Selective effects of Euterpe oleracea (açai) on Leishmania (Leishmania) amazonensis and Leishmania infantum

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1. Introduction

Leishmaniasis belongs to a complex of neglected diseases with high incidences in developing countries, especially in Brazil, Mexico, Venezuela, Peru and Colombia [1]. In Brazil, Leishmania (Leishmania) amazonensis and Leishmania infantum (=Leishmania chagasi) are re-sponsible for causing American cutaneous leishmaniasis (ACL) and American visceral leishmaniasis (AVL), respectively [2].

In the Americas, AVL is mainly caused by L. infantum (=L. chagasi) and it is considered the most severe form of leishmaniasis, causing more than 70,000 deaths annually [3,4]. AVL has varied clinical manifestations, presenting symptomatic patients and asymptomatic and spontaneous cures in rare cases [5].

L. (L.) amazonensis causes localized cutaneous leishmaniasis (LCL) and anergic diffuse cutaneous leishmaniasis (ADCL). In the Brazilian Amazon, ADCL is a severe clinical form of leishmaniasis and it has no

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effective treatment due to the extreme state of anergy that the parasite causes to the immune system, such as inducing silencing of the macrophage microbiocide response, favoring the multiplication of amastigotes, and hampering the effective action of drugs [6,7]. Pentavalent antimony (SB) compounds are indicated as the first-line drugs for leishmaniasis treatment. In addition, second-line drugs such as amphotericin B (AMP-B) and pentamidine are indicated, owing to the occurrence of SB-resistant parasites [8]. These drugs reduce the parasite burden and cure most patients. However, many side effects have been reported, and the treatment is long and expensive [9,10]. These problems have a negative impact in the compliance, especially in isolated populations as Amazonian, drastically reducing the access to an adequate treatment. Due to the limitations of anti-leishmanial agents, there is increasing interest in new substances with leishmanicidal properties obtained from natural extracts that are easily accessible to that isolated communities [11,12].

Euterpe oleracea Martius, popularly known as acai, is a palm tree found in the Varzea area of the Amazon [13]. E. oleracea has been studied by the food, pharmaceutical and cosmetics industries because of its numerous biological functions and its increased consumption worldwide [14,15]. Commercial clarified acai juice (EO) is a product that stands out on the international market, mainly because of its durability and freedom from pathogenic microorganisms [16]. The acai fruit is of great importance to the local economy. The fruit is prepared as an energy drink and marketed worldwide [13].

E. oleracea already demonstrated pro-apoptotic activity on tumor cells [17], anti-inflammatory properties [18,19], antioxidant proper-ties, cardioprotective effects [18,20], anti-plasmodial activity [21] and anticonvulsant properties [22]. In addition, in the Oyapock basin (French Guiana), E. oleracea is administered topically (poultice) or orally (juice) for the treatment of cutaneous leishmaniasis [23].

Therefore, the action of EO on the protozoan Leishmania has not been demonstrated before. Therefore, in the present study, we demonstrate for the first time that EO juice has leishmanicidal activity against two species of Leishmania (L. amazonensis and L. infantum).

2. Materials and methods

2.1. Ethics statement

This study was approved by the Committee of Ethics of Animal Experiments of the Federal University of Pará (CEP/ICB/UFPA, number 7056260916). We followed the guidelines found in the NIH Guide for the Care and Use of Laboratory Animals, and the experiments were carried out in accordance with the Brazilian animal protection law (law 11794/08) and in compliance with the National Council for the Control of Animal Experimentation (CONCEA, Brazil).

2.2. Clarified acai (E. oleracea Mart.) juice (EO) preparation

E. oleracea Martius (Arecaceae) plants were collected in the state of Pará, Brazil and identified by comparison with a voucher specimen (#768513) deposited in the Herbarium of Instituto Nacional de Pesquisas da Amazônia (INPA, Brazil). Samples of EO were prepared and provided by Amazon Dreams (Belém, Pará, Brazil). The EO was prepared as described by Souza-Monteiro et al. [22]. The doses used in the present study were chosen based on previous studies from our group.

anthocyanins and 115.8 of phenolic compounds) were chosen based on previous studies from our group.

2.3. Parasites

Promastigotes of L. (L.) amazonensis (MHOM/BR/26361) and L. infantum (=L. chagasi) (MHOM/BR/27840) were obtained in NNN medium from the Leishmaniasis Program Evandro Chagas Institute and maintained in medium RPMI 1640 supplemented with 10% Fetal Bovine Serum (FBS) at 27 °C.

