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Cytotoxic Effect of Mycotoxin Extracts from Nigerian Food on Human Blood Mononuclear Cells

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KEYWORDS Aflatoxins. Ochratoxins. Fumonisins. Ochratoxins. Toxicity

ABSTRACT Mycotoxin positive extracts from some Nigerian food commodities were exposed to human blood mononuclear (HBM) cells following methyl thiazol tetrazolium (MTT) salt assay. Human blood mononuclear cells isolated from the plasma of healthy donors were exposed to extracts derived from multi-mycotoxin dialysis clean up technique for up to 72 hours and cell viability of the mononuclear cells evaluated. Selected neutral fraction extracts, which contained high concentrations of aflatoxins (109.78µg/kg), zearalenone (531.9µg/kg) and deoxynivalenol (4.60µg/kg) induced cell viability reduction up to 72.3 percent after 48 hours, whereas, acid fraction extracts containing a high ochratoxin concentration (525.4µg/kg) reduced up to 72.4 percent after 48 hours. Food extracts containing high concentration of fumonisins (3643.6µg/kg) induced reduced viability up to 86.7 percent after 48 hours. The study showed that a combination of mycotoxins could exert a reduction in cell viability of human HBM cells thereby, resulting in immune suppression. This paper contributes to the knowledge of cytotoxic effects of extracts (containing different mycotoxins) from different Nigerian food commodities on HBM cells.

INTRODUCTION

Mycotoxins commonly occur in a wide range of fungal contaminated environment and food commodities (Campbell et al. 2004; Fandohan et al. 2005), such as cereals, seeds, fruits and vegetables (Gnonlonfin et al. 2008; Petzinger and Weidenbach 2002; Reiter et al. 2010). These chemical compounds, which are secondary metabolites produced by a certain group of fungal species (filamentous fungi) under favorable environmental conditions have continued to be a reason for concern in food safety globally (Kuiper-Goodman 1995; Wagacha and Muthomi 2008). The reason for this increasing concern can be attributed to the toxigenic, mutagenic, teratogenic, genotoxic and carcinogenic properties of these chemical compounds when ingested or inhaled by humans and animals (Chu 1991; Hussein and Brasel 2001). Out of over 300 mycotoxins reported worldwide (Chu 1991; Van Egmond et al. 2007), aflatoxins (AFS), ochratoxins (OTs), fumonisins (FBs), zearalenone (ZEA) and the trichothecenes (TCTs) are known to be of greater interests because besides occurring commonly in the environment, they have adverse health effects when humans and animals are exposed to them in large doses and over a long period of time (Ali et al. 2011; Kouadio et al. 2005; McKean et al. 2006; Wang and Groopman 1999).

Due to the predominantly hot and humid climatic conditions of Nigeria, there is always the tendency for production of mycotoxins by filamentous fungi contaminating food commodities because these climatic conditions contribute to enhancing mycotoxin production by fungi. Aflatoxins commonly produced by species of the *Aspergillus* genus (*A. flavus* and *A. parasiticus*) predominant in this part of Africa (Makun et al. 2010; Makun Hussaini Anthony et al. 2011b) are a group of toxic and carcinogenic polyketide substances reported to primarily affect the liver. The ochratoxins A (OTA) and B (OTB) commonly produced by species of the *Aspergillus* family (*A. niger* and *A. ochraceus*) and the *Penicillium* family (*P. viridicatum*) are also mycotoxins of great interest with special reference to OTA, which is a potent nephrotoxin and has been reported in association with a host kidney and upper urinary tract disorders of which the Balkan Endemic Nephropathy has been mentioned (Grollman and Jelakovic 2007; Pfhol-Leszkowicz et al. 2002; Richard 2007). Further studies have also reported OTA as a liver toxin, causing cancer in laboratory animals exposed to them (Kumar et al. 2008; Prelusky et al. 1994).

Other mycotoxins fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), zearalenone and the trichothecenes (deoxynivalenol, T-2 toxin and diacetoxyscirpenol) produced by *Fusarium* species are some chemical compounds reported to have negative health effects in humans and animals. The fumonisins (sphingolipid-like structures) are reported to be cancer promoting chemical substances, which inhibit the uptake of folic acid via the folate receptor. As a result of their sphingo-like structure, fumonisins interfere with sphingolipid metabolism resulting in their association with liver toxicity, hepatotoxicity, nephrotoxicity and oesophageal cancer in humans and animals (Dutton 1996; Richard 2007; Soriano and Dragacci 2004). Toxicity induction by trichothecenes is influenced by their ability to inhibit protein biosynthesis there resulting in immune suppression (Beardall and Miller 1994; Makun et al. 2009). Toxic effects associated with some trichothecenses include nausea, vomiting, diarrhea, acute toxicosis and hemorrhage amongst others (Hussein and Brasel 2001; Richard 2007; Zain 2011). The oestrogenic toxin zearalenone, which causes infertility in animals, has been reported in relation to human cervical cancer and outbreaks of precocious pubertal changes in children (Saenz de Rodriguez et al. 1985; Szuetz et al. 1997). A study by Khosrokhavar et al. (2009) also indicates the potential of zearalenone to stimulate growth of human breast cancer cells containing estrogen response receptors.

