

Effects of Vitamin C and E on Erythrocyte Osmotic Fragility and Some Hematological Parameters in Experimental Aflatoxicosis of Rabbits

DENEYSSEL AFLATOKSİKÖZİS OLUŞTURULAN TAVŞANLARDA C VE E VİTAMİNLERİNİN ERİTROSİT OZMOTİK FRAJİLİTESİ VE BAZI HEMATOLOJİK PARAMETRELER ÜZERİNDEKİ ETKİLERİ

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Ozet

This study was carried out to investigate the effects of supplementation of antioxidant vitamins on the osmotic fragility of erythrocytes (EOF) and some hematological parameters in rabbits with experimental aflatoxicosis. In this study, 25 home-breed rabbits were used and divided into five groups, and each group was randomized five rabbits. Experimental groups were designed as control (Group I), aflatoxin-B1 (AFB1, Group II), AFB1+C (Group III), AFB1+E (Group IV) and AFB1+C+E (Group V). The values of osmotic fragility and some hematological parameters (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, LY M % and LY M) in the samples were determined before and after the application of AFB1, vitamins C and E. The EOF in AFB1 group was statistically increased ($P < 0.05$), but the values of minimal resistance of erythrocyte hemolysis in the groups of vitamin C, E and C+E-combination were decreased, respectively ($P < 0.05$, $P < 0.05$ and $P < 0.05$). In conclusion, it was determined that vitamin C and E noted to have different effects on some hematological parameters, and antioxidant vitamins might reduce the hemolytic effects of AFB1.

Key Words: Aflatoxicosis, Osmotic fragility, Vitamin E and C, hematological parameters, Rabbits

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Summary

Bu çalışma, deneysel aflatoksikozis oluşturulan tavşanlarda antioksidan vitaminlerin eritrosit ozmotik fragilitesi (EOF) ve bazı hematolojik parametreler üzerindeki etkilerini araştırmak amacı ile yapıldı. Araştırma, her grupta 5 olmak üzere toplam 25 adet yerli ırk tavşan üzerinde yürütüldü. Çalışma; kontrol, AFB1, AFB1+C, AFB1+E ve AFB1+C+E grupları şeklinde dizayn edildi. Tüm gruplarda deneme öncesi ve sonrası eritrosit ozmotik fragilitesi ve bazı hematolojik parametreler (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, LY M % ve LY M) belirlendi. AFB1 grubunda eritrosit ozmotik fragilitesinin (eritrosit hemolizinin) istatistiksel olarak arttığı ($P < 0.05$), ancak AFB1+C, AFB1+E ve AFB1+C+E gruplarında antioksidan vitaminlerin uygulamasından sonra eritrosit hemolizinin azaldığı (sırasıyla $P < 0.05$, $P < 0.01$ ve $P < 0.05$) belirlendi. Sonuç olarak, vitamin C ve E'nin bazı hematolojik parametreler üzerinde etkili olduğu ve AFB1'in eritrositler üzerindeki hemolitik etkisini azaltabileceği gözlemlendi.

Anahtar Kelimeler: Aflatoksikosis, Ozmotik fragilite, Vitamin E ve C, Hematolojik Parametre, Tavşan

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Anatoxins are group of mycotoxins which play an important role in high incidence of the liver diseases in some regions of the world (1,2). They are extremely potent metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. AFB1, like other mycotoxins, is one of the important mycotoxins. The risk of the liver cancer is high in individuals taking AFB1 in their diets. There are hepatocarcinogenic, mutagenic and tera-

togenic effects of Aflatoxin B1 (1-4). AFB1 is transformed to epoxides and other derivatives of aflatoxins (M1, PI and Q1) by microsomes of the hepatocytes (4-6).

Vitamins C and E are important antioxidants and can inactivate free radicals (FR). FR can cause the damage of lipids and lipoproteins in cellular membrane and in tissues (7-11).

Table 1. Values of EOF and % NaCl before and after the experiment.

