Chapter 4

The Effect on Oxidative Stress of Aflatoxin and Protective Effect of Lycopene on Aflatoxin Damage

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Additional information is available at the end of the chapter

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Abstract

Aflatoxin (AF) is polysubstituted bifuranocoumarins that are secondary fungal metabolites produced by *parasiticus/flavus* group of the genus *Aspergillus*. AF is hepatotoxic, nephrotoxic, mutagenic, teratogenic, genotoxic, and immunotoxic, so the International Agency for Research on Cancer has classified AF as class I human carcinogen. AF-mediated cell injury may be associated with the release of free radicals, and these radicals initiate lipid peroxidation and a damaging process in biological systems since all cell membranes contain the polyunsaturated fatty acids (PUFAs), which are substrates for such a reaction. One of the causes for AF-induced toxicity is the oxidative stress, which leads to the improved generation of reactive oxygen species (ROS) and the oxidative DNA damage. Lycopene, a naturally occurring carotenoid, has drawn a particular attention in recent years because of its high antioxidant activity and free radical scavenging capacity and has been shown to be effective against oxidative stress due to AF. Lycopene blocks Phase 1 metabolic enzymes of AFB such as 3A4, 2A6, and 1A2.

Keywords: aflatoxin, oxidative stress, reactive oxygen species, antioxidant, lycopene

1. Introduction

Mycotoxins are toxic products generated by fungi that are present spontaneously in foodstuffs. Mycotoxins may be generated in foodstuffs at different stages from production to transfer and preservation processes. Chemical stability and persistence of mycotoxins make them long-lasting, and even after elimination of fungi, mycotoxins may exist in foodstuffs [1].

Aflatoxin (AF) is the most abundant type of mycotoxins found in foodstuffs. Chemists isolated AF from *Aspergillus flavus* and named it as AF by taking "a" from *Aspergillus* and "fla" from *flavus*. There are at least 20 intermediates of AFs generated from *Aspergillus* species [1, 2]. AFs are found in the chemical construction of the furanocoumarins, and they possess two prominent structures: one of them is difurocoumarocyclopentenone (AFB1, AFB2, AFB2A, AFM1, AFM2, AFM2A, and aflatoxicol), and the other one is difurocoumarolactone (AFG1, AFG2, AFG2A, AFGM1, AFGM2, AFGM2A, and AFB3). AFs are named as AFB or AFG referring to the blue "B" or green "G" fluorescent color emitted by them under UV light on thin-layer chromatography, while the subscript numbers 1 and 2, respectively, show major and minor compounds. Moreover, AFB1 and AFB2 metabolites that show up in body fluids are named as AFM1 and AFM2 (**Figure 1**) [3, 4].

AFs commonly contaminate cereals and cereal-based foods such as rice, maize, sorghum, millet, groundnuts, dried cassava, and many others during the storage and poor processing conditions. AFs not only contaminate foodstuff but are also found in edible tissues, milk, and eggs after consumption of contaminated feed by farm animals [1, 6]. Trout, rats, ducklings, cattle, poultry, and swine are some of the many animals that have been shown to be sensitive to AF [6]. According to AFB1 concentration, the organs may be classed as follows: gonads, liver, kidney, spleen, bursa cloacalis, thymus, endocrine glands, muscles, lungs, and brain [7]. Petr et al. [8] revealed that AFB1 was determined in the blood, kidney, liver, and testis to maximum 8–10 h after a single intraperitoneal (i.p.) injection at 0.1 mg/kg AFB1.

AFs are a group of naturally occurring food-borne poisons that have been associated with death and disease in humans and animals. They are of great worldwide concern due to their toxic effects on human and animal health [9]. Among all AFs, AFB1 is the most toxic, mutagenic, and carcinogenic to both humans and livestock and is classified into group I as human carcinogen by the International Agency for Research on Cancer [10]. The extent of the carcinogenicity of AF depends on the presence of human health factors including hepatitis B virus infection, nutritional status, sex, and age as well as the amount of AF exposure [11, 12]. In transgenic mice, it was shown that overexpression of the hepatitis B virus large peptide

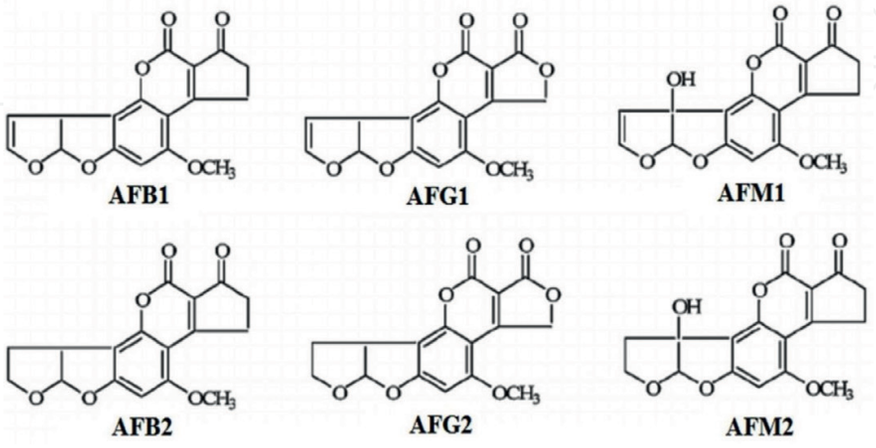

Figure 1. Chemical structure of AFs (Adapted from Marin and Taranu [5]).

envelope acted synergistically with AFB1 to have an effect on neoplastic development and other forms of chronic liver damage [13].

The immunotoxic potential of AF is known in many species, including laboratory and domestic animals [14]. In pigs, AF causes the decreases in blastogenic responses to mitogen, the reductions in complement titers, the decreases in macrophage activation, and the depression of delayed hypersensitivity responses [15]. Poultry is known to be extremely sensitive to the toxic effects of AFB1. Consumption of AFB1-contaminated feed causes a myriad of other effects either directly or indirectly associated with this toxicity: reduced feed utilization and efficiency, reduced growth rate, decreased body and organ weights [16], lowered egg production and reproductivity [17], immunosuppression [18], and increased susceptibility to disease [19].

2. Biotransformation (metabolism) of aflatoxins

AFs undergo biotransformation mainly in the liver. There are two types of biotransformations: Phase 1 and Phase 2. Phase 1 reactions are generally oxidative, reductive, or hydrolytic processes and provide a necessary chemical structure for Phase 2 reactions, which are generally conjugation reactions. Phase 1 reactions may result in activation as well as detoxification of a compound, whereas Phase 2 reactions, depending on conjugated cellular constituents, may lead either to detoxification or formation of biochemical lesions. Phase 1 is mostly mediated by the cytochrome P450 (CYP450) enzyme systems. Phase 2 metabolism involves sulfate, glucuronide, glutathione (GSH), and amino acid conjugation reactions (**Figure 2**) [20].

2.1. Phase 1: Metabolism of aflatoxins

AB1 is oxidized by CYP450 subfamilies and specific isoforms of enzymes to several products. Only one of these, AFB1 epoxide, appears to be mutagenic, and others are detoxification products. The putative AFB1 epoxide is generally accepted as the active electrophilic form of AFB1, which may attack nucleophilic nitrogen, oxygen, and sulfur heteroatoms in cellular constituents [22]. The CYP450-mediated oxidation to the extremely reactive AFB1-8,9-epoxide is considered the primary (Phase 1) bioactivation pathway for AFB1 [23]. This conversion of AFB1, to the epoxide, is the phase of reaction that enables covalent binding to cellular macromolecules (e.g., DNA and/or protein) to occur. This reaction can involve a number of isozymes of CYP450 including 1A2 and 3A4 [24]. The AFB1-8,9-epoxide reacts with the N7 atom of guanine to form a pro-mutagenic DNA adduct (AF-N7-guanine). The DNA adducts are fairly resistant to DNA repair processes, and this causes gene mutation and thus the development of cancers especially the hepatocellular carcinomas (**Figure 2**) [21, 25].

