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Original Article

Comparative evaluation of plant extract effects on peritoneal, medullary and J774 cells. G8 macrophages

Avaliação comparativa do efeito de extratos de plantas em macrófagos peritoneais, medulares e da linhagem J774.G8

F. Marques-Santos[a], M. R. R. Amendoeira[a], R. M. S. Galvão[b], L. M. Rocha[c] and R. X. Faria[d]*

[a]Fundação Oswaldo Cruz – Fiocruz, Instituto Oswaldo Cruz, Laboratório de Toxoplasmose e outras Protozooses, Rio de Janeiro, RJ, Brasil
[b]Universidade Federal do Rio de Janeiro – UFRJ, Laboratório de Estudos de Farmacologia Experimental, Rio de Janeiro, RJ, Brasil
[c]Universidade Federal Fluminense – UFF, Laboratório de Tecnologia em Produtos Naturais, Niterói, RJ, Brasil
[d]Fundação Oswaldo Cruz – Fiocruz, Instituto Oswaldo Cruz, Laboratório de Avaliação e Promoção da Saúde Ambiental, Rio de Janeiro, RJ, Brasil

Abstract

The use of medicinal plants as raw material for extracts production and pure substances isolation and subsequence development of new drugs represents a constantly growing area. However, some stages are indispensable before pharmacologically evaluating natural products such as medicines. Toxicity tests in mammalian cells are essential to initiate new drugs development or verify the substance's biocompatibility. Thus, we verified the toxicity of crude extracts and fractions with different polarities obtained from the leaves and stems of eight plant species. The toxic effect was evaluated on macrophages obtained from the bone marrow and peritoneal cavity of a *Swiss webster* mouse and J774 macrophages. G8 cell lineage. These macrophages were cultured in a 96-well plate, and the compounds were added at a concentration of 100 µg/mL for 24 hours. After this time, the supernatant was removed. The toxicity was evaluated for lactate dehydrogenase (LDH) release assay and the resazurin assay, which uses an indicator dye to measure oxidation-reduction reactions. The results showed a difference in the percentage of toxicity when comparing the same extract in different types of macrophages. This outcome indicates that these cells from different origins may exhibit different responses when exposed to the same natural compounds.

Keywords: toxicity, natural products, macrophages, plant extract, cell death.

Resumo

A utilização de plantas medicinais como matéria-prima para a produção de extratos e isolamento de substâncias puras para o desenvolvimento de novos fármacos representa uma área em constante crescimento. No entanto, existem processos a serem realizados antes de avaliar farmacologicamente produtos naturais como medicamentos. Os testes de toxicidade em células de mamíferos são fundamentais para iniciar o desenvolvimento de novas drogas ou verificar a biocompatibilidade de substâncias. Assim, verificamos a toxicidade de extratos brutos e frações com diferentes polaridades obtidos de folhas e caule de oito espécies de planta. Para comparar o efeito tóxico, os testes foram realizados em macrófagos obtidos da medula óssea e cavidade do peritônio de camundongo *Swiss webster*, bem como no macrófago da linhagem celular J774.G8. Esses macrófagos foram cultivados em placa de 96 poços e os compostos adicionados na concentração de 100 µg/mL por 24 horas. Após esse período o sobrenadante foi removido. A toxicidade foi avaliada pelos ensaios de detecção da enzima lactato-desidrogenase (LDH) e pelo ensaio de resazurina, que usa um corante indicador para medir as reações de oxidação-redução. Os resultados mostraram uma diferença na porcentagem de toxicidade quando comparamos o mesmo extrato em diferentes tipos de macrófagos. Este resultado indica que essas células de várias origens podem exibir respostas distintas quando expostas aos mesmos compostos naturais.

Palavras-chave: toxicidade, produtos naturais, macrófagos, extrato de planta, morte celular.

1. Introduction

Macrophages are immune system cells that can be found in all tissues in the body. Through phagocytosis, these cells exhibit physiological functions, including innate and acquired immune response regulation, inflammatory reaction, enzyme secretion, defense against pathogens, and infective agents in general (Lorenzi, 2006). They are also essential in stimulating specific immunological reactions, presenting antigens to lymphocytes, and acting

*e-mail: robson.xavier@gmail.com
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in tissue repair and renewal and immunity against tumors (Gentek et al., 2014).

