PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS:
Fisiologia e Farmacologia
ICB - UFMG

PÓS Graduação FISIOLOGIA E FARMACOLOGIA

NANOSYSTEMS FORMED BY AMPHIPHILIC ANTIMONY(V) COMPLEXES INCORPORATING AMPHOTERICIN B FOR THE TREATMENT OF LEISHMANIASIS

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Outlines

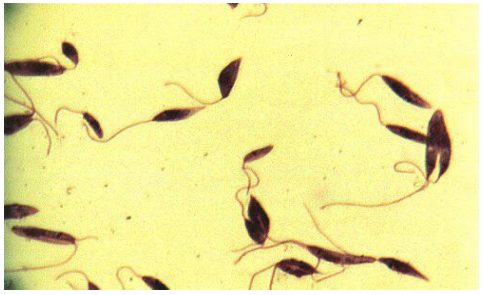
- Introduction
  - What is Leishmaniasis
  - Geographical distribution
  - Current chemotherapeutics
  - Amphotericin B (AmB) as antileishmanial drug
- Objectives of the study
- Results and Discussion
  - Preparation of the nanosystems
  - Incorporation of AmB in the system
  - Stability of the formulations in solutions on dilution
  - Efficacy of the formulations as antileishmanial drug
- Conclusions

Leishmaniasis

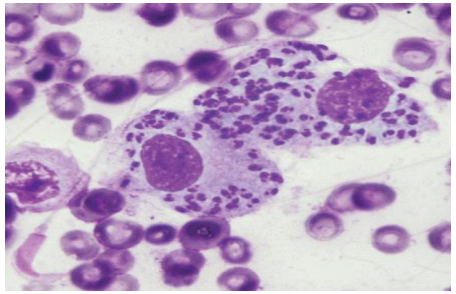
A Significant Neglected Tropical Disease

- A diverse group of diseases, also known as sandfly or black fever disease
- Infection transmitted by the bite of sandfly of genus *Phlebotamine* and *Leutzomia*.
- **Two morphological forms (Unicellular Eukaryotes):**
  1. Amastigotes (Non-motile and round)
  2. Promastigotes (Flagellated)

Motile promastigote

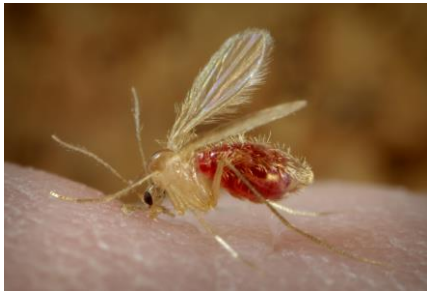


Non-motile Amastigote



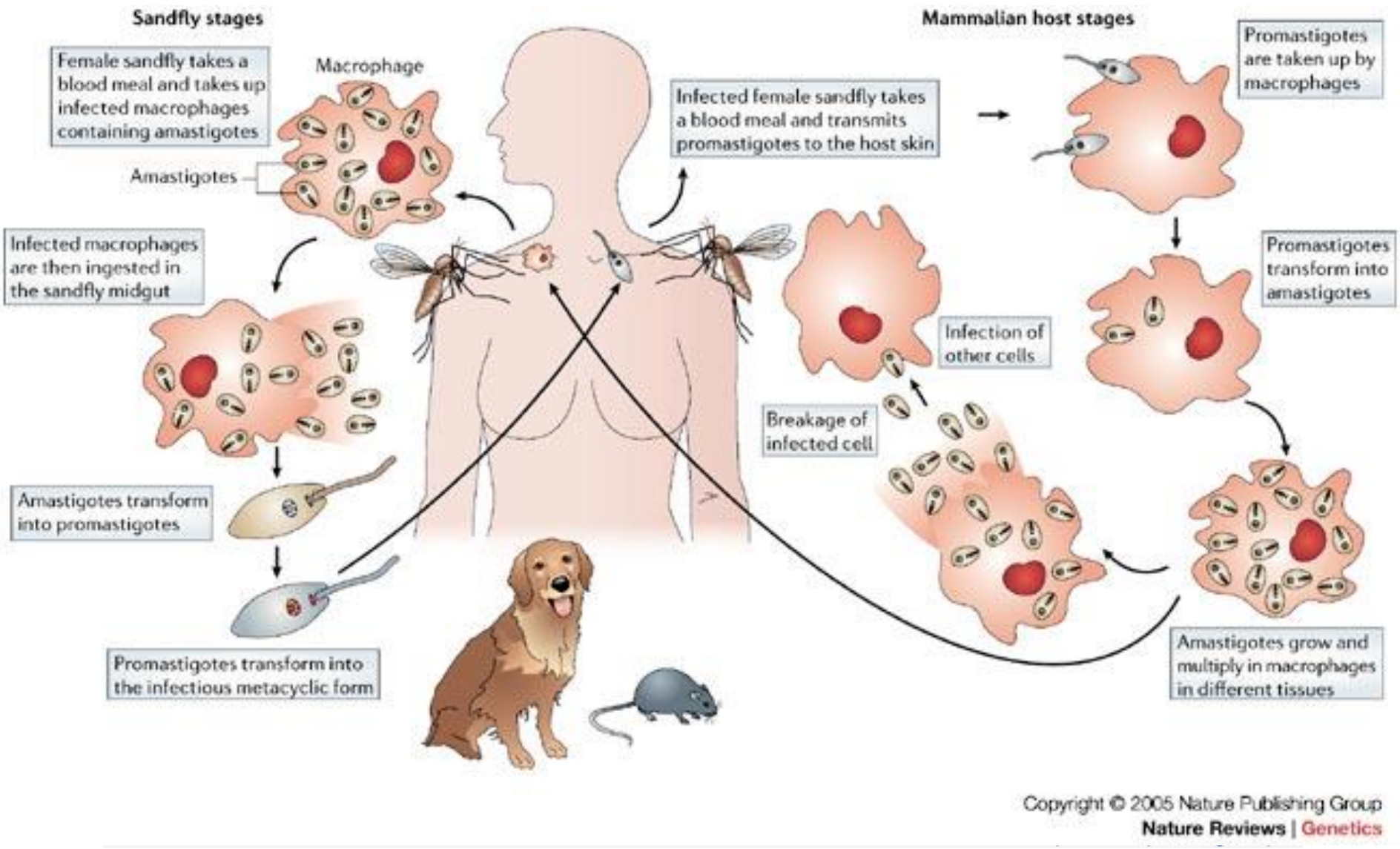
Leutzomia

Phlebotamine



Mishra, J. et al. 2007. Current Medicinal Chemistry, 14, 1153-1169.

