# PLGA and PLGA-PEG nanoparticles loaded with curcumin and their effect on aflatoxin B1 *in vitro*.

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## ABSTRACT

Aflatoxin B1 (AfB1) is a potent hepatotoxic and hepatocarcinogenic mycotoxin. Lipid peroxidation and oxidative DNA damage are the main manifestations of toxicity induced by aflatoxin B1 that could be counteracted by antioxidants. The aim of this study was to evaluate the effect of nanoparticles prepared with polylactic-co-glycolic acid (PLGA) and PLGA with polyethylene glycol (PLGA-PEG) loaded with curcumin in human hepatocytes exposed to aflatoxin B1 in vitro.

Some biomarkers associated to oxidative stress were estimated. The results showed an increase in GSH concentration in cells exposed to nanoparticles with curcumin. PLGA-PEG nanoparticles showed more significant difference compared to control. These results suggest that curcumin loaded nanoparticles can modulate cellular effects associated with oxidative stress in vitro.

***Keywords***: curcumin, nanoparticles, plga, plga-peg, aflatoxin b1.

## 1 INTRODUCTION

Aflatoxins are toxic metabolites produced for food during growth of the fungal groups Aspergillus flavus and Aspergillus parasiticus [1,2].

Aflatoxin B1 is a potent hepatotoxic and hepatocarcinogenic mycotoxin. Lipid peroxidation and oxidative DNA damage are the main manifestations of toxicity. Many parts of plants, such as curcumin, have been studied as protectors against liver damage associated with lipid peroxidation as in the toxicity induced by aflatoxin B1 [2]. Unfortunately, it has been shown that the low aqueous solubility and limited oral bioavailability are major obstacles in its development as a therapeutic agent [3].

### 1.1 PLGA and PLGA-PEG Nanoparticles

The new drug delivery systems have had a huge impact on medical technology [4]. For efficient drug release, the nanoscale range of the particles is critical, because this can facilitate the increase in drug cellular uptake, directed at target cells, which in turn, enhances the viability of therapeutic compounds [5].

PLGA is part of a family of biodegradable polymers approved by the FDA which are highly biocompatible and have been extensively studied as delivery vehicles for drugs, proteins and other macromolecules such as DNA, RNA and peptides. [1,3,6].

PLGA is a co-polymer of polylactic acid (PLA) and polyglycolic acid (PGA). Is best defined biomaterial available for drug administration with respect to design and performance [7]. However, the need for better delivery formulations that incorporate a variety of drugs and methods of administration has resulted in the development of various types of block copolymers with polyethylene glycol (PEG). This PEG layer serves as a barrier and reduces interactions with foreign molecules by steric repulsion, leading to greater storage stability.

## 2 MATERIALS AND METHODS

### 2.1 Preparation of PLGA and PLGA-PEG Nanoparticles loaded with Curcumin.

The preparation of both PLGA and PLGA-PEG nanoparticles loaded with curcumin was using the technique called nanoprecipitation. 75 mg of PLGA or PLGA-PEG and 3.8 mg of curcumin were dissolved in 10 mL of acetone, the solution was added dropwise to a solution of 1% of PVA (poly Vynil Alcohol) under agitation, the stirring was maintained for 10 min. Subsequently the organic solvent (acetone) is evaporated in a rotary evaporator. The resulting suspension was centrifuged, preparing a system containing 5 mL of glycerol, 20 mL of the nanoparticles suspension and 10 mL of deionized water, the system is centrifuged at 15000 rpm for 1 h, the supernatant was removed, collecting 5mL near to the interface and stored at 4°C protected from light.

