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CARDIAC

Copaiba oil attenuates right ventricular remodeling by decreasing myocardial apoptotic signaling in monocrotaline-induced rats

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Short title: Copaiba oil reduces myocardial apoptosis

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Conflicts of Interest

The authors declare that they have no conflict of interest.

ABSTRACT

There is an increase in oxidative stress and apoptosis signaling during the transition from hypertrophy to right ventricular (RV) failure caused by pulmonary arterial hypertension (PAH) induced by monocrotaline (MCT). In this study, it was evaluated the action of copaiba oil on the modulation of proteins involved in RV apoptosis signaling in rats with PAH. Male Wistar rats (±170g, n=7/group) were divided into four groups: control, MCT, copaiba oil, and MCT+copaiba oil. PAH was induced by MCT (60 mg/kg i.p.) and, seven days later, treatment with copaiba oil (400 mg/kg by gavage) was given for 14 days. Echocardiographic and hemodynamic measurements were performed, and the RV was collected for morphometric evaluations, oxidative stress, apoptosis, and cell survival signaling, and eNOS protein expression. Copaiba oil reduced RV hypertrophy (24%), improved RV systolic function, and reduced RV end-diastolic pressure, increased total sulfhydryl levels and eNOS protein expression, reduced lipid and protein oxidation, and the expression of proteins involved in apoptosis signaling in the

RV of MCT+copaiba oil as compared to MCT group. In conclusion, copaiba oil reduced oxidative stress, and apoptosis signaling in RV of rats with PAH, which may be associated with an improvement in cardiac function caused by this compound.

Keywords: monocrotaline; pulmonary arterial hypertension; oxidative stress; copaiba oil; apoptosis

1 - INTRODUCTION

The Amazon forest is blessed with an extraordinary biological diversity and offers a wide variety of products that could be used as sources of material with pharmacological activity to treat various diseases. Copaiba oil is among these products, especially for its medicinal properties such as antioxidant, antitumoral, anti-inflammatory agent [1-3]. No genotoxic or mutagenic effect was reported for this oil [4], and it has been used in folk

medicine since the 19th century and, nowadays, it is easily found in drugstores and widely available on international websites. However, despite its extensive use in folk medicine, there is only one study demonstrating the effect of copaiba oil on pulmonary arterial hypertension (PAH) [5].

PAH is a severe and rapidly progressive disease characterized by pulmonary vascular remodeling, elevated pulmonary arterial pressure, and right-sided heart failure [6]. Right ventricle (RV) hypertrophy occurs due to compensatory mechanisms to the increased afterload. Nevertheless, persistent overload results in RV dysfunction and failure [7]. There are some murine models used to mimic PAH, but there is no perfect preclinical model that completely replicates human PAH [8]. The monocrotaline (MCT) is the most used model (about 63% of the papers) because of its practicality, presenting similarity with numerous pathological changes observed in humans [8]. MCT is a toxic alkaloid that induces a severe PAH culminating in RV hypertrophy and failure.

Oxidative stress plays an essential role in the development of PAH with reduced systemic antioxidant defenses and increased lipid peroxidation in patients with this disease [9]. According to Reddy et al., a stressed RV due to pressure overload is more susceptible to oxidative stress [10]. Moreover, both oxidative stress [11], and ventricular remodeling [12], seen during MCT-induced PAH, are related to cardiomyocyte's apoptosis. In fact, oxidative stress contributes to pathological RV remodeling, through the activation of mitogen activated

protein kinases (MAPKs), such as c-Jun-N-terminal kinase (JNK), which stimulates apoptosis [13]. Another way to evaluate apoptosis is through Bax/Bcl-2 ratio. Bax is a pro-apoptotic protein, that is translocated to the mitochondria, where induces cytochrome c release [14]. On the other hand, Bcl-2 is an anti-apoptotic protein that attenuates cellular injury by inhibiting the Bax [15] and cytochrome c [16] translocations. Moreover, glycogen synthase kinase-3β (GSK 3β) is other protein that functions in a wide range of cellular processes, including apoptosis.

