ACTA
AMAZONICA

http://dx.doi.org/10.1590/1809-4392202102342

ORIGINAL ARTICLE

Copaiba (*Copaifera reticulata*) oleoresin reduces voluntary alcohol intake in rats

Rianne Remus PULCINELLI[1], Clara Lindenmayer Della GIUSTINA[1], Solange BANDIERA[1], Felipe Borges ALMEIDA[2], Lucas dos Reis IZOLAN[3], Mauricio Schuler NIN[1,2,4], Mirna Bainy LEAL[1,3], Rosane GOMEZ[1,3*]

[1] Universidade Federal do Rio Grande do Sul (UFRGS), Instituto de Ciências Básicas da Saúde, Programa de Pós-Graduação em Farmacologia e Terapêutica, Porto Alegre, Brazil

[2] Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Programa de Pós-Graduação em Ciências da Saúde: Farmacologia e Toxicologia, Porto Alegre, Brazil

[3] Universidade Federal do Rio Grande do Sul (UFRGS), Instituto de Ciências Básicas da Saúde, Programa de Pós-Graduação em Neurociências, Porto Alegre, Brazil

[4] Universidade Federal do Rio Grande (FURG), Departamento de Farmacologia, Rio Grande, Brazil

***** Corresponding author: rosane.gomez@ufrgs.br; https://orcid.org/0000-0002-0868-8394

ABSTRACT

Copaiba oleoresin, extracted from the *Copaifera reticulata* tree, has been used as a remedy in popular medicine in the Brazilian Amazon for various purposes, including reducing drug abuse. Yet no studies evaluated the effect of repeated administration of copaiba oil on alcohol consumption in animals. To evaluate this effect, we divided adult male Wistar rats into a) an alcohol group in which the animals had free access to choose between two bottles: one containing alcohol solution (20%) and another containing vehicle solution (0.2% saccharin); and b) a control group with access to two bottles containing vehicle solution. Rats were free to drink 24 h per day, for 35 days. Daily alcohol consumption and weekly body weight gain and food intake were monitored. From day 22, half of the rats in each group received 600 mg kg^{-1} copaiba oleoresin and the other received vehicle, subcutaneously, once a day, for three days. On day 35, rats were evaluated in an open-field test. The results showed that copaiba oil decreased voluntary alcohol intake and preference between days 2 and 6 after the last administration. Copaiba treatment also decreased the food intake and body weight gain in both alcohol and control groups without changing behaviors in the open-field test. Therefore, copaiba oil was able to reduce voluntary alcohol consumption in rats and could be tested in humans as an adjuvant to treat alcohol use disorder.

KEYWORDS: essential oil, ethanol, natural products, drug addiction

Óleo de copaíba (*Copaifera reticulata*) reduz consumo voluntário de álcool em ratos

RESUMO

O óleo extraído da árvore copaíba, *Copaifera reticulata*, tem sido usado na medicina popular na Amazônia brasileira para diversos fins, incluindo abuso de drogas. Contudo, não há estudos avaliando o efeito da administração repetida do óleo de copaíba sobre o consumo de álcool em animais. Para avaliar esse efeito, dividimos ratos Wistar machos adultos em dois grupos: a) um grupo álcool, no qual os animais tinham livre acesso a duas garrafas: uma contendo solução alcoólica (20%) e outra contendo solução veículo (sacarina 0,2%); e b) um grupo controle com acesso a duas garrafas contendo solução veículo. Os ratos podiam beber livremente, 24 horas por dia, durante 35 dias. O consumo diário de álcool, bem como o ganho de peso corporal semanal e a ingestão de alimentos foram monitorados. A partir do dia 22, metade dos ratos de cada grupo recebeu 600 mg kg^{-1} de óleo de copaíba e a outra metade recebeu veículo, por via subcutânea, uma vez ao dia, durante três dias. No dia 35, os ratos foram testados em teste de campo aberto. Os resultados mostraram que o óleo de copaíba diminuiu a ingestão voluntária e a preferência por álcool entre os dias 2 e 6 após a última administração. O tratamento com óleo de copaíba também diminuiu a ingestão alimentar e o ganho de peso corporal em ambos os grupos álcool e controle, sem alterar o comportamento no teste de campo aberto. Portanto, o óleo de copaíba foi capaz de reduzir o consumo voluntário de álcool em ratos e poderia ser testado em humanos como um adjuvante para tratar transtorno de uso de álcool.

PALAVRAS-CHAVE: óleo essencial, etanol, produtos naturais, adição a drogas

CITE AS: Pulcinelli, R.R.; Giustina, C.L.D.; Bandiera, S.; Almeida, F.B.; Izolan, L.R.; Nin, M.S.; Leal, M.B.; Gomez, R. 2022. Copaiba *(Copaifera reticulata)* oleoresin reduces voluntary alcohol intake in rats. *Acta Amazonica* 52: 53-59.

