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Aflatoxins, Carcinogens in Food, as Etiological Factors in Human Malignant Neoplasias of the Lung

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Abstract

Lung cancer is a malignant neoplasm of the lung or bronchial cells and is one of the primary causes of mortality in men and is the third leading cause of death in women worldwide. Active and passive tobacco smoking are considered the main risk factors for the development of lung cancer, but aflatoxins have also been considered important etiological factors. Aflatoxins, which are fungal secondary metabolites produced primarily by *Aspergillus spp*., are chemically bis or tetra-hydrodifuran coumarins that contaminate foods (cereals, oilseeds, spices, dry fruits and dairy products). Aflatoxins are better recognized as hepatocarcinogens, but they can cause lung cancer *via* the formation of links to DNA and by the formation of AFB_1-DNA adducts, which can remain in the DNA for years and cause mutations and eventually cancers. The ingestion of aflatoxin-contaminated foods is the most common way in which individuals are exposed to these carcinogens, but other routes of exposure include nasal aerosol inhalation of AFB_1, which damages the lung. Alveolar macrophages possess specific oxidase activity for the epoxidation of AFB_1. In the biotransformation of the lung by AFB_1, AFB_1 requires a catalyzed metabolic activation of cytochrome P450 (CYP), the levels of which are low in the lung, to exert its carcinogenic activity. AFB_1 activation in the lung is achieved by prostaglandin H-synthetize, lipoxygenases, and *CYP2A13* enzymes, the last of which catalyzes metabolic activation. *CYP2A13* also plays a critical role in human lung carcinogenesis associated with inhalation exposure to AFB_1 and is highly efficient in the activation of AFB_1 *in situ*. Aflatoxins (AFB_1 and AFG_1) cause point mutations in K-Ras and H-Ras as well as in the *p53* tumor suppressor gene, which can cause lung cancer. This article summarizes the known etiology of lung cancer with respect to the human food carcinogens AFB_1 and AFG_1, the molecular mechanisms of aflatoxins, and the known point mutations in the *K-Ras*, *H-ras* and *p53* genes. This article also discusses a possible biocontrol (creosote bush or *Larrea tridentata*), the use of which is limited by its toxicity.

Keywords: Aflatoxins; Lung cancer; Carcinogens; Mycotoxins; Point mutations; *p53* genes; Bronchial cells

Introduction

Currently, cancer is the most important group of diseases that affect human society worldwide. The incidence of cancer has increased in the last several years, primarily in underdeveloped countries, due to population growth and to different risk factors such as tobacco smoking, fatness, physical inactivity, genetics and mycotoxins, among others. In 2012, 14.1 million of new cases of cancer were reported, and 8.2 million cancer-related deaths were reported worldwide [1,2].

Lung Cancer (LC) is one of the most common causes of cancer mortality in men and is the third cause of death in women worldwide. It has been suggested that there will be 1.8 new cases worldwide, with the highest incidence seen in underdeveloped countries [1,2]. Aflatoxins can cause different types of cancers in different organs such as the lungs [3], liver [4], colon [5], rectum [5], pancreas [6], kidney [7], and cervix [8]. In the lung, malignant neoplasia may develop from either the lung parenchyma or bronchial cells. Recently, the frequency of lung cancer has increased substantially, and it is now considered one of the most frequently detected malignant tumors. The World Health Organization (2015) classified the origins of LC as epithelial, mesenchymal, lymph histiocytic, ectopic or metastatic [9].

The symptomatology varies depending on the development of the disease. Symptoms include cough, dyspnea, recurrent pneumonia, and paraneoplastic syndrome, among others. Since the symptoms are diverse and may overlap with those of other diseases, the prognosis depends on the early or late detection of the disease.

Active and passive tobacco smoking are considered the main risk factors for the development of LC [10,11], but in recent years, aflatoxins have also been considered important etiological factors [12]. Other risk factors include obesity, physical inactivity, improper nutrition, genetic load and exposure to different substances such as arsenic, asbestos and radon.

