Aflatoxin B_1 induces oxidative stress in rats: possible protective effects of onion juice (*Allium cepa* L.)

Ragaa A. Sadeek[1], Ghada Mahmoud Elbassiony[2] and Yousif A. Elhassaneen[3]

[1]Department of Home Economic, Faculty of Specific Education, Minia University, Minia, Egypt; [2]Department of Home Economics, Faculty of Specific Education, Benha University, Benha, Egypt and [3]Department of Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Shebin El-Kom, Egypt.

Abstract

Aflatoxin belongs to a group of fungal toxins known as mycotoxins. Exposure to dietary aflatoxin B_1 (AFB_1) is considered to be an important risk factor for the development of hepatocellular carcinoma for both animals and human beings. The mechanism of AFB_1 -induced cellular damage has not been fully understood, however previous *in vitro* studies suggested that AFB_1 was capable of inducing oxidative damages through the release of reactive oxygen species (ROS). Onions as one of the important *Allium* species commonly used in our daily diets are proved recently to have antioxidant properties. Therefore, the present study was conducted to investigate the possible protective effects of onion juice (OJ) in comparison with vitamin C as a standard antioxidant against AFB_1 induces oxidative stress in liver tissue of albino rats. Data of the biochemical analysis indicated that AFB_1 induced a significant ($p \leq 0.05$) increased in plasma oxidants concentration (MDA, 61.73 and123.46%; NO_2, 61.90 and 111.69% and NO_2/NO_3, 66.76 and 107.48%) and significant ($p \leq 0.05$) decreased in plasma non-enzymes antioxidant (GSH, -23.78 and -32.43% and GSSG, - 26.06and -31.69%), plasma antioxidant vitamins (vitamin A, -30.85 and - 35.82%; vitamin C, - 31.97 and - 37.18% and vitamin E, - 38.79 and -43.38%) as well as RBC's antioxidant enzymes (GSH-Px, - 23.78 and -32.43%; CAT, -17.69 and - 21.13% and SOD, -36.91 and - 47.12%) as a percent of normal group in both single and multiple doses, respectively. Injection with OJ induced significant ($p \leq 0.05$) improvement in all of these parameters by different rates. The higher amelioration effects were recorded for multiple doses treatment. In conclusion, the present data support the benefits of onion in human population at high risk to AFB_1 exposure through alleviating oxidative stress and enhancing the antioxidative defense systems associated.

Keywords: Aflatoxin B_1, chemical composition, plasma, MDA, nitric oxides, GSH fractions, antioxidant vitamins, antioxidant enzymes.

Introduction

Dietary exposure to hepatotoxic contaminants is one of the prominent risk factors for hepatocellular carcinoma **(Guyton and Kensler, 2002).** Aflatoxin B_1 (AFB_1) (Figure 1), a metabolite of *Aspergillus flavis* and *Aspergillus parasiticus*, is among the most commonly dietary mycotoxins to which humans are exposed **(Sudakin, 2003)**. At the beginning of the sixties of the past century Aflatoxin including AF B_1 were discovered after more than 100,000 young turkeys due to an epidemic known as turkey X disease in the areas surrounding London died after feeding with grain fodders containing toxic peanut flour **(Vasatkova *et al.*, 2009).** Presence of aflatoxin B_1 is largely associated with major crops such as barley, wheat, maize, groundnut, faba bean, pea, pepper and 13.33% of ground red pepper samples were positive for aflatoxin B_1 **(Wolde, 2017**).

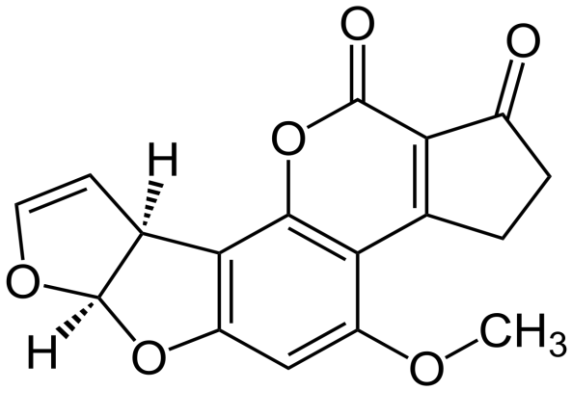

Figure (1): Molecular structure of Aflatoxin B_1 (AFB_1)

Experimental studies have shown that AFB_1 is a potent hepatotoxicant, mutagen and hepatocarcinogen **(Angsubbhakorn *et al.*, 1990; Hsieh and Atkinson,1990 and Dwivedi *et al.*,1993)**. The mechanism of AFB_1 induced cellular damage has not been well characterized. Previous in vitro studies with cultured hepatocytes suggested that AFB_1 was capable of inducing oxidative damages through the release of reactive oxygen species (ROS) **(Kodama *et al.*, 1990 and Shen *et al.*, 1995)** but the characteristics and effects of these species remain to be further investigated **(Premalatha and Sachdanandam, 1999).**

Oxidative stress was initially defined by **Sies (1985)** as a serious imbalance between oxidation and antioxidants, "a disturbance in the prooxidant–antioxidant balance in favor of the former, leading to potential damage". So, it reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA **(Toshniwal and Zarling, 1992 and Rahman *et al.*, 2012).** Living cells evolved defense system against free radicals by antioxidant molecules is crucial to the protection of cells against the development of prooxidant state. Antioxidants may protect against ROS toxicity by the prevention of ROS formation, by the interruption of ROS attack, by scavenging the reactive molecules and/or by enhancing the resistance of sensitive biological targets to ROS attack, by facilitating the repair caused by ROS and by providing co-factors for the effective functioning of other antioxidants **(Sen, 1995).** Development of life threatening diseases as cancer is linked to the availability of these antioxidants **(Gutteridge, 1994).** Low levels of antioxidants, which further increases the free radical activity, are clearly associated with cancer conditions.