CYP450 3A4, which can both activate and detoxicate AFB1, is found in the liver and small intestine. In the small intestine, the first contact after oral exposure, epoxidation, would not lead to liver cancer. CYP450 3A4 has been shown to play a major role in the activation of AFB1 due to its intrinsic activity toward this substrate, and the high level of this enzyme is present

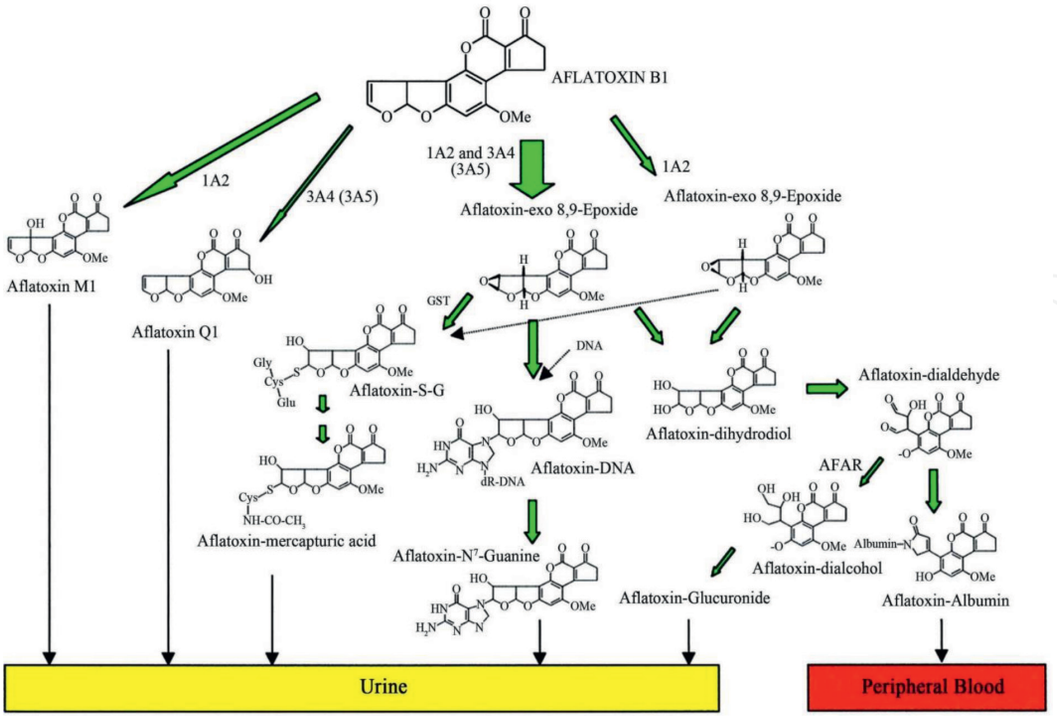

Figure 2. Metabolism of AF in the liver. 1A2, CYP1A2; 3A4, CYP3A4; 3A5, CYP3A5; GST, glutathione-*S*-transferase; AFAR, AF aldehyde reductase; AF-SG, aflatoxin-glutathione conjugate [21].

in human liver. CYP450 1A2 and some other human CYP450s also contribute, but they play a lesser role, even at relatively low AFB1 concentration [26, 27]. CYP450 3A4 forms mostly the genotoxic AFB-2,3-epoxide, whereas CYP450 1A2 forms both the exo- and nongenotoxic endoisomers [26]. CYP450 1A2 has high affinity for the bioactivation of AFB1 at low substrate concentrations following dietary exposure [21]. Some of the AFB1 intermediates go through far more metabolism in Phase 2 by binding with GSH in order to create the polar and less toxic compound that are simply excreted in urine and bile. However, AFBO and AFB1-dihydroxide intermediates led to carcinogenicity, while AFB2 causes acute toxicity, liver necrosis, and cellular metabolizing enzyme inhibition (**Figure 2**) [28].

2.2. Phase 2: Metabolism of AF role of GSH conjugation in body detoxification of aflatoxins

Phase 2 reactions that lead to the detoxification involve conjugation to glucuronic acid, sulfate, and GSH. The AFB metabolites of Phase 1 metabolism undergo Phase 2 enzymatic metabolism by glutathione-S-transferases (GSTs) that primarily catalyze conjugation reactions. After Phase 1 oxidation, AF can be readily conjugated with SH groups (in Phase 2 reactions) allowing for further detoxification and elimination of the toxin. In a number of mammalian species, the AFB1-8,9-epoxide is efficiently conjugated with reduced GSH in a reaction catalyzed by GST (**Figure 3**) [29, 30].

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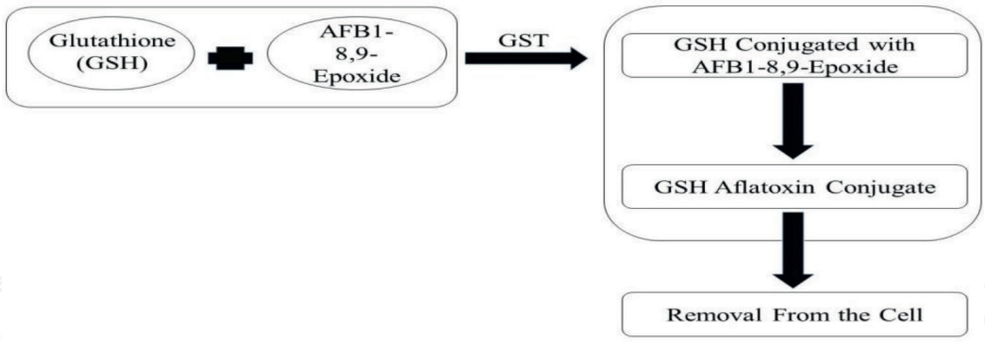

Figure 3. Metabolism of AFB1. Glutathione and glutathione-S-transferase involved in detoxification of activated AFB1.

3. Free radicals and lipid peroxidation

Free radicals are highly reactive species that have an unpaired electron, e.g., hydroxyl (·OH) and superoxide radicals (O^{-}_{2}) which have potential to cause tissue damage (**Figure 4**). Although free radicals are highly reactive and potentially damaging, they are also an integral part of some cellular processes. Extracellular secretion of free radicals by leucocytes and microphages evokes immune response against bacteria, viruses, degenerated cells, and other foreign substances. Intracellular secretion of free radicals stimulates different cell signaling pathways and triggers oxidative stress defense response as well as apoptosis [31]. Due to

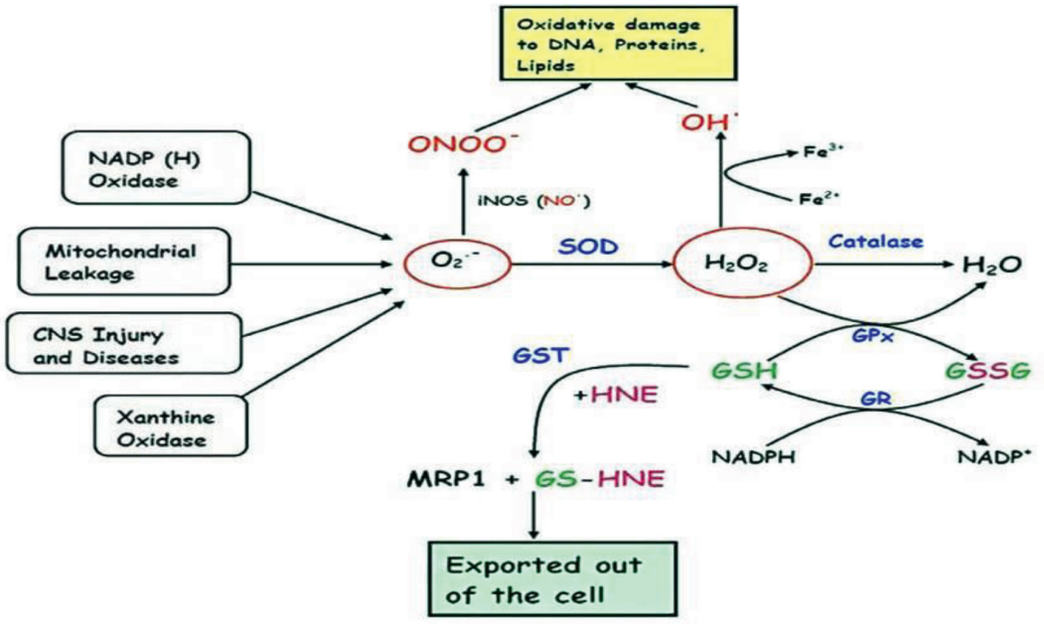

Figure 4. Different endogenous sources for ROS/reactive nitrogen species (RNS), antioxidant defense. Hydroxynonenal (HNE) is one of the end products of lipid peroxidation (adapted from Hardas [35]).

perilous nature of free radicals, cells have a counter mechanism known as antioxidant defense to keep the free radical levels under check. Unfortunately, when certain conditions promote the excess production of free radicals or deplete the antioxidant defense that leads the cell to oxidative damage, oxidative stress is said to exist. Oxygen-derived free radicals are referred as reactive oxygen species (ROS). Oxygen radicals are produced as a consequence of the normal process of reduction of oxygen to water and represent by products of oxidative cellular metabolism. The main sites of ROS produced in living organisms are mitochondrial electron transport system, peroxisomal fatty acid, CYP450, and phagocytic cells [32]. ROS can react with DNA to cause breaks in the DNA chain and mutation, which could initiate carcinogenesis. Free radicals can react with membrane lipids leading to peroxidation of polyunsaturated fatty acid (PUFA) residues (**Figure 4**) [33, 34].

The majority of lipid peroxidation events that occur within the cell are result of free radical chain reaction. Oxidative damage to lipids generally results in formation of cytotoxic aldehyde and ketone derivatives. Typically free radicals have a very short half-life; therefore, the damage caused by them is localized. Unlike free radicals, lipid peroxidation products have a longer half-life, and so they can diffuse into bilayer and can cause oxidative damage away from their site of production. For a given fatty acid, multiple aldehydic or ketonic products can arise as a result of lipid peroxidation, depending upon which allylic carbon gets attacked to initiate the chain reaction [36, 37]. Malondialdehyde (MDA) is a significant final product, which composes via the degeneration of certain primary and secondary lipid peroxidation products [38]. The MDA formation promotes the alteration of membrane fluidity and enhances of membrane fragility. Furthermore, MDA blocks particular enzyme reactions and causes mutagenicity and carcinogenicity by creating DNA adducts [39, 40].

