Effect of copaiba oil in hepatic damage induced by acetaminophen in rats[1]

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ABSTRACT

PURPOSE: To investigate the effects of copaiba oil on the hepatic damage induced by paracetamol.

METHODS Thirty six rats were distributed into six study groups (N=6): control group, that didn't receive the acetaminophen; Acetaminophen Group, that only received the acetaminophen; Prophylactic Copaiba Group 1, that received copaiba oil two hours before the acetaminophen; Prophylactic Copaiba Group 7, that received copaiba oil seven days, once by day, before the acetaminophen; Therapy Copaiba Group, that received the copaiba oil two hours afther the acetaminophen; and N-Acetyl-Cysteine Group, , that received the N-Acetyl-Cysteine two hours afther the acetaminophen. Euthanasia was performed after 24 hours. The serum levels of AST, ALT, alkaline phosphatase, ɣ GT, total bilirubin, direct bilirubin and indirect bilirubin and histological analisis were analized.

RESULTS: The prophylactic copaiba group 7, therapy copaiba group and N-Acetyl-Cysteine Group showed amounts of AST and ALT similar to the control group; and the prophylactic copaiba group 1 showed similar levels to the acetaminophen group. There was no significant difference between the groups regarding the amount of alkaline phosphatase and ɣ GT ($p>0.05$). The therapy copaiba group showed the highest levels of bilirubin and was statistically different from the other groups ($p<0.01$) and this increased the costs of direct bilirubin. Regarding histopathology, the oil of copaiba administered prophylactic or therapeutic form for 7 days could decrease the amount of necrosis and inflammatory infiltrate.

CONCLUSION: Copaiba oil administered prophylactically for seven days, and therapeutic could reduce liver damage caused by paracetamol similarly N-Acetyl-Cysteine, however, when treated with copaiba therapeutically showed increases in bilirubin, costs increasing fraction indirect.

Key words: Plant Oils. Hepatitis. Liver. Acetaminophen. Rats.

Introduction

The liver is the most important organ in the abdominal of the human body, responsible for several functions in the body, such as controlling the metabolism of lipids, carbohydrates and proteins, as well as the production and excretion of bile[1]. Moreover, the function of convert toxic substances into non-toxic products, it`s the a essential role for the survival and prevent diseases[2].

However, when liver cannot metabolize all toxic agents, this accumulate in the organ and, eventually, causes a toxic hepatitis[3]. This clinical issue is very relevant in clinical practice, especially in relation to acetominophen[4].

Acetominophen is an analgesic and antipyretic widely used in the world, and the accidental or voluntary ingestion of high doses has a great possibility to develop a toxic hepatitis[5], especially when associated with alcohol[6]. The misuse of acetaminophen is the leading cause of acute hepatitis in the United States[7].

The mechanism of hepatotoxicity caused by acetaminophen is unknown, however, it is known that there is a massive production of oxygen free radicals and a direct cytotoxic effect involved in the pathogenesis of this lesion, with a consequent necrosis or apoptosis of the hepatocytes[8,9]. This process causes a overload in the liver function, and then symptoms of cholestasis because of the inflammatory functions[10].

The various medicinal plants are assigned with hepatoprotective and anti-inflammatory effects. Studies shows an effect of the plants in reduce liver damage caused by acetaminophen and some others with similar results comparing with N-Acetyl-Cysteine, the gold standard for the treatment of this kind of liver failure[11-15]. This practice has been encouraged by the World Health Orgaznization[16].

Copaiba oil stands out among the Amazonia`s medicinal plants due to be attributed to this an important anti-inflamatory effect[17,18] and a function that promotes protection in the liver tissue[19]. Therefore, this study aims to investigate the effects of Copaiba Oil (*Copaifera officinalis*) in liver damage acetaminophen's induced.

Methods

Approved by the Ethics Committee in the Use of Animals of the State University of Para (UEPA), protocol 08/07. This study used the copaiba oil species *Copaiffera officinalis*, supplied by Brazilian Agricultural Research Corporation (EMBRAPA), previously submitted to a physicochemical analyze to define its composition.

Thirty six male Wistar rats (*Rattus norvegicus*) were used, weighing between 210 - 250 grams, provided from the Animal Colony of the Experimental Surgery Laboratory of UEPA, kept in a controlled environment, with food and water *ad libitum*. The animals were randomized distributed into six study groups, with six animals each:

-Control Group (CG): The animals were used as normal standard for biochemical and histological analysis;

-Acetaminophen Group (AG): The animals received a single dose of acetaminophen;

-Prophylactic Copaiba Group 1 (PCG1): The animals received copaiba oil, once, two hours before to receive the acetaminophen dose;

-Prophylactic Copaiba Group 7 (PCG7): The animals received copaiba, once, seven days before to receive acetaminophen;

-Therapy Copaiba Group (TCG): The animals received copaiba two hours after to receive acetaminophen;

-N-Acetyl-Cysteine Group (NG): The animals received N-Acetyl-Cysteine, two hours after to receive acetaminophen.

