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Excessive Free Radicals Due to Ingestion of Aflatoxin (S) Contaminated Food Needs More Dietary Antioxidants

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Abstract:
Aflatoxin(s) have been extensively studied with respect to their occurrence and prevalence on wide range of agricultural commodities, foods and feedstuffs. Also, toxicity and carcinogenicity on different animal species and human were studied. Survey studies collected from different developed and developing countries exhibited that foods of both plant and animal origin had the same opportunity to be aflatoxin(s)-contaminated food. Most, if not all published studies agreed that aflatoxins B1, B2, G1 and G2 were the naturally occurring forms of aflatoxins, but aflatoxin B1 was proved to be the most common and potent form of aflatoxins which occurred naturally. Ingestion of aflatoxin(s)-contaminated food had certain metabolic pathways of ingestion and biotransformation. The highly oxygenated molecule of aflatoxin B1 (AFB1) take place the biotransformation processes of reduction, hydroxylation, hydrogenation aiming to yield less toxic and more water soluble metabolites as a body defense mechanisms of detoxification and elimination. Only, the biotransformation process of epoxidation to form AFB1- 2,3 epoxide yielded a more potent and highly reactive metabolite. Such high reactive metabolite of AFB1- 2,3 epoxide take place, certain bio-reactions of conjugation and adduct to protein fragments and nucleic acids. However, the metabolic pathways of aflatoxin(s) either yielded more or less toxic metabolites accompanied with excessive released free radicals. The difference is only related to the free radicals species, means that in case of transforming to less toxic metabolites, the reactive oxygen species (ROS) are dominating, while biosynthesis of AFB1- 2,3 epoxide accompanied with reactive nitrogen species (RNS). Recently, a variety of synthetic medicine employed in the treatment of free radicals. So, it's worthy to recommend suitable intake of fresh vegetables and fruits which are very rich in antioxidants and phytoconstituents.

Keywords: *Aflatoxin(s), Free radicals, antioxidants, biotransformation, phytoconstituents*

1. Introduction

Mycotoxins are highly toxic secondary metabolites naturally produced when certain species of fungi infected agricultural commodities, foods and feedstuffs. Aflatoxins, in particular, had continuously received the attention of many research studies, because of their wide-spread occurrence and the wide range of affected foods beside their potency and toxicity to all mammals and human-being (Sherif, 2003). Aflatoxins could be produced by certain species of *Aspergillus flavus* and/ or *Aspergillus parasiticus* when invade foods and feedstuffs. The most limiting environmental factors affecting and accelerating aflatoxins production were proved to be moisture content and relative humidity (Saad, 1991). So far, more than 20 derivative and metabolite of aflatoxins was identified and evaluated. There is evidence that only aflatoxin(s) B1, B2, G1 and G2 are the natural occurring aflatoxins on foods and feeds of plant origin, while aflatoxin(s) M1 and M2 were commonly found in milk and dairy products. Thus, aflatoxins M1 and M2 in milk of certain herbs of livestock is a proved indicator that such livestock was previously fed aflatoxins-contaminated ration (Saad, 1991). AFB1 was proved to be the parent compound and the most potent and toxic form of the naturally occurring aflatoxins, besides AFB1 is the most dominating form of contamination representing more than 60% of the aflatoxins contaminated foods and feeds (WHO, 1979). However, Ingestion of aflatoxins-contaminated diet leads to yield excessive free radicals as a result of the metabolic pathways and biotransformation processes of the contaminants. The highly oxygenated molecule of AFB1 goes through definite biotransformation processes of reduction, hydroxylation, hydrogenation, demethylation and epoxidation. All metabolic pathways, except epoxidation process, are the body immune response to detoxify and eliminate aflatoxin(s), but unfortunately the bio-processes are accompanied with excessive reactive oxygen species (ROS) free radicals. yielded more toxic and potent metabolite. The highly reactive metabolite of AFB1- 2,3 epoxide might adduct proteins and nucleic acids through certain successive reactions (Groopman et al., 1985). Such consequences of metabolic processes lead to liberate excessive reactive nitrogen species (RNS) free radicals. So, food of plant origin, in particular, fresh vegetables and fruits which are rich of antioxidants and constituents are recommended to get rid of, eliminate and/ or reduce the excessive released free radicals due to aflatoxin(s) and aflatoxicosis.

