Antagonistic Effects of Selenium (Se) and Vitamin C against the Hepatotoxic Effects of AFB_1 in Rats

Salwa A. Abaskhroun, Hala E. Hamouda, Ayman A. Wagih, Rania N. Emam * Mona A. Abd El-Azem
Medical Biochemistry and *Pathology Departments,
Faculty of Medicine, Tanta University

ABSTRACT

Objective: *Aflatoxins (AFs) a group of mycotoxins, are produced by the filamentous fungi Aspergillus, particularly flavus and parasiticus. Aflatoxin B_1 (AFB_1) is the most prevalent and the most potent of these toxins, which has potent hepatotoxic and hepatocarcinogenic properties in animals and humans. Because of the wide spread of AFB_1 contaminated food and feeds and because of its hepatotoxicity, the present experimental study was carried out.* ***Aim of the study:*** *The aim of the present study was to highlight the antagonistic effects of selenium (Se) and vitamin C against the hepatotoxic effect of AFB_1 as they have a role in prevention of formation of carcinogens from precursor compounds and they are natural antioxidants.* ***Materials and methods:*** *The study was carried out on 85 white male albino rats divided into;* ***Group I (control group)****:10 rats received I.P injection of dimethylsulfoxide (DMSO) for 15 days.* ***Group II:*** *45 rats received I.P injection of AFB_1 for 15 days and then subdivided into three equal subgroups;* ***Group II a****: received regular diet,* ***group II b:*** *received Se orally for 15 days.* ***Group II c:*** *received vitamin C orally for 15 days.* ***Group III:*** *received Se orally for 15 days during and 15 days after AFB_1 injection.* ***Group IV:*** *received vitamin C orally for 15 days during and 15 days after AFB_1 injection. All groups were subjected to measurements of the following; liver function tests, serum & liver tissue levels of malondialdehyde (MDA), reduced glutathione (GSH), and the activity of serum &liver tissue glutathione S- transferase enzyme (GST) and paraoxonase1 (PON1) enzymes. Liver specimens were examined histopathologically.* ***Results:*** *The present study confirmed the hepatotoxicity of AFB_1, as marked by the significant increase of serum AST, ALT enzymes activities and decrease serum albumin which is confirmed by histopathological study of liver tissues. Serum and liver tissue MDA levels were significantly increased in AFB_1 treated animals. There was significant increase of the inducible enzyme GST activity and significant decrease of GSH level in AFB_1 treated groups. There was significant decrease in PON1 enzyme activity in both serum and hepatic tissue. Se and vitamin C were effective only when given with and after the xenobiotic treatment for another 15 days. They caused significant decrease of serum activity of AST, ALT and increase in serum albumin. They also caused a significant decrease in MDA level and GST enzyme activity with significant increase of GSH level and PON1 enzyme activity in both serum and liver tissues.* ***Conclusion:*** *All the above findings confirm the protective role of Se and vitamin C on the hepatotoxicity caused by AFB_1.*

Key Words: *Selenium (Se), malondialdehyde (MDA), reduced glutathione (GSH), glutathione S- transferase enzyme (GST) paraoxonase1 (PON1).*

INTRODUCTION

Aflatoxins (AFs) are secondary toxic fungal metabolites produced by *Aspergillus flavus and Aspergillus parasiticus*. There are four naturally occurring AFs which are structurally similar compounds namely AFB_1, AFB_2, AFG_1, AFG_2 , the most hepatotoxic being AFB_1. Aflatoxins not only contaminate our food stuffs but are also found in edible tissues, milk and eggs after consumption of contaminated feed by farm animals[1,2]. Epidemiological and experimental studies have shown that AFs are hepatotoxic, hepatocarcinogenic, mutagenic and teratogenic [3].

The liver is the primary site for biotransformation of ingested AFB_1. The parent molecule is harmless, but in the liver it is converted by members of the cytochrome P450 (CYP 450) super-family to AFB_1-8,9-epoxide. Epoxidation of AFB_1 to the exo-8, 9-epoxide is a critical step in the genotoxic pathway of this carcinogen. The epoxide is highly unstable and binds with high affinity to guanine bases in DNA to form aflatoxin-N7-guanine [4, 5]. The aflatoxin-N7-guanine has been shown to be capable of forming guanine (G) to thymine (T) transversion mutations in DNA which is regarded as a critical step in the initiation of AFB_1-induced hepatocarcinogenesis[3,6].

Generation of intracellular reactive oxygen species (ROS) occurs during the metabolic processing of AFB_1 by CYP 450 in the liver [7]. These species may attack soluble cell compounds resulting in damage to critical cellular macromolecules including lipids and proteins leading to the impairment of cell functioning and cytolysis. Peroxidation of membrane lipids initiates loss of membrane integrity, membrane bound enzyme activity and cell lysis[8]. Malondialdehyde (MDA) is a product generated during the oxidative breakdown of lipids, and is found either in free form, or bound to certain tissue structures[9].

Peroxidative damage induced in the cells is encountered by elaborate defense mechanisms, including enzymatic and non-enzymatic antioxidants [10]. The liver has been found to be the main site of these scavenger enzymes [11].

Selenium is an essential trace element. Low selenium status may contribute to the etiology of different disease conditions[12]. Epidemiological studies have suggested an inverse relationship between Se levels and different cancers[13]. Decreased Se levels were found in HBV and HCV infections, intrahepatic cholestasis, post-viral or alcoholic cirrhosis and hepatocellular carcinoma[14,15].

Vitamin C is a water-soluble chain breaking antioxidant, as it scavenges free radicals and reactive oxygen molecules, which are produced during metabolic pathways of detoxification. It also prevents formation of carcinogens from precursor compounds[16]. Tocopherol and glutathione also rely on vitamin C for regeneration back to their active isoforms. The relationship between vitamin C and glutathione is unique. Vitamin C reduces glutathione back to the active form and glutathione once reduced, it will regenerate vitamin C

from its dehydroascorbic acid (DHAA) or oxidized state. Also, it acts as an electron donor for different enzymes[17].

Glutathione pathway has been also shown to play a major role in the detoxification of AFB_1.The AFB_1 8, 9 exo and endo epoxides can be conjugated with glutathione resulting in the formation of AFB_1-mercapturate catalyzed by GST which is then excreted in urine [18].

Glutathione S-transferases (GSTs) represent a major group of detoxification enzymes. They are composed of many cytosolic, mitochondrial, and microsomal proteins. GSTs catalyse a variety of reactions and accept endogenous and xenobiotic substrates [19].

Paraoxonase 1 (PON1) is an ester hydrolase of organophosphorates and other xenobiotics that is found in several tissues, predominantly in the liver, and also in serum. PON1 activity has been observed in rats [20], and human [21]. Part of the enzyme is secreted into the circulation bound to HDL, where the rest is stored in the liver. The physiologic role played by PON_1 in liver is suggested to be the protection against oxidative stress [22].