Most tissue-resident macrophages are established in the prenatal period and maintained throughout adulthood by longevity and self-renewal by local proliferation, which is sustained independently of hematopoiesis. However, macrophages derived from adult monocytes can complement the tissue macrophage population. This second type, when leaving the bloodstream and distributing themselves through tissues, receive different names depending on their location, for example Kupffer cells (liver), osteoclasts (bone tissue), microglia (nerve tissue), peritoneal macrophages (peritoneum) and bone marrow macrophages (bone marrow) (Gentek et al., 2014; Varol et al., 2015).

In addition to acting as immunological sentinels, tissue macrophages form integral components of their normal tissue and are specialized in responding to local environmental changes to contribute to the development and specific function of their resident tissue (Gentek et al., 2014; Varol et al., 2015).

Macrophage populations isolated from a resident or overflowing peritoneal cells are generally heterogeneous in their cell types and phenotypic properties, such as the degree of activation; thus, any study related to mechanistic interpretations of activation must require a more homogeneous system. Therefore, macrophage-like cell types that maintain proliferative capacity in vitro serve as valuable tools to analyze the functional aspects of macrophages (Miyakawa et al., 1989).

Cell lines are genetically modified cells, usually isolated from cancerous tissues, that proliferate indefinitely but preserve the genotypic and phenotypic characteristics of original tissues (Cooper and Hausman, 2007).

Due to this multitasking aptitude, macrophage cell lines such as J774 cells are well-established model systems in immunology and cell biology (Lam et al., 2009). The J774 cell line was first established in 1968 from reticulocyte sarcoma (Ralph et al., 1975). Furthermore, macrophage strains can be easily maintained and genetically manipulated, as they are increasingly recognized as preferred model systems for several studies (Chingwaru et al., 2016; Digiacomo et al., 2017; Aslanyan et al., 2019; Geroldinger et al., 2019).

Studies with natural products as raw material for extract production and isolation of pure substances for new synthetic or semisynthetic drug development with anti-infective or immunomodulatory activity represent an area in constant growth (Newman and Cragg, 2016).

However, some stages should be performed to validate the safety of these natural products before pharmacological evaluation, such as toxicity tests in mammalian cells or the biocompatibility of substances (Rogero et al., 2003). Tissue macrophages obtained from biomodels have currently the preferred method used for this purpose (Hertiani et al., 2019; Jeong et al., 2019). Furthermore, with the growing need to replace the animal models with alternative methods, lineage macrophages have become more advisable by several animal use ethics committees. Nonetheless, cell types located in different body regions have been used to generate a possible different response. However, the use of cell types located in different regions of the body can generate a differentiated response (Alves and Guimarães, 2010).

In this sense, the present study aimed to compare the effect of plant extracts on peritoneal, medullary and J774 macrophages. In addition, G8 lineage macrophages contribute to the best option in choosing these models.

2. Materials and Methods

2.1. Plant extracts

Crude extracts and fractions from eight plant species from different families were tested, namely, *Erythroxylum ovalifolium* Peyr. (Erythroxylaceae), *Ocotea notata* (Nees & Mart.) Mez. (Lauraceae), *Myrsine parvifolia* A.DC. (Myrsinaceae), *Eugenia sulcata* (Spring ex Mart) O. Berg., *Eugenia pruniformis* Cambess, *Myrciaria floribunda* (West ex Willd) O. Berg., *Neomitranthes obscura* (DC.) N. Silveira. (Myrtaceae) and *Manilkara subsericea* (Mart.) Dubard. (Sapotaceae) (Chart 1).

The extracts were provided by the Natural Products Technology Laboratory (LTPN) of the Federal Fluminense University (UFF), which has authorization from SISBIO/ICMBio, under license 13659-10 and registration with the National Genetic Heritage Management System (SISGEN) A45D699 to collect plants from the Restinga de Jurubatiba National Park, located in Carapebus municipally in the state of Rio de Janeiro.

2.2. Animals

Male *Swiss webster* mice weighing 18 to 20 g were used to obtain tissue macrophages. Peritoneal macrophages were obtained under authorization from the Committee on Ethics in Animal Use of the Oswaldo Cruz Institute (CEUA-IOC) under number L-029/2018, and marrow macrophages were obtained under authorization from the Committee on Ethics in Animal Use of the Health Sciences Center (CEUA-CCS) under registration number 114/15.

2.3. Bone marrow macrophages

After euthanization, the bones (tibia and fibula) of male mice of the *Swiss webster* lineage were collected. One of the ends of each bone was cut and introduced in a 1000 µL tip located inside a 15 mL conical tube. The tubes were then centrifuged at 100 *g* for 5 minutes, and the cells were resuspended in RPMI 20/30 medium (RPMI-1640 medium supplemented with 20% fetal bovine serum and 30% RPMI-1640 medium from L929 cell culture).

