

ORIGINAL ARTICLE

Medicine Science 2020;9(2):492-5

In-vitro evaluation of aflatoxin-B1 and fumonisin-B1 cellular stress effects on SH-SY5Y cell line **Mehtap Kara,**  **Ezgi Oztas***Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Turkey*

Received 19 September 2019; Accepted 08 April 2020

Available online 16.05.2020 with doi: 10.5455/medscience.2019.08.9206

Abstract

Mycotoxins, which are produced from fungi, have harmful effects on human and animals. It has been reported in several in vivo and in vitro studies that mycotoxins caused disorders on digestive, urinary, immune, reproduction and nervous systems, however detailed molecular mechanism of toxicity needed to clarify. Mycotoxins could have potential to inducing neurotoxicity. In this study we aimed to evaluate mycotoxin aflatoxin B1 (AFB1) and Fumonisin B1's (FB1) effects on cellular stress conditions and endoplasmic reticulum stress molecules gene expressions. Cell survival analysis was conducted by MTT assay. Total-ROS analysis was performed via DC-FDA analysis with fluorescent microplate reader. IRE1 alpha and Gadd 153 which are endoplasmic reticulum stress molecules gene expressions were evaluated by quantitative (real-time) polymerase chain reaction (qPCR). According to MTT results both AFB1 and FB1 were shown 90% cell survival at 12,5-100µM concentrations range for 24h in SH-SY5Y cell line. Both 100 and 50 µM concentrations of AFB1 and FB1 were significantly induced ROS formation in SH-SY5Y cells ($p < 0.05$). Gadd153 and IRE1- α , gene expressions were not increased with AFB1 and FB1 exposure for 24h. Further detailed studies that include molecular pathways of cellular damage with longer exposure time needed to clarify mycotoxins neurodegenerative effects.

Keywords: Aflatoxin B1, fumonisin B1, oxidative stress, endoplasmic reticulum stress, neuron**Introduction**

Today, incidences of neurodegenerative diseases are increasing. Exposure to environmental toxins plays an important role in this increase and there is a growing interest in research on this subjects risk factors as medical conditions, environmental exposures, lifestyle and genetic factors, play a role in the development and progression of neurodegenerative diseases [1,2]. Most of neurodegenerative diseases such as Alzheimer's, Parkinson's, and Creutzfeldt-Jacobs are mainly include similar molecular mechanisms in their pathogenesis. Oxidative stress, folding errors in cellular proteins and endoplasmic reticulum stress caused by the accumulation of these unfolded proteins and induction of apoptosis in nerve cells are shown as common mechanisms of neurodegenerative diseases [3-5].

The endoplasmic reticulum (ER), which has key roles in vital activities in the cell, is responsible for the maturation, folding of both the cellular and secreted proteins, the control of the folded proteins and the transfer of the functions to the regions where they will demonstrate their functions.

Several different factors could cause misfolded proteins and these misfolded proteins are recognized and removed by ER. However, when the amount of inaccurate proteins increases excessively, ER is directed cell to programmed cell death by creating an unfolded protein response (UPR) [6,7].

Mycotoxins which are commonly found in various foodstuffs and feeds, are the most potent environmental toxin group which have several adverse effects on humans and animals as hepatotoxic, nephrotoxic, carcinogenic and mutagenic effects [8, 9].

There are limited data in the literature that mycotoxins may be associated with neurodegenerative diseases, but the mechanism of action of this relationship is not fully elucidated. Aflatoxin B1 (AFB1) and fumonisin B1 (FB1) are common mycotoxins with high toxicity potential. There are studies on the neurodegenerative

*Corresponding Author: Mehtap Kara, Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Turkey.
E-mail: mehtap.kara@istanbul.edu.tr

effects of AFB1 and FB1 in the literature [10]. It has been reported that FB1 inhibits sphingolipid biosynthesis that reason of “mouldy corn disease” and AFB1 cause neurodegeneration via mitochondrial disruptions [8-12].

The aim of this study was to elucidate the effects of fumonisin B1 (FB1) and aflatoxin B1 (AFB1) mycotoxins effects on cellular stress in SH-SY5Y neuron cell line.

