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RESEARCH ARTICLE

Cytotoxic Effect of Aflatoxin B1, gliotoxin, Fumonisin B1, and Zearalenone Mycotoxins on HepG2 Cell Line *in vitro*

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Abstract

Mycotoxins are biologically active secondary fungal metabolites they pose a major risk, leading to acute and chronic exposures for human and animal's health. Hepatocyte cells are an important in metabolic pathways and play a major target site for these toxins for this reason we used the HepG2 cell line in our study to investigate the cytotoxicity of aflatoxin B1, gliotoxin, fumonisin B1, and zearalenone isolated and purified from local Iraqi fungal isolates, prepared at the same starting concentration, and tested on human hepatocellular carcinoma HepG2 cell line using neutral red assay, cytotoxic effect showed a dose dependent inhibition rate increased with the increase of mycotoxins concentration but this increase was significantly higher for aflatoxin B1 treated cells ($p< 0.05$) ranged between 96.7 at maximum concentration 125ng\ml to 1.3% when HepG2 treated with lower concentration 0.12 ng/ml, at the other hand zearalenone showed the lower inhibition rate when compared with other mycotoxin used in present study ranged between 77-0.12%. All the mycotoxins used in this study showed cytotoxic effect against HepG2 cell line with a significant difference between them. The latter observation in particular raises concerns over safety margins based on toxin species, and suggests that the effects of it need to be better understood to assess health risks.

1. Introduction

Mycotoxins are secondary metabolites of microscopic filamentous molds that have adverse effects on humans, animals, and crops that result in illnesses and economic losses[1]. All mycotoxins are low molecular weight natural products produced as secondary metabolites by filamentous fungi[2]. These metabolites constitute a toxigenically and chemically heterogeneous assemblage that are grouped together only because the members can cause disease and death in human beings and other vertebrates[3]. Mycotoxins are not only hard to define, they are also challenging to classify, due to their diverse chemical structures and biosynthetic origins[4], their myriad biological effects, and their production by a wide number of different fungal species *Aspergillus flavus, Aspergillus fumigatus, Fusarium verticillioides* and *Fusarium graminearum* [5,6,7], thus, mycotoxins can be classified as hepatotoxins, nephrotoxins, neurotoxins, immunotoxins, and so forth [8,9]. Cell biologists put them into generic groups such as teratogens, mutagens, carcinogens, and allergens [10].

Gauthier *et al*., [11] used a neutral red method as a simple and cheap method and gave a clear results of mycotoxin toxicity, so we suggested this method to study the cytotoxicity of mycotoxins *in vitro*. Toxic effect of mycotoxin that are obviously naturally occurring, yet are neglected at the present time in human and animal risk assessment.

The objective of this study was to assess cytotoxic effects of aflatoxin B1, gliotoxin, fumonisin B1, and zearalenone on human hepatocellular carcinoma HepG2 cell line *in vitro* using neutral red assay, concentrations of some mycotoxins were about 10 times lower than the concentrations of the other mycotoxins as it naturally occurs in real practice, also to conduct this work as a first study in Iraq to measure the cytotoxic effect of mycotoxins isolated and purified from local pollutants fungal isolates in Iraq against HepG2 cell line *in vitro*.

2. Materials and methods

Toxins. mycotoxins in present study purified from fugal isolates, which isolated from granaries of the ministry of agriculture-Iraq. Isolation and identification of fungal isolates cultured done on Sabouraud dextrose agar(SDA) at 25-37°C to prepared the isolates for mycotoxin purification steps. Aflatoxin B1 purified from *Aspergillus flavus* according to Jesus *et al*, [12], gliotoxin purified from *Aspergillus fumigatus* according to Dheeb, [13], Fumonisin B1 purified *Fusarium verticillioides* according Fumo Prep *et al*, [14], and zearalenone was purified according to Ware GM *et al*, [15] from *Fusarium graminearum*. After purification high performance liquid chromatography (HPLC) used to diagnose the mycotoxin to its standard, standard provided from Sigma-Aldrich.

Stock solution of mycotoxins were prepared by dissolving proper amount of these toxin in DMSO to prepare 250 ng/ml stock solution of each mycotoxin then stored at -20 , then working dilutions were made by culture medium immediately when used.

Culture of HepG2 cell line. Human hepatocellular carcinoma HepG2 cell line [16]. The cytotoxicity assay done at Animal Cell Culture lab/ Biotechnology Research Centre/ AL-Nahrain University. Cells were cultured in DMEM media supplemented with 10% fetal bovine serum, L-glutamine, Non essential amino acids, HEPES, Sodium Bicarbonate, streptomycin and penicillin. Cell line grown as a monolayer in humidified 5% CO2 incubator at 37°C. The experiments were performed when cells were healthy, active and at logarithmic phase of growth [17].

