Dietary factors affecting aflatoxin Bi carcinogenicity

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ABSTRACT

This review paper describes briefly on the history of aflatoxins, the metabolism of aflatoxin B\textsubscript{1} (AFB\textsubscript{1}) that leads to the activation and detoxification of AFB\textsubscript{1}, and the findings of some of the studies relating to food nutrients and additives, and drugs on AFB\textsubscript{1} carcinogenicity and detoxification. Aflatoxins have been linked to many public health problems, especially to liver cancer incidences, in different parts of the world. Many studies have shown the potential of dietary factors modulating the formation of AFB\textsubscript{1} - DNA adduct, the initial and important step of AFB\textsubscript{1} carcinogenesis process. Among the food nutrients that have been shown to reduce the binding of AFB\textsubscript{1} to DNA are vitamin A, vitamin C and riboflavin. On the contrary, vitamin E and \(\beta\)-carotene increase the DNA binding. Choline-deficient animals when subjected to multiple doses of AFB\textsubscript{1} had higher amount of the DNA adduct being formed than the choline-sufficient animals. Carnitine supplement, feed restriction, and some vegetables and their extracts can also decrease the AFB\textsubscript{1} -DNA adduct formation. The observed and proposed mechanisms for the reduction include the inhibition of bioactivation of AFB\textsubscript{1} and induction of glutathione S-transferase activity that detoxify the activated AFB\textsubscript{1}. However, more research is needed before nutritional recommendations could be given to the public to control AFB\textsubscript{1} toxicity and carcinogenicity.

INTRODUCTION

History of Aflatoxins

Aflatoxins are a group of mycotoxins produced by the molds \textit{Aspergillus flavus} and \textit{A. parasiticus}. They are commonly found to contaminate food and feeds, such as milk, corn, peanuts, cottonseed, rice, and barley, grain-fermented beverages and edible animal tissues (Park & Pohland, 1986).

Aflatoxins were first discovered in 1960 when a series of outbreaks in poultry and fish occurred in different parts of the world. One of the worst outbreaks was the “Turkey-X” disease that caused the deaths of many turkeys, ducklings, and chicks in Britain (Blount, 1961). Consumption of aflatoxin-contaminated Brazilian groundnut meal was implicated in the disease. At the same time, feeding of contaminated corn, peas and cottonseed to farm animals and fish were reported to cause outbreaks analogous to the “Turkey-X” disease elsewhere (Palmgren & Ceigler, 1983).
Experiments conducted on the contaminated Brazilian peanut meal resulted in the isolation of A. flavus, and when the fungus was inoculated into untainted peanut meal, the fungus produced toxins similar to those found in the contaminated meal (Sargeant et al., 1961). The isolated toxins were named “aflatoxin.”

The current technology and knowledge can neither totally inhibit aflatoxins synthesis by the molds nor completely eliminate them once they are produced. As a result, the significance of aflatoxins contamination has long been recognized, and limits in agricultural commodities have been set since 1965. In 1993, the International Agency for Research on Cancer upgraded AFB₁ from a Group II to a Group I human carcinogen classification (IARC, 1993).

**Structure and Toxicity**

Aflatoxin B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁), and G₂ (AFG₂) are the four main naturally-occurring aflatoxins. The letters B and G refer to the aflatoxins’ color under UV light (B for Blue; G for Green), and the subscripts 1 and 2 refer to their relative positions on a developed thin-layer chromatography plate. The structure of aflatoxins consists of a coumarin nucleus attached to a bifuran and either pentanone (AFB₁ and AFB₂) or a six-membered lactone (AFG₁ and AFG₂) (Figure 1). AFB₁ and AFG₁ are more toxic to rats and ducklings as compared to AFB₂ and AFG₂ (Wogan, Edwards & Newberne, 1971). As for carcinogenicity, AFB₁ is more carcinogenic than AFG₁, while AFG₁ is more carcinogenic than AFB₂ (Shoenhard et al., 1981). The main target organs for AFB₁ toxic and carcinogenic effects are the liver and kidney.

