

# Metabolic Changes of Aflatoxin B1 to become an Active Carcinogen and the Control of this Toxin

Magda Carvajal-Moreno

Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México. Ciudad Universitaria, Coyoacán, 04510 México DF

**Corresponding author:** Magda Carvajal-Moreno, Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México. Ciudad Universitaria, Coyoacán, 04510 México DF, Tel: +52 55 5622 1332; E-mail: magdac@ib.unam.mx

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## Abstract

Although aflatoxins are unavoidable toxins of food, many methods are available to control them, ranging from natural detoxifying methods to more sophisticated ones. The present review englobes the main characteristics of Aflatoxins as mutagens and carcinogens for humans, their physicochemical properties, the producing fungi, susceptible crops, effects and metabolism. In the metabolism of Aflatoxins the role of cytochromes and isoenzymes, epigenetics, glutathione-S-transferase enzymes, oncogenes and the role of aflatoxins as mutagens of the tumor suppressor gene p53, and the Wnt signaling pathway are briefly explained, as well as these toxins as biomarkers.

The last section includes the Aflatoxin control methods, from the protection of the crop from the *Aspergillus* fungi, the biocontrol solution, the AFB<sub>1</sub>-DNA adduct control with the natural repair rates of adduct removal, induction to resistance to AFB<sub>1</sub>, the detoxification enzymes, recombinant yeasts, pre-exposure to Aflatoxin M<sub>1</sub>, the inhibition of AFB<sub>1</sub> lesions by different compounds, chemoprevention and protective chemical compounds, cruciferous vegetables, dietary dithiolethiones, glucoraphanin, indol-3-carbinol, oltipraz, phenols (butylated hydroxytoluene and ellagic acid), indomethacin, selenium, natural nutrients, coumarin chemoprevention, cafestol and kahweol, terpenes and monoterpenes, grapefruit juice, vitamins, traditional Chinese medical plants (*Oldenlandia diffusa* and *Scutellaria barbata*), chlorophyllin, probiotic bacteria and additives as aluminosilicates and glucomannans are described here. Finally, the aflatoxin international legislation was briefly described.

**Keywords:** Aflatoxins; Mutagens; Carcinogens; Control

## Introduction

The FAO [1] of the United Nations estimates that 25% of the world's food crops and their derivatives are contaminated with mycotoxins, which threaten human health [2]. Moreover, the Center for Disease Control from USA [3] estimates that more than 4.5 billion people in the developing world are exposed to aflatoxins (AFs).

The contamination of food supplies by naturally occurring toxins is of particular concern in the rural communities of developing countries [4]. AFs are the most frequent and toxic mycotoxins, and their metabolism and mechanisms to control them are of utmost importance.

## Aflatoxins

AFs are secondary metabolites, polyketides that chemically correspond to a bisdihydrodifuran or tetrahydrobisfuran united to a coumarin substituted by a cyclopentanone or a lactone [5-7]. AFs are divided into two subgroups [6,8,9]:

a) Bisfuran-coumarin-cyclo pentanons, which include AFs of series B (AFB<sub>1</sub>, AFB<sub>2</sub>, AFB<sub>2a</sub>), M (AFM<sub>1</sub>, AFM<sub>2</sub>, AFM<sub>2a</sub>), Q (AFQ<sub>1</sub>), P (AFP<sub>1</sub>), and aflatoxicol (AFL) that interconverts with AFB<sub>1</sub>.

b) Bisfuran-coumarin-lactones, which contain AFs of series G (AFG<sub>1</sub>, AFG<sub>2</sub>, AFG<sub>2a</sub>).

Only AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub> are naturally synthesized by toxigenic fungi. The other AFs (M<sub>1</sub>, M<sub>2</sub>, P<sub>1</sub>, Q<sub>1</sub>, G<sub>2a</sub>, B<sub>2a</sub> and AFL) are products of microbial or animal metabolism [9-13].

The liver of animals protects the organism by lowering the toxicity of AFB<sub>1</sub> via the addition of a OH- group to form hydroxylates (AFM<sub>1</sub>, AFP<sub>1</sub>, AFQ<sub>1</sub>, and AFL); this step make AFs soluble in water and facilitates their disposal via urine, feces and milk. AFB<sub>1</sub> and AFG<sub>1</sub> have a double bond at the 8,9 position that oxidizes and forms AFB<sub>1</sub>-exo-8,9-epoxide (AFBO), an unstable molecule, which produces dihydrodiol AFB<sub>1</sub> and is linked to the N<sup>7</sup>-guanine of DNA [14] to form active carcinogens called AFB<sub>1</sub>-DNA adducts. AFB<sub>2</sub> and AFG<sub>2</sub> [15] lack a double bond, which affects their toxicity. The bond changes that convert AFB<sub>1</sub> to AFB<sub>2</sub> are known [14,16], and the biotransformation and biosynthetic routes of AFB<sub>1</sub> have been described [17-20].

## Physicochemical properties

AFs are white to yellow odorless and flavorless crystalline solids that are soluble in organic solvents and insoluble in water. They fluoresce when excited under ultraviolet light, are thermo-resistant, and have a low molecular weight (MW). Furthermore, their physicochemical properties are distinct [21,22]. AFs have high points of fusion and decomposition temperatures in the range of 237°C (AFG<sub>2</sub>) to 320°C (AFP<sub>1</sub>) [23,24]. Therefore, AFs are stable at temperatures present when cooking or boiling food, milk ultrapasteurization and alcoholic fermentation.

Acid or alkaline solutions heated to temperatures higher than 100°C lead to decarboxylation with the opening of the lactone ring, which

results in the loss of the methoxy radical of the aromatic ring and a loss of fluorescence. The formation of the acid ring [25] during human digestion [26] reverses this hydrolysis.

Lime treatment of maize disguises but does not eliminate AFs. Oxidative and reductive agents react with AFs to change their molecular structure and hydrogenate AFB<sub>1</sub> and AFG<sub>1</sub> to produce AFB<sub>2</sub> and AFG<sub>2</sub> [22]. In the presence of inorganic acids, AFB<sub>1</sub> and AFG<sub>1</sub> are transformed into B<sub>2a</sub> and G<sub>2a</sub> [22,27]. AFB<sub>2a</sub> is 1000 times less mutagenic than AFB<sub>1</sub> [23].

### Producing fungi

Aflatoxins are the most toxic and frequent mycotoxins. They are produced by the mold *Aspergillus* spp., which belongs to the Kingdom Fungi, Phylum Ascomycota, Order Eurotiales, Class Eurotiomycetes, Family Trichocomaceae, and Genus *Aspergillus*. The main aflatoxigenic species are *A. flavus* [28-31], *A. parasiticus* [32-39] and *A. nomius* [40,41]. *A. tamarii* was reported to produce AFs and cyclopiazonic acid [42].

Other reports state that *A. tamarii*, *A. oryzae*, *A. versicolor*, *Penicillium commune*, and *P. griseofulvum*, as AF producers, are proven misidentifications [43]. *Aspergillus chevalieri*, *A. repens*, *Cladosporium herbarum*, *Penicillium chrysogenum* and *Phoma glomerata* remain identified as AF producers [44].

