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Original Article

β-Carboline-1-propionic acid alkaloid: antileishmanial and cytotoxic effects

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ABSTRACT

Pentavalent antimonials and amphotericin B remain as the main drugs to treat human leishmaniasis. However, the high toxicity and variable efficacy of treatment have stimulated the search for novel drug candidates. Naturally occurring alkaloids have a long history of antileishmanial activity. Here, we investigate the effects of the β-carboline-1-propionic acid alkaloid isolated from *Quassia amara* L., Simaroubaceae, against *Leishmania amazonensis* and *Leishmania infatum*. The alkaloid was isolated after liquid-liquid fractionation followed by chromatographic purification of the *Q. amara* methanol extract. The antileishmanial activity was evaluated by the microdilution method, using resazurin as the viability indicator. In addition, annexin and propidium iodide were used to detect parasites undergoing apoptosis. The anti-amastigote activity of the β-carboline-1-propionic acid alkaloid was determined by the infection of RAW 264.7 macrophages. The alkaloid displayed leishmanicidal activity against *Leishmania amazonensis* and *L. infantum* promastigotes and intracellular amastigotes with 50% inhibitory concentration ranging from 2.7 ± 0.82 to 9.4 ± 0.5 μg/ml and selectivity indexes >10. Moreover, apoptotic *Leishmania amazonensis* (19.5%) and *L. infantum* (40.4%) promastigotes were detected after 5 h incubation with the alkaloid. Finally, the β-carboline-1-propionic acid alkaloid inhibited the production of NO of infected macrophages, suggesting that the intracellular amastigote elimination occurs in a nitrosative stress-independent way. The results shown here suggest that the β-carboline-1-propionic acid alkaloid has potential as an antileishmanial agent.

Introduction

Despite the world efforts to combat leishmaniasis, which includes reservoir and vector control, treatment of infected individuals, and research on vaccine development and drug discovery, the disease continues to affect a large number of people (about 12 million) living in endemic tropical and subtropical areas (Oryan and Akbari, 2016). According to the WHO, 97 countries and territories are considered endemic for leishmaniasis. Among them, 25 were classified as high-burden countries: thirteen as high-burden countries of visceral leishmaniasis (VL) cases; eleven as high-burden countries of cutaneous leishmaniasis (CL) cases; and the last one, Brazil, which was the only country classified as a high-burden country for both VL and CL cases (WHO, 2016). Brazil has an estimated 3289 and 18,324 cases of VL and CL respectively and the population living in the endemic areas have a 32% and 59% risk of infection, respectively (WHO, 2017).

The treatment of leishmaniasis relies on the chemotherapy approach, since there are no vaccines available for humans (Srivastava et al., 2016). Since the 1940s, pentavalent antimonials (*N*-methylglucamine antimoniate and sodium stibogluconate)

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have been used to treat all clinical forms of the disease both in the New World (Central and South America) and in the Old World (Asia, Africa and Europe) (Denton et al., 2004). However, the use of these drugs is quite limited due to the high toxicity and the emergence of parasite resistant strains. Currently, the introduction of other drugs, such as amphotericin B (conventional and liposomal forms), pentamidine, paramomicin, miltefosine, or even a combination of all these drugs, have certainly improved the therapeutic arsenal to combat the disease. However, several factors, including variable efficacy, toxicity, side effects and high cost still limit the use of these drugs in the treatment of leishmaniasis (Uliana et al., 2017).

The β-carboline alkaloids (β-CA) are a group of biologically active tricyclic pyrido [3,4-b]indole ring structure molecules largely distributed in nature. The β-CA derived from plant species have been described as antitumoral (Zhang and Sun, 2015), antifungal and antibacterial (de Freitas et al., 2014; Nenaah, 2010) agents. In addition, the antiprotozoal potential of β-CA from natural origins has also been reported as having antiplasmodial (Ashok et al., 2013), antitrypanosomal (Costa et al., 2011) and antileishmanial activities (Rahimi-Moghaddam et al., 2011). Among the β-CA, β-carboline-1-propionic acid (**1**) has been found in several plant species belonging to the family Simaroubaceae, including *Eurycoma longifolia* Jack. (Kardono et al., 1991; Kuo et al., 2003), *Eurycoma harmandiana* Pierre (Kanchanapoom et al., 2001) and *Hannoa klaineana* Pierre et Engl (Lumonadio and Vanhaelen, 1985). However there are few reports concerning the biological activities of this alkaloid. Ohmoto et al. (1985) reported that the alkaloid was able to increase rabbit stomachic and intestinal blood flow rates in 10% and 35%, respectively. In the present study, we isolated the alkaloid β-carboline-1-propionic acid from the stem barks of *Quassia amara* L. (Simaroubaceae) and investigated its antileishmanial potential as well as its possible mechanisms of action.

