Effect of Free and Nanoencapsulated Copaiba Oil on Monocrotaline-induced Pulmonary Arterial Hypertension

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Abstract: Copaiba oil comes from an Amazonian tree and has been used as an alternative medicine in Brazil. However, it has not been investigated yet in the treatment of cardiovascular diseases. This study was designed to test whether copaiba oil or nanocapsules containing this oil could modulate monocrotaline (MCT)-induced pulmonary arterial hypertension (PAH). Male Wistar rats (170 ± 20 g) received oil or nanocapsules containing this oil (400 mg/kg) by gavage daily for 1 week. At the end of this period, a single injection of MCT (60 mg/kg i.p.) was administered and measurements were performed after 3 weeks. The animals were divided into 6 groups: control, copaiba oil, nanocapsules with copaiba oil, MCT, oil + MCT, and nanocapsules + MCT. Afterward, echocardiographic assessments were performed, and rats were killed to collect hearts for morphometry and oxidative stress. MCT promoted a significant increase in pulmonary vascular resistance, right ventricle (RV) hypertrophy, and RV oxidative stress. Both oil and copaiba nanocapsules significantly reduced RV hypertrophy and oxidative stress. Pulmonary vascular resistance was reduced by copaiba oil in natura but not by nanocapsules. In conclusion, copaiba oil seems to offer protection against MCT-induced PAH. Our preliminary results suggest that copaiba oil may be an important adjuvant treatment for PAH.

Key Words: monocrotaline, pulmonary arterial hypertension, oxidative stress, copaiba oil, nanocapsules, antioxidant

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INTRODUCTION

A considerable number of natural products are derived from several species in the Amazon forest, amongst which copaiba oil has become very important in Brazilian natural medicine. This oil is obtained by tapping the trunk of trees of several species of *Copaifera* sp.; it is used by native people from the northern and northeastern regions of Brazil, especially in Amazonas state.[1] This product is one of the most important renewable resources associated with natural medicine for populations of the Amazon region, and today, it can be found in markets and drugstores all over Brazil and in many countries around the world.[2] Copaiba oil is known for its antioxidant, antitumor, anti-inflammatory, and even bacteriostatic effects.[3–5]

Copaiba oil has an unctuous character, so nanoemulsions of this oil could improve patient acceptability by converting it into a more pleasant hydrophilic formulation, in addition to improving the bioavailability of its active antioxidant molecules. Through nanoemulsions, it is possible to improve the chemical stability of substances, prolong the duration of activity, and delineate the site of action.[6,7] The bioavailability of many drugs is limited. To solve this problem, many approaches have been developed, such as incorporation the drug into micro- or nanoparticles.[8] In this way, the incorporation of copaiba oil into nanopolymeric particles might improve its bioavailability.

The search for new substances to treat incurable diseases must be encouraged. Medicinal plants stand out due their active compounds that can act on pathological pathways. Copaiba oil has been used to treat stomach ulcers, as well as skin, hepatic, and intestinal disorders.[3,9,10] However, this alternative medicine has not been investigated yet in cardiovascular diseases, such as pulmonary arterial hypertension (PAH).

PAH is a rare and devastating disease, characterized by a progressive increase in pulmonary vascular resistance (PVR) and pulmonary arterial pressure.[11] Chronically elevated pressure in the pulmonary circulation can result in right ventricle (RV) hypertrophy, right-sided heart failure, and premature death.[12,13] These modifications in the RV are known as cor pulmonale, which is defined as structural and functional alterations in the heart induced by pulmonary circulatory changes. PAH is often the common link between heart and lung dysfunction in cor pulmonale.[14] This disease is a hemodynamic and pathophysiological condition that affects more than 100 million people worldwide.[15] There are several animal models to study PAH, but one of the most commonly used is the monocrotaline (MCT) model. MCT is a pyrrolizidine alkaloid that causes PAH in rats.[16] One of the pathophysiological mechanisms involved in the development of PAH is increased oxidative stress.[17] Oxidative stress mediates

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dysfunction in pulmonary vascular endothelial cells,[18] and the resulting elevation in PVR enhances RV afterload which can, in the long term, develop into RV failure.[19]

Acting as an antioxidant, copaiba oil could be a good option to treat several pathologies related to an increase in oxidative stress, such as PAH. There have been several studies investigating antioxidants as a novel treatment for PAH.[20–23] This approach is focused on drugs that are able to modulate the production of free radicals to treat PAH.

In this study, we hypothesized that copaiba oil could reduce the severity of MCT-induced PAH, through antioxidant and cardioprotective effects.