	CONTROL		AFBI		AFBI+C		AFBI+E		AFBI+C+E	
	<i>Before ex</i>	<i>After ex</i>	<i>Before ex</i>	<i>After ex</i>	<i>Before ex</i>	<i>After ex</i>	<i>Before ex</i>	<i>After ex</i>	<i>Before ex</i>	<i>After ex</i>
NaCl (%0)	58.0±0.1	59.4± 0.4	59.21±0.2	59.41 ± 0.4	59.61 ± 0.7	59.21 ± 0.4	60.01 ± 0.0	59.21 ± 0.4	58.21 ± 1.4	59.01 ± 0.2
MIRE										
% Hem	13.3± 1.6	13.41±0.9	12.01 ± 1.6'	17.21 ± 1.8	14.51 ± 1.5''	11.61 ± 1.2	15.11 ± 1.8	11.11 ± 1.2''	13.11 ± 1.2	10.01 ± 1.2''
NaCl (%0)	43.21±0.8	42.81±0.6	40.81±0.5	45.21 ± 1.9''	41.61 ± 0.7	40.41 ± 0.4	41.01 ± 0.7	40.01±0.0	40.81 ± 0.5	40.61 ± 0.2
MARE										
% Hem	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

MIRE : Minimal resistance; MARE : Maximal resistance ; Hem:Haemolysis; a:P<0.05,b:P<0.01

The purpose of the study was to investigate the effects of vitamins C and E on EOF and some other hematological parameters in experimental aflatoxicosis of rabbits.

Material and Methods

This study was carried out on male and female rabbits (home breed). All rabbits (2-3 kg body wt) were housed individually in cages in a room with 12 hours light-dark cycles and were given ad-libitum water and food. They were fed with commercially prepared diet (pellet food), and green food. Rabbits were divided randomly into five equal groups (n=5/group) in the special cages. AFB₁ (Sigma Chemical Co) was dissolved in dimethyl sulfoxide (DMSO:Sigma) and then diluted with distilled water to the required concentration and supplemented in diet. Before the study, all rabbits were normal and healthy. Experimental animals were designed according to following groups.

Rabbits in Group I served as controls and they were received pellet-food and water. Rabbits in Group II were received the AFB₁(0.1 mg/kg diet) dissolved in sterile dimethyl sulfoxide during the study. Group III were given with AFB₁ (0.1 mg/kg diet) and vitamin C (100 mg/kg diet) administered in food. Group IV were received the AFB₁(in the same concentration) and vitamin E (100 mg/kg diet). Group V were also given both AFB₁(0.1 mg/kg diet) and vitamins C and E (100+100 mg/kg diet) respectively.

Blood samples were obtained into heparinized glass-tubes from the middle ear vein of all rabbits. Hematological parameters (WBC, RBC, HGB,

HCT, MCV, MCH, MCHC, PLT, LYM% and LYM counts) were counted in automatic cell-counters (CELL-DYN 3500 R, ABBOTT) in Research Hospital at Harran University. Osmotic fragility test of all the blood samples were determined by spectrophotometrically without waiting (12).

Statistical analyses of findings in all samples were performed on computer using IBM by statistical software program (Minitab). In addition, EOF-curve of normal healthy rabbits (control group) was shown in Figure 1.

Results

Minimal and maximal resistance of erythrocyte membranes of rabbits in different sampling periods were shown in Table 1 and the values of hematological parameters were presented in Table 2.

There is no statistical difference between the values of minimum resistance of erythrocyte as well as the values of maximum resistance before and after treatment in control group (Table 1). There was statistically significant (P<0.05) difference between the values of erythrocyte minimum resistance before and after treatment in AFB₁-group. There was also significantly difference (P<0.05) between the values of erythrocyte maximum resistance before and after treatment in the same group.