In humans, oxidative stress is thought to be involved in the development of in several diseases including cancer, atherosclerosis, malaria, chronic fatigue syndrome, rheumatoid arthritis and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and Huntington's disease **(Halliwell, 1991 and Chaitanya *et al.*, 2010)**. Also, it is contributing to tissue injury following irradiation and hyperoxia as well as in diabetes and is likely to be involved in age-related development of cancer. Since high incidence of liver cancer is recorded in many areas due to unavoidable increased consumption of aflatoxin-contaminated foodstuffs, the impact of liver cancer prevention using natural plant products may be of great importance **(Nyandieka and**

Wakhisi, 1993). Dietary cancer prevention studies have shown that consumption of fruits and vegetables is associated with reduced cancer risk related to oxidative stress **(Hininger *et al.*, 1997 and Chu *et al.*, 2002).**

Onion (*Allium cepa* Linn) belongs to the *Lilliaceae* family and is grown all over the world. In Egypt, onion ranks fourth after cotton, rice, and citrus as an export crop. The total cultivated area was 36153 feddan (15184.3 ha) in 1999 and the total production was 305201 tons **(CAPMS, 2000)**. Onions represents one of the important Allium species commonly used in our daily diet have been extensively studied for their therapeutic uses as antibiotic, antidiabetic, antiatherogenic, anticancer etc. **(Augusti, 1996).** Biological action of Allium products is attributed to many bioactive compounds including phenolics, organosulfur compounds, carotenoids and vitamins **(Aly *et al.*, 2017 and Mahran, 2018).** Onion oil has been reported to effectively decrease the lipid levels in experimental animals **(Bordia *et al.*, 1975, 1977 and Bobbi *et al.*, 1984).** It has been found that administration of onion products to diabetic rats significantly reduced hyperglycemia **(Kumud *et al.*, 1990).** More recently much attention has been paid to the antioxidant properties of onions **(Helen *et al.*, 2000).** Among micronutrients, vitamin C is believed to be one of the principal biological antioxidants against lipid peroxidation **(Allen, 1991).** A number of studies have demonstrated its protective role against endogenous oxidative damage **(Duthie *et al.*, 1996 and Helen and Vijayammal, 1997).** Epidemiological studies strongly suggest that 90-100 mg vitamin C/day, and plasma concentrations of >50 µmol/L have been associated with a lower incidence and mortality from cardiovascular disease and cancer **(Gey, 1998 and Carr and Frei, 1999)**. From the previous reasons, the aim of the present study was to investigate the role of AFB_1 in induction of lipid peroxidation in serum of albino rats. Also, the possible protective effects of onion extract/juice (*Allium sepa*) in comparison with vitamin C as a standard antioxidant against AFB_1 induces oxidative stress in blood of albino rats will be in the scope of this investigation.

Material and Methods

Materials:

Onion: Onion (*Allium cepa* Linn) bulbs, Giza 20, were obtained from New Bani Suef Company for Prsevation, Dehydration and Industratzation of Vegetables, Bani Suef El-Goudida City, Nile east, Bani Suef, Egypt.

Chemicals: Vitamins standards (A, C, and E) and thiols compounds (GSH and GSSG) were purchased from Sigma Chemical Co., St. Louis, MO, USA. All other reagents and solvent were of analytical or HPLC grade were purchased from (Fisher, UK). Casein was obtained from Morgan Chemical Co., Cairo, Egypt. Some organic solvents, buffers and other chemicals of analytical grade were purchased from El-Ghomhorya Company for Trading in Drug, Chemicals and Medical Instruments, Cairo, Egypt. De-ionized water (Milli-Q 18.2 MΩ) was used in the preparation of the mobile phases, reagent solutions and standards.

Equipment's: Throughout this study a SP Thermo Separation Products Liquid Chromatograph (Thermo Separation products, San Jose, CA, USA) was used with a Consta Metvic 4100 pump, a Spectra Series AS100, Spectra System UV 1000 UV/Visible Spectrophotometer Detector, Spectra System FL 3000 and a PC 1000 system software. The columns used (Alltech, Deerfield, IL, USA) were a Spherosorb ODC-2 (5 µm, 150 x 4.6 mm I.d.) for glutathione fractions ; a reversed-phase water Adsorbosil C_{18} (5 µM, 100 mm × 4.6 mm I.d.) for vitamin C; and normal Ultrasphere Si (5 µM, 250 mm × 4.6 mm I.d.) for analysis of vitamins A and E. Also, absorbance and fluorescence for different assays were measured using Labo-med. Inc., spectrophotometer, CA and Schematzu fluorescence apparatus, Japan, respectively.

Preparation of onion juice (OJ)

Onion juice was extracted in laboratory conditions. Onion bulbs were peeled with stainless steel knifes, washed with tap water, minced in high

mixer blender (Toshiba, ElAraby Co., Benha, Egypt) and sieved through gauze. The material that passed through a 60 mesh sieve was retained the injection purpose. The volume of OJ obtained from 100 g of the vegetable was about 55 ml.

Preparation of the injected materials

AFB_1 (Sigma) was freshly prepared by dissolving in dimethyl sulfoxide (DMSO; Sigma) **(Premalatha *et al.*, 1997)** and then diluted with distilled water to the required concentration. Vitamin C was dissolved in water and administered in a dose of 10 mg/kg **Nabzdyk and Bittner, (2018).** Onion Juice (OJ) was administered in a dose of 40 ml/kg **(Shokoohi *et al.*, 2018)**. Both vitamin C and OJ were given by gastric intubation 2 hours before administration of AFB_1.

Animals

Animals used in this study, adult male albino rats (n= 54),150 ± 10 g per each were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

Basal Diet

The basic diet prepared according to the following formula as mentioned by **(AIN, 1993)** as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The diet induced obesity (DIO) prepared according to Research Diets, Inc. NJ, as follow: casein, 80 mesh (23.3%), L-cystine (0.35%), corn starch (8.48%), maltodextrin (11.65%), sucrose (20.14%), soybean oil (2.91%), lard fat (20.69%), mineral mixture (1.17%), dicalcium phosphate (1,52%), calcium carbonate (0.64%), potassium citrate.1 H_2O (1.92%), vitamin mixture (1.17%), choline bitartrate (0.23%). The used vitamins and salt mixtures components were formulated according to **Campbell, (1963) and Hegsted, (1941),** respectively.