2.4. Culture of peritoneal macrophages

Peritoneal macrophages were obtained from male BALB/c mice (Mus musculus). The animals were sacrificed and the peritoneal mac-rophages were obtained from the peritoneal cavity. Macrophages were centrifuged at 1500 rpm for 10 min at 4 °C, and the pellet was re-suspended in DMEM medium. One million cells were plated per well, in 24-well culture plate (TPP), and incubated at 37 °C and 5% CO2. After 24 h of incubation, the tests with EO were conducted.

2.5. Anti-promastigote activity by the thiazolyl blue (MTT) method

Parasites in the exponential growth phase were cultured in 96-well culture plate (TPP) and treated with EO diluted in RPMI-1640 (no phenol red; 1:12.5, 1:25 and 1:50) for 24, 48 and 72 h. After treatment, 20 μL of MTT (stock solution of 2 mg/mL) was added to each well and incubated for 4 h. After incubation, 20 μL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals, and the plate was incubated under agitation for 30 min. Absorbance was then measured at 570 nm using a microplate reader (Bio-RAD Model 450 Microplate Reader). Promastigotes treated with AMP-B (0.5 μg/mL) were used as positive control.

2.6. Anti-amastigote assay

Peritoneal macrophages isolated from the peritoneal cavities of mice were incubated separately with promastigotes forms of L. (L.) amazonensis and L. infantum (=L. chagasi) in the stationary growth phase (seven days) at a host-parasite ratio of 1:10 for 3 h, in 24-well culture plate (TPP), at 37 °C and 5% CO2. The infected cells were treated with EO (1:12.5, 1:25 and 1:50) for 72 h. After treatment, the cells were fixed in 4% paraformaldehyde in PBS at pH 7.2 for 30 min. Cells were stained with Giemsa (Sigma®) diluted to 25% in phosphate buffer (PBS) pH 8.0 for 25 min at room temperature and mounted on glass slides. Two hundred cells were counted with an Axio Scope. A Zeiss microscope and the endocytic index was determined as de-scribed by Da Silva et al. [24]. The inhibitory concentration (IC50) of promastigote and amastigote forms was determined using SigmaPlot (version 12).

2.7. Viability assay for macrophages using Annexin V-FITC

Macrophages were obtained from the peritoneal cavity of mice and treated with different dilutions of EO for 72 h. After treatment, the macrophages were incubated for 30 min with a 1:10 dilution of Annexin V-FITC (Invitrogen Molecular Probes®, 10 μL of Annexin: 90 μL of ligation buffer). After 30 min, the cells were washed in PBS (pH 7.2) and analyzed using a fluorometer (VICTOR Multilabel Plate Reader X) at a wavelength of 490 nm. The cytotoxic concentration (CC50) was de-termined using SigmaPlot (version 12), and the selectivity index (SI) was determined using the ratio CC50/IC50.

2.8. Viability assay for macrophages by MTT method

Peritoneal macrophages were treated with EO (1:12.5, 1:25 and
1:50) for 72 h in 96-well culture plate (TPP). Subsequently, the cells were incubated with MTT for 3 h at 37 °C in 5% CO₂ atmosphere, as described by Da Silva et al. [25]. The samples were reading using a microplate reader (BIO-RAD Model 450 Microplate Reader) and the results were recorded at an optical density (OD) of 570 nm.

2.9. Morphological analysis of Leishmania promastigotes treated with EO by light microscopy (LM)

Promastigotes of L. (L.) amazonensis and L. infantum (=L. chagasi) treated with EO for 72 and 48 h, respectively, were fixed in 4% paraformaldehyde/ PBS pH 7.2 and adhered on cover slips treated with poly-l-lysine. Promastigotes were stained with a 25% Giemsa (Sigma®) in PBS pH 7.2 for 25 min at room temperature and mounted on glass slides. The cells were analyzed with an Axio Scope. A1 Zeiss micro-scope.