Life Cycle – Leishmaniasis



Mishra, J. et al. 2007. Current Medicinal Chemistry, 14, 1153-1169.

Types of Leishmaniasis

21 species are known to cause disease in humans

❖ Visceral Leishmaniasis (Fatal: 90%)

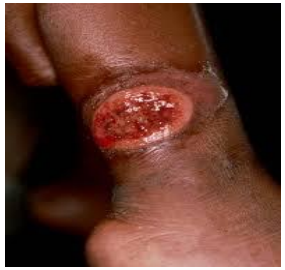
L. donovani; L. infantum

fever, substantial weight loss, spleen and liver swelling.

❖ Cutaneous Leishmaniasis

L. amazonensis; L. major; L. mexicana

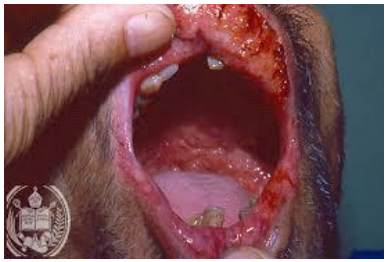
skin ulcers



❖ Mucocutaneous Leishmaniasis

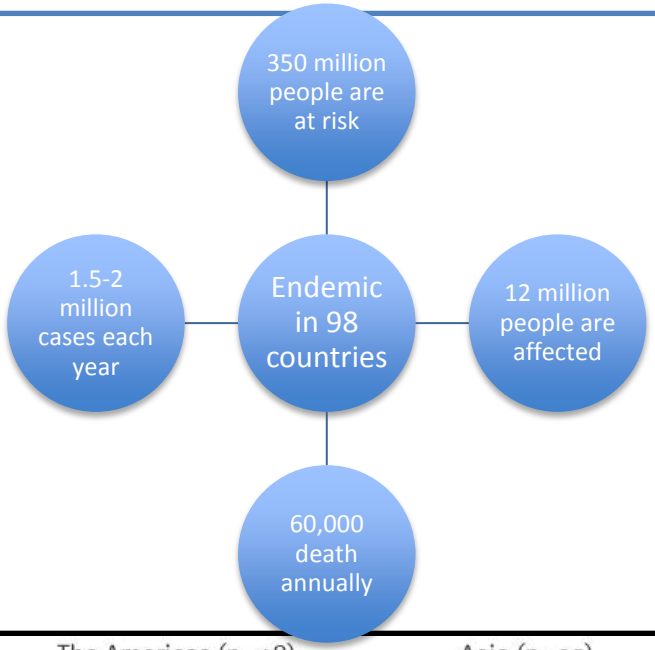
L. amazonensis; L. brazilliensis

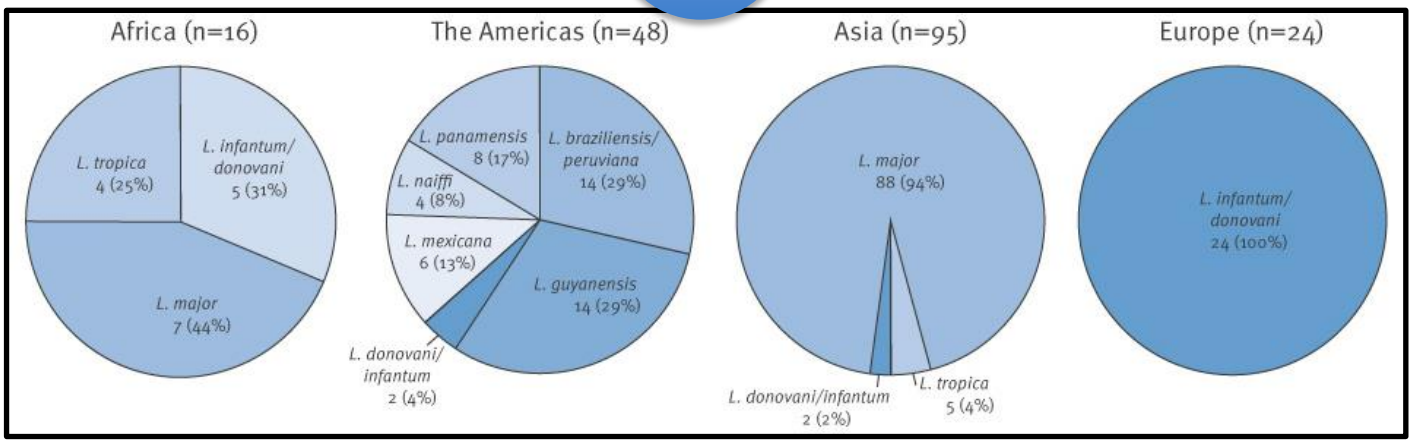
disorders of the mucous membranes



Mishra, J. et al. 2007. Current Medicinal Chemistry, 14, 1153-1169.

Risk & Geographical Distribution: *Leishmaniasis*





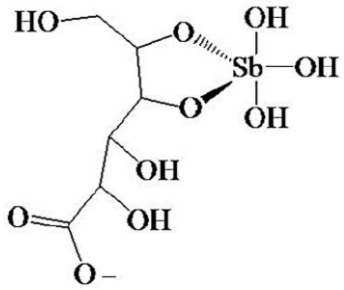
Bangladesh, **Brazil**, India, Nepal, Sudan, Afghanistan, Iran, Peru, Syria

Mishra, J. *et al*. **2007**. *Current Medicinal Chemistry*, 14, 1153-1169.

Currents Therapeutics for Leishmaniasis

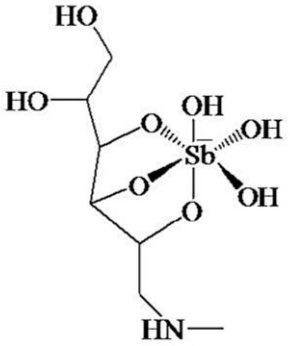
❖ Pentavalent Antimonials

- **Glucantime® and Pentostam®**



First line of drugs in developing countries for

cutaneous and visceral



❖ Mechanism of action???