![Figure 1: Structures of naturally occurring aflatoxins](image-url)
AFB$_1$ Metabolism and Carcinogenicity

AFB$_1$ is the most abundant and toxic form of all naturally occurring aflatoxins. AFB$_1$ represents 75% of all aflatoxins found in contaminated food and feeds. It is hepatotoxic (O’Brien et al., 1983), hepato-tocarcinogenic (Adamson et al., 1979), and teratogenic (Bassir & Adekunle, 1970) to various animal species. AFB$_1$ is first metabolized (Phase I metabolism) mainly by the cytochrome P-450 enzyme (CYP450) system found in the microsome. This metabolism will produce a variety of metabolites such as AFB$_1$-epoxide and hydroxylated metabolites (AFM$_1$, AFP$_1$, AFQ$_1$, and aflatoxicol).

AFB$_1$-epoxide is a very reactive and unstable metabolite of AFB$_1$ that will bind to cellular DNA, RNA, and protein. The formation of AFB$_1$-DNA adduct is highly correlated to the carcinogenic effect of AFB$_1$ in both animal and human cancer cases (Thabrew & Bababumi, 1980; Wogan et al., 1971; Alpert et al., 1971; Groopman, Cain, & Kensler, 1988). The “Virtually Safe Dose” of AFB$_1$ is estimated at 0.016 ng/kg/day (reviewed by Eaton & Gallagher, 1994).

The major AFB$_1$-DNA adduct formed with liver DNA is aflatoxin B$_1$-N$^7$-guanine (AFB$_1$-N$^7$-gua) (Essigmann et al., 1977). This adduct is unstable and subjected to decomposition. The major decomposed derivatives of AFB$_1$-N$^7$-gua in rat liver are the imidazole ring-opened AFB$_1$ formamido-pyrimidine adducts, namely AFB$_1$-N$^7$-FAPY major and minor (Croy & Wogan, 1981) (Figure 2). AFB$_1$-N$^7$-FAPY adducts are more stable, and their accumulation in liver DNA is related to the subsequent reduction of AFB$_1$-N$^7$-gua adduct level.

Hepatocellular carcinoma (HCC) is a major health problem in China where each year approximately 110,000 patients are diagnosed with it. The HCC cases in China account for almost 45% of HCC incidences in the world (Parkin, Sternward & Muir, 1984). The mortality rate for HCC is more than 95%. Excluding other risk factors, the consumption of AFB$_1$-contaminated food such as corn, soya-based products, and peanut oil was correlated...
(r = 0.55) to the HCC fatality rates in people living in ten Chinese villages that were studied. See Yu (1995) for a current review of HCC in China.

The phase I AFB\textsubscript{1} metabolites may undergo phase II biotransformation involving the enzymes glutathione S-transferase (GST), β-glucuronidase, and/or sulfate transferase which produce conjugates of AFB\textsubscript{1}-glutathione, AFB\textsubscript{1}-glucuronide, and AFB\textsubscript{1}-sulfate, respectively. The major conjugate of AFB\textsubscript{1}-epoxide identified is the AFB\textsubscript{1}-glutathione conjugate (Monroe & Eaton, 1987; O’Brien et al., 1983). This conjugation is the principal detoxification pathway of activated AFB\textsubscript{1} in many mammals. It has been accepted that cytosolic GST activity is inversely correlated to susceptibility of the several animal species to AFB\textsubscript{1} carcinogenicity (Eaton & Gallager, 1994; Neal, 1987).

The hydroxylated (AFM\textsubscript{1} and AFQ\textsubscript{1}) and O-demethylated (AFP\textsubscript{1}) metabolites of AFB\textsubscript{1} can undergo glucuronidation and sulfation. Glucuronidation, catalyzed by liver microsomal UDP-glucuronyl transferase (UDPGT), has been reported for a variety of endogenous and foreign compounds (Burchell & Coughtrie, 1989). These conjugations results in formations of water-soluble aflatoxin esters that are excreted in the urine or bile (Hseih & Wong, 1982).