The various reported toxic effects of mycotoxins from previous studies have shown that mycotoxins can have really adverse effects on both humans and animals when they are exposed to them over a long period of time. These effects contribute to reasons for classifying some mycotoxins including aflatoxin B_1 and fumonisin B_1 as human carcinogens by the international agency for research on cancer (IARC) (IARC 1993b; IARC 2002). Mycotoxins affect different human cell lines. In vitro studies have shown that there are several responses of experimental human cell lines to mycotoxins. Bouaziz et al. (2008) demonstrated that zearalenone induced apoptotic cell death in human hepatoma cells and Kouadio et al. (2005) confirmed the ability of this mycotoxin to induce oxidative stress and eventually cell death in human intestinal cells. A more recent study by Ánninou et al. (2014) reported the synergistic cytotoxic potential of mycotoxins at even the smallest concentration. Consumption of food contaminated with different mycotoxins expose individuals to individual effects as well as synergistic toxic effects of mycotoxins. From the text, there is evidence of contamination of many food commodities in Nigeria by mycotoxins and this poses a health risk to individuals even though they are consumed in low doses. There has been inadequate investigation of the toxic effects of these chemicals when ingested through food by individuals in this part of Africa with only one study by Makun Hussaini Anthony et al. (2011a), which demonstrated cytotoxicity of mycotoxins produced by a *Fusarium* strain isolated from rice on human cell lines. It is therefore imperative to investigate the cytotoxic effects of food extracts, which might contain a wide array of mycotoxins and determine health risks associated with their consumption. This paper is therefore aimed at investigating the degree of toxicity induction by mycotoxin extracts of different food samples from Nigeria on human blood mononuclear cells to understand the health risks consumers of these food commodities are exposed to.

MATERIAL AND METHODS

Chemicals

All chemicals and reagents used in this study were at least of analytical grade, unless otherwise specified and purchased from Sigma-Aldrich chemical company.

Sampling

A total of 144 food samples consisting of rice (41), maize (39), cocoa (39) and cocoa-based powder beverages (25) each weighing 200g were collected from different fields, stores and market places in towns from southeastern and southwestern Nigeria. All samples collected were destined for human consumption and were in considerably good visual condition. These samples were screened for mycotoxin contamination using various analytical methods.

Sample Preparation

All seed and grain samples were ground using a sterile laboratory mill (IKA M20, Merck, Darmstadt, Germany) and collected in plastic bags for further analysis.

Mycotoxin Standards

Aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), ochratoxin A (OTA), fumonisin B_1 (FB1), fumonisin B_2 (FB_2), zearalenone (ZEA) and deoxynivalenol (DON) standards were obtained from the Program on Mycotoxins and Experimental Carcinogenesis (PROMEC) Medical Research Council (MRC), South Africa. Standard solutions of AFs, OTA, ZEA and DON were prepared by dissolving the pure standards in HPLC grade acetonitrile to give concentrations 10ug/ml each for OTA, AFs, ZEA, and 50ug/ml for DON. Standard solutions of FBs were prepared by dissolving in HPLC grade methanol to give concentrations of 10ug/ml each for FB1 and FB2.

Mycotoxin Extraction and Cleanup from Food Samples

The method of multi-mycotoxin extraction procedure (Egbuta et al. 2013; Patterson and Roberts 1979) was used for extraction and cleanup by dialysis of ZEA, AFs, OTA and DON, whereas, strong anion exchange (SAX) columns were used for FB extraction and cleanup following the method of Shephard and Sewram (2004) with some modifications. Also, immuno-affinity columns were used for extraction and cleanup of some of the mycotoxins (AFs and FBs) following the manufacturer's instruction manual (VICAM Ltd. Watertown, USA) for confirmation of results obtained from multi-mycotoxin extraction procedure and SAX column extraction procedure.

HPLC Analyses of Extracts

High performance liquid chromatography analyses was carried out using a HPLC Spectra Physics SCM400 SYSTEM (Waters, Milford, MA, USA), Shimadzu Corporation (Kyoto, Japan) LC-20AB liquid chromatograph equipped with CBM-20A communication bus module, LC-20AB degasser, CTO-20A column oven, Nova-Pak 4μm C18 reversed phase analytical column (250×4.6 mm, 5μm), SIL-20A auto sampler, RF-10AxL fluorescence detector, RID-10A refractive index detector and SPD-M20A photodiode array detector linked to LC solutions version 1.22 Software Release. Dry mycotoxin extracts were dissolved in 500ul of acetonitrile for AFs, OTA, DON and ZEA analysis, transferred into blue cap HPLC vials for analysis. For FBs, extracts were dissolved in 1ml methanol and 50ul transferred into a HPLC vial mixed with 250ul of opthaldialdehyde (OPA) reagent prepared by dissolving 40 mg of OPA in 1 ml CH3OH and diluted with 5 ml 0.1M sodium tetraborate 125 (Na2B2O4) and 50μl mercapthoethanol. Samples were run at 1ml per minute (min-1) recording retention times against standards of known concentrations. The analysis of the different mycotoxins involved the use of specific mobile phases and methods of detection following the methods of Abdulkadar et al. (2004) and Reiter et al. (2010). Extracts containing lowest, medium and highest concentration of mycotoxins were kept for cytotoxicity analysis.

Recoveries

To confirm the efficacy of the methods used for extraction of mycotoxins from samples, different concentrations of mycotoxins were used to spike negative samples in triplicates and extracted using the methods of multi-mycotoxin extraction, SAX columns and immunoaffinity columns. 100μ/ml, 50μ/ml, 100μ/ml, 50μ/ml and 50μ/ml of AFs, OTA, FBs, ZEA, and DON were respectively spiked on samples and mixed thoroughly before extraction using the different methods. Extracts were analyzed using the HPLC apparatus.

Cytotoxicity Analyses

Cytotoxicity analyses was done using a Laminar flow (hood) containing UV light, microtitre 96- Well plates (Corning Cell WellsTM, Corning, USA), DAS Microplate Reader (modello: A2; Rome, Italy), ELISA Microplate Reader, Light microscope, Centrifuge, 5 percent CO2 humidified incubator set at 37 o143 C, Sterile Haematocytometer (Neubauer counting chamber), Sterile 96 well microtitre plates U- type with lids, histopaque 1077, MTT assay kit, Hank's Balanced Salt Solution (HBSS), tissue culture media (RPMI-1640), trypan blue solution, phosphate buffer saline (PBS) (pH 7.4), dimethyl sulphoxide (DMSO), complete culture medium (CCM) prepared by adding 50 ml of foetal calf serum (FCS) and 1 ml Penstrep-Fungizone to 500 ml RPMI-1640. Methylthiazol tetrazolium (MTT) prepared by reconstituting 5 mg of methylthiazol tetrazolium salt in 1 ml phosphate buffered

solution (PBS) and filtered through 0.22 ìm filter paper, Eppendorf tubes, sterile Pasteur pipettes, swabs, gloves, lithium heparin micropillary tubes, culture flasks, sample extracts and mycotoxin standards. Human blood mononuclear cells were exposed at different volumes (20µl, 40µl, 80µl) of extracts and FB standard against control cells, which had been exposed to the extract dissolving solvent. After 24 and 48 hours, cell viability was determined upon exposure to MTT salt solution. Samples were selected randomly based on degree of contamination by mycotoxins. Samples with high, low and no contamination were selected and used for this analysis. Extracts from neutral fraction, acid fraction and fumonisin fraction of samples were used for this analysis.