Cytotoxicity assay. To detect the growth inhibition of HepG2 cell line, culture of this cell line was incubated with different concentrations of each mycotoxin, eleven concentrations used as follows (125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95, 0.97, 0.48, 0.24, and 0.12 ng/ml) used in a triplicate form of each toxin to investigate its cytotoxic effect respectively. Negative control was achieved by incubating HepG2 cell line with the culture medium.

Neutral red assay. In order to provide a reliable basis for the cytotoxic effects of mycotoxin, it was first necessary to assess the individual effects of mycotoxins [18]. After elapsing the incubation period, withdrawal the old medium, the plates washed with PBS then 100µl/well of freshly prepared neutral red dye were added to each well then plates incubated for 2 hrs, viable cells will uptake the dye and the dead not, the plates washed by PBS to remove the excess dye, then 100µl/well of eluent buffer were added to each well for withdrawal the dye from the viable cells. Optical density for the plates were measured by using ELISA reader at 492nm wave length [19,20].

Percentage of the inhibitory rate was measured according to the formula [21] as follows:

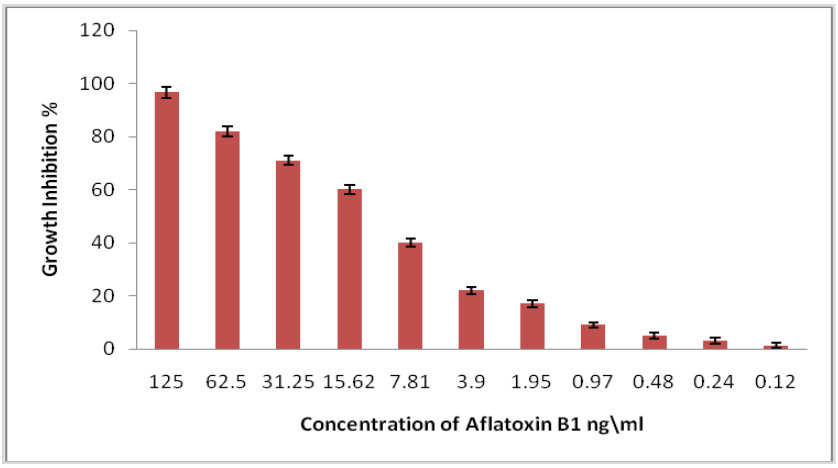
$$\text{I.R \%} = \frac{\text{O.D. of control - O.D. of test}}{\text{O.D. of control}} \times 100$$

Statistical Analysis. The obtained results (The values of the investigated parameters were given in terms of mean ± standard error) were statistically analyzed using Duncan's multiple range test in SAS software (version 17;SAS Inc., Chicago,IL, USA). The level of significance was $P > 0.05$ [22].

3. Results

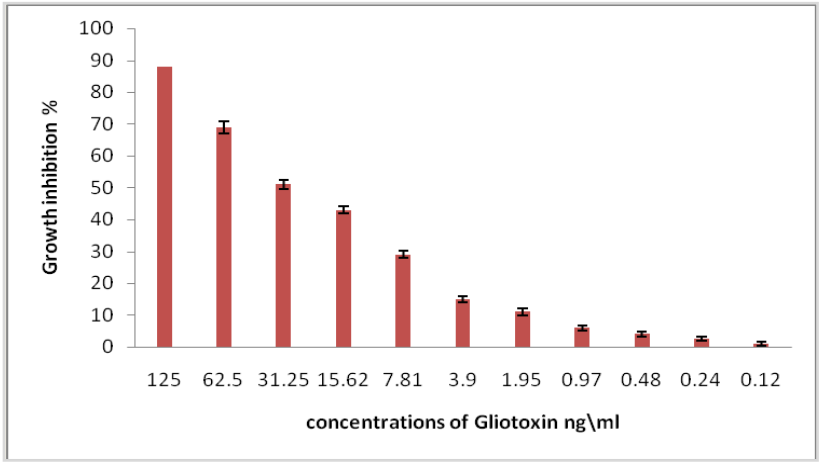
*Cytotoxic Effect of Aflatoxin B1***.** The percentage of aflatoxin B1 growth inhibition values obtained by the neutral red assay represented in Figure (1) appeared that after 24 hours incubation growth inhibition of HepG2 cell line was increased with the increase of aflatoxin B1 concentration when compared with the negative control. Aflatoxin B1 has significant differences of cytotoxic effect on HepG2 cell line ($P<0.05$), 96.7%, 82%, 71%, 60%, 40%, 22%, 17%, 9%, 5%, 3%, 1.3% these percentage of growth inhibition rate were showed at concentrations 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95, 0.97, 0.48, 0.24, and 0.12 ng/ml respectively.