Literature Review

Aflatoxins

Aflatoxins (AF) are secondary metabolites produced mainly by the fungi *Aspergillus flavus*, *A. parasiticus* and *A. nomius* [13]. More than 20 known AFs have been identified, but the basic four are aflatoxin B1 (AFB_1), aflatoxin B2 (AFB_2), aflatoxin G1 (AFG_1) and aflatoxin G2 (AFG_2) [14]. To detoxify the body from AFB_1, which is the most toxic AF, the liver adds the chemical group OH- to these compounds to form hydroxylated metabolites. These metabolites are water-soluble and can be present in urine, lymph, and milk, among other body fluids. The primary hydroxylated metabolites are aflatoxin M1 (AFM_1), aflatoxin

M2 (AFM_2), aflatoxin P1 (AFP_1) and aflatoxicol (AFL); this last compound is interconvertible with AFB_1 and can be stored as AFB_1 [15].

The chemical structure of AFs is a dihydrodifuran or tetrahydrodifuran ring joined to a coumarin by a ring of 5 to 6 carbon atoms (Figure 1). AFs are solid crystals that are odorless, flavorless and colorless and that are slightly soluble in water but very soluble in organic solvents such as methanol, acetonitrile, hexane, and chloroform [16].

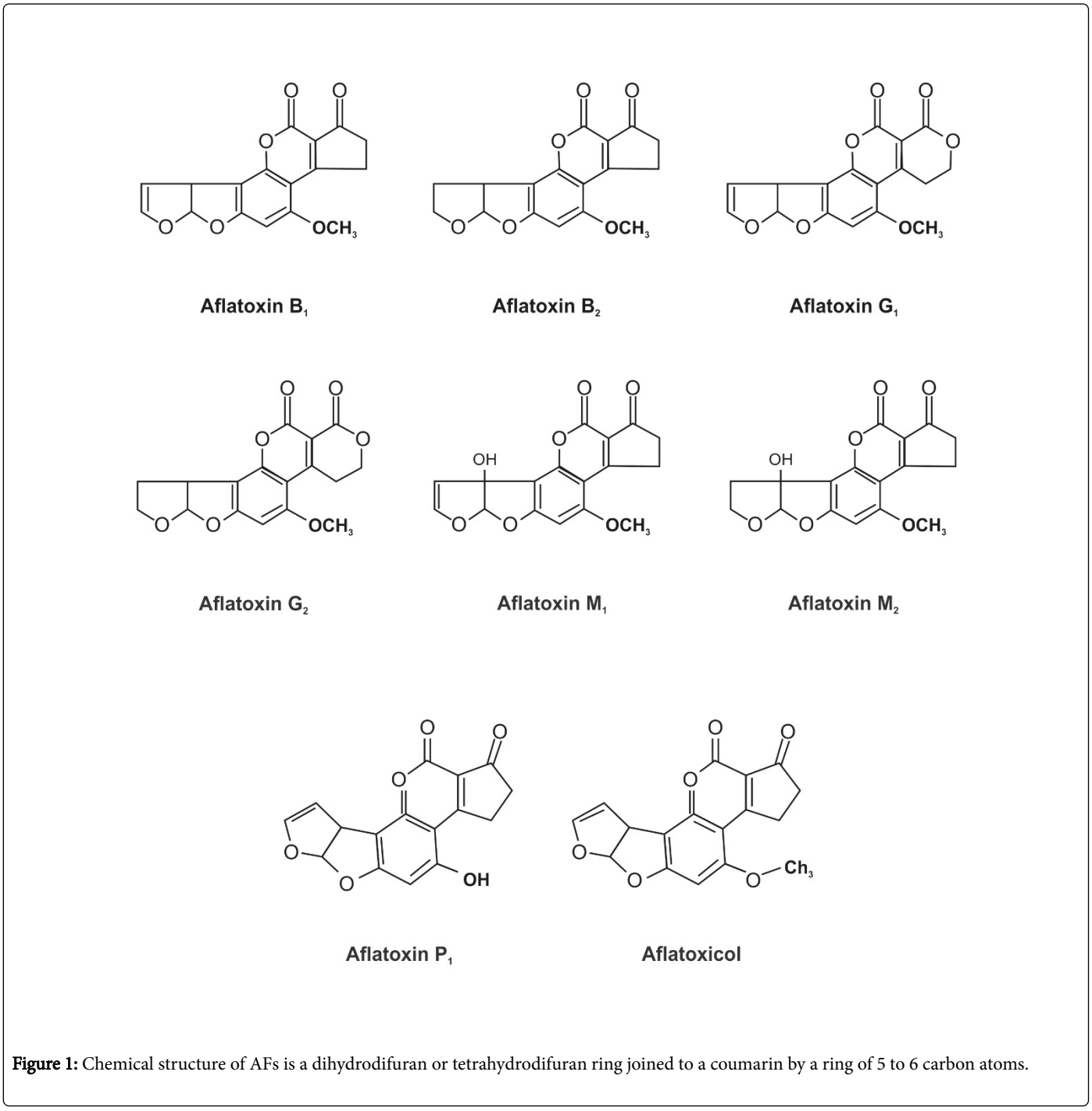

Figure 1: Chemical structure of AFs is a dihydrodifuran or tetrahydrodifuran ring joined to a coumarin by a ring of 5 to 6 carbon atoms.