Human blood mononuclear cells were isolated, purified and cultured prior to cytotoxicity assay using the methods of Cetin and Bullerman (2005) with some modifications in a sterile environment. Twelve (12) ml venous blood collected from healthy donors upon consent and ethical clearance (Academics Ethics Committee, 2010, Faculty of Health Sciences, University of Johannesburg) was transferred immediately to a 3 X 5ml heparinised tube using a sterile syringe. Human blood mononuclear cells were extracted, cell viability of 95-98 percent determined and cultured using complete culture medium prepared by mixing 5 percent PHA, 10 percent FBS, 1 percent penistrep (antibiotic) and 83.25 percent RPMI for 24 hours in 5 percent CO2 buffered and humidified incubator at 37 ^{o}C. After 24 hours of incubation, 100 µl of RPMI was added to each well of a 96-well culture plate and this was followed by the addition of 100µl of cultured cells to the wells and incubated for 16 to 24 hours to allow cells adjust to its new environment. After 24 hours, FB standards at different concentrations of 10, 20 and 40µ/ml and 50µl of dissolving solvent (0.1% methanol) were added to the wells in triplicates. Extracts of interest were dried and re-dissolved in 200µl of 0.1 percent methanol and added to the remaining wells in triplicates. Culture plates were shaken gently to enable proper mixing of the solution in the wells and incubated further in the 5 percent CO2 buffered and humidified incubator at 37 ^{o}C for 24 and 48 hours. Wells containing cells without any solvent/extract added were used as controls for determining cytotoxic activity of extract (neutral fraction and acid fraction). After 24 and 48 hours of incubation, 50µl of MTT-solution prepared by MTT salt in 0.14M PBS (pH 7.4) at 5mg/ml and filtered using a 0.22µM pore size syringe filter was added to each well, shaken gently for proper mixing and incubated for another 1 hour. After 1 hour, 50µl of twenty percent (w/v) sodium dodecyl sulphate/1M hydrochloric acid solution was added and allowed to solubilize the formazan crystals formed for 6 hours. The absorbance optical density (OD) was then read using a microplate reader at wavelengths of 540 and 620 nm. Cell viability as influenced by mycotoxin exposure was determined as:

% Cell viability = (ODM/ODN) x 100

Where, ODM is the OD value of mycotoxin-treated PHA stimulated cells and ODN is the OD value of control (no mycotoxin) PHA-stimulated cells

Subsequently, toxicity induction was calculated as:

100%- Cell viability (%) = Toxicity induction (%)

Statistical Analysis

Using SPSS statistical software version 19, a one-way analysis of variance (ANOVA) was performed to derive mean values, which were compared by least significant difference using all pairwise multiple comparison procedures (Holm-Sidak method). Mean values among treatment groups were deemed to be different if the level of probability was <0.05.

RESULTS AND DISCUSSION

HPLC Results

Mycotoxin analysis and quantification by HPLC in this study which is displayed in Table 1 showed prevalence of five different mycotoxins in all extracts of food samples at different concentration ranges from very high to very low values (Table 1). Rice and cocoa-based powder beverage samples contained highest concentration of ZEA, whereas FUMs and ZEA were of highest concentration in maize samples. Cocoa samples contained mycotoxins OTA and ZEA more in comparison with the other mycotoxins investigated. Incidences of mycotoxin occurrence in the food samples illustrated in Table 2 show that maize and rice samples were more contaminated with mycotoxins compared with the other food samples. Fumonisins (94.9%) and AFs (94.9%) occurred most in maize samples, where-

Table 1: Concentration range in µg/kg of five major mycotoxins in different Nigerian food samples

Food samples	*Mycotoxins*				
	AFs	*OTA*	*FBs*	*DON*	*ZEA*
Rice	0.0- 6.5	0.7- 180.9	0.9- 59.6	0.1- 0.7	0.7- 570.6
Maize	0.1-109.8	0.6- 79.0	10-3644	0.1- 0.7	1.8- 652.3
Cocoa	0.0- 16.0	0.3- 884.8	7.6- 440	0.2- 8.5	10.4- 531
Cocoa-based beverage	0.1- 9.8	0.3- 72.6	5.8- 208.7	0.1- 7.6	34.4-2364.7

as OTA (92.7%) and FUMs (90.2%) occurred most in rice samples.

Cytotoxicity Results

Reduced Cell Viability

Methylthiazol tetrazolium (MTT) salt analysis showed reduced cell viability of up to 76.3 percent induced by extracts of neutral fraction, which had high concentrations of mycotoxins AFs, ZEA and DON over a period of 24 hours in different dilutions. Extracts with lower concentrations of AFs, ZEA and DON showed reduced cell viability of up to ninety percent. After 48 hours of exposure, cell viability of up to 72.3 percent was reported for neutral fraction extracts with high mycotoxin contamination while extracts with low mycotoxin contamination showed cell viability of up to 88.2 percent. Mononuclear cells exposed to acid fraction mycotoxin extracts containing high concentration of OTA had reduced cell viability of up to 75.2 percent after 24 hours and 72.4 percent after 48 hours. Acid fraction extracts containing less concentrations of OTA exerted reduced cell viabilities up to 89.6 percent after 24 hours and 87.5 percent after 48 hours.

