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Noni Fruit Extract Induces Effective Control of Lesions in Mice Infected with *Leishmania amazonensis*

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Abstract: The present study evaluated the effect of *Morinda citrifolia* crude extract on mice infected with *Leishmania amazonensis*. Both infected and untreated animals displayed severe extracellular matrix destruction, hyperplasia of the white pulp of the spleen and inflammatory cell infiltration in the liver. In contrast, animals treated with *M. citrifolia* crude extract displayedintense extracellular matrix production, characterized by the predominance of mature collagen and no alterations to the analyzed organs. The results revealed regression of lesions caused by *L. amazonensis* in the groups treated with noni extract which demonstrates that *M. citrifolia* provides effective control of the lesions caused by the infection.

Key words: Experimental infection, *Morinda citrifolia*, murine leishmaniasis, susceptible mice, extracellular

INTRODUCTION

Leishmaniasis is a neglected disease with a large variety of clinical forms that is endemic in almost every tropical and subtropical area (Da Silva *et al*., 2012). Several factors have contributed to an increase in the number of cases such as HIV co-infection, the absence of effective vaccines, difficulties of vector control and resistance to the drugs used in chemotherapy such as pentavalent antimony and amphotericin B as well as the side effects caused by such drugs.

The use of natural products in the development of new drugs is not surprising as medicinal plants have historically been used to treat parasitic diseases. Plant extracts or compounds can provide a valuable source of therapeutic agents and the need for alternative treatments has led to the search for natural products that may be useful for the treatment of leishmaniasis (Rocha *et al*., 2005).

Morinda citrifolia or noni is a native plant from Polynesia and is one of the most important sources of local traditional medicine. It has been proven to be potentially effective against microorganisms such as bacteria (Bhardwaj *et al*., 2012), viruses (Ratnoglik *et al*., 2014) and fungi (Jainkittivong *et al*., 2009). Previous resaerch from our group verified the *in vitro* effect of noni crude extract on *Leishmania amazonensis* promastigotes and amastigotes. However, little data exists regarding its activity against species of Leishmania and how it interacts with the parasite. Therefore, the aim of the present study was to evaluate the effect of *M. citrifolia* on the organs of BALB/c mice experimentally infected with *Leishmania amazonensis*.

MATERIALS AND METHODS

Ethical considerations: The experiment was approved by the Ethics Committee on Animal Experimentation of Universidade Estadual do Maranhao, Brazil (protocol number 026/2009).

Preparation of *Morinda citrifolia* crude extract: *M. citrifolia* fruits were collected, according to degree of maturation on Sao Luis Island (2°31'S; 44°16'W). The fruit were washed with distilled water and stored in sterile glass flasks to obtain the crude extract. It was then lyophilized and kept at 4°C. The lyophilisate was diluted in sterile phosphate buffered saline solution for use in the experiment.

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Animals: A total of 36 BALB/c mice, divided into six groups each containing six animals were used in the experiment. During the tests, the animals were kept in a controlled environment, receiving water and food *ad libitum*.

Parasites: The experiment used *Leishmania amazonensis* strain MHOM/BR/76/MA-76 which was isolated from a patient with diffuse cutaneous leishmaniasis and maintained by serial passages in mice. Amastigote forms were removed from footpad lesions, purified by filtering and inoculated again on the footpads of the mice.

Experimental design: The animals were subcutaneously infected by injecting 10^4 *L. amazonensis* amastigotes into the right footpad. After 30 days the presence or absence of characteristic signals at the point of infection such as ulcerative lesions and oedema was checked. For the therapeutic assay, the animals were divided into six groups (G1: infected and treated for 30 days; G2: infected and treated for 60 days; G3: non-infected and treated for 30 days; G4: non-infected and treated for 60 days; G5: infected and untreated; G6: non-infected and untreated). Oral treatment was performed every 24 h for a period of 30 and 60 days, using a gavage needle. Mice received 0.1 mL of the extract at a dose of 100 mg kg^{-1}.

Histopathology: Three animals from each group were euthanized 30 and 60 days post infection for collection of footpad, spleen, liver and lymph nodes. The fragments were fixed in buffered formalin, underwent histopathological processing and were then subsequently stained with hematoxylin-eosin for evaluation of lesions and picrosirius red for collagen evaluation. The slides were examined and photographed under a light microscope and polarized light.

Statistical analysis: Considering the type of experiment, a descriptive analysis of the main histopathological findings was performed.

RESULTS AND DISCUSSION

Histopathological analysis: At day 30, the infected and untreated group showed diffuse inflammatory reactions with heavily parasitized macrophages and extensive areas of necrosis on the primary lesion (Fig. 1A). The liver exhibited inflammatory infiltrate near the terminal hepatic vein (Fig. 1B). Hyperplasia of the white pulp of the spleen (Fig. 1C) and lymph nodes was also observed. The infected and treated group presented a diffuse inflammatory reaction with rare parasitized macrophages and eosinophils on the footpad (Fig. 1D). The other organs did not display significant histological alterations.