- **Sb(V) reduces to Sb(III)**
- **Interaction with DNA or may block metabolic pathway**

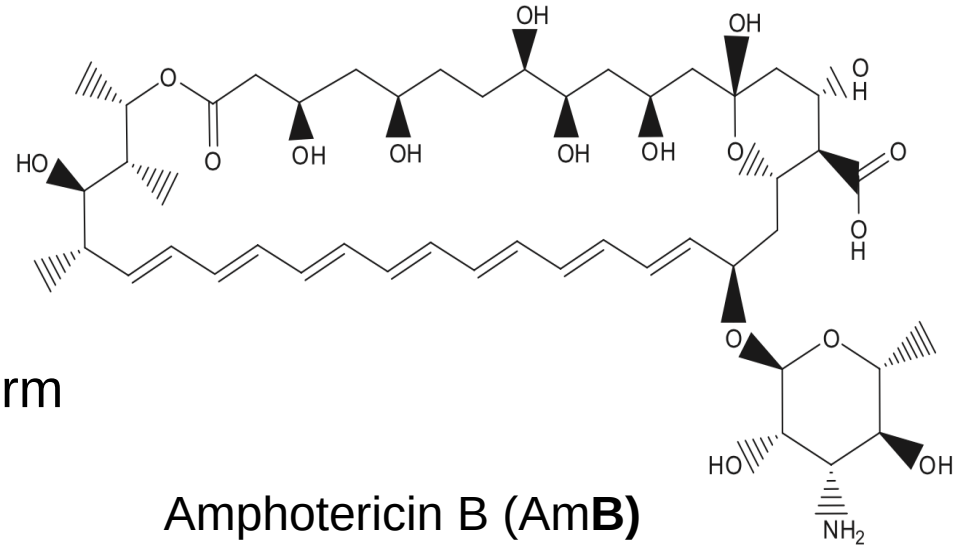
❖ Disadvantages

- **Cytotoxic, side-effects**
- **Resistance & high cost**

Frezard *et al.*, 2009 Mishra, J. *et al*. **2007**. *Current Medicinal Chemistry*, 14, 1153-1169.

Amphotericin B as antileishmanial drug

- Standard antifungal drug
- antileishmanial drug
- highly toxic in aggregated form



Amphotericin B (Am**B)**

- To reduce its cytotoxicity and to target macrophages, various strategies have been used to incorporate AmB in different nano-carriers

  - e.g. nanoemsulsions, micelles, liposomes

Objectives of the current research

NEW APPROACHES TO ANTILEISHMANIAL CHEMOTHERAPEUTICS

- **Investigate (SYNTHESIZE NOVEL DRUGS & IMPROVE EXISTING)**

- NEW

- SAFER (minimum side effects)

- COST-EFFECTIVE

- Orally Administrable (TIME & AVOID MUSCULAR PAIN)

Mishra, J. *et al*. **2007**. *Current Medicinal Chemistry*, 14, 1153-1169.

Specific Objectives

- Incorporation of AmB in nanosystem to reduce its cytotoxicity

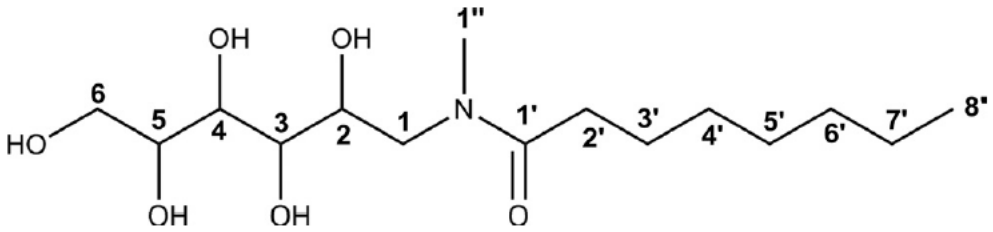
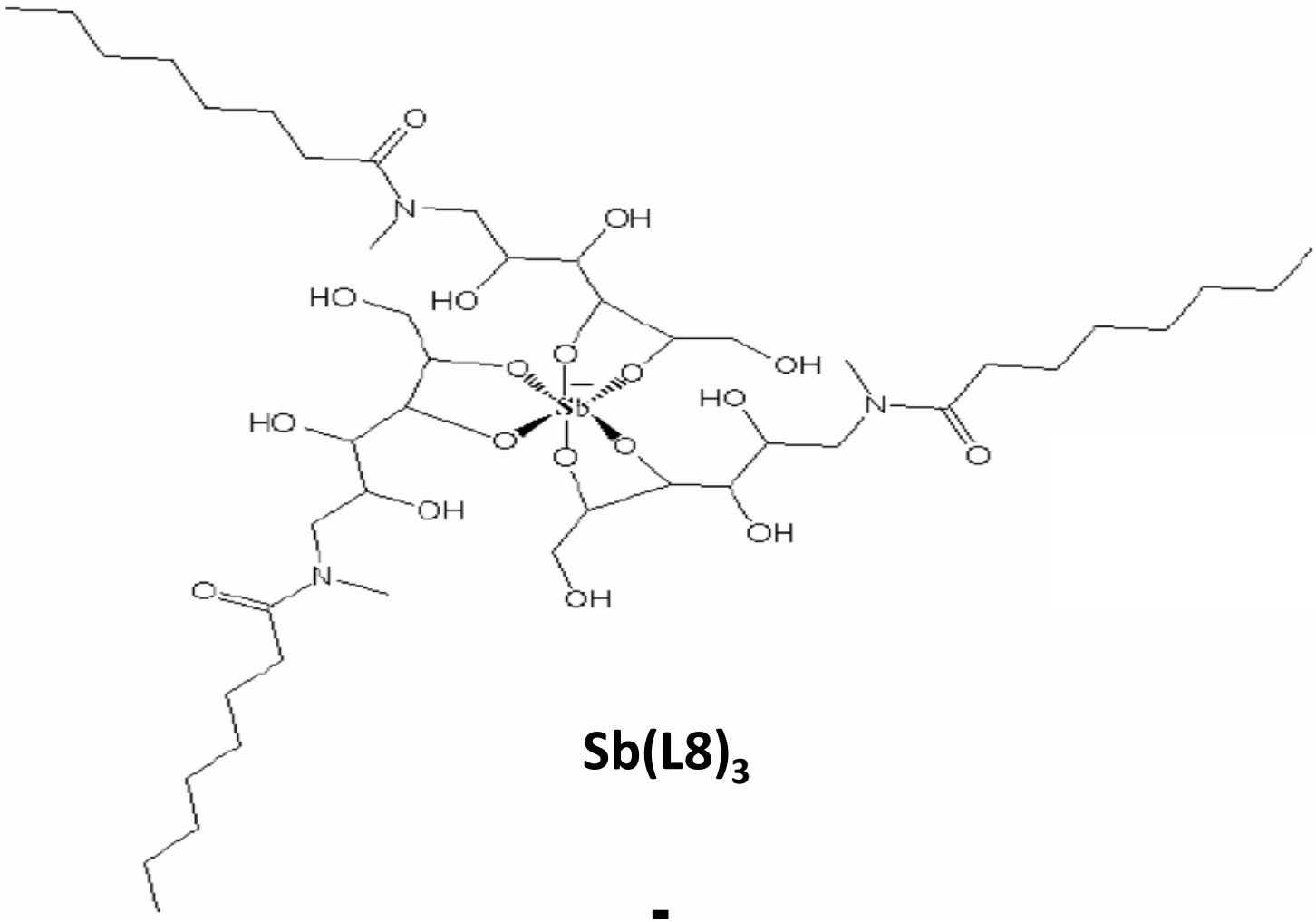