The aim of the present study was to highlight the antagonistic effect of the exogenous antioxidants Se and vitamin C against the hepatotoxic effect of AFB_1 because they have a role in prevention of formation of carcinogens from precursor compounds and they are natural antioxidants.

MATERIALS & METHODS

The current study was carried out on 85 white male albino rats of approximately 120 - 150 g body weight which were housed in wire mesh cages and were fed standard rat chew and allowed free access to water. They were kept under constant environmental conditions (25 <C and 12 h dark light cycle). The studied animals were divided into:

***Group I (control group):** 10 rats received I.P injection of dimethylsulfoxide (DMSO) (The solvent for AFB_1) in a dose of 0.5ml for 15 days.

***Group II:** 45 rats received I.P injection of AFB_1 in a dose of 0.25 mg/kg body weight dissolved in DMSO in a volume of 0.5 ml for 15 days. This group was subdivided into three equal subgroups after the end of injection period as follows; ***Group IIa*:** received regular diet without antioxidants. ***Group IIb*:** received Se orally in a dose of 0.2 mg/kg body weight for 15 days. ***Group IIc*:** received vitamin C orally in a dose of 200 mg/kg body weight for 15 days.

***Group III:** 15 rats given selenium orally in a dose 0.2 mg/kg body weight for 15 days during and 15 days after AFB_1 injection.

***Group IV:** 15 rats given vitamin C orally in a dose 200 mg/kg body weight for 15 days during and 15 days after AFB_1 injection.

At the end of the experiment, 10 rats were died, six rats from group II, two from group III and the remaining two from group IV.

The doses of AFB_1, Se and vitamin C were chosen on the basis of the previous studies [23-25].

All experiments were carried out according to the guidelines of the Ethical Committee of Tanta University, Faculty of Medicine.

Sample collection: All rats were sacrificed and blood samples were collected. Sera were separated and stored in aliquots at -70 °C till used for different estimations.

Then, the abdomen and the thorax were opened and livers were removed, washed three times in ice cold saline and blotted individually on ash-free filter paper, used for preparation of tissue homogenates.

Preparation of tissue homogenates: Each liver is then divided into two specimens. One piece was kept in 10% formalin solution and fixed for histopathological examination, and the remaining one was stored at -70 ºC till use.

Tissue samples were divided into three parts. Specimens were weighted and homogenized with a Potter-Elvenhjem tissue homogenizer (20-30 up and-down strokes). *One part* was homogenized in phosphate buffer saline (PBS) 50 mM (pH 7.4) for estimation of protein content, GST activity and GSH level, *the second* was homogenized in potassium phosphate buffer10 mM (pH 7.4) for estimation of MDA level and *the third* one in Tris- HCl 100 mM (pH 8) for estimation of PON1 activity. Homogenate was centrifuged at 7,700 x g at 4ºC for 30 minutes and the resultant supernatant was assayed for the different estimations.

Chemicals: AFB_1 as obtained from Botany Department, Faculty of Science Assiut University. Se, vitamin C, 1-chloro-2, 4-dinitrobenzene (CDNB), and paraoxon were obtained from (Sigma chemical Co. St., Louis, MO, USA). Thiobarbituric acid (TBA) and reduced glutathione (GSH) were obtained from Fluka Chemical Co. Trichloroacetic acid (TCA) were obtained from Merck Chemical Co USA.

All groups were subjected to measurements of the following;

1. **Liver function tests** including; serum ALT, AST enzymes activities, total protein and albumin using commercial kits (RANDOX, United Kingdom) according to the instructions of manufactures.
2. **Spectrophotometric determination of serum and tissue MDA levels:** This method depends on the formed MDA as an end product of lipid peroxidation which reacts with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which can be measured spectrophotometrically at 532 nm [(26)].
3. **Determination of serum and tissue GSH levels:** The method is based on the reduction of 5,5 dithiobis (2-nitrobenzoic acid) (DTNB) with reduced glutathione (GSH) to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm by using a commercial kit (Biodiagnostic, Egypt) [(27)].
4. **Determination of serum and tissue GST enzyme activity:** The activity measures the conjugation of 1-chloro-2,4-dinitro benzene (CDNB) with reduced glutathione that produces a dinitrophenyl thioether which can be detected by spectrophotometer at 340 nm. One unit of GST activity is defined as the amount of enzyme producing 1 mmol of CDNB-GSH conjugate/ min under the conditions of the

assay according to the method described by **Habig et al., (1974)** [28].

5. **Determination of serum and tissue PON1 enzyme activity:** PON1 activity towards paraoxon (O, O-diethyl-O-p-nitrophenyl phosphate) was determined by measuring the initial rate of substrate hydrolysis to p- nitrophenol, whose absorbance was monitored at 405 nm in the assay mixture [29].
6. **Estimation of protein content** was performed for liver homogenates according to **Lowry et al., (1951)**[30].
7. **Histopathological examination** of formalin fixed liver specimens.

STATISTICS:

Statistical data and analyses used in the present study were conducted, using range, the mean, standard deviation, analysis of variance [ANOVA] test followed by Tukey's post hoc test and linear correlation coefficient.

RESULTS

The present study showed significant increase of serum AST and ALT enzymes activities with decrease of serum albumin level and non significant change of total protein level (table 1). Serum and tissue MDA levels showed statistically significant increase in group IIa in comparison to their levels in group I (control group) ($P<0.001$). Their levels in group IIb and group IIc showed no significant difference when compared to their levels in group IIa and also when compared to each other ($P>0.05$). But their levels in both groups were significantly higher than the control group ($P<0.001$).Their levels in group III and group IV showed statistically significant decrease when compared to group IIa, group IIb and group IIc ($P<0.001$), with no significant difference between them and control group ($P>0.05$). Also, there was no significant difference between each other ($P>0.05$), (tables 2A and 2B).

Two endogenous antioxidants were studied, an enzyme GST and a non enzyme GSH. The two behaved differently. Serum and tissue GST enzyme activity showed statistically significant increase in group IIa in comparison to their activities in group I (control group) ($P<0.001$). The activity in group IIb and group II c showed no significant difference when compared to group II a and also when compared to each other ($P>0.05$). But the activity in both groups was significantly higher than the control group ($P<0.001$). Both serum and tissue GST activity showed statistically significant decrease in both group III and group IV when compared to group IIa, group IIb and group IIc ($P< 0.001$), with no significant difference between them and control group ($P>0.05$). Also, there was no significant difference between each other ($P>0.05$) (tables 3 A and 3 B).