### Susceptible crops

Aflatoxins contaminate cereals (maize, sorghum, rice, barley, and oats), oilseeds (peanuts, cottonseed, nuts, pistachios, almonds, hazelnuts, cacao, and coconut), dry fruits (figs, dates, and raisins), spices (black pepper, hot pepper, and cumin), and the seeds and grains of crops before and after harvest. In the field, AFs are produced in drought-stressed conditions.

AFs pass to meats, dairy products, eggs, etc., via microbial or animal metabolism. Mexico was considered the country with more liver diseases in the American continent [45], and AFs have been reported in several natural and processed foods, such as maize tortillas [46], rice [47], chilies [48], milk [49,50], eggs [51], chicken breast [52], etc.

### Effects

AFs are dangerous toxins, and their toxicity can be ranked as follows: AFB<sub>1</sub>>AFG<sub>1</sub>>AFG<sub>2</sub>>AFB<sub>2</sub> [53]. AFB<sub>1</sub> is considered the most dangerous AF of the group and is a potent teratogen, mutagen and carcinogen [54,55]. Exposure to AFs occurs primarily via the ingestion of contaminated foods [56], but it is also absorbed through the skin, and spores in the air are inhaled, causing hepatic and gastrointestinal injuries. AFs are among the most potent Group I carcinogens to humans [55], and they are acutely hepatotoxic and immunosuppressive in a variety of animals [17,57-59]. AFs can cause acute or chronic effects depending on the duration and level of exposure [60]. The ingestion of higher doses of AF can result in acute aflatoxicosis, a condition characterized by hemorrhage, vomiting, diarrhea, abdominal pain, lung edema, digestive changes, hepatotoxicity, fatty and necrotic liver [61], liver failure and death [56]. Severe acute liver injury with high morbidity and mortality has been associated with high-dose exposure to AF [62], and the ingestion of 2 to 6 mg/day of AFs for one month can cause acute hepatitis and death [63,64].

Chronic low-level AF exposure can increase the risk for cancers, mainly hepatocellular carcinoma (HCC) [65], in areas where hepatitis

B virus (HBV) infection is endemic because there is a synergism between the HBV and AFB<sub>1</sub> that increases the risk. Children younger than five years remain the most vulnerable population, with exposure damaging their immunity and causing dwarfism [66]. Other symptoms are immunosuppression [67,68], and AFs also reduce the protection given by vaccinations [69]. Furthermore, they cause miscarriages, fetal malformations [70], hepatitis B and C, cirrhosis [64,71], Reye syndrome with encephalitis and fatty liver [72], marasmus, Kwashiorkor [73], and death [74].

The worst human outbreaks of aflatoxicosis were reported in India [4,64,71], Kenya [3,74-78], Nigeria [79], Gambia [80], Uganda [81-83], Swaziland [84-85], Mozambique and Transkei [86,87], Thailand [30,88], Malaysia [89], China [59,90-93], Taiwan [94], New Zealand [95] and the Philippines [96].

Regarding the prevalence and human exposure to AFs, approximately 4,500 million persons living in developing countries are recognized to be chronically exposed to largely uncontrolled amounts of AFs [97,98].

### Metabolism

#### Role of cytochromes and isoenzymes

Cytochrome P450 enzymes (CYPs 450) are hemoproteins and electron carriers that catalyze or accelerate oxidation-reduction reactions during cellular respiration [99], and they are the main enzymes involved in the metabolic activation of AFs [100]. In the past, CYPs 450 were considered to specifically originate from the liver, but they are now known to be distributed throughout the body [101]. Nevertheless, the liver is the main organ that metabolizes xenobiotics [102].

AFB<sub>1</sub> is metabolized in the body by CYP450 isoforms such as CYP1A1 and CYP1A2, which comprise 10% of CYP450 isoforms, CYP3A4 (30%), CYP2C8 (20%), CYP3A5, and CYP3A7 [102] in the fetus. AFB<sub>1</sub> is also metabolized by glutathione S-transferase (GST) and AFB<sub>1</sub>-aldehyde reductase, leading to reactive metabolites, some of which can be used as AF exposure biomarkers [103].

CYP1A1 and CYP1A2 transform and activate procarcinogens as intermediate metabolites that link to DNA and participate in the activation of AFB<sub>1</sub> [104-107]. In humans, the CYP1A2 isoenzyme is encoded by the *CYP1A2* gene [108].

The CYP1A2 enzyme isoform is the principal metabolizer of AF at low concentrations, whereas CYP3A4 isoform acts as metabolizer for high AF amounts. The accumulation of AFB and its metabolites in the body, especially AFBO, depletes glutathione (GSH) due to the formation of high amounts of epoxides and other reactive oxygen species.

Inflammatory liver disease increases the expression of specific CYP450 isoenzymes involved in AFB<sub>1</sub> activation. The immunohistochemical expression and localization of various human CYP450 isoforms, including CYP2A6, CYP1A2, CYP3A4, and CYP2B1, have been examined. Alterations in the phenotypic expression of specific P450 isoenzymes in hepatocytes associated with hepatic inflammation and cirrhosis might increase the susceptibility to AFB genotoxicity [103].

A human cell line stably expressing human CYP3A4 has been used to study its role in the metabolic activation of AFB<sub>1</sub> and compare this

role to those of CYP1A2 and CYP2A3 [109]. The human lymphoblastoid cell line LA2/Hyg was 3- to 6-fold more sensitive to AFB<sub>1</sub>-induced mutation than the 3A4/Hol cell line. Furthermore, 3A4/Hol cells, which stably express human CYP3A4 cDNA, were 10- to 15-fold more sensitive to the AFB<sub>1</sub> mutation than 2A3/Hyg cells [109].

### Epigenetics

*Epigenetic changes* are heritable changes in gene expression that do not involve changes to the underlying DNA sequence, i.e., a change in phenotype without a change in genotype. Epigenetic changes refer to external modifications of DNA that turn genes "on" or "off." These modifications affect how cells "read" genes, resulting in changes in gene expression, cellular differentiation and growth without changing the genetic code itself. AFB, AFBO and other metabolites also affect epigenetic mechanisms, including DNA methylation, histone modifications, the maturation of microRNAs (miRNAs) and the daily formation of single nucleotide polymorphisms (SNPs). Specifically, AFB exposure may facilitate the process of change and induce G:C to T:A transversions at the third base in codon 249 of TP53, causing p53 mutations in HCC [110]. AFB also promotes tumorigenesis, angiogenesis, invasion and metastasis in HCC via epigenetic mechanisms. Chronic AF exposure leads to the formation of reactive AFBO metabolites in the body that could activate and de-activates various epigenetic mechanisms, leading to development of various cancers [103].

The effects of AFB<sub>1</sub> intake, genetic polymorphisms of AFB<sub>1</sub> metabolic enzymes, and interactions between nucleotides were studied with regard to the risk of gastric cancer in Korean populations. The probable daily intake of AFB<sub>1</sub> was significantly higher ( $p < 0.0001$ ) among gastric cancer patients than among control subjects. Only CYP1A2 was associated with the genetic polymorphisms present in gastric cancer. The effect of AFB<sub>1</sub> on gastric carcinogenesis may not be modulated by genetic polymorphisms of AFB<sub>1</sub> metabolic enzymes [111].