FIGURE 1: Cytotoxic effect of purified Aflatoxin B1 from *Aspergillus flavus* on HepG2 cell line after an incubation period of 24 hours.



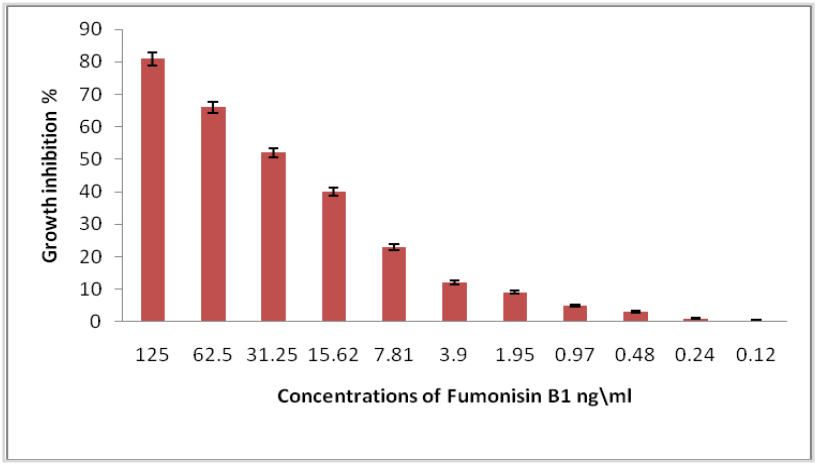
Cytotoxic Effect of gliotoxin. The analyses of effect of gliotoxin clearly indicates that significantly cytotoxic effect ($P < 0.05$) was observed on growth of HepG2 cell line when incubated with these concentrations 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95, 0.97, 0.48, 0.24, and 0.12 ng/ml with growth inhibition percentage 88%, 69%, 51%, 43%, 29%, 15%, 11%, 6%, 4%, 2.5% and 1%, respectively as shown in the Figure (2). Results showed that growth inhibition rate was a concentration dependent, there was increase in the inhibitory effect when compared with the negative control.

FIGURE 2: Cytotoxic effect of purified Gliotoxin from *Aspergillus fumigatus* on HepG2 cell line after 24 hours incubation period.



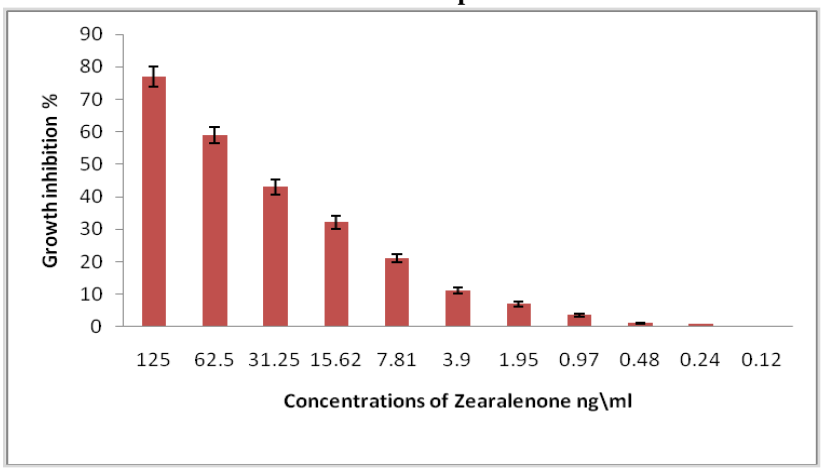
Cytotoxic Effect of Fumonisin B1. Clear cytotoxic effects were shown during the treatment of the HepG2 cells with different concentrations of fumonisin B1 (*Fusarium verticillioides* toxin), cytotoxic effect of individual toxin on the HepG2 cells was first determined by measuring the percentage of cell growth inhibition after incubation for 24 hours. The cell growth inhibition was a dose dependent manner with statistically significant effects ($P<0.05$) observed at the concentration ranged between 125 to 0.12 ng/ml Figure (3). Maximal effects were obtained at 125 ng/ml, at which growth inhibition reach to 81% when compared with the negative control, while minimal cytotoxic effect 0.4% obtained when treated with 0.21ng\ml Fumonisin B1.

FIGURE 3: Cytotoxic effect of purified Fumonisin B from *Fusarium verticillioides* on HepG2 cell line after an incubation period of 24 hours.



