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CELLULAR AND MOLECULAR BIOLOGY

Histological evaluation of the liver of mice with sarcoma-180 treated with salazinic acid

MARIA APARECIDA DA C. DE LIRA, MARLLYN M. DA SILVA, WANESSA K.M. SILVA, EMERSON P.S. FALCÃO, FRANCISCO C.A. DE AGUIAR JÚNIOR & SEBASTIÃO J. DE MELO

Abstract: Many of the drugs used to fight cancer cells induce various damage causing hepatotoxic effects which are characterized by tissue changes. The aim of the study is to know the possible effects of salazinic acid on livers of mice exposed to Sacoma-180. The tumor was grown in the animals in ascitic form and inoculated subcutaneously in the axillary region of the mouse developing the solid tumor. Treatment with salazinic acid (25 and 50 mg/kg) and 5-Fluorouracil (20 mg/kg) started 24-hours after inoculation and was performed for 7 days. To verify these effects, the qualitative method of histological criteria investigated in liver tissue was used. It was observed that all treated groups showed an increase of pyknotic nuclei in relation to the negative control. There was an increase in steatosis in all groups compared to the negative control but there was a decrease in the groups treated with salazinic acid in the 5-Fluorouracil. There was no necrosis in the salazinic acid treated groups. However, this effect was seen in 20% of the positive control group. Therefore, it can be concluded that salazinic acid did not show hepatoprotective action on mice but demonstrated a decrease in steatosis and absence of tissue necrosis.

Key words: cancer, hepatoxicity, lichen, sarcoma-180.

INTRODUCTION

Cancer is the term used to describe a set of more than 100 diseases that have in common the disordered growth of altered cells, that invade organs and tissues. The different types of cancer correspond to the various types of cells in the body. When cancer cells grow in epithelial tissues, such as skin or mucous membranes, they are called carcinomas. If the starting point are connective tissues, such as bone, muscle or cartilage, this is referred to as a sarcoma. It is called metastasis, when cancer cells in disarray spread to other regions of the body through the blood stream (INCA 2019).

These cancer groups of disease as a public health issue are the second leading cause of death in the United States. Therefore, cancer has become a major challenge for research in the search for definitive solutions, such as healing through a drug (Siegel et al. 2019). Thus, experimental animal models are important and essential to determine the antitumor potential of molecules and extracts for healing, as well as understand their action and effects throughout treatment. One such experimental model / method, is the use of tumors in mice and specifically, Sarcoma-180 (S-180), which is widely accepted due to its characteristic of forming ascitic, or solid tumors (Cai et al. 2012, Debnath et al. 2017, Ramos et al. 2019).

However, it is not enough to only determine the antitumor potential according to tumor

weight or ascitic volume. In addition, it is important to verify the effects of the drug, whether isolated or not, on the organs of potential animals, through histological and/or histomorphometric analysis. This is in order that a new drug to be developed for use, a greater safety path and possible adverse effects and achievements to be considered by the competent agencies such as ANVISA (National Health Surveillance Agency) in the case of Brazil, and the Food and Drug Administration (FDA) which is a federal agency in the United States Department of Health and Human Services responsible for oversight of drug development, manufacturing and use (Silva et al. 2012, 2019).

One of the main organs responsible for maximum activity, right after feeding, is the liver, so it is the target of every drug, especially when administered orally. In addition, in cancer patients, the liver is often reached, through metastases for example. For these reasons, the liver is often the first, to be investigated for possible changes or adverse effects such as hepatotoxicity (disease caused by toxins), followed by the spleen and kidney's (Saad-Hossne et al. 2004, Marinho et al. 2017).