4. Oxidative stress

Although ROS and reactive nitrogen species (RNS) are generated under normal physiological conditions, their levels are efficiently regulated by antioxidant enzymes and molecules to maintain the cellular redox balance. Oxidative stress is defined as a disturbance in the balance between antioxidants and prooxidants, with increased levels of prooxidants leading to potential damage. This imbalance can be due to the decrease of endogenous antioxidants, low intake of dietary antioxidants, and/or increased formation of free radicals and other reactive species. In any case, either of both circumstances occurring together or separately eventually will lead to deleterious modifications of biomolecules and multitude of downstream consequences. Oxidative stress has been implicated in vast array of conditions including cancer, arthritis, cardiovascular diseases, diabetes, aging, and neurodegenerative disorders [33, 41, 42].

4.1. Effects on oxidative stress of aflatoxin

Oxidative stress plays a major role in aflatoxicosis. Oxidative stress may be due to direct effect of AFs themselves or by their metabolites. AFB1, a mutagenic food contaminant, is widely recognized as one of the most potent hepatocarcinogens in humans and experimental animals.

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Metabolizing AFB1 increases the production of free radicals and lipid peroxides, resulting in cell damage [43, 44]. AFB1 is activated in the liver by CYP450 to AFB1-8,9-epoxide, which forms adducts with both DNA and protein. The toxic effects of AFs mostly arise from the binding of this particular epoxide derivative to DNA. AFs form after a series of highly organized oxidation-reduction reactions. Several studies provided evidences indicating that CYP450 enzymes generate superoxide hydrogen peroxide (H_2O_2) as intermediate compounds, and these ROS can cause apoptosis and other cell pathologies [45–47]. AFB1 is able to induce ROS generation, which causes oxidative stress. The genetic toxicity of AFB1 is partly due to the accumulation of ROS such as O_2^-, ·OH, and H_2O_2 radical during the metabolic processing of AFB1 by CYP450 in the liver (**Figure 5**). These species may attack soluble cell compounds as well as membranes, eventually leading to the impairment of cell functioning and cytolysis [48].

It has been reported that there is free radical generation during AFB1 metabolism, and oxidative damage is one type of damage caused by AFB1 [49, 50]. Oxidative damage induced by these ROS can, in turn, cause tissue damage by a variety of mechanisms including DNA damage, lipid peroxidation, protein oxidation, and depletion of thiols. The oxidative stress caused by AFB1 may be one of the underlining mechanisms for AFB1-induced cell injury and DNA damage, which eventually lead to tumorigenesis [37]. Studies have revealed that AFB1 alters cell cycle and apoptosis-signaling pathways in liver cell models [43, 47, 51, 52]. AFB1 can cause an increase in ROS formation in animals' target organs including rat liver, duck liver, and mouse lung [37, 44, 53]. It is indicated that AFB1 induced an important liver cell injury, as shown by the significant increase in nitric oxide, but also a strong lipid peroxidation in the liver and kidney, accompanied with a significant decrease in total antioxidant capacity in rats [53], mice [54], and chicken [55]. Also, it was shown that a strong inducible nitric oxide synthase (iNOS) and nitrotyrosine immunoreactivity were observed in the livers of chicks administered with 300 ppb of AF. Moreover, AFB1 carcinogenicity is associated with altered expression of many p53-target genes and induction of mutations, principally the p53 codon 249 hotspot mutation [13, 48].

AFs are claimed as potential risk factor of hepatocarcinoma, and the oxidative stress is considered to be a main factor in the initiation and the progression of liver cirrhosis, which is known

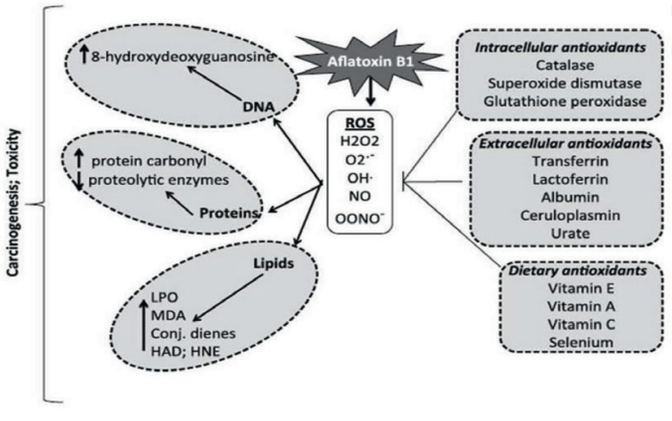

Figure 5. Effect of AFs on the oxidative stress, the alleviating role of antioxidants (adapted from Marin and Taranu [5]).

to be a pioneer of human hepatocellular carcinoma [11]. The oxidative damage caused by AF is considered to be the main mechanism leading to the subsequent hepatotoxicity [56]. AFB1 may disturb the integrity of cell membranes by stimulating phospholipid A2 to initiate lipid peroxidation in cells [57]. Animals fed with AF-contaminated diet suffer from oxidative stress as indicated by the significant increment of lipid peroxidation and the significant reduction of enzymatic antioxidant such as SOD and GSH-Px [54, 58, 59]. According to the pioneering work of Shen et al. [60], AFB1 promotes lipid peroxidation in rat liver, and lipid peroxidation is intimately linked with liver cell injury. A time- and dose-dependent increase in 8-hydroxy-2′-deoxyguanosine (8-OHdG) was observed in DNA after a single intraperitoneal injection of AFB1. It reveals that AFB1 leads to oxidative DNA damage in rat liver, which may participate in ·OH as the initiating species. Therefore, factors having an effect on formation or action of ·OH would affect the generation of 8-OHdG.

It is well known that a possible mechanism of AF cytotoxicity is the induction of oxidative stress. The induction of oxidative stress is commonly related to an imbalance between the oxidants and the antioxidant systems [49]. It is explained by its effect on mitochondria; increased lipid peroxidation; increased adduct formation with DNA, RNA, and protein; or all the three. Damage to mitochondria can lead to mitochondrial diseases and may be responsible for aging mechanisms. The damage can cause mitochondrial DNA (adducts and mutation), mitochondrial membranes, as well as disruption of energy production (production of adenosine triphosphate) [61]. The mycotoxin alters energy-linked functions of adenosine diphosphate (ADP) phosphorylation and flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD)-linked oxidizing substrates and α-ketoglutarate-succinate cytochrome reductases [62, 63]. It causes ultrastructural changes in mitochondria and also induces mitochondria-directed apoptosis [51]. AFB1 induced the production of free radicals and the reduction of antioxidant defenses in livers of murine, human lymphocytes, and bovine peripheral blood mononuclear cells [51, 64, 65].

4.2. Aflatoxin and carcinogenicity

AFB1 primarily causes hepatocellular carcinoma and cholangiocarcinoma in the liver [11]. Among various types of known AFs, AFB1 is the most potent hepatocarcinogen; however, G1 and B2 also cause cancers but with reduced potency. It causes liver tumors in mice, rats, fish, marmosets, tree shrews, and monkeys following the administration by various routes. The types of cancers described in research animals include hepatocellular carcinoma, cholangiocellular cancer, and adenocarcinoma of the gallbladder [66].

Besides, the liver tumors have also been reported to develop AF feeding in lacrimal glands, squamous cells of the tongue, esophagus, trachea, lung adenomas, osteogenic sarcoma, and carcinoma of the pancreas [66–68]. Carcinoma of the colon has been reported by many researchers [67, 69]. AF exposure contributes to the risk for development of hepatocellular carcinoma in ducklings [70]. AFB1 can cause hepatocarcinogenesis and mutation in rat liver (**Figure 6**) [71]. Ghebranious and Sell [13] proposed that some mutant proteins may act as a promoting agent for AFB1 hepatocarcinogenesis. AF and p53 expressions interact to produce malignant liver tumors transgenic in mice.

