Veterinary Parasitology 178 (2011) 235–240

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

In vitro effect of *Aloe vera*, *Coriandrum sativum* and *Ricinus communis* fractions on *Leishmania infantum* and on murine monocytic cells

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ARTICLE INFO

Article history:
Received 1 September 2010
Received in revised form
22 December 2010
Accepted 7 January 2011

Keywords:
Leishmanicidal activity
Leishmania infantum
Amastigotes
Promastigotes
Cytotoxicity

ABSTRACT

In South America, visceral leishmaniasis is a zoonosis caused by the protozoan species *Leishmania infantum* (syn. *L. chagasi*) and is primarily transmitted through the bite of the female *Lutzomyia longipalpis*. Its main reservoir in urban areas is the dog. The application of control measures recommended by health agencies have not achieved significant results in reducing the incidence of human cases, and the lack of effective drugs to treat dogs resulted in the prohibition of this course of action in Brazil. Therefore, it is necessary to search new alternatives for the treatment of canine and human visceral leishmaniasis. The objectives of this study were to evaluate the *in vitro* effect of fractions from *Aloe vera* (aloe), *Coriandrum sativum* (coriander), and *Ricinus communis* (castor) on promastigotes and amastigotes of *L. infantum* and to analyze the toxicity against the murine monocytic cells RAW 264.7. To determine the viability of these substances on 50% parasites (IC50), we used a tetrazolium dye (MTT) colorimetric assay (bromide 3-4.5-dimethylthiazol-2-yl-2,5-dephenyltetrazolium), and on amastigotes we performed an *in situ* ELISA. All fractions were effective against *L. infantum* promastigotes and did not differ from the positive control pentamidine ($p > 0.05$). However, the *R. communis* ethyl acetate and chloroform fractions, as well as the *C. sativum* methanol fraction, were the most effective against amastigotes and did not differ from the positive control amphotericin B ($p > 0.05$). The *R. communis* ethyl acetate fraction was the least toxic, presenting 83.5% viability of RAW 264.7 cells, which was similar to the results obtained with amphotericin B ($p > 0.05$). Based on these results, we intend to undertake *in vivo* studies with *R. communis* ethyl acetate fractions due the high effectiveness against amastigotes and promastigotes of *L. infantum* and the low cytotoxicity towards murine monocytic cells.

1. Introduction

Leishmaniasis comprises a group of diseases caused by several species of *Leishmania* and expresses a variety of clinical symptoms. In addition, this group of diseases is the third largest among infectious diseases transmitted by vectors, behind malaria and filariasis (Solano-Gallego et al., 2009). This complex disease is endemic in many tropical and sub-tropical areas of the Old and New World, reaching

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doi:10.1016/j.vetpar.2011.01.007

88 countries with over 350 million people at risk (WHO, 2005). Leishmaniasis is an infectious disease that affects poor people living in rural and suburban settings (Alvar et al., 2006). Visceral leishmaniasis (VL) is the most serious of these diseases because it can be fatal if not treated and is extremely important in veterinary medicine because dogs are considered the main domestic reservoir for human infection (Gramiccia and Gradoni, 2005).

In Brazil, the control measures to combat VL employed by health agencies advocate euthanasia of positively tested dogs as the primary action. However, this measure has not yielded the expected results, demonstrating the need for new alternatives to help control the disease. In Europe, the objectives of anti-*Leishmania* treatment in dogs are typically to induce a general reduction of the parasite load, to treat organ damage caused by the parasite, to restore efficient immune responses, to stabilize a drug-induced clinical improvement, and to treat clinical relapse (Oliva et al., 2010). But the drugs currently available have not proven parasitological cure effective in dogs, and therefore the use of these drugs is prohibited in Brazil (Brasil, 2008), although this position is not been accepted by the most veterinary practitioners.