In view of the great challenge of curing cancer, lichen are part of an extremely diverse group of fungi found in nature, symbiotically associated with algae and/or cyanobacteria which have been studied for a long time, as they produce secondary metabolites with large pharmaceutical properties. Uric acid for example, abundant in Alectoria, Cladonia, Usnea, Lecanora, Ramalina and Evernia, are examples of metabolites widely studied for having anti-proliferative activity (Ingólfsdóttir 2002). Kupchan & Kopperman (1975) first presented his antitumor activity against Lewis lung carcinoma in rats. Some extracts of *Cladonia convoluta*, *Cladonia rangiformis*, *Parmelia caperata*, *Platismatia glauca* and *Ramalina cuspidata* demonstrated activities against various strains of human cancer cells (Bézivin et al. 2003).

In addition to uric acid, salazinic acid (SAL) is also a secondary lichen metabolite, present in some species of the Parmeliaceae Family, such as *Parmotrema lichexanthonicum*, and presents antibiotic and cytotoxic activity against human tumor cells (Micheletti et al. 2009) and *P. cetratum* have antitumor action against neoplastic cells of mice (Alexandrino et al. 2019).

Considering the importance of qualitatively characterizing the possible effects caused by SAL in relevant organs such as the liver, this study's main objective was to histologically evaluate the liver tissue of mice exposed to S-180 and treated with SAL, as well as to analyze the activities of transaminases Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT). This is the first study that aims to determine probable reversals, promoted by SAL, to possible liver damage of mice with malignant tumors.

MATERIALS AND METHODS

Obtaining salazinic acid

SAL was provided by the Laboratório de Síntese e Isolamento Molecular, do Centro Acadêmico de Vitória, coordinated by Prof. Dr. Emerson Peter da Silva Falcão. The lichenic compound was isolated from the acetonic extract of the lichen *Parmotrema concurrens*, with more than 90% of purity level.

Experimental animals

Male Swiss albino mice (*Mus musculus*) (35-45g) were used, at 60 days of age. The animals were fasted for 8 hours before each experiment. The experimentation protocols were approved by the Animal Experimentation Ethics Commission (CEEA) of Universidade Federal de Pernambuco (UFPE) (Nº 23076.012019/2018.58), with as

standards proposed by the Conselho Nacional de Controle de Experimentação Animal (CONCEA) and by international standards established by the Guia do Instituto Nacional de Saúde para Cuidados e Uso de Animais de Laboratório.

Tumor assay

The experimental tumor, S-180 was kept in Swiss Albino mice, males, in the ascetic form with maintenance frequency of 7 days. Ascitic tumor cells (5.0×10^6 mL^{-1} cells) were inoculated subcutaneously in the right axillary region of the mouse developing the solid tumor.

Treatment started 24 hours after inoculation and was performed for 7 consecutive days. Injections of SAL were administered intraperitoneally at doses of 25 and 50 mg/kg of live weight of the animal. The animals in the negative control group were treated with saline solution + tween 80 to 5 %, as well as the animals of the positive control group treated with 5-Fluorouracil (20mg/kg of body weight). Fifty animals per group were used for the experimental model.

At the end of treatment, the animals were anesthetized with ketamine (1.25 g/kg) and xylazine (0.62 g/kg), and the liver was removed, dissected and fixed in formaldehyde solution (10 %) buffered and then submitted to histopathological analyses (Martins et al. 2016).

Qualitative study of liver histopathology

The organs were packed in a 10% buffered formaldehyde solution, remaining in this solution for a period of 48 hours. After this procedure, the samples were dehydrated in Ethyl alcohol at increasing concentrations, diaphanized by Xilol, impregnated and included in paraffin.

Tissue cuts, 4 µm thick, were submitted to the staining technique by Hematoxylin-Eosin. Histological images were captured by digital camera Moticam 2300, coupled under optical microscope, under fixed focus and field clarity, using the 40x lens and obtaining 20 fields per blade.

Histological findings were interpreted according to qualitative criteria on the appearance of pyknotic nuclei, microvesicular steatosis, tissue necrosis, hepatic fibrosis and micro hemorrhages with erythrocytes extravasation (Fontes et al. 2004).