Materials and Methods

Cell culture and MTT Test

SH-SY5Y (CRL2266) cell line was purchased from the American Type Culture Collection (ATCC, Virginia, USA), and cells were maintained as manufacturer's instructions. AFB1 and FB1 solution was prepared by dissolving in 100% of dimethyl sulfoxide (DMSO), and stored in -20°C until the day of assays conducted. Before the cell treatments mycotoxins diluted with cell culture medium to desired concentrations; and final DMSO concentration was 1%. Treatments were done at a concentration range for 24h to evaluate dose-dependent effects. All experiments were performed in triplicates in three separate days.

The SH-SY5Y cells were seeded into 96-well plates (1x10⁴ cells/100 µL cell culture medium/well). After overnight incubation cells were treated with AFB1 and FB1 at the concentration range of 12,5-100µM for 24h. Then MTT was added into each well and cells were incubated for further 3h at 37 °C in the dark. 100 µL of DMSO was added and optical densities (ODs) were measured at 570 nm using a microplate reader (Biotech, Epoch, Vermont, USA) [13].

Total ROS assay with DC-FDA

Total ROS (Reactive oxygen species) measurement of AFB1 and FB1 exposed cells were determined by Abcam DC-FDA (2',7'

–dichlorofluorescein diacetate) Cellular ROS Detection Assay Kit (ab113851) as manufacturer's instructions.

ER Stress markers gene expression analysis

RNA isolation was performed from cell lysates using the High Pure RNA isolation Kit (Roche Diagnostic GmbH), according to the manufacturer's instructions. A fixed amount of RNA from each sample was used for cDNA synthesis. cDNA was prepared using the Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostic GmbH) according to the manufacturer's instructions. The gene expression levels of IRE1 alpha and Gadd 153 were analyzed by quantitative (real-time) polymerase chain reaction (qPCR) using Light Cycler 480 machine (Roche Diagnostic GmbH) with Real Time Ready Catalog Assay (Roche Diagnostic GmbH) according to the manufacturer's instructions. PCR amplifications were performed according to manufacturer's instructions in triplicate. A reaction mixture without cDNA template was used as a negative control. The expression levels ($2\Delta\Delta C_t$) was calculated as described previously [14].

Statistical analysis

Data were analysed by one-way ANOVA followed by post hoc Dunnett's test and expressed as mean $\pm$ SD. The level of statistical significance was set at $p < 0.05$. All analyses were performed using the statistical package SPSS version 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

Cell culture and MTT Test

According to MTT results both AFB1 and FB1 express 90% viability at the concentration range of 12,5-100µM for 24h (Figure 1).

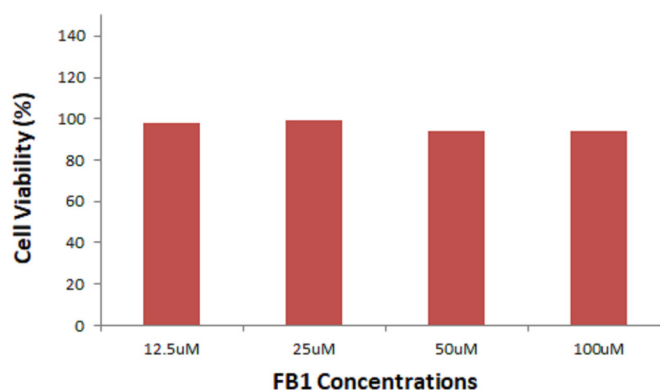
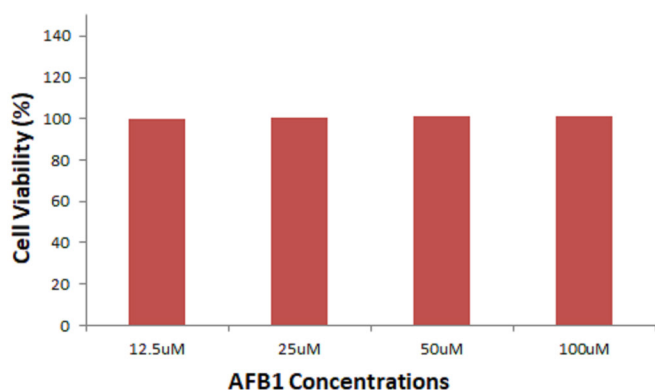


Figure 1. SH-SY5Y cells were shown approximately 90% viability with 100-50-25 and 12,5 µM concentrations of AFB1 and FB1 mycotoxins. There were no statistically difference between groups

Total ROS assay with DC-FDA

It has been observed that 100 and 50 µM concentrations both AFB1 and FB1 were significantly induced ROS formation in SH-

SY5Y cells ($p < 0,05$) (Figure 2). With AFB1 exposure, Total-ROS increased 302,7 and 313,8 fold in 50 µM and 100 µM concentrations and with FB1 exposure Total-ROS increased 330,5 and 347,2 fold.