Exposure of mononuclear cells to fumonisin standards of different concentrations- 50µg/ml, 100µg/ml and 200µg/ml showed decreased cell viability of up to 88.7 percent and 82.6 percent over 24 hours and 48 hours, respectively. Fumonisin extracts from 217 maize and rice samples containing 3643.7ppb and 59.6ppb of FB1 exerted a reduced cell viability of 90.1 percent and 92.7 percent respectively after 24 hours. After 48 hours, reduced cell viabilities of up to 86.7 percent and 88.3 percent were recorded for fumonisin extracts from maize and rice samples, respectively. Fumonisin extracts from maize and rice samples containing less concentrations of 230ppb and 10.3ppb of fumonisin respectively, exerted a reduction of cell viability up to 95.7 percent and 97.8 percent respectively, whereas cell viabilities of 92.8 percent and 94.3 percent was recorded after 48 hours. Cells, which were exposed to 0.1 percent MeOH (methanol) showed no distinctive change in cell viability.

Table 2: Incidence of mycotoxins in different food samples analysed

Mycotoxins	*% incidence*			
	Cocoa	*Cocoa-based powder beverages*	*Maize*	*Rice*
Aflatoxins	80.5	44	94.9	87.8
Deoxynivalenol	59.1	32	69.2	70.7
Fumonisins	93.2	84	94.9	90.2
Ochratoxin A	80.4	56	92.3	92.7
Zearalenone	45.9	4	64.1	80.5

Toxicity Induction

Toxicity induced on human blood mononuclear cells by the extracts of high concentration of mycotoxins (AFs, ZEA and DON) from the neutral fraction showed toxicity induction of 7.5 percent to 23.7 percent after 24 hours exposure and 9.3 percent to 23.7 percent after 48 hours. Toxicity induction by neutral fraction extracts of low concentration of mycotoxins (AFs, ZEA, and DON) showed toxicity induction from 3.2 percent to 9.1 percent for 24 hours exposure and 2.6 percent to 13.2 percent for 48 hours exposure. Extracts from neutral fractions of cocoa and maize showed higher degree of toxicity induction compared to those of rice and cocoa-based powder beverage. Toxicity induction by acid fraction extracts containing high concentration of mycotoxin OTA showed a degree of toxicity induction from 10.8 percent to 24.8 percent after 24 hours exposure and 14.4 percent to 27.6 percent after 48 hours exposure. Acid fraction extracts of low mycotoxin concentration (OTA) caused

toxicity induction from 1.8 percent to 10.4 percent after 24 hours exposure and 4.1 percent to 12.5 percent after 48 hours exposure.

Toxicity induction on cells by fumonisin standard showed 5.4 percent, 9.6 percent and 11.3 percent at 50ppb, 100ppb and 200ppb concentrations respectively after 24 hours exposure and 8.1 percent, 12.9 percent and 17.4 percent after 48 hours. Fumonisin extracts of 3643.7 ppb from maize and 59.6 ppb from rice samples showed toxicity induction up to 9.9 percent and 7.3 percent respectively after 24 hours, whereas, after 48 hours exposure, toxicity induction up to 13.3 percent and 11.7 percent was recorded. Extracts of less fumonisin contaminated (230μg/kg and 10.3gμ/kg for maize and rice respectively) samples showed toxicity induction of 1.2 percent to 4.3 percent and 3.8 percent to 7.2 percent for maize samples after 24 hours and 48 hours exposures respectively, whereas, exposure of mononuclear cells to less concentrated fumonisin extracts of rice samples for 24 hours and 48 hours showed toxicity induction of 0.8 percent to 2.2 percent and 1.5 percent to 5.7 percent respectively. Figures 1-12 show graphical illustrations of toxicity induction by mycotoxin extracts from food samples.

High mycotoxin containing rice samples included R-5 (AFs- 6.50μ/kg, ZEA- 570.6μg/kg, DON- 0.4μg/kg), R-32 (OTA- 180.9μg/kg) and R-36 (FB- 59.6μg/kg),

Low mycotoxin containing rice samples included R-18 (AFs- 0.43 μg/kg, ZEA- 68.0μg/kg, DON- 0.2μg/kg), R- 23 (OTA- 16.6 μg/kg) and R-6 (FB- 8.1μg/kg).

High mycotoxin containing maize samples were M-18 (AFs- 109.78μg/kg, ZEA- 324.4μg/kg, DON- 0.7μg/kg), M-19 (OTA- 79.0μg/kg) and M-25 (FB- 3643.6μg/kg),

Low mycotoxin containing maize samples were M-38 (AFs-96.30 μg/kg, ZEA- 125.5μg/kg, DON- 0.4μg/kg), M-38 (OTA- 12.3μg/kg) and M-36 (FB- 175.6μg/kg).

High mycotoxin containing cocoa samples were C-24 (AFs- 13.10μg/kg, ZEA- 531.9μg/kg, DON- 4.6μg/kg) and C-29 (OTA- 525.4μg/kg).

Low mycotoxin containing cocoa samples were C-16 (AFs- 4.12μg/kg, ZEA- 67.6μg/kg, DON- 0μg/kg) and C-13 (OTA- 74.3μg/kg).

High mycotoxin containing cocoa-based beverage samples included B-25 (AFs- 9.38μg/kg, ZEA- 2364.7μg/kg, DON- 1.4μg/kg) and B-4 (OTA- 72.6 μg/kg).

Low mycotoxin containing cocoa-based beverage samples included B-16 (AFs- 1.79μg/kg, ZEA- 209.2μg/kg, DON- 0.3ìg/kg) and B-21 (OTA- 20.8μg/kg).