### Glutathione S-transferase enzymes (GSTs)

In Phase I of metabolic processes water-soluble products are generated. In Phase II, GSTs allow these metabolites to combine with polar endogenous molecules to form conjugation products that are rapidly excreted [112, 113]. This reaction increases the solubility of dangerous compounds, allowing them to be excreted [114].

GSTs are a family of enzymes that protect the organism and are present in Phase II of enzymatic detoxification of many electrophilic metabolites [115,116], such as xenobiotic derivatives and endogenous molecules (antibiotics, steroids, prostaglandins and leukotrienes) [112], which exert carcinogenic and genotoxic effects [117].

GSTs were first purified from rat liver microsomes [115] in the soluble fraction in the cytoplasm (cytosolic fraction), but GSTs are also found in the nucleus, mitochondria and peroxisomes [117]. GSTs from mammals are the best-characterized enzymes that facilitate the detoxification route of dangerous components that conjugate with glutathione (GSH) [113]. GSH is an important antioxidant that prevents damage to important cellular components by reactive oxygen species, such as free radicals, peroxides, lipid peroxides and heavy metals [118].

Each subunit of GST features a specific linkage site (place-G) and an electrophilic linkage site (place-H), which is less specific and reacts with different toxic agents [118]. GSTs link to lipophilic molecules with a molecular mass >400 Daltons (hemin, bilirubin, biliary salts, steroids, thyroid hormones, fatty acids and drugs) and store and transport them to the aqueous phase of the cell [114, 119].

### Glutathione S-transferase and aflatoxins

AFB<sub>1</sub> includes the reactions of enzymatic conjugation mediated by GST to inactivate the AFBO. Spontaneously, AFBO is hydrolyzed to 8,9 dihydrodiol and conjugates with GSH to form AFB<sub>1</sub>-glutathione transferase (AFB<sub>1</sub>-SG) [120]. The conjugate AFB-SG is the most abundant biliary metabolite and is excreted by urine [89]. The induction of GST and aldehyde-AFB<sub>1</sub> reductase prevents the formation of AF-ADN and AF-protein adducts and blocks carcinogenesis in rats [121]. Specifically, the induction of GST prevent the union of AFB<sub>1</sub> and ADN in different species [122]. The dietary ingestion of antioxidants increases the levels of GST, which consequently increases the elimination of AFB-SG in the urine of treated animals [90].

### Oncogenes and the tumor suppressor gene p53

Oncogenes, such as *N-ras*, *c-myc* or *c-fos*, are over-expressed, but their mutations are rare, and evidence to directly implicate these mutations in HCC is rare [123]. A specific mutation in codon 249 of the *p53* gene is present in regions where HCC and exposure to AFs are prevalent [124]. The mutation induced by the reactive forms of AFB<sub>1</sub> in codon 249 of the *p53* gene is a "hotspot" for the mutation induced by AFB<sub>1</sub>, specifically the transversion GC→TA [125]. In Gambia, this mutation was detected in the DNA of HCC patients but was rare in control patients [126-128]. The transversion G→T or transition G→A is produced in the third base of codon 249 of the p53 gene and in the first or second base of codon 12 of the H-ras gene [129-134]. When rats, mice and fish ingest an AF-contaminated diet, some proto-oncogenes of the "ras" family are activated [135,136]. High incidences of activated Ki-ras and N-ras have been observed in liver carcinomas and adenomas induced by AFB<sub>1</sub> [135].

Expression and activation of several c-oncogenes in seven hepatocellular carcinomas from seven separate rats treated with AFB<sub>1</sub> were examined by Northern and Southern blot analyses. Both c-Ha-ras and c-myc transcripts were elevated at high levels in all hepatomas. Moreover, in one of them, T2-1 hepatoma, the c-myc gene was amplified only in a tumor part of liver without significant rearrangement. N-ras specific transcripts were not elevated in these hepatomas. The consistently increased expression or deregulation of the c-myc and c-Ha-ras genes may play an important role in the development of hepatomas induced by AFB<sub>1</sub> [137]. When male Fisher rats were exposed to AFB<sub>1</sub> and AFG<sub>1</sub>, four liver tumors were induced: three harbored activated N-ras and one exhibited the transversion G→A in codon 12 of Ki-ras [138,139].

The identification of a specific mutation in the tumor suppressor gene p53 in HCC in regions where AF exposure is high has helped to identify an AF biomarker [140]. A nonsense mutation in p53, that yields a broken, non-functional protein, provides a selective advantage for the expansion of preneoplastic or neoplastic cells. The p53 gene plays a molecular role in cancer and consequently serves as an intermediate biomarker for cancer development [141].

The suppressor p53 gene is mutated in 53% of HCC cases in Mexico, a country in which exposure to AFB<sub>1</sub> is high, whereas in populations

with low exposure to this toxin, mutations were identified in 26% of HCC cases [142]. In Senegal, where people are exposed to high concentrations of AFB<sub>1</sub> via foodstuffs, the 249 codon mutation of the p53 gene was found in 10/15 HCC tumors [143]. The mutation index of the p53 gene is higher in tumors associated with HBV compared with tumors associated with the hepatitis C virus (HCV) and non-viral HCC, independent of AF exposure [144].

### Wnt signaling pathways

The Wnt (=Wingless-related integration site in *Drosophila melanogaster*) signaling pathways are a group of signal transduction pathways that rely on proteins that pass signals from the outside of a cell to the inside of the cell via cell surface receptors [145,146].

Wnt signaling was first identified due to its role in carcinogenesis and embryonic development (cell fate specification, proliferation, migration, and body axis patterning). Its role in embryonic development was discovered when genetic mutations in proteins in the Wnt pathway produced abnormal fruit fly embryos. The genes responsible for these abnormalities also influence breast cancer development, prostate cancer, glioblastoma, type II diabetes and other diseases [145,146].

The inappropriate reactivation of the Wnt pathway as a result of mutations in the  $\beta$ -catenin gene which encodes a protein that facilitates the mobility of neoplastic cells is implicated in the development of HCC [147]. Mutations in the  $\beta$ -catenin gene can activate the transcription of Wnt target genes, such as c-myc, cyclin D1 and PPAR $\delta$ . Therefore, these mutations can promote tumor progression by stimulating cellular proliferation [147,148].

AFB<sub>1</sub> negatively regulates the Wnt/ $\beta$ -catenin signaling pathway by activating microRNA-33a (miR-33a). MicroRNAs modulate gene expression in various cancers and cardiovascular disorders, but only a few of microRNAs are associated with the pathology of AFB<sub>1</sub>. A regulatory network involving AFB<sub>1</sub>, miR-33a and  $\beta$ -catenin in human carcinoma cells showed that the level of miR-33a increases the response of HCC cells to AFB<sub>1</sub>, whereas  $\beta$ -catenin expression decreased in the same cells when they were treated at their IC<sub>50</sub> values. miR-33a decreases the expression of  $\beta$ -catenin, which affects the  $\beta$ -catenin pathway and inhibits cell growth. AFB<sub>1</sub> might decrease the response of  $\beta$ -catenin by increasing the response of miR-33a, promoting the proliferation of malignant cells [149].