Material and methods

Chemicals

The solvents used in extraction and purification procedures were of spectroscopic grade from Tedia Brazil (Rio de Janeiro, RJ, Brazil). Annexin V-FITC kit, Dulbecco's modified Eagle medium (DMEM), resazurin, and thiazolyl blue tetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO). Gibco® Fungizone® (amphotericin B, 250 μg/ml) was kindly donated by Fundação Oswaldo Cruz (Rio de Janeiro, RJ, Brazil). Fetal bovine serum (FBS) was purchased from LGC Biotecnologia (São José, Cotia, Brazil).

*Isolation of β-carboline-1-propionic acid (**1**)*

The acquisition, authentication and extraction procedures of stem barks of *Quassia amara* L., Simaroubaceae, were previously reported (Gabriel et al., 2016). The dichloromethane fraction (yield 16.6%) was subjected to a column packed with Sephadex LH-20 eluted with MeOH. The fractions obtained were united into ten major fractions on the basis of their TLC profiles after spraying Dragendorff reagent. The active fraction 8 was further chromatographed on a Sephadex LH 20 column using $CHCl_3$/MeOH (2:8) followed by MeOH. After TLC-silica gel analysis (CH_2Cl_2:MeOH, 3:7), fractions 39–42 from this column furnished an alkaloid identified by HRMS as β-carboline-1-propionic acid (4.6 mg, 0.0015% final yield). The results were also compared and in agreement with data from the literature (Chua et al., 2011).

UPLC-DAD-QTOF-HRMS analysis

Fraction 8 and β-carboline-1-propionic acid **(1)** analyses were performed using a Shimadzu UPLC-DAD-QTOF-HRMS using KINETEX C18 and KINETEX C18 PFP columns, respectively, both of 150 mm × 4.6 mm and with particles of 2.6 μm. The flows were 1.0 and 0.6 ml/min, respectively. The mobile phase was a gradient of solvent A (H_2O with 0.1% of formic acid) and B (acetonitrile) from 3 to 25% of B, 0–12 min and from 25 to 75% of B, 12–22 min. The chromatographic profile was recorded at 275 nm. The ionization source parameters (positive ESI) were as follows: nebulizer gas (nitrogen, 5.5 bar); dry gas (nitrogen, 12 l/min), 220 °C; capillary voltage, 4.5 kV; and QTOF/HRMS were used. The acetonitrile used was HPLC grade (Tedia) and water was purified with a Milli-Q system.

Parasite culture

Promastigote forms of *Leishmania (Leishmania) amazonensis* (IFLA/BR/1967/PH8) and *L. (L.) infantum* (MHOM/BR/1974/PP75) were provided by the Leishmania Type Culture Collection of Oswaldo Cruz Institute, Fiocruz/RJ/Brazil. Parasites were cultured as previously described (Rodrigues et al., 2010). In addition, parasite infectivity was assured by periodical infection of RAW 264.7 murine macrophages.

Leishmanicidal activity

The leishmanicidal activity of β-carboline-1-propionic acid **(1)** was determined using the microdilution technique as previously described (Garcia el al., 2017). The final concentrations tested ranged from 3.9 to 500 μg/ml. Fungizone® was used as the positive control (0.125–2.0 μg/ml). The microplates were incubated for 24, 48, 72 and 120 h at 26 °C in order to evaluate parasite growth and viability (Gabriel et al., 2016). The 50% inhibitory concentration (IC_{50}) was calculated based on the regression curves generated from the viability percentages. Finally, the minimum leishmanicidal concentration (MLC) of β-carboline-1-propionic acid was established after re-incubation of treated cultures in fresh medium.

Transmission electron microscopy (TEM)

Promastigote forms of *L. amazonensis* were treated with β-carboline-1-propionic acid **(1)** at concentrations of 15.6 (half minimum leishmanicidal concentration, MLC/2) and 6.6 μg/ml (IC_{50}) for 24 h, and then fixed for 1 h with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH, 7.2), plus 5 mM calcium chloride and 2% sucrose. The samples were processed as previously reported by Brasil et al. (2017). Ultra-thin sections obtained with a Leica (Nussloch, Germany) ultramicrotome were stained with uranyl acetate and lead citrate, and observed in a FEI Morgagni F 268 (Eindhoven, The Netherlands) transmission electron microscope operating at 80 kV.

Annexin V-FITC/PI staining

The apoptotic-like effect of β-carboline-1-propionic acid **(1)** was investigated according to the protocol described by Machado et al (2014). *L. amazonensis* and *L. infantum* promastigotes harvested at log phase (10^6 cells/ml) were treated with β-carboline-1-propionic acid at 31.2 and 15.6 μg/ml (MLC and MLC/2, respectively) for 5 h. The phosphatidylserine externalization was evaluated using the Annexin V-FITC apoptosis detection kit. The phosphatidylserine externalization was evaluated using the Annexin V-FITC apoptosis detection kit. Parasites were analyzed by flow cytometry

(FacsCalibur™), and analysis was performed using *Flowing Software 2.5.1*. The results were expressed as a percentage of positive cells for annexin (early apoptosis), propidium iodide (necrosis) and annexin + propidium iodide (late apoptosis), relatively to the number of cells analyzed.

