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COPAIBA OIL-RESIN IMPROVES PONDERAL DEVELOPMENT AND ATTENUATES INFLAMMATION IN ADIPOSE TISSUE IN A MODEL OF HEPATIC CIRRHOSIS

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Abstract: The aim of this study was to evaluate the effect of copaiba oil-resin on body weight, adiposity and inflammatory markers in adipose tissue in animals with liver cirrhosis. Male Wistar rats were randomly divided into two groups: Liver Cirrhosis (CH, n=7) and CH + copaiba oil-resin (CH+OC, n=7). Liver cirrhosis was induced by intraperitoneal (i.p.) administration of thioacetamide at a dose of 100mg/kg/twice a week; and copaiba oil-resin supplementation was administered, via gavage, at a dose of 200mg/kg/day for 8 weeks. The data show that copaiba oil-resin supplementation was able to improve weight gain and adiposity in animals with liver cirrhosis, in addition to increasing the levels of IL-10, an anti-inflammatory cytokine, and reducing the levels of IL-10. 1β, pro-inflammatory cytokine. These findings suggest a beneficial effect of copaiba oil-resin on adipose tissue in a liver cirrhosis model.
Keywords: liver cirrhosis, inflammation, copaiba oil-resin, adipose tissue.

INTRODUCTION

Liver cirrhosis is considered the main chronic liver disease, being a common cause of death in the world, having a high degree of morbidity and mortality (LOZANO et al., 2012). Its symptoms are nonspecific and include anorexia, weight loss, weakness and, in advanced disease, debilitation. Children with liver diseases must have their growth constantly monitored (SHEPHERD, 2004). The development of malnutrition in patients with liver cirrhosis is multifactorial and is primarily due to a decrease in food intake (MATTOS et al., 2003). As a result of low nutrient intake, there is initially a reduction in adipose tissue, which was associated with decompensation in cirrhotic patients and worse prognosis (RODRIGUES et al., 2019).

Adipose tissue, recognized as an endocrine organ for secreting a variety of

biologically active substances, participates in the process to maintain energy homeostasis, as well as promoting regulatory actions in metabolic and inflammatory systems (LOU; LIU, 2016). Changes in adipose tissue that lead to dysregulation in adipokine secretion affect liver diseases, on the other hand, liver diseases such as cirrhosis affect adipose tissue (RODRIGUES et al., 2019)

Copaiba oil-resin is extracted from the trunk of the copaiba tree, found mainly in the Amazon basin and cerrado biomes (PINHEIRO et al., 2017), and consists of a mixture of sesquiterpenes and diterpenes (LEANDRO et al., 2017). 2012). It has antioxidant, anti-inflammatory, antitumor properties, among others (AMES-SIBIN et al., 2018). However, in general, its use is done empirically and there are not any data in the literature on its effects on adipose tissue in conditions of liver cirrhosis.

Since liver cirrhosis is a catabolic disease characterized by a reduction in adipose tissue mass, and copaiba oil-resin is a byproduct used for various purposes, the objective of this study was to evaluate the effect of copaiba oil-resin on weight and adiposity. body, as well as inflammatory markers in adipose tissue in animals with liver cirrhosis.

METHODOLOGY

ANIMALS AND EXPERIMENTAL PROTOCOL

Male Wistar rats (from the Central Animal Facility of the Federal University of Mato Grosso - UFMT) were randomly assigned to two groups: Liver Cirrhosis (CH, n=7) and CH + copaiba oil-resin (CH+OC, n=7). Cirrhosis was induced by intraperitoneal (i.p.) administration of thioacetamide, a hepatotoxic drug, at a dose of 100mg/kg/twice a week for eight weeks; and copaiba oil-resin supplementation was administered at a dose of 200mg/kg/day, via gavage, for eight weeks. The CH group received vehicle, via gavage, in a volume equivalent to that offered to the CH+OC group. A control group, without interventions, was used as a reference for a healthy condition. The animals were kept in collective boxes, in an environment with controlled temperature (24±2ºC) and humidity (55±5%) and light-dark cycle (12-12hs). At the end of the experiment, the animals were sacrificed after anesthesia with thiopental (50 mg/kg) and blood and epididymal adipose tissue samples were collected. The study protocol was approved by the Ethics Committee on the Use of Animals (CEUA), # 23108.039273/2019-60, and followed the recommendations on Ethical Principles in Animal Experimentation of the Brazilian College of Animal Experimentation (COBEA), in Law 11,794/2008.