Experimental design

Biological experiments performed a complied with the rulings of the

Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council **(NRC, 1996).** Rats were housed individually in wire cages in a room maintained at 25 ± 2 ^{0}C and kept under normal healthy conditions. All rats were fed on basal diet for one-week before starting the experiment for acclimatization. After one week period, the rats were divided into **three** main experimental groups:

- Group (1): Control group, included 6 rats received no treatment.

Single dose group: It included 24 rats equally subdivided into 4 subgroups of 6 rats per each:

- Group (2): AFB_1 alone treated animals: each rat received a single dose of AFB_1 (2 mg/kg) by intraperitoneal (ip) injection.
- Group (3): Onion juice-AFB_l treated animals: each rat received a single dose of OJ , 2 hrs. prior to dosing with AFB_1 (2mg/kg. ip).
- Group (4): Vitamin C- AFB_l treated animals: each rat received a single dose of vitamin. C, 2 hrs. prior to AFB_l administration (2mg/kg, ip).
- Group (5): DMSO alone treated animals: each rat received a single ip dose of DMSO (5ml/kg). This group was designed to investigate if there is any effect of DMSO on the studied biochemical parameters.

Multiple doses group: It included 24 rats equally subdivided into 4 subgroups of 6 rats each. Each rat received treatment once a day for 7 days.

- Group (6): AFB_1 alone treated animals: each rat was injected (ip) with AFB_1 (0.5mg/kg/day for 7 days).
- Group (7): Onion juice -AFB_1 treated animals: each rat received a dose of OJ 2 hrs. prior to dosing with AFB_1 (0.5mg/kg/day for 7 days).
- Group (8): Vitamin C- AFB_1 treated animals: each rat received a dose of vitamin C 2 hrs. prior to AFB_1 administration [0.5mg/kg/day for 7 days).
- Group (9): DMSO alone treated animals: each rat received ip dose of DMSO (5ml/kg/day for 7 days).

At the end of the experiment, animals were fasted for 24 hrs then

sacrificed by cervical dislocation, blood was collected and serum was separated for estimation of total glutathione (GSH), malondialdehyde (MDA) and antioxidant vitamins. Erythrocyte residue was washed with three successive portions of sodium chloride solution (0.9 %) and then haemolysed with deionised water for 30 min. Haemolysate was then centrifuged at 3000 rpm for 30 min. and the supernatant fractions was transferred to a clean test tube for the analysis of antioxidant enzymes.

Hematological analysis

Glutathione fractions

GSH and GSSG were determined by HPLC according to the method of **McFarris and Reed (1987)**. In brief, 100 µl of aliquot were placed in 2 ml of 10% per chloric acid containing 1 m bathophenanthroline disulfonic acid and homogenized. The homogenate was cold centrifuged at 10000 rpm for 5 min and the internal standard (γ-glutamyl glutamate) was added to the supernatant. A 250 µl aliquot of acidic extract was mixed with 100 µl of 100 mM iodoacetic acid in 0.2 mM cresol purple solution. The acid solution was brought to pH 8.9 by the addition of 0.4 ml of KOH (2 M) – $KHCO_3$ (2.4 M) and allowed to incubate in the dark at room temperature for 1 hr to obtain S-carbooxymethyl derivatives. The N-nitrophenol derivatization of the samples were taken overnight at 4 ^{0}C in the presence of 0.2 ml of 1% 1-fluoro-2,4-dinitrobenzene and injected onto the HPLC system.

Antioxidant enzymes

GSH-Px and CAT activities were measured as described by **Splittgerber and Tappel, (1979) and Aebi, (1974),** respectively. SOD activity was measured by Ransod kit (Randox laboratories mmited, Germany). GSH-Rd activity was determined according to the method recommended by the International Committee for Standardization in Haematology **(ICSH, 1979).** Activities of SOD and GSH-Px enzymes were expressed in international unit per milliliter erythrocyte sediment and one

unit of SOD was expressed as the enzyme protein amount causing 50% inhibition in 2- (4-iodophenyl)-3 (4-nitrophenol) 5-phenyltetrazolium chloride ($INTH_2$) reduction rate.

Antioxidant vitamins

All vitamins (A, E, and C) were extracted and analyzed by HPLC techniques such as mentioned by **Epler *et al*., (1993), Hung *et al*., (1980) and Moeslinger *et al*., (1994),** respectively. The chromatographic conditions for vitamins A and E were flow rate, 1.5 ml/min; detection, UV absorption at 265 nm, volume of injection, 20 µl; temperature, room temperature; and the mobile phase composition was an isocratic system of isopropanol : hexane (1:99) while in vitamin C were flow rate, 1 ml/min; detection, UV absorption at 254 nm, volume of injection, 20 µl; temperature, room temperature, and mobile phase composition was an isocratic system of 100 % methanol. Retention times and absorbance ratio against those of standards were used to identify the separated vitamins. Quantitative determination of each vitamin was determined from its respective peak area and corresponding response factor. The percent recoveries of vitamins were also studied by adding each vitamin to serum after sample preparation and HPLC determination. Under such chromatographic conditions, the Mean ±SD values of vitamins A, C and E recoveries were 91.23 ± 3.9, 90.67 ± 5.72, and 88.54 ± 6.1%, respectively.

Nitrite determination

Nitrite was determined flourometric such as described by **Misko *et al*., (1993).** Ten µl of freshly prepared 2, 3-diaminonaphthalene (DAN, 0.05 mg/ml in 0.62 M HCl, protected from light) is added to100 µl of sample and mixed immediately. Nitrate standards (> 98% pure, Sigma) are routinely made fresh, dissolved in DI H_2O, and kept on ice prior to use. After 10 min incubation at 20 ^{0}C, the reaction was terminated with 5 µl of 2.8 N NaOH. The intensity of the fluorescent signal produced by the product is maximized by the addition of base. Formation of the 2,3-diaminonaphthtriazole was measured using a Schematzu fluorescence

apparatus with excitation at 365 nm and emission read at 450 nm with a gain setting at 100%.