Statistical analysis

The results of animal and liver weight analyses, together with liver enzyme activity, are expressed as the means of repetitions ± standard deviation. For variance analysis (ANOVA) the Tukey test was performed for multiple comparisons. A value of $p < 0.05$ was adopted as a significance level.

RESULTS AND DISCUSSION

All animals exposed to ascystic S-180 presented solid tumor growth in the right axillary region during the 7 days of treatment, death was not observed among the groups undergoing treatment.

On the last day of treatment, the weight of the animals was verified and no significant differences were observed in the weight of the treated groups in relation tothose of the negative control group. The mice were euthanized for organ removal. In macroscopic analysis of the collected livers, no apparent damage was seen. Overall, the organs seemed healthy, with no morphological changes or macroscopic lesions. In the analysis of liver weight, there were no significant differences between those exposed to treatment and those who only received the vehicle. Data in the Table I.

For enzymatic analysis of AST and ALT transaminases, serum samples were collected from mice. There were no significant differences

Table I. Analysis of the weight of livers and animals exposed to treatment with salazinic acid and enzymatic activity of transaminases.

	Weight (g)		Enzymatic activity (U/L)	
Groups	**Animal**	**Liver**	**ALT**	**AST**
Negative control	52,02±4,69	3,25±0,57	64,04±5,95	218,12±36,70
Positive control	50,80±5,84	3,26±0,36	47,94±8,07	216,07±38,80
SAL (25mg/Kg)	48,92±6,11	3,26±0,46	50,64±12,89	205,82±31,78
SAL (50mg/Kg)	48,34±5,07	3,51±0,28	44,87±6,37	184,11±46,93

Data expressed as mean ± SD. The statistical differences determined by ANOVA followed by the Tukey test. N= 5. p <0.05 vs. Negative control. N= number of animals per group. AST= Aspartate aminotransferase; ALT= Alanine aminotransferase; SAL= salazinic acid.

between the groups exposed to SAL treatment and the negative control group for the ALT and AST activities, Table I. These enzymes are considered markers sensitive to liver lesions (Liu et al. 2016). However, it can be confirmed that treatment with SAL did not cause lesions in the liver tissue.

The histological evaluation showed that the negative control group presented liver tissue in good condition, with well-defined nuclei and visible presence of Kupffer and endothelial cells, but 20% of the animals presented Pyknotic nuclei and 10% microvesicular steatosis.

In the group treated with 5-Fluorouracil, the presence of pyknotic nuclei, microvesicular steatosis and small regions of tissue necrosis were verified. The animals treated with salazinic acid (25 and 50 mg/kg) presented pyknotic nuclei, microvesicular steatosis and only at the dose of 50 mg/Kg were observed some regions of hepatic fibrosis. These lesions are presented in Figures 1 and 2. The presence of micro haemorrhages was not seen in analyzed groups (Table II).

It was also seen that the negative control group presented, preserved hepatic parenchyma and without severe cellular damage, hepatocytes with round central nucleus and homogeneous cytoplasm. Regarding histomorphology of hepatocytes and Kupffer cells, no cell differences were observed compared to the negative control group. The results of this study are in accordance with those evaluated by Alexandrino et al. (2019) who analyzed the acute toxicity of SAL (100 mg/Kg) and verified the low toxicity of this substance in liver histopathology.

However, Araujo et al. (2019) observed that uric acid (2000 mg/Kg), a lichenic compound, caused liver tissue lesions. The pyknotic nucleus present in hepatocytes indicates a destructive fragmentation of the nucleus, which is characterized by irregular distribution of chromatin, causing programmed cell death (apoptosis), promoting future damage, such as tissue necrosis (Kumar et al. 2010).

Hepatic steatosis is defined as an accumulation of lipids in the cytoplasm of hepatocytes, especially triglycerides. Nicotinamide adenine dinucleotide present in hepatocytes leads to disturbance of β-oxidation of fatty acids in mitochondria, which leads to lipid changes in the tissue. The excess of these free fatty acids in hepatocytes exceeds the ability of it to process and re-export them (Baraona & Lieber 1979). These changes lead to cell death and cause elevation of serum marker enzymes that are released by the liver into the bloodstream (Lowry et al. 1951).