METHODS

Drugs and Reagents

Ketamine hydrochloride was purchased from König Lab S.A., SP, Brazil, and xylazine was purchased from Virbac do Brasil I.P., SP, Brazil. MCT and all other drugs/reagents were purchased from Sigma Chemical Co, St. Louis. Copaiba oil was purchased at a drugstore, and its composition was analyzed at the Faculty of Pharmacy at the Universidade Federal do Rio Grande do Sul.

Production of Nanocapsules Using Copaiba Oil

Nanocapsules were obtained by interfacial polymer deposition using a pectin aqueous solution (6%) as the polymer, the active substance copaiba oil as the core, and tensoactives [water phase: Tween 20 (7 g), water (37 mL), and nipagin (0.1 g); organic phase: Span 80 (4 g) and copaiba oil (4 g)]. To prepare emulsion, a proportion of 1:1 of emulsion and pectin was used. Emulsion was prepared in ULTRA-TURRAX, stirring for 30 minutes. The nanocapsule solution concentration was 40 mg/mL, and it was kept in the dark until administration to rats.[1]

Characterization of Copaiba Nanocapsules

The nanocapsules were characterized in terms of particle size, polydispersion, and pH. The mean diameter over the volume distribution (d 4.3) and particle size distribution (SPAN) (Equation 1) were determined by laser diffractometry, where d 0.9, d 0.1, and d 0.5 are the particle diameters determined at 90%, 10%, and 50% cumulative undersized volumes, respectively. The pH was assessed with a microprocessor-controlled pH analyzer.[1]

$$\mathrm{SPAN} = \frac{(\mathrm{d0.9} - \mathrm{d0.1})}{\mathrm{d0.5}} \quad (1)$$

Animals and Groups

All procedures were approved by the institutional animal ethics committee. In total, 30 male Wistar rats (weighing 170 ± 20 g) from the Universidade Federal do Rio Grande do Sul were studied. They were kept at 20–22°C and with a 12:12 h dark:light photoperiod. All animals had ad libitum access to water and regular rodent chow, and the experiments were conducted in accordance with institutional guidelines and the Guide for the Care and Use of Laboratory Animals (US Department of Health and Human Services, NIH Publication No. 86-23). The animals were divided into 6 experimental groups: control (CO), copaiba oil (O), nanoemulsion of copaiba oil (N), MCT, copaiba oil + MCT (O-MCT), and nanoemulsion of copaiba oil + MCT (N-MCT). Animals received by gavage once a day for 7 days: copaiba oil (400 mg/kg) in the O and O-MCT groups[24]; a nanoemulsion of copaiba oil (400 mg/kg) in the N and N-MCT groups; and the vehicle (Tween) in the CO and MCT groups. The dose of 400 mg/kg corresponds to a volume of 0.63 mL/kg of free copaiba oil plus a volume of Tween to reach the same volume of the nanocapsules administered (10 mL/kg).

Induction of Pulmonary Hypertension

At the end of the treatment period, PAH was induced by MCT through a single in bolus injection (60 mg/kg intraperitoneal), or saline, as described elsewhere.[25]

Echocardiographic Analysis

Twenty-one days after MCT injection, echocardiography analysis was performed. Animals were anaesthetized (ketamine, 90 mg/kg; xylazine, 20 mg/kg, intraperitoneal) and placed in the left lateral decubitus position to obtain cardiac images. An EnVisor Philips system (Andover, MA) was used, with a 12–13 MHz transducer, by a trained operator with experience in small animal echocardiography.[26] The acceleration time (AT) and ejection time (ET) of pulmonary artery flow velocity tracings were measured, and the AT/ET ratio was calculated.

Morphometric Analysis and Preparation of Heart Homogenates

After cardiovascular evaluations, rats were killed by decapitation and the RV was harvested for posterior measurements, including morphometric, biochemical, and western blot analyses. The 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 minutes at 4°C.[27]

Determination of Oxidized Glutathione

To determine the oxidized glutathione (GSSG) concentration, the RV was deproteinized with 2 mol/L $HClO_4$, centrifuged for 10 minutes at 1000*g*, then the supernatant was neutralized with 2 mol/L KOH. The reaction contained 100 mmol/L phosphate buffer (pH 7.2), 2 mmol/L NADPH, 0.2 U/mL glutathione reductase, and 70 μmol/L 5,5′ dithiobis(2-nitrobenzoic acid). The absorbance was measured at 420 nm.[28]

Determination of Total Sulfhydryl Groups

The total amount of sulfhydryl groups (-SH) in the RV homogenates was determined according to Sedlak and Lindsay.[29] The optical density at 412 nm was read in

a spectrophotometer against an appropriate blank. The concentration of total sulfhydryl groups was expressed in pmol/mg protein.