INTRODUCTION

Alcohol is the most widely consumed licit psychoactive substance in the world, and alcohol use disorder represents the fifth leading cause of premature death and disability, being responsible for 4% of global mortality (WHO 2018). Alcohol activates the mesolimbic dopaminergic system, producing positive reinforcement and consumption motivation (Gilpin and Koob 2008). Alcohol can directly or indirectly modulate this reward pathway through γ-aminobutyric acid (GABA), glutamate, opioid, endocannabinoid and other neurotransmitter systems (Koob and Volkow 2016). Chronic alcohol use promotes neuroadaptations in these circuits that blunt the reward response and recruit stress mediators in several brain areas, enhancing the negative reinforcement consumption motivation that intensifies compulsive drinking behavior and addiction (Gilpin and Koob 2008; Roberto and Varodayan 2017). In this condition, alcohol produces neuroinflammation and neuronal damage associated with increased oxidative stress and cytokines such as interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor-alpha (TNFα) in the blood and brain of users (Qin *et al*. 2008; Crews and Vetreno 2014; González-Reimers *et al*. 2014).

Copaiba oleoresin is extracted from the tree trunk of several species of the genus *Copaifera* L. (Leguminosae), with *Copaifera reticulata* D. being the mostly used in popular medicine (da Trindade *et al*. 2018). The chemical composition of copaiba oleoresin varies according to the species, the season of collection, and geographical and climatic characteristics of the region where the trees grow (Herrero-Jáuregui *et al*. 2011), consisting of a mixture of sesquiterpenes (mainly β-caryophyllene) and diterpene acids (Veiga Junior *et al*. 2007; da Trindade *et al*. 2018). Its acute oral toxicity is low, with the estimated lethal dose greater than 2,000 mg kg^{-1} in mice and rats (Gomes *et al*. 2007).

Inhabitants of the Amazon region, particularly in Brazil, attribute more than 20 different therapeutic properties to copaiba oil, making it one of the most important remedies of the popular medicine in that region (da Trindade *et al*. 2018). Even though most of these therapeutic benefits are speculative, there is some evidence showing the anti-inflammatory, antinociceptive, neuroprotective, antimicrobial, and wound-healing effects of copaiba oil (Gomes *et al*. 2007; Veiga Junior *et al*. 2007; Santos *et al*. 2008). The active phytochemical constituents of copaiba oil cross the blood-brain barrier and reduce neutrophil recruitment and microglia activation after acute injury and inhibit the production of hydrogen peroxide, nitric oxide, interferon-gamma (IFN-γ), TNF-α, IL-1β, IL-6, and interleukin 17 (IL-17) in the brain and the body periphery (Veiga Junior *et al*. 2007; Guimarães-Santos *et al*. 2012). Specifically, β-caryophyllene acts as a selective cannabinoid CB_2 receptor full agonist in the endocannabinoid system inhibiting proinflammatory pathways, contributing to copaiba antinociceptive and neuroprotective effects (Gertsch *et al*. 2008; Urasaki *et al*. 2020). In mice, β-caryophyllene administration has been shown to decrease voluntary alcohol consumption and preference (Al Mansouri *et al*. 2014), but the effects of copaiba oleoresin treatment on alcohol consumption and preference is unknown. Thus, the aim of this study was to evaluate the effect of copaiba oleoresin treatment on voluntary alcohol consumption and preference in rats.

MATERIAL AND METHODS

Animals

Forty adult male Wistar rats (*Rattus norvegicus*) (270 - 280 g) from the Center for Reproduction and Experimentation on Laboratory Animals (CREAL) of Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil, were kept at 55% air humidity and a temperature of 22 ± 2 °C, under a 12-hour light/dark cycle (lights off at 7:00 PM). Animals were pair-housed in cages (40 × 33 × 17.8 cm) with an insurmountable mesh longitudinal division, allowing individual and free access to standard food (Nuvilab, Colombo, Brazil) and solutions for both rats (Figure 1) (Pulcinelli *et al*. 2020). All protocols were approved by UFRGS' ethics commission on animal use (CEUA-UFRGS protocol # 30901/2016) and followed the US Guide for the Care and Use of Laboratory Animals (NRC 2011).

