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DEVIL'S CLAW HAS ANTI-NEUROINFLAMMATORY CAPACITY THROUGH MITOCHONDRIAL RECOVERING IN MICROGLIA CELLS EXPOSED TO ROTENONE[1]

GARRA DO DIABO POSSUI CAPACIDADE ANTI-NEUROINFLAMATÓRIA ATRAVÉS DA RECUPERAÇÃO MITOCONDRIAL DE MICRÓGLIAS EXPOSTAS A ROTENONA

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ABSTRACT

Neuropsychiatric diseases, such as bipolar disorder, Alzheimer's, and Parkinson's disease, are related to mitochondrial dysfunction and chronic inflammatory activation, affecting thousands of people worldwide. Therefore, new alternative therapies with mitochondrial modulation and anti-inflammatory effects, especially involving natural agents are important to search. *Harpagophytum procumbens* is a notable candidate. It is an African plant with anti-inflammatory action. The objective of this study was to evaluate the anti-neuroinflammatory effect of *H. procumbens* ethyl acetate fraction in microglia cells. *H. procumbens* ethyl acetate fraction *per se* effect was available in SH-SY5Y and BV-2 cells during 24, 48, and 72 hours by cell viability and nitric oxide levels. Only BV-2 cells were exposed to rotenone to induce inflammation-mediated mitochondrial dysfunction and treated with curve concentration of *H. procumbens* fraction. Cellular proliferation and oxidative metabolism parameters were analyzed. *Per se* effect results revealed that significative changes are viewer depending on the time of exposure and cell line type. 0.001-400 μg/mL of *H. procumbens* treatment decreased nitric oxide and reactive oxygen species levels which have been increased by rotenone exposure, furthermore, cell viability recovered after this treatment exposure. *H. procumbens* was able to act as anti-neuroinflammatory agent, recovering mitochondrial complex I function, reducing oxidative stress, and decreasing cell proliferation.

Keywords: *Harpagophytum procumbens*, Harpagoside, Nervous system diseases, Oxidative stress.

RESUMO

Doenças neuropsiquiátricas, como transtorno bipolar, Alzheimer e Parkinson, estão relacionadas à disfunção mitocondrial e ativação inflamatória crônica, afetando milhares de pessoas em todo o mundo.

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Para isso, é importante pesquisar novas terapias alternativas com modulação mitocondrial e efeitos anti-inflamatórios, principalmente envolvendo agentes naturais. Harpagophytum procumbens é um candidato notável, sendo uma planta africana com ação anti-inflamatória. O objetivo deste estudo foi avaliar o efeito anti-neuroinflamatório da fração acetato de etila de H. procumbens em células da micróglia. O efeito per se da fração acetato de etila do H. procumbens foi avaliado em células SH-SY5Y e BV-2 durante 24, 48 e 72 horas através da viabilidade celular e níveis de óxido nítrico. Apenas células BV-2 foram expostas a rotenona para induzir disfunção mitocondrial mediada por inflamação e tratadas com uma curva concentração da fração do H. procumbens. Parâmetros de proliferação celular e metabolismo oxidativo foram analisados. Os resultados do efeito per se revelaram mudanças significativas dependentemente do tempo de exposição e do tipo de linhagem celular. O tratamento com 0,001-400 µg/mL de H. procumbens diminuiu os níveis de óxido nítrico e espécies reativas de oxigênio, enquanto aumentados pela exposição à rotenona, além disso, a viabilidade celular foi recuperada após a exposição ao tratamento. H. procumbens foi capaz de atuar como agente anti-neuroinflamatório, recuperando a função do complexo mitocondrial I, reduzindo o estresse oxidativo e diminuindo a proliferação celular.

Palavras-chave: *Harpagophytum procumbens, Harpagosídeo, Doenças do sistema nervoso central, Estresse oxidativo.*

INTRODUCTION

Neuropsychiatric diseases are a public health problem that has significance over the years. World health organization (WHO) data indicate that bipolar disorder (BD) affects about 60 million people worldwide followed by schizophrenia (SCZ) with 50 million people (WHO, 2018). However, the etiological aspects and the physiopathology of psychiatric diseases are still unclear.

Despite the pathophysiological complexity of neurological diseases, several studies have demonstrated that mitochondrial dysfunction is present in psychiatric diseases, such as BD, Parkinson's disease, SCZ, and Alzheimer's disease *(*ADAMS *et al., 2018;* CIMDINS *et al*., 2019; MACHADO *et al*., 2016).

Mitochondria is an organelle responsible for energy production, intracellular calcium homeostasis, and apoptosis (TRIGO *et al*., 2022). However, the generation of energy is also followed by reactive oxygen species (ROS) production which can be harmful to the organism in large concentrations (KIM *et al*., 2019; MACHADO *et al*., 2016; TRIGO *et al*., 2022). Subjects with mitochondrial dysfunction, specifically in complex I, present oxidative stress followed by different cellular consequences, including lipid peroxidation, protein oxidation, and DNA damage. In a study using post-mortem brain from patients with BD, a specific complex I mitochondrial dysfunction due to NDUFS7 protein and gene expression downregulation was observed (ANDREAZZA *et al*., 2013).

Additionally, some scientific studies have correlated oxidative stress and mitochondrial dysfunction to inflammatory activation in neurological diseases. For example, Kim *et al.* (2016) described mitochondrial dysfunction, inflammation, and oxidative stress in post-mortem frontal cortex samples from patients with BD. A review article states that the role of mitochondria in regulating the needs of the central nervous system is extremely important, concluding that metabolic

dysfunctions can be a determining factor for the development of psychopathologies, along with oxidative stress (MORELLA; BRAMBILLA; MORÈ, 2022).