The resuspended cells recently collected from the bone marrow were filtered in a cell strainer positioned in a 50 mL conical tube. Then, another 1 mL of RPMI 20/30 medium was passed through the cell strainer to remove any remaining cells.

The filtrate was placed in a culture bottle (175 cm^2) containing 15 mL of RPMI 20/30 medium and stored in an oven for 7 days at 37 °C in an atmosphere of 5% CO_2 for cell differentiation. This differentiation into M2-type macrophages is promoted by a mononuclear phagocyte colony-stimulating factor synthesized by mesenchymal

Chart 1. Samples of plants from the Restinga de Jurubatiba sectored according to species, family, plant part, fraction and corresponding acronym.

Species	Family	Plant Part	Fraction	Acronym
Erythroxylum ovalifolium	Erythroxylaceae	Stems	Ethanol	EIOUE
			Flavonoid-rich fraction	EIOUF
Ocotea notata	Lauraceae	Leaf	Hexane	EOHF
			Ethyl acetate	EOAF
Myrsine parvifolia	Myrsinaceae	Leaf	Ethyl acetate	EMPFA
			Ethanol	EMPF
		Stems	Ethanol	EMPC
Eugenia sulcata	Myrtaceae	Leaf	Ethanol	EBEF
Eugenia pruniformis	Myrtaceae	Leaf	Hexane	EPHF
			Ethyl acetate	EPAF
			Ethanol	EPBF
Myrciaria floribunda	Myrtaceae	Leaf	Hexane	EMFH
			Dichloromethane	EMFD
			Ethyl acetate	EMFA
			Butanol	EMFB
			Methanol	EMFM
			Ethanol	EMFE
Neomitranthes obscura	Myrtaceae	Leaf	Ethanol	ENF
		Stems	Ethanol	ENC
			Hexane	ENOH
			Dichloromethane	ENOD
			Ethyl acetate	ENOA
Manilkara subsericea	Sapotaceae	Stems	Ethanol	EMSE

cells (M-CSF) obtained from the culture medium of cells of the L929 lineage.

After a differentiation period, the adhered cells were washed with cold PBS and incubated at 4 °C for 10 minutes for cell detachment. Next, the contents were transferred to a 50 mL conical tube and centrifuged at 400 *g* for 5 minutes. The supernatant was discarded by tube version, and the cell pellet was resuspended in 5 mL of RPMI 10/5 medium (RPMI-1640 medium supplemented with 10% fetal bovine serum and 5% RPMI-1640 medium derived from cultured L929 cells).

The cells obtained were counted in a Neubauer chamber at a 1:100 dilution in 0.4% trypan blue solution (LGC Biotechnology), plated at a concentration of $2x10^5$ cells/well in a final volume of 150 µL and incubated at 37 °C in an atmosphere of 5% CO_2 for 24 hours before the experiment.

2.4. Peritoneal macrophages

Peritoneal exudate cells were obtained by washing the peritoneal cavity of male Swiss webster mice with 10 mL of PBS. This wash was subjected to centrifugation at 400 *g* for 5 minutes, and then the cell pellet was resuspended in 1 mL of RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic. Then, these cells were counted in a Neubauer chamber at a 1:100 dilution in 0.4% trypan blue solution (LGC Biotechnology) and plated in 96-well microplates at a concentration of $3x10^5$ cells/well. The microplates were kept for 1 hour in an oven at 37 °C in a humidified atmosphere containing 5% CO_2. Finally, nonadherent cells were washed and the medium was replaced.

2.5. J774. G8 lineage macrophages

The G8 macrophage strain was maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (LGC Biotechnology), 100 U/mL penicillin, 100 mg/mL streptomycin (Sigma-Aldrich), and 20 mM HEPES (Sigma-Aldrich). Cultured cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 for plating the J774. G8 cells, the medium used in the culture bottles was added a 50 mL conical tube. Adhered cells were trypsinized with trypsin-EDTA solution (1X) and placed in a conical tube with the medium removed previously.

Then, the tube was centrifuged at 400 *g* for 5 minutes, and the pellet was resuspended in 1 mL of RPMI-1640 medium. The number of cells was obtained by counting in a Neubauer chamber at a 1:100 dilution in 0.4% trypan blue solution (LGC Biotechnology). Next, cells

were plated in 96-well microplates at a concentration of $1x10^5$ and incubated at 37 °C in a 5% CO_2 atmosphere until use.