However, its activity is inhibited by protein kinase B (Akt) phosphorylating serine at position 9 [17].

In addition, Akt is an important pro-survival protein and well-established regulator of apoptosis [18]. Besides its anti-apoptotic effects, Akt also mediates the activation of eNOS, leading to increased NO production. According to the literature, serine/threonine protein kinase Akt activates eNOS by direct phosphorylation of serine 1177, thereby leading to NO production [19]. An improvement in NO production could represent a protective mechanism against the increased afterload seen in the RV of PAH patients [20].

RV dysfunction in PAH patients indicates a bad prognosis, resulting in patient's higher morbimortality [7]. Several treatments have demonstrated to increase PAH survival. However, unfortunately the cure for patients with this lethal syndrome remains far away.

In view of the above described, it can be hypothesized that copaiba oil exerts cardioprotective effects, preserving RV function, through the modulation of proteins involved in cell survival and death in an animal model of PAH.

2 - METHODS

2.1 - Drugs and reagents:

Copaiba oil was purchased at a drugstore and its composition was previously analyzed (4). Monocrotaline and all other drugs/reagents were purchased from Sigma Chemical Co.,

St. Louis. Ketamine hydrochloride was purchased from König Lab S.A., SP, Brazil and xylazine was purchased from Virbac do Brasil I.P., SP, Brazil.

2.2 - Animals, induction of pulmonary hypertension, and groups:

All procedures were approved by the institutional animal ethics committee (protocol number: 31765). In total, 28 male Wistar rats (weighing 170 ± 20 g) from Center for

Reproduction and Experimentation of Laboratory Animals (CREAL) of the Universidade Federal do Rio Grande do Sul were studied. They were kept at 20-22°C and with a 12:12h dark/light photoperiod. All animals had *ad libitum* access to regular rodent chow and water and the experiments were conducted in accordance the Guide for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services, NIH Publication No. 86-23), and with institutional guidelines.

The animals were divided into four experimental groups: control (CO), copaiba oil (O), monocrotaline (MCT), monocrotaline+copaiba oil (MCT-O). On the first day, PAH was induced by a single *in bolus* MCT injection (60 mg/kg i.p.), as described elsewhere [21]. One week after PAH induction, animals in the O and MCT-O groups received by gavage once a day for 14 days: copaiba oil (400 mg/kg)[5]. During this period, animals from CO and MCT groups received water by gavage. The dose of 400 mg/kg correspond to a volume of 0.63 mL/kg of copaiba oil.

2.3 - Echocardiographic analysis

Twenty-one days after MCT injection, echocardiographyc analysis was performed. Animals were anaesthetized (ketamine, 90 mg/kg; xylazine, 20 mg/kg, i.p.) and placed in the left lateral decubitus position to obtain cardiac images. An EnVisor Philips system (Andover, MA, USA) was used, with a 12-13 MHz transducer, by a trained operator with experience in

small animal echocardiography [22]. The following parameters were measured: diastolic and systolic RV diameter (RVDd and RVSd), area of the RV in diastole and systole and tricuspid annular plane systolic excursion (TAPSE).

2.4 - Hemodynamic Evaluation

Hemodynamic parameters were measured after echocardiographic analysis, just

before animals were sacrificed. The right jugular vein was catheterized with a PE 50 catheter connected to a transducer Strain Gauge (NarcoBiosystem Pulse Transducer RP-155, Houston, TX), connected to a pressure amplifier (HP 8805C, Hewlett Packard, Palo Alto, CA). Right ventricular end diastolic (RVEDP) and systolic pressure (RVSP), heart rate, as well as contractility and relaxation indexes (dP/dt max and dP/dt min, respectively) were measured. Data were stored on a computer equipped with an analog-to-digital conversion board (Windaq Data Acquisition System, DataQ Instruments, Akron, Ohio, USA). All the data were recorded at a

sampling rate of 1,000 Hz [23].

