Scientific Electronic Archives
Issue ID: Sci. Elec. Arch. Vol. 16 (5)
May 2023
DOI: http://dx.doi.org/10.36560/16520231722
Article link: https://sea.ufr.edu.br/SEA/article/view/1722

ISSN 2316-9281

Assessment of the effect of copaiba oil (copaifera sp.) on the kidneys of rats with hepatic dysfunction

Corresponding author
Ketlyn Caroline da Silva
Universidade Federal de Mato Grosso, Campus Sinop
ketymarc@hotmail.com

Gisele Facholi Bomfim
Universidade Federal de Mato Grosso, Campus Sinop

Danilo Henrique Aguiar
Universidade Federal de Mato Grosso, Campus Sinop

Abstract: Copaiba oil reveals therapeutic properties and has actions such as anti-inflammatory, healing, antiseptic, antitumor, antibacterial, germicidal, expectorant, diuretic and analgesic. Given the anti-inflammatory properties of copaiba oil, the present study aims to evaluate the action of the oil on possible nephrotoxic effects of rats with liver dysfunction caused by the drug thioacetamide. Wistar rats were randomly divided into four groups, consisting of the control group (C), copaiba oil group (O), thioacetamide group (TAA) and thioacetamide + copaiba oil group (TAA + O). The animals received treatment via gavage for eight weeks and were fed standard rodent chow. The kidney fragments were histologically prepared using the historresin technique and HE staining and, later, the images were photographed and processed in an image analyzer for histological characterization of the kidney tissue. The histological results showed an intact kidney tissue with a homogeneous pattern between the groups. Typical cortical and medullary layers and very distinct renal tubules with their well stained and healthy cells. The renal corpuscles showed regular contours, well-distributed anastomosed capillaries and typical macula densa. Histopathological analysis of the kidneys did not reveal alterations or any type of lesion in important structures such as renal tubules and renal corpuscles, and no lymphocytic infiltrate was observed in the renal interstitium. In contrast, the liver tissue from the parallel histological study developed cirrhosis in the group treated with thioacetamide, however, this drug did not compromise the renal histological pattern of the present study. It is concluded that the kidney tissue did not show morphological changes when submitted to thioacetamide, supposedly due to the exposure time, the dose used or that the thioacetamide has been metabolized into less toxic compounds to the kidneys to the detriment of hepatic damage. However, the parallel study of liver tissue and the present study ensure the use of copaiba oil for therapeutic purposes.
Keywords: liver dysfunction, kidney tissue, histology.

Introduction

The renal system is the primary means of elimination of unwanted products of metabolism. Products such as urea, creatinine, uric acid,hemoglobin breakdown products and metabolites of various hormones are eliminated by the kidneys through excretion. The kidneys eliminate toxins produced by the body, as well as exogenous products, such as pesticides, drugs and food additives (GUYTON & HALL, 2011).

Some medications, metals and drugs can compromise kidney function by altering Glomerular Filtration Rate (GFR) blood urea and creatinine levels. In addition to these substances, extrinsic pathological events have the potential to cause changes in nephrons, increasing oxidative stress (CALVIN et al., 2010; MAKRIS & SPANOU, 2016). It is common for critically ill patients to have Acute Kidney Disease (ARD), due to the frequent use of nephrotoxic drugs (KANE-GILL & GOLDSTEIN, 2015). Kidney damage can progress to Acute Kidney Injury (AKI) or Chronic Kidney Disease (CKD) if not repaired, leading to kidney dysfunction and multiple organ failure (EL SABBAHY & VAIDYA, 2011; HULTSTROM et al., 2018).

Medicines and toxic substances are converted by the body into highly reactive free radicals that cause severe kidney damage and have important activity in the development of nephrotoxicity (MOHAMMEED et al., 2011; OLAGUNJU et al., 2009). Thioacetamide is an organic compound that causes nephrotoxicity, as well as a hepatotoxin

(KADIR et al., 2011). Administration of thioacetamide causes renal toxicity by altering urea and creatinine values. In addition, it alters the activity of antioxidant enzymes that reduce oxidative stress and decreases the advancement of lipid peroxidation. Histopathological analyzes demonstrated impaired renal morphology (KADIR et al. 2013).

