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Pathologies associated to the consumption of aflatoxin M_1 in dairy products

Patologías asociadas al consumo de aflatoxina M_1 en productos lácteos

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Abstract:

Milk is the most traded, processed and consumed dairy product in the world, approximately 113.7 to 150 kg per capita. However, one of the food safety and human health problems worldwide is aflatoxin M_1 (AFM_1), present exclusively in milk and its derivatives, because this mycotoxin is heat-resistant to common food industrialization processes and is capable of producing hepatotoxicity, carcinogenicity, genotoxicity and immunosuppression in humans, so there are international regulations that establish parameters ranging from 0.03 to 0.250 µg/kg depending on each country. The present review aims at compiling information on mycotoxins and specifically on AFM_1 from its biotransformation from dairy cattle until the pathologies in humans associated with its consumption.

Keywords:

Aflatoxin M_1, Milk, Pathologies

Resumen:

La leche es el producto lácteo más comercializado, elaborado y consumido en el mundo, aproximadamente de 113.7 a 150 kg *per cápita*. Sin embargo, uno de los problemas de seguridad alimentaria y salud humana a nivel mundial es la aflatoxina M_1 (AFM_1), presente exclusivamente en la leche y sus derivados, debido a que esta micotoxina es termorresistente a procesos comunes de industrialización de alimentos y es capaz de producir hepatotoxicidad, carcinogenicidad, genotoxicidad e inmunosupresión en humanos, por lo que existen regulaciones internacionales en donde establecen parámetros que van de 0.03 a 0.250 µg/kg dependiendo de cada país. La presente revisión tiene como objetivo recopilar información sobre las micotoxinas y específicamente sobre la AFM_1 desde su biotransformación a partir del ganado lechero, hasta las patologías en el ser humano asociadas a su consumo.

Palabras Clave:

Aflatoxina M_1, Leche, Patologías

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INTRODUCTION

Aflatoxins (AF) are toxic compounds produced as secondary metabolites of different toxigenic fungi such as *Aspergillus flavus* and *Aspergillus parasiticus*, which produce AFB_1 in feed and food crops,[1] when these are consumed by dairy cattle or some other lactating animal, the digestive system converts part of this AF into AFM_1, with a conversion rate of 0.3 to 6.2%,[2] to be subsequently excreted in milk (approximately 0. 05 μg/kg, at an intake <40 μg per day per dairy animal fed contaminated feed), because the consumption of this feed is important to the majority of the population, it may pose a risk to human health,[3] since although AFM_1 is less carcinogenic (10% *in vivo*) than AFB_1.[4] In addition, pathologies such as hepatotoxicity, carcinogenicity, cytotoxicity, genotoxicity, and immunosuppression have been reported due to its consumption, making it of special concern in terms of food safety and human health.[5] Outbreaks of mycotoxicosis due to aflatoxins consumption, cause hundreds of deaths that can be prevented or interrupted by food analysis and treatment.[6] AF are regulated in more than 80 countries, but their legislation is not known at the international level; therefore, only permitted limits have been established for AFM_1 in milk and dairy products, ranging from 0 to 1.0 g/kg.[7] The objective of this review article is to summarize information such as definition, biotransformation, reports of aflatoxin M_1 presence in milk and pathologies associated with its consumption.

MYCOTOXINS

Mycotoxins are toxic compounds, classified as chemical hazards of biological origin, which are produced naturally by some species of molds belonging to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Claviceps* and, *Alternaria;* these mycotoxins are usually formed at the end of the exponential phase or the beginning of the stationary phase of mold growth, during storage or in the food itself. When found in warm and humid places, before or after harvest, contaminate food, feed, or raw materials used in their processing. These compounds causes diseases and disorders called mycotoxicosis, which has been shown to cause damage to the health of both humans and animals, these effects are caused by inhalation, direct contact, or ingestion. The most frequent mycotoxins that pose a risk are AF, achroatoxin A, patulin, fumonisins, zearalenone, nivalenol, and deoxynivalenol.[8,9] The first recorded data on mycotoxicosis date back to the Middle Ages and correspond to the disease called *Ergotism*, better known as St. Anthony's Fire, which affected a large number of people in Europe and was caused by the consumption of rye contaminated with alkaloids produced by *Claviceps purpurea*.[10] On the other hand, *Aspergillus flavus*, has its first record in the United Kingdom in 1960, where the death of more than 100 thousand turkeys fed with feed based on peanut flour, which came from Brazil and which contained the secondary metabolites of the fungal species,[11] these metabolites were later denominated as AF and are the first compounds identified as such.[8]