2.10. Analysis of cellular viability and cell volume of EO-treated promastigotes using Alamar blue dye

Cell volume was analyzed using the vital dye Alamar Blue (Invitrogen Molecular Probes®) after treatment of L. (L.) amazonensis and L. infantum (=L. chagasi) promastigotes with a 1:12.5 and 1:25 solution of EO for 72 and 48 h, respectively. Promastigotes non-treated and treated with EO were washed in PBS pH 7.2 and incubated with Alamar Blue for 5 h. To each tube containing Leishmania, we added 10% of marker equivalent to the total volume of the culture medium. The labeled cells were analyzed using a BD FACS Canto II flow cytometer with BD FACS Diva software, and a total of 10,000 events were acquired for each sample.

2.11. Detection of oxygen-reactive species (ROS) production in Leishmania promastigotes

After treatment with EO (1:12.5 and 1:50), promastigotes of L. (L.) amazonensis and L. infantum (=L. chagasi) were incubated with CellROX Green (Invitrogen Molecular Probes®) to detect ROS. Parasites were washed in PBS pH 7.2 and incubated with CellROX® diluted in RPMI 1640 (without phenol red) at a concentration of 7.5 μM for 45 min at 27 °C. The data were obtained using a fluorometer (VICTOR Multilabel Plate Reader X) at a wavelength of 490 nm. Promastigotes treated with AMP-B (0.5 μg/mL) were used as a positive control.

2.12. Apoptosis detection in Leishmania promastigotes treated with EO

Protozoa Leishmania were treated with EO (1:12.5, 1:25 and 1:50) for 48 h (L. infantum) and 72 h (L. amazonensis) in 24-well culture plate (TPP), washed with PBS pH 7.2 and incubated for 30 min with 10 μL of Annexin V-FITC (Invitrogen Molecular Probes®) diluted in 90 μL of Li-gation buffer (10 mM HEPES [pH 7.4], 150 mM NaCl, 5 mM KCl, 1 mM MgCl₂, and 1.8 mM CaCl₂). After incubation, the cells were washed in PBS pH 7.2, and data were obtained using a fluorometer (VICTOR Multilabel Plate Reader X) at a wavelength of 490 nm. Promastigotes treated with AMP-B (0.5 μg/mL) were used as a positive control.

2.13. Cytokine detection in non-infected and infected macrophages treated with EO

Peritoneal macrophages were separately infected with L. (L.) amazonensis and L. infantum (=L. chagasi) and treated with EO (1:12.5, 1:25 and 1:50) for 72 h in 24-well culture plate (TPP). Non-infected macrophages were also treated with EO for 72 h. As a positive control for non-infected macrophages, treatment with lipopolysaccharide (LPS-2 μg/mL) was performed for one hour. Infected macrophages treated with AMP-B (0.5 μg/mL) for 72 h were used as a positive control. After all treatments, supernatants were collected to perform dosage of the pro- and anti-inflammatory cytokines using a BD Cytoometric Bead Array Kit (CBA) for mouse Th1, Th2 and Th17. Data were obtained with an FACS Canto II flow cytometer using FACS Diva software (BD Biosciences), and the results were analyzed by FCAP Array 3.0 and expressed in pg/mL.

2.14. Statistical analyses

Data were analyzed in GraphPad Prism 6.0 using the analysis of variance (ANOVA) test followed by Tukey post hoc test. Results were considered significant with p < 0.05.

3. Results

3.1. EO has anti-leishmanial activity against promastigotes and amastigotes of Leishmania spp

EO had an anti-leishmanial effect against promastigotes of L. (L.) amazonensis (IC₅₀=1:40: presented 17.8 mg of anthocyanins and 135.4 mg of phenolic compounds) and L. infantum (=L. chagasi) (IC₅₀=1:38: presented 20.3 mg of anthocyanins and 154.5 mg of phenolic compounds). EO reduced in 39.68% (1:50), 61.04% (1:25) and 96.05% (1:12.5) the promastigotes of L. (L.) amazonensis treated for 72 h (Fig. 1A). In contrast, treatment with 0.5 μg/mL of AMP-B induced a reduction of 100% in promastigotes of L. (L.) amazonensis also after 72 h. EO also showed an anti proliferative effect against promastigotes of L. infantum (=L. chagasi) (Fig. 1B). After 72 h of treatment with EO, an important reduction of 59.12% (1:50), 78.25% (1:25) and 93.88% (1:12.5) in promastigotes of L. infantum (=L. chagasi) was observed.