Many substances are used as treatment, including copaiba oil. The cobaibeira tree is found in Brazil, in the Amazon rainforest region (ARAÚJO JÚNIOR et al., 2005). Its main denomination copaíba is of Tupi origin “cupa-yba” which means “deposit tree” and is classified in botany as belonging to the Leguminosae family and the Copaifera genus, with sixteen species found exclusively in Brazil (BRITO et al., 2000; CASCON, 2004; INDEX KEWENSIS, 1996; LLOYD, 1898; MACIEL et al., 2002; OLIVEIRA et al., 2006; RAMOS, 2006; VEIGA JUNIOR et al., 2005; VEIGA JUNIOR & PINTO, 2002). An oil-resin is extracted from the tree and is known as copaiba or balsam (LLOYD, 1898; CASCON, 2004). The Indians used copaiba oil to treat wounds and also on the umbilical stump of newborns (FRANCISCO, 2005; MACIEL et al., 2002; SALVADOR, 1975; VEIGA JUNIOR & PINTO, 2002.). Some authors cite the therapeutic properties of the oil as anti-inflammatory, and its chemical components responsible for this property are hydrocarbons and sesquiterpenes. It also has a healing action, antiseptic, antitumor, antibacterial, germicidal, expectorant, diuretic and analgesic action (ARAÚJO JÚNIOR et al., 2005; BLOISE, 2003; BRITO et al., 2000; BRITO et al., 2005; CASCON, 2004; DRUMOND et al., 2004; FRANCISCO, 2005; FREIRE et al., 2006; GOODMAN & GILMAN, 1945; GURGEL, 2004; MACIEL et al., 2002; OLIVEIRA et al., 2005; OLIVEIRA et al., 2006; PACHECO et al., 2006; PIERI, 2007; RAMOS, 2006; RIGAMONTE AZEVEDO et al., 2004; ROBBERS et al., 1996; RODRIGUES, 1989; SILVA et al., 2006; VEIGA JUNIOR & PINTO, 2002; VEIGA JUNIOR et al. al., 2005; VIEIRA, 1992).

Given the anti-inflammatory properties of copaiba oil, the present study aims to evaluate the action of the oil on the nephrotoxic effects of the organic agent thioacetamide in animals with hepatic dysfunction.

Methods

Animals and experimental protocol

All animal procedures were performed following the regulations of the National Council on Animal Experimental Control (CONCEA, Brazil). The experimental protocol was approved by the Ethics Committee on the Use of Animals (CEUA, under protocol no. 23108.039273/ 2019-60) of the Federal University of Mato Grosso. The animals used were rats male Wistar weighing about 300 g kept at constant room temperature and light cycle (12:12-h light-dark cycle). The animals were randomly divided into four groups, comprising a control group (C), control+copaiba oil (C+Copaiba), thioacetamide (TAA), thioacetamide+copaiba oil 200mg/kg (TAA+Copaiba). The animals received standard rodent chow (NUVILAB CR-1, Sorgob Industry and business Ltda, São Paulo/SP, Brazil). In the groups treated with TAA, 100mg/kg of TAA (Sigma-Aldrich®, USA) were administered intraperitoneally three times a week for 8 weeks, since TAA has hepatotoxic properties and is capable of triggering liver cirrhosis (TÚNEZ et al, 2005). The groups treated with copaiba oil received copaiba oil (200mg/kg/day) daily via gavage for 8 weeks diluted in tween 20 (3%) (GONÇALVES, 2014). Control groups were treated with vehicle, following the same protocol used in treatments with TAA and/or copaiba oil. The groups were treated as follows: Control: received vehicle via gavage and intraperitoneally; Control + copaiba oil: received vehicle intraperitoneally and copaiba oil (200mg/kg/day) for 8 weeks; Thioacetamide: TAA for 8 weeks and vehicle via gavage; Thioacetamide + copaiba oil 200mg/kg/day: TAA and copaiba oil (200mg/kg/day) for 8 weeks.