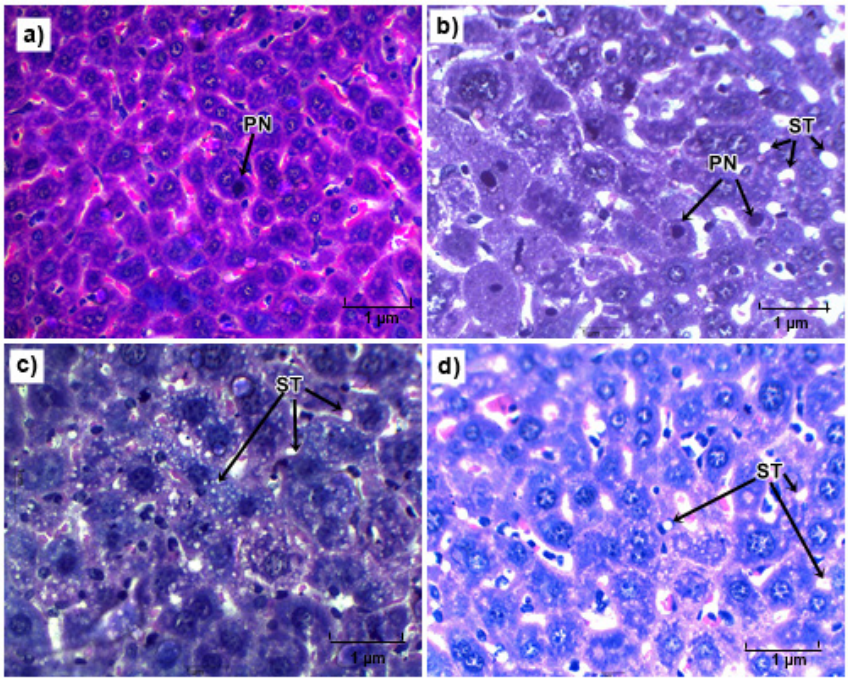

Figure 1. Photomicrograph of the histological section of the liver of mice exposed to S-180. (N=5). a) Negative control; b) 5-Fluorouracil treatment; c) and d) Treatment with SAL (25 and 50 mg / kg); PN- Pyknotic nucleus and ST- microvesicular steatosis. HE staining with 40X magnification.

However, it was considered that there is an interrelationship between metabolic processes, such as oxidative stress, endoplasmic reticulum stress, insulin resistance and inflammatory response at the hepatic level with the triggering of hepatic steatosis and its progression of chronic diseases such as inflammation, fibrosis and cirrhosis (steatohepatitis) commonly associated with non-alcoholic fatty liver disease – NAFLD (Valenzuela & Videla 2011).

NAFLD is caused by the excess of triglycerides accumulated in liver cells resulting from increased lipogenesis, which can be caused by the ingestion of various drugs and multiple defects in energy metabolism (Rolo et al. 2012). NAFLD can cause inflammation causing negative regulation of PPAR-α (Peroxisome proliferator-activated-α), causing an increase in the activation of the pro-inflammatory nuclear transcription factor, Nuclear factor-kB (NF-kB) (Valenzuela & Videla 2011).

PPARs are transcription factors of nuclear receptors that have the function of regulating energy homeostasis, lipid metabolism and inflammation (Tavares et al. 2007). They are considered sensors for fatty acids acting on gene expression controlling metabolic pathways that act in the maintenance of energy balance (Viswakarma et al. 2010). Its activation may decrease the activity of other transcription factors, such as NF-kB, thus acting directly in inflammatory processes (Wang et al. 2002).