### Biomarkers

An exposure biomarker refers to the measurement of AFs, their metabolites or interactive specific products in a compartment of the body or fluids to assess past and present exposure. The biomarkers of internal doses and from biologically effective doses of AF are generally hydroxylated metabolites, and AF-DNA adducts formed from epoxide derivatives [150].

The biomarkers identified in etiological research have been used for preventive purposes in high-risk populations because experimental studies have established time links between AF biomarker modulation and the risk of disease. The early identification of AF metabolites in human fluids [151] stimulated the development of biomarkers [152]. The availability of specific antibodies helps the detection of AF metabolites in human urine [153-155].

AFB<sub>1</sub> is biotransformed to various metabolites, especially active AFBO, which interacts with DNA, RNA and various metabolic

pathways, such as protein synthesis, the glycolytic pathway and the electron transport chain, which is involved in ATP production in cells. AFB interacts with DNA to form AFB-DNA adducts to cause DNA mutations and breakages.

CYP450 controls AF adduct formation in the metabolic route AFB<sub>1</sub>. AFBO is the electrophilic metabolite that links to N<sup>7</sup> of the guanine residues of DNA to form 8,9-dihydroxy-8-(N<sup>7</sup>) guanyl-9-hydroxy AFB<sub>1</sub> adducts (AFB<sub>1</sub>-Gua), which is the most abundant [125,156,157]. The imidazol ring on the positively charged AFB<sub>1</sub>-Gua promotes the depurination and produces an apurinic site. This ring opens to form a chemically and biologically more stable adduct, formamidopyrimidine, 2,3-dihydro-2-(N-formyl)-2',5',6'-triamino-4'-4'-oxy-N-pyrimidyl-3-hydroxy-AFB<sub>1</sub> (AFB<sub>1</sub>-FAPY) adduct present in the DNA replication several times [158,159].

One hour after injecting rats with AFB<sub>1</sub>, AFB<sub>1</sub>-Gua comprised the majority of adducts, whereas the adduct AFB<sub>1</sub>-FAPY was predominant at later time points [160]. The apurinic sites, AFB<sub>1</sub>-Gua and AFB<sub>1</sub>-FAPY, individually or collectively act as the precursors of the genetic effects of AFB<sub>1</sub>, and these two adducts develop the tumors.

Tumors were induced in rats to study the human Ha-ras proto-oncogene, which is metabolically mutated by AFB<sub>1</sub>, using an in vitro transfection of a plasmid modified with AFB<sub>1</sub>. In this experiment, G $\rightarrow$ T transversions were identified in the first and second bases of codon 12. The proto-oncogene Ha-ras mutated by AFB<sub>1</sub> was identified in its in vitro oncogenic form, but this mutation has not yet been reported in human HCC patients exposed to AFB<sub>1</sub> [161].

Therefore, identifying the presence of free AFs (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub>) is important to assess a person's exposure to AFs via food. Furthermore, measuring the metabolic hydroxylates (AFM<sub>1</sub>, AFM<sub>2</sub>, AFP<sub>1</sub> and AFL) is important as a biomarker of the internal dose. Finally, the effective biological doses in control liver and human HCC samples as well as the presence of AFB<sub>1</sub>-Gua and AFB<sub>1</sub>-FAPY adducts serve as etiologic agents of cancer.

### Control

#### Protecting harvests from *Aspergillus* fungus

AF contamination can occur before harvest when the crop undergoes drought stress at the grain filling stages and when wet conditions occur during harvest periods. AF contamination increases with insect damage, delayed harvesting and high moisture levels during storage and transportation. Therefore, additional irrigation in the fields and the control of insects reduces AF contamination. In storage, AFs can be controlled by maintaining available moisture at levels below those in the range of the growth of *Aspergillus* spp. Cultural practices, such as resistant crops and competitive exclusion using strains that do not produce AF, can block AF production.

AF destruction depends on the food water content, pH, application of propionic acid against the fungus, presence of ionic compounds, and electric charge. The degradation mechanism is not completely understood, but the lactone ring opens, allowing a decarboxylation at temperatures above 150°C that were necessary to attain partial destruction of the toxin [22]. The effects of pH (5.0, 8.0, 10.2), temperature (121°C, 130°C, 140°C) and heating time (5 s, 20 s, 15 min) on mutagenic activity (assayed by Ames test) of peanut beverages artificially contaminated with AFB<sub>1</sub>. Heat treatments at pH 8.0 were not effective in reducing the mutagenic activity. On the other hand, the

treatments pH 10.2, 130°C 20 s and pH 10.2, 121°C 15 min reduced the mutagenic activity by 78% and 88%, respectively [22].

### Biocontrol solution

The goal of the “Aflatoxin Control in Maize and Peanuts Project” is to develop and implement holistic strategies to address AF contamination in maize and peanuts. Ultimately, the project aims to develop and scale up biological control technology interventions to improve the health and income of farmers in Sub-Saharan Africa [3]. The Project applies a biocontrol solution developed by the United States Department of Agriculture (USDA) and the Agricultural Research Service (ARS) to reduce AF contamination. Specifically, it uses the ability of native atoxigenic strains of *Aspergillus flavus* to naturally outcompete their AF-producing cousins [162]. The Partnership for Aflatoxin Control in Africa (PACA) is a collaboration that aims to protect crops, livestock, and people from the effects of AFs. By combating these toxins, PACA will contribute to improve food security, health, and trade across the African continent [3].

The Agricultural Cooperative Development International and Volunteers in Overseas Cooperative Assistance (ACDI/VOCA) project is funded by the USAID and the Bill and Melinda Gates Foundation via the International Institute of Tropical Agriculture (IITA) and the UK government via the African Agricultural Technology Foundation (AATF) [163]. The AATF has been working with the USDA-ARS since 2007 to test the efficacy of Kenyan atoxigenic strains of *Aspergillus flavus* and training farmers to manage AF [163]. The biocontrol product called Aflasafe™ was applied in soil in the Alhaji Sanusi region of Zaria, Nigeria, and a similar product was developed and tested in Kenya and Senegal with encouraging results. Aflasafe™ competes with the source of AF, the fungus in the soil, before the fungus can contaminate the crop prior to harvest. Aflasafe™ reduces AF contamination in maize and groundnuts by 80-90% and improves the food production, health, livelihood and income of 4.5 million farmers and consumers while also reducing commodity losses due to AF contamination [163].

### AFB1-DNA adduct control

Several options to diminish or control AFs and the presence of AFB1-DNA adducts in an organism, which can cause a mutation that may result in carcinogenesis, are presented below. These possibilities include natural repair rates, implicated enzymes, natural products and chemicals.

