4th BIOPHARM SCIENTIFIC ANNUAL MEETING
BSAM4, Alger le 06 juin 2024

ENHANCED LEISHMANICIDAL EFFECT BY DIMETHYLFUMARATE ENCAPSULATED IN LAYERED DOUBLE HYDROXIDES ON *LEISHMANIA MAJOR* PROMASTIGOTES *IN VITRO*

Nabila Tounsi[1] *, Fouzia Touahra[2], Bahia Djerdjouri[1], and Ferroudja Bali[3]

1Laboratory of Cellular and Molecular Biology, Faculty of Biological Sciences, University of Sciences and Technology Houari Boumediene, Algiers, Algeria.
2 Research Centre in Analytical Chemistry and Physics, Algiers, Algeria.
3 Laboratory of Natural Gas Chemistry, Faculty of Chemistry, University of Sciences and Technology Houari Boumediene, Algiers, Algeria.

Abstract

In Algeria, cutaneous leishmaniasis is endemic and represents a major public health problem. Pentavalent antimonials are first-line drugs to treat leishmaniasis. However, their long-term use is limited by serious side effects and treatment failure due to the emergence of resistant Leishmanial strains. This study investigated the leishmanicidal potential of an electrophilic antioxidant, dimethyl fumarate in free form (DMF) and encapsulated in layered double hydroxides (DMF-LDH) on *Leishmania major* promastigotes *in vitro*.

Methods. MgAl LDH, used as nanovectors for DMF, were synthesized by the microwave hydrothermal method. These nanoparticles were characterized by Atomic Absorption Spectroscopy, X-ray diffraction, Fourrier transform infrared spectroscopy, and scanning electron microscopy. Promastigotes (10^6/well/ml RPMI1640) were incubated at 25 °C with increasing concentrations of DMF or DMF-LDH. At different time points, the survival rate of live promastigotes was assessed in a Malassez hemocytometer under a light microscope at 40 magnification. The survival rate of promastigotes is expressed as a percentage of the control. The concentrations of DMF and MgAl-DMF inhibiting 50% of parasite survival (IC_{50}) were determined.

Results. DMF or DMF-LDH (10–200 µg/ml) reduced parasite growth in a dose-dependent manner. After 72 h of 200 µg/ml DMF and DMF-LDH treatment, the inhibitory effect reached 51% and 94%, respectively. Free DMF or LDH-encapsulated DMF exhibited potent antipromastigote activity with IC_{50} achieved at 23.31 ± 1.33 and 10.53 ± 1.08 µg/ml, respectively.

Conclusion. Therefore, our results support the leishmanicidal effect of free DMF and suggest that the LDH-encapsulated compounds are promising biomolecules that emphasize inhibition of *L. major* promastigotes growth**.**

Keywords: Cutaneous leishmaniasis, Dimethylfumarate, Layered double hydroxides, Parasite growth.

I- Introduction

Leishmaniasis is a disease caused by obligate protozoan parasites from the genus *Leishmania* transmitted through the bites of infected sandflies. Algeria ranks second after Afghanistan for the incidence of cutaneous leishmaniasis (CL) worldwide, with more than 20,000 cases reported each year, and an incidence of 28.19 cases per 100,000 inhabitants (1).

The use of pentavalent antimonials as the primary treatment for this disease is becoming problematic due to the development of drug resistance and severe side effects. Finding alternative treatments that are effective, safe, and sustainable is crucial for combating this public health challenge in Algeria.

Layered double hydroxides (LDH) are ionic lamellar clays composed of positively charged brucite-like layers, charge-balancing anions, and water solvation molecules in the interlayer area. LDH are stable, biocompatible, and biodegradable, with a high surface-to-volume ratio and capacity to load and quick delivery of drugs (2).

Dimethyl fumarate (DM), an intermediate product of the Krebs cycle, is an immunomodulator used to treat psoriasis and multiple sclerosis and known fungicide (3). However, the antileishmanial effect of DMF has not been investigated.

In this investigation, we assessed the antileishmanial activity of of dimethyl fumarate, in free form (DMF) or encapsulated in layered double hydroxides (DMF-LDH) on *Leishmania major* promastigotes *in vitro*.

II- Materials and methods

LDH synthesis and DMF encapsulation. The microwave hydrothermal method was used for MgAl LDH preparation which are then intercalated with DMF. The LDH were characterized by x-ray diffraction (XRD), Fourier transform infrared spectra (FTIR) and scanning electron microscope (SEM) analysis.