Role of Food Extracts in Cell Viability Reduction

Cytotoxic effect of a compound on cells is the ability of that compound to bind to cell re-

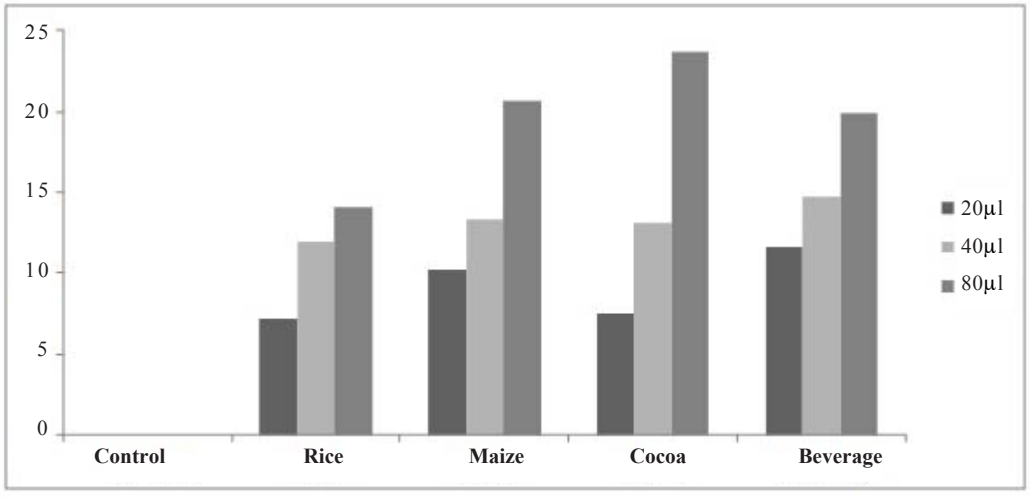

Fig. 1. Graph of toxicity induction by food extracts containing high concentration of neutral fraction mycotoxins on human blood mononuclear cells after 24 hrs. exposure

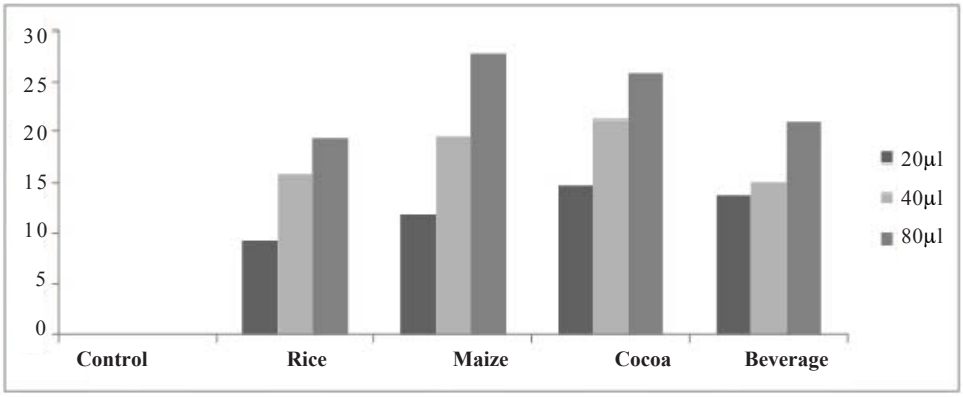

Fig. 2. Graph of toxicity induction by food extracts containing high concentration of neutral fraction mycotoxins on human blood mononuclear cells after 48 hrs. exposure

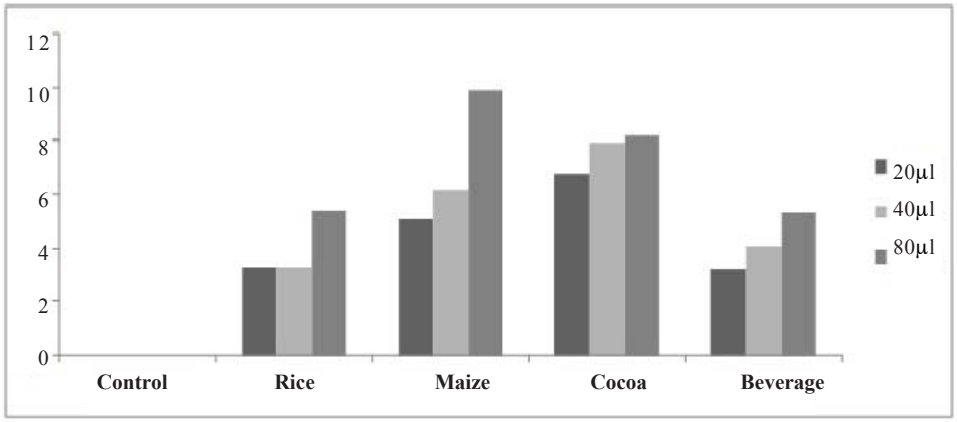

Fig. 3. Graph of toxicity induction by food extracts containing low concentration of neutral fraction mycotoxins on human blood mononuclear cells after 24 hrs. exposure

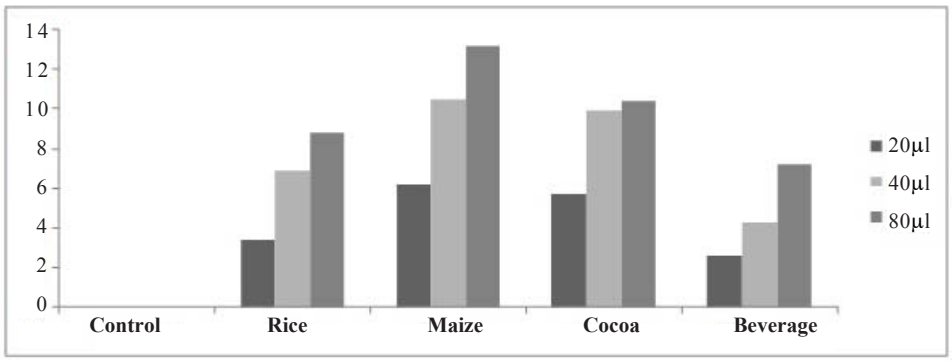

Fig. 4. Graph of toxicity induction by food extracts containing low concentration of neutral fraction mycotoxins on human blood mononuclear cells after 48 hrs. exposure