BIOCHEMICAL SERUM ANALYSIS

Liver function was assessed using serum aspartate aminotransferase (AST) (Bioclin, Cat# k048) and alanine aminotransferase (ALT) (Bioclin, Cat# k049) enzymes. In addition, C-reactive protein (CRP) levels (Bioclin, Cat# k059) were evaluated.

MORPHOLOGICAL AND NUTRITIONAL PARAMETERS

Food and caloric intake, as well as weight gain and the amount of body fat of the animals were evaluated. Epididymal, mesenteric and retroperitoneal fat deposits were dissected. The sum of the deposits is considered visceral fat. Visceral fat normalized by body weight [(epididymal+retroperitoneal+mesenteric)/ body weight x 100] was considered an adiposity index (NASCIMENTO et al., 2011).

INFLAMMATORY PROFILE

Interleukin (IL-) 6 (R3 D Systems ELISA, Cat. No. DY506), IL-1β (R3 D Systems ELISA, Cat. Number: DY201) and IL-10 (R3 D

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Systems ELISA, Cat. No. DY 522), in adipose tissue, were obtained using commercial kits specific for rats, following the manufacturer's recommendations.

STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation. Comparisons between groups were performed using Student's t test. The level of significance considered was 5%. A control group, of the same age, was used as a reference to the healthy condition and did not participate in the statistical analysis.

RESULTS AND DISCUSSION

Liver cirrhosis was induced by administering the hepatotoxic drug thioacetamide, a drug widely used for the development of pathophysiological changes in the liver, and its use to promote cirrhosis is well established in the literature (SILVA et al., 2021; GREGOLIN et al, 2021; SOUZA et al., 2021).

In the present study, we evaluated biochemical indicators of liver function, ALT and AST. ALT is found mainly in the kidney and liver, exclusively in the cytoplasm of cells, while AST is found in the mitochondrial and cytoplasmic form, present mainly in the liver, kidney, heart and skeletal muscle. Due to lesions that affect the hepatocytes, as in cirrhosis conditions, there is extravasation of aminotransferases from the tissue to the bloodstream, making possible the serum diagnosis of liver damage (WILLIAMS; HOOFNAGLE, 1988). Our data show that copaiba oil-resin supplementation did not modulate ALT and AST levels (Table 1); however, TEIXEIRA et al (2013) showed that the administration of a dose of 3.8 ml/kg of copaiba oil-resin reduced the transaminase levels of animals with liver damage induced by acetaminophen. The same was demonstrated by Noguchi et al. (2002), where the administration of 0.63 ml/kg of this oil-resin, for five days, led to a reduction in ALT and AST levels, when compared to control animals.

According to Cervoni et al. (2012), systemic inflammation is common in advanced cirrhosis. C-reactive protein plays an important role in the reabsorption of necrotic material and regulation of inflammatory processes, its levels may increase after tissue damage. Supplementation with copaiba oil-resin reduced CRP levels, corroborating its anti-inflammatory effect (LEANDRO et al., 2012; AMES-SIBIN et al., 2018).