2. Metabolism and Biotransformation of Aflatoxin(S)

The metabolism of aflatoxin B1, the most common and toxic compound of aflatoxins has different metabolic pathways of reduction, epoxidation. The principle enzyme system involved is cytochrome P-450 dependent mixed function oxidase which is located in hypatocytes (Saad, 1991). The microsomal enzyme could oxidize AFB1 to AFM1, AFB2a, AFP1, AFQ1 and/ or AFB1-2,3-epoxide. While, reductases might reduce the parent compound of AFB1 to aflatoxicol 1 and aflatoxcol 2. The obtained metabolites had a wide range of toxicity, while aflatoxin M1 had the same potency and toxicity of AFB1, aflatoxin B2a considered to be non-toxic metabolite (Saad, 1984). Only, AFB1-2, 3-epoxide represent the most toxic and activated form of aflatoxin(s) (Wong & Hsieh, 1978). The hydroxylated metabolites resulted from the first phase undergo phase 2 reactions to enhance elimination and excretion processes through conjugation to some endogenous compounds such as glucoronic acid, sulphates and glutathione. The two phases of biotransformation were actively observed in human-being leading to suggest that humans may be relatively resistant species to both the acute and chronic effects of aflatoxin(s) (Ueng et al., 1995).

Comparative studies revealed that the metabolic processes and biotransformation consequences are completely species dependent. From the available literature, it appears that AFP1 and its conjugated products are the main metabolites excreted in mice, while AFQ1 found to be the major detoxified metabolite in both Hamster and Monkey (Masri et al., 1974).

The metabolite of AFB1-2, 3-epoxide had been suspected as the most potent form of aflatoxins due to capable covalent binding to nucleic acids and protein fragments of the cell (WHO, 1981). Thus, there are 3 proposed routes for the degradation of AFB1-2,3-epoxide; 1) hydration to form AFB1-dihydrodiol (DHD), 2) conjugation to form glucoronides, sulphates or glutathione (GSH) – conjugates and 3) covalent binding to nucleophilic macromolecules such as RNA, DNA and proteins (Ramsdell et al., 1991). It was concluded that AFB1-2,3-epoxide was the precursor of AFB1-DHD during hydrolysis processes. It's now believed that AFB1-DHD is the intermediate metabolite in the formation of AFB1-protein adducts (Saad, 1984). Thus, the formation of AFB1- protein adducts inhibited protein synthesis which therefore gives rise to the various biological effects of aflatoxins. On the other hand, conjugation of the aflatoxin(s) metabolites with glucoronic acid, sulphates, mercapturic acid or glutathione resulted more soluble derivatives which are easily eliminated and excreted outside the body. So, more susceptible species such as rat and monkey produced lower levels of conjugates due to its lower possibility of formed hydroxyl groups (Groopman et al., 1984).

3. Biological Effects and Toxicity of Aflatoxin(s)

The biological effects of the common and naturally occurred contaminant of AFB1, both acute and chronic, were varied between exposed species (Hsieh et al., 1974). Acute toxicity of AFB1 is characterized by acute hepatitis, internal hemorrhage and sudden death (Ueng et al, 1995). Liver is proved to be the most affected organ in all studied species (Saad, 1991). So far, AFB1 is reported as one of the most potent hepatic-carcinogen. Fatty liver is one of the features of aflatoxicosis. The lipid content of the liver is increased the ability of the animals to synthesize lipids as demonstrated in different studies on ducklings, rats, chicken and human as well (Diaz et al., 1972). Also, AFB1 had an effect on levels of protein serum and the most sensitive component was serum albumin, which significantly decreased when digested AFB1-contaminated diet. The biological and toxic effects of AFB1 in man have been extrapolated from human incidents and etiological studies, most of these studies focused on liver diseases. Thus aflatoxins are strongly associated with liver damage leading to Encephalopathy and fatty degeneration of Rye's syndrome (Saad, 1991). Evidence is now confirmed that other organs have been known to be affected. Immunosuppresion is caused through the effects of AFB1 on thymus gland, and consequently the cell mediated immune system (Saad , 1984). As well, aflatoxicosis might have an effect on antibody formation and interference with the immune responses due to the process of protein synthesis inhibition (Stollof, 1977).