Since the discovery of AFs as etiological factors of Turkey X disease, which was responsible for the deaths of 100,000 young turkeys in Great Britain in 1960, AFs have been the most studied mycotoxins [17,18]. The first outbreak of aflatoxins, which caused the deaths of 100 people, was reported in India [19].

The fungi that produce aflatoxins grow in a large variety of foods such as cereals (maize [20], rice [21], barley, oat and sorghum [17], oilseeds (peanuts [22], pistachios [23], almonds [24], nuts [25], and cotton seeds), and spices such as hot peppers [26] and black pepper [27]. Milk and dairy products can also be contaminated with AFM_1, which is the most common hydroxylated metabolite of AFB_1 [28,29].

AFM_1 can be detected in milk 12 to 24 hours after the cow has consumed feed contaminated with AFB_1. AFM_1 can also be detected in dairy products such as cheese and in a higher concentration than in milk [30].

Aflatoxins affect the absorption and metabolism of carbohydrates, proteins, lipids, vitamins and minerals, and their toxicity affects the electron transport of the respiratory chain and the cell membranes. Changes at the cellular level caused by aflatoxins in animal and plant cells are as follows: Nucleolus appears as a ring with different layers, presence of lipid bodies, mitochondria with elongated cisterns, endoplasmic reticulum without granulations, presence of elongated and irregular nuclei and the presence of highly granular chromatin [31]. Aflatoxins can also function as antimetabolites that link to DNA, where they interfere with ARN and protein synthesis [32].

Aflatoxins and lung cancer

Aflatoxin contamination can produce acute symptoms such as hemorrhage, vomiting, abortion, and death if the ingested amounts are high. Chronic symptoms such as hepatitis, cirrhosis, immunodepression, fetal malformations, and cancer may develop if the ingested amounts are low and if exposure occurs over long periods of time. These symptoms are observed in animals such as pets as well as in humans, with the liver as the target organ [28] for aflatoxins. AFB_1 is classified by the International Agency for Research on Cancer (IARC, 2002) [33] as a Group I carcinogen, as it has been demonstrated to be carcinogenic in humans; in contrast, AFM_1 is classified as a Group 2B substance, or one that is possibly carcinogenic in humans [34]. Acute toxicity is more frequent in underdeveloped countries, especially those in Africa, but the chronic effects of aflatoxins cause cancer in many different countries worldwide [28,35].

One of the main reasons why aflatoxins are considered potent carcinogens is their ability to form mutagenic adducts in DNA. These adducts can be formed up in cells, and when they accumulate, DNA repair is difficult, and mutations may be generated [36]. AFB_1 itself is not mutagenic, as it requires bio activation by cytochrome P450 to be transformed in exo-epoxide-8, 9-AFB_1, which is a carcinogen itself. The exo-epoxide-8,9-AFB_1 links to N7 in the guanine nucleotide, which forms a DNA adduct [37,38].

Routes of exposure

Regarding the routes of exposure necessary for DNA adduct formation in rats, Zarba (1992) [39] studied the liver following either an intra-tracheal injection or nose-only aerosol inhalation exposure to AFB_1. Aerosol inhalation is an effective route of exposure for AFB_1 in rats and hamsters and results in genotoxic damage to the liver. The binding of intra-tracheal-administered [3H] AFB_1 to rat liver DNA is only marginally higher than that observed in hamster liver, which is in contrast to the large difference observed in animals that received AFB_1 intraperitoneally. A positive association was observed between the development of human lung cancer and inhalation exposure to AFB_1.

The lungs are the second most important organ after the liver with respect to their ability to retain a considerable amount of tritiated [3H] AFB_1 (66%). AFB_1 that binds to DNA in the liver approached a peak 1 hr after the intra-tracheal administration of the toxin to rats and hamsters [40]. The persistence of the binding of AFB_1 to DNA in the lung and the extent of alveolar macrophages possess specific mixed function oxidase activity for the epoxidation of AFB_1 [40].