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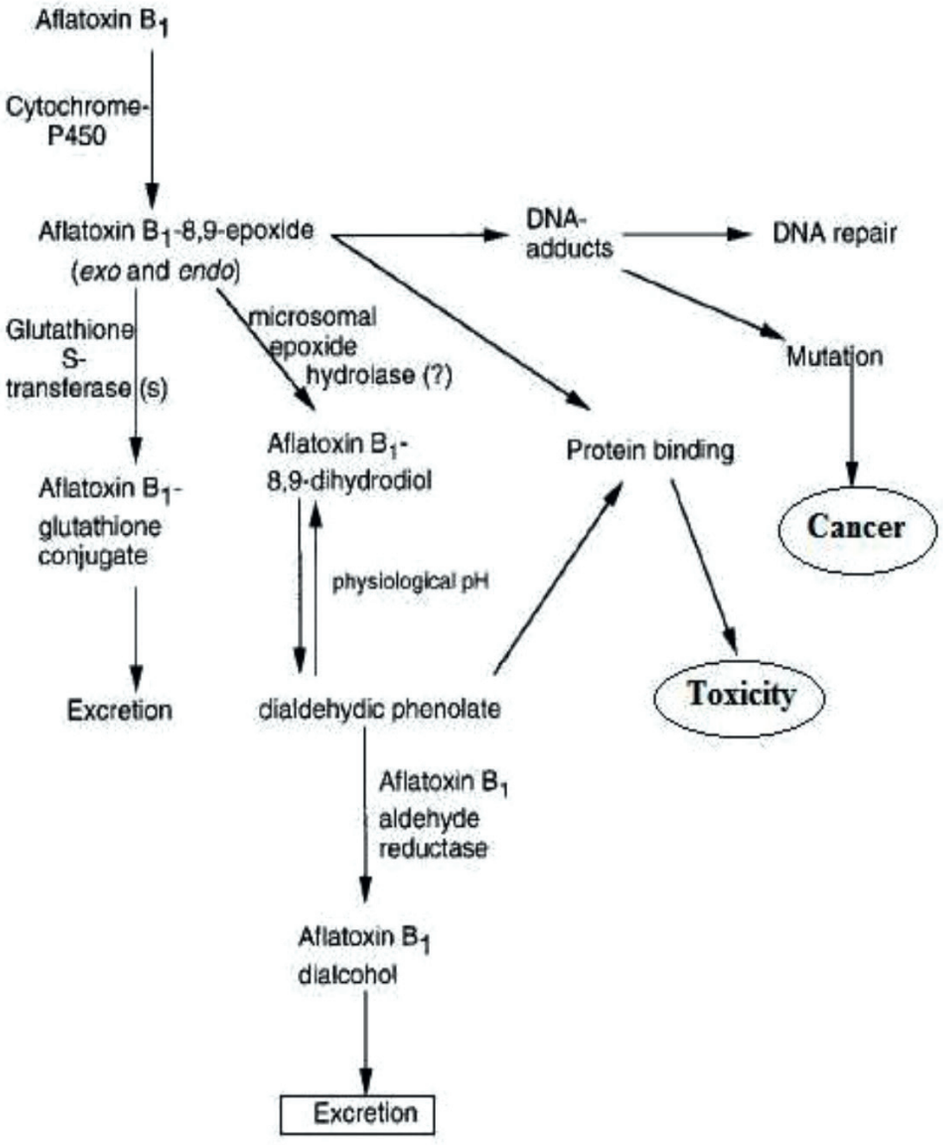

Figure 6. Overview of metabolic pathways leading to toxicity and carcinoma of AFB1 [72].

4.3. Aflatoxins and oxidative lipid damage

Polyunsaturated lipids are essential for cells, being important constituents of cell membranes, endoplasmic reticulum, and mitochondria. Thus, the disruption of their structural properties could have consequences for cellular function. Lipid peroxidation is one of the main factors responsible for structural and functional alterations of the cell membrane following oxidative stress [39] and initiation of carcinogenesis [37, 54].

It remains unknown if the mycotoxins promote the lipid peroxidation directly through the enhancement of the ROS formation or the enhancement of the tissue sensitivity to the peroxidation is the result of the compromised antioxidant defense, but it appears that both processes are taken part. AFB1-mediated cell injury may be due to the release of free radicals that initiate lipid peroxidation. The initiation of lipid peroxidation is caused by the attack of any species

that has sufficient reactivity to remove a hydrogen atom from a methylene group upon a PUFA [37, 54]. The peroxidation of PUFAs can be realized not only through nonenzymatic free radical-induced pathways but also through processes that are catalyzed by enzymes as cyclooxygenase and lipoxygenase [39]. It is shown that also 8,9-epoxide increases lipid peroxidation, followed by loss of membrane stability and the blockage of the membrane-bound enzyme activity [73]. Evaluation of the lipid damage is based on measurement of Thiobarbituric acid reactive substances (TBARS) or MDA by the TBA test and conjugated dienes. AFB1 induced an increase in the TBARS concentration in the liver [74] or in human hepatoma cells [75]. The increase of the lipid peroxide synthesis is observed not only in the liver but also in the kidney and brain [7, 35, 41]. This alteration was associated with a significant increase in conjugated diene formation. Concentrations of MDA+ 4-hydroxyalkenals as an index of lipid peroxidation are increased by AFB1 in the liver, lung, brain, and testis, but not the kidney of male Wistar rats [76]. 4-Hydroxynonenal (4-HNE), a major electrophilic by-product of lipid peroxidation caused by oxidative stress, interacts with DNA to form exocyclic guanine products, which have been shown to increase in a rat model of hepatocarcinogenesis. AFB1 induces lipid peroxidation in rat liver, which may be an underlying mechanism of carcinogenesis [44, 77].

4.4. Aflatoxins and oxidative protein damage

ROS can also lead to oxidation of amino acid residue side chains, formation of protein-protein cross-linkages, and oxidation of the protein backbone resulting in protein fragmentation, and the modified forms of proteins will accumulate in organism [78]. By its capacity to generate ROS, AFB1 can promote the ROS-mediated oxidative damages in proteins (**Figure 6**) [79].

AFB1 could inhibit some (serine) proteolytic enzymes responsible for the degradation of damaged proteins, with consequent relevant implications in hepatocarcinogenesis [79, 80]. It has been suggested that numerous action of AFs may be brought about their interactions with the proteasome, the main enzyme family account for the decomposition of most of cytosolic and nuclear proteins in eukaryotic cells. In fact, AFB1 brings about an inhibition of cellular 20S proteasomes, affecting the cellular defense against oxidative stress. Because 20S proteasome is the proteolytic machinery responsible for removing oxidized proteins, its inhibition could contribute to a higher protein carbonyl content observed in cultured hepatoma cell lysates [81].

The reduction of protein synthesis in animals treated with AFs may affect certain metal ions, which play an important role in free radical production and liberation. Inhibition of protein synthesis caused by AFs alters serum protein composition, resulting in suppression of the production of nonspecific humoral substances important to native defense [82]. At higher doses, AFB1 lowers the level of IgG and IgA in chick resulting in decreased acquired immunity. Antibodies to AFB1 have been reported in humans [83, 84].

4.5. Aflatoxins and oxidative DNA damage

Oxidative DNA damage is a general definition for all types of changes (structural or functional) of DNA, due to the interaction of ROS with DNA. The connection of ·OH to the C8 position of DNA guanine forms C8-OH-adduct radical [85], which is eventually altered to

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8-OH-guanine (8-OH-Gua) by one-electron oxidation [86]. While impaired lipids and proteins can be removed by metabolic cycle of these compounds, damaged DNA has to be fixed in situ or destroyed by apoptotic processes; conversely, mutations result in the absence of these [87]. In humans, 8-OH-Gua glycosylase is the primary enzyme for the repair of 8-OH-Gua in short-patch base excision repair. The excised form of 8-OH-Gua is a promutagenic adduct, 8-OHdG, which is excreted into urine without further metabolism and is stable for a significant time. 8-OHdG is widely considered as a key biomarker of oxidative DNA damage [60, 88].

The toxic and carcinogenic effects of AFB1 are intimately linked with its biotransformation [12]. There is a tendency for AFs especially AFB1 to convert into the epoxide and produce DNA adducts resulting in the formation of DNA strand breaks and mutations [88, 89]. It is well known that AFB1 is activated by the hepatic CYP450 enzyme system to form a highly reactive product, AFB1-8,9-epoxide, which subsequently connects to nucleophilic sites in DNA and the major adduct 8,9-dihydro-8-(N7-guanyl)-9-hydroxy-AFB1 (AFB1-N7-Gua) is formed. The formation of AFB1-DNA adducts is regarded as a critical step in the initiation of AFB1-induced hepatocarcinogenesis (**Figure 7**).

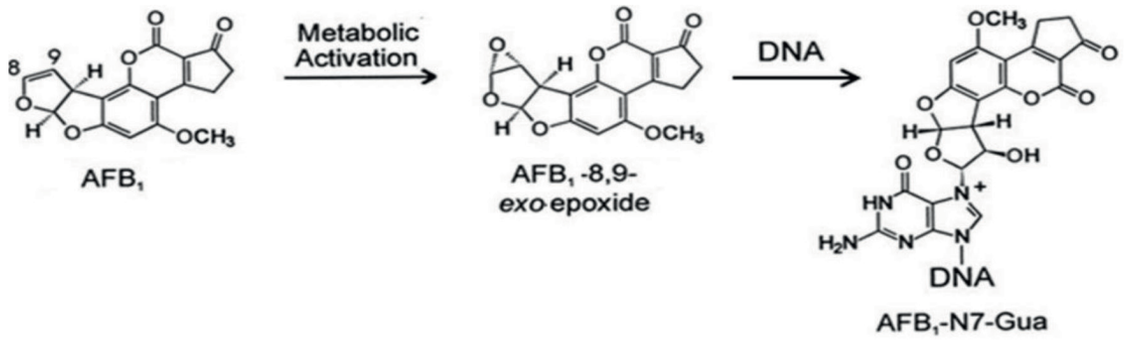

Figure 7. Metabolic activation of AFB1 (adapted from Kobertz et al. [90]).

