Study of the Cytotoxic Effects of Aflatoxin on Hematopoietic Stem Cells

Musleh Mohammed Hamada[1*], Al-Kubaisi Salah M.A.[2] and Al-Ouqaili Mushtak T.S.[3]
1. Department of Biology, College of Education for Pure Sciences, University of Anbar, IRAQ
2. Department of Veterinary Internal and Preventive Medicine, College of Veterinary Medicine, University of Fallujah, IRAQ
3. Department of Microbiology, College of Medicine, University of Anbar, IRAQ
*mohammedhamada@uoanbar.edu.iq

Abstract
Aflatoxins (AFs) are secondary metabolites having a high cytotoxic potential and have an active carcinogenic compound. This study aimed to isolate hematopoietic stem cells (Precursors of mononuclear stem cells) from human umbilical cord blood. Collection of umbilical cord blood samples from cord blood was accomplished immediately after delivery, while the placenta is intrauterine by used closed system method. The mononuclear cells (MNCs) were obtained from umbilical cord blood by using Buffy coat method. The previous cells have been Hematopoietic Stem Cells (HSCs) used in the cytotoxicity test of AF extract. The mononuclear cells were cultured in DMEM medium with 10% FCS. An experimental study was conducted in vitro on these cells to confirm the toxic effects of AF with different concentrations of AFs.

The results showed that the inhibition rate increases seriously with the increase of AF concentration. Aspergillus flavus in peanut produces the highest concentration of AF, while in soil, contamination with crude oil A. flavus produces the least concentration of AF. Additionally, the concentration of 1 µg /ml of AF extracted from the aflatoxigenic isolates which can kill 100% of hematopoietic stem cells.

Keywords: Aflatoxins, Cytotoxicity, Lymphocyte, Stem cells, Umbilical cord blood.

Introduction

HSCs can be collected from cord blood (CB), peripheral blood (PB) and bone marrow (BM). Lately, UCB was increasingly used because readily available[1]. The cell content of UCB is limited and has a higher frequency of progenitor cells compared with PB or BM[2]. UCB derived $CD34^+$ cells have also shown rapid proliferation more than their counterparts from BM[3].

These cells contribute in the formation of immune system cells that can be affected by various types of toxins such as AFs[4]. AFs (AFB1, AFB2, AFG1, AFG2) are mainly produced by *A.flavus* and *A. parasiticus* and are found in various agricultural commodities[5]. These natural toxins have been commonly classified as a carcinogen by the International Agency for Research on Cancer, (IARC). These toxins may have effects on cell immunity, reduction of lymphocyte proliferation, production of cytokines and they have many potentials such as cytotoxicity, carcinogenicity, hepatotoxicity, immunotoxicity and genotoxicity[6]. The frequent exposure of animals to low levels of AF (0.005–0.075 mg/kg body weight) for one week had shown dose-dependent decrease in the percentage of splenic CD8+ T cells and CD3−CD8a+ natural killer (NK) cells[7,8].

Young children are also exposed to dietary AF and had toxic effects on the immune system and human lymphocytes including inhibition of the lymphocyte respiration[9], Apoptosis or apoptosome describes the term Caspase mediated by cytotoxic processes which cause mitochondrial impairment on DNA fragmentation after cell membrane damage[10].

The aim of the present study was to isolate hematopoietic stem cells (precursors of polymorphonuclear stem cells) from human umbilical cord blood and study the toxic effects of AF as the precursors of mononuclear cells and detect its toxic dose.

Material and Methods

Identification of *Aspergillus flavus* isolates: Fifteen *A. flavus* isolates were obtained from two laboratories; five (33%) isolates were obtained from laboratory of College of Veterinary Medicine / University of Baghdad (in which the fungus was isolated from clinical cases), while ten (67%) isolates were obtained from laboratories of the Environment and Water Department, Ministry of Science and Technology.

Isolates were identified depending on the species level based on macroscopical and microscopical characteristics using SDA[11]; a loop was used for taking spores to implant in test tubes and then incubated at the same temperature. After incubation of 10 ml of sterile distilled water in culture tubes with *A.flavus*[12], sterile loop and suspension of spores harvested the spores were prepared in the tubes[13]. Transfer 1 ml of the spore suspension and dilute with the addition of 9 ml of sterilized distilled water to complete the size to 10 ml[14]. After that, the spores were accounted in 5 µL by use of a hemocytometer.

According to Lai et al[15], each flask contains 50 grices sterilized, inoculated with million spores and incubated at 28 ± 1°C in the dark for 21 days and shaken once or twice daily to aid in even distribution of the inocula. After fermentation, the flasks were placed in the oven at 60°C for 3 hours to

destroy the fungus and the HPLC technique determined the amount of AF.

