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ORIGINAL PAPER

Antileishmanial activity of crude extract and coumarin from *Calophyllum brasiliense* leaves against *Leishmania amazonensis*

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Abstract Infections by protozoans of the genus *Leishmania* are a major worldwide health problem, with high endemicity in developing countries. The drugs of choice for the treatment of leishmaniasis are the pentavalent antimonials, which show renal and cardiac toxicity. As part of a search for new drugs against leishmaniasis, we evaluated the in vitro leishmanicidal activity of the (−) mammea A/BB. The compound (−) mammea A/BB is a coumarin-type mammea purified from a dichloromethane crude extract of leaves of *Calophyllum brasiliense* Cambess (Clusiaceae). The isolated compound was characterized using spectral analyses by UV, infrared, nuclear magnetic resonance of ^{1}H, ^{13}C, distortionless enhancement by polarization transfer, correlation spectroscopy, heteronuclear multiple bond correlation, and heteronuclear multiple quantum coherence. The compound (−) mammea A/BB showed significant activity against promastigote and amastigote forms of *L. amazonensis*, with IC_{50} (50% inhibition concentration of cell growth) at a concentration of 3.0 and 0.88 μg/ml and IC_{90} (90% inhibition concentration of cell growth) of 5.0 and 2.3 μg/ml, respectively. The coumarin (−) mammea A/BB showed no cytotoxicity against J774G8 macrophages in culture, when it was tested at high concentrations that inhibited promastigote forms. Electron microscopy studies revealed considerable ultrastructural changes when promastigote forms of *L. amazonensis* were treated with 3.0 μg/ml of the coumarin (−) mammea A/BB for 72 h. We observed significant changes such as mitochondrial swelling with concentric membranes in the mitochondrial matrix and intense exocytic activity in the region of the flagellar pocket. Other alterations included the appearance of binucleate cells and multiple cytoplasmic vacuolization. These results showed that (−) mammea A/BB is a potent growth inhibitor of *L. amazonensis* and caused important changes in the parasite's ultrastructure. This study provided new perspectives on the development of novel drugs with leishmanicidal activity obtained from natural products.

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Introduction

Leishmaniasis is a group of infectious diseases caused by protozoan parasites of the genus *Leishmania,* which cause visceral, cutaneous, and mucosal diseases in humans. Leishmaniasis is a major and increasing public health problem, particularly in Africa, Asia, and Latin America, and is a significant cause of morbidity and mortality in

tropical and subtropical countries. More than 350 million people live in areas of active transmission of *Leishmania*. More than 12 million are infected with different species of the parasite, and more than 400,000 new cases occur annually (Grevelink and Lerner 1996; World Health Organization 2003a, b). The protozoans are transmitted to the vertebrate host by the bite of a sandfly. The promastigote in the stationary growth phase is the infective form of the parasite; it penetrates a mononuclear phagocyte, differentiates into an amastigote, and proliferates intracellularly (Grevelink and Lerner 1996). More than 50 years ago, the treatment of choice for leishmaniasis consisted of the administration of pentavalent antimonials, which cause serious side effects, principally cardiac and renal, and require long-term treatment (Croft et al. 2005). Recently, large-scale clinical resistance against the most commonly used antileishmanial drug, antimonial agents, has been reported (World Health Organization 2003a, b; Croft and Coombs 2003). The alternatives to antimonials in unresponsive cases have been amphotericin B and pentamidine; however, these cause serious toxic effects (Croft and Coombs 2003). Furthermore, antifungal agents such as imidazole and triazole derivatives inhibit ergosterol biosynthesis, and they are effective against only some species of *Leishmania* (Vannier-Santos et al. 1995; Berman 1997). A major emerging problem is the coinfection of *Leishmania* with the human immunodeficiency virus, especially because there is no effective treatment for these patients and it involves visceral leishmaniasis, one of the most severe forms of the disease (Berman 1997). In recent years, there has been growing interest in alternative therapies and the use of natural products, especially those derived from plants as sources of new chemotherapeutic compounds with better activity and fewer side effects. Many people who live in endemic areas use traditional medicine for treatment. Traditional therapy consists of oral administration of plant extracts for the systemic forms of the disease and of topical preparations for the cutaneous forms of infection (Rates 2001). In view of the present unsatisfactory situation, the study of new molecules obtained from medicinal plants for leishmaniasis treatment is highly desirable.

