The Protective Effect of Lycopene on Toxicity of Aflatoxin B1 in Rats

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Abstract
It is aimed to determine the oxidative damage which may be caused by aflatoxin (AF) B1 and the inhibitory effect of lycopene against this damage. Groups were formed as; control group, lycopene-treated group, low dose AFB1-treated group and high dose AFB1-treated group, low dose AFB1+lycopene-treated group and high dose AFB1+lycopene-treated group. Malondialdehyde, glutathione levels and antioxidant enzyme activities were measured in blood samples. It was observed a significant increase in malondialdehyde level, decrease in glutathione level and antioxidant enzyme activities in low and high dose AFB1-treated groups when compared with control group. It was determined a significant decrease in malondialdehyde level and an increase in glutathione level, antioxidant enzyme activities in low and high dose AFB1+lycopene-treated group when compared with low and high dose AFB1 group. In conclusion, it has been shown that lycopene which has antioxidant properties can be prevented from AFB1-induced toxicity.

Keywords: Aflatoxin, antioxidant enzyme, glutathione, lycopene, malondialdehyde

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1. INTRODUCTION

Aflatoxin (AF)s have high toxicity in humans and animals and are the most dangerous mycotoxins known. AFs are contaminants of improperly stored foods. Not only contaminates food, but can also be found in edible tissues, milk and eggs after contaminated feed consumption by farm animals (Verma, 2004; Yilmaz et al., 2017a, 2018). Food and feed consumption contaminated with AF is known to cause hepatotoxicity, teratogenicity, immunotoxicity, and even death in farm animals and humans. The most common and most toxic one among AFs is AFB1 (IARC, 1993; Mathuria and Verma, 2007). AFB1 was included in Group 1: Carcinogenic to humans by the International Agency for Research on Cancer in 1993. The cellular damage mechanism caused by AFB1 is not fully clarified (Mathuria and Verma, 2007; Cui et al., 2017). Marvan et al. (1988) have reported that AFB1 showed different distributions in gonads, liver, kidney, spleen, bursa fabricius, thymus, endocrine glands, lung, blood, and brain. Geyikoglu and Tuerkez (2005) suggested that blood is a valuable tissue for monitoring the genotoxicity of AFB1. Blood cells are potentially defenseless cells. Being affected by the negative effects of environmental agents is from blood towards other tissues. Therefore, blood tissue is particularly important to assess the risks in the target tissue. In this context, oxidative and genetic studies in blood tissue are very important and such studies are evaluating AFB1 toxicity (Mahmood et al., 2017).

Orally taken AFB1 is absorbed from the small intestine and is metabolized in the liver into reactive intermediate products such as AFB1-8,9-epoxide, which causes lipid peroxidation and cellular damage due to the effect of the cellular cytochrome P-450 enzyme system and the aril hydrocarbon hydroxylase enzyme (Mathuria and Verma, 2007; Yilmaz et al., 2017b; Deng et al., 2018). The AFs show their effects in particular by binding to DNA, RNA and protein formations. AFB1-8,9-epoxide, an active metabolite of AFB1, has been reported to cause cellular damage, lipid peroxidation, depletion of antioxidants, oxidative damage to DNA and also be responsible for tumor initiation (Verma, 2004; Gradelet et al., 1998; El-Bahr, 2015).

Reactive oxygen species (ROS) and lipid peroxidation have been accepted as the main mechanisms in the toxicity of AFB1. Non-enzymatic lipid peroxidation in AFB1-related toxicity occurs at a high level, resulting in increased cell membrane sensitivity, loss of the membrane-related enzyme activity and the loss of membrane integration which comprises cell lysis. Lipid peroxidation has shown a significant increase in liver, kidney, and testicle of AF-exposed mice (Halliwell, 1994; Shen et al., 1995). Oxidative damage in cells and tissues caused by AFB1-8,9 epoxide in humans and animals may cause a significant decrease in non-enzymatic antioxidant levels such as reduced glutathione (GSH) that is functionary in the antioxidant defense mechanism of the cell, and in enzymatic antioxidant activities such as catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and glucose-6-phosphate dehydrogenase (G6PD) (El-Bahr, 2015). Nakae et al. (1987) have reported that enzymatic antioxidant systems such as SOD and CAT, which neutralize oxygen radicals, prevent the lethal effect of AFB1 on liver cells.