Antileishmanial activity. Promastigotes of *Leishmania major* (10^6/well/ml RPMI1640) were incubated at 25 °C with increasing concentrations of DMF or DMF-LDH (10, 25, 50, 100 and 200 µg/ml) for 4, 18, 24, 48 and 72h. At different time points, the survival rate of live promastigotes was assessed in a Malassez hemocytometer under a light microscope at 40 magnification. The survival rate of promastigotes is expressed as a percentage of the control. The concentrations of DMF and DMF-LDH inhibiting 50% of parasite survival (IC_{50}) were determined.

III- Results and discussion

The resulting LDH analyzed by XRD patterns had three regions of the diffractograms characteristic of hydrotalcite-type phases. The shift of peak 003 towards low angles for DMF-LDH confirms the intercalation of DMF (**Fig. 1A**). The MgAl LDH absorption bands at 3420 cm^{-1} and1350 cm^{-1} indicates the vibrational bands of hydroxyl groups ν(OH) and nitrate ions respectively**.** The low-frequency region (< 1000 cm^{-1}), is attributed to the stretching vibrations of the metal-O-metal bond (**Fig.1B**). The absence of DMF a specific band in DMF-LDH spectrum attests to good DMF intercalation.

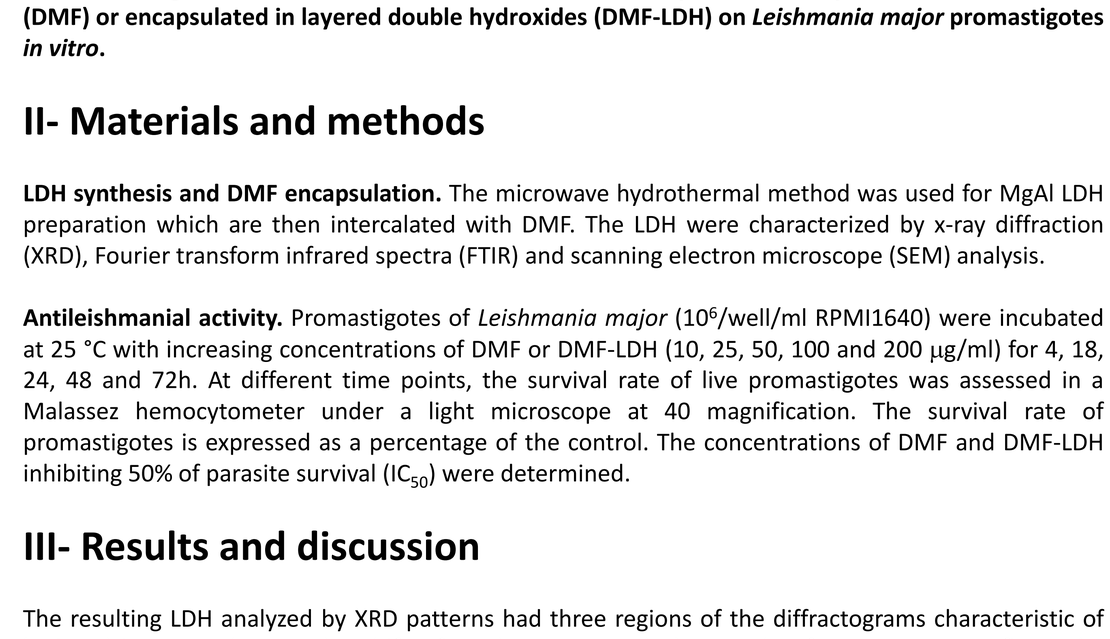

Figure 1: The x-ray diffractograms of MgAl-LDH and MDF-LDH (A), FTIR spectrum of MgAl LDH, DMF and MDF-LDH (B)

