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Potential Impacts of FB1 Exposure on Global DNA Methylation in Pancreatic Cells

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Abstract

Background and Aims: Fumonisin B1 (FB1), which emerges from the contamination of corn and similar products by *Fusarium* species, causes a high risk for human and animal health.

There are studies showing that FB1 causes carcinogenesis in the kidney and liver, but there is no study yet on the toxic effects of this molecule, which has an effect on lipid metabolism, on the pancreas. Because FB1 shows its toxic effects through nongenotoxic mechanisms, this study aimed to investigate the effects of FB1 on DNA methylation, an important epigenetic biomarker, in human pancreatic epithelial carcinoma cells (PANC-1).

Methods: PANC-1 cells treated with FB1 at concentrations of 10, 50, and 100 µM for 24 h were evaluated for global DNA methylation and expression of *DNMT1*, *DNMT3a*, and *DNMT3b* genes.

Results: It was found that exposure to 100 µM of FB1 increased global DNA methylation by 2.15-fold, however this phenomenon was not found to be associated with the changes in the gene expression of *DNMT*s.

Conclusion: The findings of this study indicate that high doses (100 µM) of FB1 resulted in global DNA methylation in PANC-1 cells. However, this increase was not associated with a change in the gene expression of *DNMT* enzymes. Consequently, further research is required to gain a deeper understanding of the epigenetic effects of FB1.

Keywords *DNMT*s · Fumonisin B1 · Global DNA Methylation · PANC-1 Cell Line

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INTRODUCTION

Fumonisins are mycotoxins generated by *Fusarium spp.* moulds, particularly *Fusarium verticillioides* (Kellerman et al., 1972; Gelderblom et al., 1991a; Rheeder, et al., 2002), which contaminates corn and other agricultural products. The results of studies conducted on laboratory animals have demonstrated that exposure to FB1 has the potential to cause liver and kidney cancers in male rats and kidney diseases in mice; furthermore, it has been shown to have adverse effects on development and reproduction (Stockmann & Savolainen, 2008; Riley et al., 1994; Voss et al.1995; Gelderblom et al., 2001; NTP, 2001; Marasas et al., 1988). According to epidemiological studies that indicate a higher incidence of esophageal cancer in countries with lower socioeconomic status and higher consumption of corn-based foods contaminated with fumonisins, the International Agency for Research on Cancer has classified FB1 as a Group 2B carcinogen (IARC 2002). Because of its structural similarity to sphinganine and sphingosine, FB1 influences sphingolipid metabolism, leading to the accumulation of sphinganine, which is important for cell growth, differentiation, proliferation, and apoptotic regulation. In addition, impairment in sphingolipid biosynthesis contributes to cancer development (Wang et al., 1991; Riley et al., 2001; Soriano et al., 2005; Voss et al., 2006).

FB1 has been reported to increase reactive oxygen species in human and rat glioblastoma cell culture and mouse hypothalamic cells (Galvano et al., 2002) and to induce lipid peroxidation in rat liver (Abel & Gelderblom, 1998; Gelderblom et al., 1996a-b; Gavino et al., 1981), monkey kidney (Abado-Becagne et al., 1998), pig kidney (Kang & Alexander, 1996), human neuroblastoma (Stockmann et al., 2004), and human intestinal (Kouadio et al., 2005) and mouse embryonic fibroblast cells (Mobio et al., 2003), resulting in oxidative damage may play a role in FB1 toxicity. In different animal species, FB1 exposure has been shown to increase apoptosis in various tissues, which may play an important role in the mechanism of tumour formation (Lim et al., 1996).

FB1 was shown to have no genotoxic effects by the tests of *Salmonella* mutagenicity test (Gelderblom & Snyman, 1991b; Park et al., 1992), *Salmonella* umu-microtest and DNA repair test in rat liver (Gelderblom et al., 1989; Gelderblom et al., 1992), DNA repair test with *Escherichia coli*, micronucleus test and mitotic activity (Gelderblom et al., 1995). Therefore, it has been suggested that epigenetic mechanisms are considered to be crucial in FB1's toxicity. In our previous studies, FB1 caused an increase in global H3K9me2/me3 levels and a decrease in global H4K20me3 and H3K9ac levels (Sancak & Özden, 2015), as well as changes in the promoter regions of tumour suppressor genes such as *p16* and *VHL* in mouse liver Clone 9 and mouse kidney NRK-52E epithelial cells (Demirel et al., 2015, Abudayyak et al., 2023). Similarly, it has been reported that 200 µM of FB1 induced changes in histone phosphorylation in HepG2 cells, with observed decreases in phosphorylated γH2AX and cleaved poly ADP-ribose polymerase (PARP) levels, associated with impaired DNA damage responses, compromising genomic stability and potentially leading to gene mutations and tumour formation (Matthaios et al., 2013). While FB1 is known to target the liver and kidneys as its primary tissues, there is no research found in the literature regarding its mechanisms of toxicity on the pancreatic cells. Since FB1 has effects on the biosynthesis of sphingolipids, it is considered that it may have effects on the pancreas, which has an important role in metabolic functions; therefore, we aimed to investigate the epigenetic effects of FB1 in PANC-1 cells. In the present study, global DNA methylation analysis was performed in PANC-1 cells for assessing the toxicity mechanism of FB1 for the first time.