Cytotoxic assay

For the cytotoxicity assay, RAW 264.7 murine macrophages (10^5 cells) previously cultivated in DMEM medium supplemented with 10% FBS were seeded in 96-well culture microplates for 6 h at 5% CO_2 atmosphere, allowing macrophage adherence. After the incubation period, the cells were treated with different concentrations of β-carboline-1-propionic acid **(1)** (3.9–500 μg/ml), and then incubated for an additional 48 h. Cell viability was assessed by the tetrazolium salt assay (MTT) (Mosmann, 1983). Cell viability was determined in an ELISA reader at 570 nm. The 50% cytotoxic concentration (CC_{50}) was calculated by analyzing the dose-response curve generated from the data. In addition, selectivity index (SI) was calculated by the ratio between the CC_{50} obtained for the host cell and the parasites IC_{50}.

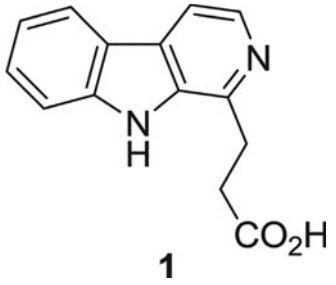
Host cell infection

RAW 264.7 macrophages previously infected (24 h) with promastigote forms of *L. amazonensis* or *L. infantum* (10 parasites/macrophage) at stationary phase (144 h in growth medium) were obtained according to protocol described by Garcia et al. (2017). The infected cultures were treated with different concentrations of β-carboline-1-propionic acid **(1)** (1.5–12 μg/ml) for 48 h. Fungizone® was used as a positive control (0.125–1 μg/ml). Subsequently, the culture supernatants were collected to evaluate NO production as previously described (Green et al., 1990). Finally, the cells were fixed with methanol, stained with Giemsa and the number of intracellular amastigotes per 100 macrophages was determined by direct counting in triplicate. The parasite load is expressed as a percentage of amastigotes compared with that for the control. The IC_{50} value for intracellular amastigotes was calculated based on the regression curves generated with the data.

Results

*Analysis of fraction 8 and β-carboline-1-propionic acid (**1**)*

The chromatographic study of the active fraction 8 by TLC after spraying Dragendorff presented orange spots indicative of alkaloids. This fraction, analyzed by UHPLC–HRMS, showed nine signals and the peak at 13.9 min was the major compound. The Sephadex LH 20 column was used to separate the fraction 8 compounds. All resulting fractions were analyzed by TLC with Dragendorff reagent detection. The fraction 39–42 obtained from this column was analyzed by LC-DAD-QTOF-HRMS and showed a major peak with an UV spectrum (275 nm) characteristic for β-carboline alkaloids (Chen et al., 2011) and a molecular ion at *m/z* 241.0978 $[M+H]^+$ ($C_{14}H_{13}N_2O_2$, calculated 241.0972). The β-carboline-1-propionic acid (Supplemental Fig. S1, SF1) was identified by these data and compared with literature (Chua et al., 2011).



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*Leishmanicidal activity of β-carboline-1-propionic acid (**1**)*

In order to evaluate the leishmanicidal activity of β-carboline-1-propionic acid, promastigote forms of *L. amazonensis* and *L. infantum* were incubated in the presence of the alkaloid. The MLC of β-carboline-1-propionic acid for both parasites was 31.2 μg/ml after 120 h of treatment. The MLC was considered the lowest concentration able to completely impair *Leishmania* growth after re-incubation of treated parasites in fresh medium. The IC_{50} values calculated for *L.* amazonensis and *L. infantum* treated with β-carboline-1-propionic acid were 6.6 and 2.7 μg/ml, respectively (Table 1). The reference drug Fungizone® displayed an IC_{50} value of 0.63 ± 0.03 and 0.8 ± 0.33 μg/ml against the parasites, respectively. Growth inhibition effects of β-carboline-1-propionic acid are demonstrated in Supplemental Fig. S2 (SF2). Moreover, even the lowest concentration tested (3.9 μg/ml) was able to reduce the promastigote numbers after 120 h of treatment.

Ultrastructure alterations

The morphological alterations of *L. amazonensis* promastigotes induced by β-carboline-1-propionic acid (**1**) at 6.6 (IC_{50}) and 15.6 (MLC/2) μg/ml were observed by TEM (Fig. 1). Some IC_{50} treated parasites presented swollen cell body (Fig. 1B) when compared to the untreated parasites (Fig. 1A), and myelin figures were often observed (Fig. 1C, inset). Parasites treated with 15.6 μg/ml (Fig. 1D–I) also displayed remarkable morphological alterations. Several vacuoles with large membranes were detected at the cell cytoplasm (Fig. 1D,E, G). Another common feature observed was the swollen kinetoplast DNA (kDNA) network (Fig. 1H,I); enlarged when compared to untreated cells. Furthermore, myelin figures were also observed (Fig. 1F).