Extraction, purification and detection of Aflatoxin: Aflatoxin has been extracted according to AOAC, procedure with few modifications as follows: 25 grams of weighed rice powder was added to 25 ml of chloroform and water (1:1 v/v). The contents of the flasks were shaken for 60 min. The crude extracts were filtered through gauze and then through Whatmann filter paper (No. 1). 25 ml of filtrate was mixed with 25 ml methanol (90%) and hexane at (1:1 v/v) in a separate funnel for 10 min. The upper layer was discharged and the methanol lower layer was evaporated to near dryness and then 25 ml chloroform/water (1:1 v/v) was added to the extract to another separate funnel. The funnel was shaken thoroughly for about 1 minute; then the lower layer chloroform was passed through filter paper containing 10 g of anhydrous sodium sulfate. The last filtrate was evaporated to near dryness and the residue was stored in small dark vials at 5°C for chemical analysis.

AF has been detected by High Performance Liquid Chromatography (HPLC) with the following conditions: column: 250x4.6mm, particle size 5 µm, ODS (C18), mobile phase acetonitrile: water 40:60 v/v, flow rate 1ml/ minute., detector UV-365nm and compared to standard AF (Sigma) as control.

Cord blood collection: Umbilical cord blood (UCB) sample was obtained from women subjected to cesarean section in Al- Jamiea hospital in Baghdad.

All UCB specimens were freshly collected from the umbilical vein. Immediately after delivery of the baby, the umbilical cord clamped breaking the link between the baby and placenta, the baby separated from the cord and the cord then cleaned in 5-8 cm area of umbilical cord with antiseptic solution and inserted the blood bag needle in the umbilical cord vein. The blood was flown by gravity into the bag containing citrate phosphate dextrose adenine-1 (CPDA-1) anticoagulant approximately 25 ml since total collections were around 100-120 ml. During compilation, blood bag was shaken gently, so that the anticoagulant is freely mixed with UCB[16]. The UCB samples were handled precisely and brought to the stem cell culture laboratory in Al-Nahrain University/ Biotechnology Research Center avoiding the direct sunlight exposure and the extremely high temperature.

Collection of mononuclear cells (MNCs) from Cord blood cell: The blood was diluted with an equivalent volume of phosphate buffer saline (PBS). Four ml of diluted serum was transferred to 10 ml round bottom tubes and layered carefully on 3 ml of Ficoll-Paque solute and centrifuged at 2200 rpm for 25 min at 4°C to isolate MNCs. By using Pasteur pipette, the MNCs rich zone (buffy coat layer) was removed and transferred into a new 10 ml round bottom tube and washed twice with PBS through centrifugation at 4°C in 2000 rpm for 8 minutes and 1000 rpm for 10 minutes[17] .

Determination of cell number and viability: The cell count and sustainability can be determined by using trypan blue stain of 0.04 %. About 100 µL from the resuspended cells were diluted 1:1 with trypan blue solution and mixed and then incubated at room temperature for two mins. To determine the cell viability, the dead cells were stained (blue color), while alive cells were not stained.[18]

A trypan blue solution is then mixed in the Eppendorf tube and was incubated for two minutes at room temperature. Using sterile Pasteur pipette, 10 µl of cell trypan blue suspension mixture was transferred to a hemocytometer chamber and covered with a coverslip firmly in place, viable cells (uncolored cells) in each of the four corner squares on either side of the center chamber were calculated, average the counts multiplied by 2×10^4 to give the number of cells/ml.[19]

Viable Cells (%) = [No. of viable cells/Total No. of cells (dead and viable)]× 100

Cultivation of the HSC from human umbilical cord blood: After determination of the cell count and viability, a number of 1×10^6 cells were cultured in a 25-mm tissue culture flask containing 5 ml of DMEM medium supplemented with 10% FCS and 100 U/ml penicillin, 100 µg /ml streptomycin and 25 µg/ml amphotericin B and plated onto a 25-mm tissue culture dish and incubated at 37°C, 5% CO_2 overnight. After three days, the nonadherent cells were harvested by centrifugation (10 min, 300g), the supernatant was aspirated and was cultured again in a similar condition until a monolayer was occurring.

Results and Discussion

It has been shown in the table 1 that there is a variation in the production of AF for the isolates that are responsible for producing AF. It was also noted from the table that the sample was taken from the peanut field, predominant overall isolates with a concentration reached to (45.03 ppm), compared to all isolates.