MATERIALS AND METHODS

Chemicals

The chemical FB1 (99% purity) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). 1000 µM of FB1 stock solution was prepared in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, Missouri, USA) and stored at –20°C. Sterile plastic components were obtained from Nest Biotechnology (Jiangsu, China), and all cell culture supplies were obtained from Wisent Bioproducts (Saint-Jean-Baptiste, Quebec, Canada). Sentromer DNA Technologies (Istanbul, Turkey) provided the gene expression primers.

Cell Culture and Treatments

PANC-1 cells were obtained from the American Type Cell Culture (ATCC) bank and were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with foetal bovine serum (FBS, 10%) and penicillin-streptomycin (100 U-100 µg/mL) in a CO_2 (5%) incubator with a humidified atmosphere at 37°C. Subculturing of the cells was performed approximately 2-3 times per week to maintain cell viability. The cells (1.5×10^6) were cultured in 25 cm^2 flasks for 24 h in a CO_2 incubator for gene expression and DNA methylation analysis. Based on our and other previous mechanistic studies in different cell types (Galvano et al., 2002; McKean et al., 2006; Domijan & Abramov, 2011; Chuturgoon et al., 2015; Demirel et al., 2015; Sancak & Ozden 2015; Arumugam et al., 2020; Karaman et al., 2023) the exposure concentrations of FB1 were selected as 10, 50, and 100 µM for 24 h, while 1% PBS was used as a solvent control for exposure assessments. Following a 24 h FB1 treatment, cells were trypsinized and cell lysate was washed with phosphate-

buffered saline (PBS) and divided into two groups for genomic DNA and total RNA isolation for the global DNA methylation and DNMTs gene expression analysis, respectively. All exposure concentrations were tested in triplicate.

Cytotoxicity

For the cytotoxicity analysis, PANC-1 cells (1×10^4 cells/well) were seeded in 96-well plates overnight. Then, the cells were exposed to FB1 at the concentration range of 31.25-1000 µmol/L, together with 1% PBS (final concentration in the culture medium) were used as solvent control for 24 h. The cell viability of FB1 was assessed by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide, a tetrazole] (Mossman, 1983; Alley et al., 1988) test. The intensity of the formed colour was quantified at 590 nm (relative to a reference spectrum of 670 nm) using a spectrophotometer with a microplate reader (Biotek-Epoch, Winooski-Vermont, USA). The obtained absorbance results were calculated as the percentage of cell viability to show the effects of FB1 treatments.

Global DNA Methylation Analysis

The PureLink Genomic DNA Kit (Invitrogen, Thermo Fisher Scientific, CA, USA) was used for the genomic DNA isolation from PANC-1 cells after treatment with the FB1 (10-100 µM) for 24 h according to the manufacturer's recommendations. Global DNA methylation (5-mC%) was analysed using the 5-mC DNA Elisa kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions as described previously (Senyildiz, & Ozden, 2015; Senyildiz et al., 2016). Briefly, 100 ng of genomic DNA and control standard solutions were added with the binding solution into the Elisa kit plate and incubated at 37°C for 30 min. After washing with buffer solutions, the DNA samples were incubated with an anti-5-mC monoclonal antibody and detection antibody. After the addition of the developer solution, the methylated DNA was quantified colorimetrically at 450 nm using a microplate spectrophotometer system (Biotek-Epoch, Winooski-Vermont, USA). The positive control (DNA containing 5% 5-mC) and negative control (100% unmethylated DNA) provided in the kit were used for the preparation of the standard calibration curve. Relative quantification was used to calculate the percentage of 5-mC (5-mC%) in the DNA samples according to the standard calibration curve.

Gene Expression Analysis

The PureLink RNA Mini Kit (Ambion, Thermo Fisher Scientific, CA, USA) was used for total RNA from PANC-1 cells based on the manufacturer's instructions. Using 500 ng of total RNA, the iScript cDNA Synthesis Kit (Bio-Rad, CA, USA) was used to perform reverse transcription (RT). To assess the gene expression of *DNMT1*, *DNMT3a* and *DNMT3b* genes, 5 µL of the 1/10 diluted RT-reaction was used as the template, and the BioLine SensiFast™ SYBR® No-Rox kit (London, UK) was used for the quantitative real-time PCR (LightCycler® 480 Instrument II, Roche Life Science). Table 1 lists the primer sequences along with the gene annealing temperatures. For each PCR test, the expression values of the selected genes were normalised using the reference gene *β-actin* in comparison to a control group using the ΔΔCt method, as previously mentioned (Karaman & Ozden, 2019; Karaman et al., 2020). Every PCR reaction had controls that had no template in which 1 µL of water was added instead of the sample cDNA.

Statistical Analysis

The results of the gene expression analysis and global methylation levels are expressed as mean ± standard deviation (SD). Statistical analysis was performed using ANOVA followed by Dunnett's multiple comparison test with the "SPSS version 21.0 for Windows" statistical software (IBM Analytics, New York, USA). *p* values less than 0.001, 0.01, and 0.05 were considered statistically significant.

RESULTS

Cytotoxicity

Cytotoxicity of FB1 at the concentration range of 31.25-1000 µM in PANC-1 cell line has been investigated. According to the MTT test, cell viability was significantly decreased by 48.18% after 24 h at 1000 µM of FB1. In the selected exposure concentrations of FB1 (10, 50 and 100 µM) cell viability was >85% (Figure 1).