Activation of NF-kB can result in oxidative stress and inflammatory processes. To minimize these causing damage in the body, the transcription factors PPAR-α and Nrf2 (nuclear factor-erythroid 2-related factor 2) come into action, acting in the inhibition of NF-kB, thus causing a decrease of metabolic disorders in the tissue, reducing the pro-inflammatory, pro-lipogenic and oxidative stress effects (Hernández-Rodas et al. 2017, Valenzuela et al. 2017, Barbosa et al. 2019). It is known that damage to liver tissue can be caused mainly by oxidative stress and inflammatory response (Rolo et al. 2012, Valenzuela et al. 2017).

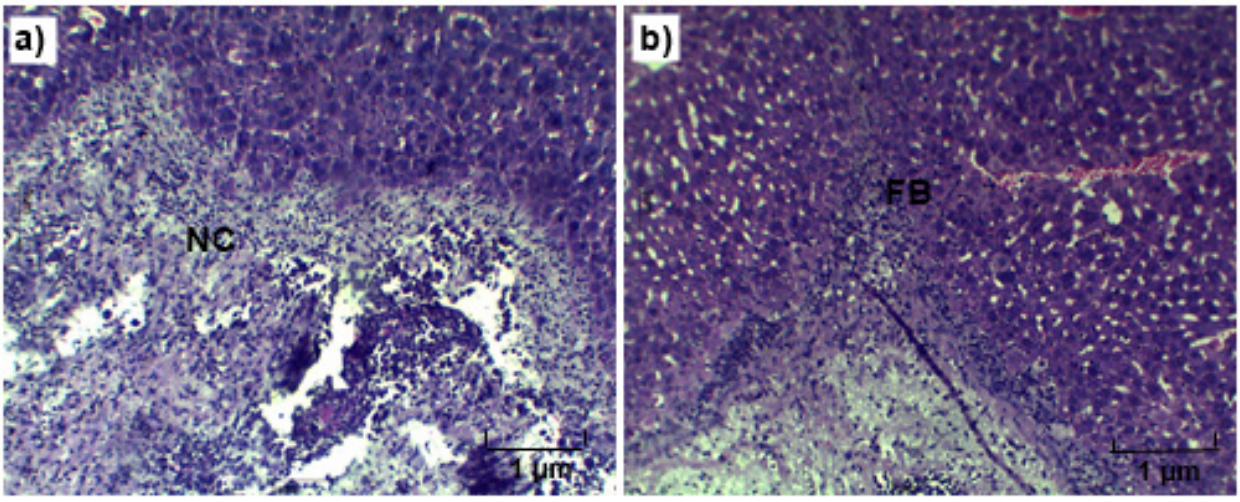

Figure 2. Photomicrograph of histological section of the liver of mice exposed to S-180. (N=5). a) treatment with 5-Fluorouracil and b) treatment with SAL (50 mg / kg). NC-Liver necrosis and FB- liver fibrosis. HE staining with 10X magnification.

Table II. Qualitative histopathological analysis of the liver of mice exposed to S-180.

Groups	Qualitative analysis criteria (%)				
	Pyknotic nuclei	Steatosis	Necrosis	Fibrosis	Micro hemorrhage
Negative control	40 (2)	20 (1)	0	0	0
Positive control	80 (4)	100 (5)	40 (2)	0	0
SAL (25 mg/Kg)	100 (5)	80 (4)	0	0	0
SAL (50 mg/Kg)	80 (4)	60 (3)	0	20 (1)	0

Data expressed as a percentage in number of animals. N=5. N= number of animals per group. Negative control= saline + tween 80 to 5 %; Positive control= 5-Fluorouracil (20 mg/Kg); SAL= salazinic acid (doses of 25 and 50 mg/Kg).

Liver damage is also associated with the use of ethanol, obesity, viral infection and the use of synthetic and natural medicines (Hewawasam et al. 2003). It is very important to understand the mechanisms of action caused by the side effects of antineoplastic agent in different organs, thus having a considerable significant role in establishing the treatment strategy. Thus, there is an adaptation to the body's response in relation to the action of anti-tumor agents and tolerance of different tissues regarding immunosuppressive state induced by chemotherapy (Crăciun & Paşca 2014).