Cytotoxic Effect of Zearalenone. Treatment of HepG2 with Zearalenone and determining their cytotoxic effects can provide useful information about the cytotoxic potential of Zearalenone. The data about cytotoxic activity of zearalenone is visible through the rate of growth inhibition, growth inhibition was a concentration dependent with statistically significant cytotoxic effects (P<0.05), 77%, 59%, 43%, 32%, 21%, 11%, 7%, 3.5%, 1%, 0.61%, 0.1% growth inhibition was shown when HepG2 cells incubated with gradual concentrations of Zearalenone, 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95, 0.97, 0.48, 0.24, and 0.12 ng/ml respectively Figure (4).

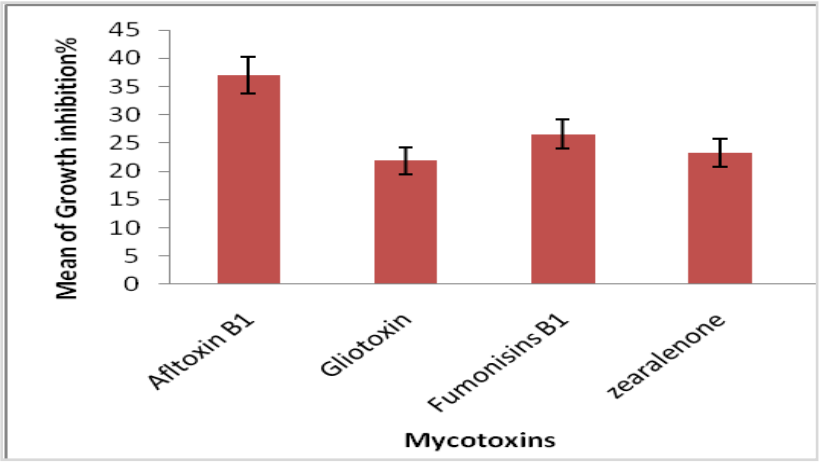
FIGURE 4: Cytotoxic effect of zearalenone from *Fusarium graminearum* on HepG2 cell line after 24 hours an incubation period.



Comparison between the mean of the cytotoxic effect of Mycotoxins on HepG2 cell line. When comparing the four tested mycotoxins on HepG2 cell line statically, the results indicated clear comparative cytotoxic effects against the HepG2 cell line when exposure to similar concentrations and same period of incubation after 24 hours and that effect was concentration dependent, however mean cytotoxic effect of these mycotoxins could be classified in increasing order of mean toxicity, we found that the toxicity aflatoxin B1> Fumonisin B1> zearalenone > gliotoxin observed Significant difference in cytotoxic effect (P<0.05) Figure (5).

The present data demonstrated that all mycotoxins gave different percentage of cytotoxic profile in all concentrations, and its generally cleare cytotxicity of all mycotoxin increase gradually with the increase of studied concentrations.

FIGURE 5: comparison between the mean of cytotoxic effect Mycotoxins on HepG2 cell line after 24 hours incubation time.



4.Discussion

Cell culture can be a more sensitive and reproducible method for preliminary screening of toxicity of mycotoxins [23,24]. Cytotoxicity assays in general are able to detect many mycotoxins that potentially inhibit the biochemical activity of numerous animal or human cell cultures [25]. Several methods have been developed to assess the cytotoxicity induced by mycotoxins, like neutral red assay [26,27,28].

Aflatoxin B1. The effects of Aflatoxin B1 on cell are directly correlated with the concentration of and the duration of the exposure [29]. Aflatoxin B1 showed the highest inhibition rate of HepG2 cell line *in vitro*. This result may be due to Aflatoxin B1 is both lipid and water soluble, which enables it to pass easily through cell membrane and into cellular organelles[30]. The cytotoxic effect of Aflatoxin B1 observed in the present experiment is in line with some other "*in vitro*" studies from the literature, [31,32,33]. These literatures results confirmed that toxicity of aflatoxin B1 attributed to number of biochemical parameters, such as binding to DNA, and inhibition of RNA and protein synthesis, it may be that the ease with which an aflatoxin interacts with a macromolecule, such as DNA, determines its biological and toxicological activity and bind covalently to macromolecules to form protein and DNA adducts [34].

The high percentage of growth inhibition may be due to Aflatoxin B1 interacted strongly with the cell, this result agreed with Corrier *et al.* [35] he studied aflatoxin B1 in a similar manner to induce cytotoxic effects in normal human lymphocytes, and may be correlates with a study by Massy *et al*., [28] which showed that when immune cells were directly treated with Aflatoxin B1 cell proliferation and antibody formation were decreased.