Table 1. Primer sequences and their annealing temperatures of the genes of *DNMTs*

Gene name	Sense (5'-3')	Antisense (5'-3')	Ta (°C)	Reference
DNMT1	CCTCCAAAAACCCAGCCAAC	TCCAGGACCCTGGGGATTTC	60	Ahmadnejad, Amirizadeh, Mehrasa, Karkhah, Nikougoftar, & Oodi, 2017
DNMT3a	GGTCACGCAAAACAGAACCC	CCTTGGTGAAACCCTTTGCG	60	Gu, Gao, Guo, Zhou, & Kong, 2017
DNMT3b	CTCTTCCTCAGCTGTGTGGG	CTGTCGGCACTGTGGTTTTG	60	Gu, Gao, Guo, Zhou, & Kong, 2017
β-actin	AACTACCTTCAACTCCAT	TGATCTTGATCTTCATTGTG	48	Rosa, Rufno, Judas, Tenreiro, Lopes, & Mendes, 2011

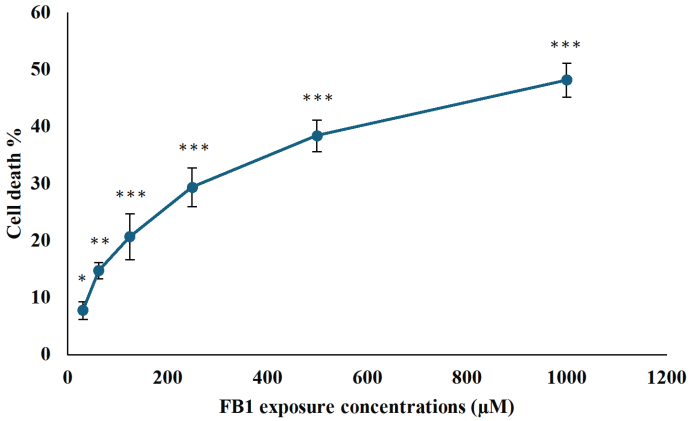


Figure 1. MTT assay results in PANC-1 cells exposed to FB1 at a concentration range of 31.25-1000 µM for 24 h. Data are presented as mean ± SD. Statistical analysis was performed by ANOVA + Dunnett's post hoc test. Statistically significant changes are indicated by *p<0.05, **p<0.01, ***p<0.001.

Effects of FB1 on global DNA methylation in PANC-1 cells

Following treatments with 10, 50, and 100 µM FB1 for 24 h, the levels of 5-mC% were measured by the Elisa method. Exposure to 50 and 100 µM of FB1 increased the 5-mC% status significantly by 1.85 and 2.15 folds (p<0.05), respectively, while there were no significant changes in the 5-mC% status at 10 µM of FB1 (1.21-fold increase) as compared to the control group (Figure 2).

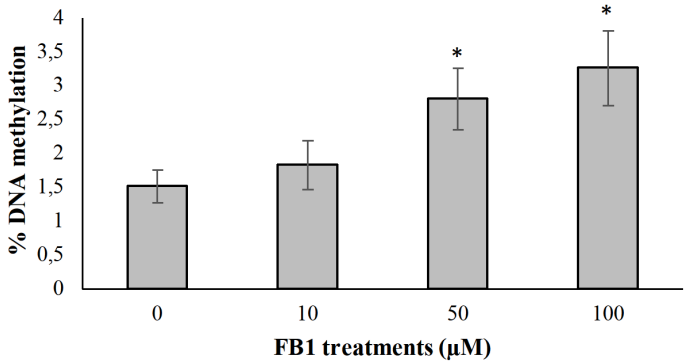


Figure 2. Alterations in the levels of 5-mC% after 10, 50 and 100 µM of FB1 exposure for 24 h in PANC-1 cells. Data are presented as mean ± SD. Statistical analysis was performed by ANOVA + Dunnett's post hoc test. Statistically significant changes are indicated by *p<0.05.

Effects of FB1 on the Gene Expression of DNMTs

Exposure to 50 and 100 µM of FB1 for 24 h significantly increased the *DNMT1* mRNA levels by 2.71-fold (p<0.01) and 5.05-fold (p<0.001), respectively, when compared with the control group (Figure 3a). In addition, the expression of *DNMT3a* and *DNMT3b* increased significantly after 50 µM (2.17-fold, p<0.01 and 6.32-fold, p<0.001, respectively) and 100 µM (2.09-fold, p<0.05 and 5.17-fold, p<0.01 respectively) of FB1 (Figure 3b-c). Nevertheless, at 10 µM of FB1 (≤35% decrease), non-significant alterations in *DNMT1*, *DNMT3a*, and *DNMT3b* were observed.