β-carboline-1-propionic acid triggers apoptosis-like promastigote death

Promastigote death of β-carboline-1-propionic acid (**1**)-treated parasites was investigated by flow cytometry to unravel possible mechanisms. Parasites treated for 5 h were exposed to annexin V-FITC and/or PI in order to evaluate apoptotic and necrotic processes. Fig. 2A–C and 2E–G are representative data from two independent experiments for the treatment of *L. amazonensis* and *L. infantum* promastigotes, respectively. In both cases, there was an increase of parasites stained with annexin (early apoptosis - EA), or double-stained parasites with annexin and PI (late apoptosis - LA). When *L. amazonensis* promastigotes were treated for 5 h with 15.6 and 31.2 μg/ml of β-carboline-1-propionic acid, 10% and 15.5% of par-

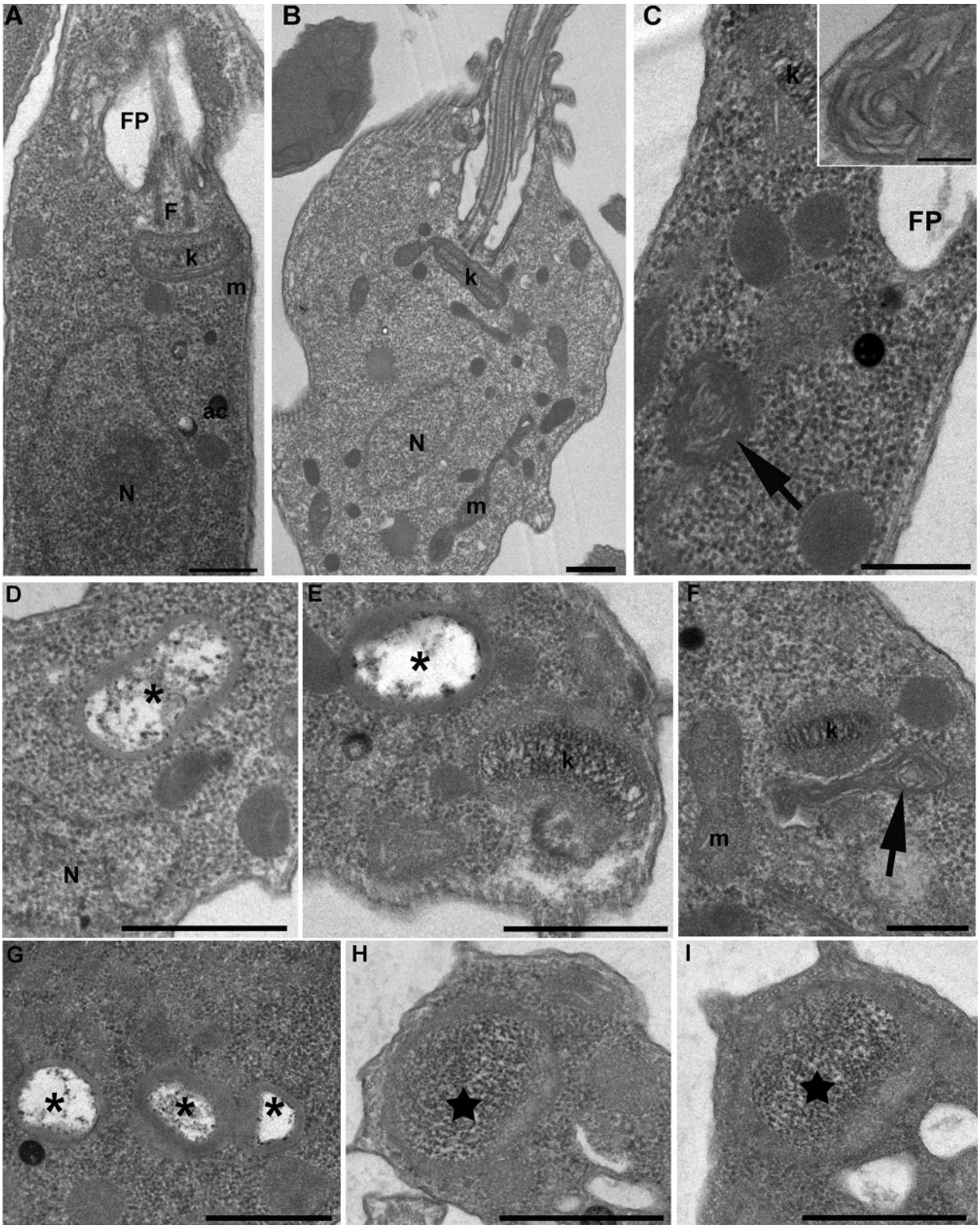

Fig. 1. Transmission electron microscopy of β-carboline-1-propionic acid (**1**)-treated promastigote forms of *Leishmania amazonensis*. A. Untreated parasite with typical elongated cell body, nucleus (N), mitochondria (m), kinetoplast (k), flagellum (F) flagellar pocket (FP) and acidocalcisome (ac). B and C. IC50 treated parasites presented swollen cell body (B) as compared to the untreated parasites (A). D–I. Treated parasites (MLC/2) displayed several vacuoles (asterisks), with large membranes that were detected at the cell cytoplasm (D, E and G); and, swollen and enlarged kDNA network (stars in H and I). Myelin figures were often observed after both treatments (arrows in C, inset, F). Bars: 0.5 μm.

