ALGINATE NANOPARTICLES AS CARRIER OF MILTEFOSINE FOR USE IN THE CANDIDIASIS TREATMENT.

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INTRODUCTION

Previous studies *in vitro* and *in vivo* indicate miltefosine (MFS) as an alternative for fungal infection treatments due to its broad spectrum of action and fungicidal effect; but it presents high renal and hepatic toxicities. Thus, the aims of this study was to produce a formulation of MFS in alginate-based nanacarrier (MFS.Alg) for toxicity reduction; and to evaluate the anti-*Candida* efficacy using invertebrate model *Galleria mellonella*.

METHODS

Alginate + Oil fase → Emulsion

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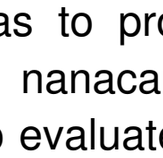
Drug encapsulation efficiency % (EE): $100\left[-\frac{\text{drug in supernatant}}{\text{starting drug}} \times 100\right]$

Drug release kinetics *in vitro*

Stability → storage at -20ºC for 7, 15, 30 and 90 days

Toxicity



Galleria mellonella larvae

Red blood cells

- Free miltefosine (MFS)
- Alginate nanoparticles (Nano.Alg)
- Encapsulated miltefosine (MFS.Alg)

Candida albicans SC5314 or IAL-40 strains

- Free miltefosine (MFS)
- Encapsulated miltefosine (MFS.Alg)

RESULTS

Table 1. Data of the physical characteristics (size, polydispersity and zeta potential) of drug-free alginate nanoparticles (Nano.Alg) and encapsulated miltefosine (MFS.Alg)

	Size (nm)	Pdi	Zeta (mV)
Nano.Alg	330.3 ± 20.6	0.35 ± 0.05	-25.3 ± 4.07
MFS.Alg	314.0 ± 32.6	0.31 ±0.05	-27.2 ± 3.01

CONCLUSION

Taken together, the alginate nanoparticles as carriers for miltefosine reduced its toxicity effect due to sustained and controlled release profile. Importantly, MFS.Alg nanoparticles were effective in the candidiasis treatment using *G. mellonella* invertebrate model promoting greater survival of infected larvae and reduced fungal burden and *Candida* filamentation. Thus, MFS in alginate-based nanocarrier as a drug controlled delivery system could be an interesting alternative to treat invasive fungal infections such as candidiasis.

RESULTS

Table 2. Stability of the size, polydispersion (Pdi) and zeta potential characteristics of the alginate nanoparticles containing encapsulated miltefosine, lyophilized with 10% trehalose and stored at -22 °C along 3 months.

	Size (nm)	Pdi	Zeta (mV)
Before lyophilization	291.6 ± 19.2	0.39 ± 0.09	-19.0 ± 3.1
After lyophilization	311.2 ± 20.1	0.38 ± 0.06	-24.9 ± 10.5
7 days	379.8 ± 11.1	0.38 ± 0.01	-25.1 ± 9.0
15 days	345.9 ± 78.8	0.43 ± 0.23	-24.0 ± 5.7
30 days	313.4 ± 9.9	0.26 ± 0.01	-22.7 ± 0.07
90 days	741.8 ± 9.9	0.89 ± 0.01	-38.1 ± 2.2

The encapsulation efficiency of miltefosine was 81.7 ± 6.64 %.

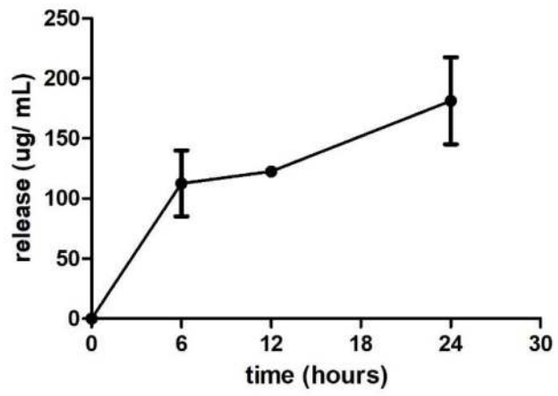


Figure 1. Release of miltefosine from the alginate nanoparticles at 37°C, with constant agitation, in 6, 12 and 24 h.

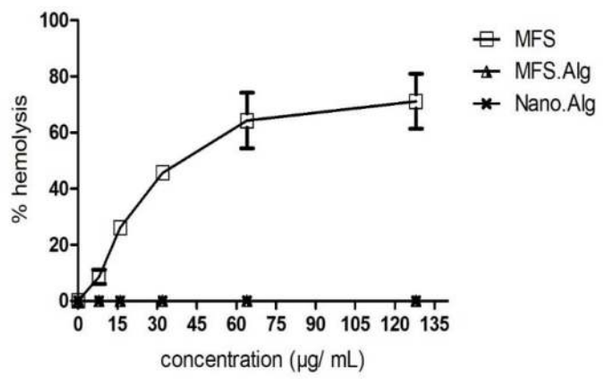


Figure 2. Hemolytic activity of free miltefosine (MFS), incorporated in the nanoparticles (MFS.Alg) and nanoparticles without the drug (Nano.Alg).