Nitrite/nitrate detection

Plasma is filtered through an ultrafree microcentrifuge filter unit (14000 rpm for 15 min) to remove the hemoglobin resulting from cell lysis. The filtrate should contain mostly nitrate (recovery greater than 90%) due to the reaction of NO with the iron-heme center of the protein. Nitrate is converted to nitrite by the action of nitrate reductase (from *Aspergillus niger*, Sigma Chemical Co., St. Louis, MO, USA) such as follow: the sample is incubated with 40 µM NADPH (to initiate the reaction) and 14 mU of enzyme in a final volume of 50 µl of 20 mM Tris buffer (pH, 7.6). The reaction is terminated after 5 min at 20 ^{0}C by dilution with 50 µl of water followed by addition of the DNA reagent for determination of nitrite. Nitrite levels in samples are then calculated by first subtracting the value of the enzyme blank (i.e., nitrate reductase plus NADPH) from the experimental and then calculating the value using a standard curve for nitrite to which NADPH has been added.

Malonaldehyde (MDA) content determination

MDA was measured as described by **Buege and Aust, (1978).** A 0.5 mL of plasma were added to 1.0 ml of thiobarbituric acid reagent, consisting of 15% TCA, 0.375% thiobarbituric acid (TBA) and 0.01% butylated hydroxytoluene in 0.25 N HCl. Twenty-five microliters of 0.1 M $FeSO_4.7H_2O$ was added and the mixture was heated for 20 min in boiling water. The samples were centrifuged at 1000 xg for 10 min and the absorbance was read at 535 nm using Labo-med. Inc., spectrophotometer against a reagent blank. The absorbance of the samples was compared to a standard curve of known concentrations of malonaldehyde.

Protein assay

Protein was determined by the method of **Lowry *et al.*, (1951)** using bovine serum albumin as the standard.

Statistical analysis

The results of all assays were reported as mean ± standard deviation for groups of six rats. Statistically significant differences between the groups were calculated using one-way analysis of variance (ANOVA).

Results and Discussion

Proximate chemical composition, bioactive compounds and antioxidant activities of onion skin powder (OSP)

The proximate composition of OJ is shown in Table (1). The results showed that the moisture content, total protein, crude fat, crude fiber, ash and total carbohydrate content were 91.13, 0.42, 0.82, 0.71, 2.05 and 4.87%, respectively. The proximate composition reported was not accordance with that observed by **Elhassaneen and Sanad (2009).** Also, OJ recorded a significant amounts of different bioactive compounds including essential oil, vitamins, total carotenoids and total phenolics which reflected the its high antioxidant activity. All of these components in OJ might be important from the nutrition point of view and high significant as an important functional food. In similar study, **Elhassaneen and Sanad (2009)** found that the Egyptian onion bulbs have been shown to contain a wide spectrum of bioactive compounds which exhibited high antioxidant activity. Also, many studies indicated that there was a positive and significant ($p \leq 0.01$) relationship between all of the bioactive compounds in OJ and the antioxidant activity in different plant parts **(Khoneem, 2009; Jaggi, 2012, Elhassaneen *et al.*, 2013 and Salama *et al.*, 2017)**. Plant-based foods generally are considered important sources of antioxidants in the diet. Antioxidants help protect cells from the potentially damaging physiological process known as oxidative stress (OS). OS is thought to be associated with the development of many chronic diseases including cancer, diabetes, rheumatoid arthritis, heart, and neurodegenerative (Parkinson's and Alzheimer's) diseases **(Chaitanya *et al.*, 2010; Elmaadawy *et al.*, 2016 and Salama *et* al., 2017)**. For the above reasons and others, OJ could be used successfully in many different therapeutic applications.

Table 1: Proximate chemical composition, bioactive compounds and antioxidant activities of OJ

Component	Content
Proximate chemical composition on w/w:	
Moisture (g/100g)	91.13 ± 3.21
Total protein (g/100g)	0.42 ± 0.19
Crude fat (g/100g)	0.82 ± 0.10
Ash (g/100g)	0.71 ± 0.12
Crude fiber (g/100g)	2.05 ± 0.07
Carbohydrate (g/100g)	4.87 ± 0.84
Bioactive compounds:	
Essential oil (g/100g)	0.30± 0.11
Vitamin C (Ascorbic acid, mg/100g)	0.63± 0.07
Vitamin A (mg/100g)	0.22 ± 0.08
Vitamin E (mg/100g)	0.18 ± 0.06
Total carotenoids (mg/100g)	0.47 ± 0.13
Total phenolics content (mg GAE.g-^{1}) methanol extract	0.85 ± 0.17
Antioxidant activity:	
Antioxidant activity (AA, %) - methanol extract	72.91±7.61

Each value represents the mean of three replicates ±SD.

Effect of AFB_1 administration (ip) on the levels of plasma oxidants concentration and the protective effect of onion juice (OJ) and vitamin C pretreatment

Oxidative stress status in rats administrated AFB_1 and pretreated with OJ and vitamin C was assessed by measuring some oxidants concentration in plasma including malonaldehyde (MDA) and nitric oxides (nitrite, NO_2 and nitrate, NO_3) content (Table 2). From such data it could be noticed that the concentrations of malondialdehyde (MDA) were increased significantly in serum following single dose (2mg/kg, ip) and multiple doses (0.5 mg/kg/day for 7 days, ip) AFB_1 treatment when compared to normal control rats. In single dose AFB_1 alone treatment, the concentration of MDA in serum was increased sharply by 61.73% when compared with control group. However, gastric instillation of onion juice (OJ) prior to

AFB_1 administration increases MDA concentrations only by 19.75% in serum with insignificant differences when compared with control group, while MDA concentrations were increased by 44.44% in serum receptively in vitamin C-fed AFB_1-toxicated animals with significant differences when compared with control group. On the other hand, when compared with AFB_1 alone group, only OJ induces significant ($P \leq 0.05$) decrease in the production of MDA in serum. In multiple doses treatment, AFB_1 induces more deleterious effects, there were much more increases in MDA concentrations in serum (123.46%) as compared with control values. Pretreatment with OJ induce excellent protection against excessive production of MDA, where its concentrations were increased only by 11.11% in serum. While pre-administration of vitamin C induce less protection as MDA concentrations was increased by 38.27% in serum. However, when compared with AFB_1 alone group, both OJ and vitamin C produce significant ($P \leq 0.05$) decrease in the production of MDA in serum. The same observations were observed for nitric oxides (nitrite, NO_2 and nitrate, NO_3) content in serum.