The genus *Calophyllum* (Clusiaceae) is composed of 180–200 tree species, most of them native to the Indo-Pacific (Stevens 1980). Members of this genus are rich and valuable sources of bioactive xanthones and coumarins (Sartori et al. 1999; Morel et al. 2002). Recently, *Calophyllum* species have received considerable attention from a pharmacological point of view because some of them produce potent inhibitors of reverse transcriptase of human immunodeficiency virus type 1 (Kashman et al. 1992; Patil et al. 1993; McKee et al. 1996, Dharmaratne et al. 2001). In addition, the methanol extract from the heartwood of *C. kunstleri* shows potent leishmanicidal activity (Takahashi et al. 2004). *C. brasiliense* Camb. (Clusiaceae) is a large tree, widely distributed in the Americas, principally in hilly forested regions of Brazil, where it is known as 'Guanandi.' This plant has been used in folk medicine for the treatment of rheumatism, varicoses, hemorrhoids, and chronic ulcers (Corrêa 1978). *C. brasiliense* has proved to be a rich source of bioactive substances, including coumarins (Ito et al. 2003; Reyes-Chilpa et al. 2004), xanthones (Sartori et al. 1999; Ito et al. 2002), triterpenoids (Reyes-Chilpa et al. 2004), and biflavonoids (Da Silva et al. 2001). Several coumarin-type mammea and xanthones from different parts of *C. brasiliense* have shown chemoprotective properties against cancer (Ito et al. 2002, 2003; Reyes-Chilpa et al. 2004). They inhibit the growth of *Staphylococcus aureus*, *S. epidermides*, *Escherichia coli*, and *Bacillus subtilis* (Reyes-Chilpa et al. 2004; Yasunaka et al. 2005). Xanthones from the heartwood of *C. brasiliense* have exhibited trypanocidal activity against the epimastigote form of *Trypanosoma cruzi* (Abe et al. 2004).

The aim of the present study was to assess the potential antileishmanial activity of *C. brasiliense*. A compound obtained from this species was purified by bioassay-guided chemical fractionation and identified. Its antiproliferative effects on the amastigote and promastigote forms of *Leishmania amazonensis* and the ultrastructural changes that it produced in promastigote forms were evaluated. In addition, the cytotoxicity of the compound to macrophages was tested.

Materials and methods

Plant material

C. brasiliense was collected on Cardoso Island, July 2000, in the state of São Paulo, Brazil. It was collected and identified by Prof. Dra. Maria Claudia M. Young. A voucher specimen (SP 363818) is deposited and authenticated at the Herbarium of the Instituto de Botânica de São Paulo, São Paulo, Brazil.

Plant extraction and purification

Leaves were dried at room temperature and powdered (985 g). The extract was prepared by exhaustive maceration in ethanol/water (9:1) at room temperature, filtered, and concentrated under vacuum at 40°C to obtain an aqueous extract and a dark green residue. The residue from the crude extract, stored in glass bottles, was dissolved with dichloromethane, and the organic solvent was completely removed at room temperature, yielding the dichoromethane extract (30.9 g). This extract was assayed for its activity against *L.*

amazonensis as described below. Subsequently, the dichloromethane extract was chromatographed in a vacuum silica-gel column with hexane, hexane/dichloromethane (1:1), dichloromethane, dichloromethane/ethyl acetate (9:1), ethyl acetate, methanol, and methanol/water (9:1). Next, the hexane fraction (5.0 g) was rechromatographed on a silica-gel column chromatograph using hexane, hexane/dichloromethane (98:2, 95:5, 90:10, 80:20, and 50:50), dichloromethane, dichloromethane/ethyl acetate (98:2, 95:5, 90:10, 80:20, and 50:50), ethyl acetate, and methanol. The hexane fraction was evaporated to a residue, which was crystallized by dichloromethane (9:1), yielding a coumarin (65 mg).