### 2.2 Determination of encapsulation efficiency.

The determination of curcumin encapsulated was performed by spectrophotometry, previously performing a calibration curve. To determine the encapsulation efficiency, 2 mL of nanoparticle suspension (both PLGA and PLGA-PEG curcumin loaded) were placed in vials to constant weight and placed in an oven at 60 ° C for 24 h to allow water evaporation, subsequently the resulting solid is weighed, and it was suspended in 2 mL of acetonitrile. The system was

gentle stirred for 4 h to allow curcumin diffuse into the solvent, finally it was centrifuged at 13000 rpm for 20 min, the supernatant was removed and diluted 1:5 with ethanol (80%) and the estimation was performed by spectrophotometry .

The encapsulation efficiency was calculated by the following equation:

$$Encapsulation\ efficiency\ (\%) = \frac{(\text{Total drug content in nanoparticles})}{(\text{Total drug amount})} x100$$

## 2.3 Characterization of Nanoparticles

Characterization of particle size and zeta potential were performed by dynamic light scattering and laser doppler electrophoresis respectively in Malvern Instruments Zetasizer device. Regarding morphology was performed by scanning electron microscopy (SEM).

## 2.4 Experimental Model

To determine the hepatoprotective effect of Curcumin nanoparticles against AfB1 induced intoxication, was used as a model cell line HepG2 [8,9].

## 2.5 Cell culture and exposure scheme.

Cells were maintained in culture medium Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 2% antibiotic at 37°C and 5% of $CO_2$. Curcumin was dissolved in dimethylsulfoxide (DMSO) and then diluted with culture medium to adjust the desired concentrations, the final concentration of DMSO in culture was not greater than 0.1%, likewise, the controls with 0.1% DMSO were included in all experiments.

Cells were seeded in 24-well plates until confluence. Initially exposure to AfB1 (25 μM) for 4 h was performed, then a second exposure with free or encapsulated curcumin at different concentrations for 4 h was done.

## 2.6 Cell viability (MTT)

Two hours before exposure time completion described above, 5 mL of MTT reactant were added in a concentration of 5 mg/mL. After the exposure time has elapsed, the culture medium was removed and added 0.3 mL of a buffer nonidet in isopropanol and left under gentle stirring for 20 min. Finally the solution was removed and the viability is determined by spectrophotometry in Elisa plate reader (mrc scientific instruments).

## 2.7 Determination of reduced glutathione (GSH)

Once the exposure time is completed, the culture medium is removed from the wells, washed 2 times with cold PBS and cells were harvested by scraping. The cell suspension was centrifuged at 12000 rpm for 10 min at 4°C, then the supernatant is removed and the button resuspended in PBS with protease inhibitor. The suspension is sonicated and 10 μL of this suspension are taken for protein determination. To the remaining suspension is added 5 μL of sulfosalicylic acid (5%) and it is incubated at room temperature for 10 min, finally centrifuged at 12000 rpm for 10 min at 4°C and the supernatant is recovered. 50 μL of this solution was taken and placed in a well of a 96-well microplate, finally 150 μL of reaction buffer are added and the GSH in the sample is quantified in Elisa plate reader at λ = 412 nm.

## 2.8 Statistical analysis.

The data are presented with standard error of the mean (SEM) analyzed using ANOVA of one via. All experiments were performed at least 6 times independently.

# 3 RESULTS AND DISCUSSION

Table 1 shows results obtained for curcumin loaded nanoparticles of PLGA and PLGA-PEG, note that the PLGA nanoparticles, despite having a larger size, have a lower encapsulation percentage.

## 3.1 Curcumin Nanoparticles Characterization.

Table 1. Results of the development of Curcumin nanoparticles of PLGA and PLGA-PEG

| Polymer | Encapsul. Efficiency (%) | Part. Size (nm) | PDI | Zeta Potential (mV) |
|---|---|---|---|---|
| PLGA | 22.26 | 261.2 | 0.095 | -1.46 |
| PLGA-PEG | 64.46 | 104 | 0.171 | -1.625 |

This PLGA encapsulation efficiency can be attributed to several factors. First, both PLGA and PLGA-PEG have hydrophobic nature, for that reason, it is easy for them to catch the hydrophobic curcumin. Second, the hydrophobic nature of curcumin, which results in minimal migration of the drug to the external aqueous phase during the nanoparticles formation [10].