AFLATOXINS

AF are classified as non-protein organic compounds, are secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticu*s fungi, which require a minimum temperature of 6 to 8 ºC, optimum of 36 to 38 ºC and maximum of 44 to 46 ºC for their growth, these fungal strains produce AF in conditions of 12 ºC as minimum temperature, [27] to 30 ºC as optimum temperature and 40 to 42 ºC as maximum [12], pH of 5, water activity less of 0.70 at a relative humidity of 80%, and *A. flavus* produces the highest amount of toxins.[13] AF are characterized by having the form of solid crystals ranging in color from white to yellow. Chemically are isocoumarin derivatives, whose basic skeleton is a furan ring attached to the coumarin nucleus (Figure 1), the molecular weight is between 312.06 and 346.06 g/mol approximately, have low solubility in water (10–30 μg/ml), but in organic solvents such as chloroform, ethanol, methanol, acetonitrile, and acetone are soluble, also not very stable to light and air when in their pure form, tend to be susceptible to alkaline hydrolysis and contact with ammonia or sodium hypochlorite solutions (pH >10.5), and their ability to adapt to heat makes them heat-resistant and at pH 3 and 10 maintain their stability.[14-17] AF is produced by *A. flavus* and *A. parasiticus* molds growing in soil, decaying vegetation and cereals, which are among the most toxic mycotoxins, mostly related to *A. flavus*. The most affected crops are cereals, oilseeds, and spices.[9,18] AF belong to a wide group of approximately 20 different types, however, the most common are B_1, B_2, G_1 and G_2, according to the blue or green fluorescence in the presence of ultraviolet light at 365 nm, AFM_1 has also been included as one of the mycotoxins of importance in human health.[15] The association of AF ingestion and the incidence of pathologies, mainly hepatocellular carcinoma (HCC).[19] One of the first cases presented, pointing to the association between the incidence of hepatocellular carcinoma (HCC) with the ingestion of AF.[19], was presented in studies conducted in China and sub-Saharan Africa,[20,21] which formed the basis for AF to be classified as a human carcinogen by the International Agency for Research on Cancer (IARC) since 1994.[22]

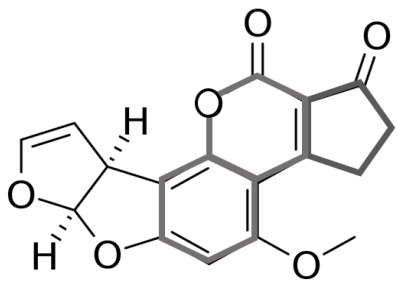

Figure 1. Basic structure of aflatoxins.
Furan ring. *Coumarin ring.*

TOXICITY OF AFLATOXINS

The toxicity of AF in humans is determined by different factors such as the bioavailability and toxicity of the mycotoxin, the synergisms between them, the amount of mycotoxin ingested daily depending on its concentration, the continuity or intermittent consumption of the contaminated food, the weight, physiological state, health and age of the individual.[9] The toxigenic effect of AF generate alteration in the synthesis of DNA, RNA, and proteins, which causes the appearance of changes in the permeability of the mitochondrial membrane, alteration of ribosomes, decrease in cellular respiration, and interruption of electron transport,[23] toxicity is the result of generalized and unspecific interactions between AF and diverse cellular proteins, which can cause the interruption of basic metabolic processes, protein synthesis and cause cell death.[24] The dose and duration of exposure to AF has been shown to play an important role in toxicology, exposure to large doses (acute aflatoxicosis) can cause pathologies and even death, while high-level exposure causes acute hepatic necrosis, which develops sequelae of cirrhosis or HCC.[25] As mentioned above, one of the mycotoxins of importance is AFM_1, which reaches milk through the metabolization of AFB_1 in dairy cattle consuming AFB_1 contaminated food or feed.

