Nimbolide inhibits the growth of Miltefosine resistant *Leishmania donovani* parasite - A Flow Cytometry based in-vitro study

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Abstract

Visceral leishmaniasis (VL) is a disease transmitted by vectors and caused by the parasitic protozoan *Leishmania donovani*. VL is a very severe manifestation of leishmaniasis and may be lethal if not treated early. The leishmania parasite developed resistance to most of the drugs used for its treatment, including Miltefosine (MIL). Therefore, it is crucial to identify novel antileishmanial compounds to prevent the development of MIL-resistant *L. donovani* parasites. The Neem (Azadirachta indica) leaf extracts containing several bioactive molecules have shown antileishmanial activities. Among those compounds, nimbolide (NB) has been shown to possess both antibacterial and anticancer properties. In the present study, we investigate the antileishmanial properties of NB against MIL-resistant and MIL-sensitive *L. donovani*. The MTT experiment and growth curve analysis demonstrate that NB has a dose-dependent inhibitory effect on promastigote parasite. A scanning electron microscopy (SEM) study has shown that NB treatment significantly influenced the size and shape of promastigote parasites. A flow cytometry study reveals that treatment with NB modified the activities of mitochondrial superoxide generation (ROS), mitochondrial membrane potential (MMP), cell cycle, and cell proliferation. Treatment with NB decreased the efficiency of intracellular amastigote infection and proliferations, which was further confirmed by a decrease in IL-10 cytokine production in human macrophages infected with *L. donovani* (THP-1 cells). Thus, studies reveal that Nimbolide has the potential to be a very effective therapeutic candidate for treating Leishmaniasis and controlling the development of MIL-resistant parasites.