Table (2): Effect of AFB_1 administration (ip) on the levels of plasma oxidants concentration and the protective effect of onion juice (OJ) and vitamin C (Vit C) pretreatment

Groups		Malondialdehyde concentration (MDA)		Nitrite (NO_2)		Nitrite/Nitrate (NO_2/NO_3)	
		nmol/mL	% of change	nmol/L	% of change	nmol/L	% of change
1) Control		0.81 ± 0.11	0.00	2.31 ± 0.27	0.00	3.61 ± 0.47	0.00
Single dose	2) AFB_1 alone	1.31 ± 0.30*	61.73	3.74 ± 0.68*	61.90	6.02 ± 0.97*	66.76
	3) OJ + AFB_1	0.97 ± 0.13#	19.75	2.55 ± 0.30#	10.39	4.06 ± 0.75#	12.47
	4) Vit. C + AFB_1	1.17 ± 0.17*	44.44	3.20 ± 0.49*	38.53	4.89 ± 0.85*	35.46
	5) DMSO	0.91 ± 0.15	12.35	2.51 ± 0.41	8.66	4.06 ± 0.80	12.47
Multiple doses	6) AFB_1 alone	1.81 ± 0.40*	123.46	4.89 ± 0.69*	111.69	7.49 ± 0.86*	107.48
	7) OJ + AFB_1	0.90 ± 0.15#	11.11	2.45 ± 0.38#	6.06	4.07 ± 0.54#	12.74
	8) Vit. C + AFB_1	1.12 ± 0.13#	38.27	3.02 ± 0.18#	30.74	4.82 ± 0.76#	33.52
	9) DMSO	0.85 ± 0.12	4.94	2.40 ± 0.34	3.90	4.00 ± 0.88	10.80

Values are expressed as mean ± SD (n=6). * Significant difference when compared to control group, # significant difference when compared to AFB_1 alone group

Many studies reported that the mechanism of AFB_1-induced cellular damage is not fully elucidated. A previous in vivo study found that AFB_1 is capable of inducing chromosomal damages **(Draz, 1999)** that were found to be due to the release of free radicals including reactive oxygen species (ROS), which led to chromosomal injuries **(Amstad *et al.*, 1984).** In addition, **Nakae *et al.*, (1987)** found that SOD and CAT could prevent the killing of cultured rat hepatocytes by AFB_1 suggesting that ROS might play a role in the cytotoxicity of AFB_1. Consequently**, Shen *et al.*, (1994)** provided an evidence of induction of lipid peroxidation by AFB_1 which could be inhibited by selenium and vitamin E (antioxidants), suggesting ROS-mediated cytotoxic response.

The process of lipid peroxidation (LP) plays an important role in the pathogenesis of numerous human diseases/disorders. The initiation of LP is carried out by free radicals such as superoxide, hydroxyl radicals etc. and other ROS like H_2O_2 , these agents extract a hydrogen atom from polyunsaturated fatty acids of membrane phospholipids **(Farber et al., 1990)** resulting in change in membrane structure, fluidity, transport and antigenic character is expected **(Bagchi *et al.*, 1993).**

The chain reaction of lipid peroxidation yields a large number of reactive compounds, resulting in the destruction of cellular membranes and other cytotoxic responses **(Premalatha and Sachdanandam, 1999)**. MDA is the most relevant of such compounds, it is one of the DNA adducts of genotoxins. It is particularly informative in studies of the effects of environmental exposures on cancer risk **(Shuker, 2002).**

Results from this study showed significant increase of MDA and nitric oxides (nitrite, NO_2 and nitrate, NO_3) concentrations in serum after single dose (2mg/kg, ip) and multiple doses (0.5 mg/kg/day for 7 days, ip) AFB_1 administration, thus providing direct *in vivo* evidence demonstrating the presence of LP caused by AFB_1 and confirming the results obtained by **Shen *et al.*, (1994); Souza *et al.*, (1999) and Yang *et al.*, (2000)**. Nitric oxide (NO) plays an important role in the communication among liver cells and regulates important liver functions. NO synthase catalyzes the

conversion of L-arginine to citrulline and highly reactive free radical species, NO **(Manahan, 1989)**. NO in turn, can react with O_2 and water to form nitrite and nitrate; with hemoglobin to form iron-nitrosyl adducts and/or nitrate in blood, with superoxide anion to make nitrate (NO_3), and with the amino and thiol groups of protein to produce nitrosylated species **(Manahan, 1989 and Misko *et al.*, 1993)**. The excess production of NO has been implicated in the pathogenesis and tissue destruction of a growing number of immunological and inflammatory diseases including hepatotoxicity **(Elhassaneen and El-Badawy, 2013; Elhassaneen *et al.*, 2014 and Mahran *et al.*, 2018).**

Effect of AFB_1 administration (ip) on the activities of antioxidant enzymes in blood and the protective effect of onion juice (OJ) and vitamin C pretreatment

Activities of antioxidant enzymes in rats administrated AFB_1 and pretreated with OJ and vitamin C was assessed by measuring GSH-Px, GSH-Rd, SOD and CAT activities (Table 3). From such data it could be noticed that the activities of antioxidant enzymes: GSH-Px, GSH-Rd, SOD, CAT were decreased significantly ($p \leq 0.05$) in - 23.78, - 26.06, - 36.91 and - 17.69%, respectively, following single dose AFB_1 treatment as compared to control rats. Pre-administration of OJ induced significant improvement in antioxidant enzyme activities in blood when compared with AFB_1 treated group. When compared with control values, OJ prevented the decrease in enzymatic activities (GSH-Px, GSH-Rd, SOD and CAT) by - 4.24, - 8.55, - 8.11 and - 1.98% in blood respectively with non-significant differences from that of control values. On the other hand, vitamin C pretreatment induce less protection in such enzymes by - 20.89, - 20.52, - 22.51 and - 12.65% in blood, respectively.

