

# Effects of andiroba (*Carapa guianensis*) oil on hepatic function of rats subjected to liver normothermic ischemia and reperfusion

## *Efeitos do óleo de andiroba (Carapa guianensis) na função hepática de ratos submetidos à isquemia e reperfusão normotérmica do fígado*

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### A B S T R A C T

**Objective:** To evaluate the effects of the Andiroba (*Carapa guianensis*) oil on liver function in rats subjected to normothermic ischemia/reperfusion injury. **Methods:** we divided 12 Wistar rats into two groups: saline (n = 6) and Andiroba (n = 6). The Andiroba group was treated with Andiroba oil (0.63 ml/kg orally) for seven days before surgery. Ischemia was induced by occlusion of the blood supply to the lateral and median lobes of the liver, using vascular clips, in both groups, for 45min, followed by reperfusion for 60 minutes later. We analyzed dosages of AST, ALT, Gamma-GT, and liver biodistribution of 99mTc phytate. **Results:** There was no significant difference in the percentage of radioactivity/gram of tissue (%ATI/g) in the right lobe of the saline group (17.53 ± 2.78) compared with the Andiroba group (18.04 ± 3.52) p = 0.461, the same occurring in the %ATI/g of the left lobe of the liver when the two groups were compared (p = 0.083). In the saline group, the %ATI/g was significantly higher in the non-ischemic right hepatic lobe (17.53 ± 2.78) when compared with the left lobe (5.04 ± 0.82) that suffered ischemia/reperfusion (p = 0.002). Significant differences also occurred when comparing the right (18.04 ± 3.52) and left (7.11 ± 1.86) lobes of the animals of the Andiroba group (p = 0.004). There was no significant difference in dosages of AST, ALT and Gamma- GT when comparing the two groups (p > 0.05). **Conclusion:** Andiroba oil did not contribute to the protection of liver function in a rat model of liver injury induced by normothermic ischemia and reperfusion.

**Key words:** Liver. Plant oils. Ischemia. Reperfusion. Rats.

### INTRODUCTION

Liver ischemia-reperfusion (I/R) involves a complex series of processes that culminate in hepatocellular injury. It is a common consequence of liver surgery, particularly after hepatectomy and liver transplantation, complicated by microcirculatory failure, followed by necrosis and cell death<sup>1</sup>. In this context, it is speculated that oxidative damage plays an important role, including the outcome of organ transplants. Current strategies for the treatment of injury caused by liver I/R are preventive<sup>2</sup> or pharmacological<sup>3</sup> treatment. Pharmacological modulation may have a more universal application; however, despite several therapeutic formulations have been studied to reduce I/R-induced mortality, none has been fully successful<sup>4,5</sup>. Thus, the development of new strategies for prevention and treatment of liver damage due to I/R is critical to improving outcomes for patients under such conditions.

*Carapa guianensis* tree is widely known and used as a medicinal plant in the Amazon Rainforest region. In Brazil it is popularly known as "Andiroba", "carapa" and "carapinha"<sup>6</sup>. It is prized for its timber and medicinal properties and is recommended by the UN program as having large pharmaceutical potential. All parts of the tree – especially its seed's oil – have been empirically used for medicinal purposes. Traditional communities in the Amazon use soap from *Carapa guianensis* seed's oil for the treatment of skin diseases, arthritis, rheumatism, infections, wounds, bruises and as an insect repellent<sup>7</sup>. Several studies have shown anti-inflammatory, analgesic and antiallergic activity of this oil<sup>8,9</sup>. It has long been used by Amazon extractivists, Indians and river-side people also in cases of bites from venomous animals, to combat helminths, protozoa, hepatitis and other diseases of the liver<sup>3</sup>. Its chemical characterization showed the presence of myristic, palmitic, linoleic, oleic and stearic acids, as

Study conducted at the Federal University of Rio Grande do Norte – UFRN in cooperation with the University of the State of Pará – UEPA, Brazil. 1. Grantee, Scientific Initiation Program Scholarship, University of Pará (UEPA) and Federal University of Rio Grande do Norte (UFRN); 2. Professor, Surgery UEPA, Pará, Brazil; 3. Assistant Professor, Potiguar University – UnP, Natal, Rio Grande do Norte State – RN, Brazil; 4. Professor, Head, Center for Experimental Surgery, Department of Surgery, Federal University of Rio Grande do Norte – UFRN. Natal-RN, Brazil.

well as arachidonic fatty acids, some tetranortriterpenoids, such as 6- $\alpha$ -acetoxy-epoxy azadiradione, oxogedunine, gedunine, andirobine, methyl angolensate<sup>10</sup>, amines, hexacosanoic and ursolic acids, naringenin, scopoletin, some with antioxidant properties, among other components<sup>11</sup>.

Therefore, the aim of this study was to evaluate the effects of the Andiroba oil (*Carapa guianensis*) on liver function in rats undergoing liver injury induced by Normothermic I/R.