Sacrifice and extract of samples

After 8 weeks of the experimental period, the animals were euthanized under anesthesia with a mixture of ketamine (113 mg kg− 1 body weight; b.w.) and xylazine (7.4 mg kg− 1 b.w.) at a dose of 0.15 mL 100 g− 1 b.w., intraperitoneally. The rats were weighed and then the renal tissue were removed and fixed in 10% buffered formaldehyde for further processing.

Histological Analysis

Initially, the material was washed with distilled water and immersion in 70% alcohol for 24 hours. Then followed with dehydration, in which water was removed from the tissues with increasing baths of 80% alcohol for 2 hours, followed by 95% alcohol for 4 hours, with 2 changes of alcohol at each concentration. Then, the samples were immersed in the mixture of plastic resin plus 95% alcohol for 6 hours. Then they were immersed in pure infiltration resin for 48 hours. Finally, the samples were soaked in historresin using a hardener in the resin solution and embedded in plastic histomolds until complete hardening. Then, after a few hours, the fragment was removed from the mold and embedded with plastic glue on a wooden block, and duly identified.

A semi-automatic rotary microtome, Leica model 2245, was used with the function of sectioning the embedded material into 3 µm cross-sections, cut on glass razors and collected on slides. The tissue slices obtained from the microtome were transferred to a container with water and then collected on a properly identified histological slide. Finally, the histological slides were placed in an oven to remove moisture for 24 hours until the beginning of the staining process.

HE staining was used in which the slides were immersed in water and then in a vat containing hematoxylin for 20 minutes. Afterwards, the slides

were washed in running water to remove excess dye and then immersed in eosin for 10 minutes. The excess dye was removed with running water and the slides were dehydrated with increasing alcohol baths (80%, 95% and 100%), cleared with xylene and mounted in permount.

Analysis of histological images

Histological sections were used for image analysis and processing using a Moticam 2.0 model analysis system. The images were photographed and processed for histological characterization of renal tissue.

Results and discussion

Histological observation of renal tissue revealed a homogeneous pattern between the studied groups. Histopathological analysis did not demonstrate altered aspects of important regions such as renal tubules and renal corpuscles, especially in the thioacetamide group. The liver tissue, from a histological study parallel to the present study, developed a condition of cirrhosis for the group treated with thioacetamide, but in the kidneys this drug did not compromise the histological pattern as shown in Figure 1. The results show in all groups a renal tissue intact with a normal pattern of the cortical and medullary layers, the renal tubules very distinct with their cells well stained and firm. The renal corpuscles showed regular contours, well positioned internally anastomosed capillaries and typical macula densa.