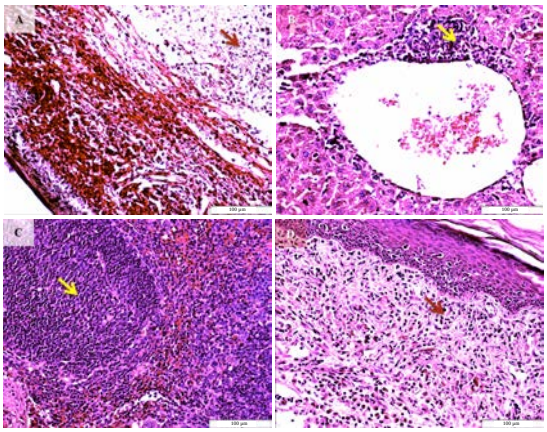

Fig. 1: Histopathological analysis of mice infected with *Leishmania amazonensis*. Infected and untreated group: A) extensive area of necrosis and diffuse inflammatory reaction with severe parasitized macrophages (arrow); B) liver showing inflammatory reaction next to the terminal hepatic vein (arrow); C) spleen with hyperplasia of white pulp (arrow). Infected and treated group and D) diffuse inflammatory reaction in dermis with predominance of eosinophil (arrow) HE

After 60 days, the infected and untreated group showed intense tissue destruction at the point of infection. The internal organs displayed the same alterations as day 30 but more markedly and associated with small apoptosis foci. The skin of treated animals displayed some inflammatory foci but no parasitized macrophages or free amastigotes. For the other organs, only small granulomas were found in the liver.

Extracellular matrix analysis: Extracellular matrix analysis of both non-treated group did not reveal enough collagen fibers to be captured under the polarized light microscope, due to the intense tissue destruction (Fig. 2a). However, in the infected and treated groups, there was intense extracellular matrix production, characterized by the predominance of mature collagen type I which presented a deep red color under polarized light (Fig. 2b). The matrix displayed the same pattern on day 60 as on day 30.

The present study analyzed the effects of *Morinda citrifolia* fruit extract on the organs of infected mice. The classical histopathological alterations of Leishmania infection were observed in accordance with the findings of several researchers.

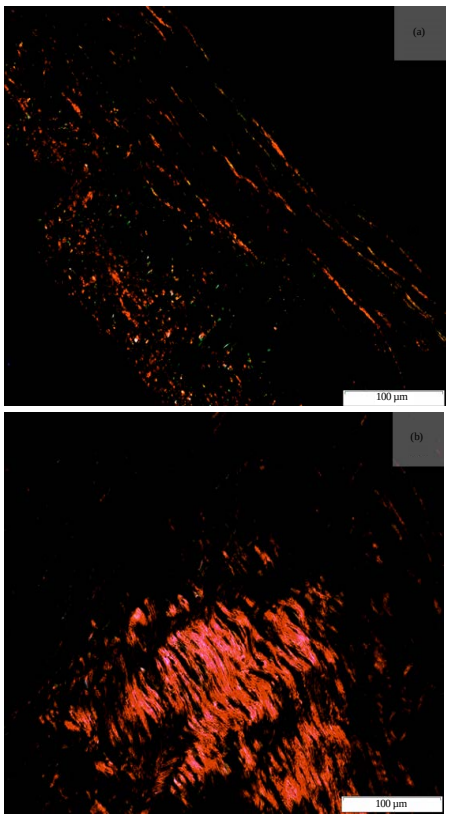

Fig. 2: Polarized light microscopy. Footpad of BALB/c mice infected with *Leishmania amazonensis*: a) untreated group and b) treated group with noni crude extract. Picrosirius Red

Experimental tests with CBA mice described the presence of mononuclear inflammatory infiltrate with intensely parasitized macrophages in the dermis. The infection of mice strains that were resistant and susceptible to *L. amazonensis* resulted in the presence of macrophages containing amastigotes and ulcerative lesions in the footpad. Other organs had no significant lesions in keeping with the findings of the present study (De Oliveira *et al*., 2010). Other researchers, studying modifications in the extracellular matrix of skin lesions and draining lymph nodes caused by *L. amazonensis* in several strains of mice showed that these changes were more evident in more susceptible animals such as BALB/c mice, especially when they had lesions containing intense inflammatory infiltrate, composed of highly parasitized macrophages (Abreu-Silva *et al*., 2004).

On the other hand, the absence of alterations in the internal organs and the regression of the skin lesions in the treated groups allow us to say that the employed treatment was satisfactory.

Further, plant extracts have been used as alternative therapies for both visceral and cutaneous leishmaniasis. The plant *Pentalinon andrieuxii*, common in the Yucatan Peninsula, exhibited a potent anti-parasitic activity and *in vivo* studies have showed that *L. mexicana*-infected mice treated by topical application of root hexane extract resulted in a significant reduction in the lesion size and parasite burden (Lezama-Davila *et al*., 2014). The metanolic extract of the African *Vernonia amygdalina* which contains high levels of flavonoids, led to a reduction in lesion size and less tissue destruction in the skin, spleen and liver of BALB/c mice infected with *L. major* (Alawa *et al*., 2012).

The impossibility of properly evaluating the alterations in the extracellular matrix was due to the severe alterations in the skin as a result of the clinical manifestation of the disease which caused the loss of normal tissue architecture.

The present study provided evidence of alterations in the collagenous constituents of the extracellular matrix caused by the infection. The intense changes that occurredin the matrix of the infected and untreated animals represented evidence that Leishmania uses collagen fibers, especially type I to evade the immune response and migrate to other organs, evidenced by the intense destruction of the matrix (Lira *et al*., 1997).

CONCLUSION

However, when comparing the results of the treated and untreated groups, it was noticed that the group treated with *M. citrifolia* extracthad a greater amount of extracellular matrix. In fact, previous studies have already demonstrated that noni fruit extract up-regulates the biosynthesis of type I collagen and glycosaminoglycans in primary cultures of human fibroblasts with the anthraquinone compound being the responsible for that (Kim *et al*., 2005). This data shows the recovery of skin tissue of groups undergoing treatment with noni. As such, the present study demonstrates that noni extract leads to the regression of lesions caused by *L. amazonensis* and that *M. citrifolia* induces effective control of the infection.

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