2.6. Detection of cell viability by resazurin assay

The resazurin assay was used to assess cell viability by adding plant extracts. Peritoneal macrophages, bone marrow, and macrophage J774. G8 cells were incubated with the extracts at 100 µg/mL for 24 hours.

After incubation, resazurin diluted in PBS at 0.15 mg/mL was added. As a positive control, 0.5% Triton X-100 was used, and as a negative control, only cells with RPMI-1640 medium were used.

After 5 hours of incubation at 37 °C, the reading was performed in a Molecular Devices SpectraMax M5 reader (Molecular Devices, USA) by detecting fluorescence in a 570 nm excitation and 595 nm emission filter.

2.7. Detection of cytotoxicity by lactate dehydrogenase enzyme assay

Peritoneal macrophages, bone marrow, and macrophage lineage J774. G8 cells were used to evaluate the toxicity of the plant extracts. These cells were plated in 96-well microplates and maintained in culture with RPMI-1640 medium for 24 hours. The following day, extracts were added at 100 µg/mL, and the cells were incubated in an oven for 24 hours. As a positive control, 0.5% Triton X-100 was used, and as a negative control, cells in the RPMI-1640 medium were used without any additional treatment.

After the cell treatment, 50 µL of the supernatant was collected and added to a new 96-well microplate. Cell toxicity disclosure was obtained following the recommendations of the LDH detection kit, CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega). The reading was performed in a Molecular Devices SpectraMax M5 reader (Molecular Devices, USA) emplying absorbance detection in a 490 nm filter with SoftMax Pro software.

2.8. Statistical analysis

Viability and toxicity assays were analyzed using ANOVA, followed by Tukey's posttest. The results are expressed as the mean ± SD, with a significance level of $p < 0.05$.

3. Results

The crude stem extract (EIOUE), as well as the flavonoid-rich fraction extract (EIOUF) of *Erythroxylum ovalifolium* (Erythroxylaceae) increased the percentage of cellular metabolic activity when tested in J774 macrophages. G8 lineage and peritoneal macrophages, respectively, compared to the negative control (Table 1). However, the low detection of lactate dehydrogenase (LDH) release, expected for both, was only observed in peritoneal macrophages (Table 2). When tested on bone marrow macrophages, the metabolic activity presented by these extracts was considered moderate, and in this group, the release of LDH was almost null (Tables 1 and 2). Thus, they constitute elements that indicate the low toxicity of the crude ethanol extract and their fraction.

Table 1. Percentage of resazurin activity according to plant extracts tested on peritoneal macrophages, J774. G8 lineage macrophages and bone marrow macrophages.

Acronym	Resazurin Activity (%)		
	Peritoneal Macrophage	J774. G8 Lineage macrophage	Bone Marrow Macrophage
CN	95.4967	98.1122	84.7841
CP	3.8588	7.3844	6.4897
EIOUE	90.0722	94.6048	41.2788
EIOUF	87.9988	107.7200	52.0351
EOHF	8.2563	90.2552	23.2395
EOAF	35.5746	92.9180	38.0301
EMPFA	91.1069	67.6218	6.9665
EMPF	5.4936	85.1251	6.3865
EMPC	5.4467	81.6705	5.7722
EBEF	57.8151	68.3694	3.6885
EPHF	4.9871	9.6646	5.4637
EPAF	5.4092	61.4832	5.7052
EPBF	5.1044	11.1602	5.5846
EMFH	59.0095	38.9501	31.3480
EMFD	21.2038	47.8778	8.8469
EMFA	5.4321	12.3165	5.5734
EMFB	41.1734	79.2093	42.2966
EMFM	5.5510	36.5478	9.8595
EMFE	68.7655	72.7864	29.6332
ENF	111.9740	98.5518	63.5491
ENC	109.2260	81.0246	59.1948
ENOH	4.8341	39.4327	5.3468
ENOD	5.2377	8.2359	5.5815
ENOA	70.6921	59.5467	58.1120
EMSE	103.7610	99.3668	61.1939

The hexane and ethyl acetate extracts of *Ocotea notata* (Lauraceae) leaves (EOHF and EOAF, respectively) also showed higher percentages of metabolic activity when tested on J774 macrophages. G8 lineage. Although cell metabolism is considered low in other types of macrophages, the EOHF extract tested on peritoneal macrophages showed the lowest percentage of viability (Table 1). The EOHF extract also showed the highest percentage of LDH release in peritoneal macrophages, which justifies the low viability observed by this extract in this type of macrophage. Unlike expected, EOHF was detected in J774. G8 cells 70% of LDH release, while in bone marrow macrophages, these values were lower than 10%. For EOAF, the J774. the G8 strain was also the most susceptible, while we observed modest toxicity in

peritoneal macrophages and an absence of toxicity in bone marrow macrophages. EMPF and EMPFC exhibited a similar profile, where both were not toxic to bone marrow macrophages and exhibited moderate toxicity in J774. G8 cells. EMPF and EMPFC caused moderate and modest toxicity in peritoneal macrophages, respectively (Table 2).