Antioxidant Enzyme Activities

Superoxide dismutase (SOD) activity, expressed as units/mg protein, was based on the inhibition of the reaction of superoxide with pyrogallol. The superoxide radical is generated by the auto-oxidation of pyrogallol in alkaline medium. SOD activity was determined by measuring the velocity of oxidized pyrogallol formation.[30]

Glutathione peroxidase (GPx) activity, expressed as nmol peroxide/hydroperoxide reduced/min/mg protein, was measured after 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 tertiary butyl hydroperoxide.[31]

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

Western Blot Analysis

RV homogenate samples were mixed with sample loading buffer and separated under reducing conditions on 12% sodium dodecyl sulfate polyacrylamide gels. Proteins were electrotransferred onto polyvinylidene fluoride membranes (Immuno-Blot 0.2 μm, BioRad). The membranes were processed for immunodetection using an Nrf2 (57 kDa) antibody. The bound primary antibody was detected using a rabbit antimouse conjugated secondary antibody, 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]

Essential Oil Analysis

Gas chromatography (GS)-mass spectrometry (MS) analysis was carried out with a Shimadzu QP5000 system. The GC column was a DB-5 fused silica capillary with a (5% phenyl)-methyl poly siloxane stationary phase, film thickness of 0.25 μm, a length of 30 m, and an internal diameter of 0.25 mm. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector and detector temperatures were set at 220 and 250°C, respectively, and the GC oven was programmed in increase in temperature from 60 to 300°C at a rate of 3°C/min. The essential oil was diluted in ethyl ether in a ratio of 1:50 (vol/vol). The identification of compounds was carried out by comparison of their relative retention times, calculated by linear interpolation relative to the retention time of a series of n-alkenes, and their mass spectra, with authentic samples or data taken from the literature,[34] as well by comparison with mass spectra recorded in the NIST 62 and NIST 12 databases (National Institute of Technology and Standards).[34]

Statistical Analysis

Data are shown as mean ± standard deviation. Statistical analysis was performed using 2-way ANOVA followed by the Student–Newman–Keuls post-hoc test. P values <0.05 were considered significant.

RESULTS

Characterization of Copaiba Nanocapsules

The particles presented a mean diameter of 139.03 nm, a pH of 3.5, and a SPAN of 0.41.

Echocardiographic Analysis

The AT/ET ratio is shown in Table 1. It was significantly ($P < 0.05$) lower in the MCT group as compared with the CO group. Copaiba oil significantly ($P < 0.05$) increased this parameter in the O-MCT group as compared with the MCT group. In the N-MCT group, the AT/ET ratio was not different from those of the MCT group and the O-MCT group.

Morphometric Evaluations

The right ventricular hypertrophy index (RV/body weight) (Table 1) was significantly ($P < 0.05$) increased in the MCT group in relation to the CO group. This parameter decreased significantly in the animals with PAH treated with both the oil and the nanoparticles.

Oxidative Stress Analysis

PAH (MCT group) promoted a significant decrease ($P < 0.05$) in total sulfhydryl groups and an increase in the GSSG concentration in RV homogenates (Table 2). Treatment with nanoparticles in rats treated with MCT reverted ($P < 0.05$) these parameters. The O-MCT group did not shown any differences in these parameters when compared with the MCT group.

Antioxidant enzyme activities (SOD and GPx) were significantly ($P < 0.05$) lower in the MCT group when compared with the CO group (Table 3). Treatment with nanoparticles or copaiba oil significantly ($P < 0.05$) recovered these enzyme activities in animals with PAH.

TABLE 1. Echocardiographic and Morphometric Evaluation

Group	AT/ET, s/s	RV, Weight/Body Weight, mg/g
CO	0.323 ± 0.030	0.545 ± 0.117
O	0.333 ± 0.030	0.568 ± 0.098
N	0.316 ± 0.050	0.533 ± 0.085
MCT	0.207 ± 0.020*	0.833 ± 0.180*
O-MCT	0.316 ± 0.050†	0.527 ± 0.062†
N-MCT	0.260 ± 0.028‡	0.482 ± 0.044‡

Data are expressed as mean ± SD (n = 5).
*$P < 0.05$ versus CO.
†$P < 0.05$ versus MCT.
‡$P < 0.05$ versus N.

TABLE 2. Total Sulfhydryl Groups and GSSG in the RV

Group	Total -SH, μmol/mg Protein	GSSG, μmol/mg Protein
CO	2.87 ± 0.36	0.003 ± 0.001
O	3.11 ± 0.34	0.004 ± 0.002
N	3.36 ± 0.34	0.003 ± 0.003
MCT	1.92 ± 0.40*	0.013 ± 0.007*
O-MCT	2.58 ± 0.21	0.014 ± 0.005†
N-MCT	2.98 ± 0.45‡	0.004 ± 0.002‡

Data are expressed as mean ± SD (n = 5).
*$P < 0.05$ versus CO.
†$P < 0.05$ versus O.
‡$P < 0.05$ versus MCT.