These problems have been calling the attention of several researchers to discover potential pharmacological targets and propose new alternatives therapies. In this sense, many efforts have been spent to find new alternatives to recover mitochondrial function and avoid or reduce neuroinflammation. Some of these studies involve natural products, includes the *Harpagophytum procumbens* (HP), popularly known as devil's claw, a native plant from South Africa (SCHAFFER *et al*., 2013; 2016). HP has proved neuroprotective, anti-inflammatory and antioxidant actions described using *in vitro* and *in vivo* models (FERRANTE *et al*., 2017; LIMA *et al*., 2023; MNCWANGI *et al*., 2012; MUZILA *et al*., 2016). HP is classified as a tuberculous root and it has significant concentrations of glycosides iridoids, such as harpagoside, which has been registered as the bioactive component responsible for the therapeutic action of this plant (DRAGOS *et al*., 2017; MNCWANGI *et al*., 2012). HP ethyl acetate fraction has been studied by our research group and it demonstrated satisfactory results to reduce oxidative stress and inflammation using *in vitro* and *in vivo* models (SCHAFFER *et al*., 2013; 2016).

Therefore, the objective of this study was to evaluate the antioxidant and anti-neuroinflammatory effects of HP ethyl acetate fraction in complex I mitochondrial dysfunction-induced microglia cells.

METODOLOGY

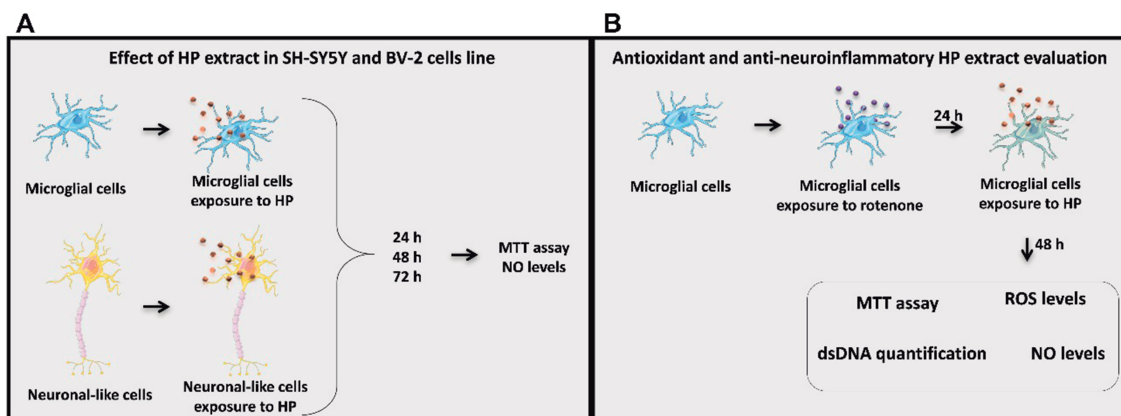
MATERIALS AND INSTRUMENTATIONS

The powdery roots obtained commercially from Quimer Comercial LTD (São Paulo, Brazil). BV-2 (ATCC® 0356) and SH-SY5Y (ATCC® 0223) cells originated from the Rio de Janeiro Cell Bank (RJ, Brazil). HEPES, MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide), and 2,7 dichlorodihydrofluorescein diacetate (DCFH-DA) were obtained from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS), penicillin/streptomycin solution, Dulbecco's Modified Eagle Medium (DMEM) containing F12 supplement, and Quant-IT PicoGreen® dsDNA kit were commercially obtained from Thermo Fisher® (Mississauga, ON, Canada).

EXPERIMENTAL DESIGN

This is an *in vitro* quantitative and experimental study where the HP ethyl acetate fraction extract was assessed about its *per se* effect in neuronal-like (SH-SY5Y) and microglial cells (BV-2). This extract was also used to evaluate its potential antioxidant and anti-neuroinflammatory effect through mitochondrial complex I recovery using BV-2 microglia cell line exposed to rotenone. The experimental design is represented in Figure 1.

Figure 1 - Explanatory illustration of the experimental design.



Description: (A) To evaluated citotoxicity of the HP extract, microglial cells and neurons were exposed to the treatment for 24, 48, and 72h, after cellular viability and NO levels were measured. (B) The antioxidant and anti-neuroinflammatory potential of the extract in cells exposed to rotenone was measured by cell viability, with the MTT and PicoGreen tests, and NO and ROS levels. Microglial cells were exposed 24h with rotenone to induce mitochondrial dysfunction, after HP extract was added for 48h, with the following measurement the tests. Elaborated by the authors (2023).

ETHYL ACETATE FRACTION OF HP PREPARATION AND CHARACTERIZATION

The powdery roots were solubilized in 70% ethanol and allowed to stand at room temperature with daily agitation. After one week, it was filtered and evaporated. The extract was resuspended in water and ethyl acetate as described by Schaffer *et al.* (2013; 2016).

The extract of HP was analyzed in reverse-phase chromatographic in the concentration of 5 mg/mL (LAGHARI *et al.*, 2011). The flow rate was 0.8 mL/min and injection volume 40 µL, the detection wavelengths were 254 nm for gallic acid, 280 nm for catechin, 325 nm for caffeic and rosmarinic acids, and 365 nm for quercetin and rutin. Results previously published by Schaffer *et al.* (2013; 2016).

CELL CULTURE

Microglia cell line (BV-2) were cultured in RPMI-1640 medium modified to contain 10 mM HEPES with 10% FBS and supplemented with 1% of antibiotic (100U/mL penicillin; 100mg/mL streptomycin). Additionally, neuron-like cell line (SH-SY5Y) were cultured using DMEM containing F12 supplement with FBS 10% and antibiotic 1%. Cells were cultured at 37ºC with 95% of oxygen and 5% CO_2 until the number of cells was sufficient for the experimental tests.

TREATMENTS

Microglial and neuronal-like cells were seeded in 96-well plates at 2.5 x 10^5 cells/mL. Initially, both cell lines were used to test the *per se* effect of the extract to identify if it could modify cellular homeostasis by itself. Cells were exposed to a concentration-response curve (0.001, 0.005, 0.01, 0.05, 0.1, 1, 10, 100, 200, 400, 500, and 1000 µg/mL) of the fraction during 24, 48, and 72 hours. For positive control (PC), 25 µM hydrogen peroxide (H_2O_2), was used (PAPPIS *et al.*, 2021). After all periods of incubation, cells were submitted to MTT and nitric oxide (NO).