The anti-leishmanial activity of EO against L. (L.) amazonensis amastigotes (IC₅₀=1:30: presented 25.1 mg of anthocyanins and 191.0 mg of phenolic compounds) was assessed by counting parasites using light microscopy. After 72 h of treatment with dilutions 1:2.5, 1:25, and 1:50 of EO, significant reductions in the number of amastigotes (68.7%, 55.9% and 52.6%, respectively) were observed (Fig. 1C). The amastigotes of L. infantum (=L. chagasi) were more sensitive to EO (IC₅₀=1:38: presented 20.3 mg of anthocyanins and 154.5 mg of phe-nolic compounds) than those of L. (L.) amazonensis. The survival index decreased by 82.9%, 72.1% and 66.2% of the parasite load after treatment with dilutions 1:12.5, 1:25 and 1:50 of EO, respectively (Fig. 1D).

EO antipromastigote activity was confirmed by labeling with the alamar blue dye. It was observed that EO at doses 1:12.5, 1:25 and 1:50 promoted a reduction in the viability of L. (L.) amazonensis and L. infantum promastigotes after treatment for 72 h and 48 h, respectively. The Alamar blue dye analysis confirmed the results observed by the MTT method (Additional file I in Supplementary material).

In addition, analysis of the viability showed no reduction of cell death in macrophages after treatment with different proportions of EO (1:3.4–1:50) compared to the untreated cells (Fig. 2A and B). Therefore, EO showed selective action against the Leishmania parasite, presenting a selectivity index of 134.11 and 166.20 against L. (L.) amazonensis and L. infantum (=L. chagasi), respectively.

3.2. Treatment with EO alters cell volume of Leishmania promastigote

Morphological analysis by light microscopy showed that promasti-gotes of L. (L.) amazonensis (treated for 72 h; Fig. 3B2 and B3) and L. infantum (=L. chagasi) (48 h of treatment; Fig. 3B2 and B3) treated with EO 1:12.5 and 1:25 showed a reduced cell body with morphologic characteristics of apoptosis.

Analysis by flow cytometry showed that promastigotes of L. (L.) amazonensis had a decreased cell volume after treatment with EO for 72 h as depicted by the presence of two histogram peaks; a red peak (left) that indicates promastigotes with a small cell volume and a black peak (right) that indicates elongated cells (Fig. 3D - EO 1:12.5 - and
Fig. 1. In vitro treatment of promastigotes and amastigotes of Leishmania (L. amazonensis and L. infantum) with EO for 72 h. Growth curves of L. (L.) amazonensis (A) and L. infantum (=L. chagasi) promastigotes (B) treated with different concentrations of EO. (C–D) Effect of EO treatment on L. (L.) amazonensis and L. infantum (=L. chagasi) amastigotes. Survival index of L. (L.) amazonensis and L. infantum (=L. chagasi) amastigotes treated with EO for 72 h. ANOVA followed by Tukey’s test were used to determine statistical significance. *p < 0.05, **p < 0.001, ***p < 0.001.

Fig. 2. Viability assay in peritoneal macrophages treated for 72 h with different concentrations of EO. No reduction in viability was observed in macrophages stained with Annexin (A) and through the MTT assay (B). ANOVA followed by Tukey’s test were used to determine statistical significance.

Fig. 3. (A) – (B) E-EO 1:25) After treatment with EO (1:12.5 and 1:25), the peak of L. (L.) amazonensis promastigotes moved to the left of the histogram (Fig. 3D and E), confirming the reduction in cell body size.

We observed an increase in the number of small cells after treatment of L. infantum (=L. chagasi) promastigotes for 48 h with EO (1:12.5), as indicated by the movement of the red peak to the left (Fig. 4D). However, cells treated with a dilution of 1:25 EO had two populations of cells: small and elongated promastigotes. This indicates that the dilution of 1:12.5 EO promoted a more pronounced reduction in cell size (Fig. 4E).

3.3. Treatment with EO increased ROS production and cell death in Leishmania promastigotes

We investigated the mechanism of action of EO on the promastigotes of L. (L.) amazonensis and L. infantum (=L. chagasi). Staining with
Fig. 3. Morphological analysis by light microscopy of L. (L.) amazonensis promastigotes treated with EO for 72 h. (A) Untreated control. (B) Cells treated with dilution of 1:12.5 EO. Note the reduction in cell size and parasite number (arrow). (C) Protozoan Leishmania treated with dilution of 1:25 EO. Note the reduction in the number of parasites. Scale bar: 10 μm. (D–E) Analysis of cell volume of L. (L.) amazonensis promastigotes stained with Alamar blue after a 72-hour incubation with EO (1:12.5 and 1:25). The red line represents promastigotes treated with dilution of 1:12.5 EO (D) and 1:25 EO (E). The black line represents the untreated control.