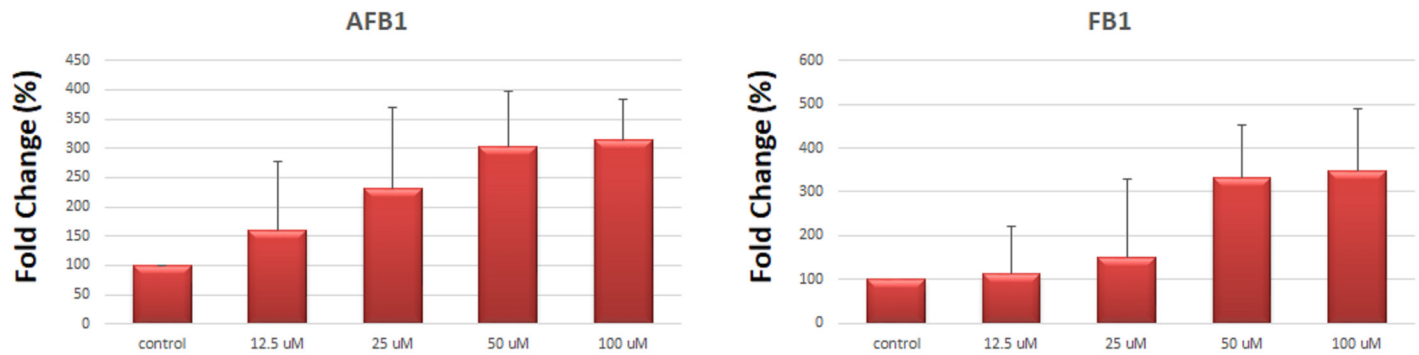


Figure 2. Total-ROS induced by AFB1 and FB1 higher concentrations (100 and 50 μ M) compared to control. (* $p < 0.05$)

ER Stress markers gene expression analysis

Gadd153 and IRE1- α , biomarkers of ER stress mechanisms and

these biomarkers gene expressions were not significantly changed with AFB1 and FB1 exposure for 24h (Figure 3).