Then, microglia cells were treated with different concentrations of rotenone (5, 15, 30, 60, 120, 200, 240, 300, 400, and 500 nM) for 24h to identify the most effective concentration in changing cellular viability. After that, cells were activated using 300 nM of rotenone and later they were treated with the same HP fraction concentration-curve described before. These treatments were used to select the best concentration of the extract able to reduce cellular imbalance through different colorimetric and fluorometric assays.

CELL VIABILITY ANALYSIS

Cell viability and/or proliferative status were measured using two methods: MTT assay and Quant-IT PicoGreen® dsDNA kit.

After treatments and incubation, the supernatant was removed and phosphate-buffered saline 1X (PBS) was added. Cells were incubated with 5 mg/mL MTT for 2h. The supernatant was removed from the wells and the cells resuspended in dimethylsulphoxide, for solubilization of the formazan crystals formed by the reduction of MTT salt by the viable cells. The absorbance was determined at 560 nm (FUKUI; CHOI; ZHU, 2010).

In the supernatant, free-double stand DNA (dsDNA) was quantified, which indicates cell apoptosis. PicoGreen® reagent was added and samples incubated for 5 minutes. Fluorescence was measured by excitation 480 nm and emission 520 nm (AHN; COSTA; EMANUEL, 1996).

REACTIVE OXYGEN SPECIES MEASUREMENT

ROS production was detected using DCFH-DA. The supernatant was incubated in black plates with 0,1 mM DCFH-DA for 1h. This fluorometric test is based on the ability of the substance to transform to dichlorofluorescein (DCF) in the presence of ROS, viewed by fluorescence under excitation of 485 nm and emission 520 nm (COSTA *et al.*, 2012).

NITRIC OXIDE MEASUREMENT

NO is an important inflammatory mediator signal. The measurement of NO present in the culture supernatant was based on the publication of Choi *et al.* (2012), which uses Griess reagent to detect organic nitrite.The supernatant with Griess reagent were incubated on 96-well plate protected from light for 15 min. The absorbance was determined at 540 nm.

STATISTICAL ANALYZES

The results were transformed to percentages for the negative control group. The data were statistically analyzed by one-way ANOVA, followed by Tukey test, using GraphPad Prism software. The data was presented as mean ± SD. Values were considered statistically significant when $P<0,05$.

RESULTS

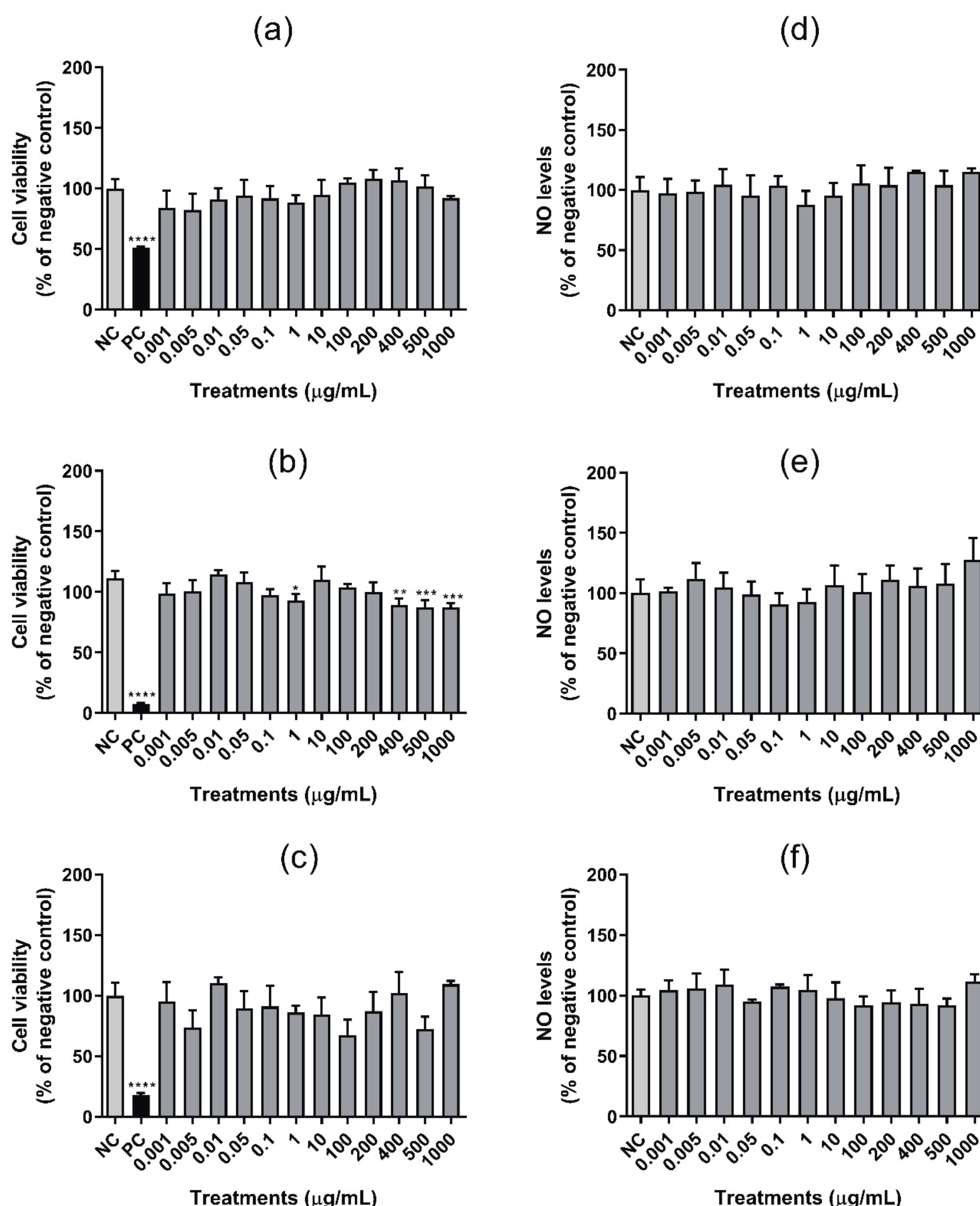
HP ETHYL ACETATE FRACTION CHARACTERIZATION

The high-performance liquid chromatography profile and compositions of HP ethyl acetate fraction were previously published by our research group (SCHAFFER *et al*., 2013; 2016). The results revealed the presence of harpagoside, gallic acid, catechin, caffeic acid, rosmarinic acid, phenol glycoside, rutin, and quercetin.