Further, liver tissue is very sensitive to lesions induced by synthetic compounds and of natural origin. This is due to its central role in the metabolism of these substances and it is possible to induce some degree of tissue damage during this experimentation and many of these compounds are involved in the mechanisms that lead to liver cell damage. For example, disorders in vital cellular organelles result in impairment of homeostatic balance, thus resulting in intracellular oxidative stress with

excessive formation of reactive oxygen species and suppressed immune response (Singh et al. 2016).

Studies have evidenced that hepatotoxicity is one of the main reasons for the withdrawal of Cancer medicines from the market. Natural products have already shown themselves to be promising in combating the toxicity of several commonly used drugs. In addition, many of these natural substances, such as resveratrol and curcumin, are now used as widely accepted chemo-preventive agents. It is known that compounds of natural origin not only reduce the risk of liver damage caused by medication, but also provide an alternative solution to remedy drug-induced hepatotoxicity response (Singh et al. 2016).

CONCLUSIONS

In conclusion, the main finding of this study is that the treatment with SAL in mice with S-180 caused apparent lesions in the liver tissue, however, at the enzymatic level no damage was seen. However, treatment with SAL caused a noticeable decrease in microvesicular steatosis compared to the positive control group. Tissue necrosis was not observed in the groups treated with lichenic acid.

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REFERENCES

ALEXANDRINO CAF, HONDA NK, MATOS MFC, PORTUGAL LC, SOUZA PRB, PERDOMO RT, GUIMARÃES RCA, KADRI MCT, SILVA MCBL & BOGO D. 2019. Antitumor effect of depsidones from lichens on tumor cell lines and experimental murine melanoma. Braz J Pharmacog 29: 449-456.

ARAUJO HDA ET AL. 2019. Usnic acid potassium salt: Evaluation of the acute toxicity and antinociceptive effect in murine model. Molecules 24: 1-17.

BARAONA E & LIEBER CS. 1979. Effects of ethanol on lipid metabolism. J Lipid Res 20: 289-315.

BARBOSA JE, STOCKLER-PINTO MB, CRUZ BO, SILVA ACT, ANJOS JS, MESQUITA CT, MAFRA D & CARDOZO LFMF. 2019. Nrf2, NF-κB and PPARβ/δ mRNA Expression Profile in Patients with Coronary Artery Disease. Arq Bras Cardiol 113: 1121-1127.

BÉZIVIN C, TOMASI S, LOHÉZIC-LE DÉVÉHAT F & BOUSTIE J. 2003. Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. Phytomedicine 10(6-7): 499-503.

CAI Z, LI W, WANG H, YAN W, ZHOU Y, WANG G, CUI J & WANG F. 2012. Antitumor effects of a purified polysaccharide from *Rhodiola rosea* and its action mechanism. Carbohydr Polym 90: 296-300.

CRĂCIUN C & PAŞCA C. 2014. Structural and ultrastructural data on side effects of cisplatin in spleen, kidney and liver of rats. Acta Metall 1: 9-22.

DEBNATH S, KARAN S, DEBNATH M, DASH J & CHATTERJEE TK. 2017. Poly-L-Lysine Inhibits Tumor Angiogenesis and Induces Apoptosis in Ehrlich Ascites Carcinoma and in Sarcoma S-180 Tumor. Asian Pac J Cancer Prev 18: 2255-2268.

FONTES CER, TAHA MO, FAGUNDES DJ, FERREIRA MV, FILHO ORP & MARDEGAN MJ. 2004. Estudo comparativo do uso de cola de fibrina e cianoacrilato em ferimento de fígado de rato. Acta Cir Bras 19: 37-42.

HERNÁNDEZ-RODAS MC ET AL. 2017. Supplementation with docosahexaenoic acid and extra virgin olive oil prevents liver steatosis induced by a high-fat diet in mice through PPAR-α and Nrf2 upregulation with concomitant SREBP-1c and NF-kB downregulation. Mol Nutr Food Res 61(12): 1-35.