FIG 1 Structure of octanoyl *N*-methyl-D-glucamide (L8).

- Kinetics and stability studies of the SbL8-AmB and SbL10-AmB formulation
- *Evaluation of the* antileishmanial activity of the new formulations *in vivo*

Preparation and characterization of amphiphilic antimony(V) based dispersions SbL8 and SbL10 (alkylmethylglucamide series)

-



Sb(L8)$_3$

For **LYSIS,**

-

Flaviana R. Fernandes et al. 2013. Antimicrobial Agents and Chemotherapy p. 4229–4236

Analysis of AmB content and drug encapsulation efficiency (EE) by an HPLC-based technique

Preparation/incoporation of AmB in SbL10 and SbL8 dispersions

AmB + SbL8/SbL10
0.05 g/L AmB + 0.2 g/L of SbL8 or SbL10
pH 7.2

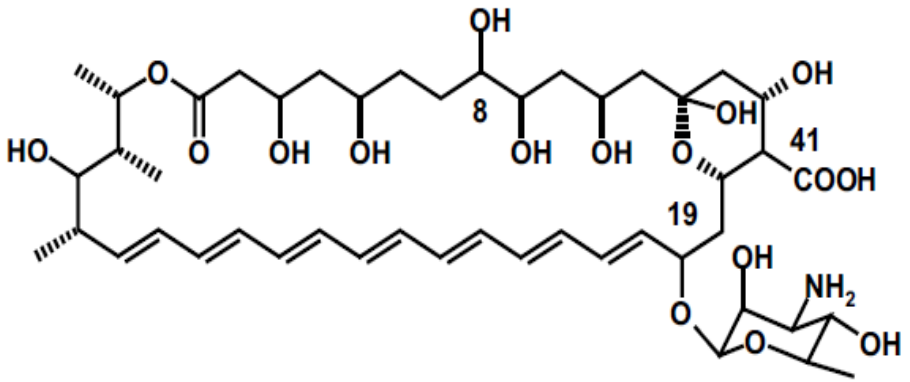
Encapsulation efficiency (EE) using reverse phase HPLC technique

EE% = [(Quantification of AmB before filtration - AmB after filtration)/AmB before filtration]x100

Leila R. C. et. al., 2014. *European Journal of Pharmaceutical Sciences*

Incorporation of AmB in SbL8 nanoassemblies

SbL8-AmB 0.2%



Amphotericin B (AmB)

Low oral absorption and high toxicity related to its aggregation state

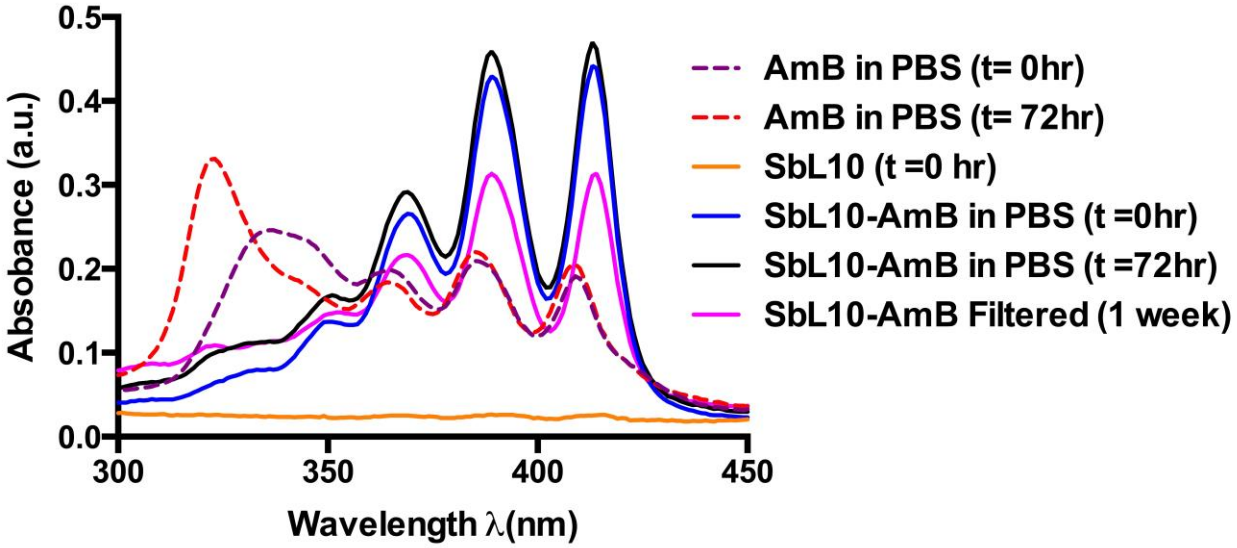
AmB incorporation efficiency (HPLC)