### Natural repair rates of adduct removal

Natural repair rates in the hamster and rat were constant over time with the removal of AFB1-Gua, accounting for the majority of adduct disappearance. Rabbits demonstrated biphasic adduct repair; all types of adducts (AFB1-FAPY) were rapidly removed during the first 12 h after treatment with AFB1, followed by a slower removal phase of primarily AFB1-Gua carcinogen activation. Overall, the repair capabilities of the tracheal epithelium vary among species (rabbit > hamster > rat) [164].

### Induction of resistance to AFB1

The induction of resistance to the binding between AFB1 and cellular macromolecules in the rat due to chronic exposure to AFB1

and AFM1 was investigated. Pre-exposure to AFM1 resulted in a small reduction in binding to nucleic acids [165].

Mixtures of genotoxins damage DNA, as evidenced by changes in DNA adduct formation by pre-existing adducts. AFB1-binding to DNA may be altered by conformational changes in the helix due to the presence of a pre-existing acetyl-amino-fluorene adduct. The use of the chemical probes hydroxylamine and diethylpyrocarbonate render AF ineffective and prevent the local denaturation of the oligomer helix. Changes in the nucleophilicity of neighboring nucleotides and local steric effects cannot be ruled out [166].

### Detoxification enzymes

Detoxification enzymes, enzyme inhibition by  $\beta$ -naphthoflavone (BNF), and CYP450 monooxygenases increased the GST activity by 133% in animals fed 50  $\mu\text{g kg}^{-1}$  AFB1, by 48% in animals pre-exposed to 50  $\mu\text{g kg}^{-1}$  AFM1, and remained at control values in rats fed 0.5  $\mu\text{g kg}^{-1}$  AFM1. BNF is an inducer of various detoxification enzymes, such as CYPs 450 and uridine-5'-diphosphoglucuronosyltransferases (UGTs) [167].

BNF is a chemopreventive agent [168]; it is a flavonoid that occurs in fruits, vegetables, teas, wine, nuts, and seeds. The biological effects of flavonoids include the reduction of cardiovascular disease risk, the inhibition of hepatocytic autophagy, antiviral activity, inhibiting the breakage and disruption of chromosomes (anticlastogenic effects), anti-inflammatory analgesic effects and an anti-ischemic effect [169]. Vitamins (C and E), minerals (zinc, selenium), and plant-based compounds (phenols, flavonoids, isoflavones, and terpenes) act as antioxidants to avoid the formation of fatty plaques in the arteries (anti-atherogenic) and exert anticarcinogenic properties.

Enzyme inhibition can also be used to control AFs: 1) The aryl hydrocarbon (Ah) receptor is a cytosolic protein and activator of transcription that increases the abundance of selective CYP450s, and 2) the ligand is a substance that binds to a specific receptor and triggers a response in the cell. It mimics the action of an endogenous ligand (such as a hormone or neurotransmitter) that binds to the same receptor [170]. Diets containing BNF inhibited *in vivo* AFB1-DNA adduct formation in 46%. Mechanisms of chemoprevention may depend on the anticarcinogen dose, and even the potent induction of phase I or phase II activities does not assure that a pathway plays a predominantly protective role *in vivo* [171,172].

BNF inhibits aryl hydrocarbon Ah receptor activation and CYP1A1 activity [173,174]. The induction of detoxification enzymes following chronic exposure to AF might contribute to the reduction of the covalent binding of AFB1 to macromolecules [165].

BNF modulates AFB1 biotransformation in isolated rabbit lung cells [175]. The cytotoxic and carcinogenic mycotoxin AFB1 is biotransformed by CYP450 to a number of relatively nontoxic metabolites as well as to the ultimately toxic metabolite AFBO. In a number of tissues and species, BNF hydroxylates AFB1 to the relatively less toxic metabolite, AFM1.

AF is also toxic and carcinogenic to respiratory tissues. The decrease in AFB1-DNA binding observed in rabbits treated with BNF is apparently due to the selective induction of CYP isozymes and related increases in AFM1 formation and not to the direct inhibition of epoxidation or enhanced conjugation of AFBO with glutathione [175].

Among the members of the mouse CYP450 2A family, CYP450 2A5 is the best catalyst of AFB1 oxidation to its 8,9-epoxide [176].

## Recombinant yeasts

The role of amino acid residues 209 and 365 of CYP450 2A5 in the metabolism and toxicity of AFB<sub>1</sub> has been studied using recombinant yeasts. In addition, replacing the hydrophobic amino acid at the 365 position with a positively charged lysine residue strongly decreased the metabolism of AFB<sub>1</sub>. The catalytic parameters of AFB<sub>1</sub> generally correlated with its toxicity to the recombinant yeasts expressing the activating enzyme and with the binding of AFB<sub>1</sub> to yeast DNA. Furthermore, high-affinity substrates and inhibitors of CYP450 2A5 efficiently blocked the toxicity of AFB<sub>1</sub> [176]. The induction of resistance to AFB<sub>1</sub> binding to cellular macromolecules in the rat by chronic exposure to AFB<sub>1</sub> and AFM<sub>1</sub> was also investigated [165].

## Pre-exposure to AFM<sub>1</sub>

Pre-exposure to AFM<sub>1</sub> resulted in a small reduction in binding to nucleic acids. In rats pre-exposed to 50 µg kg<sup>-1</sup> AFB<sub>1</sub>, GST activity increased by 133%, and labeled AFB<sub>1</sub> binding to DNA, RNA, and protein decreased by 72%, 74%, and 61%, respectively. Binding decreased by 48% in rats pre-exposed to 50 µg kg<sup>-1</sup> AFM<sub>1</sub>, and remained at control values in rats fed 0.5 µg kg<sup>-1</sup> AFM<sub>1</sub>. The induction of detoxification enzymes following chronic exposure to AF might contribute to the reduction in the covalent binding between AFB<sub>1</sub> and macromolecules [165].

The AFB<sub>1</sub> aldehyde metabolite of AFB<sub>1</sub> may contribute to the cytotoxicity of this hepatocarcinogen via protein adduction. AFB<sub>1</sub> aldehyde reductases, specifically the NADPH-dependent aldo-keto reductases in the rat (AKR7A1) and human (AKR7A2), are known to metabolize the AFB<sub>1</sub> dihydrodiol by forming a AFB<sub>1</sub> dialcohol. Using rat AKR7A1 cDNA, a distinct aldo-keto reductase (AKR7A3) from an adult human liver cDNA library was isolated and characterized [177]. The reduced amino acid sequence of AKR7A3 shares 80 and 88% identity with rat AKR7A1 and human AKR7A2, respectively. AKR7A RNA is expressed at various levels in the human liver, stomach, pancreas, kidney and liver. Based on the kinetic parameters determined using recombinant human AKR7A3 and AFB<sub>1</sub> dihydrodiol at pH 7.4, the catalytic efficiency of this reaction equals or exceeds those reported for CYP450s and GST, which are known to metabolize AFB<sub>1</sub> *in vivo*. Depending on the extent of AFB<sub>1</sub> dihydrodiol formation, AKR7A may contribute to the protection against AFB<sub>1</sub>-induced hepatotoxicity [177].