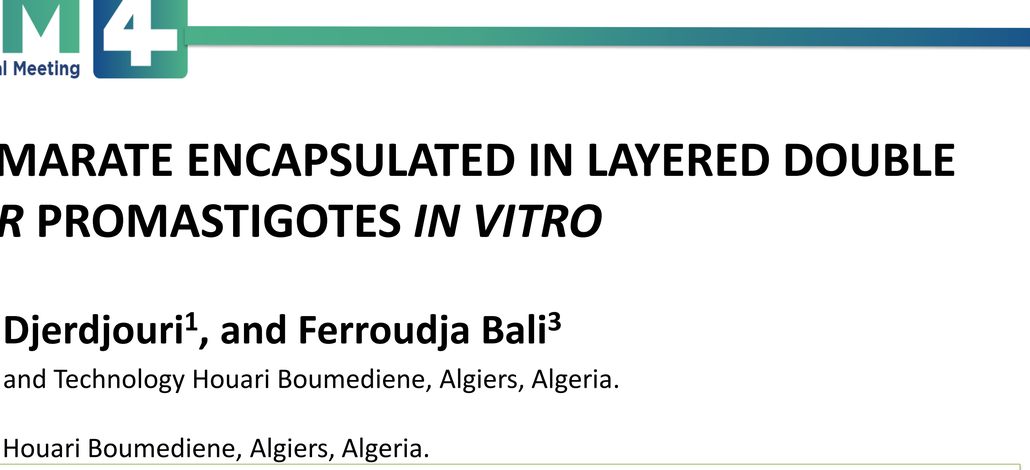

Figure 2: SEM image and EDX spectrum of MgAl LDH (left) and DMF-LDH (right)

The morphology of LDH was examined by scanning electron microscopy (SEM) coupled with energy dispersive x-ray spectroscopy (EDX). Images of the MgAl LDH (**Fig2. left**) revealed the ultrastructure of small LDH aggregates, called "desert rose". These structures are less well defined for DMF-LDH (**Fig2. right**), possibly due to the presence of DMF between the layers.

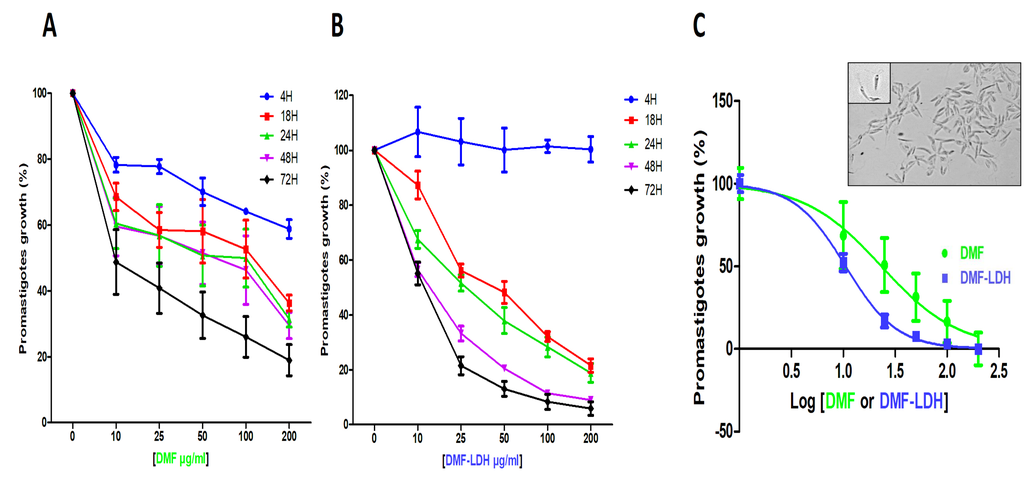

Figure 3: Promastigotes growth in presence of DMF (A), DMF-LDH (B) and IC50 of DMF or DMF-LDH at 72H (C)

DMF or DMF-LDH reduced parasite growth in a dose-dependent manner. After 72 h of 200 µg/ml **DMF** and **DMF-LDH** treatment, the inhibitory effect reached **51%** and **94%**, respectively (**Fig.3**).

DMF IC50 = **23.31 ± 1.33 µg/ml**
DMF-LDH IC50 = **10.53 ± 1.08 µg/ml**

Conclusion

As a result, free DMF exerts antileishmanical activity, and more attractive and efficient results were observed with DMF-LDH, which could be a potential alternative agent to treat leishmaniasis.
Further study of the effects of DMF-LDH *in vivo* is necessary to fully understand its potential benefits.

References

1. Izri, A., Bendjaballah-Laliam, A., Sereno, D., & Akhoundi, M. (2021). Updates on geographical dispersion of Leishmania parasites causing cutaneous affections in Algeria. *Pathogens*, *10*(3), 267.
2. Miao, L., Wei, Y., Lu, X., Jiang, M., Liu, Y., Li, P., Ren, Y., Zhang, H., Chen, W., Han, B., Lu, W. (2023). Interaction of 2D nanomaterial with cellular barrier: membrane attachment and intracellular trafficking. *Advanced Drug Delivery Reviews*, 115131.
3. Kornberg, M. D., Bhargava, P., Kim, P. M., Putluri, V., Snowman, A. M., Putluri, N., Calabresi P. A., Snyder, S. H. (2018). Dimethyl fumarate targets GAPDH and aerobic glycolysis to modulate immunity. *Science*, *360*(6387), 449-453.