Serum and liver GSH showed significant decrease in group IIa in comparison to their levels in group I (control group) ($P <0.001$). Their levels in group IIb and group IIc showed no significant difference when compared to their levels in group IIa and also when compared to each other ($P>0.05$). But their levels in both groups were significantly lower than the control group ($P <0.001$). In groups III and IV their levels showed significant increase

when compared to group IIa, group IIb and group IIc (P<0.001), with non significant difference between them and control group (P>0.05). Also, the difference between each other was insignificant (P>0.05, tables 4 A & 4B).

The activity of both serum and tissue PON1 showed statistically significant decrease in group IIa in comparison to their activities in control group (P< 0.001). Their activity in group IIb and group IIc showed no significant difference when compared to group IIa (P>0.05). But the activity in both groups was significantly lower than the control group (P< 0.001). Their activity in group III and group IV showed statistically significant increase when compared to group IIa, group IIb and group IIc (P< 0.001), with no significant difference between them and control group (P>0.05). Also, there was no significant difference between each other (P>0.05) (tables 5A and 5B).

As regards correlation matrix in the present study, there were negative correlation between serum and tissue MDA levels and serum and tissue GSH levels in all studied groups (r = -0.708, P< 0.001, r = -0.938 ,P< 0.001 respectively). Also, negative correlation was found between serum and tissue MDA levels and serum and tissue PON1 activity in all studied groups (r = - 0.661, r = -0.738 respectively, P< 0.001 for each). Positive correlation was found between serum and tissue MDA levels with serum and tissue GST activity in all studied groups. (r = 0.724, r= 0.897 respectively P< 0.001 for each), (tables 6 and 7).

Histopathological results in the present study showed no changes in the gross and histological appearance of the livers of the rats from control group, as shown in **Fig 1.** However, the livers of group IIa (AFB_1-treated group) were slightly pale, enlarged and grayish mottled in gross appearance. In addition, severe histopathological changes were observed in livers of all the rats from AFB_1-treated group. The predominant lesions were extensive vacuolar (hydropic) degeneration, in which the cytoplasm of hepatocyte contains many vacuoles with irregular border. These changes were seen in almost all lobules, and were more pronounced in centrilobular and intermediate areas. Most hepatocytes in the degenerative and necrotic regions had pycnotic nuclei, and were so swollen that several cells had ruptured. The sinusoids were shrunken or completely plugged due to swollen hepatocytes. In addition, some hepatocytes in the periportal regions had moderate to severe cytoplasmic vacuolation, indicating fatty change. In addition, some cases showed bile duct hyperplasia with focal area of necrosis and inflammatory infiltrate. Because of all these changes the normal architecture of the hepatic parenchyma was distorted. However, the livers of all animals showed no cirrhotic changes **(Fig.2 A, B)**.

Group IIb (rats given selenium after AFB_1 injection) and group IIc (rats given vitamin C after AFB_1 injection) showed no significant difference in comparison to AFB_1 injected group **(Fig. 3&4).**

In group III and IV, Se and vitamin C supplementation with and

after AFB_1 injection ameliorated or reversed the changes induced by AFB_1 in part **(Fig. 5&6)**. The lesions in the liver of rats in both groups were conspicuously less than those in the rats that received AFB_1 only. The livers from the rats in these groups showed no extensive hydropic degeneration or necrotic changes. Moreover, the architecture of lobules was not disrupted and the hepatocytes mostly had normal appearance, however only few lobules of two livers showed a focus of degenerative cells, most of which had normal nuclear appearance and mild cytoplasmic degeneration.

Table 1: Comparison between the studied groups as regards liver function tests using ANOVA test.

GROUP		Range			Mean	±	SD	ANOVA F	ANOVA P-value
AST (U/l)	GI	3.000	-	10.000	6.200	±	2.300	102.998	<0.001*
	GIIa	36.000	-	55.000	43.308	±	5.836		
	GIIb	16.000	-	31.000	22.714	±	4.548		
	GIIc	16.000	-	31.000	24.833	±	5.573		
	GIII	8.000	-	16.000	12.462	±	2.634		
	GIV	9.000	-	23.000	15.000	±	4.359		
ALT (U/l)	GI	3.000	-	9.000	5.500	±	2.068	64.205	<0.001*
	GIIa	34.000	-	62.000	47.846	±	8.947		
	GIIb	15.000	-	36.500	22.143	±	9.371		
	GIIc	17.000	-	39.000	26.917	±	7.157		
	GIII	10.000	-	18.000	13.231	±	2.713		
	GIV	10.000	-	20.000	14.154	±	2.996		
Total protein (g/dl)	GI	6.200	-	7.200	6.750	±	0.381	2.057	0.081
	GIIa	6.000	-	7.500	6.769	±	0.497		
	GIIb	6.400	-	7.300	6.843	±	0.311		
	GIIc	6.500	-	7.500	6.958	±	0.385		
	GIII	6.400	-	7.000	6.685	±	0.208		
	GIV	6.000	-	7.200	6.515	±	0.391		
Albumin (g/dl)	GI	4.000	-	5.500	4.720	±	0.525	48.348	<0.001*
	GIIa	2.500	-	3.500	2.946	±	0.338		
	GIIb	3.000	-	4.000	3.400	±	0.353		
	GIIc	3.000	-	4.000	3.625	±	0.347		
	GIII	4.000	-	5.000	4.538	±	0.340		
	GIV	4.100	-	4.800	4.431	±	0.278		

Group I: Control group (no=10).
Group IIa: AFB_1 injected group (no=13).
Group IIb: Selenium orally after AFB1 injection (no=14).
Group IIc: Vitamin C orally after AFB_1 injection (no=12).
Group III: Selenium orally during and after AFB_1 injection (no=13).
Group IV: Vitamin C orally during and after AFB_1 injection (no=13).

Table 2 (A): Comparison between the studied groups as regards serum malondialdehyde (MDA ; nmol/ml) using ANOVA test.

Groups	Serum MDA						ANOVA	
	Range			Mean	±	SD	F	P-value
GI	2.841	-	3.161	2.995	±	0.121	179.936	<0.001*
GIIa	4.379	-	5.082	4.753	±	0.274		
GIIb	4.343	-	5.290	4.627	±	0.267		
GIIc	4.299	-	5.000	4.556	±	0.230		
GIII	2.900	-	3.840	3.089	±	0.251		
GIV	3.180	-	3.500	3.253	±	0.089		

Tukey's test					
	GI	GIIa	GIIb	GIIc	GIII
GIIa	<0.001*				
GIIb	<0.001*	0.681			
GIIc	<0.001*	0.246	0.965		
GIII	0.912	<0.001*	<0.001*	<0.001*	
GIV	0.074	<0.001*	<0.001*	<0.001*	0.418

Table 2 (B): Comparison between the studied groups as regards liver malondialdehyde (MDA ; nmol/mg protein) using ANOVA test.

