with daily administration of water; **LB**: double ligature with common bile duct resection with daily administration of WEB. Letters repeated in figures 1A, 1B, 1C and 1D, of the graphs, indicate the group pairs with significant differences among them in the S.N.K. test ($p < 0.05$): **A**=SA>SB; **B**=LA>SA; **C**=LB>SB; **D**=LA>LB.

TABLE 2 - Sleeping time, aminotransferase serum activities (ALT and AST) and liver wet weight in young rats - comparisons between effects of cholestasis and of WEB, interaction between these effects and descriptive analysis

Variables	Procedure^A	Drug^B	Interaction e Comparison^C	Descriptive Analysis^D
Sleeping Time (min.)	(LA+LB)>(SA+SB) p≤0.001 significant	(SA+LA)>(SB+LB) p≤0.001 Significant	p≤0.001 significant LA>LB p<0.05 s. LA>SA p<0.05 s. LB>SB p<0.05 s. SA>SB p<0.05 s.	Medians SA=111.30 SB=97.30 LA=374.30 LB=172.80
ALT (IU/L)	(LA+LB)>(SA+SB) p≤0.001 significant	(SA+LA)≈(SB+LB) p=0.070 not significant	p=0.049 significant LA>LB p<0.05 s. LA>SA p<0.05 s. LB>SB p<0.05 s. SA≈SB p=0.249 n.s.	Medians SA=37.00 SB=38.00 LA=150.00 LB=102.50
AST (IU/L)	(LA+LB)>(SA+SB) p≤0.001 significant	(SA+LA)≈(SB+LB) p=0.141 not significant	p=0.040 significant LA>LB p<0.05 s. LA>SA p<0.05 s. LB>SB p<0.05 s. SA≈SB p=0.249 n.s.	Medians SA=10.50 SB=12.00 LA=34.00 LB=25.50
Liver wet weight (g)	(LA+LB)>(SA+SB) p≤0.001 significant	(SA+LA)>(SB+LB) p≤0.001 significant	p≤0.001 significant LA>LB p<0.05 s. LA>SA p<0.05 s. LB<SB p<0.05 s. SA≈SB p<0.05 n.s.	Means±DP SA=10.74±0.70 SB=9.41±1.79 LA=17.68±1.83 LB=7.22±1.45

Histological lesions

Cholestasis, with no administration of WEB, caused a statistically significant increase in **FM** (Means: (LA=14.13)>(SA=11.00)). WEB significantly reduced **FM** in animals without cholestasis (Means: (SB=3.53)<(SA=11.00)). WEB administration in animals with cholestasis reduced **FM** levels to those observed in animals without cholestasis and which received WEB (Means: (LB=4.60)≈(SB=3.53)<(LA=14.13)), Figure 2A.

Cholestasis caused a statistically significant increase in **NN** both in animals administered WEB (Means: (LB=2.23)>(SB=0.00)) and those which were not (Means: (LA=9.73)>(SA=0.00)). WEB significantly reduced **FM** in animals with cholestasis (Means: (LB=2.23)<(LA=9.73)), Figure 2B.

Cholestasis caused a statistically significant increase in **IF** both in animals administered WEB (Means: (LB=1.10)>(SB=0.00)) and those which were not (Means: (LA=1.37)>(SA=0.00)). WEB significantly reduced **IF** in animals with cholestasis (Means: (LB=1.10)<(LA=1.37)), Figure 2C.

Cholestasis caused a statistically significant increase in **IDP** both in animals to which WEB was administered (Means: (LB=1.27)>(SB=0.00)) and in those to which WEB was not administered (Means: (LA=1.37)>(SA=0.00)). WEB did not significantly reduce **IDP** in animals with cholestasis (Means: (LA=1.37)>(LB=1.27)) (Figure 2D).