Lycopene is one of the most important non-enzymatic antioxidant agents that are not synthesized in the human body. Since, lycopene has long chain structure and contains conjugated double bond, it shows antioxidant activity. Lycopene is a superoxide radical (O_2^•-) cleaner in biological membranes (Roa et al., 2006; Yilmaz et al., 2006; Shahi and Ahmadian, 2018). Lycopene is protective against oxidation of DNA, protein, and lipids. Clinical studies have shown that lycopene has antioxidant and free radical scavenging capacity, and tomato consumption prevents oxidative DNA damage in human leukocytes. Besides antioxidant properties of lycopene, it has been determined
to play an important role in biological processes such as signal conduction of anticarcinogen, growth factors, some hormones and cytokine, cellular communication, strengthening the bonds between cells and improving cell metabolism (Yaping et al., 2002; Roa et al., 2006; Yilmaz et al., 2006; Shahi and Ahmadian, 2018). Some researchers have reported that lycopene may be effective in preventing and slowing the growth of prostate, lung and stomach cancer (Giovannucci, 1999; Roa et al., 2006; Shahi and Ahmadian, 2018).

Therefore, to reveal the possible effects of lycopene on the toxicity caused by AFB1 in rats was aimed in this study.

2. MATERIAL and METHODS

2.1. Experimental design and biochemical analyses

A total of 3-months old 42 Wistar Albino male rats (weighing 250-300 g) were used in the study. The ethics committee approval (2015/18-176) was obtained from the Local Ethics Committee of the Animal Experiments of Firat University and experimental animals were obtained from the Experimental Research Center of Firat University. The experimental applications were conducted conveniently with the conditions for the care and use of laboratory animals (12 hours light; 12 hours darkness; 24±3°C). During the experimental applications, the rats were provided the commercial rat feed (pellet feed) and tap water ad libitum. AFB1 was dissolved in dimethylsulfoxide (DMSO)/phosphate buffer (KH2PO4/Na2HPO4, pH: 7.2), in 10% lycopene corn oil, in 1:1 ratio. AFB1 has been administered orally at a dose of 0.5 mg/kg/day for 7 days and 1.5 mg/kg/day for 3 days. Lycopene was administered orally at a dose of 5 mg/kg/day. Lycopene application was started with AFB1 application and continued for a total of 15 days.

Groups are; Group 1: Control group (rats were given no treatment) (n: 7), Group 2: Lycopene administered group (5 mg/kg/day, orally, 15 days) (n: 7), Group 3: Low dose of AFB1 administered group (0.5 mg/kg/day, orally, 7 days) (n: 7), Group 4: High dose of AFB1 administered group (1.5 mg/kg/day, orally, 3 days) (n: 7), Group 5: Low-dose of AFB1 (0.5 mg/kg/day, orally, 7 days)+lycopene (5 mg/kg/day, orally, 15 days) (n: 7) and Group 6: High-dose of AFB1 (1.5 mg/kg/day, orally, 3 days)+lycopene (5 mg/kg/day, orally, 15 days) (n: 7), respectively.

At the end of the administrations, rats were sacrificed and blood samples were taken into tubes containing anticoagulant (Ethylenediaminetetraacetic acid-EDTA). They have been centrifuged for 10 minutes at +4 °C to separate their plasmas. Plasma malondialdehyde (MDA) and whole blood were used for GSH and GSH-Px determination. After the blood samples with EDTA from which their plasma was separated were washed 3 times with saline (0.9% NaCl), CAT, SOD and G6PD activities and hemoglobin (Hb) levels were determined in erythrocytes.