Table 1
Half maximal growth inhibitory concentrations and selectivity index displayed by β-CPA after 120 h of treatment.

		β-CPA			Fungizone®		
Cells	Evolutive form	CC_{50} ±SE	IC_{50} ±SE	SI (CC_{50}/IC_{50})	CC_{50} ±SE	IC_{50} ±SE	SI (CC_{50}/IC_{50})
RAW 264.7	–	115 ± 6,8	–	–	18.4 ± 4.5	–	–
L. amazonensis	Promastigote		6.6 ± 2.75	17.4		0.63 ± 0.03	29.2
	Intracellular amastigote		8.1 ± 3.0	14.2		0.75 ± 0,03	24.5
L. infantum	Promastigote		2.7 ± 0.82	42.7		0.8 ± 0.33	23.0
	Intracellular amastigote		9.4 ± 0.5	12.2		1.14 ± 0,08	16.1

L. amazonensis, *Leishmania amazonensis*; *L. infantum*, *Leishmania infantum*; CC_{50}, 50% cytotoxic concentration; IC_{50}, 50% inhibitory concentration; β-CPA, β-carboline-1-propionic acid; SE, standard error; SI, selective index.

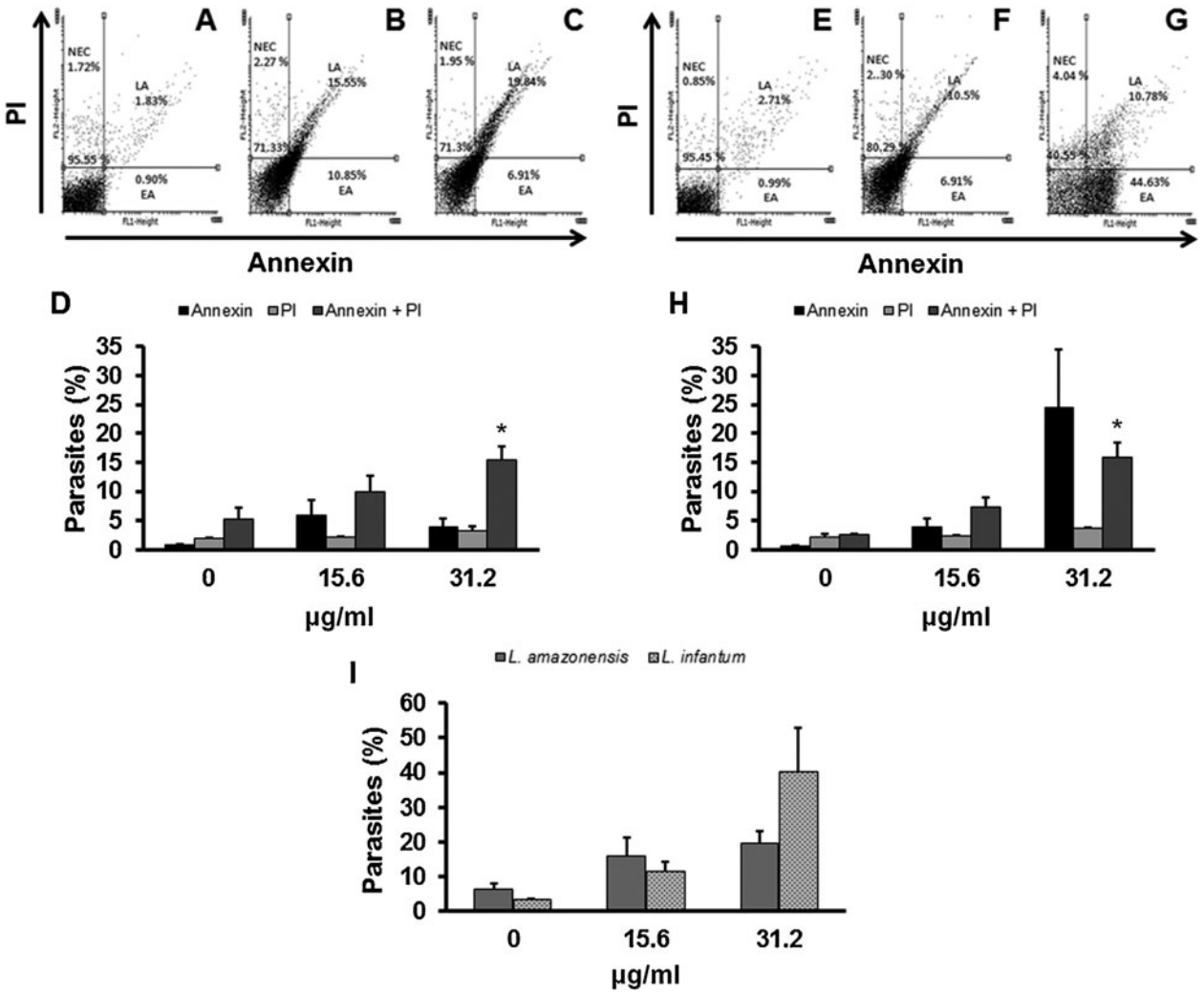

Fig. 2. Apoptosis-like cell death of *Leishmania* promastigote forms induced by β-carboline-1-propionic acid (**1**). A and E. representative flow cytometry dot-plot of untreated *Leishmania amazonensis* and *L. infantum*, respectively. B–C and F–G. representative flow cytometry dot-plot of treated *L. amazonensis* and *L. infantum* (15.6 and 31.2 µg/ml), respectively - lower left: healthy cells; lower right: early apoptotic cells; upper right: late apoptotic cells; and upper left: necrotic cells. D and H. quantitative representation of flow cytometry data as percentage early (Annexin+; PI-) and late (Annexin+; PI+) apoptotic and necrotic (Annexin-; PI+) parasites. I. quantitative representation of flow cytometry data as a percentage of the total apoptotic parasites (early apoptosis + late apoptosis). The results were expressed as mean ± standard error. Statistical analysis of the differences between mean values obtained for the experimental groups was done by one-way ANOVA with Tukey's post hoc test. The asterisk indicates significant difference compared with control group (p-value < 0.05).

asites presented double staining with annexin and PI, respectively (Fig. 2D). Promastigote forms of *L. infantum* exhibited a similar percentage of parasites displaying EA (24%) when they were exposed to 31.25 µg/ml for 5 h (Fig. 2H). However, the treatment with 15.62 µg/ml led to a smaller percentage of parasites showing LA (4%). Fig. 2I shows the total percentage of *L. amazonensis* (19.5%) and *L. infantum* (40.4%) promastigotes undergoing apoptosis (EA + LA).

Cytotoxicity for mammalian cells

β-carboline-1-propionic acid (**1**) displayed cytotoxic effect against mammalian cells with a CC_{50} value of 115 ± 6.8 µg/ml. The alkaloid selectivity index (SI) for the promastigotes of *L. amazonensis* and *L. infantum* was 17.4 and 42.7, and for the amastigote forms was 14.4 and 12.2, respectively (Table 1). These results were similar to those obtained for the reference drug Fungizone®.