At the beginning of the experiment, the animals had similar weights. After eight weeks of intervention, rats supplemented with copaiba oil-resin had higher final weight and body fat, which was accompanied by increased food intake (Table 2). As mentioned earlier, weight loss is present in individuals with liver cirrhosis, and can be caused by decreased food intake due to early satiety due to nausea and vomiting, or by changes in digestion and absorption processes (ANASTÁCIO et al., 2012). Considering that the animals, in this study, are in the growth phase, and, according to Sheperd (2004), children and adolescents must have their growth constantly monitored to avoid growth deficit, our data indicate that the supplementation with the oil-resin of copaiba was able to improve food intake promoting more adequate weight development (Figure 1).

Some pro-inflammatory cytokines such as IL-1 and IL-6 are associated with anorexia and weight loss, appearing in high levels (DELANO; MOLDAWER, 2006). The events that trigger the increase in the production of these cytokines, in a condition of liver cirrhosis, are still unclear. IL-6 is a cytokine with multiple functions, being implicated in the pathogenesis of pathological processes

Variables	Groups	
	CH	CH+OC
AST (mg/dL)	460 ± 108	406 ± 92
ALT (mg/dL)	148 ± 20	157 ± 23
PCR (mg/dL)	3,7 ± 1,04	1,8 ± 0,62*

Table 1. biochemical parameters.

CH: liver cirrhosis group; CH+OC: liver cirrhosis group supplemented with copaiba oil-resin (200 mg/kg/day for 8 weeks). AST: aspartate aminotransferase; ALT: alanine aminotransferase; CRP: C-reactive protein. Data presented as mean ± standard deviation, n = 7. Student's t test was used. * = P<0.05

Variables	Groups	
	CH	CH+OC
starting body weight (g)	322 ± 14	322 ± 25,5
final body weight (g)	369 ± 31,1	409 ± 34,5*
Weight gain (g)	47,6 ± 22	87 ± 16,3*
food intake (g)	20,4 ± 1,37	23,7± 1,12*
caloric intake (kcal)	77,8 ± 5,19	90,1 ± 4,27*
epididymal fat (g)	5,03 ± 1,00	6,59 ± 1,47*
mesenteric fat (g)	4,49 ± 0,95	6,57 ± 1,56*
retroperitoneal fat (g)	6,82 ± 1,83	9,73 ± 2,77*
visceral fat (g)	16,3 ± 3,19	22,9 ± 5,00*
adiposity index (%)	4,55 ± 0,47	5,74 ± 1,21*

Table 2. Morphological and nutritional data of the experimental groups.

CH: liver cirrhosis group; CH+OC: liver cirrhosis group supplemented with copaiba oil-resin (200 mg/kg/day for 8 weeks). Data presented as mean ± standard deviation, n = 7. Student's t test was used. * = P<0.05