The leaf extracts in ethyl acetate and ethanol; and ethanol extract of the *Myrsine parvifolia* (Myrsinaceae) stem (EMPFA, EMPF, and EMPC, respectively) showed high percentages of metabolic activity in the macrophages of J774. G8 lineage. The EMPFA extract also showed a high percentage of metabolism in the test in peritoneal macrophages (Table 1). EMPFA presented as expected low LDH release in tests with peritoneal macrophages. However, the same phenomenon occurred in the percentage of LDH released from *Ocotea notata* extracts, in which the percentage of LDH release was high in J774 macrophages.

Table 2. Percentage of lactate dehydrogenase enzyme (LDH) activity according to plant extracts tested on peritoneal macrophages, J774. G8 lineage macrophages and bone marrow macrophages.

Acronym	LDH activity (%)		
	Peritoneal Macrophage	J774. G8 Lineage macrophage	Bone Marrow Macrophage
CN	6.0843	31.1318	0.0014
CP	84.4195	97.3991	79.9864
EIOUE	5.6632	63.9811	0.0011
EIOUF	5.8300	62.0354	0.0016
EOHF	83.3246	70.0272	27.0925
EOAF	34.9958	55.7369	10.9546
EMPFA	13.9679	75.9564	9.4683
EMPF	48.2150	87.4332	20.0410
EMPC	32.7258	59.6317	11.6917
EBEF	41.0873	95.0830	18.9486
EPHF	68.9760	88.4115	24.4026
EPAF	64.4825	78.0517	50.1205
EPBF	62.4237	94.7453	17.5281
EMFH	38,8518	91.9946	0.0025
EMFD	44.2968	94.6065	17.0775
EMFA	45.676	92.4838	0.0015
EMFB	43.4363	85.3188	0.0007
EMFM	5.7391	81.2855	0.0015
EMFE	10.7745	62.9809	0.0008
ENF	11.7627	41.3497	11.4734
ENC	5.4852	89.0258	10.9835
ENOH	62.2482	99.5187	16.7785
ENOD	71.4939	98.6836	16.1623
ENOA	53.6895	86.8157	16.0888
EMSE	2.8091	45.5884	0.0012

G8 lineage, although the highest percentages of metabolic activity were also observed in these cells. In bone marrow macrophages, low percentages of LDH release and metabolic activity were detected (Table 2).

Most of the tested extracts belonged to the Myrtaceae family. Ethanol extracts from the leaves and stems of *Neomitranthes obscura* (ENF and ENC, respectively) showed the highest percentages of metabolic activity. Interestingly, the ethyl acetate stem extract of this same species (ENOA) showed high values among the three macrophage strains. The *Eugenia pruniformis* leaf extracts in hexane (EPHF) and ethanol (EPBF), *Myrciaria floribunda* leaf extracts in ethyl acetate (EMFA) and methanol (EMFM), *Neomitranthes obscura* stem extracts in hexane (ENOH) and dichloromethane (ENOD) showed the lowest percentages in different types of macrophages. The *Myrciaria floribunda* leaf extract in hexane (EMFH) indicated lower percentages in the three types of macrophages evaluated; however, this percentage was relatively higher than the extracts mentioned above (Table 1).

The *Eugenia sulcata* crude extract (EBEF) and *Myrciaria floribunda* leaf extract (EMFE) showed moderate percentages of metabolic activity in tests with peritoneal macrophages and J774. G8 lineage and modest percentages in bone marrow macrophages. On the other hand, *Myrciaria floribunda* leaf extract in butanol (EMFB) had moderate LDH release on peritoneal and marrow macrophages and elevated on J774. G8 lineage. The *Eugenia pruniformis* leaf extract in ethyl acetate (EPAF) showed low metabolic activity in peritoneal and bone marrow macrophages but not in J774. G8 lineage macrophages, the percentage was higher. Finally, the dichloromethane fraction of *Myrciaria floribunda* leaf extract (EMFD) showed low percentages of metabolic activity, with a moderate percentage in J774. G8 macrophages test (Table 1).