2.5 - Morphometric analysis and preparation of heart homogenates

After cardiovascular evaluations, rats were killed by decapitation and the RV was harvested for morphometric and biochemical measurements. RV was weighed to determine the cardiac hypertrophy index (RV weight/body weight). Then, the RV was homogenized in an ultra-Turrax blender using 1 g of tissue in 5 mL of 150 mmol/L potassium chloride added to 20 mmol/L phosphate buffer, pH 7.4. The homogenates were centrifuged at 3000 rpm for 20 min at 4°C [24]
.

2.6 - Determination of total ROS levels

ROS generation was measured by DCFH-DA fluorescence emission (Sigma-Aldrich, USA). Dichlorofluorescein diacetate is membrane permeable and is rapidly oxidized to the highly fluorescent 2,7-dichlorofluorescein (DCF) in the presence of intracellular ROS. The samples were excited at 488 nm and emission was collected with a 525 nm long pass filter. It was expressed as nmol per milligram of protein [25].

2.7 - Evaluation of lipid peroxidation

Lipid peroxidation was determined in RV homogenates by the thiobarbituric acid reactive substances (TBARS) method, as described by Ohkawa and colleagues (1979). Briefly,

samples of VD homogenate and incubated with sodium dodecyl sulfate (8.1%), acetic acid (20%), and thiobarbituric acid (0.8%) in a 100ºC water bath during 60min. Then, the supernatant was collected, and the absorbance was measured using a spectrophotometer (535 nm). Malondialdehyde was used as standard and the results are expressed as nmol per mg protein [26].

2.8 - Determination of protein oxidation (carbonyls)

Tissue samples were incubated with 2,4-dinitrophenylhydrazine (DNPH 10 mM) in a 2.5 mol/L HCl solution for 1 h at room temperature in the dark. Samples were vortexed every 15 min. Subsequently, a 20% trichloroacetic acid (w/v) solution was added and the solution was incubated on ice for 10 min and centrifuged for 5 min at 1000 g to collect protein precipitates. An additional wash was performed with 10% trichloroacetic acid (w/v). The pellet was washed three times with ethanolethyl acetate (1:1) (v/v). The final precipitates were dissolved in 6 mol/L guanidine hydrochloride solution, and incubated for 10

min at 37°C, and the absorbance was measured at 360 nm [27]. The concentration of carbonyls was expressed in nmol per mg protein.

2.9 - Determination of total sulfhydryl groups (-SH)

The total amount of sulfhydryl groups in the RV homogenates was determined according to Sedlak and Lindsay [28]. The optical density at 412 nm was read in a

spectrophotometer against an appropriate blank. The concentration of total sulfhydryl groups was expressed in nmol per mg protein.

2.10 - Antioxidant enzyme activities

Superoxide dismutase (SOD) activity, expressed as units per mg protein, was based on the inhibition of the reaction of superoxide with pyrogallol. The superoxide radical is generated by the autooxidation of pyrogallol in an alkaline medium. Superoxide dismutase activity was determined by measuring the velocity of oxidized pyrogallol formation [29].

Catalase activity was determined in heart homogenates, by following the decrease in 240nm absorption of hydrogen peroxide. It was expressed as nmol of hydrogen peroxide reduced per minute per milligram of protein[30]

Glutathione peroxidase (GPx) activity, expressed as nmol of hydroperoxide peroxide reduced per min.mg protein, was measured following NADPH oxidation at 340 nm in a reaction medium containing 0.17 mmol/L reduced glutathione, 0.2 U/mL glutathione reductase, and 0.5 mmol/L tBOOH[31].

The protein concentration was measured by the method of Lowry et al.[32] .

2.11 - Western blot analysis

RV homogenate samples were mixed with sample loading buffer and separated under reducing conditions on 12% SDS-polyacrylamide gels. Proteins were electrotransferred onto polyvinylidene fluoride (PVDF) membranes (Immuno-Blot 0.2 µm, BioRad). The membranes were processed for immunodetection using the following antibodies: eNOS, pAkt, Akt, pJNK, JNK, pGSK-3β , GSK-3β, Bax and Bcl2 (Santa Cruz Biotechnology,Santa considered significant.

