In vitro Antileishmanial Activity of Hydroalcoholic Extract, Fractions, and Compounds Isolated from Leaves of *Piper ovatum* Vahl against *Leishmania amazonensis*

Daniel RODRIGUES-SILVA¹, Celso Vataru NAKAMURA¹,², Benedito Prado DIAS FILHO¹,², Tânia UEDA-NAKAMURA¹,², Diógenes Aparício Garcia CORTEZ¹,³∗

¹Programa de Pós-graduação em Ciências Farmacêuticas; ²Departamento de Análises Clínicas; ³Departamento de Farmácia e Farmacologia, Universidade Estadual de Maringá, Maringá, Paraná, Brazil

Summary. We assessed the biological activity of a crude extract, a mixture of several fractions, and a pure compound obtained from *Piper ovatum* Vahl against promastigote and amastigote forms of *Leishmania amazonensis*. The medicinal plant *P. ovatum* is used popularly as an anesthetic and anti-inflammatory. This study included the extraction process and bioassay-guided fractionation by the adsorption chromatography and Sephadex LH-20 method. A progressive increase in the antileishmanial effect was observed in the course of fractionation. The 50% inhibitory concentration (IC₅₀) for dichloromethane-ethyl acetate (1:1 v/v) fraction was 2.1 µg/ml and 24 µg/ml; mixture of piperovatine: piperlongumine (2:3) 0.9 µg/ml and 24 µg/ml; piperovatine (1) 9.5 µg/ml and 10 µg/ml; and piperlonguminine (2) 2.5 µg/ml and 9.0 µg/ml, for promastigote and amastigote forms, respectively. Cytotoxicity analysis indicated that these toxic concentrations were much higher for J774G8 macrophages and Vero cells than for the protozoans. The mixture of piperovatine: piperlongumine (2:3) showed important antiprotozoan activity against the amastigote and promastigote forms of *L. amazonensis*, and it produced morphological changes in promastigotes and amastigotes at 0.9 mg/ml and 24 mg/ml (50% growth inhibition concentration), respectively, including intense cytoplasmic vacuolization, mitochondrial swelling, and mitochondrial damage, as revealed by transmission electron microscopy.

Key words: Antiprotozoan activity, ultrastructure alterations, *Leishmania amazonensis*, *Piper ovatum*. 

INTRODUCTION

Leishmaniasis is an insect-borne protozoan infection that affects an estimated 12 million people worldwide, causing significant morbidity and mortality in Africa, Asia and Latin America (WHO 2001). Pentavalent antimonials are the first-line treatments, but their irregular effectiveness makes them sometimes disappointing because of antimonial resistance and relapse following Amphotericin B therapy. Second-line drugs, such as Amphotericin B, are more toxic, and Amphotericin B's lipid formulation is too expensive for routine use in underdeveloped countries (Murray 2001). There is at present no effective vaccine for humans (Handman 2001). Several new alkylphosphocholine derivatives with amide or ester bonds in the alkyl chain have been...