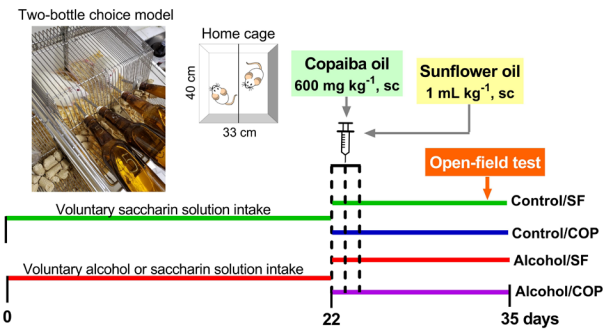

Figure 1. Two-bottle choice model and timeline for the evaluation of the effect of copaiba oleoresin on alcohol consumption in rats. Rats were left free to choose between two bottles, one containing alcohol (20%) and another containing vehicle (saccharin solution 0.2%), 24 h per day, for 35 days. On day 22, rats were administered 600 mg kg^{-1} copaiba oleoresin (COP) or 1 mL kg^{-1} sunflower oil (SF), subcutaneously (sc), once a day, for three days. Rats from the control group followed the same experimental protocol, except that they had access to two bottles containing only saccharin solution. On day 34 rats were exposed to an open-field test. This figure is in color in the electronic version.

Reagents and administration regime

For the voluntary intake protocol (Figure 1), saccharin sodium dehydrate (Sigma-Aldrich; St. Louis, MO, United States) was dissolved in purified water at a concentration of 0.2% (w v^{-1}) to produce the vehicle solution. Saccharin was added to the vehicle solution to improve the consumption, as

it is a highly palatable non-caloric substance and collaborates to produce alcohol dependence in rats. Then, 98% ethanol (Merck; São Paulo, Brazil) was diluted to 20% (w v^{-1}) in this vehicle solution. This palatable vehicle was chosen to stimulate voluntary intake in the rats. Copaiba (*Copaifera reticulata*) oleoresin (Argila Ind. e Com. de Cosméticos Ltda.; Juiz de Fora, Minas Gerais, Brazil - batch # 2336, obtained by auger extraction from copaiba originating from the Brazilian Amazon biome) was diluted in sunflower oil at a concentration of 600 mg mL^{-1}, and rats were subcutaneously injected with 1 mL kg^{-1} of this solution. Chemical composition of the copaiba oleoresin obtained by gas chromatography was: β-caryophyllene 50-65%; muurolene 12-18%; cadinene 3-6%; copaene 3-5%; β-bisabolene 2-3%; elemene 1-3%; oleic acid >1%; linolenic acid >1%. The administration regime was selected based on previous studies showing anti-inflammatory effects of copaiba oleoresin (Lopes *et al*. 2015). The subcutaneous route was chosen to minimize the stress of injections and to prolong copaiba release from the local depot.

Two-bottle choice protocol

Following acclimatization (7 days*),* the rats were randomly assigned to a alcohol and a control group. The alcohol group had free 24-hour access to two bottles, one containing the 20% alcohol solution and the other containing only the saccharin solution, for an experiment duration of 35 days (Figure 1). Rats from the control group had free access to two bottles containing only saccharin solution for the same period. Bottle placement was alternated daily to prevent side preference of consumption. Liquid intake was measured daily at 5:30 PM. The body weight of the rats was measured once a week to determine the consumption (in grams) of alcohol per kg of body weight and to fit the copaiba dosage. The alcohol preference (in percentage) was assessed by the proportion of alcohol and total fluid intake [alcohol/(alcohol + saccharin solution)] × 100. Body weight gain and food consumption were determined weekly by weight difference.

Copaiba treatment

On day 22, the animals of the alcohol and control groups were randomly assigned to four groups (n = 10 per group): two groups (control/SF and alcohol/SF) received 1 mL kg^{-1} sunflower (SF) oil via subcutaneous injection, and two groups (control/COP and alcohol/COP) received 600 mg kg^{-1} mL^{-1} copaiba oleoresin (COP) via subcutaneous injection (Figure 1). All the injections were performed once daily for three consecutive days, 60 min before the dark phase.

Open-field test

Ten days after the third day of subcutaneous administration, an open-field test was performed to assess the residual effect of copaiba on exploratory behavior and anxiety of the rats. On day 34 of the experiment, rats were accustomed to the open-field test room during two hours before the test. Rats were put individually in the lower left-hand corner (*i.e.,* the first square) of a square arena (80 × 80 × 40 cm) divided into 16 squares and left free to explore their surroundings. The test was recorded by a video camera for 5 min. The time spent in the first square before leaving it, the number of central and peripheral square crossings, the frequency of grooming and rearing actions, and the latency time for the first grooming were evaluated on the blinded videos. Anxiety-like behaviors were assessed by the central crossings, time spent in the first square, and grooming measure. Exploratory behaviors were assessed by all other parameters analyzed. At the end of each individual test, the number of fecal boli were counted and the arena was wiped with 10% ethanol.