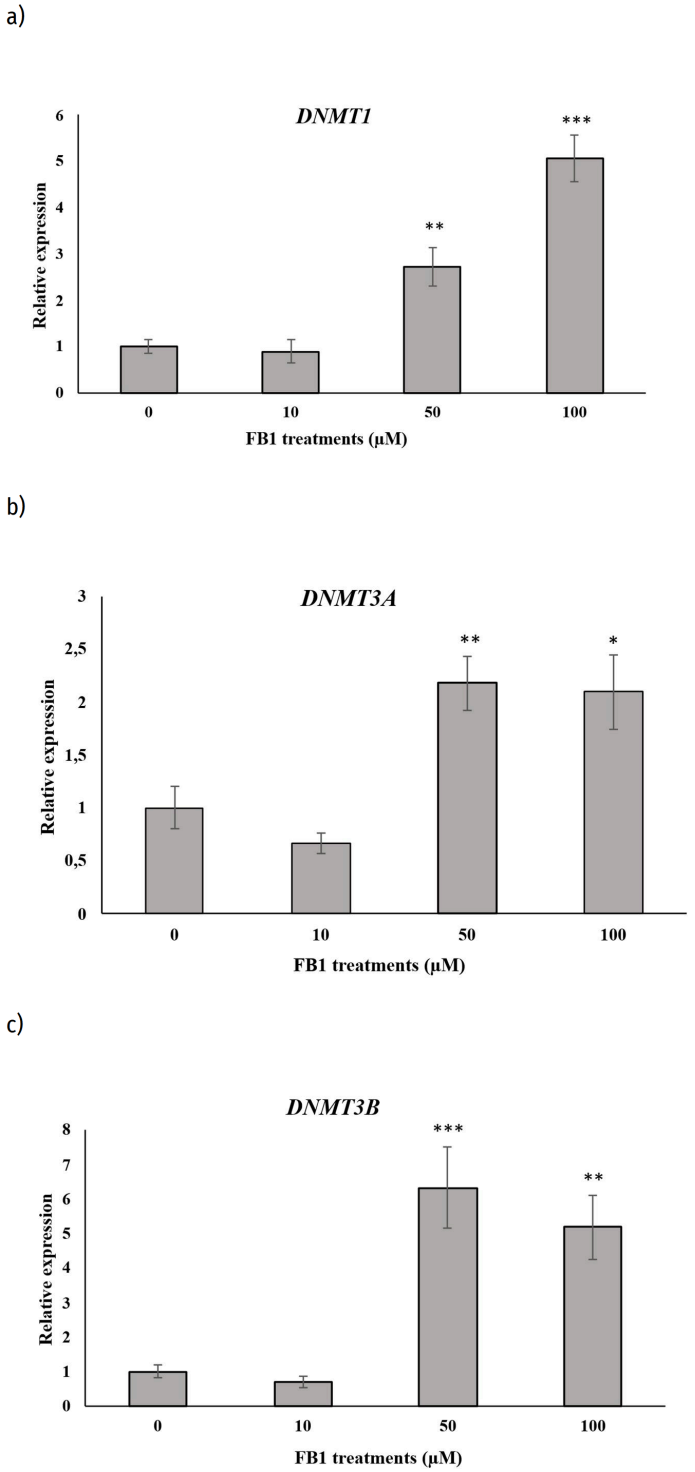


Figure 3. Alterations in the gene expression of *DNMT1* (a), *DNMT3a* (b) and *DNMT3b* (c) after 10, 50 and 100 µM of FB1 exposure for 24 h in PANC-1 cells. Data are presented as mean ± SD. Statistical analysis was performed by ANOVA + Dunnett's post hoc test. Statistically significant changes are indicated by *p<0.05, **p<0.01, ***p<0.001.

DISCUSSION

FB1 is considered a nongenotoxic carcinogen due to its lack of mutagenicity (Gelderblom & Snyman, 1991b), genotoxicity (Norred et al., 1992) and does not appear to bind directly to DNA molecules. Thus, the toxicity mechanism of FB1 may include epigenetic modifications (Gelderblom & Marasas, 2012; Demirel et al., 2015; Sancak & Ozden, 2015; Karaman et al., 2023). Therefore, we investigated the effects of FB1 on global DNA methylation in the PANC-1 cells after 24 h of exposure.

According to the cell viability analyses, IC_{50} value was investigated in the concentration range of 31.25-1000 µM after FB1 exposure for 24 h in PANC-1 cells by the MTT assay, and cell death was determined as 48% at 1000 µM exposure. Karaman et al. (2023) showed that the rates of cell death were 34.76% and 38.40% by MTT and NRU tests, respectively, at the studied highest concentration of 200 µM of FB1 in HK-2 cells. According to the XTT test, the cell viability reduction was 39% and 34% at 200 µM FB1 exposure in Clone 9 and NRK-52E cells, respectively (Demirel et al., 2015). However, IC_{50} values of FB1 were determined as 200 µM after 24 h in HepG2 cells (Chuturgoon et al., 2015); 85.5 µM/mL after 48 h in Chinese hamster ovary cells (CHO-K1) (Cetin & Bullerman, 2005); 399.2 µM after 24 h in HepG2 cells (McKean et al., 2006); and 355.1 µM after 24 h in immortalised human bronchial epithelial cells (BEAS-2B) (McKean et al., 2006). An analysis of these studies shows that the cytotoxicity of FB1 varies with the type of cell and exposure duration. The studies showed that even at very high doses, no IC_{50} value could be determined. In order not to waste material, very high doses were not used and doses frequently used as exposure doses (10, 50, 100 µM) were used.

In a study demonstrating risk assessment (Karaman et al., 2023), the harms of FB1 in foods have been shown (Marasas, 2001; Qiu & Liu, 2001; Dutton, 2009; Sun et al., 2011). This level of exposure may be encountered in daily life and, although high, the concentrations we chose to study the toxicological outcomes are considered to be within an acceptable range.