Nrf2 Protein Expression

Both copaiba oil and nanocapsules containing this oil significantly ($P < 0.05$) increased Nrf2 protein expression, not only in MCT-treated animals but also in healthy rats (Fig. 1).

GC-MS Analysis

The chemical composition of copaiba oil as identified by GC-MS is shown in Table 4 and Figure 2. The identification of these compounds was performed by comparison of their relative retention times and their mass spectra with those of the NIST62 and NIST 12 databases (National Institute of Technology and Standards). A total of 19 compounds were identified. β-caryophyllene (44.1%), β-bisabolene (12.8%), trans-α-bergamotene (7.4%), β-selinene (6.9%), and α-humulene (6.8%) were identified as the major constituents in copaiba oil.

DISCUSSION

In the present study, it was demonstrated that copaiba oil pretreatment has protective effects in a rat model of PAH. This protection was demonstrated by a decrease in PVR and RV hypertrophy reduction and by a decrease in RV oxidative stress.

This study was conducted using MCT, a PAH model that mimics this syndrome in humans.[35] PAH causes functional and structural changes in RV by mediating an increase in PVR, represented by a decreased AT/ET ratio, which was evaluated by the flow through the pulmonary artery.[36] Treatment with free copaiba oil prevented the elevation in PVR induced by MCT. Other studies have shown that β-caryophyllene, a major component found in copaiba oil, is a calcium channel blocker that produces significant vasorelaxant effects.[37,38] The vasodilatory effects of β-caryophyllene could explain the decrease in PVR found in the present study. Moreover, β-caryophyllene also inhibits cell growth.[39] In this way, it could mitigate the excessive thickening and remodeling of the distal small pulmonary arterioles caused by MCT. On the other hand, treatment with nanocapsules containing copaiba oil did not reduce PVR. However, the intervention improved other cardiac effects of PAH, such as RV hypertrophy and oxidative stress.

Other studies have shown that ROS induces cardiac hypertrophy[40] in PAH, and RV failure is associated with oxidative stress.[41] Thus, the reduction in hypertrophy may result from the antioxidant activity of copaiba oil. Since the nanocapsules did not provide benefits in terms of pulmonary vasculature but reduced cardiac hypertrophy, it seems that this formulation is more effective in heart tissue than in the pulmonary circulation. The MCT model is triggered by an acute injury in pulmonary vasculature, determining progressive increase in PVR, which affects the RV long term. Thus, we could speculate that free copaiba oil could have a readier effect, whereas the initial effect of nanoencapsulate oil could be delayed. However, during treatment, copaiba oil could be delivered constantly from the nanocapsules, promoting an improvement in RV redox environment long term. It is important to note that the nanocapsules used in this study were made of a pectin aqueous solution; according to the literature, pectin has antioxidant activity.[42] The effects of copaiba oil on oxidative stress could have been additive with the antioxidant effects of pectin, thereby potentiating it. This increased antioxidant power of nanocapsules containing copaiba oil could have cardioprotective effects and may be beneficial in the management of PAH. One limitation of this study was not having tested other nanocapsule formulations without antioxidant activity or the effect of pectin nanocapsules without copaiba oil. However, free copaiba oil and nanocapsules also improved RV antioxidant parameters. Previous studies have shown that copaiba oil possesses antioxidant properties[43] and exerts a protective effect against liver injury.[3,44] However, there have not been any studies documenting the activity of copaiba oil in cardiovascular diseases. This encouraged us to investigate its effects on RV using rats with PAH.

In this study, we evaluated some oxidative stress parameters, such as -SH groups, GSSG concentrations, and antioxidant enzyme activities. Studies in our laboratory, using this MCT model, have previously documented an association between PAH and oxidative stress in the lung[20] and in the RV.[22,45,46] In agreement with these findings, in this study, we found an increase in the GSSG concentration, ie, the oxidized form of glutathione, in the MCT group. This increase in the GSSG concentration was accompanied by a decrease in total -SH groups and in antioxidant enzyme activities in the MCT group. As demonstrated by others,[47–49] PAH decreases antioxidant defenses. However, free copaiba oil and nanocapsules

TABLE 3. Antioxidant Enzyme Activities in the Right Ventricle

Group	SOD, U/mg Protein	GPx, $nmol \cdot min^{-1} \cdot mg^{-1}$ Protein
CO	23.19 ± 2.35	125.39 ± 17.02
O	22.71 ± 3.73	146.49 ± 23.70
N	22.93 ± 2.03	113.51 ± 21.16
MCT	14.10 ± 1.85*	53.55 ± 8.57*
O-MCT	20.05 ± 1.81†	94.88 ± 15.71‡
N-MCT	20.52 ± 2.01†	146.09 ± 76.04†

Data are expressed as mean ± SD (n = 5).
*$P < 0.05$ versus CO.
†$P < 0.05$ versus MCT.
‡$P < 0.05$ versus O.