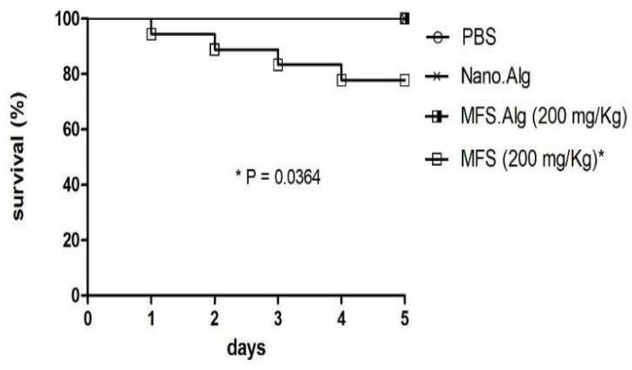


Figure 3. Toxicity in *Galleria mellonella* larvae of free miltefosine (MFS), incorporated in the alginate nanoparticles (MFS.Alg) and alginate nanoparticles without the drug (Nano.Alg).

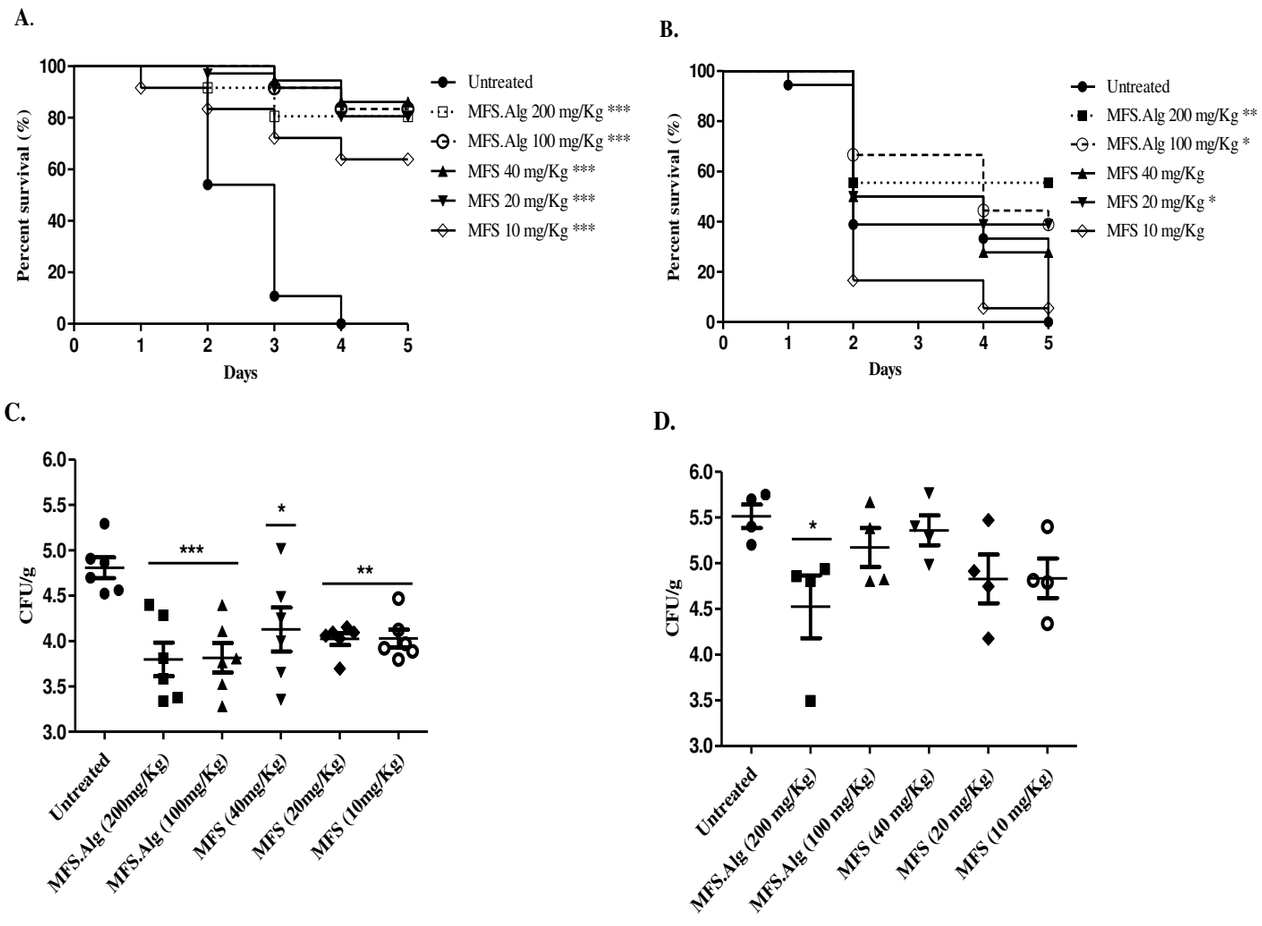


Figure 4. Antifungal efficacy of miltefosine in alginate nanocarrier (MFS.Alg) on candidiasis using invertebrate model of *Galleria mellonella*. Survival curve **(A-B)** and fungal burden **(C-D)** of larvae infected with $5x10^5$ yeasts/larvae of *Candida albicans* SC5314 **(A and C)** and *Candida albicans* IAL-40 **(B and D)** untreated and treated with MFS.Alg or free MFS. *p<0.05, **p<0.01 and ***p<0.001 when compared with untreated larvae group (ANOVA one-way).

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