On the contrary, the route of administration of the carcinogen did not affect DNA binding over time in sprout-fed animals [41,42]. Rather, it was found that the nasal mucosa of some mammalian species is susceptible to the toxic effects of AFB_1, and thus, some investigators have studied the nasal enzymes involved in the metabolic activation of AFB_1 and the metabolites produced in nasal microsomes (NMa). Members of the *P450 2A* gene subfamily play an important role in the metabolic activation of AFB_1 in rabbit and rat nasal mucosa, which suggests a molecular basis for the assessment of the health risk associated with inhalation exposure of humans to this procarcinogen. The rates of AFB_1-N7-guanine DNA adduct formation in rabbit and rat NMa are over 3- and 10-fold higher, respectively, than in liver microsomes from the same species [42]. In contrast, the rates of formation of AFM_1 (9a-hydroxy-AFB_1) and AFQ_1 (3-hydroxy-AFB_1) products, which are known to be less toxic, are lower in nasal than in liver microsomes. Nasal microsomes produce high levels of six unidentified polar metabolites that are not formed by microsomes from the liver or by those from several other tissues. Furthermore, the formation of AFB_1-DNA adducts by nasal microsomes is decreased by nicotine, a known inhibitor of P450 NMa [42].

Biotransformation of AFB_1 in the lungs

AFB_1 requires the catalyzed metabolic activation of cytochrome P450 (CYP) to exert its carcinogenic activity. The major enzymes for AFB_1 activation in the human liver are *CYP1A2* and *CYP3A4* [43]. However, in the case of the lungs, *CYP2A13* is an enzyme that is predominantly expressed in the human respiratory tract. *CYP2A13* is highly efficient and can readily activate AFB_1 to the epoxides $AFB_{1\text{-}8,9}$ and $AFM_{\text{-}8,9}$ *in situ* [44]. The catalyzed metabolic activation of *CYP2A13 in situ* may play a critical role in human lung carcinogenesis associated with inhalation exposure to AFB_1 [45]. In human cells, cytochrome P450 is expressed in the respiratory tract.

Residues Ala 117 and His 372 in cytochrome P450 2A13 are important for the epoxidation of AFB_1 and its cytotoxicity [45]. AFB_1 activation by cytochrome P450 occurs at low levels in the lungs, and thus, AFB_1 is activated by other enzymes such as prostaglandin H-synthetase (PHS) [46,47] and lipoxygenases (LOX) [48], which are expressed at high levels in the lung. The expression of genes that code for xenobiotic metabolism enzymes in human bronchial and alveolar macrophages has been reported [49].

Aflatoxins in lung cancer

Lung cancer was first associated with AF exposure (mainly with AFB_1) in the 1980s after it was found during the biopsy of malignant lung tissues of two patients with lung *Aspergillosis* [3]. Now, a positive association has been demonstrated between human lung cancer and the exposure to or inhalation of AFB_1 [45].

Mutations in the K-Ras and H-Ras oncogenes point mutations and mutations in the tumor suppressor gene p53

When rats, mice and fish ingest an AF-contaminated diet, some proto-oncogenes of the "ras" family are activated [50,51]. High incidences of activated K-ras and N-ras have been observed in liver carcinomas and adenomas induced by AFB_1 [52]. Activation of the ras proto-oncogene has also been found in AFB_1-induced tumors in mice, rats, and fish. The relationship between aflatoxin exposure and the development of human hepatocellular carcinoma (HHC) was demonstrated by studies on the *Tp53* tumor suppressor gene. A high

frequency of p53 mutations (G→T transversion at codon 249) was found to occur in HHC tissues collected from populations exposed to high levels of dietary aflatoxin in China and Southern Africa [52].