HIGH CONCENTRATIONS DECREASE NEURONAL VIABILITY WHEN EXPOSED TO HP FOR 48 H

Testing the safety profile of the HP, neuronal-like cells were exposed to a concentration-response curve of fraction, which was analyzed according to cell viability and NO production (Figure 2). None of the tested concentrations were able to produce NO during incubations of 24 h, 48 h, and 72 h (Figure 2D, 2E, and 2F). At 24 h and 72 h, cell viability change was not significant when compared with negative control (Figure 2A and 2C), however, when cells were exposed to HP for 48 h, 400-1000 µg/mL caused cell death (Figure 2B).

Figure 2 - Cell viability and NO levels in SH-SY5Y cells to analyze the HP security profile.



Description: **(a), (b), and (c)** Viability of SH-SY5Y cells exposures in a curve concentration of HP fraction during 24, 48, and 72 h, respectively. **(d), (e), and (f)** NO levels in SH-SY5Y cells exposures in a curve concentration of HP fraction during 24, 48, 72 h, respectively. NC: negative control; PC: positive control. Statistical analysis was performed comparing treatment groups with negative control, p value less than 0.05 was considered significant. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

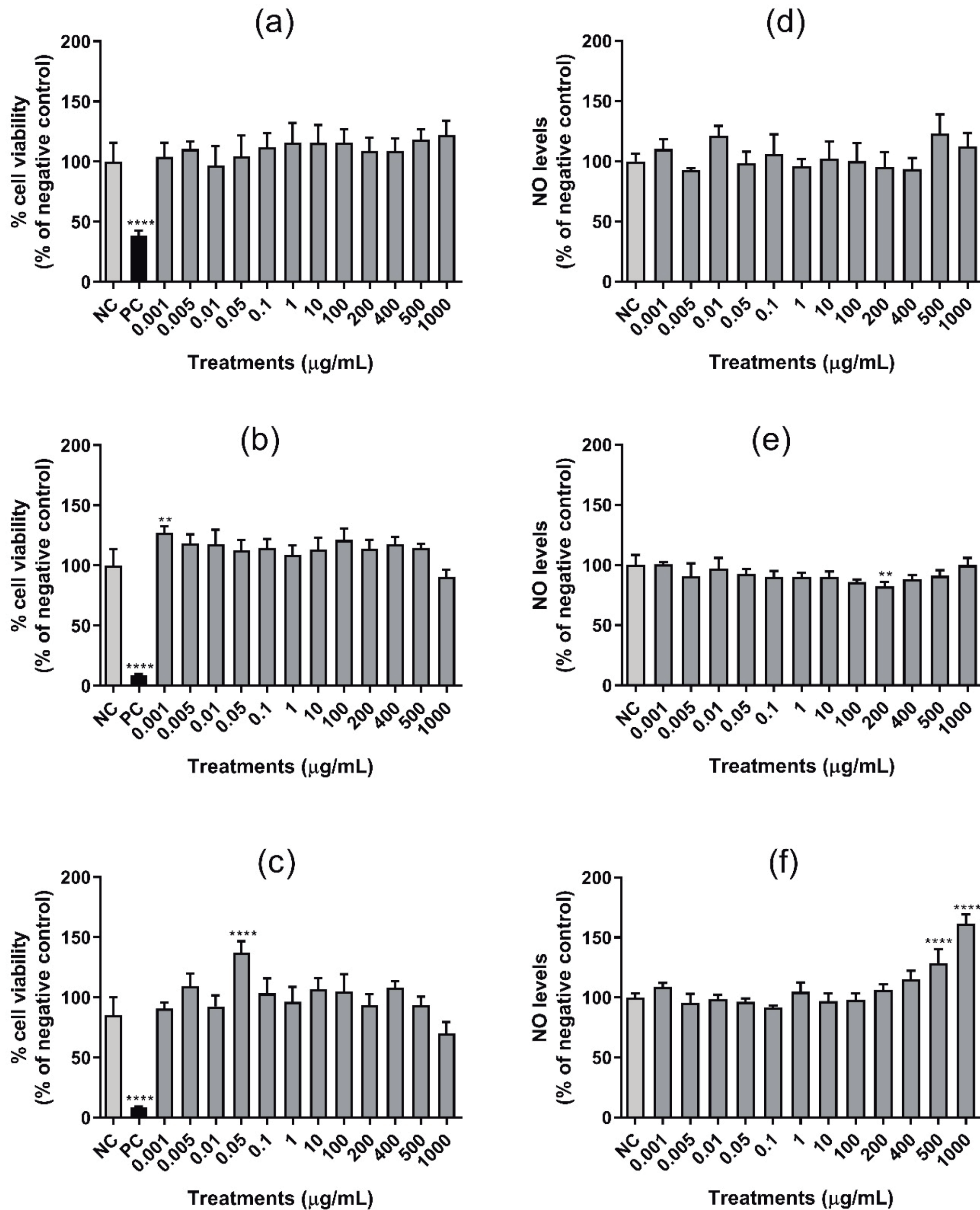
NO LEVELS ARE INCREASED WHEN MICROGLIAL CELLS WERE EXPOSED TO HP FOR 72 H

The same curve concentrations of the HP fraction tested for security profile in neuronal-like cells were used in microglia cells. During 24 h incubations, cell viability remained similar to the

negative control (Figure 3A). This also occurs in 48 h and 72 h, however, 0.001 and 0.05 μg/mL were capable to induce cell proliferation, respectively (Figure 3B and 3C).