In the Myrtaceae family extracts with higher metabolic activity in the three macrophage categories, the crude extracts of the *Neomitranthes obscura* leaves and stem (ENF and ENC, in that order) presented low LDH release, excepted for J774. G8 lineage macrophages, in which the ENC and ENF extracts showed high and moderate percentages, respectively. For the ethyl acetate fraction of the stem extract of *Neomitranthes obscura* (ENOA), only the test in bone marrow macrophages identified low LDH release, according to the metabolic activity. Most of the extracts with lower metabolic activity expressed high percentages of LDH detected in peritoneal macrophages and J774 cells. G8 lineage. *Myrciaria floribunda* leaf extracts in the hexane, dichloromethane, butanol, methanol, and ethanol fractions (EMFH, EMFD, EMFB, EMFM, EMFE, respectively), as well as the crude extract of *Eugenia sulcata* (EBEF), indicated a high percentage only in J774. G8 lineage macrophages. The ethyl acetate fraction of the *Eugenia pruniformis* leaf extract (EPAF) was the only fraction that showed high percentages of LDH activity in the three types of macrophages (Table 2).

The only tested extract from the Sapotaceae family was the crude extract obtained from the stem of *Manilkara subsericea* (EMSE). This extract revealed high percentages of metabolic activity for each type of macrophage evaluated and as expected, showed low percentages in the LDH enzyme detection test in the three groups of macrophages tested (Tables 1 and 2).

4. Discussion

The *Erythroxylum* genus contain alkaloids, flavonoids, terpenes and other metabolites with different biological and pharmacological activities (Zanolari et al., 2003; Oliveira et al., 2011; Aguiar et al., 2012). Some studies have evaluated the cytotoxic effect of other species; however, the only work in the literature that addresses the use of *Erythroxylum ovalifolium* extracts points to the potential for antivenom treatment due to its crude extract. Some fractions inhibited the hemorrhagic effect and the edematogenic activity of *Lachesis muta* venom when the extracts or products were administered before venom injection into mice (Oliveira et al., 2016). In the present study, the crude extract and the flavonoid-rich fraction obtained from the *E. ovalifolium* stem did not show toxic effects when tested on peritoneal and bone marrow macrophages; however, high percentages of LDH enzyme release were observed in J774. G8 lineage macrophages. *E. suberosum* has been studied as an antitumor and, its cytotoxic activity has already been proven in the FaDu cell line, with or without radiotherapy (Macedo et al., 2016). Some *E. caatingae* fractions showed cytotoxic activity against NCI-H292, HEp-2, and K562 cell lines using MTT.19 Therefore, the effect observed in the lineage studied diverging from that observed in macrophages obtained from mice, can be attributed to the discovery that some plant extracts have strong cytotoxic potential in cell lines of various types of cancer (Tokgun et al., 2012; Song et al., 2013).

The genus *Ocotea* (Lauraceae) is mainly distributed in tropical and subtropical regions. Some species of this genus have been described in the literature, showing antibacterial activity. At 100 µg/mL, the *O. notata* crude extract showed 95.75 ± 2.53% growth inhibition of the Mycobacterium bovis BCG strain, but this extract was toxic when evaluated in RAW 264.7 macrophage culture. The fractions of different polarities, hexane, ethyl acetate, butanol, and water, were also evaluated, with the hexane fraction showing the best performance in inhibiting the growth of mycobacterial activity. In contrast, the ethyl acetate fraction exhibited lower cytotoxicity (Costa et al., 2015). In the present study, toxic effects were also observed in J774. G8 lineage macrophages for both the hexane and ethyl acetate fraction, although high percentages of metabolic activity were observed.