The minimum resistance of erythrocytes before experiment in groups III, IV and V was statistically higher than the minimum resistance of erythrocytes after experiment (P< 0.05, 0.01 and 0.05, respectively). According to these results, vitamins

Table 2. Hematological values before and after the experiment in groups.

	CONTROL		AFB1		AFB1+C		AFB1+E		AFB1+C+E	
	Before ex X+ Sd	After ex X+ Sd	Before ex X+ Sd	After ex X+ Sd	Before ex X+ Sd	After ex X+ Sd	Before ex X+ Sd	After ex X+ Sd	Before ex X+ Sd	After ex X+ Sd
WBC	7.6±1.3	6.8±0.4	7.9+1.1	6.7+0.9	6.2+2.2	11.3+1.2 c	8.7+1.1	14.8+2.6 c	6.3+1.1	10.8+0.4 d
RBC	5.7+0.7	5.3±0.3	5.8+0.5	5.9±0.2	5.4+0.4	6.5+0.1 b	5.6± 0.9	6.9+0.4 a	5.7+0.9	5.9+0.3
HGB	12.8±0.7	12.6+0.4	13.3±0.7	13.2+0.7	12.8±1.2	14.1+0.5 a	12.6+1.7	14.9±0.6 b	12.1+1.9	13.1 ±0.7
HCT	36.7+1.9	36.2±1.3	38.0+1.7	37.7±2.3	35.7+2.9	40.1±1.0 a	36.3±4.9	42.7+1.6	35.3±4.7	38.7+1.9
MCV	65.5±5.2	67.2+2.8	66.4+4.9	64.4+3.2	67.7±4.4	61.5+1.7 a	65.4+3.2	63.6±0.5	62.8+2.9	65.5±0.4
MCH	23.6±2.9	23.2±1.3	24.9+2.8	22.3+1.1	24.1±1.6	21.4±0.5 a	22.3+1.1	22.7+ 0.3	23.5±3.4	22.4+0.3
MCHC	35.7+1.6	35.5+1.6	35.6+1.4	35.1±0.9	35.4±0.4	34.8+0.5	34.7±0.4	35.5+0.5 a	35.1+2.5	34.4±0.3
PLT	38.2±7.0	42.2± 3.4	56.6+9.5	26.4±8.5 d	51.8+13.0	64.2+7.7	52.2+10.1	30.6+5.9 £	53.0±18.7	15.4+2.3 c
LYM %	65.2±6.1	65.6+6.8	66.1+6.9	65.1 ± 10.5	60.8+7.2	68.6+1.4	65.1 + 12.2	66.7±1.3	64.4+11.8	60.8±1.5
LYM	4.9+1.1	4.9+0.4	5.1+0.9	4.2+0.6 a	4.1+2.6	6.9+1.8	6.7+1.8	10.0+1.5 b	4.1 + 1.4	6.6+0.2 b

Statistical significance: a=0.05, b=0.01, c=0.005, d=0.001

C, E and vitamin C+E-combination decrease the effects of AFB1 on erythrocyte membrane.

Differences between controls and AFB1 groups were not statistically significant for WBC, RBC, HGB, HCT, MCV, MCH, MCHC, LYM %-counts except for the values of PLT and LYM. There are differences between the values of AFB1+vitamin C groups before and after experiment, except for MCHC, PLT, LYM % and LYM-values. There was significant difference in the beginning maximal resistant value of control and of AFB1 ($P < 0.01$), and the significance for both in the end was $P < 0.005$.

Discussion

Vitamins C and E are the essential antioxidants, responsible for the protection of the cellular lipids, susceptible to peroxidation (7-11). AFB1 is also one of the important mycotoxins, transformed to toxic epoxides, in hepatocytes. Toxic epoxides may bind to DNA, RNA, tRNA and some other polynucleotids, and so they can inhibit synthesis of the proteins and suppress the immune system. These agents can cause the formation of harmful oxidative effects on hemopoietic and reticuloendothelial system (4-6,13,14). In this way, AFB1 may play a role in the high incidence of diseases in the liver and other reticuloendothelial systems of human and animals. In addition to toxic effects, it may cause the haemolysis of the erythrocytes in animals and humans.