## METHODS

This study is characterized as experimental and comparative, accomplished through cooperation between UEPA and UFRN. The Laboratory of Experimental Surgery at UEPA ceded the Andiroba oil (certified by Embrapa – Brazilian Agricultural Research Corporation) and the experiments were performed at the Center of Experimental Surgery at UFRN. All animals in this study were maintained in accordance with national law in force for Animal vivisection, Federal Law 11,794.

### Saline and Andiroba groups

Twelve male Wistar rats from the vivarium of the UFRN Center for Health Sciences were divided into two groups of six each. The animals in the Andiroba group were treated with Andiroba oil (0.63 ml/kg orally) for seven days before surgery, and the other with saline 0.63 ml/kg orally.

Anesthesia was performed using intramuscular ketamine 50mg/kg associated with intraperitoneal sodium pentobarbital at a dose of 20mg/kg. Operations followed aseptic technique, and the animals, observed in individual cages with food and water ad libitum.

In all rats a 5-cm midline laparotomy was performed from the xiphoid process. After externalizing the lobes of the liver, hepatic ischemia was induced by occlusion of the vessels supplying the median and left lobes, using vascular clips (bulldog) in both groups for 45 min. After removal of the vascular clip, there was reperfusion for 60 minutes.

### Biodistribution of <sup>99m</sup>Tc-phytate

We performed Biodistribution of <sup>99m</sup>Tc-phytate to evaluate the response of liver radioactive uptake. After the I/R period, 0.1 ml (0.66 MBq) of radiopharmaceutical phytate-per technetate (<sup>99m</sup>Tc-phytate) was injected via the jugular vein. After 30 minutes the animals were killed by an overdose of anesthetic (thiopental 100mg/kg). Samples of the right and left lobes of the liver were removed, washed in 0.9% saline solution and weighed on a precision balance. The samples were placed separately in disposable test tubes

with a height of 8 cm and 1-cm diameter and introduced into an automatic gamma counter. Results were expressed in counts per minute, duly corrected to disintegrations per minute. The specific activity of each sample was calculated dividing the radioactivity absolute count – in disintegrations per minute – by the sample weight in grams. The percentage of radioactivity in each sample of liver per gram of tissue (%ATI/g) was calculated by dividing the specific activity by the total radioactivity injected into each animal.

### Laboratory Measurements

Blood samples were obtained by cardiac puncture. The blood was centrifuged at 3000 rpm for 10 min and plasma samples were stored at -40° C for analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-GT. The measurements were performed with a spectrophotometer, with CEPA Kits.

## RESULTS

Table 1 shows the descriptive results of biodistribution of <sup>99m</sup>Tc-phytate found in the right and left lobes of the livers, as well as the research for significant differences between the saline (control) and Andiroba groups.

We observed that the values of the radioactive uptake in the right liver lobe of animals treated with saline (17.53  $\pm$  2.78) were not statistically different from those recorded in the animals from the Andiroba group (18.04  $\pm$  3.52,  $p=0.461$ ). Similarly, there was no significant difference in the values of %ATI/g of the left liver lobe when the two groups were compared ( $p=0.083$ ). However, in the group treated with saline, the radioactive uptake of <sup>99m</sup>Tc-phytate was significantly higher in the non-ischemic (right) lobe in comparison with the left lobe, which underwent I/R ( $p=0.002$ ). Significant differences also occurred when comparing the right and left lobes of the group treated with Andiroba ( $p = 0.004$ ). These data are summarized in Table 1.

In Table 2 it can be seen that there was no significant difference in plasma concentrations of AST, ALT and GGT enzymes between groups ( $p>0.05$ ), indicating that treatment of animals with Andiroba had no effect on liver function tests.

## DISCUSSION

*Carapa guianensis* was recommended by the United Nations development program as a plant species with large pharmaceutical potential<sup>7</sup>. The potential of the seeds is in their oil, extracted by hand, which is administered orally or topically, also used by the pharmaceutical industry in the formulation of cosmetics<sup>7</sup>. Surveys report the

**Table 1** - Results of the percentage of radioactivity of <sup>99m</sup>Tc-phytate per gram of liver tissue (%ATI/g), comparing right and left lobes, as well as groups saline and Andiroba.

Lobe	%ATI/g		p <sup>(1)</sup>
	Saline (control)	Andiroba	
Right	17.53 ± 2.78	18.04 ± 3.52	0.461
Left	5.04 ± 0.82	7.11 ± 1.86	0.083
p <sup>(2)</sup>	0.002	0.004	

Mean ± standard deviation

1. p of the Student t test for independent samples, comparison between treatments.

2. p of the Student t test for dependent samples, comparing the right and left hepatic lobes.

