Vitamin E (α tocopherol) attenuates toxicity and oxidative stress induced by aflatoxin in rats

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A — research concept and design; B — collection and/or assembly of data; C — data analysis and interpretation; D — writing the article; E — critical revision of the article; F — final approval of article

Abstract

Background. Aflatoxins are toxic metabolites produced by Aspergillus flavus and Aspergillus parasiticus and are classified as group I carcinogens by the International Agency for Research on Cancer (IARC).

Objectives. The purpose of this study was to investigate the possible preventive role of vitamin E (Vit E) on aflatoxin (AF) induced toxicity by using biochemical and histopathological approaches.

Material and methods. Wistar-Albino rats were divided into 4 groups as follows: control group, Vit E group (Vit E was administered), AFB 1 group (a single dose of AFB was administered), AF + Vit E group (AF and Vit E were administered). The effects of Vit E on AFB induced tissue toxicity were evaluated by using malondialdehyde (MDA), reduced glutathione (GSH) levels, antioxidant enzyme activities, and histopathological examination in tissues.

Results. AF caused the oxidative stress by the increased MDA level and the reduced GSH level, glutathione-S-transferase (GST), catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and glucose-6-phosphate dehydrogenase (G6PD) activities in tissues. Plasma aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) activities, creatinine, and urea concentrations significantly increased; whereas, chloride, phosphorus, and magnesium concentrations were insignificantly affected. Plasma glucose, protein and sodium concentrations significantly decreased. Administration of AF caused hepatotoxicity, cardiotoxicity, and nephrotoxicity. As far as histopathological changes are concerned, a statistically significant difference was found in AFB group compared to the control group. Vit E considerably reduced plasma AST, ALT, ALP, LDH activities, and urea concentration and ameliorated the deleterious effects of AF on oxidative stress markers and pathological changes.

Conclusions. This data indicated that the natural antioxidant Vit E might have a protective effect against AF-induced toxicity and oxidative stress.

Key words: oxidative stress, antioxidant, aflatoxin, vitamin E

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Aflatoxins (AF) are polysubstituted bifuranocoumarins that are secondary fungal metabolites produced by parasiticus/ flavus group of the genus Aspergillus. AF-contaminated feeds may cause many health problems among livestock. AF is a carcinogen when administered in repeated doses in many animal species. AFB1 is nephrotoxic, hepatotoxic, mutagenic, genotoxic, and immunotoxic.1–5

AFB1 mediated cell injury may be due to the release of free radicals and these radicals initiate lipid peroxidation (LPO) and a damaging process in biological systems since all cell membranes contain the polyunsaturated fatty acids which are substrates for such a reaction.6 Kodama et al. showed the formation of LPO by AFB1. Oxidative stress is thought to play an important role in AFB1 by increasing LPO and decreasing antioxidants in treated animals.7-9

Vitamin E (α-tocopherol) is a lipophilic alcohol and its food source is the root of wheat and vegetable oils. The most important part of this substance is the α-part because it constitutes 90% of the tocopherol composition of animal tissues. Many physiological functions including stabilization of membrane have been taken into consideration for this substance. This substance can absorb free radicals of oxygen and thus prevent the negative effects of LPO in the brain tissue.10–12

In the present study, the protective effect of Vit E was investigated by estimating malondialdehyde (MDA), reduced glutathione (GSH) levels and antioxidant enzymes such as glutathione-S-transferase (GST), catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and glucose-6-phosphate dehydrogenase (G6PD) activities in liver, kidney and heart of AFB1 treated rats.

**Experimental groups and sample collection**

Twenty-eight healthy male Wistar-Albino rats (300–350 g body weight) were used in this study. The protocol for the use of animals was approved by the National Institutes of Health and Committee on Animal Research. AFB1 (1.0 mL) was a single intraperitoneal (i.p.) dose administered to the animals at the dose of 2.5 mg/kg b.w. Vit E (1.0 mL) was suspended in corn oil and administered to the animals by gavage at the dose of 100 mg/kg/day for 20 days. The doses of AF and Vit E used in this study were selected according to previous studies in which Vit E was administered together with AF.13–16 The animals were randomly divided into 4 experimental groups including 7 rats in each. These groups were arranged as follows: control group, Vit E group (Vit E was administered), AFB1 group (a single dose of AFB1 was administered), AF + Vit E group (AF and Vit E were administered).

**Biochemical analysis**

Under ether anesthesia, blood samples were withdrawn by an injector from the heart of the animals and collected into tubes containing EDTA. At the end of the experiment, the rats in control and experimental groups were sacrificed by decapitation under ether anesthesia.