Cruz, CA, USA or Cell Signaling Technology, Beverly, MA, USA). The bound primary antibodies were detected using anti-rabbit or anti-mouse conjugated horseradish peroxidase (HRP)-conjugated secondary antibodies and bands were revealed by chemiluminescence. The autoradiographic images generated were quantitatively analyzed to assess protein levels with an image densitometer (Image Master VDS CI, Amersham Biosciences Europe). The molecular weights of the bands were determined by reference to a standard molecular weight marker (RPN 800 rainbow full range Bio-Rad). The results from each membrane were normalized by the Ponceau red method [33].

2.12 - Statistical Analysis

Data are shown as mean ± standard deviation. Statistical analysis was performed using two-way ANOVA followed by the Student-Newman-Keuls post-hoc test. Pearson correlation was used to study the association between variables. P values less than 0.05 were

3 - RESULTS

3.1 - Morphometric and hemodynamic parameters

MCT group showed a 65% increase in RV hypertrophy when compared to the control group. However, RV hypertrophy was significantly reduced (25%) in the MCT-O group as compared to MCT (Table 1).

No significant differences in heart rate were found among groups. However, it was

observed a significant increase in RVEDP (22%), in RVSP (100%), in dP/dtmin (84%), and in dP/dtmax (79%) in MCT animals when compared to control. The animals from the MCT O group had a significant ($P<0.05$) reduction in all these parameters (Table 1).

3.2 - Echocardiographic parameters

The echocardiographic results are shown in Table 2. In the animals from MCT group, it was found an impairment in RV systolic function, observed by the reduction of TAPSE (32%), which was prevented by the treatment with copaiba oil (MCT-O group). Monocrotaline treatment promoted an increase in the RVDd (45%), RVSd (38%), RV diastolic area (69%), and RV systolic area (66%), and copaiba oil significantly ($P<0.05$) reduces all these parameters in the MCT-O group.

3.3 - Oxidative stress parameters

MCT induced a 23% increase in ROS levels, 9% in TBARS, and 74% in carbonyls ($P<0.05$% vs

CO). By contrast, PAH animals that received copaiba oil exhibited a 9% decrease in TBARS, and 44% in carbonyl levels compared to MCT group (Figure 1). MCT promoted a reduction of 39% in total-SH levels in the MCT group as compared to CO (Figure 2A), as well as in the SOD (58%), and GPx (32%) activities (Figures 2B and 2D). Copaiba oil induced a 42% in total-SH levels and 44% increase in the SOD activity in MCT-O group

as compared with MCT (Figure 2A and 2B). No difference was found in CAT activity among groups (Figure 2C).

3.4 - Cell survival and death signaling

There was a significant ($P<0.05$) reduction in the eNOS protein expression (57%), and in the pAkt/Akt ratio (43%) in the MCT group in comparison to control (Figures 3A and 3B,

respectively). On the other hand, the expression of these proteins was found to be similar between O and CO groups. By contrast, treatment with copaiba oil was able to enhance 32% pAkt/Akt ratio, and 44% eNOS expression in the MCT-O group in comparison to MCT group ($P<0.05$).

The pGSK-3β/GSK-3β ratio was significantly decreased (53%) in the MCT group, as compared to CO (Figure 3D). MCT induced a decrease in GSK-3β phosphorylation ($P<0.05$% vs CO). By

contrast, PAH animals that received copaiba oil exhibited a significant increase in this parameter (Figure 3D).

The pJNK/JNK (Figure 3C) and Bax/Bcl2 (Figure 3E) ratios were significantly increased (22% and 40%, respectively) in the MCT group, as compared to CO. However, treatment with copaiba oil was able to reduce these apoptotic markers in 51% (pJNK/JNK) and 48% (Bax/Bcl2).

A negative correlation was found between TAPSE and Bax/Bcl2 ratio ($r=-0.860$, $P<0.05$). Conversely, a positive correlation was found between RVEDP and Bax/Bcl2 ratio ($r=0.8663$, $P<0.05$).