MDA levels in plasma samples were determined spectrophotometrically according to the method modified by Placer et al. (1966). This method is based on the reaction of MDA which is one of the aldehyde products of lipid peroxidation with thiobarbituric acid (TBA). GSH was determined by the method reported by Ellman et al. (1961). This is a spectrophotometric method based on the formation of a highly stable yellow color by sulphhydryl groups when 5,5’ dithiobis-2-nitrobenzoic acid (DTNB) is added. Aebi (1984) method was used to measure CAT activity. CAT catalyzes the breakdown of hydrogen peroxide (H2O2). The breakdown rate of H2O2 by CAT was measured spectrophotometrically by using the absorption of light by H2O2 at a wavelength of 240 nm. GSH-Px activity was determined by Beutler (1984) and SOD activity by Sun et al. (1988). Beutler (1984) method was used to measure G6PD activity.
2.2. Statistical analyses

In all statistical analyses, statistical significance between different groups was determined by using SPSS 22 software package. Shapiro-Wilk normality test was applied to determine whether the raw values of all the measured parameters showed normal distribution and as a result of the test, it was determined that the values in all parameters showed normal distribution. Based on the result of this test, one-way analysis of variance (ANOVA) was used to determine differences between groups, and post hoc Tukey test for paired comparisons. All values were calculated as mean ± standard error of the mean (mean ± SEM). The data obtained as a result of the study are expressed as mean and standard error. Statistically, $P<0.05$ and $P<0.001$ values were accepted as significant.

3. RESULTS

A significant increase in plasma MDA levels of rats to which low and high doses of AFB1 were administered was determined compared to the control group ($P<0.001$). In the groups to which low and high doses of AFB1 administered simultaneously with lycopene, it was seen that MDA levels returned normal and did not show difference according to the control group ($P<0.001$) (Figure 1). It was determined that GSH levels were decreased in the group with low and high dose AFB1 administration compared to the control group ($P<0.001$). Blood GSH levels were found to be increased in the groups to which low and high doses of AFB1 administered simultaneously with lycopene compared to low and high doses of AFB1 administered groups ($P<0.001$). When the lycopene group and the groups to which low and high doses of AFB1 administered simultaneously with lycopene were compared to the control group, there was no statistically significant difference in GSH level (Figure 1).

![Figure 1](image1.png)

**Figure 1.** Effect of lycopene on plasma MDA and GSH levels in AFB1 treated rats (Group 1: Control, Group 2: Lycopene, Group 3: Low dose AFB1, Group 4: High dose AFB1, Group 5: Low dose AFB1+Lycopene, Group 6: High dose AFB1+Lycopene ($p<0.001$))

Erythrocyte CAT, GSH-Px, SOD and G6PD activities were determined to be decreased in low and high dose of AFB1 administered groups compared to the control group and to be increased in the groups to which low and high doses of AFB1 administered simultaneously with lycopene ($P<0.05$ and $P<0.001$). When the lycopene group and the groups to which low and high doses of AFB1 administered simultaneously with lycopene were compared to the control group, there was no statistically significant difference in CAT, GSH-Px, SOD and G6PD activities ($P<0.05$ and $P<0.001$) (Figure 2).
Figure 2. Effect of lycopene on erythrocyte CAT, GSH-Px, SOD, and G6PD activities in AFB1 treated rats (Group 1: Control, Group 2: Lycopene, Group 3: Low dose AFB1, Group 4: High dose AFB1, Group 5: Low dose AFB1+Lycopene, Group 6: High dose AFB1+Lycopene (p<0.001)

4. DISCUSSION

AFB1 which is taken with nutrients and absorbed efficiently from duodenum is metabolized in the liver by conversion to AFB1-8,9-epoxide which causes lipid peroxidation and cellular damage by means of the cytochrome-P450 enzyme system (Deng et al., 2018). AFB1 has mutagenic, hepatotoxic and hepatocarcinogenic effects and causes oxidative stress (IARC, 1993; Gradelet et al., 1998; Verma, 2004). When, Petr et al. (1995) administered the hamsters 0.1 mg/kg AFB1, they have encountered free AFB1 in the blood, liver, testicles, and kidney after 8 minutes to 10 hours of intra-peritoneal injection.