Fig. 4. The morphological effects of a 48-h treatment of EO on L. infantum (=L. chagasi) promastigotes. (A) Untreated control. (B) Promastigotes treated with dilution of 1:12.5 EO. Note the reduction in the number of parasites in culture, the reduction in cell size and the parasite with two flagella (arrow). (C) Cells treated with dilution of 1:25 EO. Scale bar: 10 μm. (D–E) Cells were labeled with Alamar blue and analyzed by flow cytometry. Promastigotes of L. infantum (=L. chagasi) were treated for 48 h with EO. The red line represents EO-treated promastigotes and the black line represents the untreated control.
**ROS production**

**L. (L.) amazonensis**

![Fluorescence intensity](image)

**L. infantum (=L. chagasi)**

![Fluorescence intensity](image)

**Apoptosis Detection**

**L. (L.) amazonensis**

![Fluorescence intensity](image)

**L. infantum (=L. chagasi)**

![Fluorescence intensity](image)

Fig. 5. In vitro effects of EO on Leishmania promastigotes. Detection of Reactive Oxygen Species (ROS) production in L. (L.) amazonensis (A1) and L. infantum (=L. chagasi) (A2) promastigotes treated with EO. Phosphatidylserine detection in promastigotes of L. (L.) amazonensis (B1) and L. infantum (=L. chagasi) (B2) treated with EO. Promastigotes of L. infantum (=L. chagasi) and L. (L.) amazonensis were treated with EO for 48 and 72 h, respectively. An ANOVA followed by Tukey's test were used to determine statistical significance. *p < 0.05, ***p < 0.001.

3.4. Analysis of cytokines produced by EO-treated macrophages (non-infected and infected with Leishmania)

Detection of the cytokine using a CBA kit showed that EO induced a significant increase of IL-10 in non-infected macrophages (Additional file II in Supplementary material). In addition, there was no increase in IL-17A, TNF-α, IFN-γ, IL-6 and IL-2 secretions (data not shown).

However, EO was able to promote a great reduction of IL-17A secretion in macrophages infected with L. (L.) amazonensis and L. infantum (=L. chagasi). IL-17A is an important cytokine during the pathogenic process of the two species (Fig. 6B and D). There was no significant difference in IL-10 secretion by macrophages infected with L. (L.) amazonensis and L. infantum (=L. chagasi) and treated with EO (Fig. 6A and C).

4. Discussion

The search for new drugs to treat leishmaniasis is important because the medications currently used are expensive and cause many side effects [8]. Natural extracts are alternatives for the development of new anti-leishmanial agents. These substances are found abundantly in nature, and studies show that they exhibit selective action against...
parasites, do not reduce host cell viability, and are more available for isolated populations than traditional pharmacotherapy [24,26–28]. Studies show that there are several natural extracts that have biological functions with clinical applications [29]. Açai, a fruit widely distributed in the Amazon region, produces one of these natural extracts that has diverse biological properties [18–20,22]. In addition, there is only one study that investigates the action of E. oleracea on pathogens that cause neglected diseases, showing antiplasmodial activity [21]. However, no studies have been conducted showing the action of EO on L. (L.) amazonensis and L. infantum (=L. chagasi).

We showed, for the first time, that EO reduces the number of L. (L.) amazonensis promastigotes (39.68–96.05%) and L. infantum (=L. chagasi) promastigotes (59.12–93.88%). Interestingly, only 48 h of treatment with EO was sufficient to reduce the number of L. infantum (=L. chagasi) promastigotes, and 72 h of EO treatment was sufficient to reduce the number of L. (L.) amazonensis promastigotes. In this study, we also observed that treatment with 1:12.5 and 1:25 of EO reduced the cell size of L. (L.) amazonensis promastigotes (after 72 h of treatment) and L. infantum (=L. chagasi) (after 48 h of treatment). Flow cytometry analysis showed a decrease in cell volume in EO-treated promastigotes. A 72-h treatment of L. (L.) amazonensis with 50- and 100-μg/mL aqueous extract of Physalis angulata root caused a reduction in cell body size; the authors associated morphological alterations with anti-proliferative activity, suggesting that reduction observed in the growth curve was due to the structural changes promoted by the aqueous ex-tract [24].