Multiple doses of AFB_1 compromised the activities of antioxidant enzymes, there were more decline in the activities of antioxidant enzymes; GSH-Px, GSH-Rd, SOD and CAT in blood by - 32.43, - 31.69, - 47.12 and - 21.13%, respectively as compared with control rats.

Simultaneous administration of OJ produces almost complete protection of all antioxidant enzyme activities in blood by - 0.65, - 6.64, - 9.42 and - 2.25%, respectively with non-significant differences in all values as compared to controls. Whereas the decrease in all antioxidant enzymatic activities was prevented by - 14.66, -18.61, - 23.82 and - 12.68% respectively in blood of vitamin C pretreated rats. However, both OJ and vitamin C induce significant ($p \leq 0.05$) increase in all enzyme activities in blood when compared to AFB_1 treated rats.

Table (3): Effect of AFB_1 administration (ip) on the activities of antioxidant enzymes in blood and the protective effect of onion juice (OJ) and vitamin C (Vit C) pretreatment

Groups		Glutathione peroxidase (GSH-Px)		Glutathione reductase (GSH-Rd)		Superoxide dismutase (SOD)		Catalase (CAT)	
		U/g Hb	% of change	U/g Hb	% of change	U/g Hb	% of change	U/g Hb	% of change
1) Control		16.99±1.98	0.00	9.94±1.87	0.00	3.82±0.44	0.00	160.75±18.65	0.00
Single dose	2) AFB_1 alone	12.95±1.75*	- 23.78	7.35±1.94*	- 26.06	2.41±0.60*	-36.91	132.31±19.77*	- 17.69
	3)OJ + AFB_1	16.27±2.87#	- 4.24	9.09±1.33#	- 8.55	3.51±0.85#	- 8.11	157.57±9.64#	- 1.98
	4) Vit. C + AFB_1	13.44±2.49*	- 20.89	7.90±1.55*	- 20.52	2.96±0.17*	- 22.51	140.41±12.99*	- 12.65
	5) DMSO	16.56±1.90	- 2.53	9.52±1.84	- 4.23	3.86±0.31	1.05	153.18±5.41	- 4.71
Multiple doses	6) AFB_1 alone	11.48±2.11*	- 32.43	6.79±1.05*	- 31.69	2.02±0.24*	- 47.12	126.79±15.83*	- 21.13
	7)OJ + AFB_1	16.88±2.31#	- 0.65	9.28±1.62#	- 6.64	3.46±0.30#	- 9.42	157.13±17.76#	- 2.25
	8) Vit. C + AFB_1	14.50±2.19*#	- 14.66	8.09±1.44*#	-18.61	2.91±0.36*#	- 23.82	140.37±15.59*#	- 12.68
	9) DMSO	16.43 0±2.41	- 3.30	9.60±1.38	- 3.42	3.74± 0.43	- 2.09	158.24±11.87	- 1.56

Values are expressed as mean ± SD (n=6). * Significant difference when compared to control group, # significant difference when compared to AFB_1 alone group

In the present study, the increased concentrations of oxidants (MDA and nitric oxides) observed in AFB_1 treated rats is associated with

decreased activities of antioxidant enzymes as GSH-Px, GSH-Rd, SOD and CAT. Such decrease in the enzyme activities can lead to the excessive availability of superoxides, peroxy radicals, which in turn generates hydroxyl radical's results in the propagation of lipid peroxidation **(Sacks *et al.*, 1978)**. Earlier reports which suggested that the antioxidant enzyme activities could be suppressed by AFB_1 administration, are in support of the present results **(Rastogi *et al.*, 2001a,b).**

Supplementation of OJ prior to AFB_1 toxification significantly counteracted the increased levels of MDA and nitric oxides in serum and reversed the altered levels of antioxidant enzymes toward their near normal levels suggesting that the main effect of OJ is an accelerated removal of lipid peroxides accumulated as a result of AFB_1 toxification leading to quicker repair of the damaged cellular membranes.

Effect of AFB_1 administration (ip) on the levels of plasma glutathione fractions concentration and the protective effect of onion juice (OJ) and vitamin C pretreatment

Levels of glutathione fractions in rats administrated AFB_1 and pretreated with OJ and vitamin C was assessed by measuring GSH and GSSG levels (Table 4). From such data it could be noticed that AFB_1 toxification (2mg/kg, ip, once) induce significant depletion of GSH and GSSG contents in serum, - 30.68 and - 12.22%, respectively when compared to control group. The multiple doses of AFB_1 induced more decrease in GSH and GSSG contents in serum (- 33.91 and - 15.56%, respectively. Gastric instillation of OJ and vitamin C prior to AFB_1 dosing prevent significantly the depletion of GSH and GSSG in serum when compared with AFB_1 alone treated group and brought back their levels to near normal in serum of both single and multiple doses groups, while vitamin C protection was less for the same treatments. Such data indicated that the rates of serum GSH fractions elevation were increased with the increasing of the OJ doses. Such effect could be one of the most important reasons for increasing the GSH/GSSG ratio (redox status) in treated

groups. In general, GSH is a tripeptide-thiol (γ-glutamyl cysteinyl-glycine) that has received considerable attention in terms of its biosynthesis, regulation, and various intracellular functions **(Reed and Beatty, 1980 and Larsson *et al.*, 1983).** Among of those functions, its role in detoxifications process represent the central role through as a key conjugate of xenobiotics electrophilic intermediates and as an important antioxidant. Also, GSH plays a critical role in important cellular functions, which includes the maintenance of thiol status of proteins, the destruction of H_2O_2, lipid peroxides, free radicals and translocation of amino acids across cell membranes, the detoxification of foreign compounds and the biotransformation of drugs **(James and Hrabison, 1982)**. Furthermore, the antioxidant functions of GSH include its role in the activities of the antioxidant enzymes system such glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-Rd). In addition, GSH can apparently serve as a nonenzymatic scavenger of oxyradicals **(Halliwell and Gutteridge, 1985; Elmaadawy *et al.*, 2016 and Mahran *et al.*, 2018).**