4 - DISCUSSION

This study yielded several novel findings about the effects of copaiba oil in a model of PAH. In this study, the oral administration of copaiba oil significantly attenuates RV remodeling and apoptosis signaling in this model, representing the first report of a cardioprotective role of copaiba oil on the signaling for death and cell survival.

Our findings are in accordance with prior reports showing morphometric changes

induced by MCT [34, 35]. However, copaiba oil ameliorates RV remodeling, as can be observed by a reduction in RV weight to body weight ratio in MCT-O group. This result corroborates a previous report of our group, which was in a preventive regimen of treatment with copaiba oil [5]. RV function was verified by cardiac catheterization. As expected, MCT increased RVEDP, RVSP, dP/dt min (a parameter of relaxation), and dP/dt max (a parameter of contractility). These hemodynamic changes could be caused by an increase in pulmonary vascular resistance, characteristic of this experimental model [36], which induces an increase in RV afterload and a consequent RV remodeling, resulting in functional impairment. On the other hand, data obtained from the group MCT-O clearly demonstrate an improvement of all hemodynamic parameters by copaiba oil treatment, which could suggest an improvement of RV function. These results has a crucial relevance in the PAH treatment, since that RV dysfunction represents a bad prognostic [37], and copaiba oil could avoid it. The hemodynamic results of this study goes in line with the observations of echocardiographic and morphometric results, showing that copaiba oil markedly reduced RV systolic and diastolic areas, and RV systolic diameter. This is reflected in the TAPSE data, which is a very important measurement of RV systolic function. These effects are very important, since RV dysfunction has been reported as a risk factor in PAH [37]. In fact, according to Forfia et al [38] TAPSE has an important prognostic significance in a PAH population. In that study, authors related a three- to fourfold increased risk of death in patients with reduced TAPSE [38],

demonstrating the importance of this echo-derived assessment of RV function. In accordance with Forfia data, it was found, in the present study, a reduced TAPSE in MCT group, which was recover with copaiba treatment to PAH animals.

In this context, it has been reported that increases in oxidative stress are involved with the development of cardiac hypertrophy and its progression to heart failure [39]. Indeed, we observed an increase in oxidative stress markers in the MCT group, which was accompanied

by an increase in RV mass and RV dysfunction. These oxidative stress results are in agreement with others [5, 23, 40]. However, copaiba oil reduced protein oxidation and lipoperoxidation, and increased antioxidant defenses in PAH animals. Thus, it can be suggested that morphometric, echocardiographic, and hemodynamic improvements, observed in the present study, following copaiba oil treatment could be explained by an attenuation of oxidative stress in RV. Therefore, copaiba oil is a promising RV-targeted therapy for PAH.

In the present study, GPx activity was reduced in the MCT group, and there was no significant difference between the CO group and MCT-O. This result may indicate that copaiba oil prevents the reduction of this enzyme activity caused by MCT. Moreover, it was observed an increase in the SOD activity in the MCT-O animals. SOD is the most important enzyme that responds to ROS [41]. Our results are in accordance with others, which demonstrated that copaiba oil presents antioxidant properties, decreasing protein carbonyl groups and ROS in liver [42], and has an anti-lipoperoxidation action associated with intense antioxidant action during ischemia and reperfusion of randomized cutaneous flaps [43]. As seen in our previous study, copaiba oil is a mixture of sesquiterpenes and diterpenes, with beta caryophyllene being the main constituent of this oil [5]. This chemical composition could explain our results about oxidative stress. In fact, terpenes are potential mediators of antioxidant defense in vivo [44], which also contributed to improve total-SH levels in MCT-O

group. This result could be contributing to restore an appropriate redox balance in these animals.

Redox homeostasis is an essential balance, since an increase in oxidative stress can result in apoptotic cell death [45]. Given that copaiba oil ameliorated PAH-induced oxidative stress, it was hypothesized that it inhibited apoptosis of RV from MCT treated rats. Indeed, rats from MCT group, which are at the highest oxidative stress present higher apoptosis

markers than their normal counterparts. Moreover, it was found a reduction on oxidative stress and apoptosis pathways in the PAH animals treated with copaiba oil.