HEWAWASAM RP, JAYATILAKA KAPW, PATHIRANA C & MUDDUWA LKB. 2003. Protective effect of Asteracantha longifolia extract in mouse liver injury induced by carbon tetrachloride and paracetamol. J Pharm Pharmacol 55(10): 1413-1418.

INCA – INSTITUTO NACIONAL DE CÂNCER. 2019. Ministério da Saúde, Instituto Nacional de Câncer. https://www.inca.gov.br/o-que-e-cancer. Acesso em 27 de Julho de 2019.

INGÓLFSDÓTTIR K. 2002. Usnic acid. Phytochem 61: 729-736.

KUMAR V ET AL. 2010. Robbins E Cotran, Bases Patológicas Das Doenças. Elsevier. Brasil: Rio de Janeiro, p. 1-1479.

KUPCHAN SM & KOPPERMAN HL. 1975. Usnic Acid: Tumor Inhibitor Isolated from Lichens. Experientia 31(6): 625-752.

LIU X, ZHENG L, ZHANG R, LIU G, XIAO S, QIAO X, WU Y & GONG Z. 2016. Toxicological evaluation of advanced glycation end product Nε-(carboxymethyl) lysine: Acute and subacute oral toxicity studies. Regul Toxicol Pharmacol 77: 65-74.

LOWRY OH, ROSEBROUGH NJ, FARR AL & RANDALL RJ. 1951. Protein Measurement with the Folin Phenol Reagent. J Biol Che 193: 265-275.

MARINHO KSN, ANTONIO EA, SILVA CVNS, DA SILVA KT, TEIXEIRA VÉW, DE AGUIAR JÚNIOR FCA, DOS SANTOS KRRP, DA SILVA NH & SANTOS NPS. 2017. Hepatic toxicity caused by PLGA-microspheres containing usnic acid from the lichen *Cladonia substellata* (AHTI) during pregnancy in Wistar rats. An Acad Bras Cienc 89: 1073-1084.

MARTINS MCB, ROCHA TA, SILVA TDS, NETO MPC, SANTOS NPS, SILVA TG, AGUIAR-JUNIOR FCA, FALCÃO EPS, PEREIRA EC & SILVA NH. 2016. *In Vitro* and *in Vivo* Antineoplastic Activity of Barbatic Acid. Int Arch Med 9: 1-9.

MICHELETTI AC, BEATRIZ A, DE LIMA DP, HONDA NK, PESSOA CDÓ, MORAES MO, LOTUFO LV, MAGALHÃES HIF & CARVALHO NCP. 2009. Constituintes químicos de Parmotrema lichexanthonicum Eliasaro & Adler - Isolamento, modificações estruturais e avaliação das atividades antibiótica e citotóxica. Quim Nova 32: 12-20.

RAMOS DBM ET AL. 2019. Evaluation of antitumor activity and toxicity of *Schinus terebinthifolia* leaf extract and lectin (SteLL) in sarcoma 180-bearing mice. J Ethnopharmacol 233: 148-157.

ROLO AP, TEODORO JS & PALMEIRA CM. 2012. Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. Free Radic Biol Med 52: 59-69.

SAAD-HOSSNE R, PRADO RG & HOSSNE WS. 2004. Effect of acetylsalicylic acid and acetic acid solutions in liver of rabbits. Acta Cir Bras 19: 677-686.

SIEGEL RL, MILLER KD & JEMAL A. 2019. Cancer statistics. Cancer J Clin 69: 7-34.

SILVA DC ET AL. 2012. Polysaccharide isolated from Passiflora edulis: Characterization and antitumor properties. Carbohydr Polym 87: 139-145.

SILVA MM ET AL. 2019. Effect of acute exposure in swiss mice (*Mus musculus*) to a fibrinolytic protease produced by *Mucor subtilissimus* UCP 1262: An histomorphometric, genotoxic and cytological approach. Regul Toxicol Pharmacol 103: 282-291.