Free radicals adversely affect metabolism when they occur in a way that exceeds the protective effect of the defense system. The increase in MDA level is an important symptom of oxidative stress. MDA level is determined as an index of lipid peroxidation and lipid peroxidation is one of the main symptoms of toxicity and carcinogenesis of many carcinogens (Yaping et al., 2002; Verma and Mathuria, 2010; Cui et al., 2017). In studies conducted on mice and other animal species, AF has been shown to cause changes in markers of oxidative stress in biological materials (Fabbri et al., 1983; Shen et al., 1995; Lee et al., 2005; Yilmaz et al., 2017b). In a rat liver study, it was determined that lipid peroxidation increased and therefore MDA level was high, in 1 mg/kg dose of AFB1-administered rats (Shen et al., 1995; Verma and Mathuria, 2010). AFB1 has been reported to demonstrate its toxicity through intracellular ROS formation, such as O$_2^-$, hydroxyl radical (‘OH) and H$_2$O$_2$, during the AFB1-8,9-epoxide formation and the metabolic processing of AFB1 with cytochrome-P450 which is in the liver (Mathuria and Verma, 2007; Deng et al., 2018). Shen et al. (1995) have stated that ROS have a role in AFB1-induced cell damage. AFB1 causes mutations in the structure of DNA by reacting readily with DNA, proteins, and lipids, structure changes in proteins by binding to the structure of proteins, and thus the release of the ‘OH radical, which causes protein oxidation and lipid peroxidation (Fabbri et al., 1983; Shen et al., 1995; Verma, 2004; Verma and Mathuria, 2010). In the study, low and high dose AFB1 administration increased plasma MDA level which could result in free radical mediated toxicity. The reason why AFB1 increases the level of
MDA has been attributed to the increase in ROS and to the fact that this also increases the lipid peroxidation (Lee et al., 2005).

Antioxidant enzymes can be induced or inhibited in blood cells exposed to different toxic substances, and these enzymes play a key role in the defense of mammalian blood (Halliwell, 1994; El-Bahr, 2015). Souza et al. (1999) have reported that oxidative stress, which can be relieved by antioxidants, is the cause of AFB1-induced toxicity.

GSH plays an important role in the detoxification of AFB1. GSH prevents peroxidation, while provides elimination of free radicals and protection of cell membranes. Low GSH levels may exacerbate the toxic effects of AFB1. GSH can be easily combined with AFB1 and is also an important component of red blood cells that play a central role in the antioxidant defense of cells (Farombi et al., 2005; El-Bahr, 2015). In this study, a decrease in GSH levels in the blood of low and high dose AFB1 administered rats, was attributed to the usage of the epoxide derivatives of AFB1 in the liver (such as AFB1-8,9-epoxide) during the formation of GSH conjugate and to facilitate the elimination from the body, and to the reaction which was catalyzed with glutathione-S-transferase. However, the significant reduction in GSH levels observed in erythrocytes after AFB1 exposure results in increased lipid peroxides and thus increases the toxicity of AFB1. GSH plays a critical role in protecting tissues from the harmful effects of active AFB1, resulting in significant reductions in GSH-Px activity of GSH consumption. The decrease in GSH-Px activity on AFB1 administration may be due to a decrease in the presence of the substrate (GSH), as well as changes in ROS-induced protein structure (Larsson et al., 1994; Farombi et al., 2005). In the study, lycopene supplementation increases blood antioxidant activities and thus prevents the harmful effects of peroxidation products.