Statistical analyses

All values were expressed as means ± standard error of the mean. The normality and variance homogeneity of data distribution were assessed with the Shapiro-Wilk normality test and the equal variance test, respectively. Temporal differences in alcohol and saccharin solution intake, alcohol preference, body weight gain, and food intake were assessed using 2-way repeated measures analysis of variance (2-way RM-ANOVA). Behavioral parameters were analyzed by a 2-way analysis of variance (2-way ANOVA). Both analyses considered condition (control or alcohol) and intervention (sunflower oil or copaiba) as factors and were followed by a Bonferroni *post hoc* test when required. *P*-values lower than 0.05 were considered statistically significant. The analyses were carried out using the Sigma Plot® 12.0 software.

RESULTS

Total fluid and alcohol intake and preference

The rats in the control group drank an average of 22 mL of fluids per day, while those in the alcohol group consumed almost the double of this volume ($F_{(42,581)} = 6.21$; $P_{\text{time x treatment}} < 0.001$; $F_{(3,581)} = 20.87$; $P_{\text{condition}} < 0.001$; Figure 2a). Saccharin solution intake in the alcohol group was similar to the control group, and copaiba treatment did not affect this consumption in both groups throughout the experiment (Figure 2b), evidencing that animals in the alcohol group were not dehydrated and the stimulus to increase fluid intake in this group seemed to be related to the alcohol reward effect.

Average alcohol consumption by the alcohol group before copaiba administration was 12 g kg^{-1} day^{-1} and did not change significantly relative to the baseline in the alcohol/SF group. However, the alcohol/COP group showed a 20% reduction in overall voluntary alcohol consumption ($F_{(13,262)} = 1.85$; $P_{\text{time x treatment}} = 0.037$; $F_{(1,262)} = 4.60$; $P_{\text{treatment}} = 0.046$; Figure 3a). The copaiba treatment significantly decreased the alcohol intake between two and six days after the last subcutaneous administration ($t = 2.47$; $P_{\text{day 5}} = 0.016$; $t = 2.14$; $P_{\text{day 6}} = 0.035$;

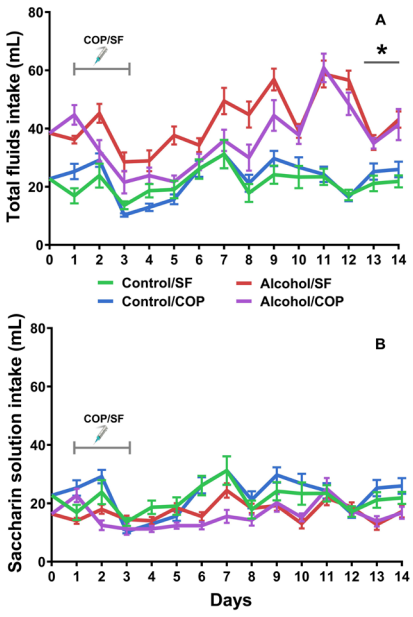

Figure 2. Effect of copaiba oleoresin (600 mg kg^{-1}, COP) or sunflower oil (SF), administered subcutaneously during three days on total volume of fluids (alcohol and/or saccharin solution) intake (A) or saccharin solution intake (B) of rats exposed to a two-bottle choice model. Zero point indicates the average daily volume of fluid consumption in the 21 days before the intervention. Saccharin solution intake in alcohol groups was similar to the control groups. Copaiba treatment did not affect the total volume of fluid intake nor saccharin solution intake in both groups. *indicates significant difference between the alcohol and control group at $P < 0.001$ according to a two-way RM-ANOVA + Bonferroni test (n = 10 rats per group). This figure is in color in the electronic version.

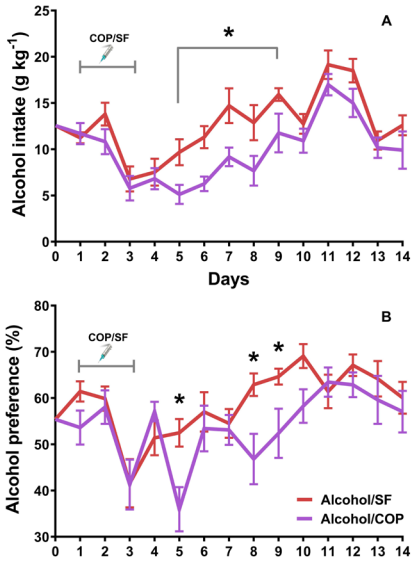

Figure 3. Effect of copaiba oleoresin (600 mg kg^{-1}, administered subcutaneously during three days) or sunflower oil (SF) on alcohol consumption (A) and alcohol preference (B) in rats exposed to a two-bottle choice model. Zero point indicates the average daily alcohol consumption and preference in the 21 days before the intervention. *indicates significant difference between the alcohol/COP and alcohol/SF group at $P < 0.05$ according to a two-way RM-ANOVA + Bonferroni test (n = 10 rats per group). This figure is in color in the electronic version.

t = 2.22; $P_{day\ 7}$ = 0.029; t = 2.81; $P_{day\ 8}$ = 0.006; t = 2.59; $P_{day\ 9}$ = 0.011; Figure 3a).