In the umbilical cord blood collection, it has been observed that close system technique gave rate 30 ml of UCB while the placenta is still in the utero, while the amount of blood fell when using the open system technique that has given rate 20 ml of UCB when the placenta is outside utero (ex utero), a possible explanation for the higher UCB volume yielded by in utero collection is that the compressing force exerted on the placenta from the uterus expels more UCB and this result is in agreement with what was said[20] .

The collection strategy is the first step for collecting cord blood good-quality, with the appropriate volume of blood and suitable for lab works and varies among banks and collection sites for the same cord blood bank[21]. In this sense, it is observed that there are many methods of collection for improving volume and total nucleated cell content of cord blood units. There are two main techniques for the collection

of umbilical cord blood from the umbilical vein and both methods have some advantages and disadvantages. It became clear through the use of both close technologies system (in a utero collection) and open system (ex utero collection)[22].

There is a highly significant correlation between the cord blood volume and the total nucleated cells (TNC) count per UCB unit, where it is found in the present study a significant increase in the number of TNC when getting them from closed system compared with open system, that gave a low rate of cell. In this study, the closed system was used and has been found effortless, easy, quick and no contamination was observed during the cord blood collection process. Fig. 1 shows the steps of blood collection from the umbilical cord vein by the closed system method, where photos were taken with the consent of the ethical committee and the patient.

Aspiration of hematopoietic stem cells: The umbilical cord blood aspirated from the umbilical vein and the blood components separated by gradient centrifugation revealed four layers: the upper translucent yellowish layer which formed about 50 – 55% of entire volume representing the plasma and lymphocyte separation solution, under which the buffy coat contained the MNCs made up about 2 - 5 % of the volume and appeared as a cloudy whitish layer, while the remaining amount settled down in the bottom half and consisted of components of the lower layer representing 40-45% of the entire volume of the blood and made up of erythrocytes as in fig. 1.

Table 1
Comparison between different samples (isolate origin) in Co. AF in HPLC

Isolates	Isolate origin	Co. AF in HPLC (ppm)
AFM1	Fish	0.00 ± 0.00
AFM2	cow lung	11.66 ± 0.75
AFM3	Spices	12.55 ± 0.87
AFM4	Rice	8.35 ± 0.63
AFM5	Animal waste	0.00 ± 0.00
AFM6	Corn Grain	33.86 ± 2.04
AFM7	Soil, black oil	0.78 ± 0.01
AFM8	Barley Grain	6.24 ± 0.58
AFM9	Wheat	29.89 ± 1.93
AFM10	Bovine Milk	0.00 ± 0.00
AFM11	Fruits	17.39 ± 1.26
AFM12	Nuts	12.66 ± 0.73
AFM13	Poultry Lung	0.00 ± 0.00
AFM14	Peanut seeds	45.03 ± 2.59
AFM15	Vegetables	0.00 ± 0.00
LSD value	---	6.593 *
* (P<0.05).		

Table 2
Inhibition rate of MNC exposed to AF measured via cytotoxicity assay

AF (µg/ml)	isolates *A. flavus* produced for AF										
	AFL2	AFL3	AFL4	AFL6	AFL7	AFL8	AFL9	AFL11	AFL12	AFL14	LSD
1	99.86	100	100	100	100	100	100	100	100	88.85	7.4*
0.5	28.72	26.18	100	89.70	17.71	100	39.73	49.05	50.74	53.28	9.7*
0.25	24.49	9.25	100	65.14	15.17	33.80	32.96	43.12	38.89	52.46	9.2*
0.12	3.32	3.32	27.88	11.79	10.94	25.34	20.25	32.96	27.03	46.51	9.8*
0.063	0	2.47	2.47	5.86	5.86	27.03	9.25	18.56	20.25	40.58	8.4*
0.031	0	0	0.78	0	4.16	16.87	5.01	14.33	16.02	32.11	9.3*
0.016	0	0	0	0	0	0	2.47	12.63	12.63	25.34	6.8*
0.0078	0	0	0	0	0	0	0	8.40	5.86	21.95	6.2*
LSD value	12.69 *	9.754 *	12.86 *	12.63*	13.02 *	11.87 *	12.52 *	10.26 *	12.37 *	10.42 *	---