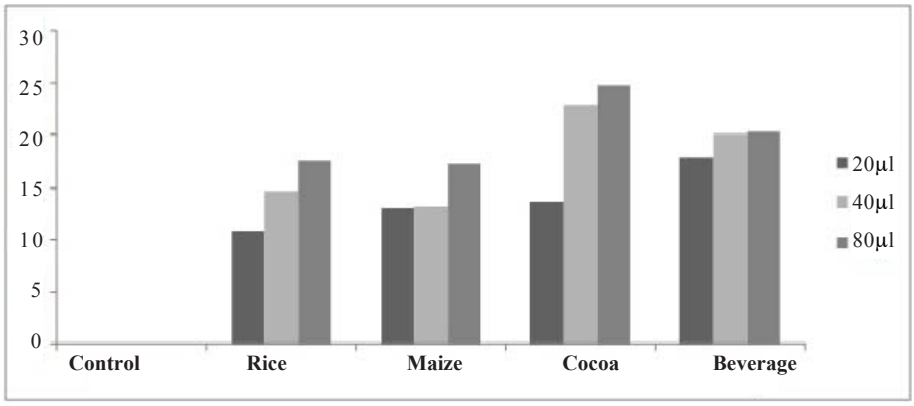

Fig. 5. Graph of toxicity induction by food extracts containing high concentration of acid fraction mycotoxins on human blood mononuclear cells after 24 hrs. exposure

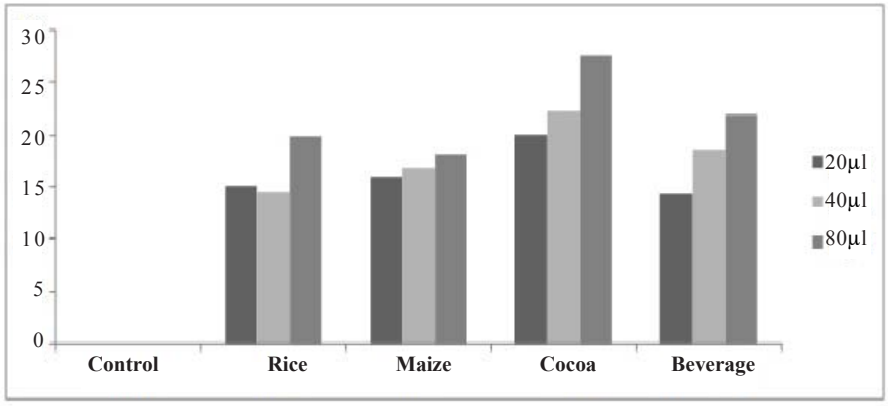

Fig. 6. Graph of toxicity induction by food extracts containing high concentration of acid fraction mycotoxins on human blood mononuclear cells after 48 hrs. exposure

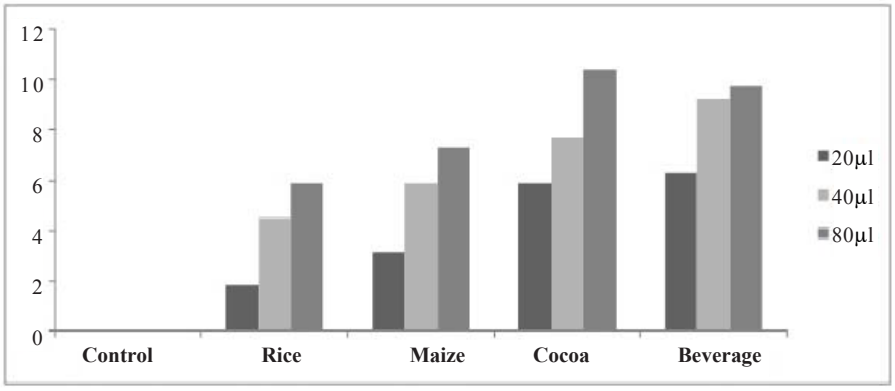

Fig. 7. Graph of toxicity induction by food extracts containing low concentration of acid fraction mycotoxins on human blood mononuclear cells after 24 hrs. exposure

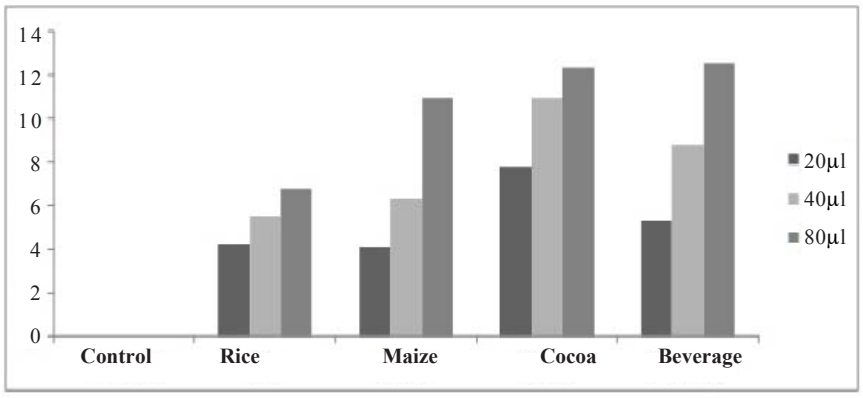

Fig. 8. Graph of toxicity induction by food extracts containing low concentration of acid fraction mycotoxins on human blood mononuclear cells after 48 hrs. exposure

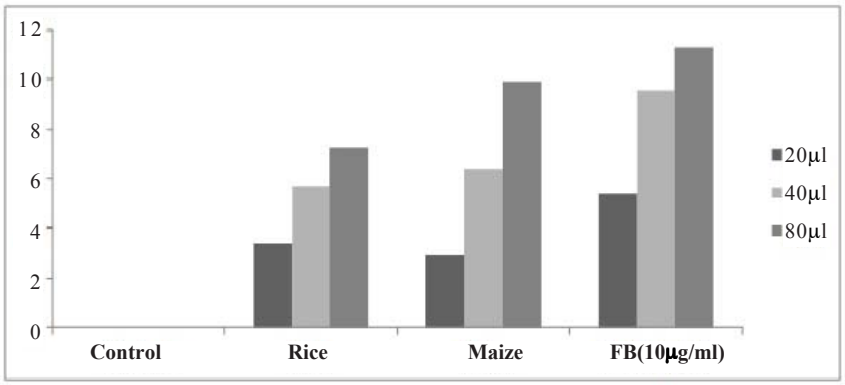

Fig. 9. Graph of toxicity induction by food extracts containing high concentration of fumonisin fraction mycotoxins on human blood mononuclear cells after 24 hrs. exposure

