EFFECT OF MYCOTOXINS ON HUMAN CELLS AND HEALTHY ANIMAL CELLS.

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Mycotoxins are a danger for humans because of their worldwide distribution and the pathological effects that they cause to animals, plants and man (ref.1,2). Tissue cultures offer a good 'in vitro' model for the study of mycotoxins in human cells from a toxicological approach. The use of HeLa cultures gives the facility of working resistant cells with a fast and easy technique. HeLa are uterine cancer cells of a woman called Helen Lane, where their name comes from. This line was established by Gey et al. (ref.3), and was one of the most important advances in the viral isolation and the observation of the toxic effects of different substances. The aims of this work were to know the effect of different mycotoxins: aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2); zearalenone (Z); deoxynivalenol (DON) and diacetoxyscirpenol (DAS) on HeLa human cells and on healthy kidney cells from Buffalo green monkey (BGM). Another aim was to obtain the lethal dose 50 (LD50) and the minimum dose that caused a reaction.

The worked subcultures were for HeLa the 69, and for BGM the 196; they were both on frozen vials at -170°C . The glass material was sterilized and the HeLa cells grown in a nutrient M-199 Microlab media; the BGM cells in Eagle media, both with antibiotics, trypsin and/or elastase to disperse the formed monolayer, and incubated in Falcon bottles for 3 days at 37°C . A supravital dye (neutral red) was added to detect which cells were alive and red coloured. The cells were counted in a hemocytometer taking the concentration to 400,000 cells/ml and applying the media to the 96 wells of each microplate. The mycotoxins were then applied, 4 replications of each dose. The mycotoxins were dissolved in dimethylsulphoxide (1%) and the doses for aflatoxins were 2.5, 5, 10, 50 and 100 ppb in their respective 1.2, 2.5, 5, 25 and 50 μl of solvent; for zearalenone and trichothecenes the applied doses were 1.2, 2, 4, 6, 8, 10, 20, 35 and 50 ppm in the same amount of μl of solvent. The effect was monitored after 24 hours for zearalenone, 18 hours for aflatoxins and 16 hours for trichothecenes.

The results are presented in Table 1 and show a sequence in the effects in direct proportion to the volumes and exposition time. The LD50 was obtained presenting differences in the toxicity of the mycotoxins. It was not possible to obtain the LD50 for DAS because all the cells died with the smallest dose of 1.2 ppm.

Table 1: Values of lethal and minimum response doses of mycotoxins on HeLa and BGM cells.

Mycotoxin	Lethal doses 50		Minimum response doses	
	HeLa	BGM	HeLa	BGM
AFB1 (ppb)	10.0	25.0	2.5	2.5
AFB2 (ppb)	-	10.0	50.0	5.0
AFG1 (ppb)	-	10.0	50.0	5.0
AFG2 (ppb)	50.0	10.0	50.0	10.0
Z (ppm)	6.0	4.0	4.0	2.0
DON (ppm)	8.0	6.0	4.0	2.0
DAS (ppm)	< 1.2	< 1.2	< 1.2	< 1.2

HeLa = Uterus cancer human cells.
 BGM = Healthy kidney cells of Buffalo green monkey.
 AFB1 = Aflatoxin B1.
 AFB2 = Aflatoxin B2.
 AFG1 = Aflatoxin G1.
 AFG2 = Aflatoxin G2.
 Z = Zearalenone.
 DON = Deoxynivalenol.
 DAS = Diacetoxyscirpenol.

The HeLa cells are less susceptible to the mycotoxins than BGM cells. The AFB1 was the most toxic one in both cultures; DAS killed all the cells in both cultures with all the doses; Zearalenone was the less aggressive one. The BGM cells were more susceptible to all mycotoxins because they come from a healthy organ and the HeLa cells were more resistant because the cancer cells are already mutated or transformed.

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