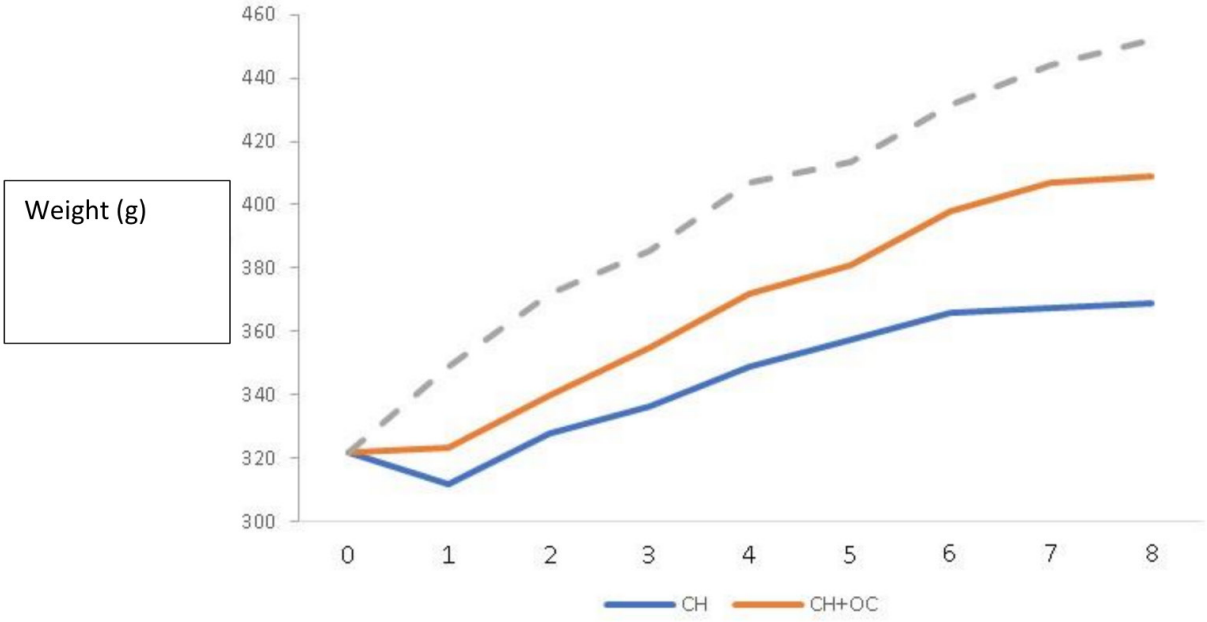

Figure 1. Evolution of weight of experimental groups. CH: liver cirrhosis group; CH+OC: liver cirrhosis group supplemented with copaiba oil-resin (200 mg/kg/day for 8 weeks). Dotted line represents values of a control group, of the same age, used only as a reference for normality.

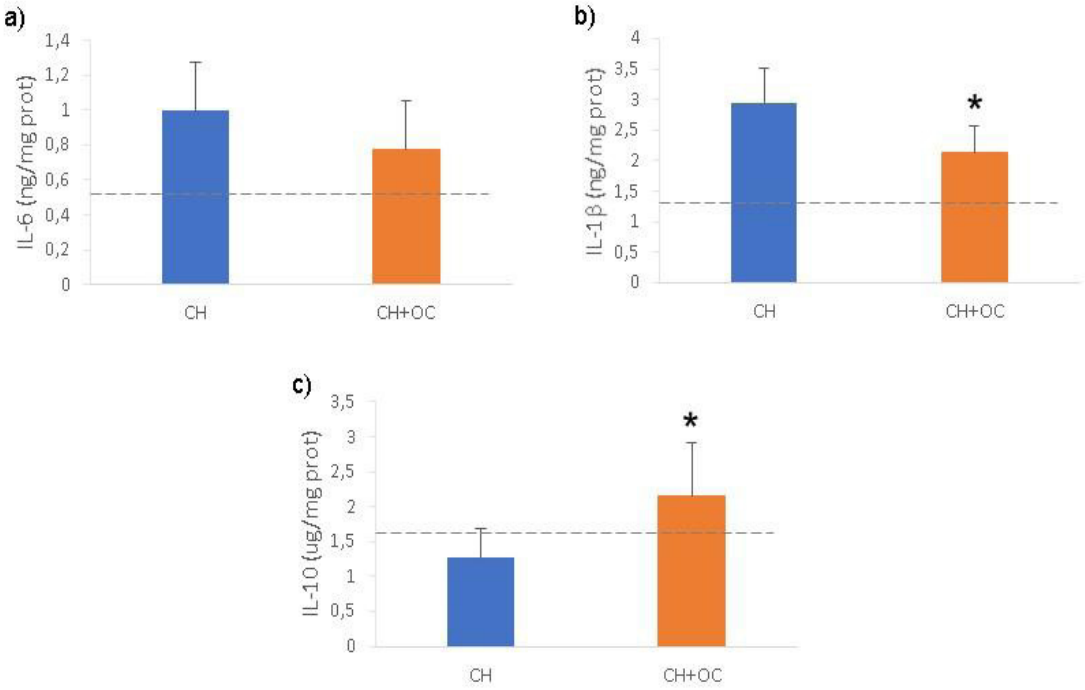

Figure 2. Protein concentration of cytokines in epididymal adipose tissue. a) Interleukin 6 (IL-6); b) Interleukin 1β (IL-1β); c) Interleukin 10 (IL-10). CH: liver cirrhosis group; CH+OC: CH supplemented with copaiba oil-resin (200 mg/kg/day for 8 weeks). Dotted line represents values of a control group, of the same age, used as a reference for normality. Data expressed as mean and standard deviation, n = 7. Student's t test was used. * = P<0.05.