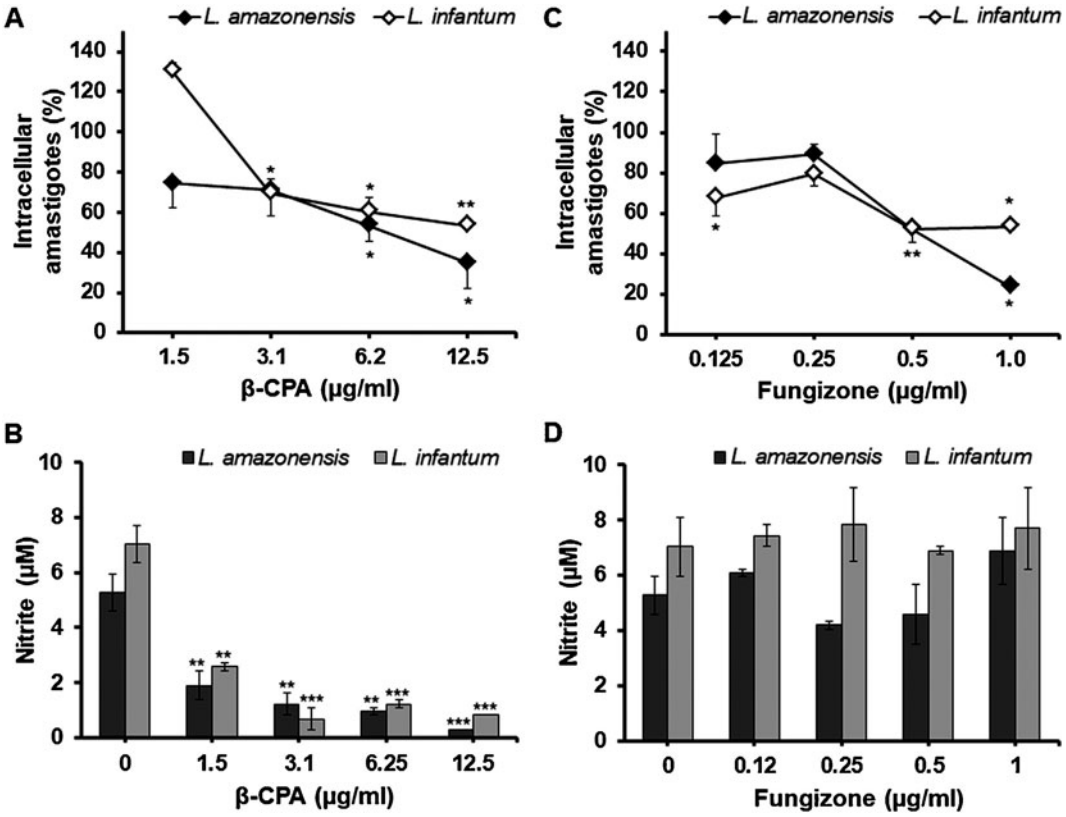

Fig. 3. Effect of β-carboline-1-propionic acid (**1**) on RAW 264.7 macrophage infection and NO production. Macrophages previously infected with *Leishmania amazonensis* or *Leishmania infantum* promastigotes were treated with β-carboline-1-propionic acid (A), or with the reference drug Fungizone® (C) for 48 h. The number of internalized amastigotes per 100 macrophages was determined by direct count under a light microscope. NO production was determined in the supernatant of *L. amazonensis* infected cultures (B) and *L. infantum* infected cultures (D) by Griess reaction. Two independent experiments were performed, and the results expressed as a percentage of amastigotes compared with that for the control ± standard error. Statistical analysis of the differences between mean values obtained for the experimental groups was done by ANOVA with Tukey's post hoc test. $^{*}p \leq 0.05$, $^{**}p \leq 0.005$, and $^{***}p \leq 0.0001$ were considered significantly different from those for the control.

Anti-intracellular amastigote activity

Infected RAW 264.7 macrophage cultures were treated with β-carboline-1-propionic acid (**1**) concentrations ranging from 1.5 to 12.5 μg/ml for 48 h. Fig. 3A shows that β-carboline-1-propionic acid was able to reduce the number of internalized amastigotes in both *L. amazonensis* and *L. infantum* infections. However, the treatments using 6.2 and 12.5 μg/ml were able to reduce *L. amazonesis* infection in 46.6% and 65.1% ($p < 0.05\%$), respectively, in relation to controls. The macrophages infected by *L. infantum* showed reductions of 39.7% and 46.5% in the number of intracellular amastigotes after treatment with the same concentrations, respectively. The IC_{50} values for intracellular *L. amazonensis* and *L. infantum* amastigotes were 8.1 ± 3.0 and 9.4 ± 0.5 μg/ml, respectively. In addition, β-carboline-1-propionic acid significantly inhibited the production of NO by infected macrophages in all systems tested (Fig. 3B).

Discussion

In a previous report we demonstrated that the dichloromethane fraction (Q2) from *Q. amara* presented antileishmanial activity (Gabriel et al., 2016). The initial analysis of that fraction revealed the presence of alkaloids as the main chemical class. In the present study, Q2 was fractionated further and the main alkaloid was isolated, and is described here as being responsible for the antileishmanial activity. In addition, we investigated the possible mechanisms of action of the alkaloid in the killing of *L. amazonensis* and *L. infantum* promastigotes and intracellular amastigotes.