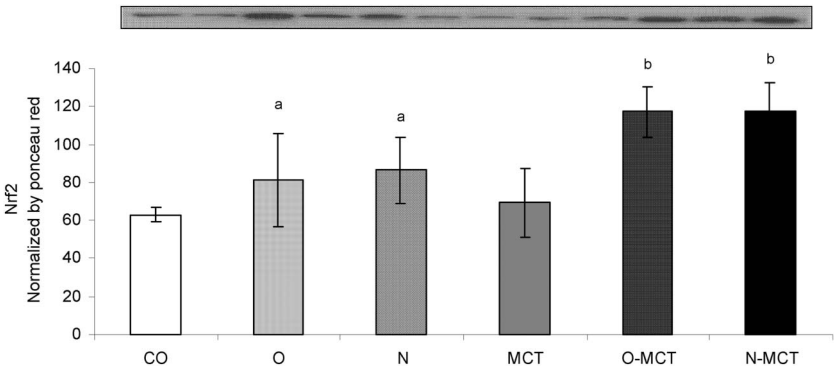

FIGURE 1. Western blot analysis of the right ventricle using an antibody against Nrf2; 1 representative gel showing 2 bands for each experimental group. a: $P < 0.05$ CO; b: $P < 0.05$ versus MCT. Data represent mean ± SD.

also conferred protection against oxidative stress by increasing SOD and GPx enzyme activities. On the other hand, although free copaiba oil increased antioxidant enzyme activities, it did not significantly change the number SH-groups or the GSSG concentration. Nevertheless, treatment with nanocapsules improved all of these parameters; this could represent a more favorable cellular redox environment, demonstrating the advantage of using this copaiba oil formulation. In fact, a study by Koriem et al[42] showed that pectin increases antioxidant enzyme activities, including those of glutathione-S-transferase, glutathione peroxidase, glutathione reductase, catalase, and SOD.

Studies in the literature have demonstrated that Nrf2, a key transcription factor, plays an indispensable role in the induction of endogenous antioxidant enzymes against oxidative stress.[50] Our results show an increase in Nrf2 protein expression in animals treated with free copaiba oil or nanocapsules containing copaiba oil. This is in agreement with other studies showing that the nuclear translocation of Nrf2 is induced by β-caryophyllene,[51,52] the principal compound found in copaiba oil. The increase in Nrf2 expression may have induced the gene transcription of antioxidant enzymes.

According to our oxidative stress results, nanocapsules may have increased the antioxidant effects of copaiba oil in heart tissue, since only this formulation decreased the GSSG concentration and increased the number of SH- groups in RV homogenates. On the other hand, treatment with free copaiba oil did not change these parameters when compared with the MCT group but rather improved the flow through the pulmonary artery. This reinforces the hypothesis that this oil could have an effect in terms of improving the vasculature, whereas the nanocapsule formulation could act directly on the heart tissue. However, this hypothesis must be tested in future studies.

According to our CG-MS analysis, β-caryophyllene was the most prevalent sesquiterpene found in copaiba oil. It is an aromatic volatile terpenoid and a bicyclic sesquiterpene with antioxidant properties. In fact, Ojha et al[53] demonstrated that β-caryophyllene restores antioxidant enzymes, inhibits lipid peroxidation, and reduces glutathione depletion. Other studies[54,55] have also shown that β-caryophyllene reduces lipid peroxidation and works as a scavenger of hydroxyl radicals and superoxide anions. In addition, a study by Calleja et al[55] showed that β-caryophyllene can act as a highly effective chain-breaking antioxidant agent and possesses excellent scavenging activity against reactive oxygen species. Moreover, the chemical structure of β-caryophyllene may provide clues into its antioxidant capacity. As a bicyclic molecule with double rings, caryophyllene has a carbon