5. Aflatoxins and the antioxidant defense

The activity of antioxidant enzymes could induce as a result to the oxidative stress or could diminish through direct or indirect action of the mycotoxins. A part of the oxidative metabolism intermediates of AFB1 composes a substrate for the Phase 2 detoxification enzymes. In a vast range of animal species, the fundamental way to detoxify the AFB1 is through the conjugation of AFBO with GSH. This way of detoxification is the principal way of AFB1 excretion in many animal species. The reaction is catalyzed by GST [89]. It is observed that in mice, the reduced sensibility to AFs is correlated with the constitutive increase of GST isoenzyme [29]. GSH and GST are effective antioxidant enzymes that take part in the protection of tissues from harmful effects of AFB1 (**Figure 3**) [90, 91]. GSH is used as a cofactor by GST that conjugates GSH with endogenous substances like estrogens, exogenous electrophiles like AFs and its metabolites, and other various xenobiotics. The increased depletion of GSH

leads to abnormally high levels of ROS in cells. AF is one of the main actors in depletion of GSH. The depletion of GSH affects metabolic processes such as catalysis of molecular oxygen (O_2) to H_2O_2 by GSH-Px, and thus the integrity of the cell membranes disrupts. Its reduction further enhances the damage to critical cellular components (DNA, lipids, proteins) by the AFB1-8,9-epoxides that form adducts. GST catalyzes the conjugation of AFB1-8,9-epoxide with GSH to form AFB1-GSH conjugate, thereby decreasing the intracellular GSH content [37]. The AF-GSH product undergoes the sequential metabolism in the liver and kidneys in which it is excreted as a mercapturic acid (AF-N-acetylcysteine) in urine [91, 92]. It has been reported that AF administration results in excessive lipid peroxidation [53] with concomitant decrease in GSH [58], increased protein oxidation, and DNA damage in rat liver. The activity of GSH-Px, which is a constituent of GSH redox cycle, decreases during AFB1 administration. The reduction in 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. The studies revealed that there were obvious increases in MDA and/or nitric oxide (NO) levels and decreases in both nonenzymatic antioxidant GSH level and enzymatic antioxidant GSH-Px, catalase (CAT), glutathione reductase (GR), and GST activities after administration with AFB1 in vivo or in vitro [41, 51, 64, 65].

The study showed that 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. 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. The 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 O_2^- 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 the 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 [49].

6. Lycopene

Lycopene is an acyclic hydrocarbon carotenoid responsible for the intense red color of tomatoes (**Figure 8**). Lycopene does not exhibit provitamin A activity since it lacks the β-ionone ring structure which is characteristic in carotenoids that are precursors for vitamin A [93, 94]. Lycopene is a natural pigment and imparts a red color in the foods containing it. In foods,

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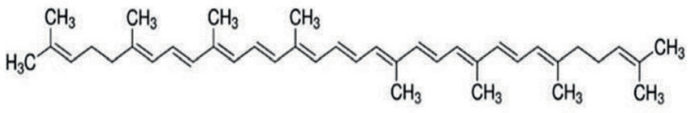

Figure 8. Structure of all-trans lycopene ($C_{40}H_{56}$).

lycopene is found predominantly in its trans-form (approximately 95.4% of total lycopene content), whereas serum and tissues contain more cis-isomers of lycopene [95–97]. Lycopene is nontoxic and Generally Recognized as Safe by the US FDA (21 CFR 73.585) and the European Union (Directive 94/36/EC) for the use as a food additive and colorant [98].

6.1. The role of lycopene as antioxidant and implications

Lycopene acts as an antioxidant by virtue of its conjugated p-electron system, which can react with oxygen radical species such as peroxy and hydroxy radicals as well as non-radical species such as ozone and H_2O_2 [99].

Lycopene has a robust antioxidant defense system, attributed to its acyclic structure, numerous conjugated double bonds, and high hydrophobicity, and thus prevents the onset of carcinogenesis and atherogenesis processes by protecting/stabilizing biomolecules such as DNA, proteins, lipids, and lipoproteins. Lycopene, as the main carotenoid in tomato products, possesses the greatest ability to quench singlet oxygen compared to the other carotenoids. It also scavenges the free radicals *via* three different mechanisms: adduct formation, electron transfer, and hydrogen atom transfer [100, 101]. Galano et al. [102] reported that lycopene and torulene are more reactive scavengers of peroxide radicals than β-carotene.

Lycopene is capable of acting as an antioxidant by virtue of its many conjugated double bonds. It is the most efficient neutralizer of singlet oxygen among all carotenoids and has also been found to be a potent scavenger of free radicals [94, 95]. The lycopene molecule reacts with free radicals to form a short-lived intermediate species, which later end up as lycopene decomposition products including apocarotenals, apocarotenones, and epoxides. Being a highly hydrophobic molecule, the greatest scavenging ability of lycopene is seen in lipophilic environments [94, 103]. After supplementing subjects with lycopene from different dietary sources, serum TBARS (a biomarker for lipid peroxidation) is significantly reduced, whereas nonsignificant reductions are observed in biomarkers for protein and DNA oxidation. Hence, lycopene may be a biologically important antioxidant by protecting membrane lipids from being oxidized which in turn preserves the integrity of cellular membranes [104].

Much of the evidence for the antioxidant function of lycopene comes from studies conducted with in vitro systems, and virtually all of them indicate lycopene to function as a superior dietary antioxidant. Being is a strong antioxidant, lycopene has been shown to reduce the amount of oxidative DNA damage and also decrease lipid peroxidation in cell culture and in rats in vivo [105–107]. Di Mascio et al. [108] compared the singlet oxygen quenching ability of various carotenoids, α-tocopherol, bile acids, and retinoic acid. They found lycopene to

be the most efficient quencher among all, with a greater than twofold quenching potency. Lycopene is the most efficient carotenoid in reducing TBARS formation by 75% compared to control in multilamellar liposomes. In a study examining the relative ability of several antioxidants in reducing carotenoid cations, it was found that lycopene was the most superior carotenoid antioxidant and the lycopene cation radical was the most stable carotenoid cation radical [109].

6.2. Protective effect of lycopene on aflatoxin damage

There are many reports indicating that lycopene is effective on inhibition of tumor formation and growth induced by chemical carcinogens in animals [107]. To sum up, in **Figure 9**, AFB1 has two important metabolic pathways: Phase 1 includes metabolism and metabolic activation, and Phase 2 is detoxification [30]. AFM1, AFQ1, AFP1, and AFB1-8,9-epoxide are important Phase 1 metabolites, and also AFB-N7-Gua and AFB-albumin complexes are specific markers formed, respectively, in the tissues and "serum or urine" during the AFB1 metabolic activation. The main Phase 2 detoxification outcome of AFB1-8,9-epoxide is AFB-N-acetyl cysteine (AFB-NAC) complex. AFB1 Phase 1 metabolism and the metabolic activation of AFB1 are inhibited by lycopene. Moreover, lycopene highly activates the enzymes responsible for Phase 2 detoxification and causes to enhance production of AFB-NAC excreted in urine. As shown in decreased urinary levels of AFP1, AFQ1, and AFM1 in lycopene-pretreated or lycopene-intervened animals, lycopene pretreatment or intervention significantly blocks Phase 1 metabolism of AFB1. This indicates that lycopene may selectively inhibit Phase 1 metabolic enzymes such as 3A4, 2A6, and 1A2. Depending on the relative potency in decreasing levels of these specific AFB1 metabolites in urine, lycopene appears to be a moderate competitive inhibitor of 3A4 and 2A6 enzymes and a weak or reversible inhibitor of 1A2

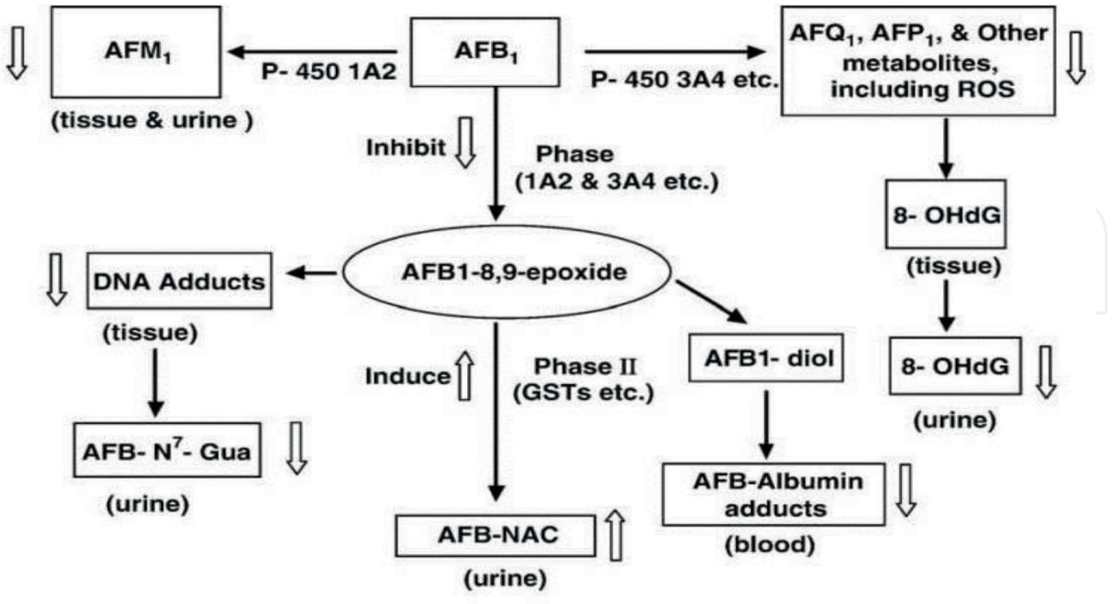

Figure 9. AFB1 metabolic activation, biomarkers, and possible mechanisms of lycopene modulation [110].

http://dx.doi.org/10.5772/intechopen.69321

enzyme (more potent inhibition of AFP1 and AFQ1 than AFM1). Reducing levels of AFB-albumin adducts in serum, reducing levels of AFB-N7-Gua excreted in urine, and reducing levels of AFB-N7-Gua adduct in the liver, DNA confirmed the inhibitory effect of lycopene on Phase 1 metabolism. These data clearly demonstrate that lycopene pretreatment or intervention effectively blocks AFB1 metabolism and also metabolic activation. AFB-NAC is the major detoxifying metabolic product of AFB1-8,9-epoxide. Lycopene pretreatment and intervention elevated significantly AFB-NAC levels in urine excretion, which suggests that activity of GSTs was greatly induced [30].