Groups	Liver MDA						ANOVA	
	Range			Mean	±	SD	F	P-value
GI	0.884	-	1.401	1.127	±	0.160	263.248	<0.001*
GIIa	3.174	-	4.679	3.767	±	0.463		
GIIb	3.082	-	4.338	3.778	±	0.429		
GIIc	3.345	-	4.261	3.826	±	0.281		
GIII	0.828	-	1.348	1.126	±	0.148		
GIV	0.914	-	1.611	1.258	±	0.183		

Tukey's test					
	GI	GIIa	GIIb	GIIc	GIII
GIIa	<0.001*				
GIIb	<0.001*	1.000			
GIIc	<0.001*	0.997	0.999		
GIII	1.000	<0.001*	<0.001*	<0.001*	
GIV	0.917	<0.001*	<0.001*	<0.001*	0.888

Group I: Control group (no=10).
Group IIa: AFB_1 injected group (no=13).
Group IIb: Selenium orally after AFB1 injection (no=14).
Group IIc: Vitamin C orally after AFB_1 injection (no=12).
GroupIII: Selenium orally during and after AFB_1 injection (no=13).
Group IV: Vitamin C orally during and after AFB_1 injection (no=13).

Table 3 (A): Comparison between the studied groups as regards serum glutathione S-transferase activity (GST; U/ml) using ANOVA test.

Groups	Serum GST						ANOVA	
	Range			Mean	±	SD	F	P-value
GI	18.20	-	78.00	44.00	±	22.57	28.982	<0.001*
GIIa	120.50	-	330.00	194.06	±	69.23		
GIIb	112.70	-	328.50	192.01	±	71.91		
GIIc	124.80	-	302.00	174.89	±	52.98		
GIII	22.60	-	81.20	50.87	±	20.40		
GIV	29.90	-	90.00	54.79	±	17.93		

Tukey's test					
	G1	GIIa	G IIb	GIIc	G III
GIIa	<0.001*				
GIIb	<0.001*	1.000			
GIIc	<0.001*	0.927	0.950		
GIII	0.999	<0.001*	<0.001*	<0.001*	
GIV	0.995	<0.001*	<0.001*	<0.001*	1.000

Table 3 (B): Comparison between the studied groups as regards liver glutathione S-transferase activity (GST ; U/mg protein/min) using ANOVA test.

Group	Liver GST						ANOVA	
	Range			Mean	±	SD	F	P-value
GI	89.20	-	132.00	112.04	±	15.50	108.993	<0.001*
GIIa	250.40	-	400.00	320.52	±	52.48		
GIIb	244.40	-	390.00	301.11	±	45.70		
GIIc	256.70	-	405.00	323.53	±	52.69		
GIII	91.10	-	128.00	109.50	±	13.48		
GIV	100.50	-	135.80	117.92	±	11.06		

Tukey's test					
	G1	GIIa	G IIb	G IIc	GIII
GIIa	<0.001*				
GIIb	<0.001*	0.758			
GIIc	<0.001*	1.000	0.651		
GIII	1.000	<0.001*	<0.001*	<0.001*	
GIV	0.999	<0.001*	<0.001*	<0.001*	0.992

Group I: Control group (no=10).
Group IIa: AFB_1 injected group (no=13).
Group IIb: Selenium orally after AFB1 injection (no=14).
Group IIc: Vitamin C orally after AFB_1 injection (no=12).
Group III: Selenium orally during and after AFB_1 injection (no=13).
Group IV: Vitamin C orally during and after AFB_1 injection (no=13).

Table 4 (A): Comparison between the studied groups as regards serum reduced glutathione level (GSH ; mg/dl) using ANOVA test.

Groups	Serum GSH						ANOVA	
	Range			Mean	±	SD	F	P-value
GI	2.920	-	3.260	3.086	±	0.120	17.001	<0.001*
GIIa	1.450	-	2.970	2.082	±	0.593		
GIIb	1.500	-	3.000	2.136	±	0.588		
GIIc	1.460	-	2.980	2.132	±	0.601		
GIII	2.770	-	3.370	3.072	±	0.180		
GIV	2.820	-	3.180	3.005	±	0.107		

Tukey's test					
	GI	GIIa	GIIb	GIIc	GIII
GIIa	<0.001*				
GIIb	<0.001*	1.000			
GIIc	<0.001*	1.000	1.000		
GIII	1.000	<0.001*	<0.001*	<0.001*	
GIV	0.998	<0.001*	<0.001*	<0.001*	0.999

Table 4 (B): Comparison between the studied groups as regards liver reduced glutathione level (GSH ; mg/g liver tissue) using ANOVA test.

Groups	Liver GSH						ANOVA	
	Range			Mean	±	SD	F	P-value
GI	3.699	-	5.634	5.068	±	0.555	139.738	<0.001*
GIIa	1.765	-	3.213	2.327	±	0.437		
GIIb	1.507	-	2.936	2.323	±	0.376		
GIIc	1.735	-	2.852	2.317	±	0.343		
GIII	4.133	-	5.723	5.121	±	0.470		
GIV	3.902	-	5.916	5.153	±	0.547		

Tukey's test					
	GI	GIIa	GIIb	GIIc	GIII
GIIa	<0.001*				
GIIb	<0.001*	1.000			
GIIc	<0.001*	1.000	1.000		
GIII	1.000	<0.001*	<0.001*	<0.001*	
GIV	0.998	<0.001*	<0.001*	<0.001*	1.000

Group I: Control group (no=10).
Group IIa: AFB_1 injected group(no=13).
Group IIb: Selenium orally after AFB1 injection (no=14).
Group IIc: Vitamin C orally after AFB_1 injection (no=12).
Group III: Selenium orally during and after AFB_1 injection(no=13).
Group IV: Vitamin C orally during and after AFB_1 injection (no=13).

Table 5 (A): Comparison between the studied groups as regards serum paraoxonase activity (PON1 ; U/ml) using ANOVA test.

Groups	Serum PON1						ANOVA	
	Range			Mean	±	SD	F	P-value
GI	65.500	-	210.000	132.310	±	51.713	11.983	<0.001*
GIIa	33.300	-	90.000	62.546	±	19.791		
GIIb	36.600	-	91.100	63.405	±	18.899		
GIIc	30.000	-	95.000	63.526	±	22.837		
GIII	64.000	-	208.000	134.152	±	45.032		
GIV	60.000	-	202.200	127.277	±	55.219		
Tukey's test								
	GI		GIIa	GIIb		GIIc	GIII	
GIIa	<0.001*							
GIIb	<0.001*		1.000					
GIIc	<0.001*		1.000	1.000				
GIII	1.000		<0.001*	<0.001*		<0.001*		
GIV	1.000		<0.001*	<0.001*		<0.001*	0.997	

Table 5 (B): Comparison between the studied groups as regards liver paraoxonase activity (PON1; U/mg protein/min) using ANOVA test.