SbL8	SbL10
84%	83%

UV absorption spectra of formulations SbL10-AmB

AmB aggregation state after dilutionof **SbL10-AmB** mixed composition

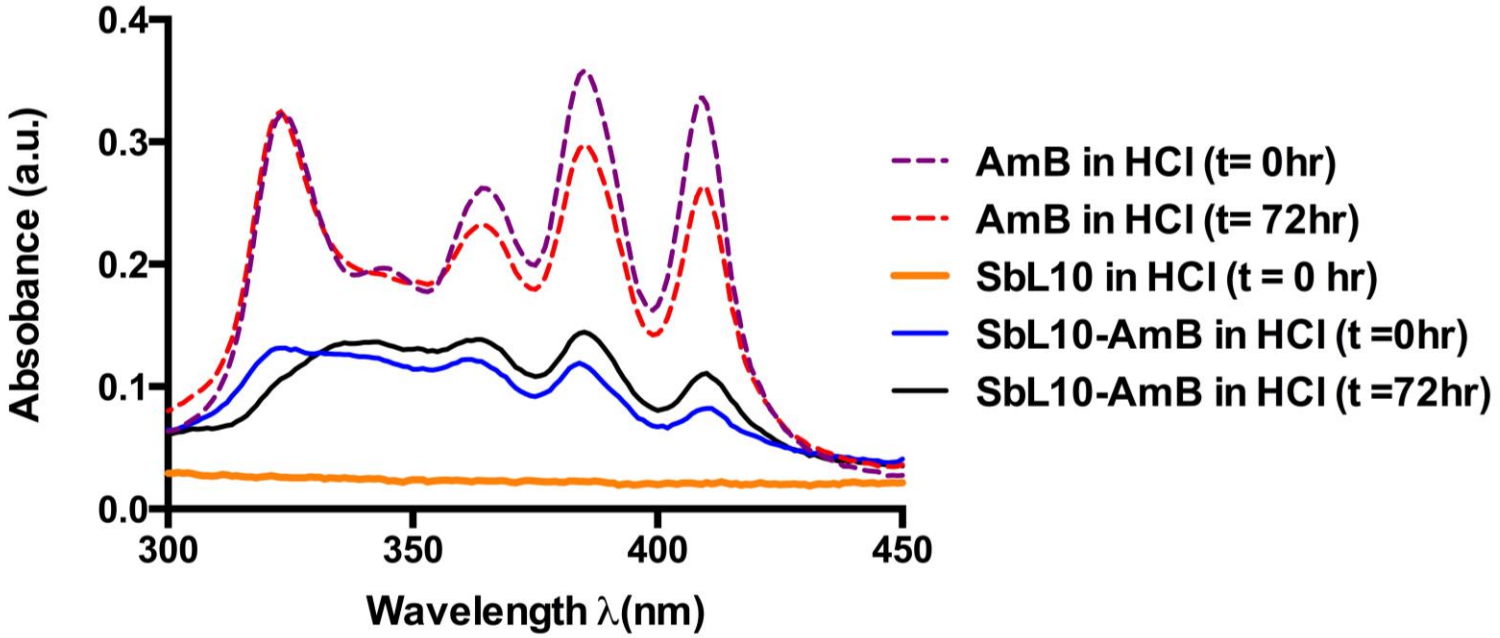
Uv-*vis* absorption spectra of AmB (Free and incorporated in SbL10) in PBS



Uv-*vis* absorption spectra of AmB (0.005 g/L) after incorporation of 0.2% solution in amphiphilic Sb(V) complex SbL10 (0.6 M) at 0 hr (Blue) and 72 hr (Black), AmB (free) at 0 hr (purple) and 72 hr (Red) in PBS solution (pH 7.2). SbL10 in PBS (Orange). SbL10-AmB filtered with 0.45 μm filter (Magneta). Quartz cuvette with an optical path of 1 cm.