NO assay revealed that the exposures at the different times did not induce its production (Figure 3D and 3E), except by the highest concentrations (500-1000 μg/mL) where statistical significance in 72 h was observed (Figure 3F).

Figure 3 - Cell viability and NO levels in BV-2 cells to analyze the HP security profile.

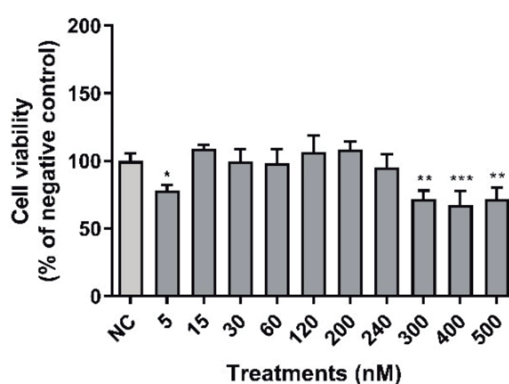


Description: **(a), (b), and (c)** Viability of BV-2 cells exposures in a curve concentration of HP fraction during 24, 48, and 72 h, respectively. **(d), (e), and (f)** NO levels in BV-2 cells exposures in a curve concentration of HP fraction during 24, 48, 72 h, respectively. NC: negative control; PC: positive control. Statistical analysis was performed comparing treatment groups with negative control, p value less than 0.05 was considered significant. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

HIGHER CONCENTRATIONS OF ROTENONE REDUCE CELL VIABILITY, THROUGH MITOCHONDRIAL IMPAIRMENT

To determine the concentration of mitochondrial toxicity of rotenone, BV-2 cells were exposed to a concentration-response curve. According to the MTT assay, four concentrations of the curve (5, 300, 400, and 500 nM) caused the decrease of cell viability during 24h, therefore 300 nM was the elected rotenone concentration to cause mitochondrial dysfunction in subsequent assays (Figure 4).

Figure 4 - Cell viability in microglia cells exposures in a curve concentration of rotenone during 24 h by MTT assay.



Description: NC: negative control. Statistical analysis was performed comparing treatment groups with negative control, p value less than 0.05 was considered significant. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

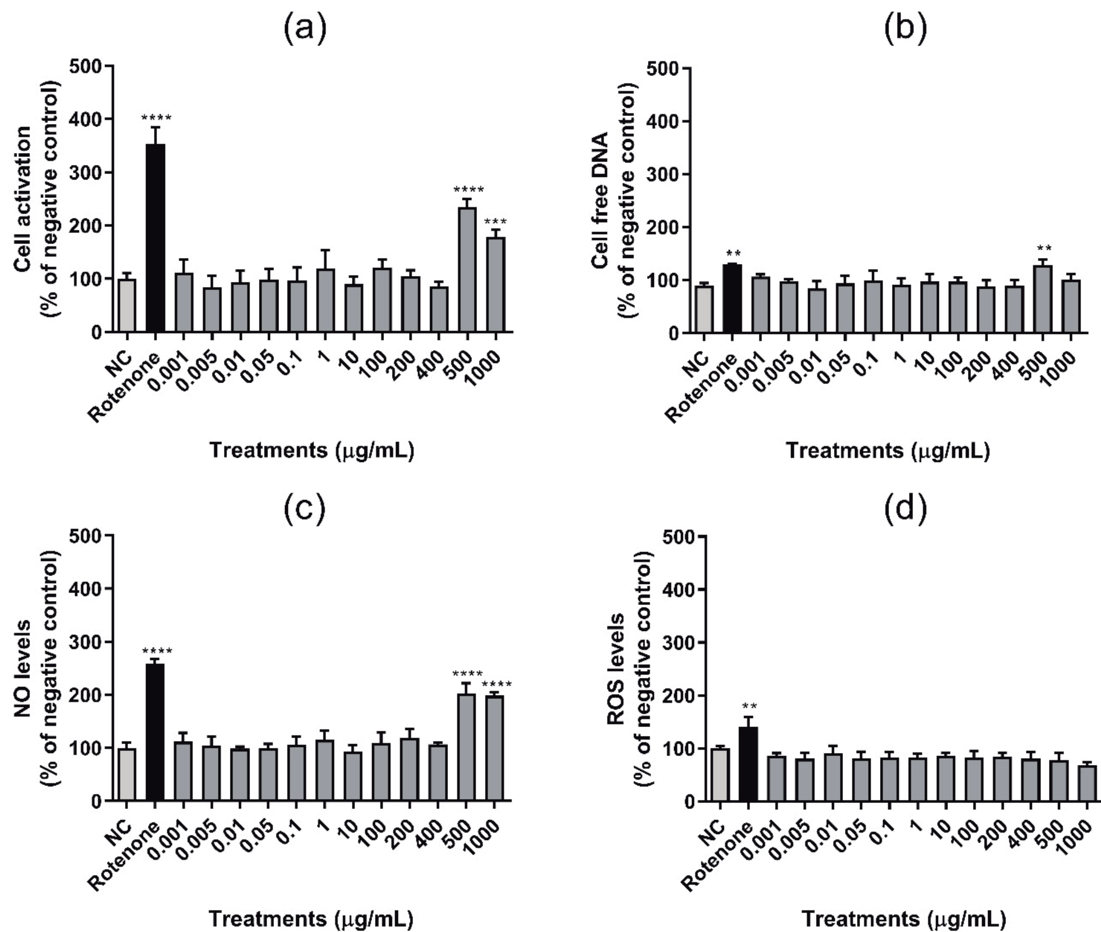
HP ETHYL ACETATE FRACTION IS ABLE TO REVERSE CELL DAMAGE CAUSED BY ROTENONE

The curve of HP ethyl acetate fraction utilized previously was also tested for the treatment of rotenone-induced mitochondrial dysfunction in BV-2 cells for a period of 48h. The capacity of HP in reverse damage and inflammation was evaluated through cell viability and oxidative metabolism (Figure 5).