TABLE 4. Chemical Composition of the Copaiba Oil Identified by GS-MS

Compound	RT, min	RI exp	% RA	Identification
α-Ylangene	24.013	1365	1.4	1
β-Elemene	24.77	1382	3.2	2
Cyperene	24.99	1386	0.2	3
β-Caryophyllene	26.09	1410	44.1	4
trans-α-Bergamotene	26.71	1424	7.4	5
α-Humulene	27.40	1439	6.8	6
(E)-β-Farnesene	27.76	1445	0.3	7
δ-Muurolene	28.40	1461	1.3	9
Germacrene D	28.56	1465	4.3	10
β-Selinene	28.80	1470	6.9	11
α-Selinene	29.17	1478	3.9	12
α-Muurolene	29.38	1483	0.4	13
(Z)- α-Bisabolene	29.53	1486	0.5	14
β-Bisabolene	29.86	1494	12.8	15
δ-Cadinene	29.92	1495	0.9	16
σ-Amorphene	30.35	1505	2.3	17
τ-Cadinol	34.87	1625	0.2	20
τ-Muurolol	35.15	1632	1.0	21
α-Muurolol	35.63	1644	0.5	22

%RA, relative peak area; RI, retention index; RT, retention time.

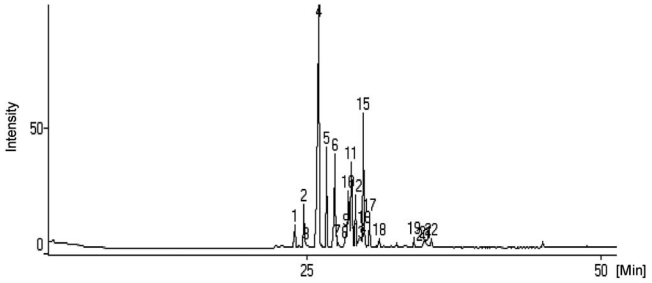

FIGURE 2. Gas chromatography mass spectrometry of copaiba oil.

bridge of the bicyclic system, which could be an adequate radical insertion site.[55] This insertion can promote opening of the bicyclic system, favoring the presence of radical structures of secondary, tertiary, and allylic carbon, which may confer a high capacity to react with free radicals, such as hydroxyl, superoxide, and peroxyl radicals.[55] In addition, the high concentration of caryophyllene in copaiba oil could explain our results regarding oxidative stress. According to the literature, β-caryophyllene upregulates the expression of Nrf2, which increases cellular GSH and ameliorates oxidative damage.[51,52] Thus, the action of this compound found in copaiba oil could, in part, explain the beneficial effects observed in this study.

CONCLUSIONS

It was demonstrated, for the first time, that copaiba oil is effective in reducing PVR and RV hypertrophy in rats treated with MCT. Although both formulations were able to reduce RV oxidative stress, the nanocapsule formulation was more effective in decreasing GSSG, whose reduced form represents the major intracellular antioxidant. Although further studies are required, our results suggest that copaiba oil might be a promising low-cost strategy to contribute to the treatment of patients with pulmonary hypertension.