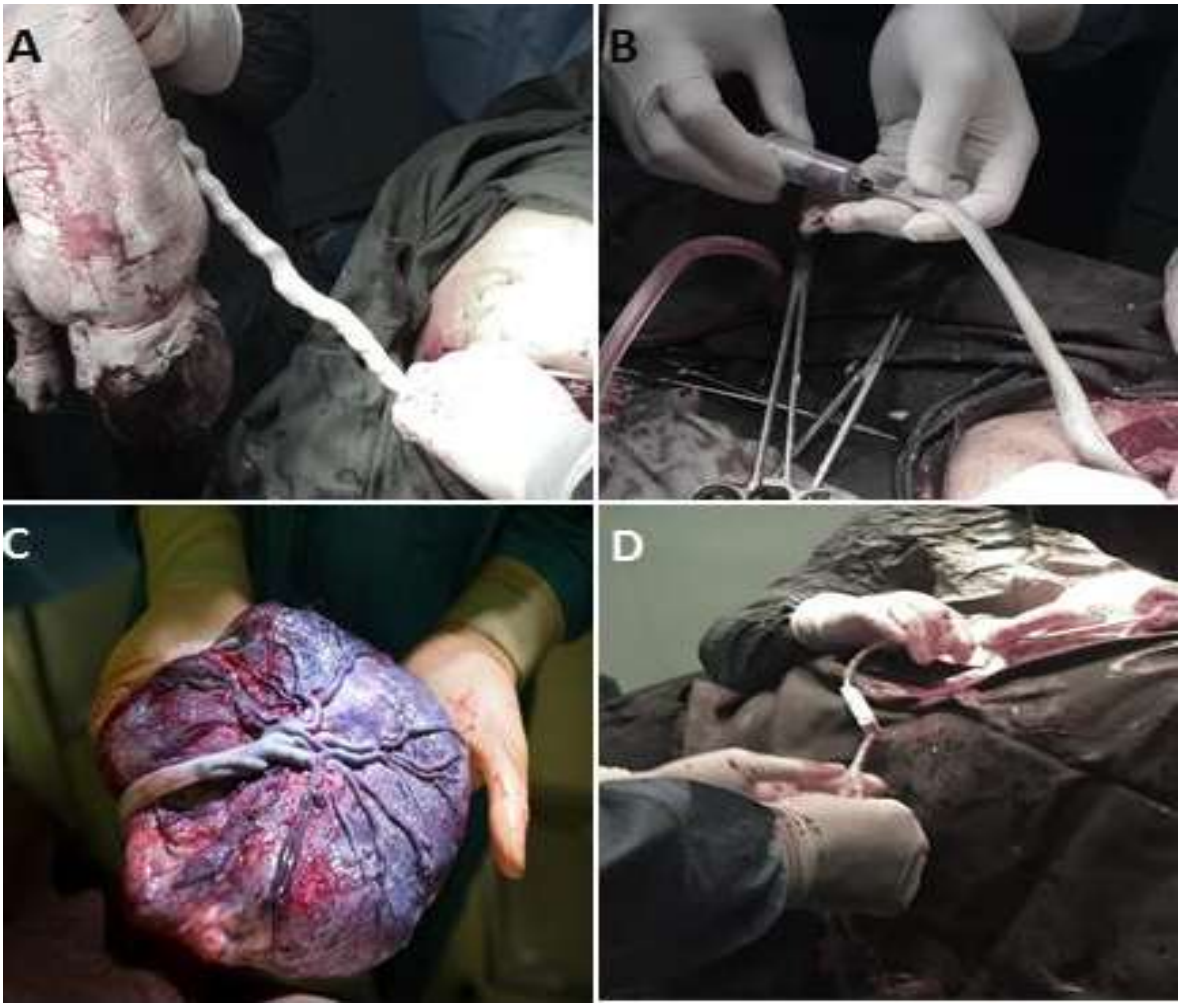

Fig. 1: Collection of umbilical cord blood during cesarean section.
(A): the umbilical cord is a conduit between the fetus and the placenta (B): Vein of umbilical cord from it collects blood while the placenta in utero (closed system) (C): The placenta out of a uterus
(D): Vein of umbilical cord from it collect blood while the placenta exutero (open system)