Circular dichroism spectra of formulations SbL10

Uv-*vis* absorption spectra of AmB (Free and incorporated in SbL10) in HCl



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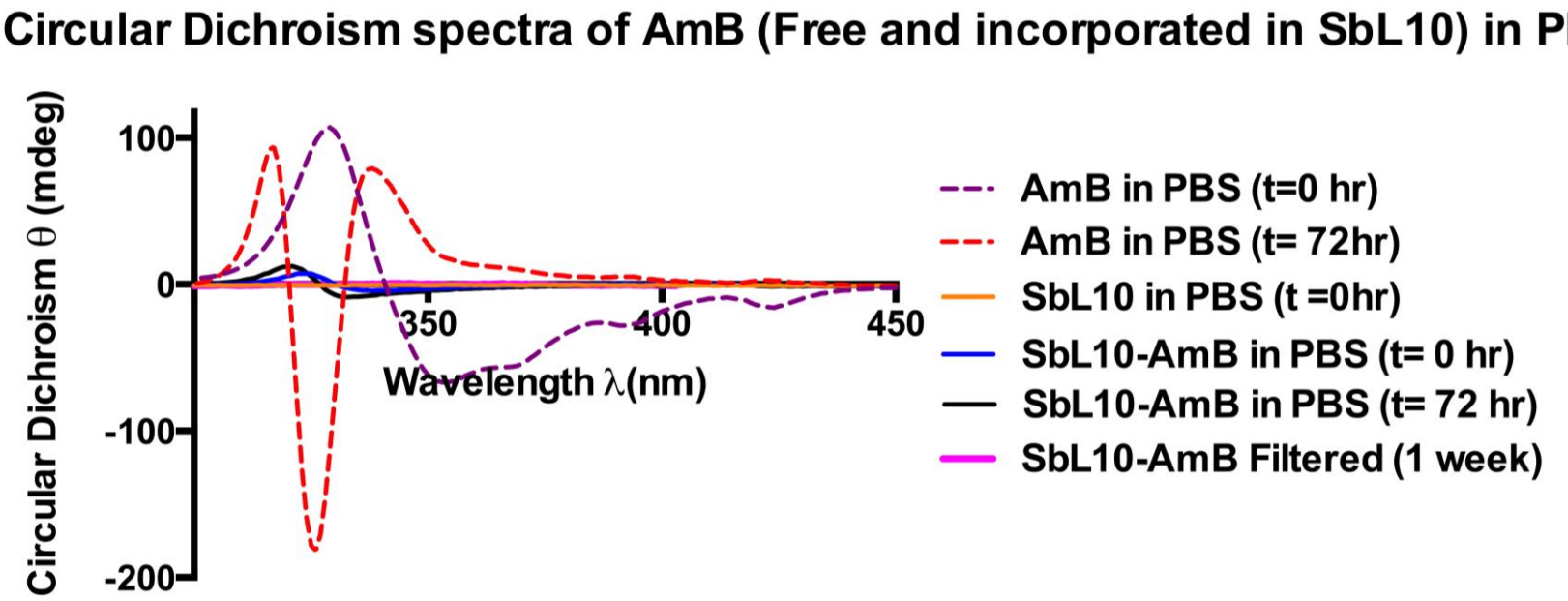
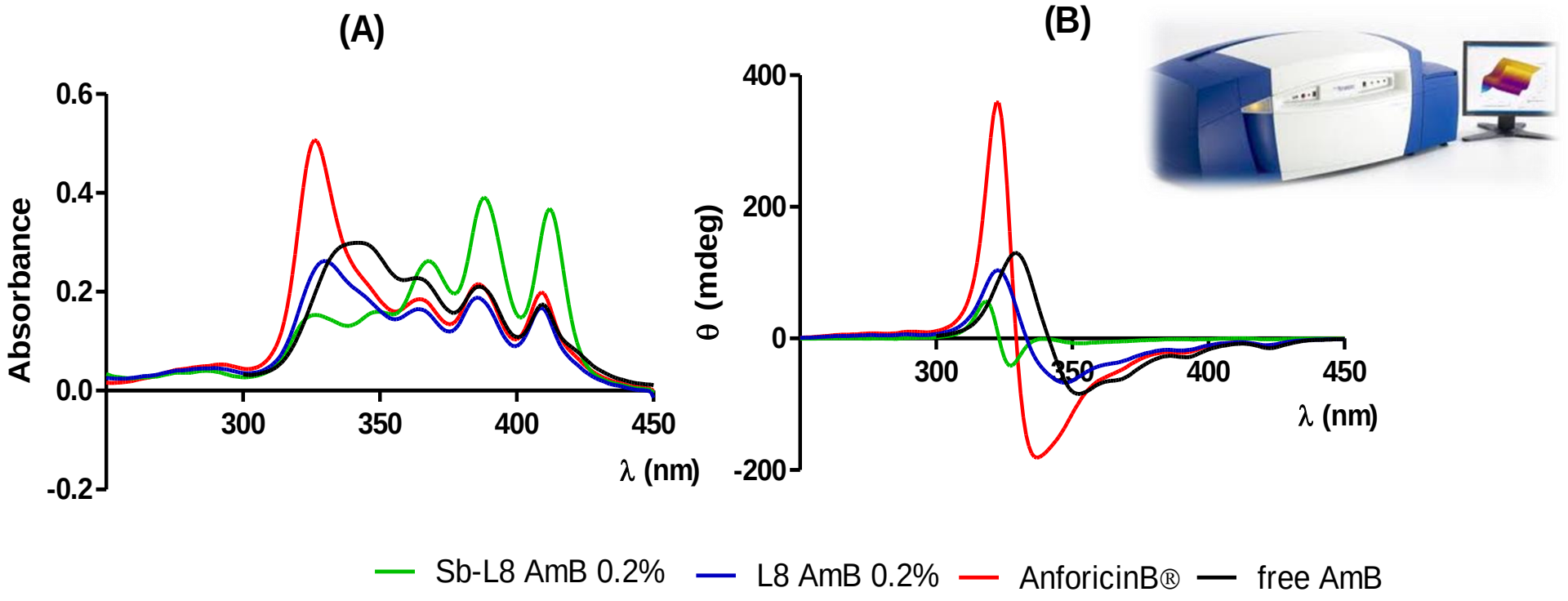
Circular dichroism spectra of formulations SbL10



Circular Dichroism spectra of AmB (0.005 g/L) after incorporation of 0.2% solution in amphiphilic Sb(V) complex SbL10 (0.6 M) at 0 hr (Blue) and 72 hr (Black), AmB (free) at 0 hr (purple) and 72 hr (Red) in PBS solution (pH 7.2). SbL10 in PBS (Orange). SbL10-AmB filtered with 0.45 μm filter (Magneta). Quartz cuvette with an optical path of 1 cm.

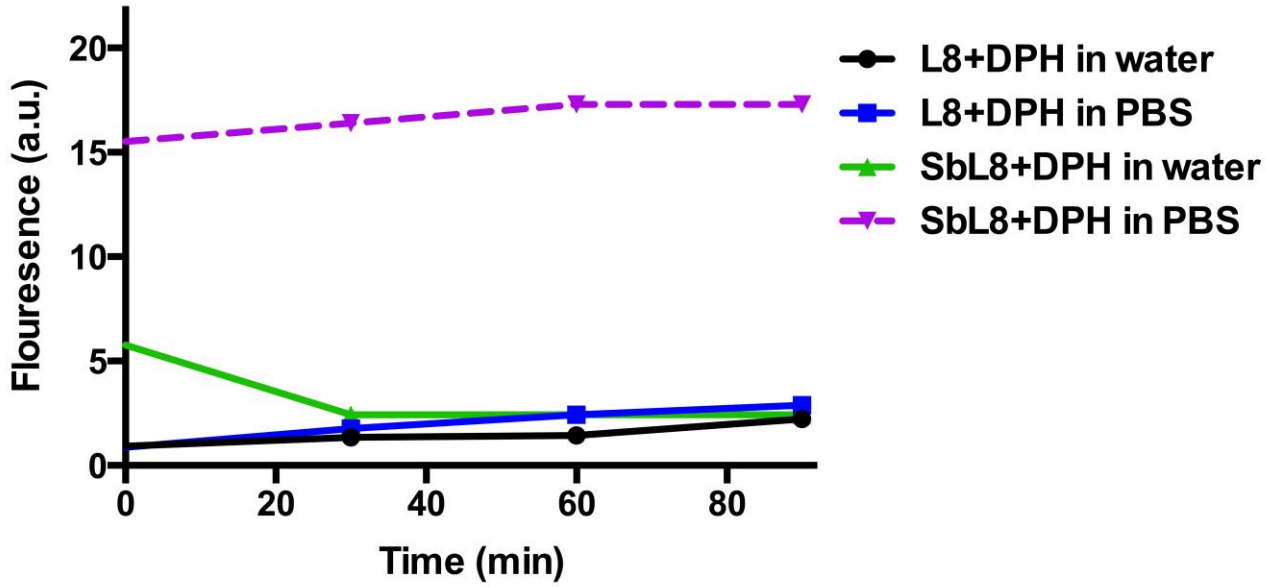
AmB aggregation state after dilution of **SbL8/AmB** mixed composition

