Zearalenone Induced Cytotoxicity and Oxidative Stress in Human Peripheral Blood Leukocytes

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clearance, Ethic Committee 2013, School of Chemistry, Autonomous University of Coahuila, México]. blood was centrifuged at 2500 ×g for 5 min at 4°C. supernatant was removed and leukocytes were isolated and resuspended in 1 mL of PBS (pH 7.4), and cell concentration was determined using a Neubauer hemocytometer. 1×10^5 cells/mL was treated with concentrations of ZEA (10, 20, 40 and 80 µg/mL) and cells treated with vehicle (DMSO) served as a negative control. All samples were incubated for 1 h at 37°C.

Trypan blue exclusion test: Following ZEA treatment, cell viability was assessed by trypan blue exclusion. 20 µL of every sample were mixed with 20 µL of the dye (1:1). Viable cells possess intact cell membranes that exclude certain dyes. In contrast, damaged cells incorporating the dye. Finally, cells were counted to determine the percentage of live cells. All tests were performed in triplicate and repeated at least three times.

Neutral red uptake assay: neutral red test is based on the ability of viable cells to incorporate and bind the supravital dye neutral red in the lysosomes [19,20]. incubation, 20 µL of neutral red and 400 µL PBS (pH 7.4) were added and incubated for 3 h at 37°C.
cells were washed twice with 400 µL of a solution (50 % ethanol, 1% acetic acid and 49 % distilled water). Absorbance was measured at 540 nm using the plate reader and the results were expressed as index of cell inhibition by the following formula:

[DO of treated cells/DO of untreated cells] × 100

Lipid peroxidation assay:

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