Alteration of the cell volume of Kinetoplastida protozoa is observed during apoptosis [30]. Apoptosis is programmed cell death (PCD) and is regulated by different biochemical and physiological processes. During PCD, mitochondrial damage and DNA fragmentation occur due to increased ROS production, PS externalization, chromatin condensing, reduction in cell size, formation of apoptotic bodies and display of plasma membrane blebs [30,31].

EO caused cell death phenotypes seems by apoptosis in promastigotes of L. (L.) amazonensis (treated for 72 h) and L. infantum (=L. chagasi) (treated for 48 h) that was associated with an increase in ROS production. EO treatment of the Leishmania protozoa reduced cell size and increased ROS production and PS externalization, which are characteristics of apoptosis in this parasite. EO has a large number of flavonoids that possess high antioxidant activity for the inhibition of ROS production [18,22]. However, we found that EO treatment is as-associated with increased ROS production in Leishmania promastigotes. EO may have selective effects on the organelles of the parasite, in-cluding the mitochondria, that promote damage, induce the release of ROS, and finally induce apoptosis.

Natural compounds are known to have biologically active sub-stance that selectively promote the death of Leishmania protozoa without damaging the host cell [32].
The mechanism of leishmanicidal action that some natural extracts exhibit is associated with the ability to induce activation of the mi-crobiodial response in macrophages and promote ROS and NO pro-duction, both of which lead to amastigote death [26,33]. However, EO seems to have an anti-inflammatory action, inducing IL-10 production while not increasing the levels of pro-inflammatory cytokines (IL-2, IL-6, TNF-α, and IFN-γ - data not shown). EO is able to inhibit ROS [20,22] and NO production [34]. The secretion of inflammatory mediators by macrophages, including ROS and NO, are important for the success in controlling Leishmania multiplication. Interestingly, EO promoted the death of the L. (L.) amazonensis and L. infantum (≈L. chagasi) amasti-gotes while in the presence of IL-10, an anti-inflammatory cytokine that does not induce the microbicide response in macrophages. However, EO modulates the secretion of IL-17, which is important for the pro-liferation of protozoa. The Leishmania parasitae modulates the host im-mune response favoring the maintenance of infection [35,36]. In vesc-eral and cutaneous leishmaniasis, IL-17 is essential for survival of the parasite [37,38]. IL-17 is a cytokine important in host defense, as it promotes the secretion of cytokines and chemokines maintaining homeostasis [39,40]. However, the action of IL-17 on macrophages infected with Leishmania is not favorable to the host because it increases the activity of arginase in macrophages, thus favoring growth of the parasite [38].

The phytochemical composition of EO is important to understand the mechanism of action of this substance. It is interesting to note that EO used in the present study consists of different flavonoids, such as cyanidin-3-rutinoside, cyanidin-3-glucoside, orientin, homoorientin, taxifolin deoxyhexose and anthocyanins [22]. In the literature, it is known that leishmanicidal activity of some plants is associated with the presence of different secondary metabolites in their composition, such as flavonoids [41,42]. The flavonoids present in EO may be responsible by leishmanicidal property observed.

These findings show that EO selectively promotes the death of L. (L.) amazonensis and L. infantum (≈L. chagasi) promastigotes and amastigotes without causing toxic effects to the host cell. Studies in-vivoing açai are of great importance because this fruit is widely consumed by natives of the Amazon region [13,43]. Studies in rodents showed that treatment with high doses of açaí pulp fruit caused no genotoxic effects [44]. In addition, clarified açaí juice is authorized for human consumption and in the present study showed that EO did not presented any significant effect on cell viability. Therefore, our results show evidence that EO has leishmanicidal activity free from cytotoxi-city in vitro, however, more in vivo studies and pharmacokinetics study are needed to develop a possible new drug against leishmaniasis.

5. Conclusions

Our results demonstrated that EO has leishmanicidal activity against promastigotes and amastigotes of L. infantum (≈L. chagasi) and L. (L.) amazonensis without cytotoxic effects for the host cell. To our knowledge, this report demonstrates, for the first time, that EO present action against two different species of Leishmania that cause American visceral and cutaneous leishmaniasis with great potential to become a new leishmanicidal agent.

Authors’ contribution

BJMS performed the experiments. BJMS, JRSM, MECL, HR, JLMN, EOS were involved in discussing the results and manuscript editing. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopharm.2017.11.089

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