HP fraction was able to decrease cellular activation of BV-2 cells with inflammation at 0,001-400 µg/mL when analyzed the cell proliferation (Figure 5A). Regarding the quantity of free dsDNA in the supernatant, a significant increase at 500 µg/mL was observed (Figure 5B).

The oxidative metabolism was also tested in this study using BV-2 cells. Rotenone significantly increased ROS production, however, ROS production was attenuated after HP treatments in all concentrations (Figure 5D). But in the case of NO production, the last two points of the tested curve (500 and 1000 µg/mL) could not prevent the nitrite generation (Figure 5C).

Figure 5 - Cellular activation, proliferation, and oxidative metabolism.
BV-2 cells were exposed by rotenone for 24 h followed by HP fraction during 48 h.

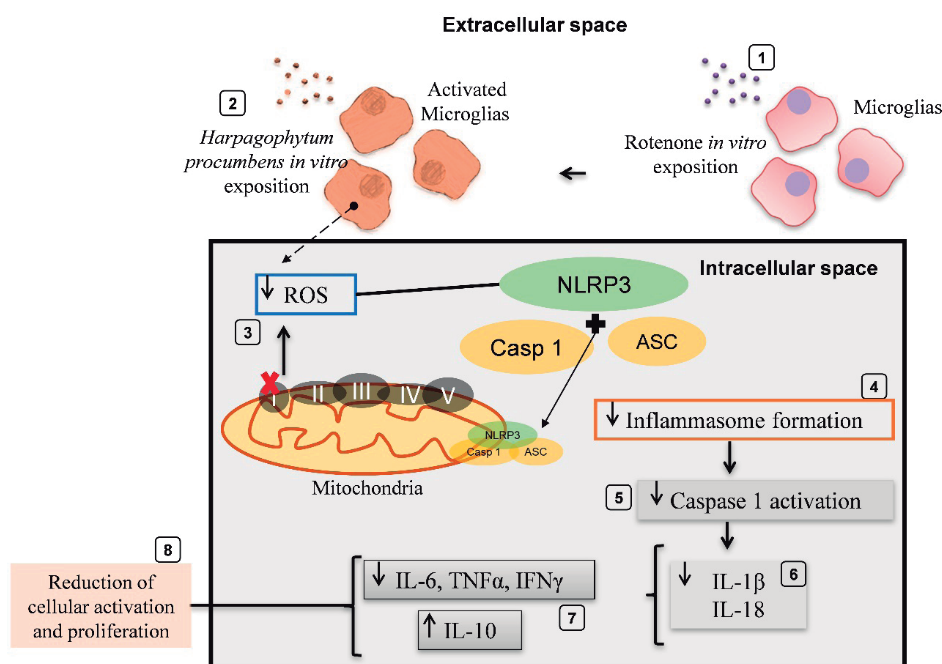


Description: **(a)** Cell viability and proliferation by MTT assay; **(b)** Cellular mortality by dsDNA PicoGreen assay; **(c)** NO levels quantification; **(d)** ROS levels by DCFH-DA fluorometric assay. NC: negative control. Statistical analysis was performed comparing treatment groups with negative control, p value less than 0.05 was considered significant. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

DISCUSSION

The present study aimed to evaluate the antioxidant and anti-neuroinflammatory capacity of an HP ethyl acetate fraction extract in an *in vitro* experimental model using the BV-2 microglia cell line. HP extract presents an anti-neuroinflammatory effect by recovering mitochondrial complex I function, reducing oxidative stress, and keeping cellular viability in a similar way to untreated cells. The results obtained are shown in Figure 6, demonstrating the possible mechanism of action of the extract in the cells with mitochondrial dysfunction.

Figure 6 - Possible mechanism of action of *Harpagophytum procumbens* in microglial cells activated by mitochondrial dysfunction.



Description: (1) The microglia cells exposed to rotenone have dysfunction in the mitochondrial complex I, (2) when activated they were treated with *Harpagophytum procumbens*, which has anti-inflammatory and antioxidant capacity. (3) Production of ROS and NO are decreased in the mitochondria by the action of the plant extract, (4) perhaps leading to a low inflammasomes production (formed by NLRP3, a redox sensor responsible for the activation of the inflammatory system through the ROS mitochondrial production, caspase 1, protein associated with apoptosis and ASC, caspase activator protein). (5) With the inactivation of caspase 1, (6) cell activating molecules, such IL-1β and IL-18, have a low expression (7) consequently low levels of inflammatory mediators, but increase IL-10, a cytokine regulator of the inflammatory response, (8) which inhibits microglial activation and proliferation. Adapted from Machado *et al.* (2019).

Medicinal plants are well classified to treat emerging diseases. Here, we study HP due to anti-inflammatory and protective activity in neuropsychiatric diseases. The production of the HP extract, as well as its characterization and composition, were previously published by Schaffer *et al.* (2013; 2016). The results indicated that the HP chemical matrix presents significant concentrations of harpagoside. Also, other components, as flavonoids were found in the plant extract that perhaps can act synergistically, contributing to its action (SCHAFFER *et al.*, 2013; SERRANO; ROS; NIETO, 2018). In our research group, fractions derived from HP were studied, but in this study, we decided to evaluate only the ethyl acetate fraction, that presented gallic acid, caffeic acid, and iridoid glycosides in high concentration (SCHAFFER *et al.*, 2013).

According to the tests performed, few changes in cell homeostasis were found to *per se* effect of the extract, and the differences were significant depending on the time of exposure and according to the concentration of the extract. There are no published studies correlating cell viability analysis of neuronal-like exposed to HP. However, plants also from Africa and with similar chemical matrix compared to HP ethyl acetate fraction did not show decrease of cell viability in both BV-2 and

SH-SY5Y (JIANG *et al*., 2014; LEE *et al*., 2019). Jiang et al. (2014), for example, evaluated the extract of *Sutherlandia frutescens* in BV-2 cells regarding NO production and viability at concentrations at 2.5-80 µg/mL. The authors did not find any significant difference in both tests when compared to the negative control. This study corroborates with our findings since HP ethyl acetate fraction extract treatment was not able to decrease cellular viability of neuronal-like cells when compared to untreated cells. Additionally, most of the tested concentration did not activate BV-2 cells, except at 500 and 1000 µg/mL where a higher NO production than the negative control was observed.

