

# DEOXYNIVALENOL INDUCES APOPTOSIS IN CELL CULTURES AND THE ANTIBIOTIC FOSFOMYCIN PROTECTS CELLS FROM NUCLEAR CHANGES

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## INTRODUCTION

Deoxynivalenol (DON) is a mycotoxin which inhibits protein synthesis and causes cytotoxicity. On the other hand, Fosfomicin (cis-1,2-epoxyphosphonic acid) (FOS) is a bactericidal broad-spectrum antibiotic used in human and veterinary medicine, which inhibits cell wall synthesis. Furthermore, FOS has some extra antimicrobial properties, including phagocytosis promotion, immunomodulatory effects and protection against the toxicity caused by certain drugs.

## MATERIALS AND METHODS

On previous studies we have demonstrated that after 4 h of incubation, FOS (550 µg/mL) was able to prevent the cytopathic effect of DON (2.8 µg/mL) on Hep2 cells (Figures 1, 2 and 3). To corroborate these results and to determine whether the effect of DON was due to the induction of apoptosis, Hep2 cells were seeded in 6 well-plates at a density of  $4 \times 10^5$  cells/well, and after 24 h, cell monolayers were treated with DON at 2.8 µg/mL or with DON (2.8 µg/mL) and FOS (550 µg/mL). Four hours after treatment, cells were fixed with 4% paraformaldehyde and stained with 4',6-diamino-2-phenylindole (DAPI). The presence of nuclear morphological changes representative of apoptosis (nuclear condensation and chromatin fragmentation) was evaluated under immunofluorescence microscope. Three to 6 visual fields were observed, the percentage of apoptotic cells was determined and the results were evaluated by ANOVA/Tukey test. Experiments were assayed by triplicate.

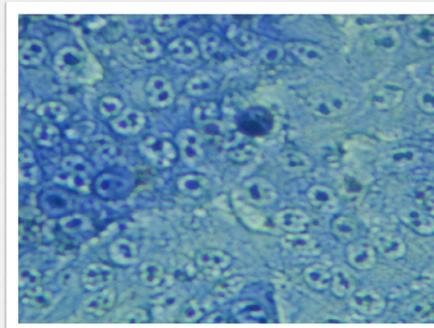


Fig. 1. Control, untreated Hep2 cells (40X - Giemsa stain).

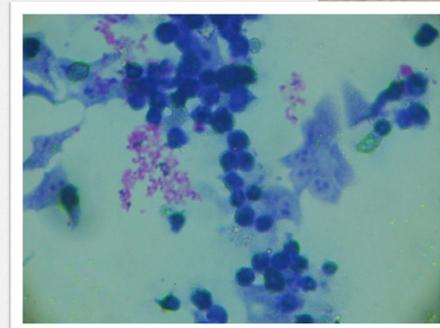


Fig. 2. Hep2 cells incubated with 2.8 µg/mL of DON in the absence of FOS (40X - Giemsa stain).

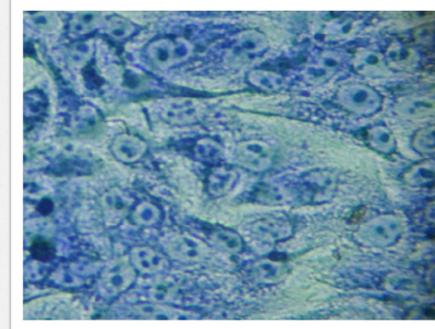


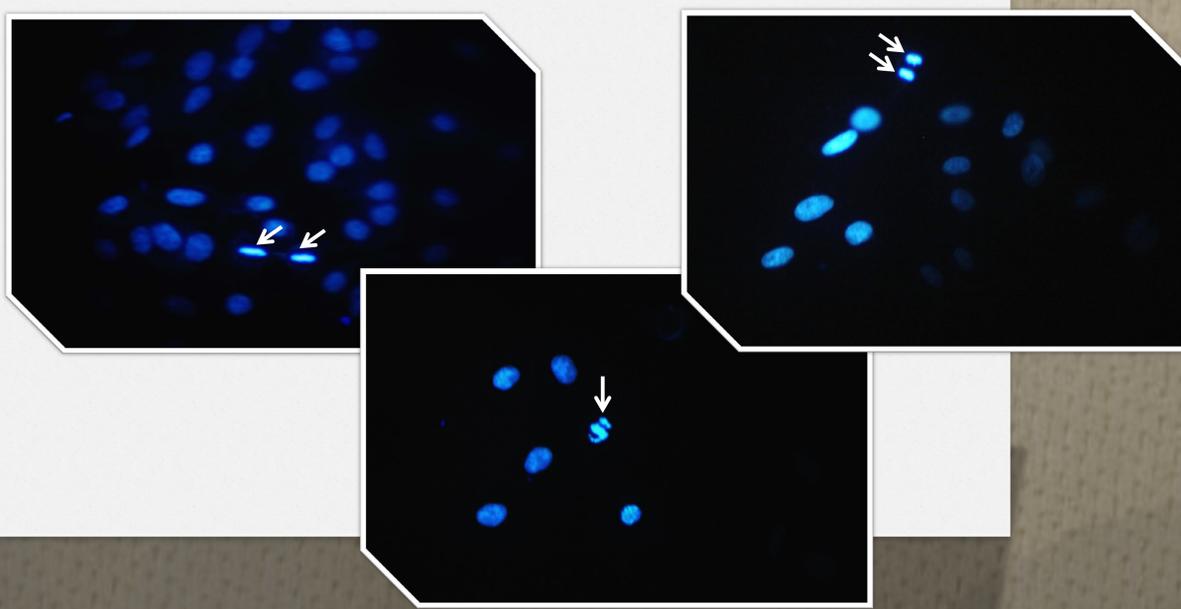
Fig. 3. Hep2 cells incubated with 2.8 µg/mL of DON and 550 µg/mL of FOS (40X - Giemsa stain).

## RESULTS

Cell cultures incubated with 2.8 µg/mL DON and 550 µg/mL FOS were similar to control wells (without antibiotic or toxin) showing the absence of cytotoxicity (Figure 4). Apoptotic cells percentage was significantly higher ( $5.64 \pm 0.27$ ) ( $p < 0.01$ ) for cells treated with DON than for cells incubated with both the mycotoxin and the antibiotic ( $0.65 \pm 0.5$ ). Figures 5, 6 and 7 show nuclear morphological changes on Hep2 cells incubated without the antibiotic, representative of apoptosis (nuclear condensation and chromatin fragmentation). Furthermore, to determine whether DON was able to induce apoptosis in different cell lines and at lower concentrations, Madin Darby bovine kidney (MDBK) and baby hamster kidney (BHK) cells were also treated with DON at 1 µg/mL for 24 h. Results show that the mycotoxin induces apoptosis in all the assayed cell lines. However, the level of induction appears to be dependent on the cell type.



Fig. 4. Hep2 cell cultures incubated with 2.8 µg/mL DON and 550 µg/mL FOS. Absence of cytotoxicity (40X - DAPI).



Figs. 5, 6 and 7. Nuclear morphological changes on Hep2 cells incubated without the antibiotic, representative of apoptosis. Arrows indicate apoptotic cells. (40X - DAPI).

## DISCUSSION AND CONCLUSIONS

This work reveals that DON induces nuclear changes in cell cultures which are indicative of apoptosis and that FOS is able to prevent the cellular effect induced by the mycotoxin in Hep2 cells. Further studies are needed to confirm that the protective effect of the antibiotic occurs in all cell lines and its implications under *in vivo* conditions.