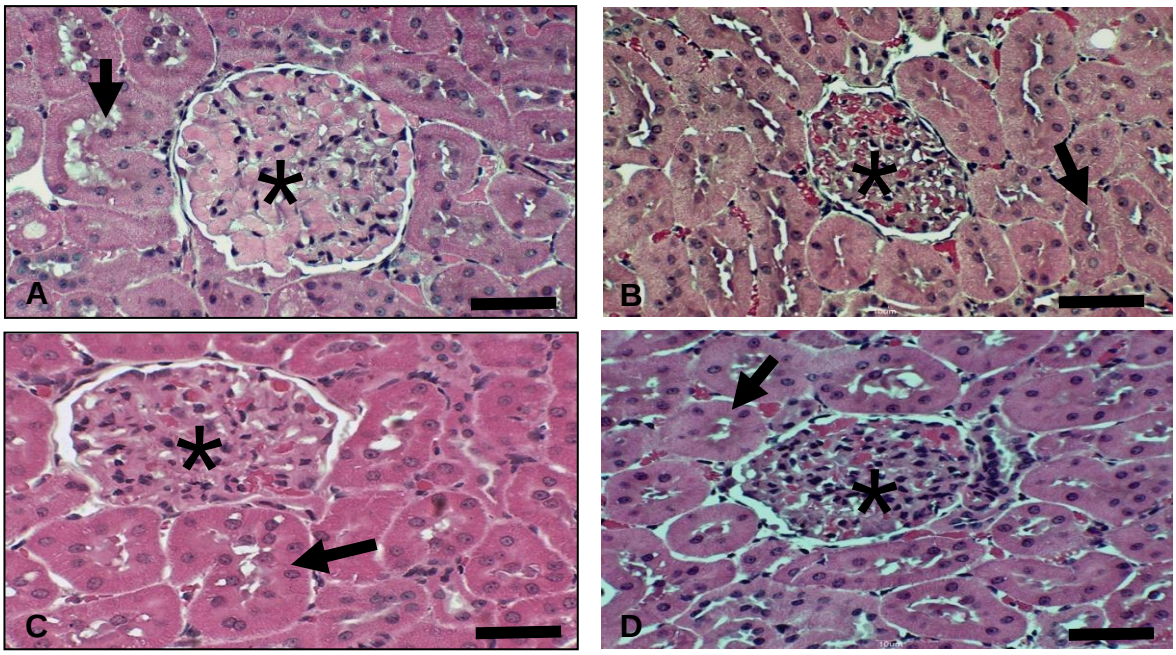

Figure 1. Frontal section photomicrograph of the kidneys showing a histological pattern. A. Control Group (C). B. Oil Group (O). C. Thioacetamide (TAA) group. D. Thioacetamide + Oil Group (TAA+O). Typical renal corpuscles (asterisks). Typical renal tubules (arrows). HE. Bar = 50

There was no lymphocytic infiltrate in the renal interstitium or any type of lesion, only small changes of technical effect, caused by the preparation of the material. In the research by Schyman et al. (2018) markers of necrosis were identified as a possible kidney injury after treatment with intraperitoneal injection of TAA, however histological analysis revealed no injury after 24 hours. Previous study by Igarashi et al. (2015) showed that thioacetamide causes renal necrosis after four days. The mechanisms of kidney damage are dependent on the time of exposure to the toxicant and on the dose. Activation of lesion pathways increases with time after exposure to high dose of TAA, however for low dose, activation of necrosis pathways was reduced by 24 hours compared to 8 hours (SCHYMAN et al., 2018). Therefore, the animals in the present study, despite having presented cirrhosis in the liver tissue, did not develop kidney injuries from the administration of thioacetamide, possibly due to the exposure time and the dose used. Another hypothesis is that thioacetamide has been eliminated or metabolized into less toxic compounds, allowing the rats to recover from kidney injuries (SCHYMAN et al., 2018).

As there were no changes in renal tissue with the administration of thioacetamide in the present study, it was not possible to identify the protective effects of copaiba oil, however, the study by Brito et al. (2005) demonstrated that after subjecting rats to ischemia and reperfusion syndrome, they exhibited damage to the renal parenchyma, and subsequent treatment with copaiba oil ameliorated the kidney damage and decreased urea and creatinine levels, which are markers of functionality. . Oliveira et al. (2013) used copaiba tree leaf extract and identified

potentially antioxidant activity, which prevented the formation of lithiasis, after induction of injury by the toxic ethylene glycol. Lima Silva et al. (2009) showed intense antioxidant activity and anti-inflammatory activity in a model involving skin flaps. Given these findings in the literature, there is a need for further studies to be carried out on the use of this oil to better clarify its phytotherapeutic effects (BRITO et al. 2005).