## Inhibition of AFB1 lesions by different compounds

AFB<sub>1</sub>-induced tumors or preneoplastic lesions in experimental animals can be inhibited by co-treatment with the compounds described here.

Fischer 344 rats readily develop liver cancer when exposed to AFB<sub>1</sub>, but the dietary administration of the antioxidant ethoxyquin (EQ) provides protection against hepatocarcinogenesis [178]. Chemoprotection by EQ is accompanied by the overexpression of enzymes that detoxify activated AFB<sub>1</sub>. AF-protein adducts form following the metabolism of AFB<sub>1</sub> to the dialdehydic form of AFB<sub>1</sub>-dihydrodiol. The dialdehyde can be detoxified by reduction to a dialcohol via the catalytic actions of an enzyme present in the hepatic cytosol from rats fed EQ-containing diets [178].

The enzyme responsible for catalyzing the formation of dihydroxy-AFB<sub>1</sub> has been purified from the livers of rats fed diets supplemented with EQ. This enzyme is a soluble monomeric protein, and this

inducible enzyme has been designated AFB<sub>1</sub>-aldehyde reductase (AFB<sub>1</sub>-AR), a previously unrecognized enzyme that could provide protection against the cytotoxic effects of AFB<sub>1</sub> resulting from the formation of protein adducts. The importance of AFB<sub>1</sub>-AR and the GST Yc2 subunit in conferring resistance to AFB<sub>1</sub> has also been discussed [178].

## Chemoprevention and protective chemical compounds

Cancer chemoprevention is the use of agents to inhibit, delay or reverse carcinogenesis. Many classes of agents, including anti-estrogens, anti-oxidants, anti-inflammatories, and other diet-derived agents, have shown promise in this context [179]. Some phytochemicals (benzyl isothiocyanate, coumarin, or indole-3-carbinol), synthetic antioxidants, and other drugs (butylated hydroxyanisole, diethyl maleate, ethoxyquin, BNF, Oltipraz, phenobarbital, or trans-stilbene oxide) have been shown to increase hepatic aldo-keto reductase activity toward AFB<sub>1</sub>-dialdehyde and GST activity toward AFBO in both male and female rats.

## Cruciferous vegetables

Several compounds, such as dietary dithiolethione (DTT), glucoraphanin, indole-3-carbinol and Oltipraz, are described below.

## Dietary dithiolethiones (DTTs)

DTTs are a class of organosulfur compounds present in cruciferous vegetables. At concentrations of 0.03%, DTTs were demonstrated to potentially protect against AFB<sub>1</sub> hepatocarcinogenesis, and they also reduced the levels of hepatic AFB<sub>1</sub> (AFB)-DNA adducts by 80% following acute or subchronic treatments with AFB (250 µg kg<sup>-1</sup> daily) by increasing the hepatic activity of the Phase II enzyme GST without affecting the CYP450 levels or Phase I enzyme activities. The elimination of the major DNA adduct, AFB-Gua, was markedly reduced in animals fed DTT [180].

Cruciferous vegetables (e.g., Brussels sprouts, cabbage) contain several agents, including dithiolethiones, which appear to inhibit carcinogenesis; however, the specific dietary compounds that produce the protective effects have not yet been identified [181].

- **Brussels sprouts** significantly ( $P < 0.001$ ) decreased hepatic AFB<sub>1</sub>-DNA binding by 50-60% and increased hepatic and intestinal GST activities [182].
- Glucoraphanin, the principal glucosinolate in broccoli sprouts, can be hydrolyzed by gut microflora to sulforaphane, a potent inducer of carcinogen detoxification enzymes. In a randomized, placebo-controlled chemoprevention trial, they demonstrated that drinking hot water infusions of 3-day-old broccoli sprouts, which contained defined concentrations of glucosinolates, altered the presence of AF and phenanthrene. Individuals receiving broccoli sprout glucosinolates exhibited decreased AF-DNA adduct excretion. The effects of glucosinolate-rich broccoli sprouts on urinary levels of AF-DNA adducts and phenanthrene tetraols were reported in a randomized clinical trial in He Zuo township, Qidong, People's Republic of China [183,184].
- The inclusion of indole-3-carbinol (I3C), a component of cruciferous vegetables, in experimental diets inhibited *in vivo* AFB<sub>1</sub>-DNA adduct formation in 68%, and the addition of BNF (dexamethasone, a corticosteroid) further increased this inhibition to 51% [171]. AFB<sub>1</sub>-induced tumors or preneoplastic lesions can be

inhibited in experimental animals by cotreatment with several compounds, including I3C and the well-known Ah receptor agonist BNF. This study examines the influence of these two agents on the AFB<sub>1</sub>-glutathione detoxification pathway and AFB<sub>1</sub>-DNA adduction in rat livers [171].

- Oltipraz [5- (2-pyrazinyl)- 4-methyl-1, 2-dithiole-3-thione; RP 35972] is a synthetic, substituted 1,2-dithiole-3-thione previously used in humans as an antischistosomal agent. Animal studies have demonstrated that Oltipraz is a potent inducer of Phase II detoxification enzymes, most notably GST. Dietary concentrations of Oltipraz markedly inhibit AFB<sub>1</sub>-induced hepatic tumorigenesis in rats. The levels of hepatic AF-DNA adducts, urinary AF-N<sup>7</sup>-guanine, and serum AF-albumin adducts decreased when the biliary elimination of AF-glutathione conjugants increased, thus providing predictive biomarkers that can be used to measure a chemopreventive effect. In other animal experiments, Oltipraz was found to inhibit chemically induced carcinogenesis in bladder, colon, breast, stomach, and skin cancer models. In addition, Oltipraz has been shown to be non-mutagenic and act as a radioprotector and chemoprotective agent against carbon tetrachloride and acetaminophen toxicity [181].

Oltipraz protects against AFB<sub>1</sub>-induced hepatocarcinogenesis in rats when fed before and during carcinogen exposure; however, this type of exposure-chemoprotection is not directly relevant to most human populations. GST catalyzes the detoxification of AFBO and was found to be rapidly induced in the livers of animals after the beginning of Oltipraz intervention. The significant protection against presumptive preneoplastic tumors suggests that Oltipraz may substantially inhibit the cytotoxic and autopromoting action of repeated exposure to AFB<sub>1</sub> and support the utility of intervention trials with Oltipraz in individuals chronically consuming AFB<sub>1</sub>-contaminated foods, particularly in regions with high incidences of liver cancer [185]. Oltipraz was reported as a useful agent for the modulation of gene expression in subjects at risk for colorectal cancer [186].

## Phenols

Butylated hydroxytoluene (BHT) and ellagic acid (EA) are described below.