Copaiba oil was administered by gavage at a dose of 0.63 mL/kg in group PCG7 and 3.8 mL/kg in groups PCG1 and TCG. The oil was provided by the EMBRAPA, as crude oil. The acetaminophen and N-acetyl-cysteine were obtained with a concentration of 400mg/mL and 300mg/mL, respectively. The acetaminophen and N-acetyl-cysteine were administered by gavage at a dose of 2 g/kg and 1.2 g/kg, respectively[11,12].

After 24 hours from the acetaminophen administration, the animals were anesthetized using ketamine (70 mg / kg) and xylazine (10 mg / kg) intraperitoneally. After that was performed to collect 5 ml by vena cava inferior to measurement of aspartate aminotransferase (AST), alanine aminotrasferase (ALT), alkaline phosphatase, gamma glutamyltraspeptidase (ɣGT), total bilirubin (TB) and its fractions, Direct (DB) and indirect (IB).

Followed by collecting the median lobe of the liver, this was fixed in formalin 10% and used for histopathological analysis by hematoxylin and eosin staining. The presence of necrosis, inflammatory infiltrate and vascular congestion were analyzed by a semi-quantitative scale: 0 - absent, 1 - mild, 2 - moderate, 3 - severe[14,15].

ANOVA test was used to compare the biochemical results and the Kruskal-Wallis test to compare the histopathological results. Was adopted a significance level of 5% to reject the null hypothesis.

Results

The mean serum levels of AST and ALT in each group are shown in Table 1. On these significant differences between the CG and the groups AG and PCG1, ($p<0.01$) also was no significant difference between the AG and the groups PCG7, GCT and NG.

TABLE 1 - Mean and standard deviation of serum AST and ALT according to the groups.

Group	AST	ALT
CG	131.66 ±17.71	74.16 ±15.18
AG*	373.00 ±103.91	311.00 ±137.77
PCG1*	303.00 ±214.85	226.83 ±159.90
PCG7#	225.00 ±95.29	164.33 ±62.05
TCG#	198.33 ±91.99	168.16 ±41.84
NG#	175.33 ±97.08	104.50 ±73.90

Source: Protocol search
*p<0.01 relative to CG (ANOVA)
#p<0.01 relative to AG (ANOVA)

Regarding the measurement of alkaline phosphatase and ɣGT (Table 2), no significant difference between groups was showed. Regarding total bilirubin and fractions (Table 3) there was a statistical difference ($p <0.01$) between TCG and all other groups in the dosage of total bilirubin and indirect, but there was no statistical difference between the groups regarding the estimation of direct bilirubin.

TABLE 2 - Mean and standard deviation of serum alkaline phosphatase and ɣ GT according to the groups.

Group	alkaline phosphatase	ɣ GT
CG	150.33 ±88.14	6.83 ±1.83
AG	195.16 ±58.43	7.16 ±1.60
PCG1	145.66 ±30.91	7.83 ±2.40
PCG7	181.50 ±29.04	6.00 ±1.26
TCG	112.50 ±65.49	5.66 ±1.03
NG	145.00 ±52.82	7.50 ±1.87

Source: Protocol search
p>0.05 (ANOVA)

TABLE 3 - Mean and standard deviation of serum total bilirubin and fractions according to the groups.

Group	total bilirubin*	direct bilirubin	indirect bilirubin*
CG	0.0783 ±0.04	0.0283 ±0.01	0.0500 ±0.02
AG	0.2133 ±0.11	0.0433 ±0.02	0.1700 ±0.11
PCG1	0.2567 ±0.09	0.0617 ±0.02	0.1950 ±0.09
PCG7	0.1633 ±0.08	0.0450 ±0.02	0.1183 ±0.06
TCG	0.5100 ±0.46	0.1033 ±0.12	0.4067 ±0.35
NG	0.1167 ±0.04	0.0385 ±0.03	0.0733 ±0.03

Source: Protocol search
*p<0.01 TCG relative to outhers groups (ANOVA)

Histopathological analysis (Table 4) showed no areas of necrosis, inflammatory infiltrate and vascular congestion in the control group. In AG, all animals showed grade 3 areas of necrosis, inflammatory infiltrate and vascular congestion, and the group PCG1 showed similar results but an animal of this group had grade 2 necrosis and inflammatory infiltrate.