BIOTRANSFORMATION OF AFLATOXIN M_1

After ingestion by dairy cows through contaminated feed, AFB_1 is partially metabolized and bio-transformed to AFM_1.[26] A part of AFB_1 consumption by dairy cattle is transformed by some microorganisms to form aflatoxicol (highly toxic).[27] The remaining part that is not degraded by the rumen is absorbed in the small intestine because it has lipophilic properties and is of low molecular weight and then transported through the portal bloodstream.[28] Once it reaches the liver, it is metabolized by mixed-function microsomal oxidase enzymes (CYP450 superfamily enzyme) to a reactive epoxide intermediate (8,9-epoxide), which is responsible for DNA mutation and can bind RNA and proteins, which can lead to cellular dysregulation. [25] This hepatic biotransformation is also responsible for subjecting AFB_1 to reduction, epoxidation, hydroxylation, and demethylation. The hydroxylation phase produces AFM_1,[29,30] which is subsequently distributed to the mammary gland via the bloodstream, to be excreted in the milk.[31,32] This excretion may take place by passive diffusion, but active transport, mediated by outward transporters of the ABC family, expressed in the epithelial cells of the mammary gland, is probably the principal means of excretion.[33]

CHARACTERISTICS OF AFLATOXIN M_1

AFM_1, the 4-hydroxy-metabolite of fungal AFB_1, is excreted in the milk of all lactating species, including cows.[34] This AF has a chemical structure similar to AFB_1, but with an OH group attached to the tetrahydrofurans (Figure 2) and possesses toxic effects even at low concentrations (≤1 ng/kg/day).[34] Its molecular weight is 328 g/mol and it has a melting point of 299 ºC is resistant to temperatures between 260 and 320 ºC without decomposing,[35] and remains stable in milk and dairy products after industrial processes, such as pasteurization and ultra-pasteurization. It has been mentioned that AFM_1 may be associated with the casein fraction of milk, because it increases its hydrophobicity during the proteolysis stage in cheese making, this condition is necessary for AFM_1 to bind.[4,36] It was previously mentioned that according to the IARC, mycotoxins are found in group 1, the same agency classifies AFM_1 as a possible human carcinogen, which indicates that there is some evidence of causing cancer in humans but it has not yet been conclusive,[37] therefore, different countries have established maximum permissible limits for AFM_1 in milk and milk products (Table 1), in order to carry out pertinent regulations, since the evidence indicates the presence of this mycotoxin in products in order to carry out regulations, since the evidence points to the presence of this mycotoxin in products of popular consumption such as liquid milk (Table 2). The range of contamination goes from 0. 009–1020.00 ng/L, 0.01–5.16 µg/kg, 9.0–12.00 pg/kg and 0.53–0.207 ppb. Mexico has an established limit of 0.05 µg/kg and according to the AFM_1 content in raw milk reported by Rangel-Muñoz et al.,[38] their results exceed what is established in the country. Considering the maximum permissible limit of the European Union (0.05 µg/kg), countries such as Africa, Brazil, China, Egypt, El Salvador, Spain, Ethiopia, India, Iran, Malawi, Mexico, Pakistan, and Serbia exceed this regulation. In addition to the fact that they are close to the TD50 (10.38 µg/kg body weight/day),[39] the substantial public health problem and the possible development of pathologies caused by AFM_1 consumption are exposed.

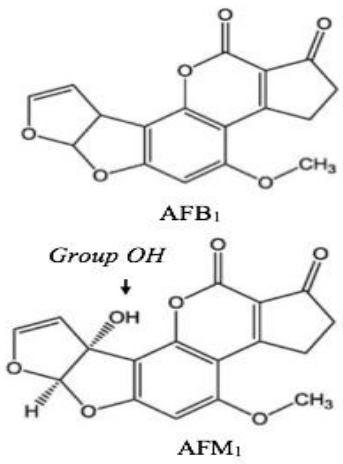

Figure 2. Difference between AFB_1 and AFM_1 in their chemical structure.

Table 1. Maximum permissible limit of AFM_1 in different countries.