**AFB\textsubscript{1} and Diet Interactions**

Species differences, nutritional manipulations, health status, drugs, and chemical treatments affect AFB\textsubscript{1} biotransformations, and thus, its potency. There are many reports on the effects of various foods or nutrients and xenobiotics on AFB\textsubscript{1}-macromolecule adducts formation. Obviously, the major objectives of these studies were to determine if and how those nutrients or xenobiotics could affect adducts formation, especially DNA adduct. Tables 1 and 2 summarize some of the effects of nutritional factors and drugs or xenobiotics on the formation of AFB\textsubscript{1}-DNA adduct.

**Table 1:** Influences of dietary nutrients on AFB\textsubscript{1}-DNA adducts formation.

<table>
<thead>
<tr>
<th>Compound*</th>
<th>Test System</th>
<th>Increase (↑) / Decrease (↓)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Protein</td>
<td>Rat liver</td>
<td>↑</td>
<td>Mandell et al. (1992)</td>
</tr>
<tr>
<td>Low fat (high carbohydrate)</td>
<td>Rat liver</td>
<td>↑</td>
<td>Nyathi et al. (1993)</td>
</tr>
<tr>
<td>Fat (saturated or unsaturated)</td>
<td>Rat liver</td>
<td>NSE*</td>
<td>Marzuki &amp; Norred (1984)</td>
</tr>
<tr>
<td>Essential oils</td>
<td>Rat liver microsome</td>
<td>↓</td>
<td>Hashim et al. (1994)</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>Rat liver</td>
<td>↓</td>
<td>Bhattacharya et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>Rat liver microsome</td>
<td>↓</td>
<td>Aboobaker et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>Rat liver</td>
<td>NSE*</td>
<td>Chen et al. (1982)</td>
</tr>
<tr>
<td></td>
<td>Woodchuck hepatocyte</td>
<td>↓</td>
<td>Yu et al. (1994)</td>
</tr>
<tr>
<td>β-carotene</td>
<td>Woodchuck hepatocyte</td>
<td>↑</td>
<td>Yu et al. (1994)</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>Rat liver</td>
<td>↓</td>
<td>Gradelet et al. (1998)</td>
</tr>
<tr>
<td>Vitamine E</td>
<td>Woodchuck hepatocyte</td>
<td>↑</td>
<td>Yu et al. (1994)</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>Rat liver</td>
<td>↓</td>
<td>Webster et al. (1996)</td>
</tr>
<tr>
<td>Vitamin B\textsubscript{6}</td>
<td>Rat liver microsome</td>
<td>NSE*</td>
<td>Bhattacharya et al. (1984)</td>
</tr>
<tr>
<td>Thiamin</td>
<td>Rat liver microsome</td>
<td>NSE*</td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Woodchuck hepatocyte</td>
<td>↓</td>
<td>Yu et al. (1994)</td>
</tr>
<tr>
<td>Lipotropes (deficient)</td>
<td>Rat liver</td>
<td>↓</td>
<td>Campbell, Hayes &amp;</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Compound</th>
<th>Test System</th>
<th>Increase (↑) / Decrease (↓)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carnitine</td>
<td>Rat liver</td>
<td>↘</td>
<td>Newbem (1978)</td>
</tr>
<tr>
<td>Choline (deficient)</td>
<td></td>
<td></td>
<td>Sachan &amp; Ayub (1992)</td>
</tr>
<tr>
<td>Rat liver</td>
<td></td>
<td></td>
<td>Bhattacharya et al. (1984)</td>
</tr>
<tr>
<td>- Single AFB$_1$ dose</td>
<td></td>
<td></td>
<td>Schrager et al. (1990)</td>
</tr>
<tr>
<td>- Multiple AFB$_1$ doses</td>
<td></td>
<td></td>
<td>Schrager et al. (1990)</td>
</tr>
<tr>
<td>Copper</td>
<td>Rat liver microsome</td>
<td>↑</td>
<td>Bhattacharya et al. (1984)</td>
</tr>
<tr>
<td>Selenium (Excess or deficient)</td>
<td>Chick liver</td>
<td>NSE</td>
<td>Chen et al. (1982)</td>
</tr>
<tr>
<td>Selenium</td>
<td>Hamster ovary cells</td>
<td>NSE</td>
<td>Shi et al. (1995)</td>
</tr>
<tr>
<td>Feed restriction</td>
<td>Rat liver</td>
<td>↓</td>
<td>Pegram et al. (1989)</td>
</tr>
<tr>
<td>Indole-3-carbinol</td>
<td>Trout liver</td>
<td>↓</td>
<td>Dashwood et al. (1989)</td>
</tr>
<tr>
<td>R-goitrin</td>
<td>Rat liver</td>
<td>↓</td>
<td>Chang &amp; Bjeldanes (1987)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Rat liver microsome</td>
<td>↓</td>
<td>Firozi et al. (1996)</td>
</tr>
<tr>
<td>Garlic compounds</td>
<td>Rat liver S-9 fraction</td>
<td>↓</td>
<td>Tadi, Teel &amp; Lau (1991)</td>
</tr>
<tr>
<td>Green tea</td>
<td>Rat liver</td>
<td>↓</td>
<td>Guozhong et al. (1997)</td>
</tr>
<tr>
<td>Coffee extracts</td>
<td>Rat liver fractions</td>
<td></td>
<td>Cavin et al. (1998)</td>
</tr>
</tbody>
</table>