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REFERENCES

1. Kanis LA, Prophiro JS, Vieira Eda S, et al. Larvicidal activity of copaifera sp. (leguminosae) oleoresin microcapsules against aedes aegypti (diptera: Culicidae) larvae. *Parasitol Res.* 2012;110:1173–1178.
2. Veiga Junior VF, Rosas EC, Carvalho MV, et al. Chemical composition and anti-inflammatory activity of copaiba oils from copaifera cearensis huber ex ducke, copaifera reticulata ducke and copaifera multijuga hayne–a comparative study. *J Ethnopharmacol.* 2007;112:248–254.
3. Brito MV, Costa FD, Vasconcelos DM, et al. Attenuation of copaiba oil in hepatic damage in rats. *Acta Cir Bras.* 2014;29:776–780.
4. Gelmini F, Beretta G, Anselmi C, et al. GC-MS profiling of the phytochemical constituents of the oleoresin from Copaifera langsdorffii Desf. and a preliminary in vivo evaluation of its antipsoriatic effect. *Int J Pharm.* 2013;440:170–178.
5. Leandro LM, Vargas Fde S, Barbosa PC, et al. Chemistry and biological activities of terpenoids from copaiba (copaifera spp.) oleoresins. *Molecules*. 2012;17:3866–3889.
6. Patel P, Pol A, More S, et al. Colloidal soft nanocarrier for transdermal delivery of dopamine agonist: ex vivo and in vivo evaluation. *J Biomed Nanotechnol.* 2014;10:3291–3303.
7. Sethi K, Roy I. Organically modified titania nanoparticles for sustained drug release applications. *J Colloid Interf Sci.* 2015;456:59–65.
8. Gursoy RN, Benita S. Self-emulsifying drug delivery systems (sedds) for improved oral delivery of lipophilic drugs. *Biomed Pharmacother.* 2004;58:173–182.
9. Silva JJ, Pompeu DG, Ximenes NC, et al. Effects of kaurenoic acid and arginine on random skin flap oxidative stress, inflammation, and cytokines in rats. *Aesthet Plast Surg.* 2015;39:971–977.
10. Comelli Junior E, Skinovski J, Sigwalt MF, et al. Rupture point analysis of intestinal anastomotic healing in rats under the action of pure copaiba (copaifera iangsdorfii) oil. *Acta Cir Bras.* 2010;25:362–367.
11. Humbert M, Morrell NW, Archer SL, et al. Cellular and molecular pathobiology of pulmonary arterial hypertension. *J Am Coll Cardiol.* 2004;43:13S–24S.
12. Badesch DB, Champion HC, Sanchez MA, et al. Diagnosis and assessment of pulmonary arterial hypertension. *J Am Coll Cardiol.* 2009;54:S55–S66.
13. Farber HW, Loscalzo J. Pulmonary arterial hypertension. *N Engl J Med.* 2004;351:1655–1665.
14. Weitzenblum E, Chaouat A. Cor pulmonale. *Chron Respir Dis.* 2009;6:177–185.
15. dos Santos Fernandes CJ, Jardim CV, Hovnanian A, et al. Survival in schistosomiasis-associated pulmonary arterial hypertension. *J Am Coll Cardiol.* 2010;56:715–720.
16. Gomez-Arroyo JG, Farkas L, Alhussaini AA, et al. The monocrotaline model of pulmonary hypertension in perspective. *Am J Physiol Lung Cell Mol Physiol.* 2012;302:L363–L369.
17. Tabima DM, Frizzell S, Gladwin MT. Reactive oxygen and nitrogen species in pulmonary hypertension. *Free Radic Biol Med.* 2012;52:1970–1986.
18. Grobe AC, Wells SM, Benavidez E, et al. Increased oxidative stress in lambs with increased pulmonary blood flow and pulmonary hypertension: role of nadph oxidase and endothelial no synthase. *Am J Physiol Lung Cell Mol Physiol.* 2006;290:L1069–L1077.
19. Chaouat A, Naeije R, Weitzenblum E. Pulmonary hypertension in copd. *Eur Respir J.* 2008;32:1371–1385.
20. Ludke AR, Mosele F, Caron-Lienert R, et al. Modulation of monocrotaline-induced cor pulmonale by grape juice. *J Cardiovasc Pharmacol.* 2010;55:89–95.
21. Morales-Cano D, Menendez C, Moreno E, et al. The flavonoid quercetin reverses pulmonary hypertension in rats. *PLoS One*. 2014;9:e114492.
22. Mosele F, Tavares AM, Colombo R, et al. Effects of purple grape juice in the redox-sensitive modulation of right ventricular remodeling in a pulmonary arterial hypertension model. *J Cardiovasc Pharmacol.* 2012;60:15–22.
23. Chaumais MC, Ranchoux B, Montani D, et al. N-acetylcysteine improves established monocrotaline-induced pulmonary hypertension in rats. *Respir Res.* 2014;15:65.
24. Guimaraes-Santos A, Santos DS, Santos IR, et al. Copaiba oil-resin treatment is neuroprotective and reduces neutrophil recruitment and microglia activation after motor cortex excitotoxic injury. *Evid Based Complement Alternat Med.* 2012;2012:918174.
25. Farahmand F, Hill MF, Singal PK. Antioxidant and oxidative stress changes in experimental cor pulmonale. *Mol Cell Biochem.* 2004;260:21–29.
26. Tavares AM, da Rosa Araujo AS, Baldo G, et al. Bone marrow derived cells decrease inflammation but not oxidative stress in an experimental model of acute myocardial infarction. *Life Sci.* 2010;87:699–706.
27. Llesuy SF, Milei J, Molina H, et al. Comparison of lipid peroxidation and myocardial damage induced by adriamycin and 4′-epiadriamycin in mice. *Tumori.* 1985;71:241–249.
28. Akerboom TP, Sies H. Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. *Methods Enzymol.* 1981;77:373–382.
29. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with ellman's reagent. *Anal Biochem.* 1968;25:192–205.
30. Marklund SL. Superoxide dismutase isoenzymes in tissues and plasma from New Zealand black mice, nude mice and normal balb/c mice. *Mutat Res.* 1985;148:129–134.
31. Flohe L, Gunzler WA. Assays of glutathione peroxidase. *Methods Enzymol.* 1984;105:114–121.
32. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the folin phenol reagent. *J Biol Chem.* 1951;193:265–275.
33. Klein D, Kern RM, Sokol RZ. A method for quantification and correction of proteins after transfer to immobilization membranes. *Biochem Mol Biol Int.* 1995;36:59–66.
34. Adams RP. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*. Carol Stream, IL: Allured Pub Corp; 2007.
35. Doggrell SA, Brown L. Rat models of hypertension, cardiac hypertrophy and failure. *Cardiovasc Res.* 1998;39:89–105.
36. Chan SY, Loscalzo J. Pathogenic mechanisms of pulmonary arterial hypertension. *J Mol Cell Cardiol.* 2008;44:14–30.