Akt activation inhibits cardiomyocyte apoptosis and promotes cell survival [46]. In the present study, the ratio of expression of phosphorylated Akt to total Akt was significantly lower in RV samples from PAH animals, being normalized by copaiba oil treatment. Moreover, the production of NO is stimulated by eNOS via Akt- phosphorylation at Ser1177 [47]. In fact, it was also observed a reduction in the eNOS expression in the MCT group. Conversely, copaiba oil increases both, Akt phosphorylation and eNOS expression. NO is very important in the regulation of excitation-contraction coupling in cardiomyocytes [48]. An increase in the eNOS expression by copaiba oil, probably through Akt signaling pathway activation, can be enhancing NO production, which could be implicated in the regulation of RV function observed in this study. However, a limitation of this study is that the proteins involved in calcium handling were

not evaluated. These proteins are directly involved in the regulation of cardiac contractility.

In addition, phosphorylated Akt inhibits GSK-3β[17], another protein quantified in this study. It was observed that copaiba oil increases GSK-3β phosphorylation, diminishing the apoptotic activity of this protein. Thus, both Akt and GSK-3 β results demonstrate the anti apoptotic effect of copaiba oil in the RV of PAH rats.

It was also quantified the expression of JNK, which is a stress-activated protein kinase, from the MAPK family, and plays an important role in many cellular events, including apoptosis [49]. It was observed an increase on the p-JNK/JNK ratio in the MCT group, and this was abolished by copaiba oil treatment, probably due to its antioxidant properties.

The Bax/Bcl2 ratio represents that cells are more prone to apoptosis [50]. Other studies

showed that overproduction of ROS increases the Bax/Bcl-2 ratio [51]. In the present study, Bax/Bcl2 ratio is increased in the MCT group and it was reduced in the MCT-O. These results suggest that the Bax/Bcl-2 ratio is involved in the apoptosis induced by MCT. However, copaiba oil may prevent signaling for apoptosis, demonstrating a protective role in cardiomyocytes.

Cardiac apoptosis leads to continuous loss of contractile units, contributing to heart failure [52]. Conversely, copaiba oil was able to protect RV against apoptosis. Indeed, we found a

negative correlation between TAPSE and the Bax/Bcl2 ratio, and a positive correlation between RVEDP and the Bax/Bcl2 ratio. These results suggest that an increase in the apoptosis signaling is related to RV function impairment. On the other hand, is notable the efficacy of copaiba oil to protect RV in PAH.

5 - CONCLUSION

Our data strongly suggest that the increase of ROS and oxidative stress is involved in the development of MCT-induced PAH and RV remodeling, being cardiomyocyte's apoptosis a crucial event to the progression to heart failure (Figure 4). Copaiba oil, by reducing oxidative stress, may attenuate apoptosis signaling and contribute to RV function preservation. However, other effects of copaiba oil than the antioxidant action cannot be ruled out, and may also contribute to its cardioprotection.

PAH remains a progressive and incurable pathology, which progresses to RV dysfunction and failure, besides of the current therapeutics. Copaiba oil emerges as a complementary therapy, that has the potential to impact on the pathobiology of adverse RV remodeling, and could be of great advance in the management of this disease.

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7 - FIGURE CAPTIONS

Fig 1: Effects of copaiba oil on oxidative stress in MCT-induced pulmonary hypertension. A)

Total reactive oxygen species concentration; B) Thiobarbituric acid reactive substances; C)

Carbonyl. Data are expressed as mean ± sd. a $p<0.05$ vs CO; b $p<0.05$ vs MCT. CO= control, O= Copaíba oil, MCT= Monocrotaline, MCT-O=Monocrotaline+Oil