*NSE, no significant effect

Table 2: Influences of food additives and drugs on AFB$_1$-DNA adducts formation.

* BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DDB; dimethyl-4-4' dimethoxy-5, 6, 5'-6'-'dimethylenedioxy biphenyl-2,2' dicarboxylate.

**NSE no significant effect

Fat-soluble vitamins
A number of vitamins and vitamin analogs have been tested on \(\text{AFB}_1\)-macromolecule adduct formation. Bhattacharya and coworkers (1984; 1987) reported comprehensive studies on the effects of various vitamins on in vitro adducts formation.

Vitamin A supplementation in rats inhibited \(\text{AFB}_1\)-DNA binding (Bhattacharya, Prabhu & Aboobaker, 1989). The protective effects of retinoids such as retinol, retinal, retinoic acid, and retinal esters on \(\text{AFB}_1\) carcinogenicity were due to inhibition of \(\text{AFB}_1\)-DNA adduct formation by affecting the CYP450 systems resulting in less epoxide being formed (Bhattacharya et al., 1984; Aboobaker et al., 1997). Retinal had the same inhibitory effect on the formation of \(\text{AFB}_1\)-protein adducts (Bhattacharya et al., 1989). Vitamin A has been shown to induce the activity of glutathione S-transferase, thereby enhancing the detoxification of \(\text{AFB}_1\)-epoxide. On the other hand, vitamin A deficiency decreased glutathione S-transferase activity.

A combined deficiency of vitamin E and selenium decreased \(\text{AFB}_1\) binding to DNA, RNA, and protein (Chen et al., 1982). Vitamin E and menadione (a water-soluble synthetic vitamin K) have been found to prevent \(\text{AFB}_1\)-induced mutagenesis in the Ames bacterial system (Raina & Gurto, 1985). \(\beta\)-carotene and vitamin E increased DNA adduct formation in woodchuck hepatocytes (Yu et al., 1994).

**Water-soluble vitamins**

Riboflavin, riboflavin-5’-phos-phate (FMN), and flavin adenine dinucleotide (FAD) inhibited \(\text{AFB}_1\)-DNA adduct formation in vitro. Riboflavin was reported as the most effective of the three vitamins (Bhattacharya et al., 1984). It has been recently suggested that the mechanism for the riboflavin effect is its ability to induce the enzymes involved in repairing damaged DNA (Webster, Gaude & Bhattacharya, 1996).

Vitamin C, vitamin \(\text{B}_6\) and thiamin had no significant effect on DNA adduct production (Bhattacharya et al., 1984). However, vitamin C, \(\text{B}_6\), and folic acid inhibited mutagenesis in bacterial systems (Bhattacharya et al., 1984; Bhattacharya et al., 1987). The inhibition by vitamin C was not as great as with the fat-soluble vitamins.

In a study employing woodchuck hepatocytes to find the role of vitamins A, C, and E, and \(\beta\)-carotene on the initiation of \(\text{AFB}_1\)-induced carcinogenesis, the workers found that vitamin A was more effective than vitamin C in inhibiting DNA adduct formation. In contrast, vitamin E and \(\beta\)-carotene enhanced the binding (Yu et al., 1994). However, a current study reported that carotenoids were effective in lowering \(\text{AFB}_1\)-DNA adduction in rats. This reduction was due to the enhancement of the detoxification of the activated \(\text{AFB}_1\) (Gradelet et al., 1998). Therefore, these results suggest that different antioxidant vitamins may effect \(\text{AFB}_1\)-DNA binding differently.

