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In vitro leishmanicidal activity of Artennua®

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Background

Canine leishmaniosis (CanL) due to *Leishmania infantum* is a zoonotic disease prevalent in more than 80 countries worldwide. The clinical manifestations of this infection are closely influenced by the dog's immune response [1]. Even though current chemotherapy can reduce parasitic load and improve the quality of life, parasitological cures are rarely achieved and clinical recurrences of CanL often occur after therapy. Moreover, the available protocols can cause significant side effects and resistance to several anti-*Leishmania* conventional drugs has been reported [2]. Thus, there is an urgent need for new, effective, and safe drugs for the treatment of CanL, and the use of medicinal plants could be a promising approach. *Artemisia annua* is the unique natural source of artemisinin, a molecule recommended by the World Health Organization for the treatment of malaria. Due to its antiparasitic and immunomodulatory properties, it is considered whether this plant could be an alternative for the treatment of CanL, without the significant side effects of conventional treatments [3]. Artennua® is a standardized version of *A. annua*, with higher concentrations of artemisinin and flavonoids, which exhibit synergistic effects of artemisinin by increasing its bioavailability [3]. Therefore, the aim of this study was to evaluate the leishmanicidal activity of Artennua® *in vitro*.

Materials and methods

In vitro L. infantum promastigote and amastigote susceptibility assays were carried out using a colorimetric assay based on resazurin (Alamar Blue®: Invitrogen, Life Technologies, Madrid, Spain) to obtain the half maximal inhibitory concentration (IC50) [4,5]. Artennua® A (dried leaves of *A. annua* with 1.21% of artemisinin), Artennua® B (dried leaves of *A. annua* with 0.75% of artemisinin) and a commercial product (30:1 *A. annua* extract with 0,16% of artemisinin, old formulation of Luparte®: Lupovet GmbH, Müllheim/Baden, Germany) were dissolved in dimethyl sulfoxide. Serial dilutions of the compounds and the promastigote form of the parasite were prepared. The most active compound versus promastigotes was also tested against the amastigote form. J774A.1 murine macrophages infected with amastigotes were cultured and serial dilutions were also prepared. For parasite rescue, controlled lysis of *L. infantum* amastigote-infected macrophages was performed. In both assays, IC50 was determined by nonlinear regression analysis with 95% confidence limits. Previously, cytotoxicity was also measured in J774A.1 murine macrophages using serial dilutions of Artennua® starting at a concentration of 400 µg/mL. Cell viability was determined by the resazurin method.

Results

Mean values ± standard deviation (SD) of IC50 for Artennua® A and B and the commercial extract against promastigotes were 54.62 ± 6.94 µg/mL, 274.13 ± 55.71 µg/mL and 433.3 ± 117.6 µg/mL, respectively. These results revealed that the leishmanicidal effect of the compounds against *L. infantum* promastigotes was strongly dependent on artemisinin concentration. The mean value ± SD of IC50 for Artennua® against amastigotes was 69.71 ± 12.8 µg/mL. This IC50 value is similar to that of other plants screened against *L. amazonensis* [6]. No cell toxicity was observed at any of the tested dilutions, suggesting that Artennua® is not cytotoxic for murine macrophages up to 400 µg/mL.

Conclusion

The present study shows that Artennua® with 1.21% artemisinin proved to have an *in vitro* leishmanicidal activity against promastigotes and amastigotes of *L. infantum*, with very encouraging IC50 values. Furthermore, Artennua® was not cytotoxic for murine macrophages even at high concentrations, suggesting selective activity against the parasite. Therefore, based on the results of this study, Artennua® appears to be a potential drug to be used in the treatment of CanL. However, further investigations are still needed to confirm the anti-*Leishmania* potential of Artennua® *in vivo*.

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