The expression of both the Ras gene and Ras protein is commonly associated with alterations in tumors that increase invasiveness and metastasis and that decrease apoptosis. Molecules of this group function as regulatory-interrupters of signaling pathways such as the integrity of the cytoskeleton, proliferation, differentiation, attachment and migration. Activation of the proto-oncogene *Ras*, which occurs by point mutations in hot spots of codons 12, 13 and 61, is an early event in carcinogenesis and has been studied in lung tumorigenesis in mice. The pattern of point mutations that activate the *K-ras* oncogene reveals the nature of aflatoxin carcinogens [50]. The proto-oncogenes *Kras-2* in the lung and *Hras-1* in the liver, which are both expressed in mice, are the most common induced or spontaneous mutations reported. The proto-oncogene *Kras-2* and the tumor suppressor gene *Tp53* exhibit the major genetic alterations reported in the human lung. The incidence of *Kras-2* mutations was similar to that of asbestos-induced human lung tumors, while the incidence of Tp53 mutations differed significantly [51]. AFB_1, hepatitis B and C viruses, and vinyl chloride induced *Tp53* mutations in human liver tumors but with different mutation spectra. Mutation spectra serve as "fingerprints" of exposure and is based on chemical structure [51].

Oncogenes such as *N-ras*, *c-myc* and *c-fos* are over-expressed, but mutations in these genes are rare [53]. A specific mutation in codon 249 of the *Tp53* gene is present in individuals who live in regions where HCC and exposure to AFs are prevalent [54] and is considered to be a "hotspot" for mutations induced by AFB_1 [38]. The transversion G→T or the transition G→A occurs in the third base of codon 249 (AGG – AGT) of the *Tp53* gene and results in a change of amino acids from arginine to serine. This mutation is described as a dactylic fingerprint that shows AFB_1 exposition [55]. Another fingerprint that is indicative of AFB_1 presence is located in the first or second base of codon 12 of the *H-ras* gene [56-59].

An early event in the carcinogenesis of the lung is the transversion mutation G-T in the proto-oncogene *K-ras*. This mutation is caused by the association of AFB_1 (8-hydroxi-2'-desoxiguanosin) with DNA. In one study, tumors grew larger after exposure to polyethylene glycol-conjugated catalase, which was not protective against AFB_1-induced carcinogenicity in the mouse lung even though it prevented DNA oxidation [60]. The presence of AFB_1 in human bronchial epithelial cells has been observed along with a concomitant decrease in expression of the tumor suppression gene *p53* [61].

Although AFB_1 is best known as a hepatocarcinogen, the respiratory system can also be a target. In lung cells isolated from rabbits and mice, AFB_1 is biologically activated by cytochrome P450 and *K-ras* mutations occur primarily in non-ciliated bronchiolar epithelial (Clara) cells. In agreement with the DNA adduct, AFB_1-induced AC3F1 mouse lung tumors contain point mutations in guanine residues of *K-ras* [12].

AFB_1-induced mouse lung tumors overexpress p53 protein and demonstrate *p53* point mutations, which suggests that AFB_1 induces a carcinogen-specific response. In contrast, the human lung bio-activates AFB_1 primarily by prostaglandin H synthase and/or lipoxygenase-catalyzed co-oxidation, with activity concentrated in macrophages. Although glutathione S-transferase M_1-1 ($GSTM_1$) has high specific activity for AFB_1 epoxide conjugation, lung tissues from $GSTM_1$-null individuals show diminished rates of conjugation compared with tissues from $GSTM_1$-positive individuals. AFB_1-induced tumorigenesis in mice demonstrates unique properties, and the processes of bio activation show significant interspecies differences [12].

Of the 26 mutations found in micro-dissected regions from adenomas and carcinomas, 9 were G:C3 A:T transitions, 11 were A:T3G:C transitions, and 5 were transversions (2 G:C3T:A, 2 T:A3A:T, and 1 A:T3C:G), whereas 1 deletion mutation was identified. The concordance between immunostaining and the molecular detection of *p53* alterations was 72% when laser capture microdissection was used *versus* 17% when whole tumor analysis was used. The high mutation frequency and heterogeneous staining pattern suggest that *p53* mutations occur relatively late in AFB_1-induced mouse lung tumorigenesis and emphasize the value of the analysis of different staining regions of paraffin- embedded mouse lung tumors [62].

In humans, the *p53* or *TP53* tumor suppressor gene, which is sometimes referred to as the "guardian of the genome", is located on the short arm of chromosome 17 (17p13) and codes for a nuclear transcription factor of 53 k Da [63]. It controls DNA damage, stops the cell cycle when mutations occur and plays an important role in apoptosis and senescence when the damage cannot be repaired. The *p53* protein activates DNA repair enzymes so that they can correct the detected damage, and it also induces certain proteins such as GADD45 (=growth arrest and DNA damage) that cooperate in the DNA repair process. The *p53* protein activates the transcription of the miR34 microRNA family, which comprises small RNA molecules that prevent the translation of specific mRNAs to stop the cell cycle and apoptosis [64].