In the PLGA-PEG nanoparticles, PEG chains are oriented toward the external aqueous phase, enveloping the PLGA-curcumin complex. This PEG layer serves as a barrier and reduces interactions with foreign molecules by steric repulsion as well as giving a hydrophilic property, so these PEG chains provide greater stability, reducing interaction and preventing migration of curcumin outwards, giving greater load stability [7].

## 3.2 Nanoparticles Morphology

The morphology was performed by scanning electron microscopy (Jeol SEM JSM6010LA).

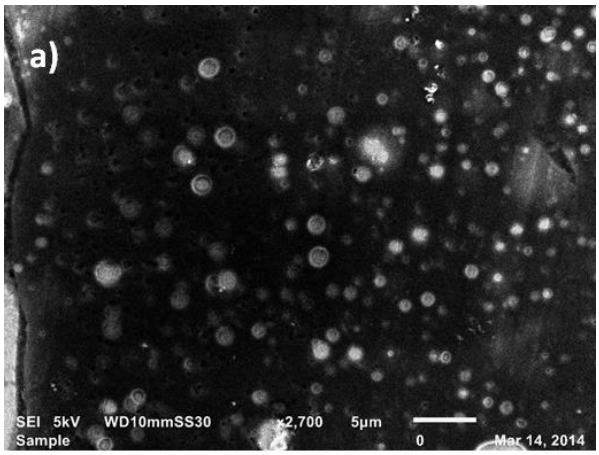


Figure 1: PLGA Curcumin Nanoparticles SEM Micrography.

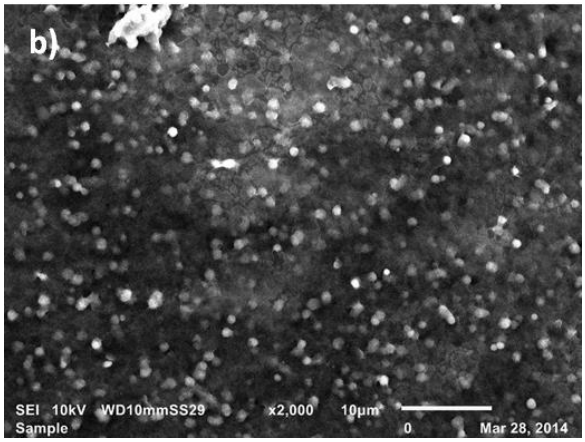


Figure 2: PLGA-PEG Curcumin Nanoparticles SEM Micrography.

In SEM micrographs for Curcumin Nanoparticles (Figure 1 and 2) we can see spherical nanoparticles with an uniform size distribution for both PLGA and PLGA-PEG nanoparticles, having a polydispersity index (PDI) of 0.095 and 0.171 respectively.

## 3.3 Cell viability (MTT)

Figure 3 shows the results of the exposure of HepG2 cells against a concentration of 25 µM of AfB1 for 4 h, and subsequent exposure to free curcumin and Curcumin Nanoparticles at concentrations of 0.2, 2, 5 and 10 µM. This results showed viability above of 100% in both Curcumin Nanoparticles (PLGA and PLGA-PEG).