**Amino acids**

There are conflicting reports on the effects of different amino acids on \(\text{AFB}_1\) carcinogenesis. A diet marginally deficient in methionine (which was also deficient in choline and lacking in folacin) depressed DNA and RNA adducts formation in rat liver. Protein adduct formation was
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not affected by the diet. The inhibition of AFB₁-nucleic acid adducts in the marginally lipotrope-deficient diet was due to the decrease in the activation of AFB₁ and not due to an increase in glutathione levels (Campbell, Hayes & Newberne, 1978). A choline and methionine-deficient diet fed rats showed no significant AFB₁ dose-response changes in serum biochemical parameters or liver pathology compared to the complete amino acid diet (Mehta et al., 1993). These two studies treated the rats with a single dose of AFB₁.

Schrager et al (1990) found that a single dose of AFB₁ did not affect the DNA adduct concentration in both choline-deficient and control animals. However, when multiple doses of AFB₁ were administered, the AFB₁-DNA adduct levels were significantly higher in the rats fed a choline-deficient diet than in the rats fed with a control diet. An earlier report also found that marginally deficient lipotrope diets induced AFB₁ tumorigenesis in rats (Rogers & Newberne, 1969).

L-carnitine supplementation in rats has been found to decrease AFB₁-DNA binding in rats (Sachan & Ayub, 1992). Carnitine, a quaternary amine whose structure is quite similar to choline, can also affect AFB₁ binding to hepatic RNA and protein (Table 3). The total amount of AFB₁ present in the liver and kidney were not significantly different between the carnitine supplemented and control animals. However, the concentrations of AFB₁ were higher in the plasma of carnitine supplemented rats than the non-supplemented rats. Recently, we found that carnitine inhibited the microsomal activation of AFB₁ and on the binding of activated AFB₁ to endogenous (calf thymus) DNA (Ayub & Sachan, unpublished data). This maybe the mechanisms for carnitine reducing the AFB₁-DNA adducts formation. We had also shown that carnitine ameliorated the earlier signs of acute toxicity of AFB₁, such as the elevation of total lipid concentration in the liver and the decrease in total lipids and triacylglycerol concentrations in the plasma (Sachan & Ayub, 1991; Sachan & Ayub, 1992).

Sulfur-containing amino-acids such as cysteine, N-acetyl cysteine, cystine, methionine, and glutathione inhibited AFB₁ mutagenicity in microbial systems. Cysteine and N-acetylcysteine were more potent inhibitors than glutathione. The investigators suggested that the inhibition was due to amino acids affecting the synthesis of AFB₁-epoxide (Shetty Francis & Bhattacharya, 1989).

### Table 3: Effects of L-carnitine supplement on aflatoxin B₁-macromolecules adduct formation in rat liver 6-h post-aflatoxin B₁ administration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control pmol/mg macromolecules</th>
<th>L-Carnitine pmol/mg macromolecules</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB₁-DNA</td>
<td>6.8 ± 28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7 ± 2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.9</td>
</tr>
<tr>
<td>AFB₁-RNA</td>
<td>21.6 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.3 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.8</td>
</tr>
<tr>
<td>AFB₁-Protein</td>
<td>1.2 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
</tr>
</tbody>
</table>

1 Values are mean ± SEM, n = 5.
2 Diet contained 0.4% L-carnitine (w/w) and given to animals for 6 weeks.
3 Different letters indicate significant difference between groups (p <0.05).

(Source: Sachan & Ayub 1992)
**Protein**

Weanling rats fed a low protein diet (5% casein) had fewer AFB1-induced preneoplastic foci in their livers than rats fed a high protein diet (20% casein) (Youngman & Campbell, 1992). The enhanced development of the preneoplastic foci (g-glutamyl transpeptidase-positive foci) by the high protein diet was reversed when the animals were put on a low protein diet. Thus, it was concluded that the low protein diet prevented lesions caused by AFB1.