When exposed to the concentration-response curve of rotenone, BV-2 cells presented a slight mortality index when treated with 5 nM of rotenone and a significant decrease in the cellular viability from 300 nM after 24h of exposition. In this sense, this specific concentration has been chosen as the better chemical model of mitochondrial complex I dysfunction model for microglia cells and to develop the additional experiments. Rotenone is used in BV-2 cells to generate mitochondrial dysfunction followed by inflammation. This pesticide is known to compromise the complex I of the electron transport chain (KIM *et al*., 2019).

Then, BV-2 cells were exposed to 300 nM of rotenone for 24h followed by treatment with the concentration-response curve of HP extract for 48 h. The obtained results showed that cells of the rotenone positive control presented a significant increase in cellular proliferation compared to the negative control. It can be explained by mitochondrial dysfunction, especially involving complex I impairment, increasing ROS production, causing oxidative stress. This oxidative stress acts as a damage-associated molecular pattern (DAMP), perhaps activating the nod-like receptor pyrin containing 3 (NLRP3) wish in association with the ASC protein is capable to activate pro-caspase 1. Caspase 1 activation can increase the production of several pro-inflammatory cytokines and decrease interleukin-10 (IL-10) that is an anti-inflammatory molecule (TSCHOPP; SCHRODER, 2010).

After rotenone exposure, cells were treated with the concentration-response curve of HP. The results showed that the used extract can similarly decrease cellular proliferation compared to the negative control. HP has anti-inflammatory effect in animals with induced osteoarthritis (WACHSMUTH *et al*., 2011). Currently, an *in vivo* model showed that HP could act as a significant antioxidant compound in fluphenazine-induced vacuous chewing movements in rats (SCHAFFER *et al*., 2016). All these findings corroborated with the results here described.

As expected, rotenone exposure increased BV-2 NO production when compared to untreated cells. On the other hand, most of the HP concentrations were able to recover these levels similar to the negative control. NO is a gas that is involved in several physiological metabolic processes. It is already known that high levels of NO are observed under inflammatory responses, also influencing oxidative metabolism (MONTEIRO; SILVA; STERN, 2004). There are some studies developed using natural products with the capacity to modulate NO production. Machado *et al.* (2019), for example, showed that LPS-induced macrophage activation treated with freeze-dried hydroalcoholic açaí

extract presented reduced NO levels when compared to the LPS positive control. In this sense, the HP controlling NO production could be a mechanism through which this extract performs its effect.

Finally, it was possible to observe that rotenone at 300 nM was able to induce increased total levels of ROS. These results were also expected since rotenone is a known chemical molecule that causes mitochondrial complex I dysfunction, followed by decreased cellular ATP synthesis and high levels of superoxide anion. This free radical usually is metabolized by the endogenous antioxidant pathway, conducting to increased levels of hydrogen peroxide (COSTA *et al*., 2012). This cellular condition probably is the cause of cellular mortality as well as NO production. Fortunately, when microglial cells were activated through rotenone exposure followed by HP ethyl acetate treatments it was possible to observe a recovery of ROS status for all tested concentrations. These results are in concordance with several articles already published that describe HP antioxidant activity (LIMA *et al*., 2023; LOCATELLI *et al*., 2017; SCHAFFER *et al*., 2013). Also, with these results, it is possible to suggest that HP ethyl acetate could present an anti-inflammatory effect through mitochondrial complex I functional recovery.

This present study is the first one showing the HP ethyl acetate fraction extract acting as antioxidant and anti-inflammatory through mitochondrial modulation. Nevertheless, this is an *in vitro* study, using basic and preliminary methodological tests, complementary research that evaluate the activity of the mitochondrial complexes, mainly complex I, which is inhibited by rotenone, are extremely important to confirm the mechanism of action of the HP extract. Also, in future research it is necessary to use *in vivo* experimental models to confirm our findings.

CONCLUSION

The roots of HP have been studied due to its anti-inflammatory and antioxidant effects. In this paper, it was shown for the first time the HP ethyl acetate fraction extract has anti-neuroinflammatory capacity through mitochondrial complex I function recovers in the BV-2 microglial cell line. HP was capable to normalize cellular proliferation and oxidative metabolism.

It is already known that several neuropsychiatry illnesses are related to chronic neuroinflammatory responses. In this sense, our results suggest that HP extract could be an important natural alternative to focus on drug development, especially considering its chemical matrix.

REFERENCES

ADAMS, S. D. *et al.* An investigation into closed-loop treatment of neurological disorders based on sensing mitochondrial dysfunction. **Journal of NeuroEngineering and Rehabilitation**, v. 15, n. 1, p. 1-18, 2018.

AHN, S.; COSTA, J.; EMANUEL, J. . PicoGreen quantitation of DNA: effective evaluation of samples pre- or post-PCR. **Nucleic Acids Research**, v. 24, n. 13, p. 2623-2625, 1 jul. 1996.

ANDREAZZA, A. C. *et al.* Specific subcellular changes in oxidative stress in prefrontal cortex from patients with bipolar disorder. **Journal of Neurochemistry**, v. 127, n. 4, p. 552-561, 2013.

CHOI, W. *et al.* The regulatory effect of veratric acid on NO production in LPS-stimulated. **Cellular Immunology**, v. 280, n. 2, p. 164-170, 2012.

CIMDINS, K. *et al.* Amyloid Precursor Protein Mediates Neuronal Protection from Rotenone Toxicity. **Molecular Neurobiology**, v. 56, n. 8, p. 5471-5482, 5 ago. 2019.