Structure elucidation

The structure of the isolated compound was identified by chromatography–mass spectrometry (Micromass Quattro LC); nuclear magnetic resonance (NMR; Gemini 2000 BB; Varian), ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz), distortionless enhancement by polarization transfer, correlation spectroscopy (400 MHz), heteronuclear multiple bond correlation and heteronuclear multiple quantum coherence analysis in $CDCl_3$; infrared analysis (Bomem-MV 100; Hartmann & Braun-Michelson); UV analysis (CARY 1E UV-Vis; Varian); and by comparison with literature data (Crombie et al. 1987; Gasparotto-Júnior et al. 2005).

Parasites

We used promastigote forms of *L. amazonensis* (MHOM/BR/75/Josefa) originally isolated from a human case of diffuse cutaneous leishmaniasis. This strain has been maintained by weekly transfers in Warren's medium (brain–heart infusion plus hemin and folic acid) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco Invitrogen, New York) at 25°C in a tissue flask.

Axenic amastigote cultures

Axenic amastigote forms of *L. amazonensis* were obtained by in vitro transformation of infective promastigotes (Ueda-Nakamura et al. 2001) and maintained by weekly transfers in Schneider's insect medium (Sigma Chemical, St. Louis, MO) at pH 4.6, supplemented with 20% heat-inactivated FBS at 32°C in a tissue flask.

Cells

J774G8 murine macrophages were maintained in tissue flasks in RPMI 1640 medium (Gibco Invitrogen) with sodium bicarbonate and l-glutamine and supplemented with 10% FBS at 37°C in a 5% CO_2–air mixture.

Antileishmanial activity

Promastigote forms of *L. amazonensis* in log phase of growth (10^6 parasites/ml) were grown on a 24-well plate in Warren's medium supplemented with 10% heat-inactivated FBS in the absence or in the presence of different concentrations of the dichloromethane extract, hexane fraction (160 to 10 μg/ml), or isolated compound (40 to 0.625 μg/ml) at 25°C, to evaluate parasite survival. Axenic amastigote forms of *L. amazonensis* in log phase of growth (10^6 parasites/ml) were grown on a 24-well plate in Schneider's *Drosophila* medium supplemented with 20% heat-inactivated FBS in the absence or in the presence of different concentrations of the dichloromethane extract, hexane fraction (80 to 1 μg/ml), or isolated compound (40 to 0.625 μg/ml) at 32°C, to evaluate parasite survival. In all tests, 0.5% dimethyl sulfoxide (DMSO; Sigma Chemical), a concentration that was used to dissolve the highest dose of the samples but that had no effect on cell proliferation, and the medium alone were used as controls. Amphotericin B was used as the reference drug, and it was assayed at concentrations of 0.924 to 0.023 μg/ml for promastigote and amastigote forms. The percentage of inhibition was determined daily after promastigote and amastigote forms were counted in a hemocytometer (Improved Double Neubauer). Each experiment was performed in triplicate on three different occasions, and the results were expressed as percentage of inhibition in relation to the control cultured in medium alone. The 50% inhibitory concentration (IC_{50}) and the 90% inhibitory concentration (IC_{90}) were determined by logarithm regression analysis of the data obtained.