Country	Maximum limit µg/kg	Food
Austria	0.05[a]	Milk and dairy products
Belgium	0.05[a]	Milk
Bulgaria	0.50[a]	Milk
China	15.00[a]	Milk and dairy products
Czech Republic	0.10[a] 0.50[a]	Milk for children Milk for adults
European Union	0.05[b] 0.025[b]	Milk Infant and follow-on formula
France	0.03[a] 0.05[a]	Milk for children Milk for adults
Germany	0.05[a]	Milk
Honduras	0.02[a] 0.05[a] 0.25[a]	Baby food Dairy products Cheese
Hungary	0.05[a]	Milk and dairy products
India	30.00[a]	Milk and dairy products
Indonesia	5.00[a]	Milk cheese
Iran	0.05[a] 0.50[a] 0.01[a] 0.20[a]	Pasteurized, raw or sterilized milk and milk derivatives Powdered milk Baby milk Cheese
Israel	0.05[a]	Milk and dairy products
Italy	0.01[a]	Baby food
Korea	0.05[a]	Milk and dairy products
Latvia	0.50[a]	Milk and dairy products
Moldova	0.50[a]	Milk
Malta	0.05[a]	Milk
Mercosur	0.50[a] 5.00[a]	Liquid milk Powdered milk
Mexico	0.05[a]	Milk, milk formula and combination products
Morocco	0.05[a] 0.03[a] 0.50[a] 0.30[a]	Dairy products Milk for < 3 years Powdered milk Powdered milk for < 3 years
Netherlands	0.05[a] 0.02[a] 0.20[a]	Milk Butter Cheeses
Peru	0.50[a]	Milk
Poland	0.05[a]	Dairy products
Romania	0.50[a]	Milk and dairy products
Russia	0.50[a]	Milk and dairy products
Saudi Arabia	0.20[a]	Milk and dairy products
Senegal	0.50[a]	Milk and dairy products
Singapore	0.50[a]	Dairy products
Slovakia	0.50[a] 0.10[a]	Milk and dairy products Infant formulas and milk-based baby foods
Switzerland	0.05[a] 0.25[a] 0.02[a]	Milk and dairy products Cheese Infant and follow-on formula
Sweden	0.050[a]	Baby food
Syrian Arab Republic	0.20[a] 0.05[a]	Liquid milk Powdered milk
Taiwan Province of China	0.50[a] 5.00[a]	Liquid milk Powdered milk
Turkey	0.05 [a] 0.5 [a] 0.25 [a] 0.05[a]	Liquid milk Powdered milk Cheese Milk-based baby food
Ukraine	0.50[a]	Milk, dairy products y baby food

United States	0.50[a]	Milk
Venezuela	0.50[a] 5.00[a]	Liquid milk Powdered milk
Vietnam	0.50[a]	Milk and dairy products

[a]: FAO Food and Nutrition Paper,[40]. [b]: Commission Regulation (EC),[41]

Table 2. Presence of AFM_1 in milk in different countries.

Country	Quantity	Food
Africa	0.05–0.1 µg/kg.[42]	Milk
Africa	4.8–26.11 ng/kg.[43]	Milk
Algeria	95.59–557.22 ng/L.[44]	Raw milk
Bangladesh	22.79–1489.28 ng/kg 18.11–672.18 ng/kg 25.07–48.95 ng/kg.[45]	Raw milk Pasteurized milk UHT milk
Brazil	0.01–0.81 µg/kg.[46]	Commercial milk
Brazil	12.84–18.72 ng/L.[47]	Raw milk
Brazil	150–1020 ng/kg.[48]	UHT milk
Chile	0.015 µg/kg.[49]	Milk
China	0.022–0.049 µg/kg[50]	Milk
China	25.5–60.0 ng/L.[51]	Raw milk
Costa Rica	0.042–154 ng/mL.[52]	Raw milk
Ecuador	0.023–0.751 µg/kg.[53]	Raw milk
Egypt	0.02–0.19 µg/kg.[54]	Raw milk
Egypt	0.053–0.207 ppb.[55]	Raw milk
Egypt	0.18–0.41 µg/L.[56]	Raw milk
Egypt and South Africa	8.52–78.06 ng/L.[57]	Commercial milk
El Salvador	22.5–10.3 µg/kg.[58]	Raw milk
Ethiopia	0.031–5.16 µg/L. [59]	Raw milk
India	0.013–0.396 µg/L.[60]	Milk
India	0.027–2.281 µg/kg 0.01–1.116 µg/kg.[61]	Pasteurized milk
		Commercial milk
India	0.598–1.29 µg/L.[62]	Raw milk
India	0.80–3.2 µg/L.[63]	Commercial milk
Iran	0.005–0.098 µg/kg 0.005–0.120 µg/kg.[64]	UHT milk Pasteurized milk
Iran	0.011–0.321 µg/L.[65]	Raw milk
Iran	10–150 ng/L.[66]	Commercial milk Raw milk
Iran	11.7–106.6 ng/L.[67]	Pasteurized milk
Italy	0.009–0.015 µg/kg.[68]	Raw milk
Italy	0.009–0.026 ng/Kg.[69]	Commercial milk
Italy	0.71–3.63 ng/kg 0.85–44.39 ng/Kg.[70]	UHT Milk Pasteurized milk
Italy	7.19–22.53 ng/kg.[71]	Raw milk
Kenya	290.3–500 ng/kg.[72]	Commercial milk
Malawi	0.42–1.02 µg/L.[73]	Raw milk
Mexico	0.021–18.5 µg/kg.[38]	Raw milk
Mexico	9.0–12.00 pg/mL.[74]	Raw and pasteurized milk
Pakistan	0.03 ng/ml 0.35 ng/ml 0.11 ng/ml.[75]	Raw milk UHT milk Pasteurized milk
Pakistan	0.187–0.346 µg/L.[76]	Raw milk Processed milk
Pakistan	0.3–1.0 µg/L.[77]	Raw milk
Serbia	0.012–0.53 µg/L.[78]	Raw milk
Serbia	0.024–0.319 µg/kg 0.038–0.231 µg/kg.[79]	UTH milk Pasteurized milk
Serbia	0.076–0.069 µg/kg.[80]	Raw milk