Before copaiba treatment, the average alcohol drinking preference was 56%. This value remained unchanged in alcohol/SF animals, but decreased by 30% in the alcohol/COP group two days after the last copaiba administration (t = 2.29; $P_{day\ 5}$ = 0.025; Figure 3b). Alcohol preference also decreased in this group on day 8 and day 9 (t = 2.03; $P_{day\ 8}$ = 0.047; t = 2.32; $P_{day\ 9}$ = 0.023; Figure 3b). The two-way RM-ANOVA showed a significant interaction between group and days ($F_{(14,289)}$ = 1.91; $P_{time\ x\ treatment}$ = 0.026; Figure 3b), but there was no overall reduction of alcohol preference in the alcohol/COP group.

Open-field test

We observed a significant main effect of condition as rats of both the alcohol/SF and alcohol/COP groups showed significantly more central crossings ($F_{(1,37)}$ = 6.53; $P_{condition}$ = 0.015; Figure 4b) and less time spent in the first square ($F_{(1,36)}$ = 8.25; $P_{condition}$ = 0.007; Figure 4d) compared to control groups. However, no significant difference was observed between the copaiba and sunflower groups. No difference was observed in other behavioral parameters in the open-field test (Figure 4).

Food consumption and body weight gain

Although it was not our main goal, we found that copaiba oleoresin reduced food consumption and body weight gain in both the control/COP (t = 3.44; $P_{week\ 1}$ = 0.007; and t = 7.12; $P_{week\ 1}$ < 0.001, respectively), and alcohol/COP group (t = 9.27; $P_{week\ 1}$ < 0.001; and t = 8.31; $P_{week\ 1}$ < 0.001, respectively) compared to their SF counterparts (Table 1). Total body weight gain was 26% and 37% lower in the control/COP and alcohol/COP group, respectively (Table 1). We did not observe changes in the health of rats, nor recorded any deaths due to treatment throughout the experiment.

DISCUSSION

Our results showed that copaiba oleoresin decreased voluntary alcohol consumption and preference in rats. Before copaiba treatment, alcohol groups showed saccharin solution intake similar to control groups, but exhibited 56% preference for alcohol drinking. Copaiba administration did not affect the consumption of the saccharin solution in both groups, but decreased the daily alcohol intake by 20% and the alcohol preference by 30% in the alcohol/COP group. These data provide evidence that decreased alcohol consumption and preference of copaiba-treated animals was not due to an overall fluid intake reduction.

β-caryophyllene, found in the copaiba oleoresin, is a selective cannabinoid CB_2 receptor full agonist (Gertsch *et al*. 2008; Machado *et al*. 2018). Because β-caryophyllene composed 52% (~ 310 mg kg^{-1} day^{-1}) of our copaiba oleoresin,