Conclusion

It is concluded that the renal tissue did not show morphological changes when subjected to thioacetamide, supposedly due to the exposure time, the dose used or that the thioacetamide has been metabolized into less toxic compounds to the kidneys to the detriment of liver damage. Nevertheless, the parallel study with liver tissue and the present study ensure the use of copaiba oil for therapeutic purposes

References

ARAÚJO JÚNIOR, F. A. et al. Efeito do óleo de copaíba nas aminotransferases de ratos submetidos à isquemia e reperfusão hepática com e sem pré-condicionamento isquêmico. Acta. Cirúrgica Brasileira, v. 20, n.1, p. 93-9, 2005. Acesso em: https://doi.org/10.1590/S0102-86502005000100013

BLOISE, M.I. Óleos vegetais e especialidades da floresta amazônica. Cosmetics & Toiletries, 2003. v.15, n.5, p. 46-9. Acesso em:

https://www.cosmeticsonline.com.br/2011/ed.php?edicao=Setembro/Outubro_2003

BRITO, M.V.H. et al. Copaiba oil effect on urea and creatinine serum levels in rats submitted to kidney ischemia and reperfusion syndrome. Acta Cirúgica Brasileira, v.20, n.3, p.243-6, 2005. Acesso em: https://doi.org/10.1590/S0102-86502005000300009

BRITO, N. M. B et al. Aspectos morfológicos e morfométricos do colo uterino de ratas ooforectomizadas após aplicação de óleo de copaíba. Revista Brasileira de Ginecologia e Obstetrícia, v. 22, n. 8, p. 489-93, 2000. Acesso em: https://www.scielo.br/j/rbgo/a/JShn5ZdrGvz8WnWTz5j5qSt/?format=pdf&lang=pt

CALVIN, A. D.; MISRA, S.; PFLUEGER A. Contrast-induced acute kidney injury and diabetic nephoropathy. Nat. Ver. Nephrol., v. 6, p. 679-688, 2010. DOI: 10.1038/nrneph.2010.116

CASCON, V. Copaíba- Copaifera spp. In: CARVALHO, J.C.T. Fitoterápicos antiinflamatórios: aspectos químicos, farmacológicos e aplicações terapêuticas. Ribeirão Preto. Tecmedd, p. 480, 2004. Acesso em: https://doi.org/10.1590/S1516-05722009000400016

SANTANA, M. D. R; CASTRO, R. D. ; ALMEIDA, R. V. D. ; PERREIRA, M. S. V. ; PADILHA, W. W. N. . Estudo comparativo in vitro da atividade antibacteriana de produtos fitoterápicos sobre bacterias cariogenicas. Pesquisa Brasileira em Odontopediatria e Clínica Integrada, João Pessoa, v. 4, n.jan/abr, p. 33-38, 2004.

EL SABBAHY, M.; VAIDYAN, V. Ischemic kidney injury and mechanisms of tissue repair. WIREs Syst. Biol. Med., v. 3, p. 606-618, 2011. DOI: 10.1002/wsbm.133

FRANCISCO, S. G. Uso do óleo de copaíba (Copaifera officinalis) em inflamação ginecológica. Femina, v. 33, n. 2, p. 89-93, 2005.

FREIRE, D.B. et al. Efeito dos óleos vegetais de andiroba (Carapa sp.) e copaíba (Copaifera sp.) sobre forídeos, pragas de colméias, (Díptera: Phoridae) na Amazônia central. Acta Amazônica, v.36, n.3, p.365-8, 2006.

GONÇALVES, E. S. Avaliação da segurança de uso do óleo de Copaifera multijuga Hayne (Fabaceae). 2014. 140f. Tese (Doutorado em ciências farmacêuticas). Universidade Federal do Pernambuco, Recife, 2014. Acesso em:

https://repositorio.ufpe.br/handle/123456789/11946

GOODMAN, L.; GILMAN, A. As bases farmacológicas da terapêutica. Rio de Janeiro. Editora Guanabara, v.2, p. 1461, 1945.