Plasma was used to measure MDA level as a marker of LPO and to determine aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) activities, glucose, protein, creatinine, urea, sodium, chloride, phosphorus, and magnesium concentrations. Tissue samples were quickly removed and perfused with ice-cold saline for biochemical and histopathological evaluation. The tissues were homogenized in distilled water by using a Potter-Elvehjem Homogenizer. The homogenate was centrifuged (at 3,500 rpm for 15 min to MDA, GSH, GST, CAT, SOD analyze and at 14,000 rpm for 55 min to GSH-Px and G6PD at +4°C).

MDA and GSH levels, GST, CAT, GSH-Px, SOD and G6PD activities were analyzed in liver, kidney and heart tissues. While MDA level was measured according to the method developed by Placer et al., the GSH level was determined by the method developed by Ellman et al.17,18 GST activity was measured by the method developed by Habig et al.19 CAT activity was carried out by using Aebi’s method.20 GSH-Px activity was measured by the Beutler method.21 The protein concentration determination was based on the method of Lowry et al.23 The plasma glucose, protein, creatinine, urea, sodium, chloride, phosphorus and magnesium were

**Material and methods**

**Chemicals**

AFB1 (5 mg, Code 11293) was purchased from Cayman Chemical Company (Michigan, USA), Vit E (α-tocopherol from vegetable oil, Code T3634), GSH, glutathione reductase, thiobarbituric acid, hydrogen peroxide, nicotinamide adenine dinucleotide phosphate (NADPH) and other reagents were supplied from Sigma (St. Louis, MO, USA).

**Preparation of AFB1 and Vit E**

AFB1 was dissolved in dimethyl sulfoxide (DMSO), diluted with distilled water, and then administered to the experimental animals.8 Vit E (α-tocopherol ampoule) was dissolved in corn oil and administered at the dose of 100 mg/kg.

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measured by using an AutoAnalyzer (Olympus AU 600, Tokyo, Japan).

**Histopathological examination**

Necropsy of the rats was performed and liver, kidney, and heart tissue samples were fixed at 10% neutral buffered formalin. Paraffin embedded blocks were routinely processed. 5-μm thick sections were stained with hematoxylin-eosin and examined under a microscope. Then random 10 microscopic fields were examined in ×40 magnification.

**Statistical analysis**

The results are expressed as mean ± standard error. Statistical significance between the different groups was determined by using a one-way analysis of variance (ANOVA) in the SPSS 21 software package. Post hoc test was performed for between-group comparisons by using the Tukey multiple comparison test. The level of significance was set at p < 0.001.

**Results**

**MDA and GSH levels**

Figures 1–3 show the tissue MDA levels, GSH levels and the activities of antioxidant enzymes such as GST, CAT, GSH-Px, SOD, and G6PD in the control and experimental groups. The data indicated that the AFB1 group had a significantly higher MDA level than the control group in all the tissues. The Vit E group had a significantly lower MDA level than the AFB1 group. Treatment with AFB1 and Vit E provided apparent normalization in MDA level compared to the AF group. GSH level significantly decreased in the AFB1 group compared to the control group. Upon supplementation of Vit E to the AFB1 group, a significant increase was observed in the GSH level compared to that of the AFB1 group. The GSH level was restored in the AFB1 and the Vit E group.

**GST, CAT, GSH-Px, SOD and G6PD enzyme activities**

A significant reduction was found in GST, CAT, GSH-Px, SOD and G6PD activities in the AFB1 group compared to that of the control group. Upon simultaneous supplementation of Vit E to the AFB1 group, significant increases were observed in antioxidant enzyme activities compared to the AFB1 group. With the addition of Vit E to AF, normal values were reached (p < 0.001, p < 0.05).

**Biochemical parameters**

Fig. 4 shows the biochemical parameters such as AST, ALT, ALP, LDH, glucose, protein, creatinine, urea, sodium, chloride, phosphorus and magnesium in the control and experimental groups. Plasma AST, ALT, ALP, LDH, creatinine, and urea concentrations were significantly higher after administration of AFB1 compared to the control group. Administration of AFB1 alone induced a significant decrease in plasma glucose, protein, sodium concentration, however; it did not induce a significant effect on plasma chloride, phosphorus, and magnesium concentrations. Plasma AST, ALT, ALP, LDH activities, glucose, protein and urea concentrations in AFB1 + Vit E group were lower than those determined in AFB1 group. Plasma creatinine concentration in AFB1 + Vit E group was lower than AFB1 group, but this value could not reach the control level. Administration of AFB1 alone did not cause a significant change in plasma chloride, phosphorus, and magnesium concentrations (p < 0.001, p < 0.05).