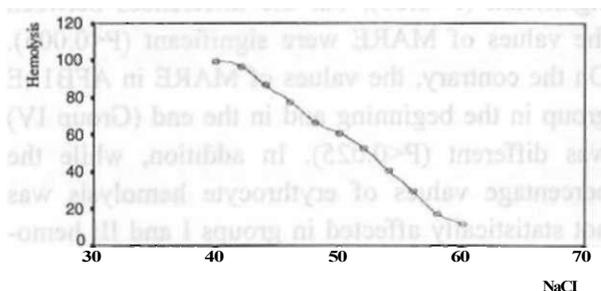


Figure 1. EOF curve of the control group.

The interrelationships between EOF, hematological parameters and some antioxidants ((β -carotene, vitamins C and E) have been investigated (3,7-9,14,15), and reported that zinc deficiency increased osmotic fragility of erythrocytes in rats (7). Minimum resistance of erythrocytes (MIRE) in groups III, IV and V were significantly increased, but in groups II and III, MIRE have not changed. While maximum resistance of erythrocytes (MARE) in groups II (AFB1) was decreased, other groups (I, III, IV and V) were not effected (Figure I). Our findings may be confirmed by the results of Kraus et. al. (7).

While the numbers of erythrocytes, leukocytes, HCT, HGB and neutrophil, basophil and eosinophil increased, the numbers of lymphocytes, monocytes decreased in experimental aflatoxicosis of the rats. In addition, the effect of aflatoxin on some hematological parameters due to animal spe-

cies and administration time of the AFB1 was reported (3). But, the effects on some hematological parameters of AFB1 has not been confirmed with results of some other studies (2). On the other hand, since this subject has been started to be studied in recent years, the interrelationships between protective effects of antioxidant vitamins and toxic effects of AFB1 are not clear yet (2,14,16,17). It was reported that changes in erythrocyte and leukocyte values were nonspecific for aflatoxicosis (2).

The differences between the values of MIRE and MARE in the beginning and in the end of the study in controls (Group I) are not statistically significant ($P>0.05$), but the differences between the values of MARE were significant ($P<0.005$). On the contrary, the values of MARE in AFB1+E group in the beginning and in the end (Group IV) was different ($P<0.025$). In addition, while the percentage values of erythrocyte hemolysis was not statistically affected in groups I and II; hemolysis of erythrocytes in groups III, IV and V were significantly decreased ($P<0.05$, 0.01 and 0.05, respectively). While the values of MARE and MIRE decreased in AFB1 groups, the values of MIRE in the groups of III, IV and V were statistically increased (Table 1).

In this study, our findings are in accordance with studies performed using antioxidants (2,7,9,16). These results can be attributed to the antioxidant functions of vitamins C and E. Indeed, these vitamins are known with their antioxidant ability to inhibit oxidative processes of lipids and lipoproteins in cell membranes. In addition, vitamin C may protect the membranes by regenerating vitamin E (7,17). Moreover, it has been shown that vitamin E modified the susceptibility to lipid peroxidation by influencing the microsomal environment (18).

The values of PLT in AFB1 group in the end of the study was statistically decreased ($P<0.001$), while other values of the hematological parameters (WBC, RBC, HGB, HCT, MCV, MCH, MCHC) were not changed. Findings in this study are agreement with the results of the some other au-

thors (2,3).

According to these results, WBC, PLT and lymphocyte counts were decreased in AFB1 group. It was determined that vitamins C, E and C+E combinations affected the counts of the hematological parameters. Therefore, these antioxidant vitamins may be administered for protective purpose in aflatoxicosis. And so, corrective treatment could be taken by receiving these essential substances. However, there is a need for further detailed studies in order to assess possible effects of antioxidant vitamins on aflatoxicosis.

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