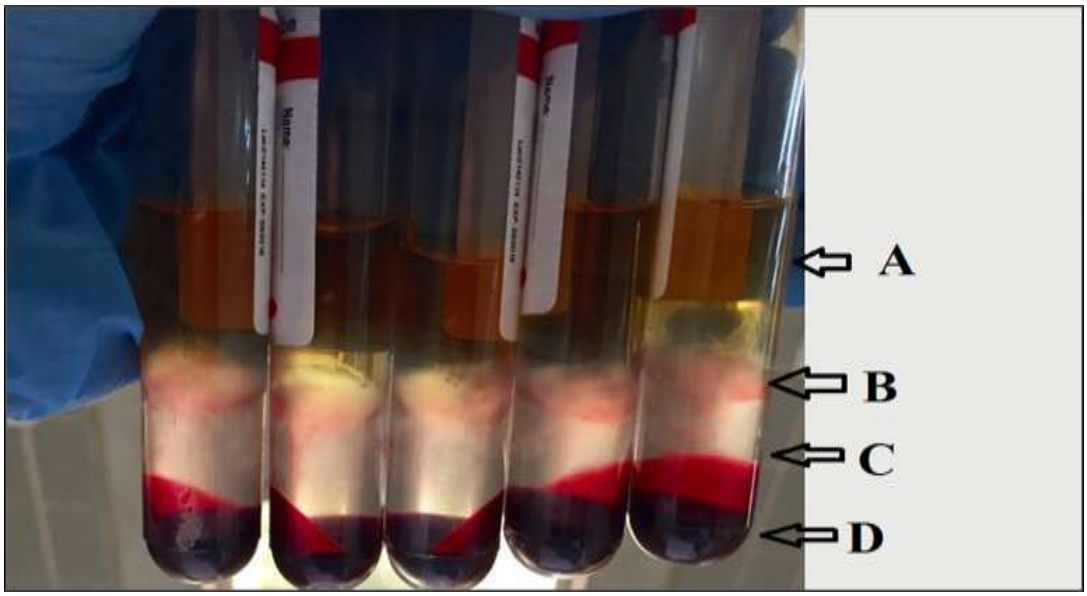

Fig. 2: Separation tube of blood four layers after gradient centrifugation, the upper one (A) represents the supernatant plasma, (B) represents the medium cloudy layer (Buffy coat) representing the MNCs, (C) represents the layer of the Ficoll-Paque and (D) represents the remainder cells which settled in the lower layer.

Due to a large number of thrombocytes and erythroid progenitors in cord blood, all erythroid cells and thrombocytes retained in the buffy coat and those due to the erythroid cells which are nucleated, remained in the buffy coat layer[25] which can be removed by washing 2– 3 times. By trypan blue stain, the MNCs viability and cell count were determined.

Three days later, after centrifugation, 50% of the medium was changed; the floating cells were discarded during the change process while the MNC cells began to form a cluster 3 – 5 days after the first cultivation. One week later, the HSC formed about 80% confluence as in fig. 3 and at this time the culture medium was discarded after centrifugation and the cells washed with PBS and passed into a new flask with the same cultivation conditions and so on until the cells were seeded into a microwell plate at a density of $2x10^4$ cells/well and cultured in 200 µl DMEM supplemented with 10% FCS of each well, then incubated until they are treated with different concentrations of AF.

Cytotoxic effects of Aflatoxins: From table 2, it has been observed that the concentration of 1 µg for all isolates was significant in giving the highest rate of inhibition compared with the other concentrations. The masses 0.5, 0.25, 0.125 µg have given low rates of inhibition reaching to 28.72, 24.49, 3.32% respectively, but the other concentrations did not affect the inhibition of cells. This suggests that the presence of higher concentrations of AF can lead to a significant reduction in the number of lymphocytes. In addition to that, the decline of the lymphocytes' mitochondria activity is possibly due to the weakness of mitochondria. It was also observed that the prevalence of the concentration 0.5 µg significant in inhibiting cells with a range of (26.18) % of the isolate.

The concentrations 0.5 and one µg of the isolate AFM4 have given an inhibition percentage of (100%), while the inhibition percentage in the concentration 0.125 had decreased to 27.88% which outperformed significantly on other concentrations even though some concentrations in this isolate did not give any percentage of inhibition. It was found that the concentration 0.5 µg of isolate AFM6 had excelled significantly in the inhibition of cells with a rate of 89.70% compared to the concentration 0.25 µg in which the inhibition rate fell considerably to 65.14.