Spain	0.05–0.50 µg/L.[81]	Commercial milk
Spain	9.0–18.8 ng/kg.[82]	Raw milk
Uruguay	0.005–0.08 µg/L.[83]	Commercial milk

PATHOLOGIES CAUSED BY AFLATOXIN M_1

AFM_1 can cause DNA damage, genetic mutation, chromosomal abnormalities, and cell transformation in mammals *in vitro*, in insects, lower eukaryotes, and bacteria.[3] The metabolic and toxic effects are mainly observed in the liver,[84,85] although it has also been reported in the lung after exposure by inhalation and diet, and it is also a pathogenic factor in weight insufficiency, hypo-immunity, neurological damage, and even high infant mortality,[86,87] in general, the main repercussions of AFM_1 are hepatotoxicity, carcinogenicity, genotoxicity, and immunosuppression.

Hepatotoxicity. Although AFM_1 is less mutagenic and genotoxic than AFB_1, it possesses cytotoxicity in hepatocytes *in vitro* and it has been demonstrated that its acute toxicity in several species is similar to that of AFB_1,[3] in addition this AF also possesses hepatotoxic effects and is relatively stable in the processes of pasteurization, storage and processing of milk.[88] Marchese et al.,[89] highlights damage in HepG2 cells (human hepatoma cell line), carrying out the analysis of the effects of AFM_1 on the metabolomic and cytochemical profile in a hepatoblastoma cell line, it was found that AFM_1 is able to block the cell cycle in the G0/G1 phase, it also induces the modulation of lipid, glycolytic and amino acid metabolism, increases the levels of proinflammatory cytokines such as IL-6, IL-8 and TNF-**α** and decreases the levels of IL- 4.

Carcinogenicity. Although AF ingestion has classically been associated with primary liver cancer or HCC and bile duct hyperplasia,[90] organs such as kidney, pancreas, bladder, bones, viscera, among others, have also been reported to develop cancer after mycotoxin exposure.[91] The carcinogenic effect of AFM_1 metabolites is produced from DNA intercalation and base alkylation by the epoxide moiety, resulting in mutations in the p53 gene, which is important in preventing cell cycle progression in DNA mutations or programmed cell death signaling.[25] The risk of cancer due to exposure to the various forms of AF is based on cumulative lifetime dose, thus IARC identifies AFM_1 as carcinogenic (group 2B), therefore, its regulation is limited to very low concentrations, this mycotoxin can cause cancer after prolonged exposure or at high doses (≥1 ng/kg/day), such as liver cancer and breast cancer (causing cell death, cell cycle detection and apoptosis), which are the most common causes of death in men and women, respectively.[92–94] On the other hand, taking into account the findings of Creppy, [95] in the weighted average of consumption, only an intake of <1 ng/body weight contributes to the risk of liver cancer, as indicated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)[96] so it could be said that the development of pathologies associated with AFM_1 would be present almost everywhere in the world.