Gliotoxin. The biological activity of any chemical compound is based on active groups and an internal bridge, gliotoxin have an internal disulfide bridge that can bind and inactivate proteins via a sulfide:thiol exchange [35] therefore the cytotoxic effect of gliotoxin maybe attributed to its disulfide bridge. The disulfide bridge allows the cross linking with proteins via cysteine residues and generate deleterious reactive oxygen species (ROS) through the redox cycling between the reduced and oxidized form. This mechanism of ROS generation is believed to be responsible for the toxicity of gliotoxin [36]. The ROS generated as a result was reported to facilitate the release of cytochrome c and apoptosis inducing factors from mitochondria, leading to caspase activation, as well as other events that mediate cell death [37].

Cytotoxic effect of gliotoxin in our result may be attributed to that gliotoxin induce morphological changes in the cells, these changes in normal cell line are due to a loss in the adherence of the cells to their plastic container[38], which is characteristic of an apoptotic process[39], this phenomenon has been described for gliotoxin on different cell types: thymocytes, lymphocytes, spleen cells, or macrophages [40]. Gliotoxin inhibited oxidative burst of human neutrophils, gliotoxin also causes damage to the ciliated respiratory epithelium *in vitro* and this property might assist *A. fumigatus* in the colonization of the respiratory mucosa . Furthermore, Nierman [41] have shown by the genome-wide gene expression profile analysis that gliotoxin genes are upregulated in germination during initiation of infection in mice [37].

Fumonisin B1. Toxicity of fumonisin B1 was represented by inhibition of cell growth, this exhibition may be its act as proliferation enhancing and growth inhibiting effects [42,43,44] in a panel of cultured cell lines, and its characterized as a tumor initiator and a tumor promoter [45], these activities help us to explain the cytotoxic effect of fumonisin B1 in our results. The cytotoxic effect of fumonisin B1 may also due to presence of an amino group and the location of the hydroxyl on the CI4/CI5of FBI may play an important role in both the toxic and cancer initiating activities of fumonisin B1. It has been shown that the presence of the amino group facilitates the conjugation of fumonisin B1 via gluteraldehyde to protein carriers [46].

The mechanisms of fumonisin BI cytotoxicities are complex, it's possible to interpret these mechanisms through its activity as a competitive inhibitor of sphingosine and sphinganine *N* aceyltransferase, which are key components in the pathways for *de novo* biosynthesis of sphingolipids [47]. The effects of sphingolipids on cell growth are complex. Free sphingoid bases both stimulate and inhibit cell growth and therefore the accumulation of sphinganine in FBI treated cells is likely to play a role in the cytotoxic affect of cell growth and [48]. Our results agreed with the results of studies on renal epithelial cell line, LLC-PKI, have shown a close association between the concentrations of fumonisin B1 that inhibit sphingolipid biosynthesis and cause growth inhibition and toxicity. This inhibition is supposed to be an early event in the cytotoxicity of fumonisin B1 [49].

However previous studies have shown that fumonisin BI is more cytotoxic at low concentrations. The cell viability of a rabbit kidney cell line (RK13) was reduced to 22% by treatment with 0.51µM fumonisin BI [50].

zearalenone. Cytotoxic activity following HepG2 cell line exposure to zearalenone at present study, agree with the results in previous studies by other investigators on different cell line[51,52], our results could be attributed to its ability to induce lipid peroxidation, DNA damage, DNA fragmentation, DNA methylation [50]. Zearalenone toxicity, may be attributed that it's biotransformed predominantly into a- Zearalenone (a-ZOL) and to lesser extent, into b-zearalenol (b- ZOL) in the glucuronide forms [51,52,53]. Accumulation of a-ZEA might cause inhibition of cell proliferation in our system, which might be mediated by apoptotic mechanisms of cell death [54]. Zearalenone, on the other hand, was moderately toxic affect at the less concentration [54,50].

Comparison between the cytotoxic effect of Mycotoxins on HepG2 cell line.

Although all mycotoxins used in our experiment are secondary metabolites of fungi, they have totally distinct chemical properties and structures, which lead to their different toxicological mechanisms [49]. Aflatoxin B1 shows much higher cytotoxicity than other mycotoxin used in present experiment, while zearalenone show the lower percentage of growth inhibition.

The reasons for differences in the sensitivity of the HepG2 cell line to aflatoxin B1, gliotoxin, Fumonisin B1, and zearalenone may be explained by differences in the modes of action and metabolic transformation of the toxins. Metabolic transformation may either turn the parent compounds to more or less toxic products [10, 55,56,57,58].
However, the present results give clear view about the cytotoxicity of mycotoxin, these results it's important for risk assessments. To understand the nature of these cytotoxicity pathways and the site of action of these mycotoxin a further detailed study needed on the molecular, metabolic, and signal pathways, also to develop more effective detoxification and remediation strategies aiming at understanding mycotoxin impact on animal and human health [57,58].