Mandell *et al.*, (1992) reported that AFB1-induced hepatocarcinogenicity can occur in both low-and high-protein fed weanling animals. Moreover, the low protein diet (5% lactalbumin) also caused severe liver histopathological changes or sub-acute toxicity symptoms such as necrosis and bile duct proliferation due to AFB1. Also, the protein-deficient animals had a more rapid decrease in glutathione S-transferase activity than the protein-sufficient (20% lactalbumin) animals. High protein fed animals did not show the sub-acute toxicity responses induced by AFB1. Therefore, the authors suggested that protein deficiency is more likely to enhance, rather than protect the liver against, AFB1 toxicity and carcinogenicity.

**Fat and Essential Oils**

Different types and amounts of fat may have different effects on the carcinogenesis of AFB1. High polyunsaturated oil (corn oil) increased the incidence of liver cancer in rats caused by AFB1 as compared to rats fed with saturated oil (beef fat). The cancer incidence was higher when the corn oil was fed with or after the exposure to AFB1 than when the oil was fed before the AFB1 dose. An increase in induction of AFB1 activation by corn oil was suggested for the high cancer incidence (Newberne, Weiger & Kula, 1979). However, in a similar study, dietary saturated (coconut oil) and unsaturated (corn oil) fats were found to have no significant effect on the adduct formation and the production of AFB1-epoxide in rat livers (Marzuki & Norred, 1984).

In another report, the metabolism and mutagenicity of AFB1 were not significantly different between mice fed with beef fat and olive oil diets (Brennan-Craddock *et al.*, 1990). With respect to the levels of dietary fat, a low-fat (high carbohydrate) diet increased the AFB1-DNA binding more than a high fat diet (Nyathi *et al.*, 1993). The protective effect of a high fat diet may be due to a decrease in the uptake of AFB1 into hepatocytes or a reduction of AFB1-epoxide production.

Hashim *et al.* (1994) investigated the capability of essential oils extracted from nutmeg, ginger, cardamom, celery, xanthoxylum, coriander, cumin, and black pepper to inhibit AFB1-DNA adducts formation mediated by liver microsomal enzymes. All the essential oils tested were suppressive to the adducts formation, and the inhibition was dose-dependent. The modulating effect of these oils was through their ability to inhibit the activation of AFB1.

**Trace Elements**

Copper inhibited AFB1-DNA binding in vitro (Bhattacharya *et al.*, 1984). A deficiency and an excess of selenium decreased the adduct formation in rats. In chicks, however, excess of selenium did not change the concentration of adducts formed (Chen *et al.*, 1982). Recently,
selenium was demonstrated to have no effect on DNA adduct formation in ovary cells and did not effect AFB$_1$ mutagenesis (Shi, How & Ong, 1995).

Copper, manganese, zinc, and selenium were effective in preventing in vitro AFB$_1$-induced mutagenesis. Copper was the most potent among the elements tested. To a lesser extent, iodine, molybdenum, cobalt, and iron were antimutagenic. The investigators suggested that the inhibition was due to interaction of trace elements with the microsomal enzymes (Francis, Shetty & Bhattacharya, 1988).

**Feed Restriction**

The potentially protective effects of caloric restriction on cancer-causing compounds has promoted considerable interest and investigation. It has been reported that rats fed with 60% of the food consumed by ad libitum animals had lower AFB$_1$ microsomal activation, lower AFB$_1$-adducts, faster plasma clearance, and increased urinary excretion of AFB$_1$ than the ad libitum fed animals. The authors concluded that 40% feed restriction may decrease AFB$_1$ carcinogenicity (Pegram, Allaben & Chow, 1989).

Similarly, about more than 50% reduction in AFB$_1$-DNA binding was found when rats were fed 40% caloric restricted diet (Gao & Chou, 1992; Chou *et al.*, 1997). The restriction also decreased the hepatic DNA double strand damage induced by AFB$_1$. Induction in glutathione S-transferase activity in feed restriction will enhance AFB$_1$-epoxide conjugation to GSH and thus reduce AFB$_1$-DNA adducts formation (Chen *et al.*, 1995).