SOD protects cells from oxidative damage by reducing the damaging free radical $O_2^•$ to $H_2O_2$ and $O_2$. Consisted $H_2O_2$ is not only fragmentized by CAT and GSH-Px enzymes but also may interact by lipid peroxidation at the same time (Johnson and Giulivi, 2005). Antioxidant enzymes such as CAT and GSH-Px are the first line of defense against their ROS and a decrease in their enzyme activity was observed by AFB1 administration. The first of the reasons why oxidative stress caused by AFB1 decreases CAT and GSH-Px activities, may be the fact that their AFB1-induced ROS have an inhibitory effect on enzyme activity. The second reason may be the fact that their ROS bind to proteins, causing structural changes in proteins and, consequently, causing oxidation of proteins (Mathuria and Verma, 2007; Verma and Mathuria, 2010; Cui et al., 2017). GSH-Px, in the presence of GSH, removes $H_2O_2$ and lipid peroxides by catalyzing the conversion of lipid peroxide to hydroxy acids. In this reaction catalyzed by GSH-Px, it is clear that GSH is essential for enzyme activity. Reduction of GSH-Px activity due to AFB1 may be due to changes in protein structure by ROS as well as reduced availability of substrate (GSH). It can also be attributed to its efficient use with GSH for detoxification of $H_2O_2$. As a matter of fact, various researchers have reported a decrease in GSH-Px activity in parallel with GSH in aflatoxicosis, similar to the findings in this study (Farombi et al., 2005; Mathuria and Verma, 2007; Verma and Mathuria, 2010). Karabacak et al. (2015) have found changes in MDA, SOD, CAT, and GSH-Px activities in blood, liver, kidney, brain, spleen tissues by administrating 400 mg/kg/day dose of AF to rats. They have reported that these changes may be directly related to the fact that AF causes production of free radicals and may be due to the consumption of antioxidants during the conversion of free radicals into less harmful or harmless metabolites.

Many chemicals have been used to prevent the harmful effects of AF and successful results have been obtained from some applications. However, the number of commercially used ones among
these is extremely limited (Neeff et al., 2018). Many conducted studies have proved that carotenoids, especially lycopene, inhibit low-density lipoprotein oxidation and lipid peroxidation. It has also been reported that consumption of diets lacking in terms of carotenoid causes a significant increase in MDA levels (Yilmaz et al., 2006; Shahi and Ahmadian, 2018). Lycopene has an important role in the prevention of oxidative stress locally in cell membranes, in membrane lipids. It has been reported to provide its effect by affecting the thickness durability and fluidity. Antioxidant activities of lycopene are associated with its task of clearing free radicals such as O$_2^-$ and ‘OH (Yaping et al., 2002; Yilmaz et al., 2006; Shahi and Ahmadian, 2018). Several experimental studies have been conducted on the role of lycopene in cancer prevention or treatment. Tang et al. (2007) in their study have shown that lycopene is effective in inhibiting tumor formation and growth caused by chemical carcinogens in animals. Lycopene intake has been shown to significantly suppress the growth of brain and breast tumors (Rao and Rao, 2018). Taş et al. (2010) have shown that lycopene reduces the damage of AFB1 in epididymal epithelial cells and has protective and therapeutic effects on oxidative stress caused by AF. However, Gradelet et al. (1998) have reported that lycopene treatment (300 mg/kg) via diet had no effect on the formation of AFB1-induced liver foci in rats.

It was determined that antioxidant activity decreased in AFB1-administered rats, and when AFB1 was administered with lycopene which has antioxidant effect, MDA, GSH levels and antioxidant enzyme activities approached control group in general. Improvement in blood MDA concentration and antioxidant activity suggests that lycopene plays a role in reducing the AFB1-induced oxidative stress and its damage. This can be explained by the fact that lycopene has the ability to prevent oxidative stress and free radicals by limiting free radical production and increasing the antioxidant defense system (Yilmaz et al., 2017a). The increase in antioxidant enzyme activity after lycopene application shows the protective and antioxidant role of lycopene against AFB1 toxicity. This is due to the inhibitory effect of lycopene on MDA and its stimulatory effect on GSH, CAT, GSH-Px, SOD, G6PD. These results suggest that lycopene may reduce AF toxicity.

As a result, the present study showed that lycopene has a potential protective effect against AFB1-induced toxicity through various mechanisms in different cellular processes.

Conflict of interest: The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES