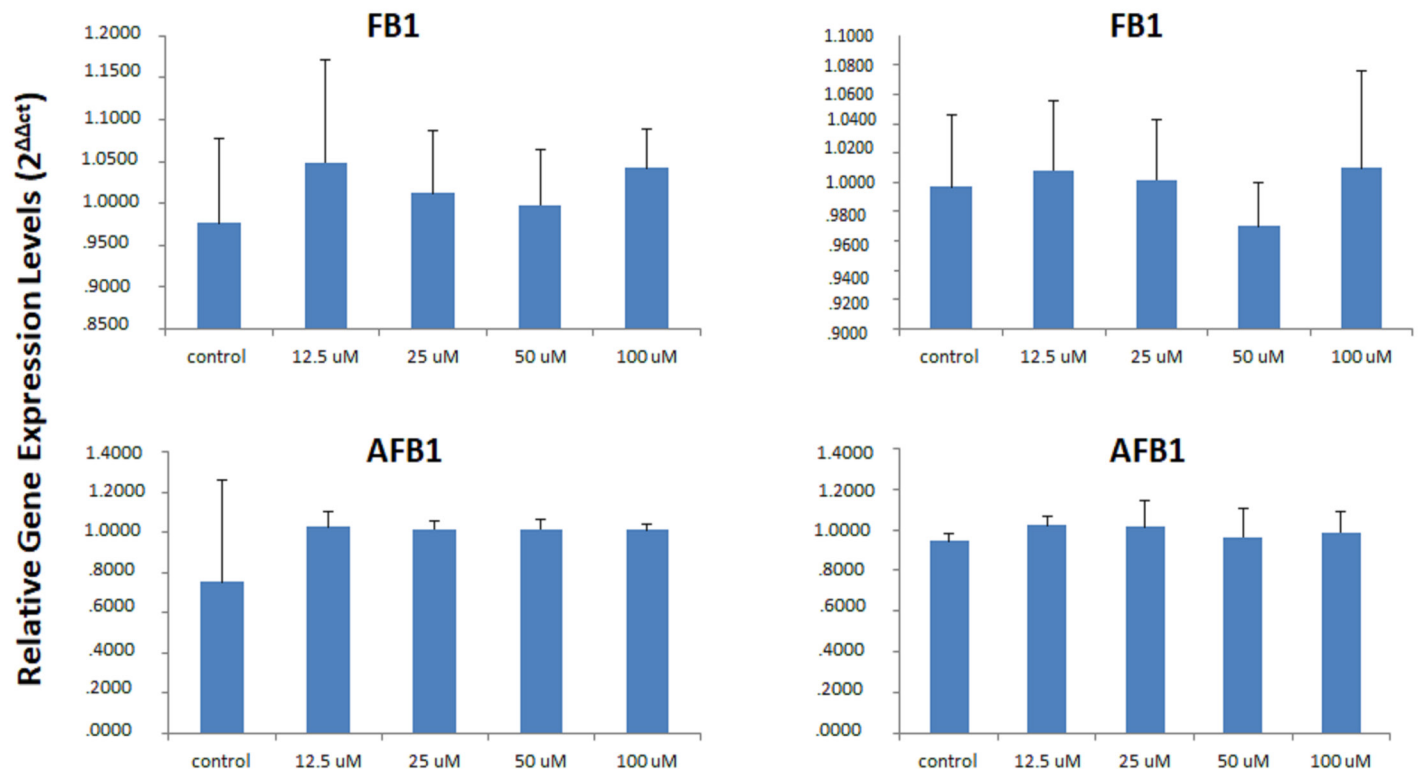


Figure 2. Relative gene expression level of AFB1 and FB1 of IRE1alpha and Gadd153. There were no significant difference between groups ($p > 0.05$) for Gadd153 and IRE1 alpha in both AFB1 and FB1 exposure in SH-SY5Y cell line

Discussion

Neurodegenerative diseases are multifactorial progressive disorders which affect about 30 million individuals worldwide. Several multifactorial effect such as abnormal intracellular protein dynamics and protein aggregation, oxidative stress and free radical formation, mitochondrial dysfunction and bioenergetics deterioration, cause neurodegeneration. There are studies suggesting that chronic mycotoxin exposure may cause

neurodegenerative diseases. Although there are many studies on the mechanism of neurodegeneration, a molecular target for treatment has not yet been determined [15,16]. Several studies have reported that mycotoxins induce neurotoxicity in experimental animals [10]. There are also in-vitro studies on the toxic effects of mycotoxins in various neuronal cell lines. It has been reported that exposure to FB1 disrupts axonal growth and myelination and these changes are observed before cell death. It has been observed that FB1 disrupts myelin formation and delays oligodendrocyte maturation, but has

no cytotoxic properties [17-20]. Stockmann-Juvala et al. reported that FB1 exposure reduced the survival rate of SH-SY5Y cells by approximately 80% for 48 hours exposure [20]. The toxic effects of FB1 on SH-SY5Y cells are controversial and different effects have been reported in different studies. Studies investigating the neurotoxic effects of aflatoxin are limited in the literature [21,22]. There is no literature study investigating the relationship between the ER stress mechanism and mycotoxins, which play an important role in triggering cell death in the neurodegeneration mechanism. In current study, according to MTT results both AFB1 and FB1 express 90% viability at the concentration range of 12,5-100 μ M for 24h. It has been observed that 100 and 50 μ M concentrations both AFB1 and FB1 were significantly induced ROS formation in SH-SY5Y cells ($p < 0.05$). Gadd153 and IRE1- α , biomarkers of ER stress mechanisms, gene expressions were not increased with AFB1 and FB1 exposure for 24h.

Conclusion

In summary, this study demonstrates that AFB1 and FB1 not reduce cell viability for 24h exposure. However ROS formation was induced with high concentrations. ER stress was not induced via AFB1 and FB1 exposure. Further and detailed in-vitro and/or in-vivo studies with different exposure durations needed to clarify neurotoxic effects of AFB1 and FB1 mycotoxins.

Competing interests

We declare that we have no conflict of interest.

Financial Disclosure

This study received no financial support.