UV/Vis Absorption and Circular Dichroism Spectra



Kinetics studies/Stability in solution: Variation of flouresecence intensity of DPH probe after interaction with nanosytems SbL8

Variation of floursecence intensity of DPH ($5x10^{-7}$ M) probe:

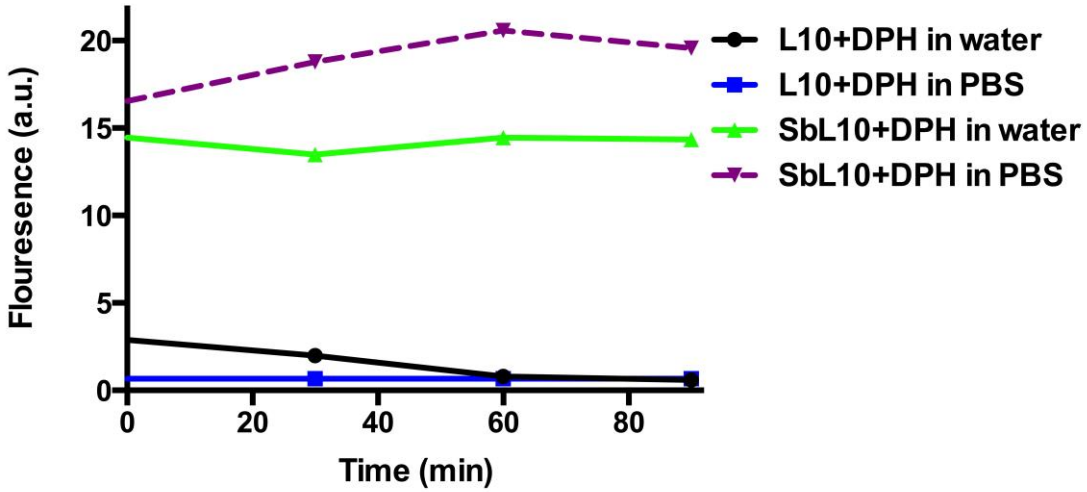


Variation of floursecence intensity of DPH ($5x10^{-7}$ M) probe:
Variation of DPH flouresecence intensity after interaction with L8 and SbL8 (diluted from 50 mM to 0.5 mM) dispersions in deionized water, PBS (pH 7.2) and HCl (0.5 M) at 37 °C ***vs.*** Time (min.) obtained in kinetics mode.

References:

Kinetics studies/Stability in solution: Variation of flouresecence intensity of DPH probe after interaction with nanosytems SbL10

Variation of floursecence intensity of DPH ($5x10^{-7}$ M) probe:



Variation of floursecence intensity of DPH ($5x10^{-7}$ M) probe:
Variation of DPH flouresecence intensity after interaction with L10 and SbL10 (diluted from 50 mM to 0.1 mM) dispersions in deionized water, PBS (pH 7.2) and HCl (0.5 M) at 37 °C ***vs.*** Time (min.) obtained in kinetics mode.

References:

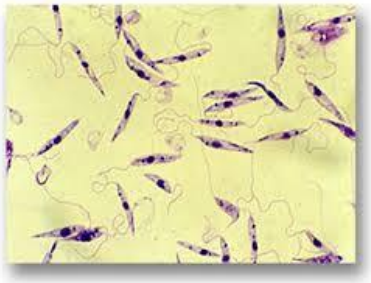
Infection and treatment protocol of mice infected with *L. amazonensis* and *L. infantum*

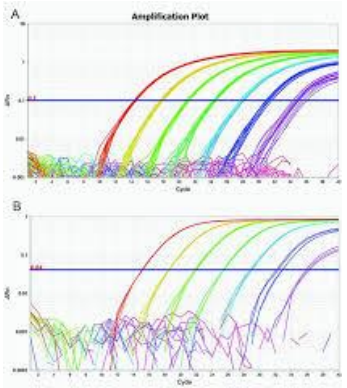
SbL8/SbL10 - 170 mg Sb/kg

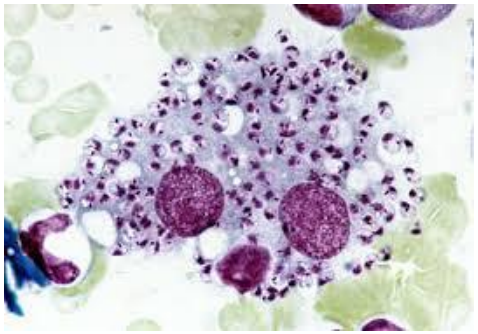
AmB-Doc – 14 mg/kg Oral

AmB-Doc – 1 mg/kg IP (Control)

SbL8/SbL10 – Sb 170 mg/kg + AmB 14mg/kg







Day 1 – Infection:
intraperitoneal inoculum of
10^7 metacyclic *Leishmania infatum/amaz*
promastigotes .