- Butylated hydroxytoluene (BHT), also known as dibutylhydroxytoluene, also known as dibutylhydroxytoluene is a lipophilic organic derivative of phenol that exhibits antioxidant properties. Specifically, BHT inhibits tumor formation due to AFB<sub>1</sub> by inducing liver GSH-S-transferases. The permitted dose of BHT, added to processed food as a preservative, does not affect the biotransformation of AFB<sub>1</sub> [187]. The effects of low- and high-dose dietary BHT on microsome-mediated AFB<sub>1</sub>-DNA binding were compared [187].
- The anticarcinogenic effect of BHT pretreatment on the metabolism and genotoxicity of AFB<sub>1</sub> in primary cultures of rat hepatocytes was due to hepatic detoxification mechanisms. Specifically, the intracellular concentrations of reactive metabolites were reduced, and fewer covalently bound adducts were formed [188].
- Ellagic acid (EA), a plant phenol found in various fruits, raspberries and nuts, was examined for its ability to inhibit AFB<sub>1</sub> mutagenesis and DNA damage in cultured rat and human tracheobronchial tissues [189]. In the presence of a rat liver S9 microsomal preparation, EA (1.5 µg/plate) inhibited the number of mutations induced by AFB<sub>1</sub> (0.5 µg/plate) by 50%. EA at a dose of

1000 µg/plate inhibited the mutation frequency > 90%. In tissues, the major AFB<sub>1</sub>-DNA adducts were AFB<sub>1</sub>-Gua and AFB<sub>1</sub>-FAPY, and their formation was reduced by 28-76% in the presence of EA. EA acts as a naturally occurring inhibitor of AFB<sub>1</sub>-related respiratory damage in rats and humans [189].

## Indomethacin

Indomethacin is a nonsteroidal anti-inflammatory drug that produced a 63-100% decrease in [3H] AFB<sub>1</sub>-DNA binding in macrophages from five of seven patients, whereas nordihydroguaiaretic acid inhibited [3H] AFB<sub>1</sub>-DNA adduct formation by 19, 40 and 56% in macrophages from three of seven patients [190].

## Selenium

Selenium effectively inhibited AFB<sub>1</sub>-induced DNA damage, exerting an anticarcinogenic effect against AFB<sub>1</sub>. Selenium pretreatment inhibited AFB<sub>1</sub>-DNA binding and adduct formation by increasing the level of reduced GSH in the liver of treated animals [191].

## Natural nutrients

The medicinal herb *Thonningia sanguinea*, which is prophylactically used against bronchial asthma in Ghana, exhibits antioxidative and hepatoprotective actions against acute AFB<sub>1</sub> hepatotoxicity in Fischer 344 rats [192].

## Coumarin chemoprevention

Coumarin is a natural benzopyrone that is a potent inducer of AFB<sub>1</sub>-aldehyde reductase, the GST A5 and P1 subunits, and NAD(P)H:quinone oxidoreductase in the rat liver [193]. The consumption of a coumarin-containing diet provides substantial protection against the initiation of AFB<sub>1</sub> hepatocarcinogenesis in the rat [193].

## Cafestol and kahweol (C&K)

These diterpenes are two potentially chemoprotective agents present in green and roasted coffee beans; they act as blocking agents by modulating multiple enzymes involved in carcinogen detoxification [194]. Significant inhibition was detected at 2300 mg kg<sup>-1</sup>, and the reduction of DNA adduct formation to nearly 50% of the control value was maximized by 6200 mg kg<sup>-1</sup> of dietary C&K. Two complementary mechanisms may account for the chemopreventive action of cafestol and kahweol against AFB<sub>1</sub> in rats. A decrease in the expression of the rat activating CYP450s (CYP2C11 and CYP3A2) was observed, which was accompanied by a strong induction of the expression of the GST subunit GST Yc2, which detoxifies AFB<sub>1</sub>. These coffee components may broadly inhibit chemical carcinogenesis [194].

## Terpenes

A potent protection against AF-induced tumorigenesis through induction of Nrf2-regulated pathways by the triterpenoid 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl] imidazole was reported [195].

## Monoterpenes

Salvia, amaranth seeds and eucalyptus reduced adduct formation in rats exposed to AFB1 [196].

## Grapefruit

The influence of grapefruit juice intake on AFB1-induced liver DNA damage was examined in F344 rats administered 5 mg kg<sup>-1</sup> AFB1 by gavage. Grapefruit juice extract inhibited AFB1-induced mutagenesis by inhibiting the metabolic activation potency of AFB1 in the rat liver [197].

The hepatic GST activity and glutathione content in the portal blood and the liver concentrations of AFB1 did not significantly differ between grapefruit juice intake rats and the controls, but fewer revertant colonies were observed in the Ames test using *Salmonella typhimurium* TA98. A significant decrease in the hepatic CYP3A content, but not the CYP1A and CYP2C contents, was observed in the microsomes of grapefruit juice-treated rats compared with non-treated rats [197].

## Vitamins

Whereas lycopene and an excess of vitamin A showed no effect,  $\beta$ -carotene,  $\beta$ -apo-8'-carotenal, astaxanthin and canthaxanthin, and a highly carcinogenic polycyclic aromatic hydrocarbon called 3 methylcholanthrene (3-MC) were highly efficient in reducing the number and size of liver preneoplastic foci [198].

Both  $\beta$  carotenoids and 3-MC decreased AFB1-induced DNA *single-strand binding* protein and the binding of AFB1 to liver DNA and plasma albumin *in vivo*. *In vitro*, these compounds increased AFB1 metabolism to AFM1, a less genotoxic metabolite. These carotenoids exert their protective effect by directing AFB1 metabolism towards detoxification pathways. By contrast,  $\beta$ -carotene did not protect hepatic DNA from AFB1-induced alteration, and caused only minor changes in AFB1 metabolism. Thus, its protective effect against the initiation of liver preneoplastic foci by AFB1 appears to be mediated by other mechanisms [198]. The intake of 300 mg of ascorbic acid by gavage protected guinea pigs from the acute toxicity of AFB1 [199].

Finally, human hepatocytes (HepG2) cells pretreated with lycopene and  $\beta$ -carotene are protected from the toxic effects of AFB1 at both the cellular and molecular levels [200].

## *Oldenlandia diffusa* and *Scutellaria barbata*

*Oldenlandia diffusa* and *Scutellaria barbata* have been used in traditional Chinese medicine to treat liver, lung and rectal tumors. They inhibited mutagenesis, DNA binding and the metabolism of AFB1 bioactivation [201]. Specifically, they exerted antimutagenic and antitumorigenic effects on AFB1 by inhibiting the CYP3-mediated metabolism of AFB1 [201].

*Oldenlandia diffusa* (= *Hedyotis diffusa*) is from the Rubiaceae family, found in the southeastern provinces of China-Guangxi, Guangdong and Fujian-growing at low altitude in moist fields. It is dried in sunlight to make tea or used fresh. The part of the plant used in herbal formulas is the rhizome. An early use of this herb was to treat poisonous snake bites, to cure childhood malnutrition, acute appendicitis, peritonitis and cancer tumors, especially from stomach, esophagus, rectum, ovary, pleura, liver and lung and, when used

externally, it is effective for vesicles and ichthyosis. It is bitter, neutral, non-toxic, and used to clear heat, remove toxin, and alleviate pain [202-210].

*Scutellaria barbata* is a species of flowering plant in the mint family, Lamiaceae. It is native to Asia. Its English common name is barbed skullcap.