GURGEL, C.B.F.M. A fitoterapia indígena do Brasil colonial (os primeiros dois séculos). In: ENCONTRO REGIONAL DE HISTÓRIA, 11., 2004. Anais eletrônicos. Rio de Janeiro: UFF, 2004. Disponível em: http://www.uff.br/ichf/anpuhrio/Anais. Acesso em: 24 de agosto de 2021.

GUYTON, A. C.; HALL, J. E. Tratado de Fisiologia Médica. 12. ed. Rio de Janeiro: Elsevier, 2011. 321-322 p.

HULSTROM, M. BECIROVIC-AGIC, M.; JONSSON, S. Comparison of acut kidney in injury of different etiology reveals in-common mechanisms of tissue damege. Physiol Genomics., v. 50, p.127-141, 2018. DOI: 10.1152/fisiogenômica.00037.2017

IGARASHI, Y.; NAKATSU, N.; YAMASHITA, T.; ONO, A.; OHNO, Y.; URUSHIDANI, T.; et al. Open TG-GATEs: a large-scale toxicogenomics database. Nucleic Acids Res. 43, D921–D927. 2015.DOI: 10.1093/nar/gku955

INDEX KEWENSIS. Supplement XX. Oxford. Claredon Press, p. 346, 1996.

KADIR, F. A.; KASSIM, N. M.; ABDULLA, M. A.; YEHYE, W. A. Effect of oral administration of ethanolic extract of Vitex negundo on thiocetamide-

induced nephrotoxicity in rats. BMC Complem. and Altern. Med., 13:294, 2013. DOI: 10.1186/1472-6882-13-294

KADIR, F. A.; OTHMAN F.; ABDULLA, M. A; HUSSAN, F., HASSANDARVISH, P. Effect of Tinospora crispa on thioacetamide-induced liver cirrhosis in rats. Indian J. Pharmacol., v. 43 (1): p. 64-68, 2011. DOI: 10.4103/0253-7613.75673

KANE-GILL, S. L.; GOLDSTEIN, S. L. Drug-induced acute kidney injury: a facus on risk assessment for prevention. Crit Care Clin. v. 31 (4): p. 675-84, 2015. DOI: 10.1016/j.ccc.2015.06.005

LIMA SILVA, J. J; GUIMARÃES, S. B.; DA SILVEIRA, E. R.; DE VASCONCELOS, P. R.; LIMA, G. G.; TORRES, S. M.; DE VASCONCELOS R.C. Effects of Copaifera langsdorffii Desf. on ischemia-reperfusion of randomized skin flaps in rats. Aesthet Plast Surg., 2009. 33:104–109. DOI: 10.1007/s00266-008-9263-2

LLOYD, J. U. Copaifera officinalis. Chicago: The Western Druggist, p.13 ,1898.

MACIEL, M. A. et al. Plantas medicinais: a necessidade de estudos multidisciplinares. Química Nova, v.25, n.3, p.429-38, 2002. Acesso em: https://doi.org/10.1590/S0100-40422002000300016

MAKRINS, K.; SPANOU, L. Acute Kidney injury: Definitin, pathophysiology, and clinical phenotypes. Clin. Biochem Rev., v. 37. p. 85-98, 2016.

MOHAMMED A. A, MAHMOOD AMEEN A, SALMAH I, ZAHRA A. A. Hepatoprotective effects of Orthosiphon stamineus extract on thioacetamide-induced liver cirrhosis in rats. Evidence-Based Complementary and Alternative Medicine 2011, 2011. DOI: 10.1155/2011/103039

OLAGUNJU, J., ADENEYE, A.; FAGBOHUNKA, B. et al. Nephroprotective activities of the aqueous seed extract of Carica papaya Linn. In carbono tetrachloride induced renal injured Wistar rats: a dose-and time-dependent study. Biol Med., v. 1 (1): p. 11-19, 2009.

OLIVEIRA, E. C. P. et al. Identificação da época de coleta do óleo-resina de copaíba (Copaifera spp.) no município de Moju-PA. Revista Brasileira de Plantas medicinais, v.8, n.3, p.14-23, 2006.