5.Conclusion

All purified mycotoxins showed clear cytotoxic effect increased with the increase of concentrations, and Aflatoxin B1 show the higher cytotoxic activity between mycotoxins used in present study, suggests that the cytotoxic effect of it need to be better understood to assess health risks spatially about mycotoxin which pollute the grain used for human and animal food in Iraq and world.

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References

[1] Wild, C.P., Gong, Y.Y., "Mycotoxins and human disease: a largely ignored global 578 health issue". Carcinogenesis 31, 71–82. 2010.
[2] Smith, J. E. and Moss, M. Mycotoxins. "Formation, Analysis and Significance". John Wiley and Sons, Chichester. 1985.
[3] Forgacs, J., and W. T. Carll. 1962. "Mycotoxicoses". Adv. Vet. Sci. 7:273–382.1962.
[4] Graniti, A. "The evolution of the toxic concept in plant pathology", p.1–18. In R. K. Wood, A. Ballio, and A. Graniti (ed.), Phytotoxins in plant diseases. Academic Press, New York, N.Y.1972.
[5] Betina, V. "Bioactive molecules vol. 9. Mycotoxins: chemical, biological and environmental aspects". Elsevier, Amsterdam, The Netherlands. 1989.
[6] Betina, V. (ed.). "Mycotoxins: production, isolation, separation, and purification". Elsevier, Amsterdam, The Netherlands. 1984.
[7] Jonsyn-Ellis, F. E. "Seasonal variation in exposure frequency and concentration levels of aflatoxins and ochratoxins in urine samples of boys and girls". Mycopathologia 152:35–40.2000.
[8] Hsieh, D."Potential human health hazards of mycotoxins" p. 69–80. *In*S. Natori, K. Hashimoto, and Y. Ueno (ed.), Mycotoxins and phytotoxins. Third Joint Food and Agriculture Organization W.H.O. United Nations Program International Conference of Mycotoxins. Elsevier, Amsterdam, the Netherlands. 1980.
[9] Barrett, J."Mycotoxins: of molds and maladies. Environ". Health Perspect. 108:A20–A23,2000.
[10] Kuiper-Goodman, T., Mycotoxins: risk assessment and legislation. *Toxicology Letters.,* Volume 82-83; pp. 853-859, 1995
[11] Gauthier, T., Wang, X., Sifuentes Dos Santos, J., Fysikopoulos, A., Tadrist, S., Canlet, C., 469 Artigot, M.P., Loiseau, N., Oswald, I.P., Puel, O., 2012. "Trypacidin, a spore-borne 470 toxin from Aspergillus fumigatus, is cytotoxic to lung". PLoS One 7, 2012.
[12] De Jesus AE, Gorst-Allman CP, Horak RM, Vleggaar R."Large-scale purification of the mycotoxins aflatoxin B1, B2 and G1". J ChromatogrOct 19;450(1):101-4, 1988.
[13] Dheeb, B.I."Study on the pathogenicity, cytotoxicity and virulence factors of some clinical of *Aspergillus fumigatus* isolates and the use of RAPD markers to distinguish them. Ph.D thesis, Tikreet University, 2013.
[14] Szekeres A, Lorántfy L, Bencsik O, Kecskeméti A, Szécsi Á, Mesterházy Á, Vágvölgyi C. "Rapid purification method for fumonisin B1 using centrifugal partition chromatography". Epub 30(1):147-55,2013.
[15] Ware GM, Zhao Y, Kuan SS, Carman AS."Preparative method for isolating alpha-zearalenol and zearalenone using extracting disk". J AOAC Int. 82(1):90-4, 1999.
[16] Mersch-Sundermann, V.; Knasmüller, S.; Wu, X. J.; Darroudi, F.; Kassie, F. "Use of a human-derived liver cell line for the detection of cytoprotective, antigenotoxic and cogenotoxic agents". *Toxicology* 198 (1–3): 329–340, 2004.
[17] Freshney R. I. "Culture of animal cells: A manual for basic technique" 4th Ed. Wiley- Liss, A John wiley and Sons, Inc. Publication, New York, 2000.
[18] Heussner, A.H., Dietrich, D.R., O'Brien, E. 2008. "In vitro investigation of individual and combined cytotoxic effects of ochratoxin A and other selected mycotoxins on renal cells". Toxicol. In Vitro 20, 332–341,2008.
[19] Freshney, R.I.Culture of animal cells. 6th Edition . Wiley- Liss. New York, 2010.
[20] Mahony, D. E.; Gilliatt, E.; Dawson, S.; Stockdale, E. and Lee, H. S. "Vero cell assay for rapid detection of Clostridium perfringins enterotoxin" Appl. Environ. Microbiol. J., 55:2141-2143, 2011.
[21] Wang, B.; Relling, M. V. and Storm, M. C. "Evaluation of immunologic cross reaction of antiasparaginase antibodies in acute lymphoblastic leukemia (ALL) and lymphoma patients. Leukemia. J., 17:1583-1588, 2012.
[22] SAS users."Guide personal compute"r (ver.7) inst. Inc. Cary. Nc. USA, 2004.
[23] Buckle, A.E., Sanders, M.F. " An appraisal of bioassay methods for the detection of mycotoxins—a review". Lett. Appl. Microbiol. 10, 155–160, 1994.
[24] Ekwall, B. "Screening of toxic compounds in tissue culture. Toxicology" 17,127–142, 1980.
[25] Cetin, Y., Bullerman, L.B.," Cytotoxicity of Fusarium mycotoxins to mammalian cell cultures as determined by the MTT bioassay". Food Chem. Toxicol. 43, 755–764,2005.
[26] Gutleb, A.C., Morrison, E., Murk, A.J., "Cytotoxicity assays for mycotoxins produced by Fusarium strains: a review. Environ". Toxicol. Pharmacol. 11, 309–320, 2002.
[27] Reubel, G., Gareis, M., Amselgruber, W.,"Cytotoxicity evaluation of mycotoxins by an MTT-bioassay". Mycotoxin Res. 3, 85–96, 1987.