Cytotoxicity assay

A suspension of 5×10^4 J774G8 macrophage cells in RPMI 1640 medium supplemented with 10% FBS was added to each well in 96-well microplates. The plates were incubated in a 5% CO_2–air mixture at 37°C to obtain confluent growth of the cells. After 24 h, the medium was removed, and several concentrations of purified compound (1,000 to 0.5 μg/ml) were added to each well containing the cells, and the plates were incubated for 48 h. The nonadherent cells were removed by washing with the RPMI 1640 medium, and the adhered macrophages were fixed with 50 μl/well of 10% trichloroacetic acid at 4°C for 1 h; after that, the well plates were washed with water, and 50 μl/well of sulforhodamine B (0.4% w/v) in 1% acetic acid solution was added; the microplate was then maintained at 4°C for 30 min. Next, the sulforhodamine B was removed, and the microplate was washed five times with 1% acetic acid, then 150 μl/well of 10 mM unbuffered Tris-base solution (Sigma) was added, and it was homogenized for 15 min.

Next, the absorbance of each individual well, minus the blank value, was calculated automatically. Each experiment was performed in triplicate on three different occasions, and the percentage of viable cells was calculated in relation to controls cultured in the medium alone. The 50% cytotoxicity concentration (CC_{50}) was determined by logarithm regression analysis of the data obtained.

Ultrastructural analysis

Promastigote forms of *L. amazonensis* treated with the isolated compound (IC_{50} and IC_{90}), 0.5% DMSO, or medium alone were collected by centrifugation after 72 h incubation, washed in 0.01 M phosphate-buffered saline at pH 7.2, and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at 4°C. The cells were postfixed in a solution containing 1% osmium tetroxide and 0.8% potassium ferrocyanide in 0.1 M cacodylate buffer, washed in the same buffer, dehydrated in acetone, and embedded in Epon® resin. Ultrathin sections obtained in a Reichert Ultracut E ultramicrotome were stained with uranyl acetate and lead citrate and examined in a Zeiss 900 transmission electron microscope.

Statistical analysis

All experiments were performed in triplicate. The means and standard deviations of at least three experiments were determined. The differences between mean values obtained for experimental groups were analyzed by means of the Student's *t* test. *P* values of 0.05 or less were regarded as significant.

Results

Structure elucidation

The dichoromethane extract of *C. brasiliense* leaves yielded the coumarin (−) mammea A/BB. The spectral data were in good agreement with the literature values (Gasparotto-Júnior et al. 2005).

Antileishmanial activity

The effects of the dichloromethane extract obtained from *C. brasiliense* leaves on the growth of *L. amazonensis* were tested. This extract inhibited growth of the promastigote and amastigote forms, with IC_{50} of 40.0 and 3.7 μg/ml, and it showed IC_{90} of 73.0 and 20.0 μg/ml (Fig. 1a and b), respectively, after 72 h of incubation. The effects of the hexane fraction of the dichloromethane extract from *C. brasiliense* were tested against both forms of the parasite. The IC_{50} of the hexane fraction was 17.0 μg/ml for promastigote forms and 3.2 μg/ml for amastigote forms, and the IC_{90} was 35.0 μg/ml for promastigotes and 17.0 μg/ml for amastigotes (Fig. 1a and b), after 72 h of incubation. Fractionation of the hexane fraction led to purification of the coumarin (−) mammea A/BB, which showed IC_{50} of 3.0 μg/ml against promastigote and 0.88 μg/ml against amastigote forms and IC_{90} of 5.0 and 2.3 μg/ml (Fig. 1a and b), respectively, after 72 h of treatment. When cultures of promastigote and amastigote forms of *L. amazonensis* were treated with the IC_{90} of the dichloromethane extract, hexane fraction, and (−) mammea