**Cruciferous Vegetables**

Cruciferous vegetables have been shown to enhance detoxification of xenobiotics by inducing xenobiotic-metabolizing enzymes in animals and humans (Salbe & Bjeldanes, 1989). High consumption of vegetables such as broccoli, cabbage, cauliflower, and Brussels sprouts has been related to a reduced risk of bladder, colon, and rectum cancers (Grahams, 1983).

Brussels sprouts significantly decreased AFB$_1$-DNA binding and increased the GST activity in rats. Indole-3-carbinol, a compound found in cruciferous vegetables, did not have much effect on the DNA binding and GST activity (Salbe & Bjeldanes, 1989). The same investigators also found that the route of administration, intragastric or intraperitoneal, did not have a different effect on AFB$_1$-DNA binding. Thus, they concluded that the small intestine did not play an important role in AFB$_1$ metabolism. However, in another study that utilized trout, 1000 and 2000 ppm of indole-3-carbinol were shown to strongly depress AFB$_1$-DNA adducts formation (Dashwood *et al.*, 1989). R-goitrin, another compound found in cruciferous vegetables, also exhibited anticarcinogenic properties such as inhibition of AFB$_1$-DNA binding, induction of GST activity, and enhancement of biliary excretion of AFB$_1$ in rats (Chang & Bjeldanes, 1987).

**Plant Flavonoids and Phenolic Compounds**

Five major derivatives of plant flavonoids, namely flavone, flavonol, isoflavone, and flavanol, have been tested on activation of AFB$_1$ and AFB$_1$-DNA adducts formation (Bhattacharya &
Most of the flavonoid derivatives significantly inhibited adduct formation, and flavonols being the most potent. Flavonols also showed greater inhibition of AFB\(_1\) mutagenicity in bacterial system (Goeger \textit{et al}, 1988).

Phenolic compounds may have protective effects against AFB\(_1\)-induced mutagenicity. Gallic acid, chlorogenic acid, caffeic acid, dopamine, p-hydroxybenzoic acid, and salicylic acid decreased mutation caused by AFB\(_1\) in bacterial system containing rat-liver microsomes. The inhibition occurred when the compounds and AFB\(_1\) were administered concurrently (San & Chan, 1987). Using the S9 liver fraction that contains the metabolic enzymes, ellagic acid (a compound found in strawberries, grapes, and walnuts) has been shown to be antimutagenic against AFB\(_1\) in bacterial assay (Loarca-Pina \textit{et al}., 1996). The inhibition was greatest when the acid was incubated together with AFB\(_1\).

Curcumin, a phenolic compound extracted from tumeric, was recently reported to inhibit the production of AFB\(_1\)-epoxide by affecting CYP450 enzyme function (Firozi, Aboobaker, Bhatacharya, 1996). The inhibition became higher as the curcumin concentration was increased in the incubation mixture. However, the suppression was reversed when the CYP level in the mixture was higher.

Diterpenes cafestol and kahweol extracted from coffee were shown to inhibit AFB\(_1\)-DNA covalent binding in rat liver fractions. The decrease in activation of AFB\(_1\) and induction of GST expression were the suggested mechanisms of the inhibition (Cavin \textit{et al}., 1998). Green tea drink fed to rats also inhibit AFB\(_1\)-DNA binding by affecting AFB\(_1\) metabolism (Guozhong \textit{et al}., 1997).

**AFB\(_1\) and Food Additives/Drugs Interactions**

**Food Additives**

Rats fed a butylated hydroxyanisole (BHA)-containing diet had lower AFB\(_1\)-DNA binding, higher GST activity, and higher biliary excretion of AFB\(_1\) (Chang & Bjeldanes, 1987). Animals treated with butylated hydroxytoluene (BHT) before or together with AFB\(_1\) had lower cancer incidences than the animals administered AFB\(_1\) alone (Dragon and Pitot, 1994). However, in trout, BHA did not effect liver tumor incidence, AFB\(_1\)-DNA binding, or AFB\(_1\)-glutathione conjugation (Goeger \textit{et al}., 1988). In vitro system, both BHA and BHT inhibited AFB\(_1\)-DNA binding (Bhattacharya \textit{et al}., 1984). Another antioxidant, ethoxyquin, also suppressed AFB\(_1\) carcinogenesis by inducing the activity of glutathione S-transferase activity (Kensler \textit{et al}., 1986).