AFB1 also induces formation of ROS [44], lipid peroxidation, and formation of 8-OHdG in vivo and in vitro [60]. Lycopene could increase the activity of GSH-Px, GST, and GR in several animal models including rats [111]. The antioxidant capacity of lycopene is at extremely high levels and lessens not only the oxidative damage of DNA in particular rates but also lessens lipid peroxidation both in vitro and in vivo [105–107]. It has also been documented that lycopene intervention reduces the 8-OHdG levels of urine even in recurring exposures to AFB1 (**Figure 10**).

Administration of lycopene alleviates the negative effects of AF. Lycopene removes free radicals produced by AF while improving the body's antioxidant enzymes such as GSH, GSH-Px, and CAT to prevent the oxidative damage caused by AF, enhancing the body antioxidant capacity, reducing the levels of lipid peroxidation, and maintaining cell membrane permeability. For this reason, natural antioxidant lycopene can be regarded as a good therapeutic agent against aflatoxicosis [112].

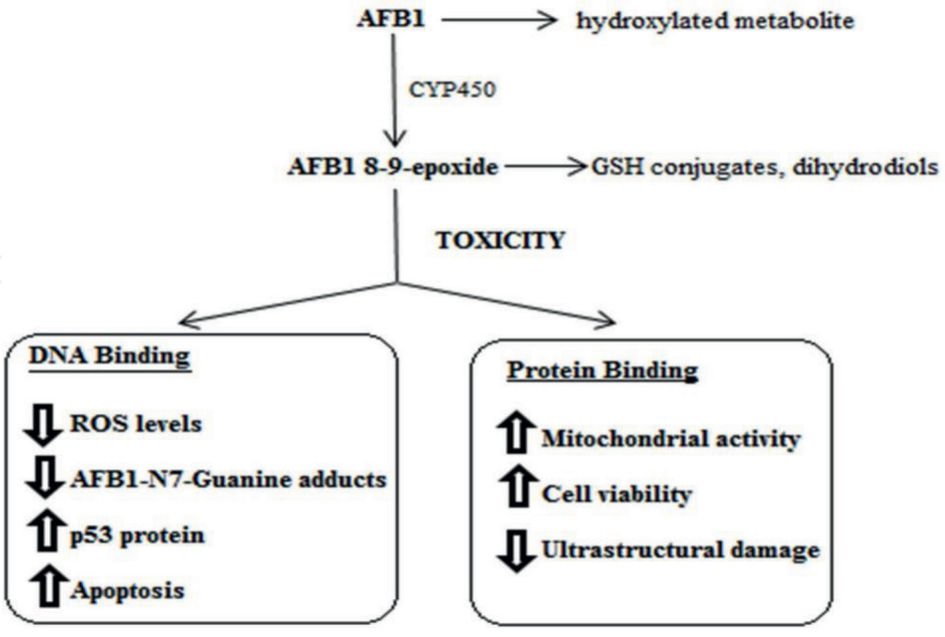

Figure 10. Inhibition of toxicity and cancer by lycopene in AFB1-exposed cells. Chemoprotective effects of lycopene effects are shown by arrows: ↑, increase; ↓, decrease (adapted from Reddy et al. [52]).

7. Conclusion

Current concepts derived from intensive research on biotransformation, mechanisms of toxicity, the effect on oxidative stress of AF, and protective effect of lycopene on AF damage were summarily presented in this chapter. AFB1 exerts its effects after conversion to the reactive compound AFB1 epoxide by means of CYP450-dependent enzymes. This epoxide can form derivatives with cellular macromolecules, including proteins, RNA and DNA. Biomonitoring of AFB1 metabolites such as AFB1-N7-guanine has demonstrated that AFs constitute an important risk factor for hepatocellular carcinoma in highly exposed populations. Oxidative stress formed due to AF is associated with biochemical disturbances in oxidant/antioxidant balance system, which may cause AF toxicity. When administered together with AF, lycopene was determined that it exhibited strong positive effect on AF-induced oxidative stress parameters. It could be concluded that the lycopene being a nontoxic, highly promising natural "eco-friendly" antioxidant compound has a protective effect against AF toxicity. When administered together with AF, lycopene was determined that it exhibited a strong positive effect on AF-induced oxidative stress parameters.

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References

[1] Coulombe RA. Aflatoxins. In: Sharma RP, Salunkhe, editors. Mycotoxins and Phytoalexins. Boca Raton: CRC Press; 1991. pp. 103-143. ISBN 0849388333

[2] Mushtaq M, Sultana B, Anwar F, Khan MZ, Ashrafuzzaman M. Occurrence of aflatoxins in selected processed foods from Pakistan. International Journal of Molecular Sciences. 2012;**13**:8324-8337

[3] Bbosa GS, David Kitya D, Lubega A, et al. Review of the biological and health effects of aflatoxins on body organs and body systems: Aflatoxins-recent advances and future prospects. Intechopen Publisher. 2013;**12**:239-265

[4] Reddy SV, Waliyar F. Properties of aflatoxin and its producing fungi. Aflatoxins. http://www.icrisat.org/aflatoxin/aflatoxin.asp, (2012).

[5] Marin DE, Taranu I. Overview on aflatoxins and oxidative stress. Toxin Reviews. 2012;**31**(3-4): 32-43

[6] Fink-Gremmels J. Mycotoxins: Their implications for human and animal health. Veterinary Quarterly. 1999;**21**:115-120

[7] Marvan F, Vernerova E, Samek M, et. al. Aflatoxin B1 residues in the organs of young poultry. Biologicke Chem Vet (Praha). 1983;**24**:85-92

[8] Petr T, Barta I, Turek B. In vitro effect of mutagenic activity of aflatoxin B1. Journal Hygiene Epidemiology Microbiology Immunology. 1995;**34**:123-128

[9] Hartley RD, Nesbitt BF, O'kelly J. Toxic metabolites of *Aspergillus flavus*. Nature, London. 1963;**198**(4885)

[10] IARC. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. Lyon: IARC Scientific Publication, No. 82; 2002

[11] Liu ZM, Li LQ, Peng MH, et al. Hepatitis B virus infection contributes to oxidative stress in a population exposed to aflatoxin B1 and high-risk for hepatocellular carcinoma. Cancer Letters. 2008;**263**:212-222

[12] Wu HC, Wang Q, Yang HI, et al. Aflatoxin B1 exposure, hepatitis B virus infection, and hepatocellular carcinoma in Taiwan. Cancer Epidemiology Biomarkers & Prevention. 2009;**18**:846-853

[13] Ghebranious N, Sell S. Hepatitis B injury, male gender, aflatoxin, and p53 expression each contribute to hepatocarcinogenesis in transgenic mice. Journal of Hepatology. 1998;**27**(2):383-391

[14] Bondy G, Pestka JJ. Immunomodulation by fungal toxins. Journal of Toxicology and Environmental Health. 2000;**3**:109-143

[15] Mocchegiani E, Corradi A, Santarelli L, et al. Zinc, thymic endocrine activity and mitogen responsiveness (PHA) in piglets exposed to maternal aflatoxicosis B1 and G1. Veterinary Immunology and Immunopathology. 1998;**62**:245-260

[16] Han XY, Hung QC, Li WF. Changes in growth performance, digestive enzyme activities and nutrient digestibility of cherry valley ducks in response to aflatoxin B1 levels. Livestock Science. 2008;**119**:216-220

[17] Trucksess MW, Stoloff L, Young K. Aflatoxical and aflatoxin B1 and M1 in eggs and tissue of laying hens consuming aflatoxin-contaminated feed. Poultry Science. 1983;**62**: 2176-2182

[18] Jiang Y, Jolly PE, Ellis WO. Aflatoxin B1 albumin adduct levels and cellular immune status in Ghanaians. International Immunology. 2005;**17**:807-814

[19] Sumit R, Ji EK, Roger CJ. Aflatoxin B1 in poultry: Toxicology, metabolism and prevention. Research in Veterinary Science. 2010;**89**:325-331

[20] Monosson, E. Biotransformation. National Library of Medicine (NLM): The Encyclopeadia of earth. http://www.eoearth.org/view/article/150674, (2012).