Group	Liver PON1						ANOVA	
	Range			Mean	±	SD	F	P-value
GI	202.000	-	464.400	280.374	±	92.741	16.146	<0.001*
GIIa	90.800	-	210.000	147.162	±	43.599		
GIIb	93.000	-	212.000	147.172	±	44.552		
GIIc	91.000	-	211.500	152.900	±	43.877		
GIII	200.000	-	466.000	297.746	±	84.620		
GIV	198.000	-	455.500	276.946	±	75.188		
Tukey's test								
	GI		GIIa	GIIb		GIIc	GIII	
GIIa	<0.001*							
GIIb	<0.001*		1.000					
GIIc	<0.001*		1.000	1.000				
GIII	0.989		<0.001*	<0.001*		<0.001*		
GIV	1.000		<0.001*	<0.001*		<0.001*	0.966	

Group I: Control group (no=10).
Group IIa: AFB_1 injected group(no=13).
Group IIb: Selenium orally after AFB1 injection (no=14).
Group IIc: Vitamin C orally after AFB_1 injection (no=12).
Group III: Selenium orally during and after AFB_1 injection(no=13).
Group IV: Vitamin C orally during and after AFB_1 injection (no=13).

Table 6: Correlation matrix between serum MDA level (nmol/ml), serum GSH level (mg/dl) , serum GST activity(U/ml) and serum PON1 activity(U/ml).

		Serum GSH	Serum GST	Serum PON1
Serum MDA	**r**	-0.708	0.724	-0.661
	P-value	<0.001*	<0.001*	<0.001*

Table 7: Correlation matrix between liver MDA level (nmol/mg protein/min), liver GSH level (mg/g liver tissue) , liver GST activity (U/mg protein/min) and liver PON1 activity (U/mg protein/min).

		Liver GSH	Liver GST	Liver PON1
Liver MDA	**R**	-0.938	0.897	-0.738
	P-value	<0.001*	<0.001*	<0.001*

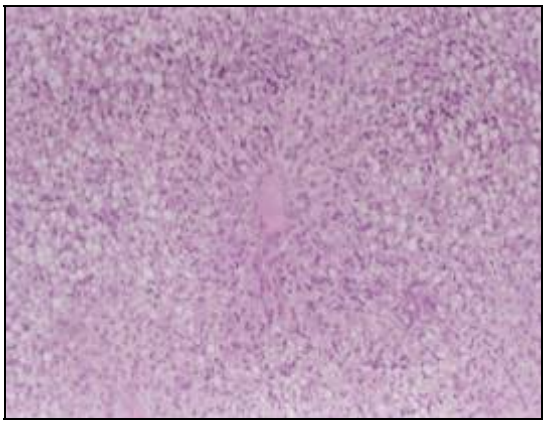

Fig. 1: No histopathological changes in the livers of control group.

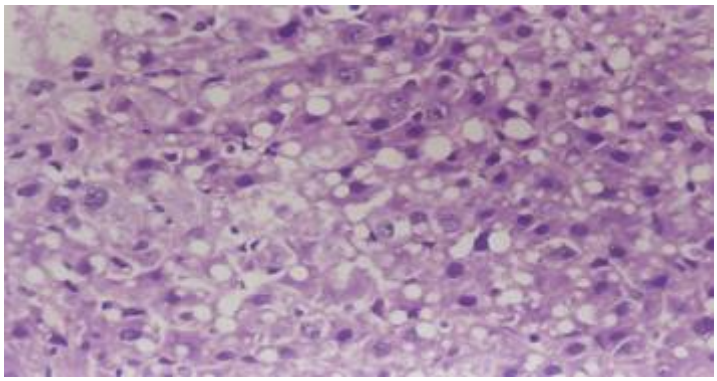

Fig.2 A: Extensive vacuolar (hydropic) degeneration of hepatocytes from group IIa (AFBI injected rats) (H&E x 200) .

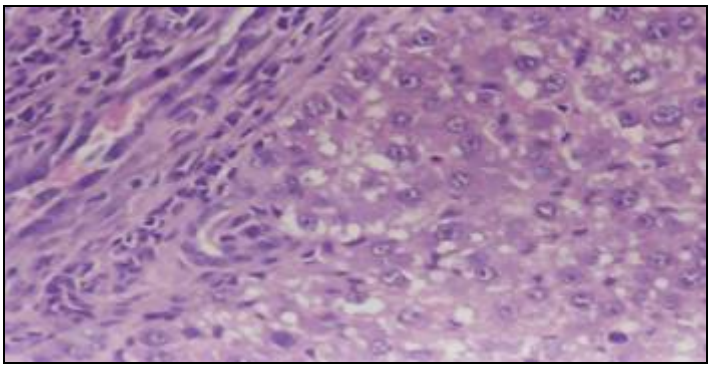

Fig.2 B: Some necrotic hepatocytes, heavy inflammatory infiltrate with some vacuolar degeneration of hepatocytes in group IIa (AFB_1 injected rats) (H&E x 200).

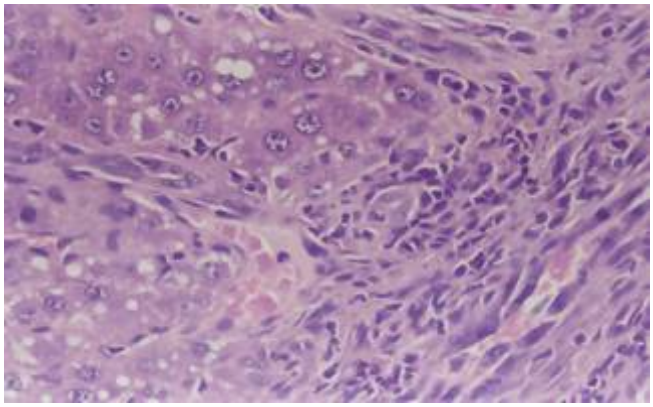

Fig.3: Mild vacuolar degeneration of hepatocytes, inflammatory infiltrate and some necrotic liver cells from group IIb (rats given selenium after AFB_1 injection) (H&Ex 200) .

