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Ultrastructural Alterations in Amastigotes of *Leishmania amazonensis* Treated with Essential Oil Extracted from *Ocimum gratissimum*.

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Several *Leishmania* species are causative agents of a wide spectrum of cutaneous, mucocutaneous and visceral human diseases named Leishmaniasis. This endemic disease affects millions of people in the world and chemotherapy is limited to few drugs, such as the pentavalent antimonials and pentamidine. Plants extracts has been extensively studied in the search of new compounds with antimicrobial effects. The essential oil of *Ocimum gratissimum,* a plant used in folk medicine in the treatment of many diseases, showed to be effective against bacteria and helminths [1, 2]. The aim of this study was to investigate the effect of the essential oil from *O. gratissimum* on amastigote forms of *Leishmania amazonensis*, the intracellular form of the parasite in mammalian hosts.

Promastigote forms of *L. amazonensis* were transformed to amastigotes *in vitro* and then maintained as axenic cultures in Schneider's Drosophila Medium (Sigma), pH 4.6 with 20% Fetal Bovine Serum (Gibco) at 32° C. Different concentrations of the essential oil (100 to 1000 μg/ml), extracted from the leaves of *O. gratissimum*, were diluted in 2% Polyethylene glycol, and tested on axenic amastigotes for 48 h. Cell counting was performed using Neubauer chamber and cell viability was checked using erythrosine B. Samples of axenic amastigotes treated with sub-inhibitory concentration of the essential oil during 48 h were fixed for 1 h with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2. Post-fixation was carried in 1% osmium tetroxide solution in 0.1 M cacodylate buffer containing 0.8% potassium ferrocyanide and 5 mM calcium chloride. Cells were then dehydrated in acetone and embedded in Epon. Ultrathin sections were stained with 5% aqueous uranyl acetate and lead citrate and observed in Zeiss EM-900 transmission electron microscope.

Axenic amastigotes growth was inhibited by the essential oil (IC_{50} = 135 μg/ml). Ultrastructure changes are demonstrated in Figure 1. In contrast to control cells that exhibited normal appearance (Fig. 1A), treated cells showed more than two nucleuses (Fig. 1B), suggesting interference in the cell division. In addition, mitochondrion swelling was frequently observed in treated amastigotes (Figs. 1C and D). These results demonstrate that the essential oil from *O. gratissimum* has antiproliferative effect against amastigote forms of *L. amazonensis,* providing new perspectives on drug development against leishmaniasis.

References

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[3] This research was supported by CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico.

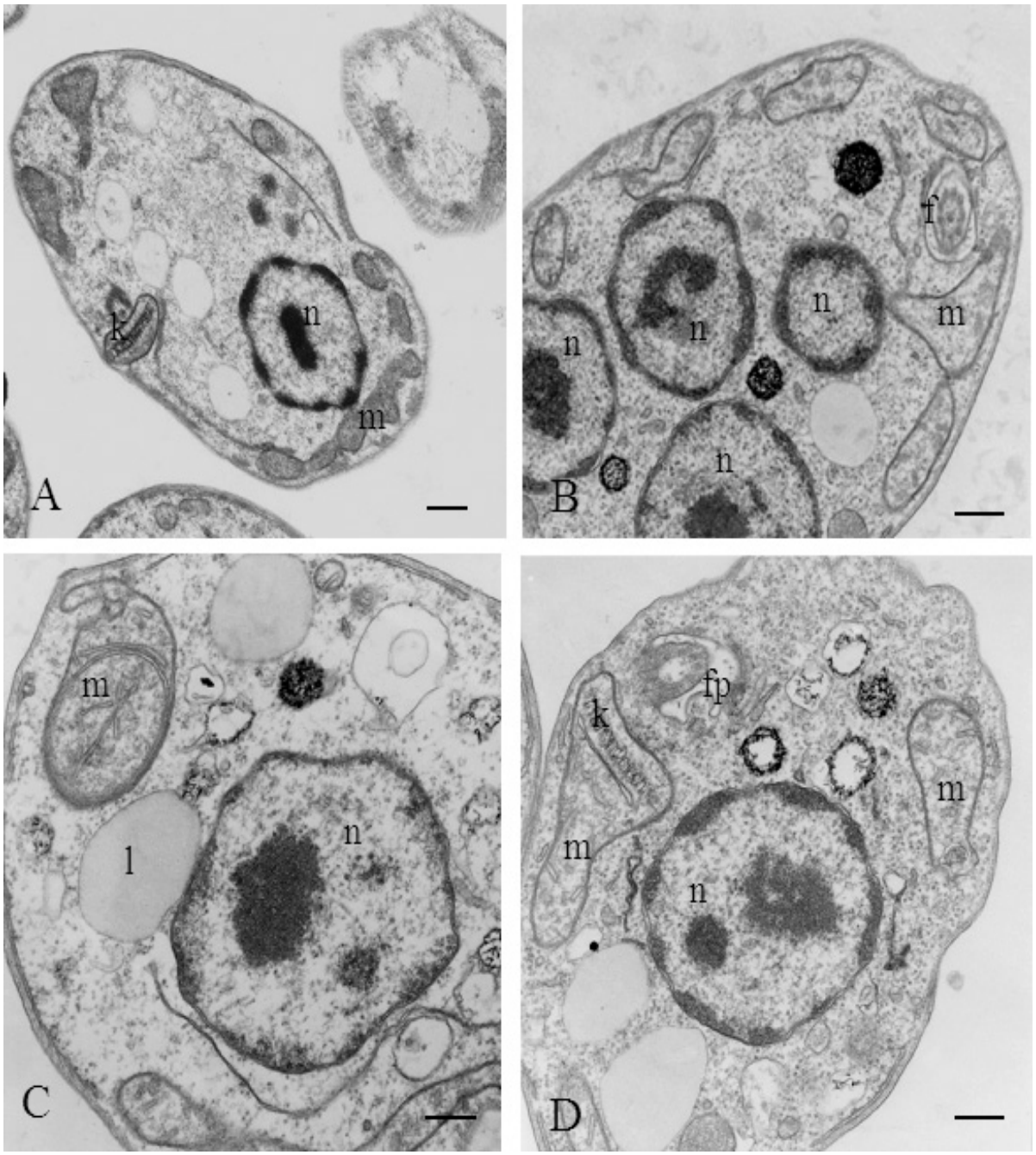

Figure 1. Transmission electron microscopy of amastigote forms of *Leishmania amazonensis*. **(A)** Untreated cell; **(B, C,** and **D)** Cells treated with essential oil IC_{50} of 135 μg/ml - f, flagellum; fp, flagelar pocket; l, lipid inclusion; k, kinetoplast; m, mitochondrion; n, nucleus. Bars = 1 μm

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