[28] Widestrand, J., Lundh, T., Pettersson, H., Lindberg, J."Cytotoxicity of four trichothecenes evaluated by three colorimetric bioassays". Mycopathologia 147,149–155, 1999.
[29] Baptista,A.S., Abdalla, C. L. Aguiar et al., "Utilization of diets amended with yeast and amino acidsfor the control of aflatoxicosis,"World Journal of Microbiology and Biotechnology,vol. 24, no. 11, pp. 2547–2554, 2008.
[30] Massy, T.E, Stewart, R.K, Daniels, *l.M*, Liu, L. "Minireview: Biochemical and Molecular Aspects of Mammalian Susceptibility to Aflatoxin B1 Carcinogenicity" Proceedings of the Society for Exp Bio and Med e., 208(3); pp. 213-227, 1995.
[31] Hamuforid, V., Ligocka, D., Buysschaert, M. Horsmans, Y., Lison, D. European Journal of Clinical Pharmacology., Volume 59; pp. 29-33, 2003.
[32] Song, Q., Lees-Miller, S.P., Kumar, S., Zhang, Z., Chan, D.W., Smith, G.C., Jackson, S.P., Alnemri, E.S., Litwack, G., Khanna, K.K., Lavin, M.F. "DNA-dependent protein kinase catalytic subunit: a target for an ICE-like protease in apoptosis"EMBO Journals., Volume 15(13); pp. 3238-46,1996.
[33] Eton, D.L., Gallagher, E.P. Mechanisms of aflatoxin carcinogenesis. Annual Review of Pharmacology and Toxicology., Volume 34; pp. 135-172. Review, 1994.
[34] Corrier, D.E. "Mycotoxicoses: mechanisms of immunosuppression" Ve Immuno and Immunopa. , Volume 30; pp. 7f:.S7, 1991.
[35] Kwon-Chung ,K. and Janyce Sugui A. J. "What do we know about the role of gliotoxin in the pathobiology of *Aspergillus fumigatus?"*Med Mycol. 47(Suppl 1): S97–103.1, 2009.
[36] Collins, J.A.; Schandl, C.A., Young,K.K.,Vesely,J.,Willingham,M.C. "Major DNA fragmentation is a late event in apoptosis".J. Histochem. Cytochem. 45 (7), 923–934, 2007.
[37] Golden, M.C., Hahm, S.J., Elessar, R.E., Saksonov, S., Steinberg, J.J. "DNA damage by gliotoxin from *Aspergillus fumigatus*. An occupational and environmental propagule: adduct detection as measured by 32P DNA radiolabelling and two-dimensional thinlayer chromatography". Mycoses 41, 97–104, 1998.