[21] Wild CP, Turner PC. The toxicology of aflatoxins as a basis for public health decisions. Mutagenesis. 2002;**17**(6):471-481

[22] Swenson DH, Miller EC, Miller JA. Aflatoxin B1-2, 3-oxide: Evidence for its formation in rat liver in vivo and by human liver microsomes in vitro. Biochemical and Biophysical Research Communications. 1974;**60**(3):1036-1043

[23] Swenson DH, Lin JK, Miller EC, Miller JA. Aflatoxin B1-2, 3-oxide as a probable intermediate in the covalent binding of aflatoxins B1 and B2 to rat liver DNA and ribosomal RNA in vivo. Cancer Research. 1977;**37**(1):172-181

[24] Aoyama T, Yamano S, Guzelian PS, Gelboin HV, Gonzalez FJ. Five of 12 forms of vaccinia virus-expressed human hepatic cytochrome P450 metabolically activate aflatoxin B1. Proceedings of the National Academy of Sciences. 1990;**87**(12):4790-4793

[25] Wild CP, Yin F, Turner PC, et al. Environmental and genetic determinants of aflatoxin-albumin adducts in the Gambia. International Journal of Cancer. 2000;**86**:1-7

[26] Ueng YF, Shimada T, Yamazaki H, Guengerich FP. Oxidation of aflatoxin B1 by bacterial recombinant human cytochrome P450 enzymes. Chemical Research in Toxicology. 1995;**8**(2):218-225

[27] Crespi CL, Penman BW, Leakey JA, et al. Human cytochrome P450IIA3: cDNA sequence role of the enzyme in the metabolic of promutagens comparison to nitrosamine activation by human cytochrome P450IIE1. Carcinogenesis. 1990;**11**(8):1293-1300

[28] Dhanasekaran D., Panneerselvam A., Thajuddin N., Shanmugapriya, S. Aflatoxins and aflatoxicosis in human and animals. In: Guevara-Gonzalez RG, Editor. Aflatoxins-Biochemistry and Molecular Biology. Rijeka (Croatia): Intechopen Publisher, 2011:221-254

[29] Neal GE, Green JA. The requirement for glutathione S-transferase in the conjugation of activated aflatoxin B1 during aflatoxin hepatocarcinogenesis in the rat. Chemico-Biological Interactions. 1983;**45**(2):259-275

[30] Wang JS, Shen X, He X, et al. Protective alterations in phase 1 and 2 metabolism of aflatoxin B1 by oltipraz in residents of Qidong, People's Republic of China. Journal of the National Cancer Institute. 1999;**91**(4):347-354

[31] Simkó M, Gazsó A, Fiedeler U, Nentwich M. Nanoparticles, free radicals and oxidative stress. Nano Trust Dossiers. 2011;**12**:1-3

[32] Ray PD, Huang BW, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signalling. 2012;**24**:981-990

[33] Sies H. Oxidative stress: From basic research to clinical application. The American Journal of Medicine. 1991;**91**(3):31-38

[34] Halliwell B, Cross CE. Oxygen-derived species: Their relation to human disease and environmental stress. Environmental Health Perspectives. 1994;**102**(10):5

[35] Hardas Sarita S. Investigations of Oxidative Stress Effects and Their Mechanisms in Rat Brain After Systemic Administration of Ceria Engineered Nanomaterials. University of Kentucky, Theses and Dissertations-Chemistry, Paper 7. http://uknowledge.uky.edu/chemistry-e ds/7, 2012.

[36] Muller FL, Lustgarten MS, Jang Y, Richardson A, Remmen HV. Trends in oxidative aging theories. Free Radical Biology and Medicine. 2007;**43**:477-503

[37] Shen HM, Shi CY, Lee HP, Ong CN. Aflatoxin B1-induced lipid peroxidation in rat liver. Toxicology and Applied Pharmacology. 1994;**127**:145-150

[38] Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radical Biology and Medicine. 1990;**9**:515-540

[39] Halliwell B, Chirico S. Lipid peroxidation: Its mechanism, measurement, and significance. American Journal of Clinical Nutrition. 1993;**57**:715-725.

[40] Marnett LJ. Lipid peroxidation: DNA damage by malondialdehyde. Mutation Research. 1999;**424**:83-95

[41] Yilmaz S, Kaya E, Comakli S. Vitamin E, α tocopherol, attenuates toxicity and oxidative stress induced by aflatoxin in rats. Advances in Clinical and Experimental Medicine. 2017. In press.

[42] Halliwell B, Gutteridge JMC. Cellular responses to oxidative stress: Adaptation, damage, repair, senescence and death. Free Radicals in Biology and Medicine. 2007;**4**:187-267

[43] Josse R, Dumont J, Fautre A, Robin M, Guillouzo A. Identification of early target genes of aflatoxin B1 in human hepatocytes, inter-individual variability and comparison with other genotoxic compounds. Toxicology and Applied Pharmacology. 2012;**258**:176-187

[44] Shen HM, Shi CY, Shen Y, Ong CN. Detection of elevated reactive oxygen species level in cultured rat hepatocytes treated with aflatoxin B1. Free Radical Biology and Medicine. 1996;**21**:139-146

[45] Bondy SC, Naderi S. Contribution of hepatic cytochrome P450 systems to the generation of reactive oxygen species. Biochemical Pharmacology. 1994;**48**:155-159

[46] Shimamoto NA. A pathophysiological role of cytochrome p450 involved in production of reactive oxygen species. Yakugaku Zasshi. 2013;**133**:435-450

[47] Sun X, Ai M, Wang Y, et al. Selective induction of tumor cell apoptosis by a novel P450-mediated reactive oxygen species (ROS) inducer methyl 3-(4-nitrophenyl) propiolate. Journal of Biological Chemistry. 2013;**288**:8826-8837

[48] Shen HM, Ong CN. Mutations of the p53 tumor suppressor gene and ras oncogenes in aflatoxin hepatocarcinogenesis. Mutation Research/Reviews in Genetic Toxicology. 1996;**366**(1):23-44

[49] Kodama M, Inoue F, Akao M. Enzymatic and non-enzymatic formation of free radicals from aflatoxin B1. Free Radical Research Communications. 1990;**10**:137-142

[50] Amstad P, Levy A, Emerit I, Cerutti P. Evidence for membrane-mediated chromosomal damage by aflatoxin B1 in human lymphocytes. Carcinogenesis. 1984;**5**:719-723

[51] Meki ARM, Abdel-Ghaffar SK, El-Gibaly I. Aflatoxin B1 induces apoptosis in rat liver: Protective effect of melatonin. Neuroendocrinology Letters. 2001;**22**(6):417-426

[52] Reddy L, Odhav B, Bhoola K. Aflatoxin B1-induced toxicity in HepG2 cells inhibited by carotenoids: Morphology, apoptosis and DNA damage. Biological Chemistry. 2006; **387**(1):87-93

[53] El-Nekeety AA, Mohamed SR, Hathout AS, et al. Antioxidant properties of thymus vulgaris oil against aflatoxin-induce oxidative stress in male rats. Toxicon. 2011;**57**(7):984-991

[54] Kanbur M, Eraslan G, Sarica ZS, Aslan O. The effects of evening primrose oil on lipid peroxidation induced by subacute aflatoxin exposure in mice. Food and Chemical Toxicology. 2011;**49**:1960-1964

[55] Sirajudeen M, Gopi K, Tyagi JS, et al. Protective effects of melatonin in reduction of oxidative damage and immunosuppression induced by aflatoxin B1-contaminated diets in young chicks. Environmental Toxicology. 2011;**26**(2):153-160

[56] Preetha SP, Kanniappan M, Selvakumar E, Nagaraj M, Varalakshmi P. Lupeol ameliorates aflatoxin B1- induced peroxidative hepatic damage in rats. Comparative Biochemistry and Physiology. 2006;**143**:333-339

[57] Rastogi S, Dogra RK, Khanna SK, Das M. Skin tumorigenic potential of aflatoxin B1 in mice. Food and Chemical Toxicology. 2005;**44**:670-677

[58] Abdel-Wahhab MA, Hassan NS, El-Kady AA, et al. Red ginseng extract protects against aflatoxin B1 and fumonisins-induced hepatic pre-cancerous lesions in rats. Food and Chemical Toxicology. 2010;**48**:733-742

[59] Abdel-Aziem SH, Hassan AM, Abdel-Wahhab MA. Dietary supplementation with whey protein and ginseng extract counteracts oxidative stress and DNA damage in rats fed an aflatoxin-contaminated diet. Mutation Research. 2011;**723**:65-71

[60] Shen HM, Ong CN, Lee BL, Shi CY. Aflatoxin B1-induced 8-hydroxydeoxyguanosine formation in rat hepatic DNA. Carcinogenesis. 1995;**16**:419-422

[61] Thrasher JD. Are chlorinated pesticides a causation in maternal DNA (mtDNA) mutations? Archives of Environmental Health. 2000;**55**:292-294

[62] Sajan MP, Satav JG, Bhattacharya RK. Alteration of energy-linked functions in rat hepatic mitochondria following aflatoxin B1 administration. Journal of Biochemical and Molecular Toxicology. 1996;**11**(5):235-241

[63] Obasi SC. Effects of scopoletin and aflatoxin on B1 on bovine hepatic mitochondrial respitatory complexes, 2: A-ketoglutarate cytochrome c and succinate cytochrome c reductases. Zeitschrift für Naturforschung C. 2001;**56**(3-4):278-282

[64] Bernabucci U, Colavecchia L, Danieli PP, et al. Aflatoxin B1 and fumonisin B1 affect the oxidative status of bovine peripheral blood mononuclear cells. Toxicology in Vitro. 2011;**25**:684-691

[65] Kotan E, Alpsoy L, Anar M, Aslan A, Agar G. Protective role of methanol extract of Cetraria islandica (L.) against oxidative stress and genotoxic effects of AFB1 in human lymphocytes in vitro. Toxicology and Industrial Health. 2011;**27**:599-605

[66] Butler WH, Greenblatt M, Lijinsky W. Carcinogenesis in rats by aflatoxins B1, G1, and B2. Cancer Research. 1969;**29**(12):2206-2211

