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New phytotherapeutic approaches in the treatment of canine leishmaniasis: leishmanicidal and immunomodulatory activity of phytocomplexes in in vitro models of Leishmania infantum

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Introduction:
Leishmaniasis is a zoonic parasitic disease with a generally chronic course, caused by protozoa of the genus Leishmania. Although there are different treatments, drugs currently available have significant side effects, high cost, and long-term toxicity. Therefore, the development of novel and effective drugs is an important and urgent need. Natural products are an important source of bioactive molecules for the development of new drugs., as natural compounds have easy availability, low cost, and reduced side effects. In the past medicinal plants and natural products have been used for phytotherapeutic purposes due to their immunostimulant, antiseptic, and antioxidant properties and some secondary metabolites of natural products, such as artemisin, eugenol, quercetin, resveratrol, and beta-sitosterol, are already known in the literature for their leishmanicidal and immunomodulatory actions. The present study aims to evaluate phytolium, full of artemisinin, eugenol, quercetin, resveratol compounds, in order to understand their mechanism of action and anti-leishmania action.
Materials and methods:
Leishmania infantum (MHOM/TN/80/IPT1) were grown at 25° C, pH 7.18 in 10 ml of RPMI-PY medium [1] and 4x106 cells/ml were treated with scalar concentrations (0.5, 1, 2, 4 mg/ml) of a phytocomplex preparation extracted from Artemisia Annua and Eugenia caryophyllata plants, called 'phytolium'. Parasites were counted using a Bürker counting chamber after 48 hrs and percentage of viability (%) was compared with control cultures. Potential cytotoxic action of 'phytolium' was checked by LDH citocolorimetric assay on U937 (human Caucasian histiocytic lymphoma) cell line, previously cultured in RPMI 1640 supplemented with 10% fetal bovine serum, treated with 25 ng/ml of PMA for 18h at 37°C and 5% CO2 to induce the macrophages differentation. The percentages was compared with control considered as 100% citotoxicity. All data were obtained from three independent experiments and the results are shown at mean ± standard deviation or as a representative experiments.
Results:
Figure 1A shows the inhibiting action of phytocompounds on parasite growth of Leishmania infantum starting from the lower dose (0,5 mg/ml) of concentration compared with the control (100% viability).
In addition, the potential phytocompound citotoxicity was tested in U937, showing an absence of cell damage compared with the control (100% citotoxicity) (Fig.1B).
Conclusions:
The natural compounds can be an important complementary therapy in the fight against parasites, owing to their natural origin, safety, and low cost compared to synthetic drugs. In conclusion, we firstly demonstrated that “phytolium”, can be endowed with activity against L. infantum and its low toxicity on normal cells makes this compound interesting for further clinical and biological studies. The next research step will select the activities of phytocomplexes and investigate their action on leishmaniasis disease and to study their action on macrophages infected with L. infantum. Furthermore, it would be very interesting to isolate or synthesise structurally related compounds in order to establish structure-activity relationships.