associated with inflammation (KOJIMA et al., 2013). Approximately one third of plasma IL-6 is attributed to production in white adipose tissue (KWON and PESSIN, 2013). Activation of IL-6 appears to decrease appetite and food consumption, and, in cancer patients, induce muscle degradation even when there is adequate nutrition (PFITZENMAIER et al., 2003; LEE et al., 2004; ONESTI; GUTTRIDGE, 2014, 2014).). In addition, an association has been demonstrated between increased IL-6 and nutritional risk in pediatric patients with chronic liver disease of various causes (SANTETTI et al., 2015). Furthermore, a study carried out by Wunsch et al. (2013), demonstrated a positive correlation between IL-6 and clinical severity in liver diseases. The anti-inflammatory action of copaiba oil-resin has been reported; however, in this study, copaiba oil-resin supplementation did not significantly modulate IL-6 levels (Figure 2).

IL-1β is an important pro-inflammatory cytokine that leads to insulin resistance, in addition to interfering with the endocrine and immune functions of adipose tissue in a paracrine fashion (Wu; Cheung; Cheng, 2020). Under normal conditions, IL-1 levels are low, however, IL-1 binds to its receptors with high affinity, thus low concentrations can activate a biological response (SPULBER, 2010). Elevated levels of IL-1β may be related to chronic oxidative stress. Reactive oxygen species released by adipose tissue can promote unregulated production of this interleukin (FURUKARA et al., 2004). Kaurenoic acid, a diperten present in copaiba oil-resin, has been shown to have an anti-inflammatory effect against ischemia-reperfusion injury in a skin flap model in rats, decreasing the expression of TNF-α and IL-1 (SILVA et al. al., 2015). Our data show that oil-resin supplementation was effective in reducing IL-1β levels in the adipose tissue of animals with liver cirrhosis (Figure 2), indicating a decrease in the inflammatory state in the tissue. No studies were found that evaluated the effect of copaiba oil-resin and IL-1β levels.

Interleukin 10 is a cytokine produced mainly by activated M2-type macrophages and type 2 (Th2) T helper cells, but also by adipose tissue (CHOI et al., 2007; ITOH et al., 2011; YE, 2013). It is the main immunoregulatory cytokine with anti-inflammatory properties, which suppresses the signal transduction of pro-inflammatory cytokines, TNF, IL-6 and IL-1, thus leading to a lower recruitment of macrophages to the tissue (MOCELLIN et al, 2003). In this sense, Gotoh et al. (2012) showed that IL-10 treatment improves inflammation in adipose tissue and liver, in addition to improving hepatic lipid and glucose metabolism in obese mice. However, the exact role of IL-10 in adipose tissue biology and energy homeostasis is still unknown. In the present study, copaiba oil-resin supplementation was efficient in increasing IL-10 levels in the adipose tissue of animals with cirrhosis.

CONCLUSION

This study presents unprecedented data on the effects of copaiba oil-resin on the adipose tissue of animals with liver cirrhosis. Supplementation with copaiba oil-resin promoted weight gain and adiposity, improving the development of cirrhotic animals. Copaiba oil-resin anti-inflammatory activity has been demonstrated in adipose tissue, in addition to improving systemic inflammation, decreasing CRP levels. Thus, our data suggest a beneficial effect of copaiba oil-resin on the development and inflammatory state in cirrhotic animals.

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