In groups PCG7, TCG and NG all had grade 3 vascular congestion, however in relation to necrosis and inflammatory infiltrate, the PCG7 4 animals showed grade 2 and grade 1 two animals, the TCG, one animal showed grade 3, two grade 2 animals and three grade 1; Gnac the three animals had grade 2 and grade 1 three animals.

TABLE 4 - Mean histological grades of necrosis, inflammatory infiltrate and vascular congestion according to the groups.

Group	Necrosis	inflammatory infiltrate	vascular congestion
CG	0.00	0.00	0.00
AG*	3.00	3.00	3.00
PCG1*	2.83	2.83	3.00
PCG7#	1.66	1.66	3.00
TCG#	1.66	1.66	3.00
NG#	1.50	1.50	3.00

Source: Protocol search
*p<0.05 relative to CG (necrosis and inflammatory infiltrate) (Kruskal Wallis)
#p<0.05 rerlative to AG (necrosis and inflammatory infiltrate) (Kruskal Wallis)

Discussion

The acetaminophenin the dose used, showed hepatotoxic effects perceived by increased dosage of ALT and AST in the control group[11,12,14], and the copaiba oil showed no effects when administered prophylactically during a day, however for 7 days

and prophylactic therapy showed similar results to those animals treated with N-Acetyl-Cysteine, showing a hepatoprotective potential of this oil.

One mechanism of liver injury caused by acetaminophenin is the production of free radicals leading to apoptosis and necrosis[3,7,12]. The oil of copaiba demonstrably can attenuate the production of free radicals due to its anti-inflammatory, and the effect of copaiba oil in liver transaminases had already been identified by Noguchi *et al*.[20], suggesting a hepatoprotective real potential of this oil.

The use of the copaiba oil's dose of 3.8 ml/Kg, used in PCG1 and TCG, was based on the total amount of oil administered in PCG7 and toxicity studies on copaiba oil. So, we tried to match the dose offered in all groups that received copaiba.

The use of standard dosage copaiba (0.63 mL/Kg) does not significantly alter liver function, displaying only a fraction increases from direct bilirubin, but without showing signs of cholestasis[19,20]. However the use of higher doses (6 mL/Kg), changes the amount of AST, alkaline phosphatase and ɣGT, showing signs of cholestasis without providing other signs of histopathological changes[21,22]. Showing a direct cause and effect of this oil.

Most studies that use medicinal plants show effects similar to those found in this study[11-15], however all perform prophylactic treatment of toxic hepatitis, similar to PCG1 and PCG7. However, studies should focus on the analysis of the therapeutic effect of the oils, whereas hardly identifies the patients who abuse acetaminophenin before they show hepatitis.

Regarding alkaline phosphatase and ɣ GT, it is known that toxic hepatitis caused by paracetamol not generally leads to hepatic congestion[3,12,15], even treatment with N-Acetyl-Cysteine, however, due to the groups treated with copaiba balsam expected a certain degree of congestion, especially on PCG1 and TCG, because the high dose administered could lead to biliary stasis, as found in a study of Botelho *et al.*[21]. Probably, this difference was not found due to the shorter exposure time and amount of oil administered.

The amount of total bilirubin was higher in TCG compared to the other groups, however all other groups showed an increase in average bilirubin compared to control, this increase probably occurred on the hepatic load due to the death of several hepatocytes, however the TCG, beyond that under load, the amount of oil delivered to the liver overload has deteriorated, similar to study of Botelho *et al.*[21].

In TCG hardly copaiba damaged liver function, whereas the levels of AST and ALT were normal, and the major increase in bilirubin was indirect because its fraction, showing that the injury occurred probably due to liver overload and not due to cholestasis[22].

The histopathologic results confirmed the toxic effects of paracetamol at the dose used, and confirm that copaiba administered two hours before the paracetamol showed no protective effect. Also, it is evident that the oil copaiba groups PCG7 and TCG have protective effects similar to NG, as both failed to reduce vascular congestion, even reducing the area of necrosis and inflammatory infiltrate.

Copaiba oil administered seven days before and therapeutically liver damage from paracetamol showed hepatoprotective effects, however, was only analyzed the effects within 24 hours after injury, thus, further studies should be conducted to understand whether longer periods in the oil copaiba can cause harm liver, as well as studies to identify the active principle and / or how this oil works to reduce liver damage[18].

Conclusion

Copaiba oil administered prophylactically for seven days and prophylactic could reduce liver damage caused by paracetamol similarly N-Acetyl-Cysteine, however, when treated with copaiba therapeutically showed increases in bilirubin, costs increasing fraction indirect.

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