**Drugs**

The activities of CYP, GST, and UDPGT enzymes can be induced by several drugs or xenobiotics. Enzyme inducing drugs such as pheno-barbital (anti-seizure drug) and Aroclor 1254, given before or together with AFB\(_1\), reduced the number of neoplasms as compared to animals given AFB\(_1\) only (Dragan and Pitot, 1994). Although phenobarbital enhanced AFB\(_1\) activation, it also induced GST activity and thus increased AFB\(_1\)-glutathione conjugation.
Therefore, the overall hepatic binding of AFB\textsubscript{1} to DNA is reduced (Loury, Hseih & Brard, 1984; Lotlikar \textit{et al}, 1989). Other inducers such as ethoxyquin (Kensler \textit{et al}., 1986), and oltipraz (Primiano \textit{et al}., 1995), have also been shown to inhibit AFB\textsubscript{1}-induced carcinogenesis by inducing GST activity. In a study employing human hepatocytes, oltipraz was also reported to lower the production of AFB\textsubscript{1}-epoxide by inhibiting the CYP1A2 and CYP3A4 activities (Longouet \textit{et al}., 1995).

A compound isolated from a Chinese herb, dimethyl-4,4’ dimethoxy-5,6,5’,6’-dimethyleneedioxy biphenyl-2,2’-dicarboxylate (DDB), is a drug used for its liver protective effects. Pretreatment of rats with DDB inhibited liver damage caused by AFB\textsubscript{1}. DDB also induced the activity of glutathione S-transferase and therefore enhanced detoxification of AFB\textsubscript{1}-epoxide (Liu \textit{et al}., 1995). Crocetin, a carotenoid isolated from the seeds of Cape jasmine, has been reported to elevate the cytosolic glutathione S-transferase activity and glutathione concentration in a fibroblast cell line treated with AFB\textsubscript{1} (Wang, Shiah & Lin, 1991\textit{a}). Another Chinese herbal drug, geniposide, isolated from a fruit of a species of gardenia, can also inhibit AFB\textsubscript{1}-induced DNA binding. Induction of glutathione S-transferase and gamma glutamyl cysteine synthase (involved in glutathione synthesis) activities, and suppression of AFB\textsubscript{1}-induced unscheduled DNA synthesis were the suggested mechanisms of action of geniposide (Wang, Wang & Lin, 1991\textit{b}; Wang, Lai & Wang, 1992).

Cortisol pretreatment in rats markedly increases the acute hepatotoxicity of AFB\textsubscript{1} (Chentanez \textit{et al}., 1988). The toxicity effects, such as higher mortality rates, increased in liver triacylglycerol, and elevated AFB\textsubscript{1} binding to DNA and protein, were dose-dependent. These cortisol effects may by due to increased metabolism of AFB\textsubscript{1} to its epoxide derivative.

Ethanol, when given to animals together with or prior to aflatoxin, increased the aflatoxin hepatotoxicity and DNA binding (Toskulkoa & Glinsukon, 1986; Toskulkoa, Lohokachonpan & Glinsukon, 1991; Sahaphong, Toskulkoa & Glinsukon, 1992). The alcohol pretreatment increased the activation of AFB\textsubscript{1} but not the GST activity. This explains the increased binding of AFB\textsubscript{1} to DNA. On the other hand, when given after AFB\textsubscript{1} administration, ethanol showed no influence on AFB\textsubscript{1}-DNA binding (Messlbeck Campbell & Roe, 1984).

**CONCLUSION**

Aflatoxins are a real public health problem and research should be continued to prevent their presence in food, and to inhibit their harmful effects. Much progress has been achieved in showing the importance of dietary factors in modulation of AFB\textsubscript{1} toxicity and carcinogenicity. Obviously, there are still a wide range of dietary components that can be explored and investigated. Additionally, the more important research areas would be in explaining the protective mechanisms and formulating the effective “dose” before any true public health measures could be recommended.

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Dietary factors and aflatoxin B, carcinogenicity


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