37. Bigliani MC, Rossetti V, Grondona E, et al. Chemical compositions and properties of schinus areira L. essential oil on airway inflammation and cardiovascular system of mice and rabbits. *Food Chem Toxicol.* 2012;50: 2282–2288.
38. Rasheed HM, Khan T, Wahid F, et al. Chemical composition and vasorelaxant and antispasmodic effects of essential oil from rosa indica L. Petals. *Evid Based Complement Alternat Med.* 2015;2015:279247.
39. Park KR, Nam D, Yun HM, et al. Beta-caryophyllene oxide inhibits growth and induces apoptosis through the suppression of pi3k/akt/mtor/s6k1 pathways and ros-mediated mapks activation. *Cancer Lett.* 2011;312:178–188.
40. Takimoto E, Kass DA. Role of oxidative stress in cardiac hypertrophy and remodeling. *Hypertension*. 2007;49:241–248.
41. Redout EM, Wagner MJ, Zuidwijk MJ, et al. Right-ventricular failure is associated with increased mitochondrial complex ii activity and production of reactive oxygen species. *Cardiovasc Res.* 2007;75:770–781.
42. Koriem KM, Arbid MS, Emam KR. Therapeutic effect of pectin on octylphenol induced kidney dysfunction, oxidative stress and apoptosis in rats. *Environ Toxicol Pharmacol.* 2014;38:14–23.
43. Teixeira LF, Boas RV, Oliveira PC, et al. Effect of natural antioxidants on the lipase activity in the course of batch and continuous glycerolysis of babassu oil. *Bioprocess Biosyst Eng.* 2014;37:1717–1725.
44. Teixeira RK, Yamaki VN, Yasojima EY, et al. Effect of copaiba oil in hepatic damage induced by acetaminophen in rats. *Acta Cir Bras.* 2013; 28:526–530.
45. Siqueira R, Campos C, Colombo R, et al. Influence of estrogen on pulmonary arterial hypertension: role of oxidative stress. *Cell Biochem Funct.* 2011;29:543–548.
46. Souza-Rabbo MP, Silva LF, Auzani JA, et al. Effects of a chronic exercise training protocol on oxidative stress and right ventricular hypertrophy in monocrotaline-treated rats. *Clin Exp Pharmacol Physiol.* 2008;35: 944–948.
47. Mata M, Sarrion I, Milian L, et al. PGC-1alpha induction in pulmonary arterial hypertension. *Oxid Med Cell Longev.* 2012;2012:236572.
48. Leichsenring-Silva F, Tavares AM, Mosele F, et al. Association of the time course of pulmonary arterial hypertension with changes in oxidative stress in the left ventricle. *Clin Exp Pharmacol Physiol.* 2011;38:804–810.
49. Lu Z, Xu X, Hu X, et al. Extracellular superoxide dismutase deficiency exacerbates pressure overload-induced left ventricular hypertrophy and dysfunction. *Hypertension*. 2008;51:19–25.
50. Sekhar KR, Yan XX, Freeman ML. Nrf2 degradation by the ubiquitin proteasome pathway is inhibited by kiaa0132, the human homolog to inrf2. *Oncogene*. 2002;21:6829–6834.
51. Assis LC, Straliotto MR, Engel D, et al. Beta-Caryophyllene protects the C6 glioma cells against glutamate-induced excitotoxicity through the Nrf2 pathway. *Neuroscience*. 2014;279:220–231.
52. Lou J, Cao G, Li R, et al. Beta-caryophyllene attenuates focal cerebral ischemia-reperfusion injury by nrf2/ho-1 pathway in rats. *Neurochem Res.* 2016;41:1291–1304.
53. Ojha S, Javed H, Azimullah S, et al. Beta-caryophyllene, a phytocannabinoid attenuates oxidative stress, neuroinflammation, glial activation, and salvages dopaminergic neurons in a rat model of parkinson disease. *Mol Cell Biochem* 2016.
54. Vinholes J, Rudnitskaya A, Goncalves P, et al. Hepatoprotection of sesquiterpenoids: a quantitative structure-activity relationship (QSAR) approach. *Food Chem.* 2014;146:78–84.
55. Calleja MA, Vieites JM, Montero-Melendez T, et al. The antioxidant effect of beta-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. *Br J Nutr.* 2013;109: 394–401.