SINGH D, CHO WC & UPADHYAY G. 2016. Drug-induced liver toxicity and prevention by herbal antioxidants: An Overview. Front Physiol 6: 1-18.

TAVARES V, HIRATA MH & HIRATA RDC. 2007. Peroxisome proliferator-activated receptor gamma (PPARγ): molecular study in glucose homeostasis, lipid metabolism and therapeutic approach. Arq Bras Endocrinol Metab 51: 526-533.

VALENZUELA R, ILLESCA P, ECHEVERRÍA F, ESPINOSA A, RINCÓN-CERVERA MA, ORTIZ M, HERNÁNDEZ-RODASMC, VALENZUELA A & VIDELA L. 2017. Molecular adaptations underlying the beneficial effects of hydroxytyrosol in the pathogenic alterations induced by a high-fat diet in mouse liver: PPAR-α and Nrf2 activation, and NF-κB down-regulation. Food Funct 7: 1-35.

VALENZUELA R & VIDELA LA. 2011. The importance of the long chain polyunsaturated fatty acid n-6/n-3 ratio in development of non-alcoholic fatty liver associated with obesity. Food Funct 2: 644-648.

VISWAKARMA N, JIA Y, BAI L, VLUGGENS A, BORENSZTAJN J, XU J & REDDY JK. 2010. Coactivators in PPAR-Regulated Gene Expression. PPAR Res 2010: 250126.

WANG N, VERNA L, CHEN NG, CHEN J, LI H, FORMAN BM & STEMERMAN MB. 2002. Constitutive activation of peroxisome proliferator-activated receptor-gamma suppresses pro-inflammatory adhesion molecules in human vascular endothelial cells. J Biol Chem 277: 34176-34181.

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MARIA APARECIDA DA C. DE LIRA[1]
https://orcid.org/0000-0002-2662-1003

MARLLYN M. DA SILVA[2]
https://orcid.org/0000-0002-1768-9831

WANESSA K.M. SILVA[3]
https://orcid.org/0000-0001-7802-1854

EMERSON P.S. FALCÃO[1]
https://orcid.org/0000-0003-2346-8477

FRANCISCO C.A. DE AGUIAR JÚNIOR[3]
https://orcid.org/0000-0001-8676-4826

SEBASTIÃO J. DE MELO[4]
https://orcid.org/0000-0002-4134-6169

[1]Universidade Federal de Pernambuco, Centro Acadêmico de Vitória, Laboratório de Síntese e Isolamento Molecular, Rua Alto do Reservatório, s/n, 55608-680 Vitória de Santo Antão, PE, Brazil

[2]Universidade Federal de Pernambuco, Centro Acadêmico de Vitória, Laboratório de Nanotecnologia, Biotecnologia e Cultura de Células, Rua Alto do Reservatório, s/n, 55608-680 Vitória de Santo Antão, PE, Brazil

[3]Universidade Federal de Pernambuco, Centro Acadêmico de Vitória, Laboratório de Biotecnologia e Fármacos, Rua Alto do Reservatório, s/n, 55608-680 Vitória de Santo Antão, PE, Brazil

[4]Universidade Federal de Pernambuco, Departamento de Antibióticos, Avenida Prof. Moraes Rego, 1235, 50740-560 Recife, PE, Brazil

Correspondence to: **Maria Aparecida da Conceição de Lira**
E-mail: lira.macbio@gmail.com

Author contributions

Maria Aparecida da Conceição de Lira, designed the study and realized the experiments; Marllyn Marques da Silva, contributed in performed the histological analyses; Wanessa Karina de Moura Silva, assisted in the preparation of experiments; Emerson Peter da Silva Falcão, supervised the lichenic acid isolation technique; Francisco Carlos Amanajás de Aguiar Júnior, was responsible for monitoring the histological analysis and Sebastião José de Melo, supervised all experiments.