*Scutellaria* refers to banzhilian, the whole plant of *Scutellaria barbata*, and should not be confused with "scute," the common name referring to huangqin, the root of *Scutellaria baicalensis*. These are in the mint family. Though both are from the same genus, the former, for which the tops are used, has essential oils among the active components, while the latter relies primarily on flavonoids, particularly baicalin and baicalein [211-214].

The Chinese name for the herb refers to "half twigs" (banzhi): the stems of the plant are half covered with leaves and half a flower stalk, hence the name. The term lian is used to describe the lotus, which is most likely mentioned here just to indicate that the plant is valued, not for any other relation. *Scutellaria* had been used as a folk medicine and is not mentioned in any classic herbals. It was first described formally in a modern science journal (Jiangsu Botanicals Journal). It was reported in the National Collection of Medicinal Herbs that: "the herb is slightly bitter and cool, used to clear heat, remove toxin, and vitalize blood to remove blood stasis, and it has anticancer actions; it is used for tumor, appendicitis, hepatitis, ascites due to cirrhosis, and pulmonary abscess" [211-214].

The plant is a small-leaved mint, producing bright purple flowers. Like *Oldenlandia*, it grows in moist flatlands, particularly at the edges of rice paddies and ditches, in southeastern China, though it is also found further West, to Sichuan, and further north, to Shaanxi, and at altitudes up to 2,000 feet. The tops are collected in late spring or early June, and carefully dried.

*Scutellaria* is much less studied than *Oldenlandia*, so there is only limited information available about it. However, it is considered of potential value and has been shown in laboratory studies to provide some of the same mechanisms of anticancer action as *Oldenlandia* mentioned above [211-214]. It is a common practice to combine it with *Oldenlandia*, especially for treatment of cancer, though it is sometimes used alone or with other herbs.

## Anti-Cancer Formulations

In the book Anticancer Medicinal Herbs, some therapies are mentioned with *Oldenlandia* and *Scutellaria* as main ingredients for cancers of the specified areas as indicated below. The listing by cancer site is what the formula had been applied for at the hospital where it was being used:

- Stomach: Combine *Oldenlandia* (90 g) and *Imperata* (60 g) or use *Scutellaria* (30) and *Imperata* (30).
- Esophagus, rectum, and stomach: *Oldenlandia* (70 g) and *Coix lacryma-jobi* (30 g); plus other herbs in small quantities.
- Esophagus: *Oldenlandia* (60 g), *Scutellaria* (60 g), *Cycas leaf* (60 g), *Imperata* (60 g), cotton root (60 g).
- Rectum: *Oldenlandia* (60 g), *Scutellaria* (15 g), *Solanum* (60 g), *lonicera stem* (60 g), *Viola* (15 g).
- Ovary: *Oldenlandia* (30 g), *Scutellaria* (50 g), *Solanum* (50 g *S. nigrá*; 30 g *S. lyrati*), turtle shell (30 g).
- Pleura (metastasize to): *Scutellaria* (120 g), *Taraxacum* (30 g).



- Liver, rectum, lung: *Oldenlandia* (60 g) and *Scutellaria* (60 g).
- Liver: *Oldenlandia* (60 g), *Scutellaria* (60 g), *Cycis* (18 g), *Phragmites* (30 g) [211-214].

### Chlorophyllin

Chlorophyllin is another natural product that has been reported as useful to reduce aflatoxin-DNA adducts in individuals at high risk for liver cancer [215].

### Probiotic bacteria

Some selected strains of probiotic bacteria can form tight complexes with AFB1 and other carcinogens and can block the intestinal absorption of AFB1 to reduce the urinary excretion of AFB1-Gua, a marker of the biologically effective dose of AF exposure. Increases in the urinary excretion of AFB1-Gua adduct are associated with an increased risk of liver cancer. A probiotic supplement has been shown to reduce the biologically effective dose of AF exposure and may thereby offer an effective dietary approach to decrease the risk of liver cancer [216].

### Additives: Aluminosilicates and glucomannans

The most frequently used method to decontaminate grains for feed are the addition of aluminosilicates, zeolites and glucomannans. Aluminosilicates are oxides of silicon and aluminum associated with cations, such as calcium, magnesium, sodium, potassium, etc. The dosage for synthetic aluminosilicates is 1 kg/ton, and the dosage for natural aluminosilicates is 3 to 5 kg/ ton of feed [217]. Glucomannan comprises 40% of the dry weight of the roots of the Konjac plant, and it is also a constituent of the bacterial, plant and yeast cell walls, where it differs in the branches or glycosidic linkages in the linear structure [218-220].

### Legislation

AFs are highly regulated worldwide, with strict limits permitted in human commodities and animal feed.

The current worldwide regulations for AFs vary depending on whether the country setting the limits is an importer or exporter. In 76 countries, the AFt tolerance limits are 0-35  $\mu\text{g kg}^{-1}$ , whereas 61 countries legislate AFB1 to be between 1-20  $\mu\text{g kg}^{-1}$  [221].

The European Union legislated the level of AFB1 and AFt in corn to be 5  $\mu\text{g kg}^{-1}$  and 10  $\mu\text{g kg}^{-1}$ , respectively, for further treatment [222].

The Food and Drug Administration (FDA) analyzes products via a formal compliance program and exploratory surveillance activity [30]. The FDA regulatory levels for AFt ( $\mu\text{g kg}^{-1}$ ) apply 20  $\mu\text{g kg}^{-1}$  to all products for humans, except for milk; the limit for corn for immature animals and dairy cattle is 20  $\mu\text{g kg}^{-1}$ ; the limit for corn or peanuts for breeding beef cattle, swine and mature poultry is 100  $\mu\text{g kg}^{-1}$ ; the limit for corn or peanuts for finishing swine is 200  $\mu\text{g kg}^{-1}$ ; the limit for corn or peanuts for finishing beef cattle is 300  $\mu\text{g kg}^{-1}$ ; the limit for cotton seed meal as a feed ingredient is 300  $\mu\text{g kg}^{-1}$ ; the limit of all other feed stuffs is 20  $\mu\text{g kg}^{-1}$ , and that for milk (AFM1) is 0.5  $\mu\text{g kg}^{-1}$  [222].

### Conclusion

Although aflatoxins are “unavoidable” toxins in food, and the most important mutagens and carcinogens due to their frequent ingestion

and the big amount of contaminated foods, many methods are available to control them, ranging from natural detoxifying methods to more sophisticated ones. The metabolic routes of aflatoxins were mentioned here, including the CYP 450 isoenzymes and the formation of biomarkers. Physicians must be well informed to help people with uncommon and easy ways to control aflatoxins, which have produced serious outbreaks worldwide. The easy ways can be to reduce the ingestion of risky foods such as oilseeds, dairy products, spices, chili pepper and dry fruits, to prefer wheat instead of maize products. In the field the biocontrol method using non mutagenic *Aspergillus* spp strains have given good results. The role of government is crucial in monitoring the food products that are available for the human population, as well as the importations of foods with undetectable amounts of aflatoxins.

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