Fig 2: Effects of copaiba oil on antioxidant defenses in MCT-induced pulmonary hypertension. A) Total sulfhydryl groups; B) Superoxide dismutase activity; C) Catalase activity; D) Glutathione peroxidase activity. Data are expressed as mean ± sd. a $p<0.05$ vs CO; b $p<0.05$ vs MCT. CO= control, O= Copaíba oil, MCT= Monocrotaline, MCT O=Monocrotaline+Oil

Fig 3: Effect of copaiba oil on expression of eNOS and signaling proteins for apoptosis and cell survival in MCT-induced pulmonary hypertension. A) eNOS expression; B) p-Akt/Akt ratio; C) p-JNK/JNK ratio; D) p-GSK-3β/ GSK-3β ratio E) Bax/Bcl2 ratio. Data are expressed as mean ± sd. a $p<0.05$ vs CO; b $p<0.05$ vs MCT, c $p<0.05$ vs O. CO= control, O= Copaíba oil, MCT= Monocrotaline, MCT-O= Monocrotaline+Oil

Fig 4: Copaiba oil attenuates right ventricular remodeling by decreasing myocardial

apoptotic signaling in monocrotaline-induced rats.

Table 1: Morphometric and hemodynamic results

	CO	MCT	O	MCT-O
BW (g)	307±16	290±12	307±8	296±17
RVW/BW (mg/g)	0.61±0,04	1.01±0.06 [a]	0.59±0.04	0.76±0.08 [b c]
RVEDP (mmHg)	5.55±0.59	6.79±1.18[a]	5.50±0.50	5.68±0.40 [b]
RVSP (mmHg)	29.21±2.62	59.09±3.12[a]	30.09±2.18	51.81±3.30[bc]
HR (bpm)	264±13	248±6	244±18	D 250±3 5

dP/dt min (mmHg/s)	-848±109	-1565±145[a]	-83	-1217±206[bc]
dP/dt max (mmHg/s)	1310±125	2347±102[a]	1116±120[a]	1226±73[b]

Data are expressed as mean ± SD. a $p<0.05$ vs CO; b $p<0.05$ vs MCT; c $p<0.05$ vs O. CO= control, O= Copaíba oil, MCT= Monocrotaline, MCT-O= Monocrotaline+Oil. RVW = right ventricular weight, BW = body weight, RVEDP= right ventricular end diastolic pressure, RVSP right ventricular systolic pressure, HR= heart rate, dP/dt max = right ventricular contractility index, dP/dt min = right ventricular relaxation index.

Table 2: Echocardiography results

	CO	MCT	O	MCT-O
TAPSE (cm)	0.237±0.035	0.153±0.027[a]	0.201±0.01	0.245±0.03 [bc]
RVDd (cm)	0.259±0.078	0.376±0.031[a]	0.300±0.005	0.336±0.034
RVSd (cm)	0.241±0.019	0.332±0.035 [a]	0.244±0.006	0.293±0.034 [b c]
RV diastolic area (cm^2)	0.151±0.020	0.256±0.049 [a]	0.205±0.038	0.162±0.014 [b]
RV systolic area (cm^2)	0.117±0.013	0.194±0.046 [a]	0.143±0.031	0.131± 0.017 [b]

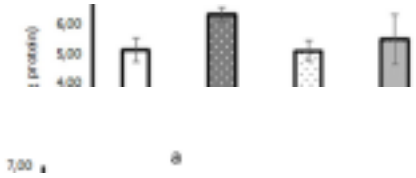
Data are expressed as mean ± SD. a $p<0.05$ vs CO; b $p<0.05$ vs MCT; c $p<0.05$ vs O.

CO= control, O= Copaiba oil, MCT= Monocrotaline, MCT-O=Monocrotaline+Oil.

RV=right ventricle, RVDd = RV diastolic diameter, RVSd = RV systolic diameter,

TAPSE = tricuspid annular plane systolic excursion.

Figure 1 A



A

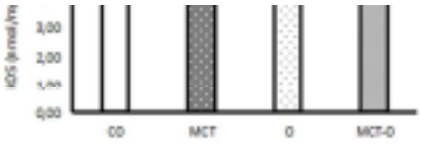


Figure 1 B

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