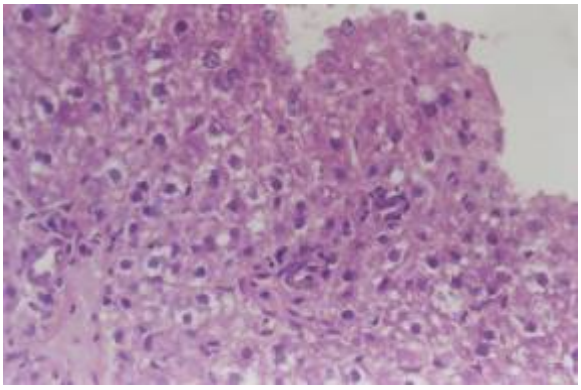

Fig.4: Some necrotic hepatocytes, inflammatory infiltrate with few vacuolar degeneration from group IIc (rats given vitamin C after AFB_1 injection) (H&E x 200)

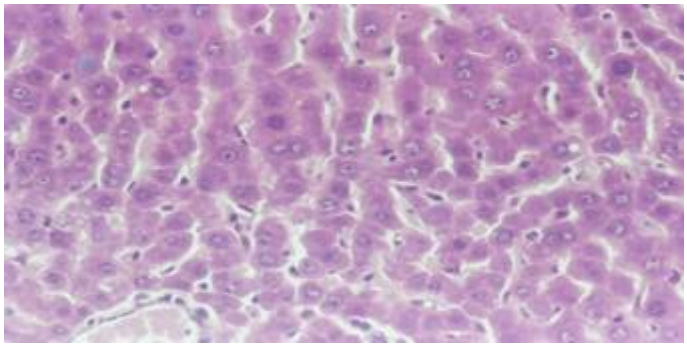

Fig.5: Minimal vacuolar degeneration of hepatocytes with few necrotic cells together with regenerating surrounding cells from group III (rats given Se with and after AFB_1 injection) (H&E x 200) .

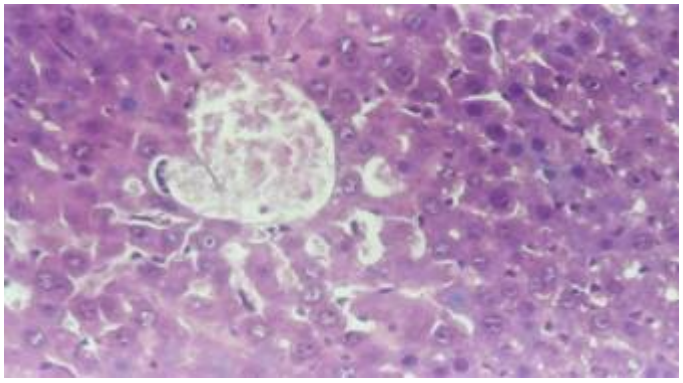

Fig. 6: Few vacuoles in some hepatocytes with regenerating of surrounding cells from group IV (rats given vitamin C with and after AFB_1 injection) (H&E x 200).

DISCUSSION

Aflatoxins (AFs) are a group of mycotoxins of difuranocoumarin derivatives produced by *Aspergillus flavus* and *A. parasiticus. T*he most hepatotoxic being AFB_1 and is usually predominant in foods [(2)]. *Aspergilli* are common in nature, and can colonize and contaminate various foods and feeds under favorable conditions of temperature and humidity [(31)].

The main target organ of Afs intoxication is the liver, and acute toxic hepatitis with high mortality rate has been reported [(3,32)]. In addition, in humans adverse effects in immune system [(33)], and gastrointestinal tract have been shown [(34)]. Other effects have been described, as interference with metabolic processes of various essential micronutrients [(33)]. An assessment of the health risk possibly related to AFs exposure is difficult as the toxicokinetics in humans is complex and knowledge is incomplete. In addition to intensity and duration of exposure, several

factors, such as age and health conditions, seem to influence susceptibility to AFs effects [35]. So, it is easier to asses this in experimental animal models.

The aim of the present study was to highlight on the antagonistic effect of the exogenous antioxidants Se and vitamin C against the hepatotoxic effect of AFB_1 because they have a role in prevention of formation of carcinogens from precursor compounds and they are natural antioxidants.

Aflatoxin B_1 toxicity may ensue through the generation of intracellular reactive oxygen species (ROS) during the metabolic processing of AFB_1 by CYP 450 in the liver [7]. Lipid peroxidation is one of the main manifestations of oxidative damage initiated by ROS and it has been linked with altered membrane structure and enzyme inactivation [36].The increase in lipid peroxides might result from increased production of free radicals and/or a decrease in antioxidant status where it has been implicated in AFB1-induced hepatotoxicity [37].

The present study showed that serum and liver tissue MDA tissue levels were significantly increased in AFB_1 treated rats (group IIa) when compared with control one (group I). The animal groups, receiving Se and vitamin C after AFB_1 treatment (groups IIb and IIc respectively), showed the same increment in MDA levels. Groups III and IV receiving the antioxidants during and 15 days after AFB_1 injections showed decrease of MDA levels back to the control level.

The significant increase in serum and hepatic MDA levels observed in AFB_1 -treated groups in the present study is in agreement with other previous studies of **Farombi et al. (2005)** [38] and **Umarani et al. (2008)**[39] who reported increased MDA formation and lipid hydroperoxide accumulation in the livers of AFB_1 -treated rats. **Gesing et al. (2008)**[40] also documented that AFB_1 increased lipid peroxidation in the liver, lung, brain and testis, but not the kidney. On the other hand**, Gyamfi and Aniya (1998)** [41] did not find any changes in hepatic MDA level in rats given AFB_1 that sacrificed after 48 h. This could be explained by the short duration of their experiment.

The increase in MDA level may be due to the fact that AFB_1 is metabolized by cellular CYP450 to form the reactive intermediate, AFB_1 -8,9-epoxide, which then reacts with macromolecules such as lipid leading to lipid peroxidation and cellular injury[42]. In addition, lipid peroxidation occurs as a result of the inability of the animals to compensate for the free radicals generated by AFB_1. Due to the high susceptibility of lipid membranes to peroxidation, the free radicals easily peroxidated the lipid membranes, and MDA is generated as a final product of peroxidation. MDA also causes peroxidation itself, and accelerates peroxidation by means of synergy with free radicals. Changes in tissue or blood MDA levels may point out the development and severity of peroxidation[43].

The cotreatment with either Se or vitamin C offered substantial hepatoprotective effects when administrated during and after AFB_1

injections. The two antioxidants were ineffective when given only after the end of AFB_1 administration. This may be explained by the fact that the AFB_1 binds with plasma proteins especially albumin to form AFB_1 -albumin adduct. As the half life of albumin is 20 days, so there will be accumulation of the xenobiotic [(44)].

Serum and hepatic GST activity showed significant increase in the AFB_1 treated groups (IIa, IIb, IIc) in comparison to control group. On the other hand, there was a concomitant significant decrease in serum and hepatic GSH in these groups compared with the control group.