In the present study, a significant increase was observed in the global DNA methylation status as 1.85-fold and 2.15-fold after 50, and 100 µM of FB1 exposure, respectively. Kouadio et al. (2007) showed that a significant increase in the 5-mC% ratio from 4.5% to 9% was observed after 24 h exposure to FB1 at 10, 20 and 40 µM concentrations in human intestinal Caco-2 cells. It was reported that DNA hypermethylation was significantly induced (1.58-fold) in rat C6 glioma cells by 4 h FB1 exposure at 18 µM FB1 concentrations but no change at higher concentrations (27 and 54 µM) (Mobio et al., 2000). In addition, in our previous study, exposure to 100 µM of FB1 for 24 h significantly increased the 5-mC% level (2.23-fold), but had no effect on the levels of 5-mC% in 10 and 50 µM in HK-2 cells (Karaman et al., 2023). In another study in NRK-52E and Clone 9 cells, global DNA methylation ranged from 4% to 5% and there were no significant dose-related effects of FB1 on global DNA methylation (Demirel et al., 2015).

We also observed an increase in the expression of genes encoding enzymes involved in DNA methylation (*DNMT1*, *DNMT3a*, and *DNMT3b*), which are consistent with the levels of 5-mC%. Karaman et al. (2023) showed that FB1 caused a decrease in *DNMT1* gene expression, whereas *DNMT3a* and *DNMT3b* expression increased significantly at 100 µM FB1 in HK-2 cells. Chuturgoon et al. (2014) showed that in HepG2 cells exposed to 200 µM of FB1 for 24 h, global DNA hypomethylation increased significantly, the activities and gene expressions of *DNMT1*, *DNMT3a*, and *DNMT3b* decreased significantly, and the activity of MBD2 demethylase increased significantly. In a study by Sugiyama, Kinoshita, Furusawa, Sato, & Honma (2021), significant increases in global DNA methylation and *DNMT* activities were observed in human embryonic kidney (HEK293) cells following exposure to 100 µmol/L FB1. According to these results, FB1 affects global DNA methylation and expression of maintenance *DNMTs* (*DNMT1*) and de novo (*DNMT3a*, *3b*) at different levels and in different cell cultures, according to the response to stress conditions. It is thought that the increase in global DNA methylation and the consequent increase in the expression of *DNMT* genes may be related to the decrease in TET (The ten-eleven translocation) proteins. TET proteins regulate the expression of key differentiation and fate-determining genes via enzymatic activity-dependent DNA methylation of the promoters and enhancers of target genes (Joshi et al., 2022). Therefore, further research is needed for a detailed evaluation of these processes. In conclusion, this is the first study to evaluate the effect of FB1 on DNA methylation in PANC-1 cells. FB1 may cause an increase in the global DNA methylation, which is also related to the increase in the expression levels of the genes involved in DNA methylation (*DNMT1*, *DNMT3a* and *DNMT3b*). Our findings will contribute to literature on elucidating the role of epigenetic changes in the toxicity mechanisms of FB1 in PANC-1 cells.

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REFERENCES

Abado-Becognee, K., Mobio, T. A., Ennamany, R., Fleurat-Lessard, F., Shier, W. T., Badria, F., & Creppy, E. E. (1998). Cytotoxicity of fumonisin B1: Implication of lipid peroxidation and inhibition of protein and DNA syntheses. *Archives of Toxicology*, 72(4), 233–236. https://doi.org/10. 1007/s002040050494

Abel S., & Gelderblom, W. C. (1998). Oxidative damage and fumonisin B1- induced toxicity in primary rat hepatocytes and rat liver in vivo. *Toxicology*, 131(2-3), 121–31. https://doi.org/10.1016/S0300-483X(98)00123-1

Abudayyak, M., Karaman, E. F., Guler, Z. R., & Ozden, S. (2023). Effects of perfluorooctanoic acid on endoplasmic reticulum stress and lipid metabolism-related genes in human pancreatic cells. *Environmental Toxicology and Pharmacology*, 98, 104083. https://doi.org/10.1016/j.etap.2023.104083

Ahmadnejad, M., Amirizadeh, N., Mehrasa, R., Karkhah, A., Nikougoftar, M., & Oodi, A. (2017). Elevated expression of DNMT1 is associated with increased expansion and proliferation of hematopoietic stem cells co-cultured with human MSCs. *Blood Research*, 52(1), 25–30. https://doi.org/10.5045/br.2017.52.1.25

Alley, M. C., Scudiere, D. A., Monks, A., Hursey, M. L., Czerwinski, M. J., Fine, D. L., Abbott, B. J., Mayo, J. G., Shoemaker, R. H., & Boyd, M. R. (1988). Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Research*, 48(3), 589–60. https://pubmed.ncbi.nlm.nih.gov/3335022/

Arumugam, T., Ghazi, T., & Chuturgoon, A. (2020). Fumonisin B1 epigenetically regulates PTEN expression and modulates DNA damage checkpoint regulation in HepG2 liver cells. *Toxins*, 12(10), 625. https://doi.org/10.3390/toxins12100625

Chuturgoon, A. A., Phulukdaree, A., & Moodley, D. (2014). Fumonisin B1 induces global DNA hypomethylation in HepG2 cells - An alternative mechanism of action. *Toxicology*, 315, 65–69. https://doi.org/10.1016/j.tox.2013.11.004

Chuturgoon, A. A., Phulukdaree, A., & Moodley, D. (2015). Fumonisin B1 inhibits apoptosis in HepG2 cells by inducing Birc-8/ILP-2. *Toxicology Letters*, 235(2), 67–74. https://doi.org/10.1016/j.toxlet.2015.03.006