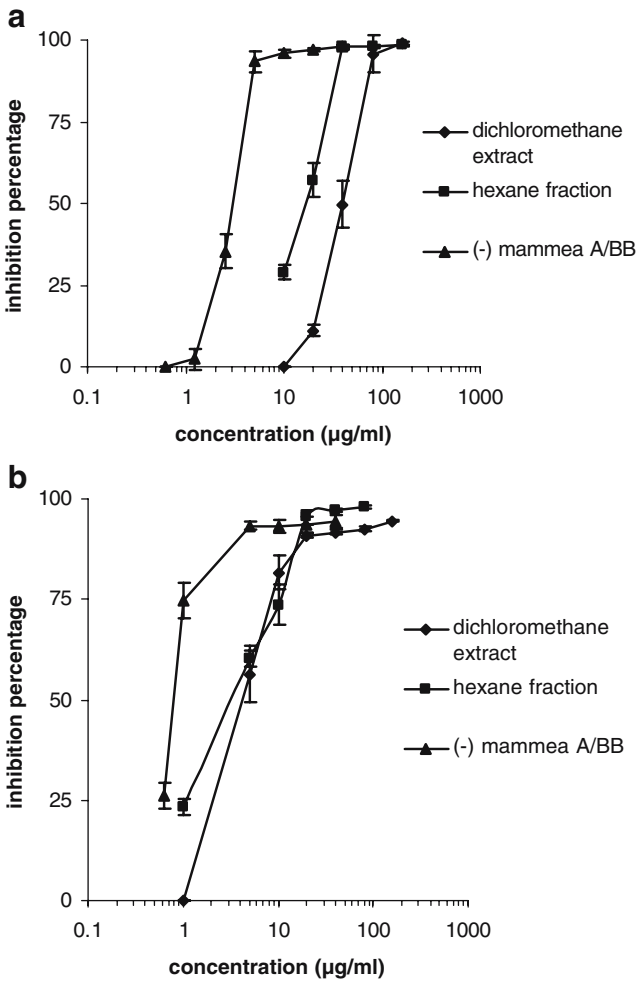

Fig. 1 **a**, **b** Effects of dichloromethane extract, hexane fraction, and (−) mammea A/BB on the growth of *L. amazonensis* promastigote forms (**a**) and amastigote forms (**b**). The promastigote forms were incubated in Warren's medium supplemented with 10% heat-inactivated FBS at 28°C for 72 h. The amastigote forms were incubated in Schneider's medium supplemented with 20% heat-inactivated FBS at 32°C for 72 h. The samples were added to the cultures at 0 h, and the cells were counted daily. The *bars* indicate standard deviations. All results were significant at $P \leq 0.05$ (compared to the control group, Student's *t* test)

A/BB, they showed rapid and extensive cell lysis after 24 h of incubation. The dilution agent (0.5% DMSO) had no effect on the parasite proliferation and cellular morphology. In addition, Amphotericin B showed IC_{50} of 0.058 and 0.231 μg/ml against promastigote and amastigote forms, respectively, after 72 h of treatment.

Cytotoxicity assay

J774G8 murine macrophages were treated with increasing concentrations of the pure isolated compound, to evaluate the safety of this compound for mammalian cells. After 48 h of treatment, cell viability was checked by a sulforhodamine B colorimetric assay. When macrophages were treated with (−) mammea A/BB, CC_{50} was 25.8 μg/ml. The pure compound cytotoxicity for J774G8 macrophages and its activity against the protozoans were compared using the selectivity index (SI) ratio (CC_{50} for J774G8 macrophages/IC_{50} for protozoans). When this value is greater than 1, the compound is more selective for activity against parasites than macrophages; when the value is less than 1, the compound is more selective for activity against macrophages. The (−) mammea A/BB was more selective against parasites than mammalian cells: It showed a SI ratio of 8.6 for promastigote and 29.3 for amastigote forms.

Transmission electron microscopy

Transmission electron microscopy analyses of treated and untreated *L. amazonensis* promastigote forms were performed to determine ultrastructural changes caused by the pure compound at 3.0 (IC_{50}; Fig. 2b–e) and 5.0 μg/ml (IC_{90}; Fig. 2f). The photomicrographs of the promastigote forms shown in Fig. 2 illustrate parasites with different degrees of damage after 72 h of incubation. The promastigote forms treated with 3.0 μg/ml of coumarin (−) mammea A/BB showed significant morphological alterations: the appearance of binucleate cells and multiple cytoplasmic vacuolization (Fig. 2b) and considerable mitochondrial swelling (Fig. 2b–d) with the concentric membranes in the mitochondrial matrix (Fig. 2b and d), indicating damage and significant change in this organelle. There was also intense exocytic activity in the region of the flagellar pocket, which appeared in the form of concentric membranes within the pocket (Fig. 2e). The promastigotes treated with 5.0 μg/ml of (−) mammea A/BB showed binucleate cells, and other cell organelles were destroyed. No ultrastructural changes were observed in untreated promastigote forms or in cells cultured with 0.5% DMSO, which showed a normal mitochondrial profile containing the kinetoplast; the nucleus and flagellum showed normal morphology (Fig. 2a).