The significant increase in serum and hepatic GST activity in the AFB_1 treated groups is in agreement with the studies of **Sotomayor et al. (2003)** [(45)], **Liebert et al. (2006)**[(46)] and **Kheir Eldin et al. (2008)**[(47)].

On the other hand, **Premalatha and Sachdanandam (2000)**[(48)] and **Rastogi et al. (2001)**[(49)] observed the reduction of GST activity in rats treated with AFB_1 and suggested that the GST activity was first stimulated by oxidative stress evoked by AFB_1 and then after several weeks its activity was reduced as a result of AFB_1 hepatotoxicity.

The significant decrease in levels of serum and hepatic GSH in the AFB_1 groups in the present study is in agreement with the finding of **Liebert et al., (2006)**[(46)] **Saleha et al. (2007)**[(50)] and **Kheir Eldin et al. (2008)**[(47)] who reported significant reduction in the levels of hepatic GSH and serum protein thiols in the AFB_1 treated group.

Contrary to these findings, **Gyamfi and Aniya (1998)** [(41)] observed a slight insignificant increase in the hepatic GSH level in rats administered I.P. AFB_1 sacrificed after 48 h. Similarly, **Abdel-Wahhab and Aly (2003)**[(51)] reported no significant changes in hepatic GSH levels in rats fed AFB_1 contaminated diet for 15 days.

The decrease of GSH level in serum and liver tissue homogenate may be attributed to the conjugation of AFB_1 active metabolite, AFB_1-8,9 epoxide, with GSH, a reaction that is catalyzed by the induced enzyme GST [(52,53)].

Selenium and vitamin C had protective effects on both serum and hepatic tissue GST enzyme activity and GSH level only in groups III and IV. Our results showed normalization of hepatic GSH. This is in agreement with the study of **Kheir Eldin et al. (2008)**[(47)], who showed that Se and vitamin E had the same effect.

The decrease of serum and hepatic GST activity in antioxidants treated groups during and after AFB_1 injection (groups III and IV) could be explained by decrease in oxidative stress through the antioxidant properties of Se and vitamin C.

To our knowledge this is the first study of the relation between serum and liver PON1 and AFB_1 hepatic intoxication in experimental rats. Therefore, the results of the present work are compared with those of the hepatotoxin, CCl_4 on PON1 activity.

The present study showed that serum and liver tissue PON1 activity were significantly decreased in AFB_1 injected rats (group IIa) and those having either Se or vitamin C after AFB1 injections (groups IIb and IIc, respectively) in comparison to control

group (group I). The two exogenous antioxidants, Se and vitamin C, administrated during and after AFB_1 treatment had a protective effect on PON1 activity, which was back to normal in groups III and IV.

The present study showed that both serum and liver PON1 behaved similarly. A common in-vitro identity of both enzymes is supported by biochemical studies showing properties shared by serum and hepatic PON1, optimum pH, affinity for substrate (Km), heat inactivation and calcium requirement [(21)]. The present results also, show that PON1 activity inversely correlated with MDA level in all studied groups. These results are in agreement with those of **Ferre et al. (2001)[(22)]** who found that PON1 activity decreased while lipid peroxidation increased in CCl_4 administered rats while the addition of zinc, which possesses antioxidant and antifibrogenic properties, was associated with enhanced PON1 activity and normalization of lipid peroxidation.

The decreased PON1 enzymatic activity in the intoxicated animals could be due to hepatic dysfunction. Supporting this hypothesis is the observation of an inhibition of microsomal PON1 activity in rats with chronically administered CCl_4 [(22)]. In a recent study, decreased PON1 activity in sera of patients with chronic liver disease was suggested to be related to the degree of liver damage [(54)].

Various in-vivo and in-vitro studies in animals and humans have provided evidence that antioxidants can increase PON1 activity, possibly by protecting the enzyme from the oxidative stress-induced inactivation. This supports the present finding that Se and vitamin C caused an increase of PON1 activity in the AFB_1 treated rats (groups III and IV) to normal control level (group I). Also, it has been showed in human study positive correlation was found between the dietary and medical intakes of vitamin C and PON1 activity [(55)].

In our work all the studied parameters behaved similarly in both serum and liver tissue in all the studied groups. Se and vitamin C, were only effective when given through out the experiment i.e during and 15 days after AFB_1 administration. They did not cause any significant effect on any of the studied parameters when given only after AFB_1 injection. This may be due to AFB_1 intoxication is caused by AFB_1 8,9 epoxide which form adducts with cellular molecules which are still present few weeks after no dosing of AFB_1 [(56)].

The two antioxidants were effective in combating lipoperoxidation of hepatocytes as manifested by decreasing the production of MDA. They also caused increase of the endogenous antioxidant GSH to normal level, as well as normalization of the activity of the inducible GST enzyme .They also, caused an increment of PON1 activity to nearly normal value.

In the present study, liver cell damage caused by AFB1 administration was confirmed by the significant elevation of the activity of serum AST and ALT enzymes (groups IIa, IIb and IIc) which may be due to leakage of these cytoplasmic enzymes from liver cells into the

blood stream as a result of damage of plasma membranes by lipoperoxidation [57]. Se and vitamin C during and after AFB1 treatment (groups III and IV, respectively) caused significant decrease of serum activities of transaminases. This is because these two antioxidants may have a protective effect on the hepatic cell membrane combating lipoperoxidation.

The serum total protein concentration in all treated groups (II,III,IV) did not show any significant change compared with control group. While serum albumin showed a significant decrease in the treated groups (IIa,IIb,IIc). Se and vitamin C caused increase of serum albumin when given only with and after AFB_1 injection (groups III and IV). The decrease of serum albumin may be explained by decrease of its synthesis by the liver[44] .The discrepancy of the results between total protein and albumin could be because there may be an increase of other serum proteins e.g globulins, that were not determined .

Histopathological study confirmed the biochemical findings in this study. Livers of AFB_1 treated rats showed marked degenerative changes, necrosis and disrupted architecture of hepatic lobules. Selenium or vitamin C-treated animals showed less histopathological abnormalities including hydropic degeneration, bile duct proliferation and peripheral fibrosis as compared with AFB_1 treated rats, thereby confirming their protective role against hepatotoxicity induced by AFB_1.

Conclusion and Recommendations:

All the above findings confirm the protective role of the two antioxidants studied on the hepatotoxicity caused by AFB_1.

Because of the protective role of antioxidants selenium and vitamin C against the hepatotoxicity of AFB1 in the present experimental work together with other published studies, it is highly recommended to eat well balanced diets that contain sufficient antioxidants selenium and vitamin C or take antioxidants supplementation daily as a way to counteract deleterious effects of this common hepatotoxin, AFB_1.