Table (4): Effect of AFB_1 administration (ip) on the levels of plasma glutathione fractions concentration and the protective effect of onion juice (OJ) and vitamin C (Vit C) pretreatment

Groups		Reduced glutathione concentration (GSH)		Oxidized glutathione concentration (GSSG)		GSH/GSSG ratio	
		µmol /L	% of change	µmol /L	% of change	Value	% of change
1) Control		9.91 ± 1.12	0.00	0.90 ± 0.22	0.00	11.01 ± 1.12	0.00
Single dose	AFB_1 alone	6.87 ± 0.79*	- 30.68	0.79 ± 0.15*	- 12.22	8.70 ± 0.82*	- 20.98
	3) OJ + AFB_1	9.08 ± 0.76#	- 8.38	0.91 ± 0.08#	1.11	9.97 ± 0.68#	- 9.45
	4) Vit. C + AFB_1	8.80 ± 0.55#	- 11.20	0.93 ± 0.10#	3.33	9.47 ± 1.14#	-13.99
	5) DMSO	9.50 ± 0.93	- 4.14	0.96 ± 0.12	6.67	9.90 ± 0.87	10.08
Multiple doses	6) AFB_1 alone	6.55 ± 0.1.02*	- 33.91	0.76 ± 0.29*	- 15.56	8.60± 0. 59*	- 21.89
	7) OJ + AFB_1	9.38 ± 0.77#	- 5.35	0.94 ± 0.10#	4.44	10.01± 0.69#	- 9 .08
	8) Vit. C +AFB_1	8.85 ± 1.08#	- 10.70	0.90 ± 0.09#	0.00	9.78 ± 0.87#	- 11.17
	9) DMSO	9.60 ± 0.24	- 3.13	0.98 ± 0.11	8.89	9.80 ± 1.32	- 10.99

Values are expressed as mean ± SD (n=6). * Significant difference when compared to control group, # significant difference when compared to AFB_1 alone group

Effect of AFB_1 administration (ip) on the activities of antioxidant vitamins in serum and the protective effect of onion juice (OJ) and vitamin C pretreatment

Concentration of antioxidant vitamins in rats administrated AFB_1 and pretreated with OJ and vitamin C was assessed by measuring vitamins A, E and C levels (Table 5). From such data it could be noticed that the concentrations of vitamin A, E, C in serum were significantly ($p \leq 0.05$) decreased in AFB_1 treated groups as compared to control rats. The levels of vitamins were decreased by - 30.85, - 38.79 and - 31.97% respectively) in serum in single dose AFB_1 treatment. More decline was observed in multiple doses AFB_1 administration in serum which - 35.82, - 43.38 and - 37.18%, respectively. Gastric instillation of OJ and vitamin C prior to AFB1 dosing prevent significantly the depletion of vitamins A, E and C in serum when compared with AFB1 alone treated group and brought back their levels to near normal in serum of both single and multiple doses groups, while vitamin C protection was less for the same treatments.

Table (5): Effect of AFB_1 administration (ip) on the activities of antioxidant vitamins in serum and the protective effect of onion juice (OJ) and vitamin C (Vit C) pretreatment

Groups		Vitamin A		Vitamin E		Vitamin C	
		µmol/L	% of change	µmol/L	% of change	µmol/L	% of change
1) Control		2.01±0.41	0.00	22.89±3.06	0.00	60.65±8.64	0.00
Single dose	2) AFB_1 alone	1.39±0.23*	- 30.85	14.01±1.43*	- 38.79	41.26±6.72*	- 31.97
	3) OJ + AFB_1	1.83±0.33#	- 8.96	18.66±4.03#	-18.48	54.76±4.72#	- 9.71
	Vit. C + AFB_1	1.61±0.20	- 19.90	16.01±2.17*	- 30.06	48.19±5.85*	- 20.54
	DMSO	1.92 ± 0.47	- 4.48	19.29±0.70	- 15.73	54.83±7.04	- 9.60
Multiple doses	6) AFB_1 alone	1.29±0.25*	- 35.82	12.96±3.01*	- 43.38	38.10±4.10*	- 37.18
	7) OJ + AFB_1	1.86±0.59#	- 7.46	19.37±3.43#	- 15.38	53.16±6.76#	- 12.35
	8) Vit. C + AFB_1	1.67±0.44	- 16.92	15.62±2.87*	- 31.76	47.99±3.95*#	- 20.87
	9) DMSO	1.90±0.29	- 5.47	19.82±3.23	- 13.41	58.04±9.62	- 4.30

Values are expressed as mean ± SD (n=6). * Significant difference when compared to control group, # significant difference when compared to AFB_1 alone group

Data of the present study reported that the reducing in antioxidant enzymes defense potential of erythrocytes was contrary with significant decreasing ($p \leq 0.05$) in antioxidant vitamins in rats plasma as a consequence of AFB_1 toxification. Vitamin A has been shown to prevent or delay carcinogenesis induced by chemicals through selective inhibition of the metabolic activation pathway, scavenging of the reactive intermediate and/or interaction with DNA leading to protection from reactive intermediates **(Decoudu *et al.*, 1992)**. Vitamin E is the most significant antioxidant of its kind in animal cells and it can protect against carcinogenesis and tumor growth **(Das, 1994).** It is the major lipid soluble peroxyl radical scavenger which can limit lipid peroxidation by terminating chain reactions initiated in the membrane lipids **(Wiseman, 1996).**Vitamin C is a water-soluble antioxidant that removes free radicals from cytosol by reacting directly with them. The availability of vitamin C is a determined factor in controlling and potentiating many aspects of host resistance to cancer **(Allen, 1991).**

In the present study, the administration of AFB_1 either single or multiple doses significantly decreases the levels of non-enzymatic antioxidants (GSH fractions and vitamins A,E and C) in serum. Significant depletion of GSH may be due to its utilization by excessive amount of free radicals **(Allen, 1991).** This depletion not only compromise cellular defenses against attack by reactive molecules but also has profound effects on normal hepatocellular functions **(Premalatha and Sachdanandam 1999).** Similar results were obtained by **Yang *et al.*, (2000).**