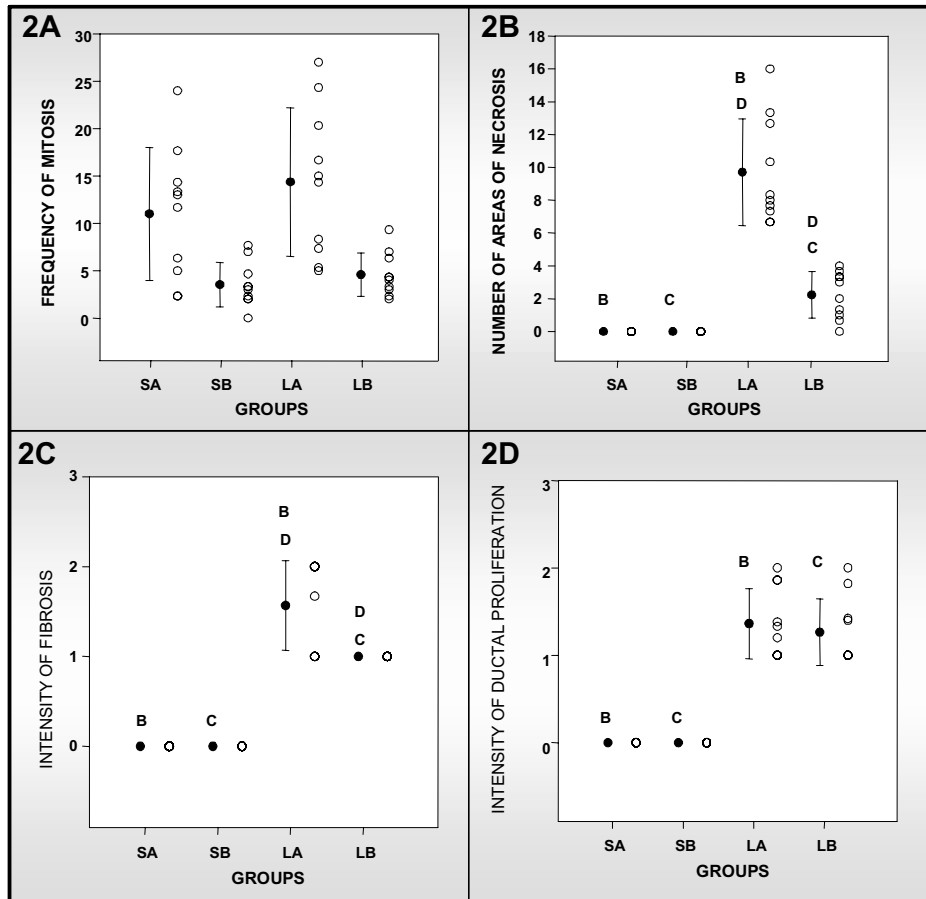


FIGURE 2 – Means (of 30 readings – 3 per animal), standard deviations and individual values of histological lesions: Frequency of mitosis (**2A**), number of areas of necrosis (**2B**), Intensity of fibrosis (**2C**) and Intensity of ductal proliferation (**2D**) in rats, in groups of 10, submitted to one of the following treatments: **SA**: sham operation with daily administration of water; **SB**: sham operation with daily administration of WEB; **LA**: double ligature with common bile duct resection with daily administration of WEB; **LB**: double ligature with common bile duct resection with daily administration of WEB. Letters repeated in figures 2A, 2B, 2C and 2D, of the graphs, indicate the group pairs with significant differences among them in the Wilcoxon test ($p < 0,05$): **A**=SA>SB; **B**=LA>SA; **C**=LB>SB; **D**=LA>LB.

Pearson's correlation between the groups with cholestasis

In animals with cholestasis not receiving WEB (LA) there was a significant positive linear correlation between **IDP** and **IF** ($r_p = 0.7852$; $p = 0.0071$) and a significant negative lin-

ear correlation between **IDP** and **FM** ($r_p = -0.8198$; $p = 0.0037$), see Table 3 and Figure 3.

In animals with cholestasis with WEB administration (LB) there was a significant linear positive correlation between **NN** and **IDP** ($r_p = -0.8541$; $p = 0.0017$), see Table 4 and Figure 4.

TABLE 3 - Pearson's correlations between pairs of histological variables in group LA

Group LA	Number of areas of necrosis	Intensity of fibrosis	Intensity of ductal proliferation
Frequency of mitosis	c=0.3336 p=0.3462	c= -0.8198 p=0.0037	c= -0.7182 p=0.0193
Number of areas of necrosis	-----	c= -0.5542 p=0.0964	c= -0.1932 p=0.5927
Intensity of fibrosis	-----	-----	c=0.7852 p=0.0071

Shaded areas indicates a statistically significant linear correlation between pairs of variables ($p < 0.05$).

TABLE 4 - Pearson's correlation between pairs of histological variables in group LB

Group LB	Number of areas of necrosis	Intensity of fibrosis	Intensity of ductal proliferation
Frequency of mitosis	$r_p=0.2742$ $p=0.4433$	♦	$r_p= -0.2264$ $p=0.5293$
Number of areas of necrosis	-----	♦	$r_p= -0.8541$ $p=0.0017$

Shaded area indicate a statistically significant linear correlation between pairs of variables ($p<0.05$)
 • The intensity of fibrosis of all animals was identical (1), thus correlation was impossible to perform.