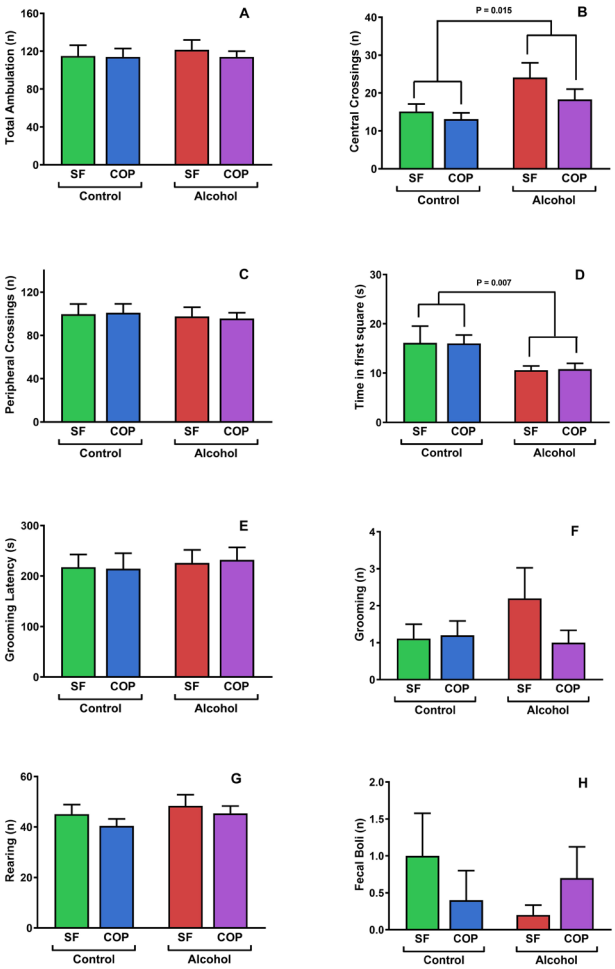

Figure 4. Behavioral responses of rats in an open-field test 10 days after the last of three days of subcutaneous injections of copaiba (600 mg kg^{-1}) or sunflower oil and exposition to a two-bottle choice model of alcohol voluntary intake. A – total ambulation; B – central crossings; C – peripheral crossings; D – time spent in the first square; E – grooming latency; F – grooming frequency; G – rearing frequency; H – number of fecal boli. Significant differences according to a two-way ANOVA (n = 10 rats per group). This figure is in color in the electronic version.

Table 1. Effect of the subcutaneous administration during three days of 600 mg kg^{-1} copaiba (COP) or 1 mL kg^{-1} sunflower (SF) oil on daily food intake and weekly body weight gain in rats exposed to a two-bottle choice model of voluntary alcohol intake. Two-way RM-ANOVA + Bonferroni's test; n = 10/group; *indicates a significant difference between of the COP and respective SF groups at $P < 0.05$.

Food intake (g day^{-1})				
	Control/SF	Control/COP	Alcohol/SF	Alcohol/COP
Baseline	28.6 ± 2.0	28.6 ± 2.0	27.2 ± 1.5	27.2 ± 1.5
Week 1	27.2 ± 2.0	24.1 ± 0.9*	28.4 ± 2.9	20.1 ± 2.0*
Week 2	25.6 ± 2.6	28.6 ± 1.4	26.8 ± 1.8	24.2 ± 1.6
Body weight gain (g week^{-1})				
	Control/SF	Control/COP	Alcohol/SF	Alcohol/COP
Baseline	26.4 ± 3.0	26.4 ± 3.0	22.7 ± 5.0	22.7 ± 5.0
Week 1	12.8 ± 3.7	-6.5 ± 7.4*	15.4 ± 5.2	-6.5 ± 11.0*
Week 2	17.6 ± 6.6	20.7 ± 4.9	20.0 ± 5.3	20.2 ± 6.8

it may have contributed to decreasing alcohol intake and preference in our rats after subcutaneous administration. This dosage is three times the highest dosage of β-caryophyllene that was shown to decrease alcohol drinking in mice (Al Mansouri *et al*. 2014). Moreover, robust results show that copaiba oil inhibits inflammatory response decreasing dose-dependently NF-kB, IL-1β, IL-6, IL-17, TNF-α, and IFN-γ levels in the CNS of rats (Veiga Junior *et al*. 2007; Guimarães-Santos *et al*. 2012; Gelmini *et al*. 2013). Specifically, β-caryophyllene inhibits proinflammatory pathways, including the toll-like receptor complex CD14/TLR4/MD2, which suppresses cytokine expression (Gertsch *et al*. 2008). Because alcohol-induced neuroinflammation has been related to increased voluntary alcohol consumption and worsening of withdrawal symptoms and relapse in rodents and humans (Kelley and Dantzer 2011; Robinson *et al*. 2014; Schneider *et al*. 2017), we cannot ignore that this mechanism would also contribute to antiaddictive properties of copaiba oleoresin.

In the present study, rats showed a significant reduction in alcohol intake and preference between two and six days after the last copaiba oleoresin administration. This long-lasting effect may be related to the subcutaneous route of administration, that produces extended release and absorption, in addition to slower elimination, since its main active molecules are lipophilic (Herrero-Jáuregui *et al*. 2011). Indeed, evidence shows that the residual therapeutic effect of copaiba subcutaneous administration persisted for up to five days after the last dose in mice (Lopes *et al*. 2015). Here, this extended-release effect from the subcutaneous tissue lasted for six days, since animals in the alcohol/COP group returned to their basal daily alcohol consumption on day 7 of the last copaiba administration. Nevertheless, the general analysis of consumption in all days after the intervention showed a 20% reduction of overall daily alcohol consumption in copaiba-treated animals, evidencing the magnitude of effect on alcohol consumption.

Regarding behavioral tests, no residual effect of copaiba was observed in the open-field test 10 days after the last subcutaneous administration. On this day, we observed only the alcohol effect on the rats' behavior. Both alcohol groups increased central crossings and reduced the time spent in the first square, which are indicative of an anxiolytic-like effect (Prut and Belzung 2003). An anxiolytic phenotype for alcohol-treated animals was expected since it is a central nervous system depressant drug exerting a positive modulatory effect on $GABA_A$ receptors (Gilpin and Koob 2008).