Cytotoxicity. A study *in vitro* reported that AFM_1 causes toxicity in human MCL-5 lymphoblastoid B cells and cHol cells, expressing or not human cytochrome P450 enzymes.[97] One study found that AFM_1 did not significantly inhibit the differentiated or undifferentiated phase of Caco-2 cell growth when membrane damage was detected simultaneously by monitoring lactose dehydrogenase (LDH) release.[98] In models of differentiated and undifferentiated Caco-2 cells, where they were exposed to a dose range of 0. 01 to 1 mg/mL of AFM_1, caused marked cytotoxicity and decreased survival, furthermore, this mycotoxin also caused cell membrane damage,[99,100] indicating that it could damage intestinal cells at the cell membrane and affect DNA integrity, evidencing that, prolonged consumption of AFM_1 containing foods may pose a risk of injury to the intestinal tract,[99] as reported by Gao et al.,[101] on the toxicity of AFM_1, on the intestinal barrier, resulting in dysfunction of intestinal integrity in Caco-2 cells differentiated through clathrin-mediated endocytosis into TJ proteins. Huiying et al.,[102] investigated the effects of AFM_1 on renal cells, showed that long-term administration of AF, causes renal damage through oxidative stress and apoptosis. On the other hand, it was analyzed whether L-proline alleviates renal injury caused by AFM_1 in a mouse model, to which different mycotoxins were administered, including AFM_1 at dose of 3.5 mg/kg, the mycotoxin in question, activates pathways related to oxidative stress and causes renal damage; however, L-proline significantly alleviated oxidative damage and decreased apoptosis.[103] Nan et al.,[104] studied lactoferrin as an inhibitor of AFM_1-induced cytotoxicity and DNA damage in Caco-2, HEK, HepG2 and SK-N-SH cells, AF inhibited cell growth, induced an increase in lactate dehydrogenase level, as well as caused genetic damage and evidenced cytotoxicity and DNA damage, in the mentioned cell lines. Yanan et al.,[105] with Caco-2 cells, analyzed the expression of inflammation-related genes, revealed the inflammatory response caused by mycotoxins such as AFM_1, and demonstrated a decrease in gene expression of proinflammatory cytokines. Finally, the toxicity of AFM_1 in human cell lines has been shown to not require CYP450 activation, unlike AFB_1.[97]

Genotoxicity. The AFM_1 metabolite can increase the percentage of apoptotic cells,[106] and produce DNA damage, which leads to a prolongation of the G1 and G2 phases of the cell cycle,[107] in addition, AFM_1 can reduce the expression of tight junction proteins,[30] causing a time and concentration-

dependent reduction in cell viability,[99] significantly decreasing the S phase of the cell cycle.[107] Correlation between AF exposure and the appearance of guanine-aflatoxin N7 adducts in serum and urine has been demonstrated. As a consequence of DNA adduction, mutations occur during DNA repair or replication and, if the mutations are in critical genes, can significantly alter cellular functions. Studies suggest a high correlation between AFM_1 exposure and point mutations at a specific location, the third base of codon 249, of the p53 tumor suppressor gene.[24, 108] AFM_1 does not require cytochrome P450 activation to exert cytotoxicity, therefore cells can be more easily affected by this mycotoxin in milk, and AFM_1 does not need to pass through the blood circulation and can enter milk directly.[94]

Immunosuppression. AFM_1 inhibits phagocytosis and protein synthesis by interrupting the formation of DNA, RNA and proteins in the ribosome; the absorption of amino acids is altered and their hepatic retention is increased.[109] In innate immunity AFM_1 reduces macrophage viability, proliferation, cytotoxicity and phagocytic activity, as well as their expression of cytokines such as TNF-a, IL-1, and IL-6, and inducible nitric oxide synthase that mediate intracellular destruction of pathogens in phagocytosis.[110] Regarding adaptive immunity, an *in vitro* study in human Jurkat lymphoblastoid T cell line, AFM_1 inhibited T cell proliferation, but did not cause apoptosis or necrosis, however, it significantly increased the expression of IL-8 involved in innate immunity, while adaptive was not affected.[111]

CONCLUSION

The development of pathologies caused by ingestion or exposure to AFM_1 is a topic that is little studied, when compared to what has been reported for AFB_1. In-depth studies are still required in the field of breast cancer and other target organs such as the kidney and lungs, as well as the intestine, although there are reports on mutagenicity and teratogenicity by exposure to AF, the exact mechanism of these pathologies closely related to AFM_1 in humans is not yet reported.

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