**Histopathological examination**

The histological changes in the tissues were assessed as defined in Fig. 5–7. Control and Vit E groups had a normal histological appearance in liver, kidney, and heart tissues. As histopathological changes, a statistically significant difference was found in the AFB1 group compared to the control group (p < 0.05).

While severe necrotic hepatocytes and hydropic degeneration were observed in liver tissues of the AFB1 group, it was determined that these changes reduced in AFB1 with Vit E group (Fig. 5). Administration of AFB1 caused severe tubular necrosis, tubular degeneration, and hyaline droplets in kidney tissues. However, administration of Vit E ameliorated severe tubular necrosis and degeneration, and hyaline droplets (Fig. 6). Administration of AFB1 caused a severe hemorrhage and degenerative changes in heart tissues. Rats treated with AFB1 + Vit E had lower hemorrhage and degenerative changes (Fig. 7).

**Discussion**

One of causes for AFB1 induced toxicity is the oxidative stress which caused improved reactive oxygen species (ROS) generation and oxidative DNA damage. Previous studies, using different animal species, indicated that AF induced changes in oxidative stress markers. This study was conducted to assess the effect of AF on oxidative stress signs in rat liver, heart and kidney tissues. According to AFB1 concentration, the organs were classed as follows: gonads, liver, kidney, spleen, bursa cloacalis, thymus, endocrine glands, muscles, lungs, and the brain. Petr et al. revealed that AFB1 was determined in...
Fig. 1. Effect of Vit E on oxidant-antioxidant status in liver tissue of AFB1 treated rats. MDA (nmol/g tissue), GSH (µmol/mL), GST (U/mg protein), CAT (k/mg protein) GSH-Px (U/g protein), SOD (U/mg protein), G6PD (U/g protein)
Fig. 2. Effect of Vit E on oxidant-antioxidant status in kidney tissue of AFB-treated rats. MDA (nmol/g tissue), GSH (µmol/mL), GST (U/mg protein), CAT (k/g protein), GSH-Px (U/g protein), SOD (U/mg protein), G6PD (U/g protein).
Fig. 3. Effect of Vit E on oxidant-antioxidant status in heart tissue of AFB1 treated rats. MDA (nmol/g tissue), GSH (µmol/ml), GST (U/mg protein), CAT (k/g protein) GSH-Px (U/mg protein), SOD (U/mg protein), G6PD (U/g protein)
the blood, kidney, liver, and testis from minutes to maximum 8–10 h after a single i.p. injection at 0.1 mg/kg AFB1.

The results showed that the administration of AFB1 produced a marked oxidative impact as evidenced by a significant increase in MDA in the liver, kidneys and heart of AF-treated rats. These alterations might have been triggered either by the direct effects of AFB1 or by the metabolites formed by AF and the free radicals, which were generated during the formation of these metabolites. This result is compatible with the results reported previously about rat tissues. The initiation of LPO by AFB1 is noted as one of the principal appearances of ROS-induced oxidative damage. The mechanism of free radical damage also includes ROS-induced peroxidation of polyunsaturated fatty acids in the cell membrane lipid bilayer which causes a chain reaction of LPO, thus damaging the cellular membrane, causing further oxidation of membrane lipids and proteins, and leading to DNA damage. It is revealed that AFB1, with the help of microsomal cytochrome p-450 mediated oxidation, is bio-transformed into AFB1–8,9-epoxide, which is a reactive intermediate and highly toxic.

The present study also showed that a significant increase in the oxidative stress was accompanied by a concomitant decrease in the enzyme activities involved in the disposal of superoxide anions and peroxides, namely CAT and SOD, as well as GSH levels and its related enzymes (GST, GSH-Px). A significant increase observed
in tissue MDA levels in AFB1 treated animals indicated that AF led to the generation of high level of free radicals, which could not be tolerated by the cellular antioxidant defense system. A significant decrease in these enzyme activities could be explained by their consumption during the conversion of free radicals into less harmful or harmless metabolites. The enzyme activities decreased by AFB1 can be attributed to the lower ability of the tissue, which cannot scavenge free radicals and prevent the action of LPO. Similar results were also reported in previous studies.8,36–38

GSH and GST are effective in providing protection from the harmful effects on the tissues of AFB1.39 GST catalyzes the conjugation of AFB1–8, 9-epoxide with GSH to form AFB1-epoxide-GSH conjugate, thereby decreasing the intracellular GSH content.40,41 The activity of GSH-Px, which is a constituent of GSH redox cycle decreased during AFB1 administration. The reduction in the GSH-Px activity by AFB1 may be due to a decrease in
the availability of GSH and also alterations in their protein structure by ROS.32,39,42,43

The decreased G6PD activity as GSH metabolizing enzymes in AFB1-treated animals occurs as a result of decreased supply of reduced NADPH for the conversion of oxidized glutathione to GSH. Under conditions of oxidative attack, the NADP+/NADPH ratio changes in favor of NADP+, indicating decreased G6PD activity.44 The present study also showed a similar result in this enzyme activity and administration of Vit E significantly improved the G6PD activities.