COSTA, F. *et al.* Influence of Val16Ala SOD2 polymorphism on the in-vitro effect of clomiphene citrate in oxidative metabolism. **Reproductive BioMedicine Online**, v. 24, n. 4, p. 474-481, abr. 2012.

DRAGOS, D. *et al.* Phytomedicine in joint disorders. **Nutrients**, v. 9, n. 1, p. 1-18, 2017.

FERRANTE, C. *et al.* Protective Effects Induced by Microwave-Assisted Aqueous Harpagophytum Extract on Rat Cortex Synaptosomes Challenged with Amyloid β-Peptide. **Phytotherapy Research**, v. 31, n. 8, p. 1257-1264, 2017.

FUKUI, M.; CHOI, H. J.; ZHU, B. T. Mechanism for the protective effect of resveratrol against oxidative stress-induced neuronal death. **Free Radical Biology and Medicine**, v. 49, n. 5, p. 800-813, 1 set. 2010.

JIANG, J. *et al.* Sutherlandia frutescens ethanol extracts inhibit oxidative stress and inflammatory responses in neurons and microglial cells. **PLoS ONE**, v. 9, n. 2, p. 1-9, 2014.

KIM, H. K. *et al.* Nod-like receptor pyrin containing 3 (NLRP3) in the post-mortem frontal cortex from patients with bipolar disorder: A potential mediator between mitochondria and immune-activation. **Journal of Psychiatric Research**, v. 72, p. 43-50, 2016.

KIM, Y. *et al.* Mitochondria, Metabolism, and Redox Mechanisms in Psychiatric Disorders. **Antioxidants & Redox Signaling**, v. 1, p. ars.2018.7606, 2019.

LAGHARI, A. H. *et al.* Determination of free phenolic acids and antioxidant activity of methanolic extracts obtained from fruits and leaves of Chenopodium album. **Food Chemistry**, v. 126, n. 4, p. 1850-1855, 2011.

LEE, H. J. *et al.* Neuroprotective effects of Scrophularia buergeriana extract against glutamate-induced toxicity in SH-SY5Y cells. **International Journal of Molecular Medicine**, v. 43, n. 5, p. 2144-2152, 2019.

LIMA, V. B. *et al.* Ethyl Acetate Fraction of Harpagophytum procumbens Prevents Oxidative Stress In Vitro and Amphetamine-Induced Alterations in Mice Behavior. **Neurochemical research**, v. 48, n. 6, p. 1716-1727, 1 jun. 2023.

LOCATELLI, M. *et al.* Optimization of Aqueous Extraction and Biological Activity of Harpagophytum procumbens Root on Ex Vivo Rat Colon Inflammatory Model. **Phytotherapy Research**, v. 31, n. 6, p. 937-944, 2017.

MACHADO, A. K. *et al.* Upstream pathways controlling mitochondrial function in major psychosis: A focus on bipolar disorder. **Canadian Journal of Psychiatry**, v. 61, n. 8, p. 446-456, 2016.

MACHADO, A. K. *et al.* Açaí (Euterpe oleracea Mart.) has anti-inflammatory potential through NLRP3-inflammasome modulation. **Journal of Functional Foods**, v. 56, n. November 2018, p. 364-371, 2019.

MNCWANGI, N. *et al.* Devil's Claw - A review of the ethnobotany, phytochemistry and biological activity of Harpagophytum procumbens. **Journal of Ethnopharmacology**, v. 143, n. 3, p. 755-771, 2012.

MONTEIRO, H. P.; SILVA, E. F.; STERN, A. Nitric oxide: A potential inducer of adhesion-related apoptosis - Anoikis. **Nitric Oxide - Biology and Chemistry**, v. 10, n. 1, p. 1-10, 2004.

MORELLA, I. M.; BRAMBILLA, R.; MORÈ, L. Emerging roles of brain metabolism in cognitive impairment and neuropsychiatric disorders. **Neuroscience & Biobehavioral Reviews**, v. 142, n. January, p. 104892, nov. 2022.

MUZILA, M. *et al.* Alteration of Neutrophil Reactive Oxygen Species Production by Extracts of Devil's Claw (Harpagophytum). **Oxidative Medicine and Cellular Longevity**, v. 2016, p. 1-9, 2016.

PAPPIS, L. *et al.* Randia ferox (Cham & Schltdl) DC.: phytochemical composition, in vitro cyto- and genotoxicity analyses, **Natural Product Research**, v. 36, p. 4170-4176, 4 ago. 2021.

SCHAFFER, L. F. *et al.* Harpagophytum procumbens prevents oxidative stress and loss of cell viability in vitro. **Neurochemical Research**, v. 38, n. 11, p. 2256-2267, 2013.

SCHAFFER, L. F. *et al.* Harpagophytum Procumbens Ethyl Acetate Fraction Reduces Fluphenazine--Induced Vacuous Chewing Movements and Oxidative Stress in Rat Brain. **Neurochemical Research**, v. 41, n. 5, p. 1170-1184, 2016.

SERRANO, A.; ROS, G.; NIETO, G. Bioactive Compounds and Extracts from Traditional Herbs and Their Potential Anti-Inflammatory Health Effects. **Medicines**, v. 5, n. 3, p. 76, 2018.

TRIGO, D. *et al.* Mitochondria, energy, and metabolism in neuronal health and disease. **FEBS Letters**, v. 596, n. 9, p. 1095-1110, 1 maio 2022.

TSCHOPP, J.; SCHRODER, K. NLRP3 inflammasome activation: The convergence of multiple signalling pathways on ROS production? **Nature Reviews Immunology**, v. 10, n. 3, p. 210-215, 2010.

WACHSMUTH, L. *et al.* Micro-morphometrical assessment of the effect of harpagophytum procumbens extract on articular cartilage in rabbits with experimental osteoarthritis using magnetic resonance imaging. **Phytotherapy Research**, v. 25, n. 8, p. 1133-1140, 2011.

World Health Organization (WHO). **Mental disorders.** (2018). [Access on 10 May 2019]; Available from: https://www.who.int/en/news-room/fact-sheets/detail/mental-disorders.