[67] Ward JM, Sontag JM, Weisburger EK, Brown CA. Effect of lifetime exposure to aflatoxin B1 in rats. Journal of the National Cancer Institute. 1975;**55**(1):107-113

[68] Dickens JW, Pattee HE. The effects of time, temperature and moisture on aflatoxin production in peanuts inoculated with a toxic strain of *Aspergillus flavus*. Tropical Science. 1966;**8**:11-22

[69] Newberne PM, Butler WH. Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals: A review. Cancer Research. 1969;**29**(1):236-250

[70] Sell S, Xu KL, Huff WE, et al. Aflatoxin exposure produces serum α-fetoprotein elevations and marked oval cell proliferation in young male Peking ducklings. Pathology. 1998;**30**:34-39

[71] Lee CC, Liu JY, Lin JK, Chu JS, Shew JY. p53 point mutation enhanced by hepatic regeneration in aflatoxin B 1-induced rat liver tumors and preneoplastic lesions. Cancer Letters. 1998;**125**(1):1-7

[72] Bammler TK, Slone DH, Eaton DL. Effects of dietary oltipraz and ethoxyquin on aflatoxin B1 biotransformation in non-human primates. Toxicological Sciences. 2000;**54**(1):30-41

[73] Toskulkao C, Glinsukon T. Hepatic lipid peroxidation and intracellular calcium accumulation in ethanol potentiated aflatoxin B1 toxicity. Journal of Pharmacobio-Dynamics. 1988;**11**(3):191-197

[74] Naaz F, Javed S, Abdin MZ. Hepatoprotective effect of ethanolic extract of Phyllanthus amarus Schum. et Thonn. on aflatoxin B1-induced liver damage in mice. The Journal of Ethnopharmacology. 2007;**113**:503-509

[75] Lee JK, Choi EH, Lee KG, Chun HS. Alleviation of aflatoxin B1-induced oxidative stress in HepG2 cells by volatile extract from Allii Fistulosi Bulbus. Life Sciences. 2005;**77**:2896-2910

[76] Gesing A, Karbownik-Lewinska M. Protective effects of melatonin and N-acetylserotonin on aflatoxin B1-induced lipid peroxidation in rats. Cell Biochemistry and Function. 2008;**26**(3):314-319

[77] Marquez A, Villa-Treviño S, Guéraud F. The LEC rat: A useful model for studying liver carcinogenesis related to oxidative stress and inflammation. Redox Report. 2007;**12**:35-39

[78] Berlett BS, Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. The Journal of Biological Chemistry. 1997;**272**:20313-20316

[79] Peng T, Li LQ, Peng MH, et al. Is correction for protein concentration appropriate for protein adduct dosimetry? Hypothesis and clues from an aflatoxin B1-exposed population. Cancer Sciences. 2007;**98**:140-146

[80] Peng T, Li LQ, Peng MH, et al. Evaluation of oxidative stress in a group of adolescents exposed to a high level of aflatoxin B1–a multi-center and multi-biomarker study. Carcinogenesis. 2007;**28**:2347-2354

[81] Amici M, Cecarini V, Pettinari A, et al. Binding of aflatoxins to the 20S proteasome: Effects on enzyme functionality and implications for oxidative stress and apoptosis. The Journal of Biological Chemistry. 2007;**388**:107-117

[82] Thaxton JP, Tung HT, Hamilton PB. Immunosuppression in chickens by aflatoxin. Poultry Science. 1974;**53**(2):721-725

[83] Tung HT, Wyatt RD, Thaxton P, Hamilton PB. Concentrations of serum proteins during aflatoxicosis. Toxicology and Applied Pharmacology. 1975;**34**(2):320-326

[84] Wang JS, Abubaker S, He X, et al. Development of aflatoxin B1-lysine adduct monoclonal antibody for human exposure studies. Applied and Environmental Microbiology. 2001;**67**(6):2712-2717

[85] Steenken S. Purine bases, nucleosides, and nucleotides: Aqueous solution redox chemistry and transformation reactions of their radical cations and e- and OH adduct. Chemical Reviews. 1989;**89**:503-520

[86] Kasai H, Yamaizumi Z, Yamamoto F, et al. Photosensitized formation of 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in DNA by riboflavin. Nucleic Acids Symposium Series. 1992;**27**:181-182

[87] Luo H, Tang L, Tang M, et al. Phase IIa chemoprevention trial of green tea polyphenols in high-risk individuals of liver cancer: Modulation of urinary excretion of green tea polyphenols and 8-hydroxydeoxyguanosine. Carcinogenesis. 2006;**27**(2):262-268

[88] Valavanidis A, Vlachogianni T, Fiotakis C. 8-Hydroxy-2′ -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. Journal of Environmental Science and Health Part C Environmental Carcinogenesis & Ecotoxicology Reviews. 2009;**27**:120-139

[89] Eaton DL, Gallagher EP. Mechanisms of aflatoxin carcinogenesis. Annual Review of Pharmacology And Toxicology. 1994;**34**:135-172

[90] Kobertz WR, Wang D, Wogan GN, Essigmann JM. An intercalation inhibitor altering the target selectivity of DNA damaging agents: Synthesis of site-specific aflatoxin B1 adducts in a p53 mutational hotspot. Proceedings of the National Academy of Sciences. 1997;**94**(18):9579-9584

[91] Larsson P, Busk L, Tjalve H. Hepatic and extrahepatic bioactivation and GSH conjugation of aflatoxin B1 in sheep. Carcinogenesis. 1994;**15**:947-955

[92] Gross-Steinmeyer K. Eaton DL. Dietary modulation of the biotransformation and genotoxicity of aflatoxin B(1). Toxicology. 2012;**299**:69-79

[93] Rao AV, Rao LG. Carotenoids and human health. Pharmacological Research. 2007;**55**(3): 207-216

[94] Gerster H. The potential role of lycopene for human health. Journal of the American College of Nutrition. 1997;**16**(2):109-126

[95] Rao AV, Agarwal S. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: A review. Nutrition Research. 1999;**19**(2):305-323

[96] Nguyen ML, Schwartz SJ. Lycopene: Chemical and biological properties. Food Technology (USA). 1999:**53**;38-45.

[97] Nguyen ML, Schwartz SJ. Lycopene stability during food processing. Proceedings of the Society for Experimental Biology and Medicine. 1998;**218**(2):101-105

[98] Trumbo PR. Are there adverse effects of lycopene exposure? The Journal of Nutrition. 2005;**135**(8):2060-2061

[99] Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. New York: Oxford University Press. Inc; 1999

[100] El-Agamey A, Lowe GM, McGarvey DJ, et al. Carotenoid radical chemistry and antioxidant/pro-oxidant properties. Archives of Biochemistry and Biophysics. 2004;**430** (1):37-48

[101] Krinsky NI, Yeum KJ. Carotenoid–radical interactions. Biochemical and Biophysical Research Communications. 2003;**305**(3):754-760

[102] Galano A, Francisco-Marquez M. Reactions of OOH radical with β-carotene, lycopene, and torulene: Hydrogen atom transfer and adduct formation mechanisms. The Journal of Physical Chemistry B. 2009;**113**(32):11338-11345

[103] Stahl W, Sies H. Lycopene: A biologically important carotenoid for human. Archives of Biochemistry and Biophysics. 1996;**336**(1):6

[104] Rao AV, Agarwal S. Effect of diet and smoking on serum lycopene and lipid peroxidation. Nutrition Research. 1998;**18**(4):713-721

[105] Matos HR, Di Mascio P, Medeiros MH. Protective effect of lycopene on lipid peroxidation and oxidative DNA damage in cell culture. Archives of Biochemistry and Biophysics. 2000;**383**(1):56-59

[106] Yilmaz S, Atessahin A, Engin S, Karahan I, Ozer S. Protective effect of lycopene on adriamycin-induced cardiotoxicity and nephrotoxicity. Toxicology. 2006;**218**(2-3):164-171

[107] Velmurugan B, Bhuvaneswari V, Burra UK, Nagini S. Prevention of N-methyl-N′-nitro-N-nitrosoguanidine and saturated sodium chloride-induced gastric carcinogenesis in Wistar rats by lycopene. European Journal of Cancer Prevention. 2002;**11**(1):19-26

[108] Di Mascio P, Kaiser S, Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Archives of Biochemistry and Biophysics. 1989;**274**(2):532-538

[109] Mortensen A, Skibsted LH. Relative stability of carotenoid radical cations and homologue tocopheroxyl radicals. A real time kinetic study of antioxidant hierarchy. FEBS Letters. 1997;**417**(3):261-266

[110] Tang L, Guan H, Ding X, Wang JS. Modulation of aflatoxin toxicity and biomarkers by lycopene in F344 rats. Toxicology and Applied Pharmacology. 2007;**219**(1):10-17

[111] Breinholt V, Lauridsen ST, Daneshvar B, Jakobsen J. Dose-response effects of lycopene on selected drug-metabolizing and antioxidant enzymes in the rat. Cancer Letters. 2000;**154**(2):201-210

[112] Karaca A. The Effect of Lycopene on Hepatotoxicity of Aflatoxin B1 in Rats. Director: Seval Yilmaz. University of Firat, Health Sciences Institute-Biochemitry, Elazig/Turkey, Master's Thesis; 2015

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