So far, the biological activity of *Q. amara,* as well as other plants belonging to the family Simaroubaceae, is attributed mainly to their quassinoid content. Little attention has been given to other substances present in these plant-derived extracts, such as sterols, aliphatic and aromatic acids, and indole alkaloids. Nevertheless, β-CA isolated from natural sources or obtained synthetically are promising molecules with diverse pharmacological activities, including antileishmanial action (Mishra et al., 2009; Ashok et al., 2015). Ashok et al. (2016) reported the anti-*L. infantum* activity of sixteen novel series of tetrahydro-β-carboline derivatives. The authors described promising results comparable with the reference drug amphotericin B. Here, the phytochemical analysis performed on *Q. amara* fractions led to the isolation of β-carboline-1-propionic acid (**1**). The alkaloid strongly inhibited the growth of *L. amazonensis* and *L. infantum* promastigotes (Table 1). The biological activity of this alkaloid is poorly described in the literature. Kardono et al. (1991) demonstrated that the methoxylated form, 7-methoxy-beta-carboline-1-propionic acid, displayed significant antimalarial activity. The authors also tested β-carboline-1-propionic acid (**1**) but no antimalarial activity was detected. Thus, to our knowledge, the present study is the first report of β-carboline-1-propionic acid activity against protozoa parasites.

The psychoactive β-CA harmane, harmine, and harmaline displayed antileishmanial activity against promastigote and intracellular amastigote forms of *L. infantum*, with IC_{50} values of 19.2, 3.7, 116.8 μM and 0.27, 0.23, 1.16 μM, respectively. In addition, harmaline was described as an inhibitor of parasite protein kinase C (PKC), which could lead to a reduction in the number of intracel-

lular amastigotes (Di Giorgio et al., 2004). In a study conducted by Lala et al. (2004), harmine was isolated from *Peganum harmala* L. and tested against *L. donovani in vitro* and *in vivo*. The *in vitro* assay demonstrated an IC_{50} of 24 μg/ml when promastigotes were treated for 24 h. Interestingly, the authors demonstrated that the mode of action of harmine may be related to non-specific membrane damage suggestive of necrosis. In the present study, promastigote forms of *L. amazonensis* and *L. infantum* treated with β-carboline-1-propionic acid **(1)** (MLC and MLC/2) were subjected to the phosphatidylserine externalization assay. We observed that 20.9% of *L. amazonensis* promastigotes underwent apoptosis (EA + LA) after 5 h of exposure. Better results were observed for β-carboline-1-propionic acid-treated *L. infantum* promastigotes, which presented 40.3% of the total cellular population displaying apoptosis (Fig. 2).