Ethical approval

No ethic approval is needed to this research.

References

- Kovacs, G. Current concepts of neurodegenerative diseases. *Eur Med J Neurol.* 2014;1:78-86.
- Ramanan, V. K., and Saykin, A. J. Pathways to neurodegeneration: mechanistic insights from GWAS in Alzheimer's disease, Parkinson's disease, and related disorders. *Am J Neurodegener Dis.* 2013;2:145-175.
- Skovronsky DM, Lee VMY, Trojanowski JQ. Neurodegenerative diseases: new concepts of pathogenesis and their therapeutic implications. *Annu Rev Pathol Mech Dis.* 2006;1:15-70.
- Zeeshan HMA, Lee GH, Kim HR, et al. Endoplasmic reticulum stress and associated ROS. *Int J Molec Sci.* 2016;17:327.
- Hosoi T, Ozawa K. Endoplasmic reticulum stress in disease: mechanisms and therapeutic opportunities. *Clinical Sci.* 2010;118:19-29.
- Sano R, Reed JC. ER stress-induced cell death mechanisms. *Biochim Biophys Acta.* 2013;1833:3460-70.
- Kim I, Xu W, Reed JC. Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. *Nat Rev Drug Discov.* 2008;7:1013-30.
- Streit E, Schatzmayr G, Tassis P, et al. Current situation of mycotoxin contamination and co-occurrence in animal feed-Focus on Europe. *Toxins.* 2012;4:88-809.
- Zain ME. Impact of mycotoxins on humans and animals. *J Saudi Chem Society.* 2011;15:129-44.
- Doi K, Uetsuka K. Mechanisms of mycotoxin-induced neurotoxicity through oxidative stress-associated pathways. *Int J Mol Sci.* 2011;12:5213-37.
- González-Hunt CP, Leung MC, Bodhicharla RK, et al. Exposure to mitochondrial genotoxins and dopaminergic neurodegeneration in *Caenorhabditis elegans*. *PloS one.* 2014;9:e114459.
- Lizárraga-Paulín EG, Moreno-Martínez E, Miranda-Castro SP. Aflatoxins and their impact on human and animal health: An emerging problem. *Intech Open Access Publisher.* 2011.
- Oztas E, Abudayyak M, Celiksoz M, et al. Inflammation and oxidative stress are key mediators in AKB48-induced neurotoxicity in vitro. *Toxicol In Vitro.* 2019;55:101-7.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods.* 2001;25:402-8.
- Sheikh S, Haque E, Mir SS. Neurodegenerative diseases: multifactorial conformational diseases and their therapeutic interventions. *Journal of neurodegenerative diseases.* 2013.
- Anyanwu EC, Kanu I. Biochemical impedance on intracellular functions of vitamin B12 in chronic toxigenic mold exposures. *Scientific World J.* 2007;7:1649-57.
- Axelsson V, Holback S, Sjögren M, et al. Gliotoxin induces caspase-dependent neurite degeneration and calpain-mediated general cytotoxicity in differentiated human neuroblastoma SH-SY5Y cells. *Biochem Biophys Res Commun.* 2006;345:1068-74.
- Domijan AM. Fumonisin B1: A Neurotoxic Mycotoxin/Fumonisin B1: Neurotoksični Mikotoksin. *Arch Indus Hygiene Toxicol.* 2012;63:531-44.
- Osuchowski MF, Sharma RP. Fumonisin B1 induces necrotic cell death in BV-2 cells and murine cultured astrocytes and is antiproliferative in BV-2 cells while N2A cells and primary cortical neurons are resistant. *Neurotoxicol.* 2005;26:981-92.
- Stockmann-Juvala H, Mikkola J, Naarala J, et al. Oxidative stress induced by fumonisin B1 in continuous human and rodent neural cell cultures. *Free radical research.* 2004;38:933-42.
- Tanaka T, Mizukami S, Hasegawa-Baba Y, et al. Developmental exposure of aflatoxin B1 reversibly affects hippocampal neurogenesis targeting late-stage neural progenitor cells through suppression of cholinergic signaling in rats. *Toxicol.* 2015;336:59-69.
- Trebak F, Alaoui A, Alexandre D, et al. Impact of aflatoxin B1 on hypothalamic neuropeptides regulating feeding behavior. *NeuroToxicol.* 2015;49:165-73.