In an in vitro study of *Myrsine africana*, low cytotoxic behavior of fruit extracts and higher antioxidant values were observed, as well as a larger zone of inhibition concerning different types of bacteria (Gul et al., 2021). In our study with extracts from the leaves and stem of *Myrsine parvifolia*, we observed, in contrast to what was expected, a phenomenon of direct correspondence between the percentages of metabolic activity and cell death by necrosis, except the ethyl acetate fraction of the leaf extract in peritoneal macrophages, which demonstrated high metabolic activity, and low cytotoxicity. However, in this direct link, we found higher percentages in J774. G8 lineage macrophages and low percentages in bone marrow macrophages. Corrêa et al. (2019) evaluated the capacity of extracts from *Myrsine parvifolia* leaves to reduce the inflammatory process (edema, increased vascular permeability, and leukocyte migration) induced by *Bothrops jararaca* venom. In this single study evaluating the extracts of this species, they observed that after oral administration of extracts (100 mg/kg) in mice by different protocols, the group pretreated with hydroethanolic extract and dichloromethane reduced total edema (40 and 52%, respectively), the increase in vascular permeability (32.4 and 32.2%, respectively) and the influx of leukocytes into the pleural cavity (42 and 39%, respectively). In contrast, the group treated with hexane extract showed only a reduction in edema (37%) induced by *B. jararaca* venom. The partial inhibition values observed in this study may be associated with possible moderate toxicity observed in the J774 cells. G8 lineage and peritoneal macrophages for the leaves and stem extracts (Corrêa et al., 2019).

The Myrtaceae family has great diversity and is found mainly in tropical and temperate areas of the globe (Judd et al., 2009). Ethnobotanical studies conducted on several family species indicate numerous popular uses, such as treating diarrhea, sore throat, gout, rheumatism, flu, urinary tract diseases, and diabetes (Boscolo and Senna-Valle, 2008)

The genus *Eugenia*, belonging to the Myrtaceae family, the most commonly used folk medicine in Restinga Jurubatiba National Park in Carapebus city, RJ, Brazil (28) Regarding the constituents and biological activities of *E. pruniformis*, these remain scarce, and only the essential oil and antileishmanial activity of the terpenoids of this species have been previously reported (Albuquerque et al., 2012, 2020) For the species *E. sulcata*, there is only one study on its insecticidal and acaricide potential; however, according to the authors, other chemical and physiological studies are needed to determine whether these substances can be helpful in the control of arthropods and vector transmission in the field (Feder et al., 2019). Therefore, we evaluated the metabolic activity and cell death by necrosis in these two species in the present study. In general, we observed high cytotoxicity in the studied extracts, except for *E. pruniformis* leaf extracts in the hexane and ethanol fractions and the *E. sulcata* crude extract when evaluated in bone marrow macrophages. Furthermore, the percentages of metabolic activity were higher in the ethyl acetate fraction of the *E. pruniformis* leaf extract, only in the evaluation of the effect at J774. G8 lineage cells.

Myrciaria floribunda is a native plant species from north to south Brazil of the Atlantic Forest. The lyophilized extract of ethyl acetate from the leaves of *M. floribunda* exhibited antiproliferative activity against cancer cell lines in vitro, with the MCF-7 cell line (breast cancer) being more sensitive to this extract. In contrast, normal cells were less inhibited from growth and presented a strong antioxidant potential (Tietbohl et al., 2017). The antimicrobial activity of the *M. floribunda* leaf extract was also evaluated, with the methanol extract being active against *Staphylococcus aureus* and *Escherichia coli* but inactive against *Candida albicans* and *Candida krusei* (Azevedo et al., 2019). In our study, extracts from *M. floribunda* leaves in ethyl acetate and methanol showed the lowest percentages of metabolic activity in different types of macrophages; however, the cytotoxic potential of these extracts was more evident

in J774. G8 lineage cells previous corroborating studies showed, more significant inhibition of cell line growth. The evaluation of cytotoxicity of different extracts from other species of the genus *Myrciaria* has already been reported. The aqueous extract of the *M. dubia* tegument showed cytotoxic effects on Caco-2, A549, and HepG2 cancer cells but no cytotoxicity to IMR90 cells (Willemann et al., 2020). The action of the dichloromethane fraction of *M. dubia* leaf extract was evaluated against the protozoa *Plasmodium falciparum*, *Leishmania amazonensis*, *Leishmania braziliensis*, and *Leishmania chagasi* and against the human hepatoma strain HepG2, indicating the absence of toxicity and more excellent selectivity against parasites (Correia et al., 2016). In the present study, the dichloromethane fraction of *M. floribunda* leaf extract showed low percentages of metabolic activity, with a slightly higher percentage being observed in the J774 test. G8 lineage macrophages; however, it also presented a high percentage of LDH enzyme release and was considered more toxic in this macrophage category. The optimized extract of *M. cauliflora* exerted antiproliferative and cytotoxic effects against A549 and HCT8 cells and antimicrobial, antihemolytic and antihypertensive effects in vitro (Fidelis et al., 2020). In cytotoxicity assays using the resazurin technique, *M. plinioides* essential oils showed moderate activity (Faleiro et al., 2017). In general, as pointed out in the literature, the toxicity of extracts can vary according to the factors corresponding to the fractions of different polarities used, and the origin of the cells studied.