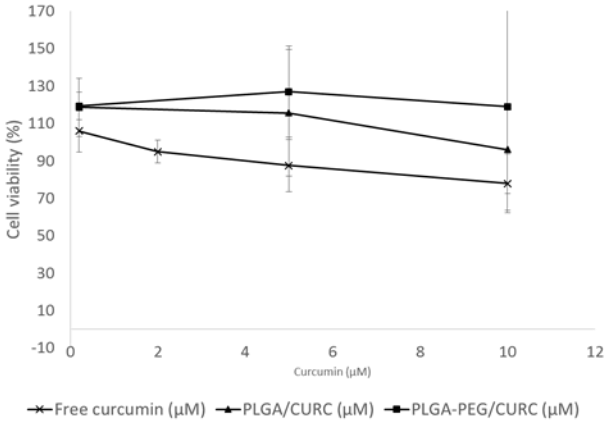


Figure 3: Viability of HepG2 cell line post exposure for 4 h to free curcumin and Curcumin Nanoparticles at different concentrations (0.2, 2, 5 and 10 µM), after an exposure with AfB1 (25 µM) for 4h.

Figure 3 shows the means of the treatments studied, which shows that there were significant differences between the values of cell viability treatments of free curcumin and treatments with Curcumin Nanoparticles for both PLGA and for PLGA-PEG, denoting those differences starting in 0.2 µM, increasing cell viability, even on the treatment of major concentration of free curcumin.

These results clearly show the hepatoprotective effect of curcumin against mycotoxin intoxication. However, due to the decrease in cell viability generated by free curcumin and AfB1 be totally different processes, as mentioned above, curcumin can change the morphology of the cell surface and lead to a pro-apoptotic process, this process is dose-dependent [11,12]; on the contrary, the process of cytotoxicity of aflatoxin, wherein the oxidative stress is the main toxic effect [1], so further studies are needed to define more accurately the hepatoprotective effect of curcumin over AfB1 intoxication.

## 3.4 Determination of reduced glutathione (GSH)

In Figure 4 the results for the quantification of GSH in HepG2 cells are shown. Although no statistically significant difference between treatments are seen, except between free curcumin at a concentration of 2 µM and 20 µM and Curcumin Nanoparticles of PLGA at 0.2 µM and 20 µM compared with the control, in the figure 4 we observed a tendency wherein the amount of GSH present in the sample significantly increases when concentration of free curcumin and encapsulated curcumin in PLGA and PLGA-PEG nanoparticles increases, since concentrations of 0.2, 2 and 20 µM.

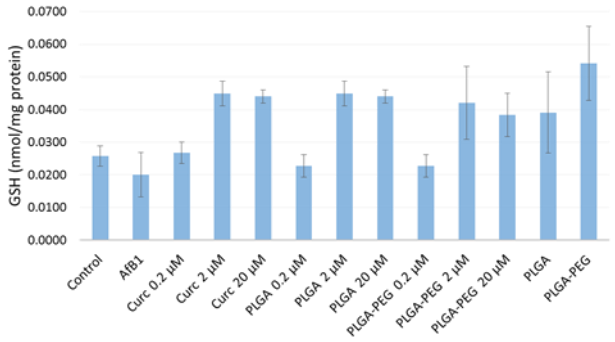


Figure 4: Quantification of GSH in HepG2 cells exposed to Curcumin Nanoparticles post an exposure of AfB1 25 µM for 4h. Error bars correspond to standard error of the means.

This is consistent with previous studies by Pou Kuan Leong et. al [13]. Where exposure of AML12 cells to curcumin was performed, finding an increase in GSH levels, coupled with a decrease in lipid peroxidation. The greatest effect of curcumin on the stimulation of GSH production may be related to their ability to directly induce dissociation of Keap 1 protein, a Nrf2 repressor, with an antioxidant modulation [13].

## 4 CONCLUSIONS

PLGA and PLGA-PEG nanoparticles loaded with curcumin were prepared, obtaining better results in terms of particle size and loading of curcumin with the polymer PLGA-PEG. The cell viability assay showed that the encapsulated curcumin has a better protective effect against AfB1 intoxication than free curcumin, showing an increased intracellular concentration of GSH, having a tendency in increasing GSH in cells treated with Curcumin Nanoparticles. Based on these results, we can say that the Curcumin Nanoparticles prepared with PLGA and PLGA-PEG have potential as a treatment for AfB1 intoxication.

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