Discussion

The leishmanias are responsible for considerable morbidity and mortality in many countries, principally in the tropics and subtropics. Because the drugs currently available for treatment of leishmaniasis are unsatisfactory because of their limited efficacy, frequent side effects, and increasing drug resistance, new, safer, and more efficacious drugs are urgently required (World Health Organization 2003a, b; Croft et al. 2005). In this regard, medicinal plants offer enormous prospects for discovering new compounds with therapeutic properties.

In the present study, we report for the first time a novel pharmacological activity of the dichloromethane extract from the leaves of *C. brasiliense*, which showed important activity against *L. amazonensis* in vitro. The dichloromethane extract from this plant inhibited protozoan growth with an IC_{50} of 40.0 and 3.7 μg/ml for promastigote and amastigote forms, respectively. These results led us to fraction this extract. The hexane fraction showed a greater inhibitory effect than did the dichloromethane extract, with an IC_{50} of 17.0 μg/ml for promastigote and 3.2 μg/ml for amastigote forms. Fractionation of the hexane fraction led to purification of the coumarin-type mammea, which was identified by chemical analysis as (−) mammea A/BB. In a previous study, this coumarin was isolated for the first time from *C. brasiliense* leaves, and it showed activity against *Biomphalaria glabrata* snails (Gasparotto-Júnior et al. 2005). The coumarin (−) mammea A/BB displayed better antileishmanial effect than the hexane fraction and the dichloromethane extract, with an IC_{50} of 3.0 μg/ml for promastigote and 0.88 μg/ml for amastigote forms. Previous studies have shown that mammea-type coumarins have several important pharmacological properties, such as anticancer, insecticidal, and molluscicidal (Crombie et al. 1987; Ravelonjato et al. 1992; Guilet et al. 2001).

In preceding studies, species of the genus *Calophyllum* displayed activity against trypanosomatids. The methanol extract from *C. kunstleri* heartwood showed potent leishmanicidal activity against *Leishmania major* (Takahashi et al. 2004). Three xanthones, denominated jacareubin, 6-deoxyjacareubin, and 1,3,5,6-tetrahydroxy-2-(3-methyl-2-butenyl) xanthone, isolated from heartwood of *C. brasiliense*, exhibited activity against epimastigote forms of *T. cruzi* (Abe et al. 2004).

Cytotoxicity assays showed that the action of the isolated compound is more specific for protozoans, and it is not toxic to macrophages. Cytotoxicity tests with natural products are important because of the interest in alternative therapies and the therapeutic use of medicinal plants. Development of drugs of plant origin is of interest because the conventional treatments for many diseases can be