β-carboline-1-propionic acid (**1**) was able to induce major ultrastructure damages on *L. amazonensis* promastigotes when parasites were treated at 6.6 and 15.6 μg/ml (IC_{50} and MLC/2, respectively). Interestingly, there were some evidences of apoptosis, such as cytoplasm vacuolization, the presence of myelin-like figures and swollen kDNA networks (Fig. 1). In a previous report, the indole alkaloid voacamine was able to induce intense cytoplasm disorganization and the presence of autophagic vacuoles, followed by alterations in the kinetoplast, mitochondrial membrane and cristae in *L. donovani* and *L. amazonensis* promastigotes (Chowdhury et al., 2017). In that study, ultrastructure damage was attributed to the interaction of the indole alkaloid with parasite topoisomerase IB, an enzyme involved in DNA metabolism. Indeed, other indole alkaloids have been described as *Leishmania* topoisomerase inhibitors including harmine (Cao et al., 2005) and anthocephaline (Kumar et al., 2015). In addition, there are strong evidences that several β-CA have important DNA intercalation capacity (Ashok et al., 2015; Cao et al., 2005).

Several studies have investigated the interaction between infected macrophages and new antileishmanial agents able to promote the reduction of intracellular amastigotes or their total elimination. Here, β-carboline-1-propionic acid (**1**) was tested against non-infected macrophages in order to establish its cytotoxicity prior to the anti-amastigote assays. The alkaloid was toxic for these cells with a CC_{50} value of 115 ± 6.8 μg/ml. Thus, in order to avoid its toxicity for the host cells, the interaction assays were performed with concentrations below the CC_{50}. There was a significant ($p < 0.05$) reduction in amastigotes of both *Leishmania* species when infected macrophages were treated with 6.2 or 12.5 μg/ml of β-carboline-1-propionic acid. *L. amazonensis* intracellular amastigotes were more sensitive to β-carboline-1-propionic acid when compared to the viscerotropic species *L. infantum* (Fig. 3A). Interestingly, the production of NO was inhibited by β-carboline-1-propionic acid treatment (Fig. 3B). This result was quite intriguing due to previous evidence that the alkaloid-rich fraction from *Q. amara* stimulates the production of NO by infected macrophages (Gabriel et al., 2016). We believe that the presence of other minor substances found in that fraction may be responsible for macrophage activation. Moreover, Di Giorgio et al. (2004) demonstrated that the β-CA harmaline had no effect on NO production despite a reduction of the parasite load observed in the infected macrophage cultures (IC_{50} = 1.16 μM). However, the β-CA 7-methoxy-(9*H*-β-carbolin-1-il)-(*E*)-1-propenoic acid, isolated for the first time from the *Eurycoma longifolia* Jack (Simaroubaceae), strongly inhibited the production of NO by the LPS-induced RAW 264.7 line. The authors observed that the alkaloid down-regulated the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) (Ngoc et al., 2016). More recently, Liu et al. (2019) tested a set of seventy five alkaloids, including natural β-CA and canthinone-type alkaloids from Simaroubaceae plants, against the RAW 264.7 line in order to evaluate their effects on NO production. Despite the potent inhibition activity (>80%) described for fourteen β-CA, β-carboline-1-propionic acid inhibited the production of NO in only 7.26%. The low efficacy of β-carboline-1-propionic acid on NO production may be related to the concentration used in the screening test (100 μM).

Conclusion

We reported the leishmanicidal activity of β-carboline-1-propionic acid (**1**) alkaloid isolated from *Q. amara*. The alkaloid led promastigote forms of *L. amazonensis* and *L. infantum* to death by apoptosis. The inhibition effect on the production of NO by infected macrophages observed here suggests that the alkaloid displays an NO-independent mechanism of action for the elimination of intracellular amastigote forms. Taken together, the results presented herein pave the way for further pharmacological evaluations of β-carboline-1-propionic acid alkaloid as a promising antileishmanial agent.

Authors'contributions

IAR and DSA designed the work. IAR wrote the manuscript. RSG and ARG performed the antileishmanial assays. ACFA, ICL and JDC performed the phytochemical assays and data analysis. RSG and HASS performed the apoptosis assay and data analysis. CMA performed the transmission electron microscopy. ABV and CSA critically revised the manuscript. All the authors have read the final manuscript and approved the submission.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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Appendix A. Supplementary data

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