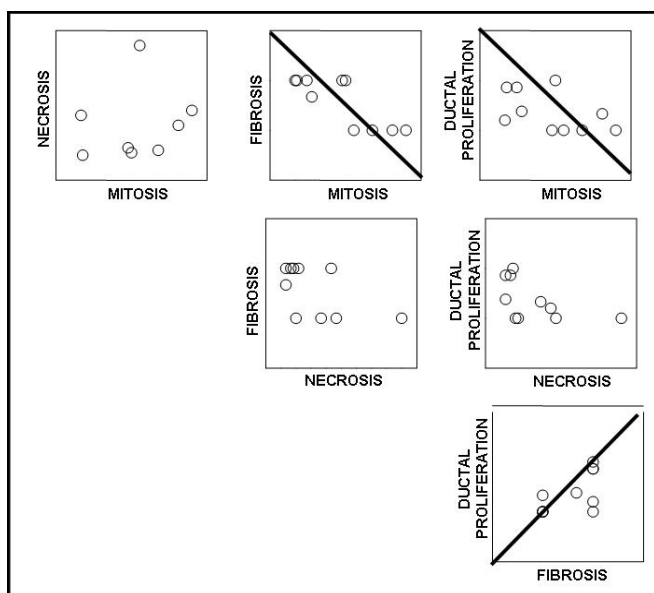


FIGURE 3 – Histological variables in group LA (ligature of the common bile duct with administration of distilled water) Graphs of dispersion showing the results of analysis of Pearson's linear correlation

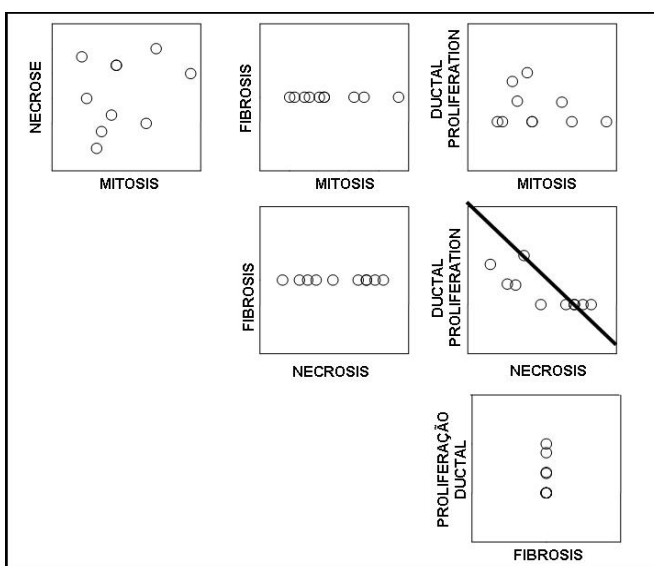


FIGURE 4 – Histological variables in group LB (ligature of the common bile duct with administration of WEB). Graphs of dispersion showing the results of analysis of Pearson's linear correlation

Discussion

Effects of cholestasis

In order to characterize the effects of cholestasis after ligature and resection of the common bile duct (LRBD) we summarized the following alterations: an increase in the aminotransferase serum activities (AST and ALT), a prolongation of sleeping time after pentobarbitol, an increase in liver wet weight and the presence of the following histological lesions – necrosis, fibrosis, ductal proliferation and an increase in the number of hepatocyte mitoses.

The sleeping time test induced by pentobarbitol, in hypnotic doses, was proposed as a simple, non-invasive test of liver function in rats. It reflects the velocity of pentobarbitol disintoxication by the hepatocyte cytochromes P-450¹⁰. The hepatocytic lesion in cholestasis induced by LRBD leads to a reduction in the isoenzymes of the cytochrome P-450¹¹. The sleeping time test induced by pentobarbitol, has been used to evaluate liver function in several experimental models: biliary cirrhosis secondary to extra-hepatic biliary obstruction^{30,31} and by carbon tetrachloride^{13,14,15,32,33}. In the literature we found no reports on sleeping time in young rats with obstructive cholestasis by LRBD.

The aminotransferase serum activities (AST and ALT) are indicators of the velocity of hepatocellular damage and have been applied in the study of several experimental models for hepatopathies^{34,8}. Our results are in agreement with other findings in the literature: experimental cholestasis by a-naphthylisothiolcyanate – ANIT^{34,35}, experimental cholestasis by LRBD in adult rats^{36,37,9,8}, cholestasis induced by endotoxins³⁸ and cholestasis associated with total experimental parenteral nutrition in adult rats³⁹. In the literature we found no reports on aminotransferase serum activities (AST and ALT) in young rats with obstructive cholestasis by LRBD.