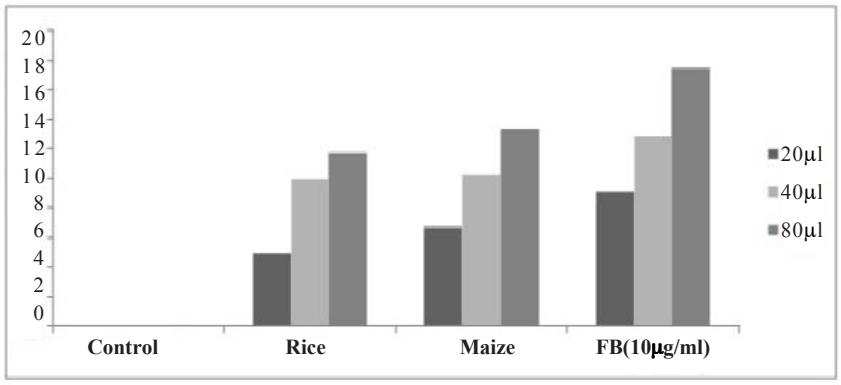

Fig. 10. Graph of toxicity induction by food extracts containing high concentration of fumonisin fraction mycotoxins on human blood mononuclear cells after 48 hrs. exposure

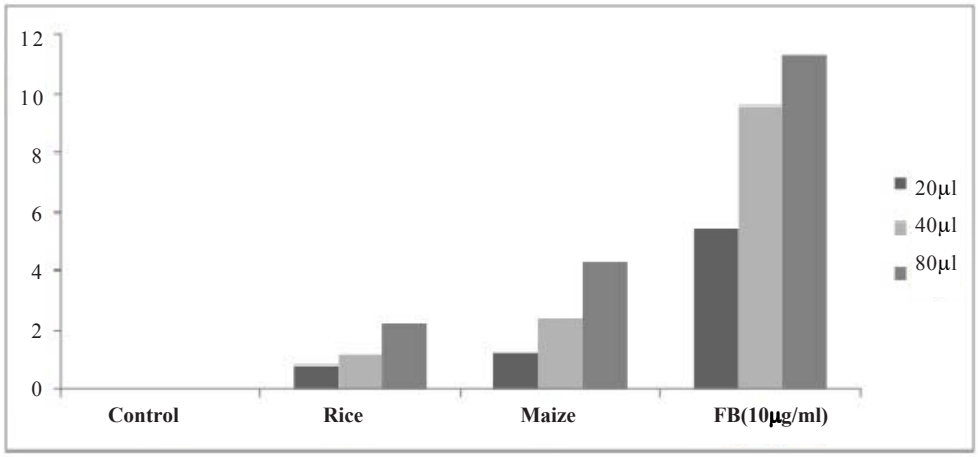

Fig. 11. Graph of toxicity induction by food extracts containing low concentration of fumonisin fraction mycotoxins on human blood mononuclear cells after 24 hrs. exposure

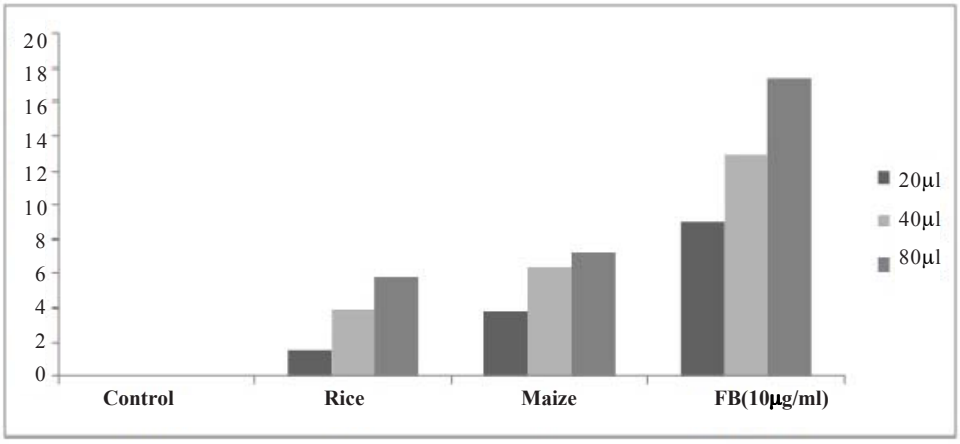

Fig. 12. Graph of toxicity induction by food extracts containing low concentration of fumonisin fraction mycotoxins on human blood mononuclear cells after 48 hrs. exposure

ceptors or penetrate the cell membrane and thereby induce an alteration of the behavior of the cells, as well as, alter viability of the cells since compounds that have cytotoxic effects often compromise cell membrane integrity thereby causing necrosis (Los et al. 2013). Data obtained from cytotoxicity assay of neutral fraction extracts of maize, rice, cocoa and cocoa-based beverage samples showed a time and dose dependent decrease in cell viability of human blood mononuclear cells. As was indicated in the results, higher doses (80µl) of mycotoxin extracts induced greater reduction in cell viability when compared to lower doses (20µl) of the same extracts. Reduction in cell viability progressed along with the length of time of exposure to the highly concentrated extracts in comparison with untreated mononuclear cells. Whereas, extracts from samples, which were less contaminated with AFs, ZEA and DON induced a reduction in cell viability but not as much as highly contaminated samples. Although there is limited information on the combined cytotoxic effects of these three mycotoxins (AFs, ZEA and DON) detected in the neutral fractions on human blood mononuclear cells, reports of combined cytotoxic ef-

fects of ZEA and DON as well as AFs and FB1 have been reported (Bernabucci et al. 2011; Kouadio et al. 2005) on human intestinal cell line and bovine peripheral blood mononuclear cells to induce reduction in cell viability with respect to the concentration and length of time of exposure.