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التأثيرالمعاكس للسيلينيوم و فيتامين ج ضد تأثيرالأفلاتوكسين ب١ على كبد فئران التجارب

سلوى أخنوخ ابسخرون، هالة السيد حموده، أيمن عبد الفتاح وجيه، رانيا عبد اللطيف امام*مروى عبد العظيم
قسمى الكيمياء الحيوية الطبية *والباثولوجيا - كلية الطب- جامعة طنطا

مقدمة البحث:
الأفلاتوكسين هو مجموعة من السموم الفطرية التي يمكن أن تكون موجودة في عدد كبير من المواد الغذائية والأعلاف و تنتج من فطرالأسبيرجيلاس. الافلاتوكسين ب١ هو أكثر هذه السموم شيوعا و أهمية لما له من تأثير سام على الكبد كما أنه يتسبب فى احداث أورام الكبد السرطانية فى الانسان والحيوان.
يتعرض الأفلاتوكسين للتحول الكيميائى فى الكبد و تنتج مجموعة من الوسائط الضارة والتى ترتبط بمركبات هامة مثل الدهون ويؤدى ذلك الى تأكسدها و تدمير الخلايا الكبدية. كما تتحد هذه الوسائط مع الحمض النووى وتسبب طفرات جينية وأورام سرطانية. تمتلك الخلية مجموعة من المركبات البيولوجية التى لهاخصا ئص مضادة للاكسدة وتساهم في حماية الخلايا والأنسجة ضد الآثار الضارة للشقائق الحرة.
ان مضادات الأكسدة هى مجموعة من المركبات الطبيعية أو المخلقة وتقلل من تكون الشقائق الحرة كما تقلل من تفاعلها مع المركبات الهامة و يتم ذلك عن طريق مضادات الاكسدة الانزيمية واللا انزيمية.
ولذلك فان الهدف من البحث هي القاء الضوء على فاعلية مضادات الأكسدة مثل السيلينيوم و فيتامين ج ضد التاثير السام للأفلاتوكسين ب١ على الكبد.

خطة البحث:
أجريت الدراسة على أربع مجموعات من فئران التجارب:
١-**المجموعة الأولي**: مجموعة ضابطة و تشمل ١٠ فئران.
٢- **المجموعة الثانية** تم حقنها بالأفلاتوكسين ب١ ثم قسمت لثلاث مجموعات فرعية بعد نهاية الحقن :
٢أ-تشمل ١٥ فأرا أعطيت وجبات عادية بدون مضادات أكسدة.
٢ب-تشمل١٥فأرا أعطيت سيلينيوم عن طريق الفم بعد الحقن بالأفلاتوكسين ب١ .
٢ج-تشمل فأرا أعطيت فيتامين ج عن طريق الفم بعد الحقن بالأفلاتوكسين ب١.
٣-**المجموعة الثالثة**: تشمل ١٥ فأرا أعطيت سيلينيوم اثناء وبعد الحقن بالأفلاتوكسين ب١ .
٤-**المجموعة الرابعة**:تشمل ١٥ فأرا أعطيت فيتامين ج اثناء و بعد الحقن بالأفلاتوكسين ب١ .

وتم قياس مايلى لمجموعات الدراسة الأربع :-
*وظائف الكبد
*قياس مستوى ثنائى ألدهيد المالون فى مصل الدم و الكبد
*قياس مستوى الجلوتاثيون المختزل فى مصل الدم و الكبد .
*قياس نشاط الأنزيمات المضادة للأكسدة فى مصل الدم و الكبد :
الجلوتاثايون الناقل ، الباروكسوناز-١
وتم أخذ عينات من كبد كل مجموعات الدراسة للفحص الهستوباثولوجى.

وقد أسفرت الدراسة عن النتائج الآتية:
قد أكدت الدراسة الحاليه التأثير السام للأفلاتوكسين على الكبد و الذى تسبب فى زيادة نشاط انزيمات الكبد(ALT وAST) ، و نقص الالبومين ويرجع ذلك الى تدمير خلايا الكبد. وقد أكد ت نتائج الفحص الهستوباثولوجى للكبد هذا التأثير الضار للأفلاتوكسين
وجدت زيادة ذات دلالة إحصائية فى مستوى ثنائى الدهيد المالون فى مصل الدم والكبد لمجموعة الفئران المحقونة بالافلاتوكسين . ويعتبر ثنائى الدهيد المالون هو الناتج النهائى لأكسدة الدهون وهو العامل الأساسى فى احداث التأثير السام للأفلاتوكسين على الكبد.
وقد تم دراسه اثنان من مضادات الأكسدة الموجودة فى الجسم وهما نشاط انزيم الجلوتاثيون الناقل و مستوى الجلوتاثيون المختزل و قد وجد أنهما مختلفان. فيوجد زيادة ذات دلالة إحصائية فى نشاط انزيم الجلوتاثيون

الناقل فى مصل الدم والكبد لمجموعة الفئران المحقونة بالافلاتوكسين بينما يوجد نقص ذو دلالة إحصائية فى مستوى الجلوتاثيون المختزل فى مصل الدم والكبد لمجموعة الفئران المحقونة بالأفلاتوكسين . تم تفسير ذلك لارتباط الأفلاتوكسين ايبوكسيد بالجلوتاثيون المختزل عن طريق أنزيم الجلوتاثيون الناقل. وقد ظهر أيضا نقص ذا دلالة إحصائية فى نشاط إنزيم الباروكسوناز1 فى مصل الدم والكبد لمجموعة الفئران المحقونة بالافلاتوكسين و ذلك بسبب قلة تصنيعه فى الكبد.

وقد تم أعطاء بعض من مجموعات الفئران اثنان من مضادات الأكسدة و هما السيلينيوم وفيتامين ج ليضادوا التأثيرات الضارة للأفلاتوكسين على الكبد. و قد وجد أنهما ذا تأثير فعال و أيضا مماثل عندما أعطوا اثناء وبعد الحقن بالأفلاتوكسين . و كلاهما يتسبب فى تقليل نشاط انزيمات الكبد و زيادة نسبة الالبومين كما وجد نقص فى مستوى ثنائى الدهيد المالون فى مصل الدم والكبد و كذلك فى نشاط انزيم الجلوتاثيون الناقل. ووجدت زيادة فى مستوى الجلوتاثيون المختزل فى مصل الدم والكبد وكذلك فى نشاط انزيم البروكسوناز١.

وقد أكدت هذه النتائج التأثير الوقائى لمضادات الأكسدة المستخدمة فى الدراسة ضد التأثير السام للأفلاتوكسين على الكبد.

ولذلك يوصى بتناول الغذاء المتكامل والذى يحتوى على كميات كافية من السيلينيوم و فيتامين ج وذلك لمواجهة التاثيرات الضارة للأفلاتوكسين ب١ .