Supplementation of Vit E to AFB1 significantly reduced MDA level and eliminated the possibility of oxidative stress due to the administration of AFB1 to rats. The antioxidative function of Vit E is mainly due to its reaction to membrane phospholipid bilayers to break the chain reaction initiated by hydroxyl radicals.11,45 Vit E may inhibit free radical generation by direct scavenging of the free radicals and subsequent transformation of the antioxidant species into less toxic product.46,47 Vitamin E as a therapy for AF-treated rats can be effective to significantly increase GSH, CAT, GSH-Px and GST activities. Vit E increases the GSH levels, resulting in an increase in SOD activity, and thereby preventing the deleterious effect of superoxide radicals. The restoration of antioxidant enzyme activities and the GSH level of Vit E indicates that they play an important role in mitigating AFB1 induced oxidative stress and subsequent damage to the tissue.48 The results of the present study revealed that AF was potent hepatotoxic, nephrotoxic, and cardiotoxic and Vit E showed protection against AF-induced nephrotoxicity, hepatotoxicity, and cardiotoxicity.

The liver is considered the principal target organ for AF.1 The result of the study clearly indicated that the administration of AF caused a significantly higher level of plasma AST, ALT and ALP in rats. Increased activities of these enzymes are well known to be diagnostic indicators of hepatic injury. ALP is a membrane bound enzyme and its alteration is likely to affect the membrane permeability and produce a derangement in the transport of metabolite. The results of the present study indicated a significant improvement in these marker enzyme activities in plasma, which is compatible with the previous studies.49–52 In the present study, LDH levels increased significantly in AFB1-treated rats which indicated the presence of myocardial infarction, cardiac injury, and toxicity to cardiac tissue and was compatible with the previous studies suggesting that exposure to AFB1 causes heart defects.53–55 Increased levels of these enzymes might also be due to the leakage of enzymes from the kidney as a result of necrosis induced by AFB1. Administration of Vit E significantly reduced these enzyme levels. The morphological changes included severe necrotic hepatocytes and hydropic degeneration. The histopathological and biochemical actions may be due to its antioxidant effects. Histopathologically, the livers of AFB1 treated rats were observed to be extensive hepatocellular necrosis, fatty infiltration, and bile duct proliferation.56 Vayalil57 showed that the date fruit extract (Phoenix dactylifera L. Arecaceae) induced liver protection against aflatoxicosis occurred via decreased liver enzyme activity as well as decreased free radical propagation, and also by its lowering the pathological lesions resulting from AFB1.

Histopathologically, the liver and kidney treated with
AFTB1 and Vit E showed less morphological changes compared to the changes seen in AFTB1 alone, which was an indication of partial protection. The results support the presence of hepatoprotective and nephroprotective roles of Vit E and this might be due to the membrane stabilizing and antioxidant activity of Vit E.

A significant decrease was found in plasma glucose and protein levels in AF-treated rats. This result is supported by the results of Abdulmajeed.58-59 who indicated that aflatoxicosis affects the cellular energy supply of rat hearts by causing its inhibitory effects on some markers of the energy metabolism due to a decrease in glucose and glycogen contents of the heart tissue and a reduction in the activities of some glycolytic enzymes such as phosphoglucoisomer and glyceraldehyde-3-phosphate dehydrogenase.

Decreased biosynthesis and secretion of proteins might be due to formation of AF adducts with DNA, RNA and protein. AFs were previously shown to lower the total protein concentration in serum of rabbits and broilers.58,59 Several studies have reported that the intoxication of AFTB1 causes nephrotoxicity.60 Administration of AF caused a significantly higher concentration of plasma creatinine and urea. This result is compatible with the previously mentioned microscopic changes that were evident in AFB1 + Vit E group in the present study.

In conclusion, results of the present suggest that AF has a harmful and stressful effect on hepatic, renal and cardiac tissue. Treatment of Vit E may alleviate AF toxicity by the reduction of oxidative damage of AF in liver, renal, and cardiac tissues and alterations of cardiac energy metabolism. For this reason, Vit E can be regarded as a good therapeutic agent against aflatoxicosis.

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