Results from the cytotoxic effects of mycotoxin contaminated extracts in the present paper also agree to an extent with other papers mentioned, as there was a reduction in cell viability of mononuclear cells on exposure to mycotoxin contaminated neutral fraction extracts in comparison with the unaffected cell viability of untreated mononuclear cells (Bernabucci et al. 2011; Kouadio et al. 2005). Reports on the individual effects of AFs, ZEA and DON have shown that they induce cytotoxic effects on lymphocytes, monocytes and other cell lines in both humans and animals. *In vitro* studies on cytotoxic effect of AFs on human blood lymphocytes and monocytes showed that the longer cells were exposed to AFs, the more their cytotoxic effects (Cusumano et al. 1996; Riley and Norred 1996). Cusumano et al. (1996) reported that as little as 0.1ng/ml was able to induce up to fifteen percent cytotoxicity on human monocytes. Mwanza M (2007) also reported that AFs, DON and ZEA had individual cytotoxic effects on human blood lymphocytes after 24 hours with reduced cell viabilities up to fifty percent, thirty-seven percent and seventy-four percent, respectively. ZEA and DON have also been reported in previous papers to induce cytotoxicity on different human cell lines individually but in combination, the cytotoxic ability of ZEA was reduced (Cetin and Bullerman 2005; Kouadio et al. 2005; Visconti et al. 1991). Reduction in cell viability of the exposed cells in the paper conformed to other cytotoxicity studies involving mycotoxins.

Extracts from acid fractions of maize, rice, cocoa and cocoa-based powder beverages that had highest OTA concentration level induced a reduced cell viability of up to 83.8 percent, 82.4 percent, 75.2 percent and 79.7 percent respectively after 24 hours exposure and up to 81.8 percent, 80.1 percent, 72.4 percent and 78.1 percent respectively after 48 hours exposure at highest concentration (80μl). Low OTA contaminated extracts induced decreased cell viability after 24 hours and 48 hours exposure and this data is in agreement with previous studies on the cytotoxic effects of OTA on human blood lymphocytes and other human and animal cell lines (Bouaziz et al. 2008; Cosimi et al. 2009; Lioi et al. 2004; Russo et al. 2005; Scibelli et al. 2003; Thuvander et al. 1995) at different concentrations over a period of hours and days with concentrations as low 1.2 μg/ml reducing cell viability to 77.6 percent in bovine lymphocyte cultures *in vitro* (Lioi et al. 2004) and as low as 12 μg/g reducing cell viability by fifty percent in human hepatoma cells (Bouaziz et al. 2008). There is also the possibility of other toxins not screened for being present in the extracts subjected on the mononuclear cells during the present study that could have contributed to inducing reduced cell viability.

Human blood mononuclear cells exposed to FB contaminated extracts of maize and rice samples showed sensitivity to extracts as well as standards. The degree of sensitivity increased with increased dose and duration of exposure. Reduced cell viability of up to 86.7 percent and 88.3 percent after 48 hours exposure was recorded for FB contaminated maize and rice extracts, respectively. Less contaminated samples also exerted reduction in cell viability as duration of exposure increased. This data is in agreement with a paper by Gutleb et al. (2002), who reported that 50μg/kg of FB_1 resulted in eighty percent cell viability and another study by Cetin and Bullerman (2005) indicated a reduced cell proliferation by fifty percent at exposure of cells to high concentrations of 94.8μg/ml and 98.38μg/ml. Also, a paper by Mwanza M. et al. (2009) showed a decrease in cell viability of human cells up to 16.3 percent and 23.5 percent after 96 hours exposure to fumonisin standards of 5 μg/ml and 20ig/ml, respectively. Fumonisin extracts from maize samples induced toxicity on mononuclear cells in comparison to rice samples, which could be attributed to high contamination of maize samples by FB compared to rice samples. Lioi et al. (2004) also reported a decrease in cell proliferation on exposure to high levels of FB1, which can also be related to reduce cell viability, one of the mechanisms of immunosuppression by mycotoxins.

In this paper, there was a time and dose dependent response of cells to mycotoxin extracts from food samples, which could be explained by the ability of mycotoxins to induce negative health effects when exposure is prolonged (Wagacha and Muthomi 2008; Zain 2011). Mycotoxins like many other cytotoxic compounds possess the ability to penetrate cell membranes,

thereby disrupting them and inducing different abnormal cell functions (Doi and Uetsuka 2011). This can be one explanation for toxicity induced by food extracts in this study with reports of mycotoxins such as DON associated with alteration of cell cyles (Tiemann et al. 2003), AFs affecting membrane potential of mitochondria thereby resulting in apoptosis and necrosis (Al-Hammadi et al. 2014; Liu et al. 2014), and OTA inducing oxidative stress in cells (Doi and Uetsuka 2011).

CONCLUSION

It is usually common to find more than one mycotoxin occurring in a particular food or environment and this paper has shown more than one mycotoxin present in food extracts can result in adverse effects. This paper was able to show that consumption of mycotoxin contaminated food commodities over time can result in negative health conditions such as immune suppression that could lead to more serious effects. Reduction in cell viability, which eventually results in immunosuppression among other negative health effects of these mycotoxins is an issue that must be taken to heart when considering intervention strategies to combat mycotoxin contamination of food in Nigeria.

RECOMMENDATIONS

It is therefore recommended that more intensive research be conducted on other food commodities to have more information on mycotoxin occurrence and mycotoxin control/reduction, as well as more studies on the health implications in relation to dietary exposure of people in Nigeria to mycotoxins in agricultural commodities in order to tackle the mycotoxin problem in the country. More research into cellular activities of human cells when exposed to these toxins in the food commodities can also be looked into to determine more health risks consumers are exposed to.

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