In our study, the percentage of metabolic activity and detection of LDH enzyme release in macrophages from three different origins under crude leaf extract and *Neomitranthes obscura* stem extracts in the ethanol, ethyl acetate, hexane, and dichloromethane fractions showed variations. The percentage of metabolic activity in the hexane and dichloromethane fractions was low, while in all other extracts, higher percentages were observed. Furthermore, low detection of the LDH enzyme was observed in all extracts when we evaluated its effects on bone marrow macrophages, including the hexane and dichloromethane fractions, which, as mentioned above, showed reduced metabolic activity. These data indicate the need to assess the cytotoxicity of this species within the specific parts of this plant and in the fractions of different polarities of its extracts. However, no study in the literature has pointed out these variations in *N. obscura*, and there is only one study that indicates that high concentrations of the methanol fraction of leaf extract induced maximum mortality of 46.66% in *A. aegypti* larvae after 48 hours (LC50 = 25 ppm) (Carneiro et al., 2021).

Manilkara subsericea is endemic to the Brazilian Restinga of the Rio de Janeiro state and has a large variety and potential of biological activities, such as anticholinesterases, antimicrobials, antiparasitics, insecticide, acaricide, molluskicide and antivenom in *Lachesis muta* snake bites, already existing in the literature (Feder et al., 2019; Muelas-Serrano et al., 2000; Fernandes et al., 2011, 2013, 2014; Oliveira et al., 2014; Faria et al., 2018). In this sense, the high metabolic activity and low cytotoxicity found in all types of macrophages obtained in this study corroborate the promising potential of extracts from this plant.

In general, for the comparative evaluation of the action of extracts and fractions, plants from different families on peritoneal macrophages, bone marrow macrophages, and J774. G8 lineage macrophages, we used resazurin, a colorimetric indicator with redox properties. This dye has a blue color with a minor intrinsic fluorescence. When in contact with viable cells, cellular reductases, such as NADH dehydrogenase, reduce resazurin to resorufin, acquiring a pink color and fluorescence. According to Al-Nasiry et al. (2007), this is a possible way to assess whether extracts interfere in any way with metabolic activity (Al-Nasiry et al., 2007).

Nevertheless, natural products could cause only a transient and nonlethal effect, requiring evaluation by the LDH release assay. The identification of this enzyme in the medium is proportional to the number of cells killed by an in vitro necrosis process, where there is rupture of the cell membrane and LDH extravasation (Pereira et al., 2015).

Thus, the expected result would be that in evaluating the action of extracts on different types of macrophages, the percentages of metabolic activity obtained through the detection of resazurin were inversely proportional to the percentages detected by the release of the LDH enzyme; however, some phenomena observed could be justified by the descriptions below.

Different behaviors of the action of some extracts on each type of macrophage can be explained by the distinct intrinsic characteristics presented by each extract, such as the homogeneity of macrophage populations of lineages to the detriment of more heterogeneous populations of tissue macrophages (Miyakawa et al., 1989).

Furthermore, according to Lam et al. (2009), J774 cells, when removed from adherent cell culture flasks by scraping, might not be genuinely passive but in a preactive state, manifested by easy excitability widespread observed mechanical properties. In this sense, the J774. the G8 cell line showed a reduction in cell viability by themselves, and for this reason, when the percentages of activity in the extracts were compared to the negative control, an overestimation of the values could have occurred (Lam et al., 2009).

Another justification could be that J774 cells do not present the intrinsic suppression mechanism observed in long-term stimulated peritoneal macrophages (Miyakawa et al., 1989).

5. Conclusion

The data obtained in the present study showed a difference in the percentage of toxicity when comparing the action of the same extract on different macrophage types.

This result indicates that these cells from various origins may exhibit different responses when exposed to the same compounds. Elements are influenced by metabolic pathways and constituents of the plasma membrane. In our analysis, the cell lineage with neoplastic characteristics was more susceptible to the toxic action of the extracts.

Additionally, primary marrow macrophages were the least susceptible. This factor is interesting both in predicting a possible tolerance of the extracts when administered in vivo and in studies that may focus on the antineoplastic action of these extracts.

Thus, a factor extremely relevant is understanding the origins of the variations between the different cell types to establish which of these strains will be experimentally more receptive and, in this way, can be transported to mobile cell behavior in humans.

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