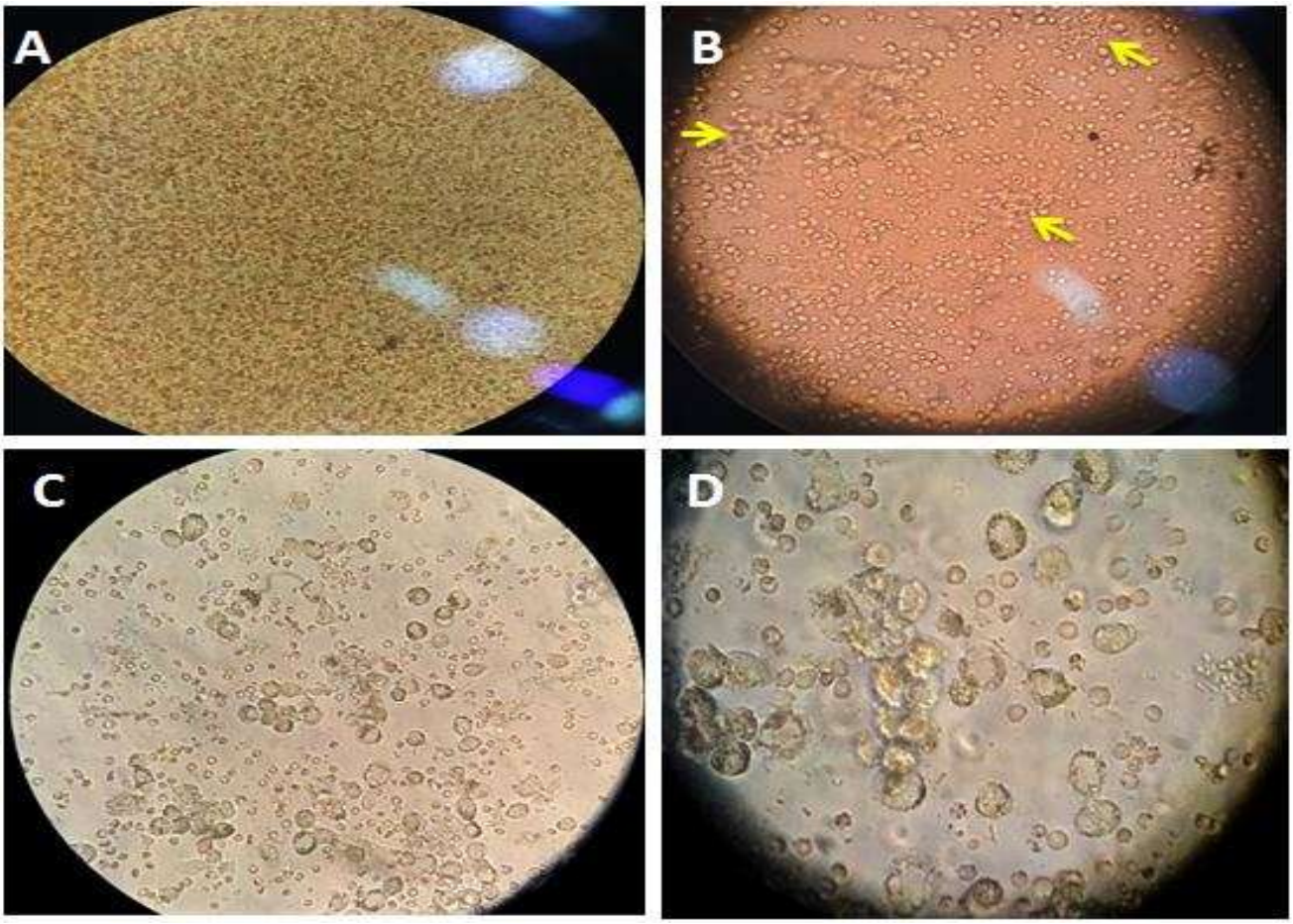

Fig. 3: Morphology of MNC isolated by the gradient centrifugation, which cultured in DMEM revealed by inverted microscope. (A) The maximized appearance of the cells 24 hours after cultivation. (B) The presence of the cells one week after the cultivation, where several cells began to attach and to form many clusters (arrows). (C) The maximized appearance of a cluster of cells, which revealed the attachment of round cells. (D) The optimized presence of the cells two weeks after cultivation

The results also showed that the isolate AFM7 had given less inhibition percentage of the cells compared to all isolates when the AF concentration was 0.5 µg with an average of (17.71) %. It had been clarified in this isolate that there are significant differences among the concentrations used in inhibition, despite the gradual decreasing in the inhibition percentage of cells with the reduction of AF concentration, whereas the concentrations 0.5, 0.25, 0.125, 0.0625 µg have been insignificantly different in the inhibition rate of cell and the results indicate that this isolate is producing low amounts of AF, which is one of the most toxins involved in the immune-disruptive process and affects lymphocytes mainly, when it was detected by using HPLC system and according to previous results reported in the table 2. This result is consistent with what reported by Mehrzad et al[26].

The concentration 0.5 µg of AF of the isolate AFM8 had excelled in giving the highest percentage of inhibition which reached 100% compared to other concentrations, which differed among them significantly where the inhibition rate of cell had decreased with the decreasing of AF concentration, it was also noted that the concentration 0.5 µg of AF of the isolate AFM9 had given inhibition rate of 39.73% which was not significantly different from concentration 0.25 µg which gave the percentage inhibition of 32.96 where they both excelled significantly at all other concentrations that belong to this isolate, in which the inhibition rate of cells was gradually decreased, but the interpretation of this is due to the effect of AF on DNA that cause abnormal changes in it[27].