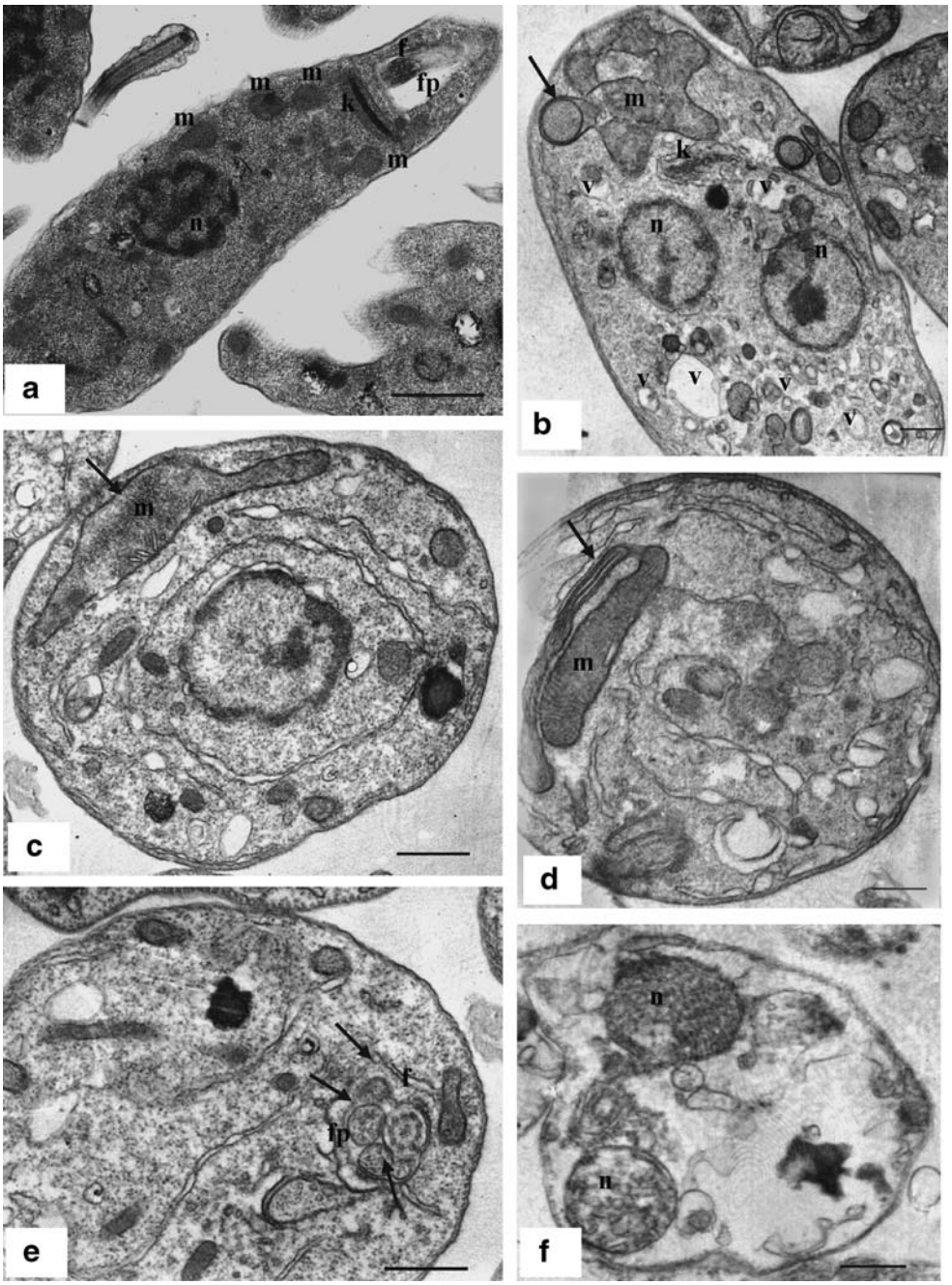

Fig. 2 **a**–**f** Transmission electron microscopy of *L. amazonensis*: promastigote forms cultured in medium alone or with 0.5% DMSO (**a**), treated with coumarin (−) mammea A/BB at 3.0 (IC_{50}; **b**, **c**, **d**, and **e**) and 5.0 μg/ml (IC_{90}; **f**). **a** Parasite showing normal morphology; **b**, **c**, **d** parasites showing some alterations in the mitochondrion, such as intense swelling(**b** and **d**) and the presence of several concentric membranes (*arrows*); **e** parasites showing intense exocytic activity. The *arrows* indicate the vesicles located in the flagellar pocket. **b** The promastigotes also showed multiple cytoplasmic vacuolization and two nuclei. **f** Note promastigote form with two nuclei and other cell organelles destroyed; *n* nucleus, *f* flagellum, *fp* flagellar pocket, *k* kinetoplast; *m* mitochondrion, *v* vacuole. *Bars*, 1 μm

inefficient or ineffective or can result in side effects. Many people worldwide have no access to conventional pharmacological treatments but depend on folk remedies. The widespread use of folk medicines suggests that natural products are harmless, but traditional use is no proof of their safety (Edzard 1998). In this context, both the efficacy and safety of natural products require investigation.