Hepatomegaly is an important clinical characteristic in cholestasis. The liver weight increases as a result of the depositing of conjunctive tissue, the formation of regenerative nodules and ductal proliferation⁴⁰. Our results are in agreement with the findings of Aller et al, 1993⁴¹ and Sokal et al., 1996⁴², who studied rats submitted to LRBD, and who also observed an increase in liver weight in these animals.

The presence of necrosis⁸, hepatic fibrosis^{40,9,8} and ductal proliferation^{21,8} in the biliary cirrhosis secondary to ligation of the common bile duct model has been confirmed in recent works. As our study has pointed out, Aggerbeck et al., 1983²², studying the model of cholestasis by LRBD in adult rats also observed an increase in the number of mitoses in cholestatic animals.

In animals with cholestasis without the administration of WEB there was a positive correlation between the intensity of the fibrosis and the intensity of the ductal proliferation. This correlation is to be expected, as in this experimental model these two lesions are intensely related to each other²¹.

The frequency of mitosis presented negative correlation with both intensity of fibrosis and ductal proliferation. Perhaps this correlation can be explained by the deviation of oval cell (originated from stem cells) transformation into cholangiocytes, diminishing the oval cell transformation into hepatocytes. Although it is known that oval cells are totipotent, in that they can transform themselves into either cholangiocytes or hepatocytes^{43,44,45}, there is no evidence in the literature that the transformation into cholangiocytes diminishes the transformation into hepatocytes, which our study suggests with the possibility of totipotent oval cells.

WEB effects in the absence of cholestasis

The administration of WEB in animals without cholestasis resulted in a shorter sleeping time, which is in agreement with the work of Fischman et al., 1991⁴⁶, who studied the effects of water extract of *Coleus barbatus* in adult rats, concluding that the animals which received the extract presented a 20 to 25% reduction in sleeping time induced by pentobarbitol. Therefore, WEB must have a stimulatory effect on the cytochrome P450. No reports on the effects of WEB on the cytochrome P450 were found in the literature.

WEB also reduced the number of mitoses in the absence of cholestasis. No reports on the effects of WEB and a reduction in the number of mitoses were found in the literature.

WEB effects on cholestasis

We were able to show that WEB, at the dosage applied, in biliary cirrhosis secondary to extra-hepatic biliary obstruction in young rats has a hepatoprotective effect characterized by the following actions: it diminished the increase in aminotransferase serum activities (ALT and AST); shortened sleeping time induced by pentobarbitol; prevented the increase in liver wet weight; reduced the number of necrotic areas, the intensity of hepatic fibrosis and hepatocytic regeneration (measured by the number of mitoses).

This hepatoprotective action may be mediated by *forskolin*, which is one of the most important components of *Coleus barbatus*⁵. *Forskolin* stimulates adenylate cyclase and the production of the secondary messenger cAMP⁴⁷, which has a hepatoprotective action against glycochenodeoxycholic acid in hepatocyte cultures⁴⁸. The accumulation of this acid appears to be the most significant factor in the hepatocytic damage of

cholestasis. We were unable to show the effect of WEB on ductal proliferation, perhaps due to the small sample size.

The effect of WEB on the reduction of the frequency of mitoses in animals with cholestasis suggests a reduction in the hepatocytic regeneration, which is in agreement with the reduced intensity of necrosis. No reports were found in the literature on the use of WEB in the reduction in the number of mitosis in cholestasis.

Although the group with cholestasis with WEB administration did not present a greater ductal proliferation than the group with cholestasis with distilled water administration, there was a negative correlation between ductal proliferation and the number of necrotic areas. This negative correlation could indicate that one process inhibits the other, which is not the case, or that a third factor inhibits one and stimulates the other, which suggests that the WEB, possibly through the action of *forskolin*, inhibits necrosis and stimulates ductal proliferation, as show above.

We present this hypothesis even though it was not possible to demonstrate any difference between the ductal proliferation with or without the administration of WEB, perhaps due to the sample size. Knowing that *forskolin* stimulates ductal proliferation in the presence of the reduction of vagal tonus⁴⁹, further studies should be conducted to test for the existence of vagal hypotonia in this cholestasis model and if found not to be the case, if this action also occurs in the presence of normal vagus tonus.

Conclusions

The administration of water extract of *Coleus barbatus* exerts an effect on animals without cholestasis (sham surgery) reducing the frequency of mitoses and the sleeping time induced by pentobarbitol. In cholestasis, the water extract of *Coleus barbatus* exerts a hepatoprotective effect, demonstrated by the reduction of: the sleeping time with pentobarbitol, aminotransferase seric activity of AST and ALT, liver wet weight, hepatocytic regeneration (measured by the number of mitoses), the number of necrotic areas, the intensity of fibrosis and, further, by the negative correlation between the number of necrotic areas and the intensity of ductal proliferation.

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