[38] Jordan, T.W., Pedersen, J.S., " Sporidesmin and gliotoxin induce cell detachment and perturb microfilament structure in cultured liver cells" J. Cell Sci. 85, 33–46, 1968.
[39] Shepherd, V.L., Vandre, D.D., Eltin, g.J.J., Montgomery, R.,"Effects of cesalin on the ultrastructure and biological properties of cultured mammalian cells" Cancer Res. 40, 225–231, 1980.
[40] Nierman, WC; Fedorova, N."3rd Advances Against Aspergillosis. Miami Beach, Florida: Subtelomeric diversity as a major force in evolution of *Aspergillus* secondary metabolism and virulence pathways"; p. 77 ,2008.
[41] Pardo J, Urban C, Galvez E.M, "The mitochondrial protein Bak is pivotal for gliotoxin-induced apoptosis and a critical host factor of *Aspergillus fumigatus* virulence in mice"J. Cell Biol ;174:509–519, 2008.
[42] Tolleson, W.H., Dooley, K.L., Sheldon, W.G., Thurman, J.D., Bucci, T.J., Howard, P.C. Chapter 21: "The mycotoxin Fumonisin induces apoptosis in cultured human cells and in livers and kidneys of rats". In: Ed L Jackson. Fumonisins in Food. Plenium Press: New York, 1996.
[43] Lim, C.W., Parker, H.M., Vesonder, R.F., Haschek, W.M."Intravenous fumonisin B1 induces cell proliferation and apoptosis in the rat". Natural Toxins., Volume 4(1); pp. 34-41,1999.
[44] Dombrik-Kurtzman, M.A., Bennett, G.A., Richard, J.L. "An optimized MIT bioassay for determination of cytotoxicity offumonisins in turkey lymphocytes" Journal of AOACInternational., Volume 77(2); pp. 512-516, 1994.
[45] Rumora, L., Kovacic, S., Rozgaj , R., Cepelak, 1., Pepeljnjak, S., Zanic Grubisic, T. "Cytotoxic and genotoxic effects of fumonisin B1 on rabbit kidney RK13 cell line" Archives of Toxicology. Volume 76(1); pp. 55-61, 2004.
[46] Azcona-Olivera, J.L, Abouzied, M.M" Plattner, R.D., Norred, W.P., Pestka, J.J. "Generation of antibodies reactive with fumonisins BI, B2 and B3 by using cholera toxin as the carrier-adjuvant" Applied Environmental Microbiology., Volume 58(1); pp. 169-173, 1998.

[47] Wang, E, Riley, R.T, Meredith, F.I, Merill, A.H. "Fumonisin Br consumption by rats causes reversible dose dependent increases in urinary sphinganine and sphingosine"Journal of Nutrition., Volume 129; pp.214-220, 2008.
[48] Merrill, A.H., Liotta, D.C., Riley, R.T. " Fumonisins: fungal toxins that shed light on sphingolipid function. Trends in Cell Biology" Volume 6(6); pp. 218 - 223,1996.
[49]Yoo, H.S., Norred, W.P., Wang, E., Merril, A H., Riley, R.T. " Fumonisin inhibition of of de nova sphingolipid biosynethesis and cytotoxicity are correlated in LLC-PK1 Cells. Toxicology and App lied Pharmacology. , Volume 114; pp 9-15, 1992.
[50] Rumora, L., Kovacic, S., Rozgaj , R., Cepelak, 1., Pepeljnjak, S., Zanic Grubisic, T."Cytotoxic and genotoxic effects of fumonisin B1 on rabbit kidney RK13 cell line". Archives of Toxicology. ,. Volume 76(1); pp. 55-61, 2002.

[51] Kouadio, J.H., Mobio, T.A., Baudrimont, I., Moukha, S., Dano, S.D., Creppy, E.E., "Comparative study of cytotoxicity and oxidative stress induced by deoxynivalenol, zearalenone or fumonisin B1 in human intestinal cell line" Caco-2. Toxicology 213, 56–65, 2005.
[52] JECFA"Opinion of the scientific committee on food on Fusarium toxinszearalenon (ZEA)".
[53] Malekinejad, H., Maas-Bakker, R., Fink-Gremmels, J., "Species differences in the hepatic biotransformation of zearalenone". Vet. J. 172, 96–102, 2006.
[54] Mirocha, C.J., Pathre, S.V., Robison, T.S.,"Comparative metabolism of zearalenone and transmission into bovine milk. Food Cosm. Toxicol. 19, 25–30, 1981.
[55] Luongo, D., De Luna, R., Russo, R., Severino, L.,"Effects of four Fusarium toxins (fumonisin B1, [alpha]-zearalenol, nivalenol and deoxynivalenol) on porcine whole-blood cellular proliferation. Toxicon 52, 156–162, 2008.
[56] Cetin, Y., Bullerman, L.B., "Cytotoxicity of Fusarium mycotoxins to mammalian cell cultures as determined by the MTT bioassay" Food Chem. Toxicol. 43, 755–764, 1981.
[57] Dutton, "Fumonisins, mycotoxins of increasing importance: Their nature and effects" Pharmacology and Therapeutics. , Volume 70; pp. 137- 161,1996.
[58] Reubel, G.H., Gareis, M., Amselgruber, W.M.,"Effects of the Fusarium mycotoxins zearalenone and deoxynivalenol on the mitochondrial methylthiazol tetrazolium-cleavage activity of monolayer cells". Toxicol. In Vitro 3, 311–316, 1989.