Cetin, Y., & Bullerman, L. B. (2005). Cytotoxicity of Fusarium mycotoxins to mammalian cell cultures as determined by the MTT bioassay. *Food and Chemical Toxicology*, 43(5), 755–764. https://doi.org/10.1016/0278-6915(92)90038-M

Demirel, G., Alpertunga, B., & Ozden, S. (2015). Role of fumonisin B1 on DNA methylation changes in rat kidney and liver cells. *Pharmaceutical Biology*, 53(9), 1302–1310. https://doi.org/10.3109/13880209.2014.976714

Domijan, A. M., & Abramov, A. Y. (2011). Fumonisin B1 inhibits mitochondrial respiration and deregulates calcium homeostasis—implication to mechanism of cell toxicity. *The International Journal of Biochemistry & Cell Biology*, 43(6), 897–904. https://doi.org/10.1016/j.biocel.2011.03.003

Dutton, M. (2009). The African Fusarium/maize disease. *Mycotoxin Research*, 25, 29–39. https://doi.org/10.1007/s12550-008-0005-8

Galvano, F., Russo, A., Cardile, V., Galvano, G., Vanella, A., & Renis, M. (2002). DNA damage in human fibroblasts exposed to fumonisin B1. *Food and Chemical Toxicology*, 40(1), 25–31. https://doi.org/10.1016/S0278-6915(01)00083-7

Gavino, V. C., Miller, J. S., Ikharebha, S. O., Milo, G. E., & Cornwell, D. G. (1981). Effect of polyunsaturated fatty acids and antioxidants on lipid peroxidation in tissue cultures. *Journal of Lipid Research*, 22(5), 763 – 9. https://pubmed.ncbi.nlm.nih.gov/7288284/

Gelderblom, W. C. A., Marasas, W., Thiel, P., Semple, E. & Farber, E. (1989). Possible non-genotoxic nature of active carcinogenic components produced by *Fusarium moniliforme*. *The American Association for Cancer Research*, 30, 144 – 147.

Gelderblom, W. C., Kriek, N. P., Marasas, W. F., & Thiel, P. G. (1991a). Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B1, in rats. *Carcinogenesis*, 12(7), 1247–1251. https://doi.org/10.1093/carcin/12.7.1247

Gelderblom, W. C., & Synman, L. (1991b). Mutagenicity of potentially carcinogenic mycotoxins produced by *Fusarium moniliforme*. *Mycotoxin Research*, 7(2), 46–52. https://doi.org/10.1007/BF03192165

Gelderblom, W. C., Semple, E., Marasas, W. F., & Farber, E. (1992). The cancer-initiating potential of the fumonisin B mycotoxins. *Carcinogenesis*, 13(3), 433–437. https://doi.org/10.1093/carcin/13.3.433

Gelderblom, W. C., Snyman, L., Van der Westhuizen, L., & Marasas, W. F. (1995). Mitoinhibitory effect of fumonisin B1 on rat hepatocytes in primary culture. *Carcinogenesis*, 16(3), 625–631. https://doi.org/10.1093/carcin/16.3.625

Gelderblom, W. C., Smuts, C. M., Abel, S., Snyman, S. D., Cawood, M. E., Van der Westhuizen, L., Swanevelder, S. (1996a). Effect of fumonisin B_1 on protein and lipid synthesis in primary rat hepatocytes. *Food and Chemical Toxicology*, 34(4), 361–369. https://doi.org/10.1016/0278-6915(96)00107-X

Gelderblom, W. C., Snyman, S., Abel, S., Lebepe-Mazur, S., Smuts, C. M., Van der Westhuizen, L., Marasas, W. F., Victor, T. C., Knasmüller, S., & Huber, W. (1996b). Hepatotoxicity and carcinogenicity of the fumonisins in rats: A review regarding mechanistic implications for establishing risk in humans. *Advances in Experimental Medicine and Biology*, 392, 279–96. https://pubmed.ncbi.nlm.nih.gov/8850624/

Gelderblom, W. C. A., Abel, S., Smuts, C. M., Marnewick, J., Marasas, W. F. O., & Lemmer, E. R. (2001). Fumonisin induced hepatocarcinogenesis: mechanisms related to cancer initiation and promotion. *Environmental Health Perspectives*, 109(Suppl 2), 291–300. https://doi.org/10.1289/ehp.01109s2291

Gelderblom, W. C. A., & Marasas, W. F. A. (2012). Controversies in fumonisin mycotoxicology and risk assessment. *Human & Experimental Toxicology,* 31(3), 215-35. https://doi.org/10.1177/0960327110395338

Gu, H., Gao, J., Guo, W., Zhou, Y., & Kong, Q. (2017). The expression of DNA methyltransferases 3A is specifcally downregulated in chorionic villi of early embryo growth arrest cases. *Molecular Medicine Reports*, 16(1), 591–596. https://doi.org/10.3892/mmr.2017.6650

Joshi, K., Liu, S., Breslin, S. J. P., & Zhang, J. (2022). Mechanisms that regulate the activities of TET proteins. *Cellular and Molecular Life Sciences*, 79(7), 363. https://doi.org/10.1007/s00018-022-04396-x

Kang, Y. J., & Alexander, J. M. (1996). Alterations of the glutathione redox cycle status in fumonisin B1-treated pig kidney cells. *Journal of Biochemical Toxicology*, 11(3), 121–6. https://doi.org/10.1002/(SICI)1522-7146(1996)11:3<121::AID-JBT3>3.0.CO;2-M