The significantly decreased vitamin E levels might be due to its excessive utilization for quenching enormous free radicals produced in these conditions. Vitamin E is thought to act as a chain breaking antioxidant by donating its labile hydrogen atom from phenolic hydroxyl radical groups to propagating lipid peroxyl and alkoxyl radical intermediates of lipid peroxidation, thus terminating the chain reaction **(Wiseman, 1996).** The tocopheroxyl radical formed during this reaction can be reduced primarily by ascorbic acid, GSH and other thiols. In biomembranes, vitamin E has been found to have potent antioxidant

activity due to its ability to penetrate to a precise site into the membrane which may be the important feature of protection against highly reactive radicals **(Packer *et al.*, 1979).** In addition, the decreased levels of vitamin C might be due to its role in protection of cell membranes and lipoproteins particles from oxidative damage by regenerating the antioxidant form of vitamin E **(Buettner, 1993 and Beyer, 1994).** Thus, vitamin C and E act synergistically in scavenging a wide variety of ROS. Additionally, vitamin C induces its effect through alteration of activities of hepatic microsomal enzymes, thus interferes with the formation of toxic metabolites of AFB_1 **(Nyandieka and Wakhisi, 1993).**

Conclusion

The antioxidant defense system, which plays a critical role in carcinogenesis process, is severely altered after AFB_1 administration. The evidence comprises enhanced oxides (MDA and nitric oxides) production and decreased enzymatic (GSH-Px, GSH-Rd, SOD and CAT) and non-enzymatic (GSH fractions and vitamins A, E and C) antioxidants levels that scavenge LO products. These deleterious effects were controlled by the administration of OJ and vitamin C, however the effect of OJ was more pronounced than that of vitamin C in AFB_1-treated animals indicating its capacity to induce effectively the *in vivo* antioxidant defense system. The present data support the benefits of onion in human population at high risk to AFB_1 exposure through alleviating oxidative stress and enhancing the antioxidative defense systems associated.

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الأفلاتوكسين ب١ تحدث إجهاد تأكسدى قى الفئران: تأثيرات وقائية محتملة لعصير البصل (*Allium cepa* L.)

رجاء أحمد صديق[١] ، غادة محمود البسيون[٢]، يوسف عبد العزيز الحسانين[٣]

[١] قسم الاقتصاد المنزلى– كلية التربية النوعية– جامعة المنيا – المنيا– مصر
[٢] قسم الاقتصاد المنزلى– كلية التربية النوعية– جامعة بنها– بنها– مصر
[٣] قسم التغذية وعلوم الأطعمة– كلية الاقتصاد المنزلي– جامعة المنوفية– شبين الكوم– مصر

المستخلص

ينتمي الأفلاتوكسين إلى مجموعة السموم الفطرية المعروفة بإسم mycotoxins، ويعتبر التعرض للأفلاتوكسين B_1 (AFB_1) عامل خطر مهم لتطور سرطان الكبد لكل من الحيوانات والإنسان، إن ميكانيكية التلف الخلوي الناتج عن التعرض لسموم AFB_1 ما زالت غير مفهومة. ولكن معظم الدراسات السابقة التي أجريت في المعامل تشير إلى أن AFB_1 قادرًا على إحداث إجهاد تأكسدي من خلال إطلاق أنواع الأكسجين النشطة (ROS) . يعتبر البصل واحد من أهم أنواع عائلة (Allium) الشائع استخدامها في وجباتنا اليومية يمتلك الكثير من الخصائص المضادة للأكسدة لذلك ، أجريت هذه الدراسة للتحقيق في الآثار الوقائية المحتملة لعصير البصل (OJ) مقارنة بفيتامين C كمضاد للأكسدة القياسي ضدAFB_1 والتي تحدث إجهاد تأكسدي لأنسجة الكبد في فئران الألبينو. أشارت بيانات التحليل الكيميائي الحيوي إلى أن AFB_1 تسبب في زيادة كبيرة ($p\leq0.05$)في تركيز مؤكسدات البلازما (MDA ٧٣, ٦١ و ١٢٣,٤٦ ٪; NO2 ٦١,٩٠ و ٦٩, ١١١ ٪ وNO3 / NO2 ٦٦,٦٧ و ١٠٧,٤٨ ٪) وانخفاض في مضادات الأكسدة غير الإنزيمية في البلازما (GSH – ٢٣,٧٨ و –٣٢,٤٣ ٪ و GSSG–٠٦, ٢٦ و –٦٩, ٣١٪) ، الفيتامينات المضادة للأكسدة في البلازما (فيتامين A – ٣٥,٨٢ و –٣٢,٤٣ ٪، فيتامين C – ٣١,٩٧ و – ٣٧, ١٨ ٪ وفيتامين E – ٣٨, ٧٩ و – ٤٣,٣٨ ٪) وكذلك إنزيمات RBC المضادة للأكسدة (GSH–Px – ٢٣,٧٨ و – ٣٢, ٤٣ ٪; CAT– ١٧,٦٩ و – ١, ١٣ ٢ ٪ وSOD – ٦,٩١ ٣ و – ٤٧, ١٢ ٪) كنسبة مئوية من المجموعة العادية في كل من الجرعة الواحدة والجرعات المتعددة على التوالي. الحقن بـ(OJ) أدى إلى تحسن معنوي ($p\leq0.05$) في جميع المعاملات السابقة بمعدلات مختلفة، كما سجلت أعلى التأثيرات التحسينية للعلاج بالجرعات المتعددة. في الختام ، تدعم البيانات الحالية فوائد البصل للأشخاص الأكثر تعرضا لخطر الـ AFB_1من خلال تخفيف الإجهاد التأكسدي وتعزيز أنظمة الدفاع المضادة للأكسدة المرتبطة به.

الكلمات المفتاحية: الأفلاتوكسينB_1 ، التركيب الكيميائي، البلازما، MDA ، أكاسيد النيتريك ، GSH، الفيتامينات المضادة للأكسدة ، الإنزيمات المضادة للأكسدة.