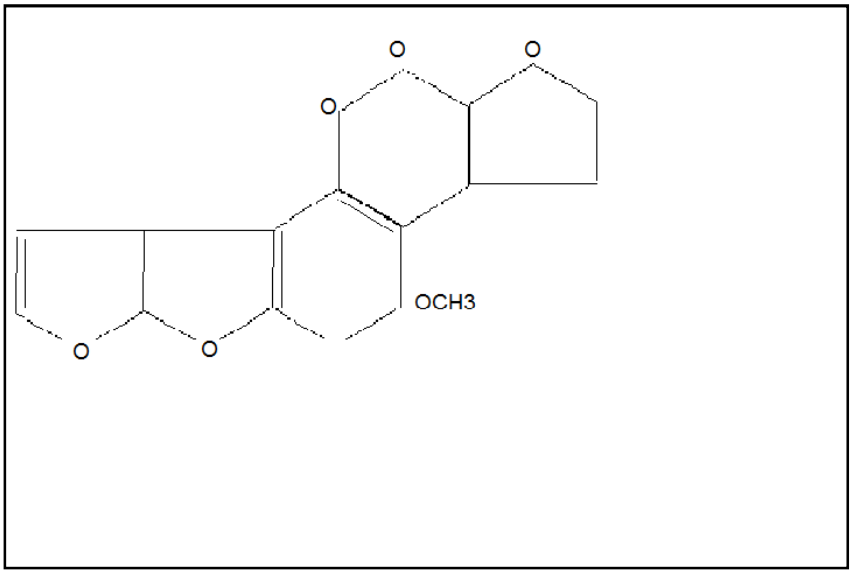

Figure 1: The highly oxygenated molecule of aflatoxin B1

Food Contaminants/ molecular wt.	Biotransformed metabolites/ molecular wt.	Expected free radicals	Toxicity vs. AFB1
AFB1 (312)Main	Aflatoxicol1(314)	O``	Less toxic
AFB2 (314)	Aflatoxicol2(314)	OH`	Less toxic
AFG1 (328)	AFB2a (330)	RO`	Less toxic
AFG2 (330)	AFG2a (330)	HO2`	Less toxic
AFM1 328) Dairy	AFP1 (298)	RO`	Less toxic
AFM2 (330)	AFQ1 (328)	ROO`	Less toxic
AFB1 (312)	AFB1 (312)	----	The same
AFB1 (312)	AFB1-2,3 epoxide (328)	OH``	More toxic
AFB1 (312)	AFB1- Di-hydro-Diol (DHD)	O`` RO``	Less toxic
	AFB1-DNA add.	NO``	More toxic
	AFB1-RNA add.	HNO2``	More toxic
	AFB1-Protein adduct	N2O3``	More toxic

Table 1: Aflatoxin(s) intake, biotransformed metabolites, expected released free radicals and toxicity vs. AFB1

4. Prospective Free Radicals

Recently, within the last decade free radicals and their harmful effects were discovered and reported. Free radicals are dangerous substances produced normally during the normal metabolic pathways in the body (sisein, 2014). Overproduction of the free radicals due to ingestion of naturally contaminated food might be responsible for tissue injury (SaikatSen et al., 2010). Oxidative damage of cell membranes due to unsaturated lipids peroxidation of the cell walls leads to death of cell. Undoubtedly, oxygen is an obligatory element for life of all living organisms. Oxygen has double-edged properties, being essential for survival and also, it can aggravate the damage within the cell by oxidative processes. It's worthy to mention that the most sensitive biological molecules of RNA, DNA and protein enzymes are more exposed to oxidative damage (Sisein, 2014). Free radicals are simply defined as molecular fragments with one or more unpaired electrons in the outer orbital shell and are capable of independent existence. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have different degrees of reactivity. Continuous oral exposure to aflatoxins-contaminated diet leads to generate excessive free radicals, generally involved in chain reactions. However, since free radicals are formed as an initiation step, the second step is followed to regenerate repeatedly free radicals as a result of chain reactions. So, there is a need for termination step to destruct such free radicals (Saikatsen et al., 2010). A variety of synthetic medicine employed in the treatment of different disadvantages capable to generate excessive amounts of free radicals in the body.

5. Antioxidants and Phytoconstituents

Antioxidants and phytoconstituents are a variety of components act against free radicals to neutralize them. These components include; 1) endogenous enzymatic antioxidants like, superoxide dismutase (SOD), 2) non-enzymatic metabolic and nutrient antioxidants, 3) metal binding proteins like ferritin and 4) phytoconstituents and phytonutrients (Mandal et al., 2009). Fortunately, the body produces different endogenous antioxidants to protect and maintain tissues and cells. Such endogenous antioxidants classified into 2 main groups, enzymatic and non-enzymatic. The enzymatic group includes superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx). While, the non-enzymatic group include the vitamins of C and E and reduced glutathione. The enzyme superoxide dismutase (SOD) has an important role acting as a first line defense system against all reactive oxygen sources (ROS) including those released during biotransformation processes of AFB1, which scavenges superoxide radicals to H2O2. Also, glutathione, as a tripeptide, is a powerful antioxidant capable of scavenging ROS directly or enzymatically via glutathione peroxidase (Percival, 1998).

However, the exogenous antioxidants, which can 'not be produced internally, and should externally supply to the body through food, plays an important role to protect body cells and tissues. Such exogenous antioxidants provided trough diet or supplements included; trace elements (zinc – manganese – selenium), flavonoids, phenols, tannins, coumarins, lignins, carotenes and terpenoids. Omega-3 and omega-6 fatty acids and vitamins of C and E (Mark, 2013). It's worthy to mention that many synthetic antioxidant compounds have shown toxic and/ or mutagenic effects, which have stimulated the interest of many researchers and investigators to search for natural antioxidants (SaikatSen, 2010). Foods of plant origin, in particular, fresh vegetables and fruits which are very rich in minerals, vitamins and phytoconstituents could act as free radical scavengers and inhibitors of lipid peroxidation might be the choice. In other words, suitable intake of fresh vegetables and fruits is recommended to avoid the disadvantages of synthetic antioxidants and the risk of excessive free radicals accompanied the metabolic and biotransformation processes of the natural contaminants of aflatoxins.

6. References

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