Karaman, E. F., & Ozden, S. (2019). Alterations in global DNA methylation and metabolism-related genes caused by zearalenone in MCF7 and MCF10F cells. *Mycotoxin Research*, 35(3), 309–320. https://doi.org/10.1007/s12550-019-00358-8

Karaman, E. F., Zeybel, M., & Ozden, S. (2020) Evaluation of the epigenetic alterations and gene expression levels of HepG2 cells exposed to zearalenone and alpha-zearalenol. *Toxicology Letters*, 326, 52–60. https://doi.org/10.1016/j.toxlet.2020.02.015

Karaman, E. F., Abudayyak, M., & Ozden, S. (2023). The role of chromatin-modifying enzymes and histone modifications in the modulation of p16 gene in fumon-

isin B1-induced toxicity in human kidney cells. *Mycotoxin Research*, 39(3), 271–283. https://doi.org/10.1007/s12550-023-00494-2

Kellerman, T. S., Marasas, W. F., Pienaar, J. G., & Naudé, T. W. (1972). A mycotoxicosis of equidae caused by Fusarium moniliforme sheldon. A preliminary communication. *Onderstepoort Journal of Veterinary Research*, 39(4), 205-8. https://pubmed.ncbi.nlm.nih.gov/4664322/

Kouadio, J. H., Mobio, T. A., Baudrimont, I., Moukha, S., Dano, S. D., Creppy, E. E. (2005). Comparative study of cytotoxicity and oxidative stress induced by deoxynivalenol, zearalenone or fumonisin B1 in human intestinalcell line Caco-2. *Toxicology*, 213: 56–65. https://doi.org/10.1016/j.tox.2005.05.010

Kouadio, J. H., Dano, S. D., Moukha, S., Mobio, T. A., & Creppy, E. E. (2007). Effects of combinations of Fusarium mycotoxins on the inhibition of macromolecular synthesis, malondialdehyde levels, DNA methylation and fragmentation, and viability in Caco-2 cells. *Toxicon*, 49(3), 306–317. https://doi.org/10.1016/j.toxicon.2006.09.029

Lim, C. W., Parker, H. M., Vesonder, R. F., & Haschek, W. M. (1996). Intravenous fumonisin B1 induces cell proliferation and apoptosis in the rat. *Natural Toxins*, 4(1), 34–41. https://doi.org/10.1002/19960401NT5

Marasas, W. F., Kellerman, T. S., Gelderblom, W. C., Coetzer, J. A., Thiel, P. G., & Van der Lugt, J. J. (1988). Leukoencephalomalacia in a horse induced by fumonisin B1 isolated from *Fusarium moniliforme*. *Onderstepoort Journal of Veterinary Research*, 55(4), 197-203. https://pubmed.ncbi.nlm.nih.gov/3217091/

Marasas, W. F. (2001). Discovery and occurrence of the fumonisins: a historical perspective. *Environmental Health Perspective,* 109(2): 239–243. https://doi.org/10.1289/ehp.01109s2239

Matthaios, D., Hountis, P., Karakitsos, P., Bouros, D., & Kakolyris, S. (2013). H2AX a promising biomarker for lung cancer: a review. *Cancer Investigation*, 31(9), 582–99. https://doi.org/10.3109/07357907.2013.849721

McKean, C., Tang, L., Tang, M., Billam, M., Wang, Z., Theodorakis, C. W., Kendall, R. J., & Wang, J. S. (2006). Comparative acute and combinative toxicity of aflatoxin B1 and fumonisin B1 in animals and human cells. *Food and Chemical Toxicology*, 44(6), 868–876. https://doi.org/10.1016/j.fct.2005.11.011

Mobio, T. A., Anane, R., Baudrimont, I., Carratú, M. R., Shier, T. W., Dano, S. D., Ueno, Y., & Creppy, E. E. (2000). Epigenetic properties of fumonisin B1: cell cycle arrest and DNA base modifcation in C6 glioma cells. *Toxicology and Applied Pharmacology*, 164(1), 91–96. https://doi.org/10.1006/taap.2000.8893

Mobio, T. A., Tavan, E., Baudrimont, I., Anane, R., Carratú, M. R., Sanni, A., et al. (2003). Comparative study of the toxic effects of fumonisin B1 in rat C6 gliomacells and p53-null mouse embryo fibroblasts. *Toxicology*, 183, 65–75. https://doi.org/10.1016/S0300-483X(02)00441-9

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1-2), 55-63. https://doi.org/10.1016/0022-1759(83)90303-4

National Toxicology Program (NTP) (2001). Toxicology and Carcinogenesis Studies of Fumonisin B1 (CAS No. 116355-83-0) in F344/N Rats and B6C3F1 mice (Feed Studies). *National Toxicology Program technical report series*, 496, 1–352. https://pubmed.ncbi.nlm.nih.gov/11852482/

Norred, W. P., Plattner, R. D., Vesonder, R. F., Bacon, C. W. & Voss, K. A. (1992). Effects of selected secondary metabolites of Fusarium moniliforme on unscheduled synthesis of DNA by rat primary hepatocytes. *Food and Chemical Toxicology*, 30(3), 233 – 237. https://doi.org/10.1016/0278-6915(92)90038-M