Day 7 or 35
Infection confirmation
(imprint)

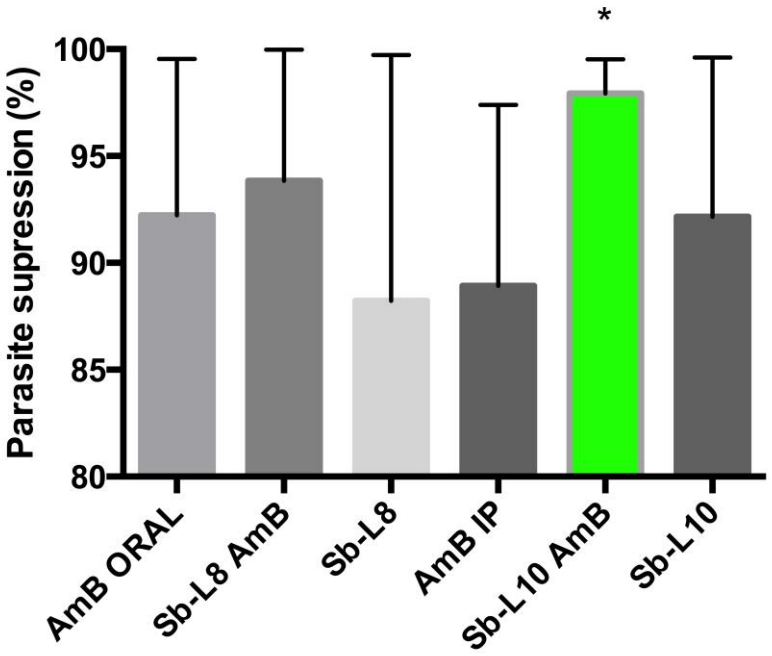
Beggining of
treatment

Day 28 or 65–
Last day of
treatment

Day 38 –
Euthanasia

Analysis
of qPCR

Antileishmanial activity of **amphotericin B/SbL10 or AmB/SbL8** mixed composition by oral route in a murine model of VL (in vivo)

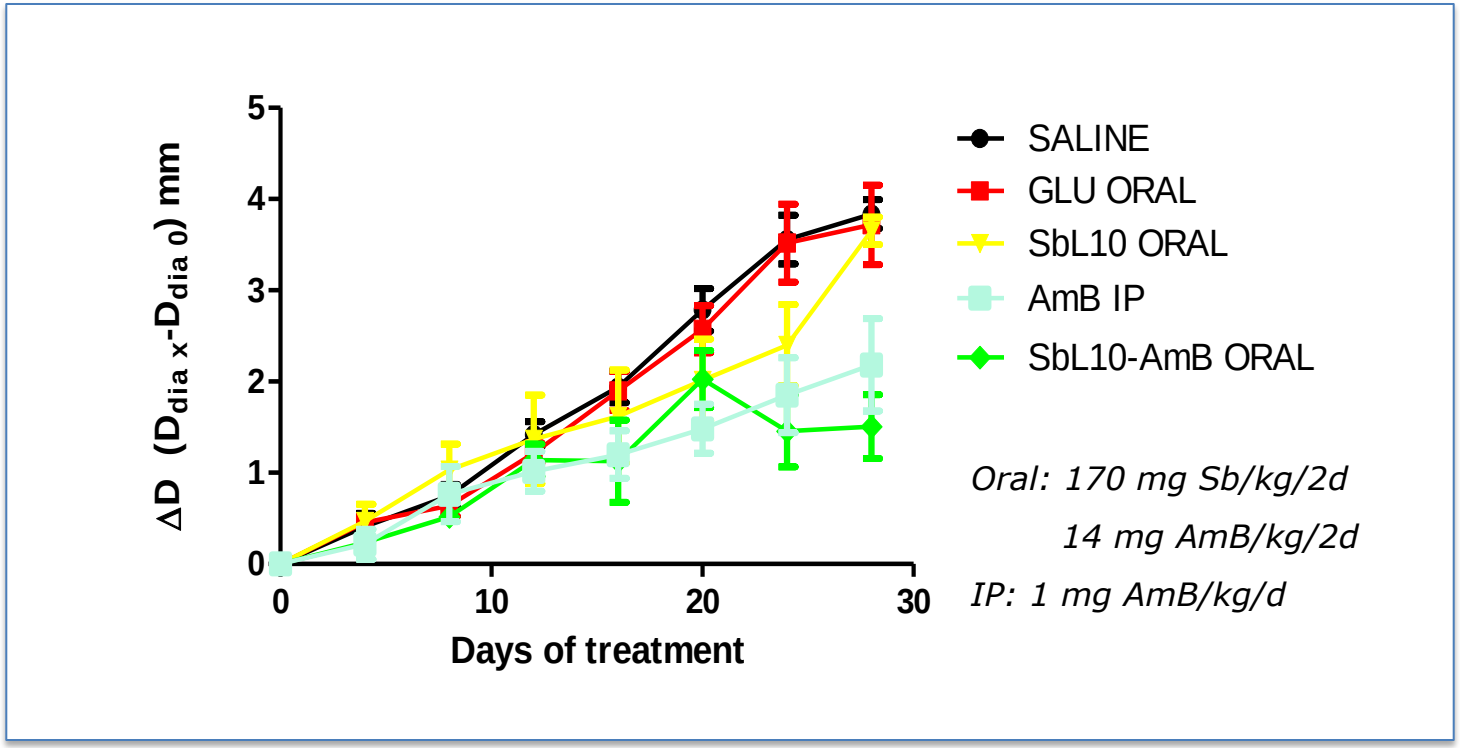


qPCR of Balb/c mice live parasite load infected with *Leishmania chagasi* and treated with following formulations: Anforicin® oral 13,6mg of AmB/kg/day; Sb-L8-Anfotericin oral, 170mg of Sb/kg/day and 13,6mg of AmB/kg/day; Sb-L8 oral 170mg of Sb/kg/day, AmB intreperitoneal 0,9mg/kg in each 4 days; Sb-L10-AmB oral 170mg of Sb/kg/day and 13,6mg of AmB/kg/day and Sb-L10 170mg of Sb/kg/day. One-way ANOVA with Bonferroni post test, *p<0,05.

Nanoparticle system of Sb-L10 AmB was the most effective treatment in reducing live parasite load in infected mice.

Summation or Synergism

Antileishmanial activity of **amphotericin B/SbL10** mixed composition by oral route in a murine model of CL



Conclusions

- AmB is highly admissible in the monomeric form in the SbL8 and SbL10 dispersions
- SbL8-AmB and SbL10-AmB formulations are stable in solution upon dilution at neutral pH as well at acid pH
- SbL10-AmB is less cytotoxic than SbL8-AmB formulation *in vivo*
- This study established for the first time the potential of mixed SbL10-AmB and SbL8-AmB formulations for the oral treatment of both cutaneous and visceral leishmaniasis, indicating their potential for further development and applications.

Thank you