The results also showed that the prevalence of the concentration 0.5 excelled significantly in the isolate AFM11 with an inhibition rate reaching to 49.05% compared to all concentrations, while it does not differ significantly from the concentration 0.25 µg which inhibited cells at a rate of 43.12%, as well as the concentration 0.125 had excelled significantly in inhibiting cells compared to all concentrations in which the inhibition ratio is decreased. The reason for that is because AF affects the plasma membrane and leads to the loss of optional permeability feature and this explains the reason for the water exodus out of the cells and that leads eventually to cell death[28].

The data in table 2 showed that the concentration of AF 0.5 µg of the isolate AFM12 might be prevalent significantly to give the highest percentage inhibition of cells amounting to 50.74 % but had an insignificant difference when compared with a concentration of 0.25 µg in which the inhibition rate fell to 38.89 %, while differing significantly from all other concentrations in which the inhibition rate decreased significantly ($P < 0.05$) until the concentration of 0.007813 which gave lesser inhibition of cell rate of 5.86 %.

The AFM14 concentrations have outperformed 0.5, 0.25, 0.125 µg giving the highest percentage of cell inhibition 46.51, 52.46, 53.28, respectively while the rate of inhibition of cells gradually decreased with the low concentration of AF. Continuous exposure to AF and various concentrations may cause immunosuppression[29], another mechanism of action of AFs involves the inhibition of DNA synthesis[30].

The study suggested that HPLC is a standard and superior technique in identifying and analyzing AF with high sensitivity and accuracy. Further, the concentration 1 µg /ml of AF extracted from the aflatoxigenic isolates can kill 100% of hematopoietic stem cells.

References

1. Rohrabaugh S.L., Campbell T.B., Hangoc G. and Broxmeyer H.E., Ex vivo Rapamycin treatment of human cord blood CD34+ Blood Cells enhances their engraftment of NSG mice, *Blood Cells Mol Dis*, **46**, 318–320 **(2012)**

2. Basford C., Forraz N., Habibollah S., Hanger K. and Mcguckin C., The Cord Blood Separation League Table : a Comparison of the Major Clinical Grade Harvesting Techniques for Cord Blood Stem Cells, *Int. J. Stem Cells*, **3**, 32–45 **(2010)**

3. Delaney C., Ratajczak M.Z. and Laughlin M.J., Strategies to enhance umbilical cord blood stem cell engraftment in adult patients, *Expert Rev Hematol*, **3**, 273–283 **(2011)**

4. Malik A., Ali S., Shahid M. and Bhargava R., Occupational exposure to Aspergillus and aflatoxins among food-grain workers in India, *Int. J. Occup. Environ. Health*, **20**, 189–193 **(2014)**

5. Calvo A.M. and Cary J.W., Association of fungal secondary metabolism and sclerotial biology, *Front Microbiol.*, **6**, 1–16 **(2015)**

6. Al-hammadi S., Marzouqi F. and Al-mansouri A., The Cytotoxicity of Aflatoxin B1 in Human Lymphocytes, *Sultan Qaboos*, **14**, 65–71 **(2014)**

7. Chen K. et al, Protective role of sodium selenite on histopathological lesions, decreased T-cell subsets and increased apoptosis of thymus in broilers intoxicated with aflatoxin B 1, *Food Chem. Toxicol.*, **59**, 446–454 **(2013)**

8. Jiang M. et al, Effects of Aflatoxin B 1 on T-Cell Subsets and mRNA Expression of Cytokines in the Intestine of Broilers, *Int. J. Mol. Sci.*, 6945–6959, doi:10.3390/ijms16046945 **(2015)**

9. Maria S., Maria S. and Alegre P., In-Vitro Cytotoxicity of Aflatoxin B1 to Broiler Lymphocytes of Broiler Chickens, *Brazilian J. Poult. Sci.*, **16**, 307–312 **(2014)**

10. Tait S.W.G. and Green D.R., Mitochondrial Regulation of Cell Death, *Cold Spring Harb Perspect Biol.*, **9**, 1–15 **(2013)**

11. Oueslatia S., Blesab J., Moltób J.C., Ghorbela A. and Mañesb J., Presence of mycotoxins in sorghum and intake estimation in Tunisia, *Food Addit. Contam.*, Part A, **11**, 37–41 **(2014)**

12. Ho H.L. and Sali S.A., Biodiversity, Bioprospecting and Development Bioprocessing of Agricultural Residuals for the Optimum Production of Extracellular Xylanase by Aspergillus brasiliensis in Solid State Fermentation, *Biodiversity, Bioprospecting Dev.*, **1**, 1–15 **(2014)**

13. Eissa M.E., Abd M., Naby E. and Beshir M.M., Bacterial vs. fungal spore resistance to peroxygen biocide on inanimate surfaces, *Bull. Fac. Pharmacy, Cairo Univ.*, **52**, 219–224 **(2014)**