Park, D. L., Rua, S. M., Mirocha, C. J., Abd-Alla, E. S., & Wenig, C.Y. (1992). Mutagenic potentials of fumonisin contaminated corn following ammonia decontamination procedure. *Mycopathologia*, 117, 105–108. https://doi.org/10.1007/BF00497285

Qiu, M., & Liu, X. (2001). Determination of sphinganine, sphingosine and Sa/So ratio in urine of humans exposed to dietary fumonisin B1. *Food Additives & Contaminants*, 18, 263–269. https://doi.org/10.1080/02652030117470

Rheeder, J. P., Marasas, W. F., & Vismer, H. F. (2002). Production of fumonisin analogs by Fusarium species. *Applied and Environmental Microbiology,* 68(5), 2101– 2105. https://doi.org/10.1128/AEM.68.5.2101-2105.2002

Riley, R. T., Hinton, D. M., Chamberlain, W. J., Bacon, C. W., Wang, E., Merrill, A. H. Jr., & Voss K. A. (1994). Dietary fumonisin B1 induces disruption of sphingolipid metabolism in Sprague-Dawley rats: a new mechanism of nephrotoxicity. *The Journal of Nutrition*, 124(4), 594–603. https://doi.org/10.1093/jn/124.4.594

Riley, R. T., Enongene, E., Voss, K. A., Norred, W. P., Meredith, F. I., Sharma, R. P., Spitsbergen, J., Williams, D. E., Carlson, D. B., & Merrill, A. H. Jr. (2001). Sphingolipid perturbations as mechanisms for fumonisin carcinogenesis. *Environmental Health Perspectives*, 109(2), 301–308. https://doi.org/10.1289/ehp.01109s2301

Rosa, S. C., Rufno, A. T., Judas, F., Tenreiro, C., Lopes, M. C., & Mendes, A. F. (2011). Expression and function of the insulin receptor in normal and osteoarthritic human chondrocytes: modulation of anabolic gene expression, glucose transport and GLUT-1 content by insulin. *Osteoarthritis and Cartilage*, 19(6), 719–727. https://doi.org/10.1016/j.joca.2011.02.004

Senyildiz, M., Ozden, S. (2015). Alteration in global DNA methylation after bisphenol a exposure in MCF-7 cells. *Istanbul Journal of Pharmacy,* 45(2), 153–164.

Senyildiz, M., Karaman, E. F., Bas, S. S., Pirincci, P. A., & Ozden, S. (2016). Alteration on global and gene-spesific DNA methylation and global histone modifications in HepG2 cells in response to BPA. *Istanbul Journal of Pharmacy*, 46(2), 97–114. https://dergipark.org.tr/en/pub/iujfp/issue/27053/284679

Sancak, D., & Ozden, S. (2015). Global histone modifcations in Fumonisin B1 exposure in rat kidney epithelial cells. *Toxicology In Vitro,* 29(7), 1809–1815. https://doi.org/10.1016/j.tiv.2015.07.019

Soriano, J. M., Gonzalez, L., & Catala, A. I. (2005). Mechanism of action of sphingolipids and their metabolites in the toxicity of fumonisin B1. *Progress in Lipid Research*, 44(6), 345–356. https://doi.org/10.1016/j.plipres.2005.09.001

Sugiyama, K. I., Kinoshita, M., Furusawa, H., Sato, K., & Honma, M. (2021). Epigenetic effect of the mycotoxin fumonisin B1 on DNA methylation. *Mutagenesis*, 36(4), 295–30. https://doi.org/10.1093/mutage/geab019

Sun, G., Wang, S., Hu, X., Su, J., Zhang, Y., Xie, Y., Zhang, H., Tang, L., & Wang, J. S. (2011). Co-contamination of aflatoxin B1 and fumonisin B1 in food and human dietary exposure in three areas of China. *Food Additives & Contaminants: Part A Chem Anal Control Expo Risk Assess.*, 28(4), 461–470. https://doi.org/10.1080/19440049.2010.544678

Stockmann-Juvala, H., Mikkola, J., Naarala, J., Loikkanen, J., Elovaara, E., Savolainen, K. (2004). Oxidative stress induced by fumonisin B1 in continuous human and rodent neural cell cultures. *Free Radical Research*, 38, 933–942. https://doi.org/10.1080/10715760412331273205

Stockmann- Juvala, H., & Savolainen, K. (2008). A review of the toxic effects and mechanisms of action of fumonisin B1. *Human & Experimental Toxicology*, 27(11), 799–809. https://doi.org/10.1177/0960327108099525

Voss, K. A., Chamberlain, W. J., Bacon, C. W., Herbert, R. A., Walters, D. B., & Norred, W. P. (1995). Subchronic feeding study of the mycotoxin fumonisin B1 in B6C3F1 mice and Fischer 344 rats. *Toxicological Sciences*, 24(1), 102–110. https://doi.org/10.1093/toxsci/24.1.102

Voss, K. A., Gelineau-van Waes, J. B., & Riley, R.T. (2006). Fumonisins: current research trends in developmental toxicology. *Mycotoxin Research*, 22(1), 61–69. https://doi.org/10.1007/BF02954559

Wang, E., Norred, W. P., Bacon, C. W., Riley, R. T., & Merrill, A. H. Jr. (1991). Inhibition of sphingolipid biosynthesis by fumonisins. Implications for diseases associated with Fusarium moniliforme. *Journal of Biological Chemistry*, 266(2), 14486–14490. https://pubmed.ncbi.nlm.nih.gov/1860857/