OLIVEIRA, R. B.; COELHO, E. B.; RODRIGUES, M.R.; COSTA-MACHADO, A. R. M.; SOUSA, J. P. B.; BERRETTA, A. A.; BASTOS, J. K. Effect of the Copaifera langsdorffii Desf. Leaf Extract on the Ethylene Glycol-Induced Nephrolithiasis in Rats. Evidence-Based Complementary and Alternative Medicine. 2013. p. 10. DOI: 10.1155/2013/131372

OLIVEIRA, U.D. et al. Avaliação do ciclo celular de Aspergillus nidulans exposto ao extrato da planta Copaifera officinalis L. Revista Saúde e Biologia, v.1, n.2, p.42-7, 2005.

PACHECO, T. A. R. C. et al. Antimicrobial activity of copaíba (Copaifera spp) balsams. Revista Brasileira de Plantas Medicinais, v.8, p.123-4, 2006.

PIERI, F.A. Efeito (in vitro/ in vivo) do óleo de copaíba (Copaifera officinalis) sobre bactérias formadoras de placa dental em cães (Canis lupus Familiaris). Dissertação (Mestrado em Ciência Animal) -Universidade José do Rosário Vellano. Alfenas. p. 85, 2007.

RAMOS, M. F. S. Desenvolvimento de microcápsulas contendo a fração volátil de copaíba por spray-dryving: estudo de estabilidade e avaliação farmacológica. Ribeirão Preto. Universidade de São Paulo, p. 132, 2006.

RIGAMONTE AZEVEDO, O.C. et al. Copaíba: ecologia e produção de óleo-resina. Rio Branco. EMBRAPA, MAPA, 2004. 28p. Acesso em: https://ainfo.cnptia.embrapa.br/digital/bitstream/CPAF-AC/10517/1/doc91.pdf

ROBBERS, J. E. et al. Pharmacognosy and pharmacobiotechnology. Baltimore. Williams & Wilkins, p. 100, 1996.

RODRIGUES, R. M. A flora da Amazônia. Belém: Editora Cejup, p. 463,1989.

SALVADOR, V. História do Brasil: 1500-1627. 6.ed. São Paulo. Melhoramentos, 1975. 63p.

SCHYMAN, P.; PRINTZ, R. L.; ESTES, S. K.; BOYD, K. L.; SHIOTA, M.; WALLQVIST, A. Identification of the Toxicity Pathways Associated With Thioacetamide-Induced Injuries in Rat Liver and Kidney. Front. Pharmacol. 9:1272. 2018. DOI: 10.3389/ffar.2018.01272

SILVA, F. H. et al. Estudo do óleo essencial e extrato hidrometanólico de Copaifera langsdorffii Desf (Caesalpinaceae) do cerrado e mata atlântica. REUNIÃO NACIONAL DA SOCIEDADE BRASILEIRA DE QUÍMICA. Águas de Lindóia, 2006.

TÚNEZ, I.; MUNOZ, M. C.; VILLAVICENCIO, M. A. et al. Hepato- and neurotoxicity induced by thioacetamide: protective effects of melatonin and dimethylsulfoxide,
Pharmacol. Res. 52, p. 223–228, 2005. DOI: 10.1016/j.phrs.2005.03.007

VEIGA JUNIOR, V. F. et al. Plantas medicinais: cura segura? Química nova, v.28, n.3, p.519-28, 2005. Acesso em: https://doi.org/10.1590/S0100-40422005000300026

VEIGA JUNIOR, V. F.; PINTO, A. C. O Gênero Copaifera L. Nova Química, v. 25, n. 2, p. 279-

86,2002. Acesso em: https://doi.org/10.1590/S0100-40422002000200016

VIEIRA, L. S. Fitoterapia da Amazônia. 2.ed. São Paulo. Editora Agronômica Ceres, p. 347, 1992.