14. Miyamoto K., Murakami T. and Kakumyan P., Formation of 1-octen-3-ol from Aspergillus flavus conidia is accelerated after disruption of cells independently of Ppo oxygenases and is not a main cause of inhibition of germination, *Peer J*, 1–12, doi:10.7717/peerj.395 **(2014)**

15. Lai X., Zhang H., Liu R. and Liu C., Potential for aflatoxin B1 and B2 production by Aspergillus flavus strains isolated from rice samples, *Saudi J. Biol. Sci.*, **22**, 176–180 **(2015)**

16. Musleh M.H., Molecular Study of Aflatoxin and its Toxic Effect on Hematopoietic Stem Cells, M.Sc. Thesis, Department of Biology, College of Education for Pure Sciences, University of Anbar, doi:10.13140/RG.2.2.33673.54889/1 **(2017)**

17. Al-Ouqaili M.T.S., Musleh M.H. and Al-Kubaisi S.M.A., Detection of Aflatoxigenic and Non-Aflatoxigenic Isolates of Aspergillus flavus Isolated From Some Clinical and Environmental Sources by HPLC and PCR Techniques, *J. Biotechnol. Res. Cent.*, **12**, 40–48 **(2018)**

18. Cadena-herrera D. et al, Validation of three viable-cell counting methods : Manual , semi-automated and automated, *Biotechnol. Reports*, **7**, 9–16 **(2015)**

19. Sivakumaran N., Rathnayaka I.R. and Shabbir R., Umbilical Cord Blood Banking and its Therapeutic Uses, *Int. J. Sci. Res. Innov. Technol.*, **5**, 160–170 **(2018)**

20. Yuen S.L., Kong H., Ys L., Mbbs C. and Fhkam O., Umbilical Cord Blood for Transplantation : from Collection Quality to Its Use in Cerebral Palsy, *HKJGOM*, **9**, 43–48 **(2009)**

21. Al-Ouqaili M.T.S., Musleh M.H. and Al-Kubaisi S.M.A., Depending on HPLC and PCR, detection of aflatoxin B1 extracted from *Aspergillus flavus* strains and it's cytotoxic effect on AFB treated-hematopoietic stem cells obtained from human umbilical cord, *Asian Journal of Pharmaceutics*, **12(3)**, 1048- S1054 **(2018)**

22. Allan D.S., On T., Casper R.F. and On T., Umbilical Cord Blood: Counselling, Collection and Banking, *Obstetrics and Gynaecology Canada*, **2015**, 832–844 **(2015)**

23. Bassiouny M.R., El-Chennawi F. and Mansour A.K., Optimal method for collection of umbilical cord blood: an Egyptian trial for a public cord blood bank, *Transfusion*, **1**, 1–6 **(2014)**

24. Volpe G. et al, Four phases of checks for exclusion of umbilical cord blood donors, *Blood Transfus*, **9**, 286–291 **(2011)**

25. Kekarainen T., Mannelin S., Laine J. and Jaatinen T., Optimization of immunomagnetic separation for cord blood-derived hematopoietic stem cells, *BMC Cell Biol.*, **10**, 1–10 **(2006)**

26. Mehrzad J., Devriendt B., Baert K. and Cox E., Aflatoxins of type B and G affect porcine dendritic cell maturation in vitro, *Immunotoxicology*, **6901**, 1–7 **(2014)**

27. Ghaderi M., Allameh A., Soleimani M., Rastegar H. and Ahmadi-Ashtiani H.R., A comparison of DNA damage induced by aflatoxin B1 in hepatocyte-like cells, their progenitor mesenchymal stem cells and CD34(+) cells isolated from umbilical cord blood, *Mutat. Res.*, **719**, 14–20 **(2011)**

28. Ribeiro D.H.B., Ferreira F.L., da Silva V.N., Aquino S. and Corrêa B., Effects of aflatoxin b(1) and fumonisin b(1) on the viability and induction of apoptosis in rat primary hepatocytes, *Int. J. Mol. Sci.*, **11**, 1944–1955 **(2010)**

29. Murugesan G.R. et al, Prevalence and effects of mycotoxins on poultry health and performance and recent development in mycotoxin counteracting strategies 1, *Poult. Sci.*, **94**, 1298–1315 **(2015)**

30. Iheanacho H., Cytotoxic Effects of Aflatoxin B1 Standard in Relation to Aflatoxin Extracts from South African Compound Feeds on Human Lymphocytes, *Int. J. Biomed. Data Min.*, **3**, 1-5 **(2015)**.