Additionally, the copaiba treatment reduced food intake and body weight gain during the week of intervention in both the control/COP and alcohol/COP groups, with the decreased feeding lasting longer to the next week in alcohol/COP animals. It is possible that the decreased food consumption in our treated rats is related to β-caryophyllene or other

compounds in the copaiba oleoresin that acts as a cannabinoid CB2 receptor agonist. The anti-obesity effect of the CB2 receptor agonist was demonstrated in diet-induced obese mice (Youssef *et al*. 2019). Also, we cannot discard the possibility that the lower alcohol consumption in the alcohol/COP group may reflect a generalized effect of copaiba on caloric intake, rather than its selective effect as an antiaddictive substance.

Finally, we need to consider that our study presents some limitations. We do not have results from female rats and cannot translate them to this gender. Moreover, we do not have a water control group to compare with the saccharin vehicle group. These groups (water-SF and water-COP) would reveal the effect of saccharin in our results, deviating our focus and breaking the replacement, reduction, and refinement principles (3R's) for the use of animals in research.

CONCLUSIONS

Our study showed that subcutaneously administered copaiba oleoresin reduces voluntary alcohol intake and preference in rats. We suggest that this effect is due to the synergistic interaction among different phytochemical compounds in the copaiba oleoresin with the endocannabinoid system, attenuating dopamine release in the nucleus accumbens and decreasing the positive reinforcement from alcohol intake. Additionally, since alcohol use disorder presents significant inflammatory hallmarks, we do not discard that copaiba reduces alcohol consumption related to its neuroprotective and anti-inflammatory effects. Further studies will elucidate the pharmacological mechanisms involved in the copaiba oleoresin reducing effect on alcohol consumption. Finally, because alcohol use disorder is related to premature death and disability, and copaiba is used in folk medicine, with a safe profile, we suggest translating our results from bench to bedside and investigate the potentially beneficial effect of copaiba oleoresin in alcohol-dependent individuals.

ACKNOWLEDGMENTS

This work was financially supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The authors acknowledge Fabian Laszlo Flegner, from Empório LASZLO (Belo Horizonte, Brazil), for kindly providing the copaiba oleoresin used in this study.

REFERENCES

Al Mansouri, S.; Ojha, S.; Al Maamari, E.; Al Ameri, M.; Nurulain, S.M.; Bahi, A. 2014. The cannabinoid receptor 2 agonist, β-caryophyllene, reduced voluntary alcohol intake and attenuated ethanol-induced place preference and sensitivity in mice. *Pharmacology, Biochemistry, and Behavior,* 124: 260–268.

Crews, F.T.; Vetreno, R.P. 2014. Neuroimmune basis of alcoholic brain damage. *International Review of Neurobiology,* 118: 315–357.

Gelmini, F.; Beretta, G.; Anselmi, C.; Centini, M.; Magni, P.; Ruscica, M.; *et al*. 2013. GC-MS profiling of the phytochemical constituents of the oleoresin from Copaifera langsdorffii Desf. and a preliminary in vivo evaluation of its antipsoriatic effect. *International Journal of Pharmaceutics,* 440: 170–178.

Gertsch, J.; Leonti, M.; Raduner, S.; Racz, I.; Chen, J.-Z.; Xie, X.-Q.; *et al*. 2008. Beta-caryophyllene is a dietary cannabinoid. *Proceedings of the National Academy of Sciences of the United States of America,* 105: 9099–9104.

Gilpin, N.W.; Koob, G.F. 2008. Neurobiology of alcohol dependence: focus on motivational mechanisms. *Alcohol Research & Health: The Journal of the National Institute on Alcohol Abuse and Alcoholism,* 31: 185–195.

Gomes, N.M.; Rezende, C.M.; Fontes, S.P.; Matheus, M.E.; Fernandes, P.D. 2007. Antinociceptive activity of Amazonian Copaiba oils. *Journal of Ethnopharmacology,* 109: 486–492.

González-Reimers, E.; Santolaria-Fernández, F.; Martín-González, M.C.; Fernández-Rodríguez, C.M.; Quintero-Platt, G. 2014. Alcoholism: a systemic proinflammatory condition. *World Journal of Gastroenterology,* 20: 14660–14671.

Guimarães-Santos, A.; Santos, D.S.; Santos, I.R.; Lima, R.R.; Pereira, A.; de Moura, L.S.; *et al*. 2012. Copaiba oil-resin treatment is neuroprotective and reduces neutrophil recruitment and microglia activation after motor cortex excitotoxic injury. *Evidence-Based Complementary and Alternative Medicine,* 2012: 918174.