The cellular concentration of *p53* should be well regulated because although it can suppress tumors, a high level of *p53* can accelerate aging due to excessive apoptosis. A defective *p53* protein could allow abnormal cells to proliferate, which may lead to cancer, as 50% of all human tumors have *p53v* mutations and 80% of *p53* point mutations in human cancers are located in the DNA binding area of the protein. *p53* belongs to the same family of transcription factors as *p63* and *p73*. The *p53* protein is ubiquitously expressed in all tissues, while *p63* and *p73* are tissue-specific. All members of this transcription factor family have isoforms that function as activators, while others function as dominant negatives [65]. The *p53* protein is a phosphoprotein formed by 393 amino acids and 3 domains:

1) One that activates transcription factors,

2) Another that recognizes the central domain of a DNA-specific sequence,

3) A final-carboxyl domain (C-).

The primary negative regulator of the tumor suppressor *p53* is the protein *Mdm2* (=murine double minute 2), which can degrade *p53*. *Mdm2* acts as an E3 ubiquitin ligase [65], which marks *p53* for degradation by a proteasome [66]. *Mdm2* expression is also regulated by *p53*, and thus, *p53* can be maintained at low levels once the cellular damage has been repaired [67].

The ability of *p53* to induce apoptosis in the presence of DNA damage has important therapeutic implications. The two main treatment modalities for cancer are irradiation and chemotherapy; these both cause damage to DNA, which activates apoptosis in tumor cells. The malignant tumors that retain *p53* will be cured by these treatments, but the tumors that present *p53*-mutated alleles will be resistant because they will exhibit complications in the activation of apoptosis. Therefore, therapeutic modalities that increase *p53* activity

in malignant tumors that retain *p53* and those that selectively destroy tumor cells that lack *p53* are needed [64].

Approximately 40% of lung cancers present G>T mutations in codons 157, 158, 245, 248, 249 and 273 in the *TP53* gene. Other types of cancers in which the *p53* gene is mutated are Li-Fraumeni syndrome, lymphatic leukemia and Hodgkin disease, cancers of the skin, breast, head, neck, esophagus, liver, stomach, colon, bladder, cervix, ovary, and prostate as well as glioblastoma and pancreatic adenocarcinoma [68].

The *p53* tumor suppressor gene is implicated in the regulation of nucleotide excision repair (NER). AFB_1 causes different mutations in cells, while *p53* regulates the repair of those mutations [69].

Aflatoxin G_1 (AFG_1) is implicated in lung cancer, as its oral administration induces chronic alveolar inflammation, which is associated with lung tumorigenesis in mice, 3 to 6 months after treatment. The proliferation and angiogenesis of AT-II cells were induced in inflammatory tissues. Oxidative stress and cyclooxygenase 2 (COX-2) were increased in the alveolar epithelium of AFG_1-treated mice. Oral gavage of AFG1 induced lung epithelial hyperplasia and adenocarcinoma in Balb/c mice 12 months after treatment [70].

The up-regulation of nuclear factor-κB (NF-κB), a key transcription factor that is thought to play a major role in carcinogenesis, regulates many important signaling pathways involved in tumor promotion. In addition, the expression of signal transducer and activator of transcription 3 (p-STAT3), and COX-2 was also induced in lung adenocarcinoma, which establishes a link between AFG1-induced chronic inflammation and lung tumorigenesis. Oral administration of AFG1, which could induce chronic lung inflammation, may provide a pro-tumor microenvironment that contributes to lung tumorigenesis [10].

Epidemiological studies have revealed an association between AFB_1 exposure and lung cancer in humans. The bio activation and detoxification of AFB_1 was observed in human lung tissue obtained from patients undergoing clinically indicated lobectomy. [3H] AFB_1 was bio activated to a DNA binding metabolite by human whole lung cytosols and AFB_1 in a concentration-dependent manner. The inhalation of AFB_1 was supposed to cause primary lung cancer, and different tobacco sources:

a) Virginia of Jujuy,

b) Brasilero and

c) Black of Salta, presented AFB_1-positive determinations.

The bronchial tissues obtained by lung biopsies presented positive AFB_1 in lung epidermoid cancer. Additionally, 12 patients with chronic bronchitis who smoke presented an AFB_1 level less than that in the epidermoid lung cancer group [71].