New alternatives of control are used like the anti-sand fly measures with deltamethrin impregnated collars or permethrin-based spot-on that have higher efficacy (Maroli et al., 2010). Moreover, the use of plants to obtain new drugs has increased due to the need to control parasites with multidrug-resistance and to maximize the control of endemic diseases like VL (Croft and Coombs, 2003; Sharief et al., 2006). The use of secondary metabolites from certain plants was effective in *in vitro* studies on different forms of *Leishmania* spp., demonstrating the feasibility of obtaining new compounds to combat this parasite (Chen et al., 1993; Schinor et al., 2007; Soares et al., 2007).*Aloe vera* belongs to Liliaceae family and was selected for this study due to the effectiveness of the leaf extract against *Leishmania donovani in vitro* and its ability to reduce more than 90% of the *in vivo* parasite burden in the spleen, liver, and bone marrow of BALB/c mice (Dutta et al., 2007, 2008). *Ricinus communis* L., known as castor bean, is a plant belonging to the Euphorbiaceae family that has bioactivity in mammalian cells, including effects as an antioxidant, anti-inflammatory, antibacterial and antiviral (Beattie et al., 2005; Ghosh, 2005). While the oil of *Coriandum sativum*, the coriander of the family Umbelliferae, shows pronounced antibacterial and antifungal effects (Matasyoh et al., 2009).

Thus, the objectives of this study were to evaluate the *in vitro* effect of fractions of *A. vera*, *C. sativum,* and *R. communis* against promastigotes and amastigotes of *L. infantum* and their cytotoxicity on the murine monocytic cells, RAW264.7.

2. Materials and methods

2.1. Obtaining the fractions

The leaves of *R. communis* were collected on the campus of State University of Ceará in Fortaleza in northeast Brazil, and the leaves of *A. vera* and the seeds of *C. sativum* were purchased commercially. The samples were dried at room temperature for 7 days to *R. communis* and *C. sativum* and 14 days for *A. vera*. The leaves were cut and the seeds were crushed in an industrial blender, the material was then immersed in 96% ethanol for a week. After passing the extracts through a rotary evaporator, they were subjected to liquid chromatography with silica filtration and repeated washings with hexane, chloroform, ethyl acetate, and methanol. The solvent of each organic phase was rotary-evaporated to obtain hexane, chloroform, ethyl acetate, and methanol fractions of each plant (Degani et al., 1998; Conegero et al., 2003).

2.2. Phytochemical tests

The qualitative phytochemical tests of phenols, tannins, catechins, leuco-anthocyanidins, flavonoids, steroids, terpenes, quinones, saponins, and alkaloids were performed according to Matos (2009) and Siddiqui et al. (2009). These tests are based on the visual observation of the colorimetric changes or the precipitate formation after an addition of specific reagents.

2.3. Cultivation of L. infantum

Promastigotes of *L. infantum* strain MHOM46/LC/HZ1 were grown in M199 (Cultilab®) plus 10% fetal calf serum (FCS) (Cultilab®), HEPES (Sigma–Aldrich®), bovine Hemin (Inlab®), sodium bicarbonate (Sigma–Aldrich®), gentamicin (40 mg/ml) (Inlab®), and 5% human male sterile urine. Cultures were maintained in a BOD incubator at 23.6 °C and peal was done every three or four days.

Amastigotes were cultured with murine monocyte RAW 264.7 cells in 96-wells microplates. Murine monocyte RAW 264.7 cells were counted in a Neubauer chamber and the concentration used was 1×10^4 cells/well. The promastigotes were added at a ratio of 10:1 parasites to cell. These cells were cultured in Dulbecco's (Cultilab®) plus 5% fetal bovine serum, sodium bicarbonate, and gentamicin (Inlab®) at a concentration of 40 mg/ml. The bottles were kept ajar for cultivation under glass with 5% CO_2 at 36.6 °C.

2.4. Tests on promastigotes of L. infantum

Promastigotes were counted in a Neubauer chamber and the concentration used was 1×10^5 promastigotes/well. The fractions of each plant were dissolved in milli Q water, and they were evaluated at 6.25, 12.5, 25, 50, and 100 μg/ml according to Tempone et al. (2005). After 24 h incubation of parasites with the fractions, a MTT test was performed to determine the IC50 of each plant fraction on the promastigotes. The positive control was 40 μg/ml pentamidine and the negative control was M199 media only (Cultilab®). All fractions were tested in triplicate. The results were read with a Multiskan MS (UNISCIENCE®) microplate reader at a wavelength of 570 nm.

2.5. Tests on amastigotes of L. infantum

The fractions of plants were dissolved in milli Q water, and they were used at concentrations of 6.25, 12.5, 25,