Haass-Koffler, C.; Bartlett, S. 2012. Stress and addiction: contribution of the corticotropin releasing factor (CRF) system in neuroplasticity. *Frontiers in Molecular Neuroscience,* 5: 91. doi: 10.3389/fnmol.2012.00091

Herrero-Jáuregui, C.; Casado, M.A.; das Graças Bichara Zoghbi, M.; Célia Martins-da-Silva, R. 2011. Chemical variability of Copaifera reticulata Ducke oleoresin. *Chemistry & Biodiversity,* 8: 674–685.

Kelley, K.W.; Dantzer, R. 2011. Alcoholism and inflammation: neuroimmunology of behavioral and mood disorders. *Brain, Behavior, and Immunity,* 25 Suppl 1: S13-20.

Koob, G.F.; Volkow, N.D. 2016. Neurobiology of addiction: a neurocircuitry analysis. *The lancet. Psychiatry,* 3: 760–773.

Lopes, L.N.; Santos, F.A.F.; Oliveira, L.C.M.; Percário, S.; Barros, C.A.V. de; Brito, M.V.H. 2015. Copaiba oil effect on induced fecal peritonitis in rats. *Acta Cirurgica Brasileira,* 30: 568–573.

Machado, K. da C.; Islam, M.T.; Ali, E.S.; Rouf, R.; Uddin, S.J.; Dev, S.; *et al.* 2018. A systematic review on the neuroprotective perspectives of beta-caryophyllene. *Phytotherapy Research, PTR* 32: 2376–2388.

Prut, L.; Belzung, C. 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *European Journal of Pharmacology,* 463: 3–33.

Pulcinelli, R.R.; de Paula, L.F.; Nietiedt, N.A.; Bandiera, S.; Hansen, A.W.; Izolan, L.D.R.; *et al*. 2020. Taurine enhances voluntary

alcohol intake and promotes anxiolytic-like behaviors in rats. *Alcohol (Fayetteville, N.Y.),* 88: 55–63.

Qin, L.; He, J.; Hanes, R.N.; Pluzarev, O.; Hong, J.-S.; Crews, F.T. 2008. Increased systemic and brain cytokine production and neuroinflammation by endotoxin following ethanol treatment. *Journal of Neuroinflammation,* 5: 10. doi.org/10.1186/1742-2094-5-10

Roberto, M.; Varodayan, F.P. 2017. Synaptic targets: Chronic alcohol actions. *Neuropharmacology,* 122: 85–99.

Robinson, G.; Most, D.; Ferguson, L.B.; Mayfield, J.; Harris, R.A.; Blednov, Y.A. 2014. Chapter Two - Neuroimmune pathways in alcohol consumption: Evidence from behavioral and genetic studies in rodents and humans. *International Review of Neurobiology*, 118: 13–39.

Santos, A.O.D.; Ueda-Nakamura, T.; Dias Filho, B.P.; Veiga Junior, V.F.; Pinto, A.C.; Nakamura, C.V. 2008. Antimicrobial activity of Brazilian copaiba oils obtained from different species of the Copaifera genus. *Memorias do Instituto Oswaldo Cruz,* 103: 277–281.

Schneider, R.; Bandiera, S.; Souza, D.G.; Bellaver, B.; Caletti, G.; Quincozes-Santos, A.; *et al*. 2017. N-acetylcysteine Prevents Alcohol Related Neuroinflammation in Rats. *Neurochemical Research,* 42: 2135–2141.

da Trindade, R.; da Silva, J.K.; Setzer, W.N. 2018. Copaifera of the Neotropics: A Review of the Phytochemistry and Pharmacology. *International Journal of Molecular Sciences,* 19: 1511–1544.

Urasaki, Y.; Beaumont, C.; Workman, M.; Talbot, J.N.; Hill, D.K.; Le, T.T. 2020. Fast-Acting and Receptor-Mediated Regulation of Neuronal Signaling Pathways by Copaiba Essential Oil. *International Journal of Molecular Sciences,* 21: 2259–2274.

Veiga Junior, V.F.; Rosas, E.C.; Carvalho, M.V.; Henriques, M.G.M.O.; Pinto, A.C. 2007. 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. *Journal of Ethnopharmacology,* 112: 248–254.

WHO. 2018. World Health Organization. *Global status report on alcohol and health*. (http://www.who.int/substance_abuse/publications/global_alcohol_report/en/). Accessed on May 26, 2021.

Youssef, D.A.; El-Fayoumi, H.M.; Mahmoud, M.F. 2019. Beta-caryophyllene alleviates diet-induced neurobehavioral changes in rats: The role of CB2 and PPAR-γ receptors. *Biomedecine & Pharmacotherapie,* 110: 145–154.

RECEIVED: 04/10/2021
ACCEPTED: 31/01/2022
ASSOCIATE EDITOR: Emiliano Barreto