Natural herbs as biocontrols for lung cancer caused by AFB_1

The use of plants for medicinal purposes was first recorded in Chinese, Egyptian, and Assyrian texts on herbal therapies. In 1985, the World Health Organization estimated in 1985 that 65% to 80% of the world's population rely on herbs for their medical needs [72], which is not surprising given that herbs serve as the basis for many drugs.

Larrea tridentata (creosote bush), which is known as greasewood in the United States (Arizona, California, Nevada, Texas and New Mexico) and as "gobernadora" or "hediondilla" in Mexican desert areas (San Luis Potosi, Coahuila, Chihuahua, Durango, Sonora, Zacatecas, Baja California), also grows in the arid zones of Argentina and Bolivia [73,74].

Aqueous extracts of *creosote* bush (*chaparral tea*) are used to treat a wide variety of disorders including chicken pox, skin sores, diabetes, kidney and gallbladder stones, cancer, venereal disease, tuberculosis, colds, and rheumatism [74,75].

Creosote bush has shiny leaves with a thick resinous coating, a strong odor and a sour flavor. The resin-coated leaves yield many flavonoid aglycones, essential oils, halogenic alkaloids as well as several lignans such as the antioxidant nordi-hydroguaiaretic acid (NDGA) [76]. NDGA and other phenols of the leaf surface function as antimicrobial agents and as protection against herbivores, UV radiation and water loss. As such, they are potentially important in the preservation of desert species.

The cytosolic activation of [3H] AFB_1 was correlated with 5-lipoxygenase (LOX) activity and was inhibited by the LOX inhibitor NDGA, which indicates that LOXs are largely responsible for the observed cytosolic activation of AFB_1 [10]. Indomethacin produced a 63-100% decrease in [3H] AFB_1-DNA binding in macrophages from five of seven patients, while the antioxidant NDGA from *Larrea tridentata* [77] inhibited [3H] AFB_1-DNA adduct formation by 19, 40 and 56% in macrophages from three patients. Cytochrome P450 has a minor role in the bioactivation of AFB_1 in the human lung [10].

NDGA inhibits the growth of several human cancer types and is useful in cancer therapy including that for breast, prostate, lung, esophageal and skin cancers. These encouraging results suggest a possible chemotherapeutic role for NDGA [73,78-80].

The exact mechanisms by which NDGA exerts its anti-tumorigenic and anti-proliferative effects are still unknown. LOX NDGA suppresses tumor growth *via* the inhibition of metabolic enzymes as well as receptor tyrosine kinase-phosphorylation, which is overactive in certain cancer cells [81].

Toxicity

Creosote bush *Larrea tridentata* J.M. Coult. and its primary metabolite NDGA have been useful in traditional medicine, industry and research, but neither creosote bush nor NDGA has been tested at the clinical level [73]. The toxicity of creosote bush was demonstrated, although the reported toxic doses in humans and experimental animals always exceeded the traditional use of the plant. Creosote bush extracts with NDGA that are used in healing practices are applied as either a topical paste or are consumed as a tea prepared by boiling the extracts in water. This extraction process may transform the compound into a weaker, stronger or even a toxic derivate [82]. Creosote bush was utilized commercially in the United States as a preservative for fats and butter, but it was removed from the FDA's list of safe agents because it induced cystic nephropathy in the rat. However, *Larrea*-containing products have remained on the dietary-supplement market. Although the consumption of low doses of such products appears to be harmless, high doses have been associated with dermatitis, nephrotoxicity (including renal cell carcinoma), biliary toxicity, and hepatotoxicity (including fulminant liver failure) in humans [83-85]. An interesting finding is that a structural modification of NDGA changed its toxicity *in vivo*, and at the beginning, the LD50 of NDGA was 75 mg kg^{-1}, whereas its derivative M4N was well-tolerated at 1000 mg/kg (both given by intraperitoneal injection) [86]. Moreover, other tetra-O-

substituted NDGA analogs also showed decreased toxicity *in vitro*, and therefore, the utility of this compound as a medicine is hopeful [87].

Conclusion

This article summarizes the known etiology of malignant lung neoplasia with respect to AFB_1 and AFG_1 as human carcinogens, the molecular mechanisms, mutations in the *K-ras* and *H-ras* oncogenes, point mutations and mutations in the p53 tumor suppressor gene and metabolic pathways. This article also discusses a possible biocontrol using *Larrea tridentata*, which is known as creosote bush, and its toxicological aspects.

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