**Table 2** - Descriptive values and statistical testing of the analysis of liver function tests between groups.

Liver tests	Group		p <sup>(1)</sup>
	Saline (Control)	Andiroba	
AST (U/L)	117 ± 4.6	115.0 ± 0.77	0.066
ALT (U/L)	68.2 ± 2.8	66.4 ± 1.14	0.226
Gama-GT (U/L)	2.86 ± 0.35	2.88 ± 0.17	0.90

Mean ± standard deviation

1. p of the Student t test for independent samples.

antimicrobial and anti-inflammatory potential of Andiroba oil, which can act as an adjuvant factor in the treatment of inflammations of various organs<sup>12</sup>.

The radiopharmaceutical <sup>99m</sup>Tc-phytate is used as evidence of hepatic function and in the diagnosis of liver disease through imaging tests, depending on its biodistribution<sup>7</sup>. A recent study in rats showed that splenectomy caused changes in the uptake of <sup>99m</sup>Tc-phytate by the liver, coinciding with deterioration of liver function<sup>13</sup>.

In the present study, we evaluated the effects of Andiroba oil on liver function in rats subjected to Normothermic induced ischemia-reperfusion hepatic injury. Pretreatment of animals with the oil was not sufficient to alter the deterioration of liver function assessed by uptake of <sup>99m</sup>Tc-phytate. The effect of Andiroba oil presented itself neutral in protecting liver function, probably indicating that, at the concentration used, it did not minimize the damage caused by I/R. When comparing the radiation in the left hepatic lobe (which suffered ischemia) of the control group with those who used Andiroba, we found that there was greater uptake of the radiotracer in the experimental group than in the saline one, but the difference between groups was not statistically significant. This finding indicated that the Andiroba oil has not

contributed to prevent or reduce deterioration of liver function by I/R.

A statistically significant difference in uptake was found when comparing the right and left lobes, suggesting that the ischemic lobe was highly affected in their ability to capture <sup>99m</sup>Tc-phytate. Some studies have shown that the Andiroba oil does not cause or increase mortality when applied in the abdominal cavity of mice<sup>14,15</sup> in periods of up to seven days. In this study the oral route was used. In one of the rare studies on the acute and subacute toxicity of Andiroba oil, Costa-Silva et al. used Wistar rats and found increased serum ALT levels and increased absolute and relative liver weight of treated animals, which may indicate liver toxicity<sup>16</sup>. In another study in mice, it was shown that the acute toxicity of liver oil of andiroba is practically nil when administered orally<sup>17</sup>. In this study there was a slight increase in dosage of gamma-GT after treatment with Andiroba oil, but the comparison of means showed no significant difference from a statistical standpoint. There was also a trend of reduction of ALT and AST in animals treated with Andiroba oil, but without statistical significance.

The data obtained indicate that treatment with Andiroba oil did not contribute to the protection of liver function in a rat model of induced normothermic ischemia and reperfusion liver injury.

## R E S U M O

**Objetivo:** avaliar os efeitos do óleo da andiroba (*Carapa guianensis*) na função do fígado de ratos submetidos à isquemia/reperfusão hepática normotérmica. **Métodos:** foram utilizados 12 ratos Wistar, distribuídos em dois grupos: solução salina (n=6) e andiroba (n=6). O grupo andiroba foi tratado com óleo de andiroba (0,63ml/kg, VO) durante sete dias antes do procedimento cirúrgico. A isquemia foi induzida por oclusão da vascularização dos lobos mediano e lateral do fígado, usando clip vascular, nos dois grupos, por 45min, com posterior reperfusão por 60min. Analisaram-se as dosagens de AST, ALT, Gama-GT e biodistribuição hepática do fitato- $Tc^{99m}$ . **Resultados:** não houve diferença significativa no percentual de radioatividade/grama de tecido (%ATI/g) no lobo direito do grupo salina (17,53±2,78) quando comparado com o grupo andiroba (18,04±3,52), com  $p=0,461$ , o mesmo ocorrendo no %ATI/g do lobo esquerdo do fígado quando os dois grupos foram comparados ( $p=0,083$ ). No grupo salina o %ATI/g foi significativamente mais elevado no lobo hepático direito não isquemiado (17,53±2,78), em comparação com o lobo esquerdo (5,04±0,82), que sofreu isquemia/reperfusão ( $p=0,002$ ). Diferença significativa também ocorreu na comparação entre os lobos direito (18,04±3,52) e esquerdo (7,11±1,86) dos animais do grupo andiroba ( $p=0,004$ ). Não houve diferença significativa nas dosagens de AST, ALT e Gama-GT comparando-se os dois grupos ( $p>0,05$ ). **Conclusão:** o óleo de andiroba não contribuiu para a proteção da função hepática em modelo de lesão induzida por isquemia e reperfusão normotérmica do fígado de ratos.

**Descritores:** Fígado. Óleos vegetais. Isquemia. Reperfusão. Ratos.

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