Significant mitochondrial swelling with concentric membranes in the mitochondrial matrix and intense exocytic activity in the region of the flagellar pocket were observed at the ultrastructural level when promastigote forms of *L. amazonensis* were treated with 3.0 μg/ml of coumarin (−) mammea A/BB for 72 h. Similar mitochondrial swelling has been reported for *L. amazonensis* treated with inhibitors of ergosterol synthesis, ketoconazol and terbinafine (Vannier-Santos et al. 1995), 22,26 azasterol (Rodrigues et al. 2002), and other compounds, derivatives of azasterol (Lorente et al. 2004). Previous studies have demonstrated that the presence of ergosterol and its analogs is essential for maintenance of a normal structural organization of the mitochondrial membrane in trypanosomatids (Rodrigues et al. 2001). Biochemical studies have shown that, contrary to what is known for

mammalian cells, there are large amounts of endogenous and exogenous sterols in the mitochondrial membranes of trypanosomatids (Vannier-Santos et al. 1995; Rodrigues et al. 2001). This indicates that the mitochondrion of trypanosomatids is an important target in leishmaniasis chemotherapy. Previous studies have demonstrated ultrastructural changes in mitochondrial morphology of promastigote forms of *L. amazonensis* treated with different leishmanicidal agents such as lincochalcone A (Zhai et al. 1995), a purified chalcone from *Piper aduncum* inflorescences (Torres-Santos et al. 1999), a purified indole alkaloid, obtained from the stem of *Peschiera australis* (Delorenzi et al. 2001), linalool-rich essential oil from *Croton cajucara* (Rosa et al. 2003), and eugenol-rich essential oil from *Ocimum gratissimum* (Ueda-Nakamura et al. 2006). Another important ultrastructural change observed in treated promastigotes was intense exocytic activity in the region of the flagellar pocket; these changes appeared as concentric membranes within the pocket. Similar changes were observed in promastigote forms of *L. amazonensis* treated with inhibitors of ergosterol synthesis, such as 22,26 azasterol (Rodrigues et al. 2002) and other derivatives of azasterol (Lorente et al. 2004). The intense exocytic activity may occur as a result of secretion into this region of abnormal lipids, which accumulate as a consequence of the drug action (Rodrigues et al. 2002), or may indicate a process of exacerbated protein production by the cells as they attempt to survive (Tiuman et al. 2005). Elucidation of the mechanism of action of this coumarin is important for the development of this compound into an antileishmanial drug.

Plants are an important source of therapeutic agents in the search for new and selective agents for the treatment of tropical diseases caused by protozoans. In recent years, natural products of different biosynthetic origins and several groups of compounds have been isolated and have shown activity against different species of *Leishmania* (Torres-Santos et al. 1999; Delorenzi et al. 2001; Ferreira et al. 2004). The antileishmanial activity of plant extracts has been attributed to compounds belonging to diverse chemical groups, such as isoquinoline alkaloids, indole alkaloids, quinones, and terpenes (Araújo et al. 1998). Several studies have shown that natural products represent a source of diverse compounds in drug discovery and the development of novel antiprotozoal agents. In conclusion, the results presented in this study indicated that the coumarin isolated from *C. brasiliense* showed important leishmanicidal activity in vitro and no toxicity for macrophages. This may be a potential new drug for the treatment of this leishmaniasis, which could become available for low-income populations. This study is a part of a continued search for new drugs with high activity and low side effects against diseases associated with protozoan parasites, such as leishmaniasis.

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