# Hepatocellular carcinoma as a consequence of exposure to aflatoxins

# O carcinoma hepatocelular como consequência da exposição às aflatoxinas

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**ABSTRACT**
Aflatoxins are toxic substances produced by microscopic fungi of the genus Aspergillus, having as main representatives A. flavus, A. parasiticus and A. nomius (TRABULSI et al., 2015). According to Oliveira and Germano (1997), this group of chemical compounds has 17 representatives, being the most relevant for the health area the types B1, B2, G1 and G2. Trabulsi et al. (2015) state that aflatoxins were discovered in the 1960s, after an investigation into the deaths of hundreds of birds in England, which were fed feed from Brazil and Africa. Since then, it has been found that these substances have, in addition to a high toxicity, a great mutagenic, carcinogenic, and teratogenic power (KEW, 2013). Aflatoxin B1 (AFB1) has been described as the most potent natural carcinogen known (MURRAY; ROSENTHAL; Pfaller, 2017).

**Keywords**: Hepatocellular carcinoma, Exposure, Aflatoxins.

**RESUMO**
As aflatoxinas são substâncias tóxicas produzidas por fungos microscópicos do gênero Aspergillus, tendo como principais representantes o A. flavus, A. parasiticus e A. nomius (TRABULSI et al., 2015). Segundo Oliveira e Germano (1997), esse grupo de compostos químicos possui 17 representantes, sendo os de maior relevância para a área da saúde os tipos B1, B2, G1 e G2. Trabulsi et al. (2015) afirmam que as aflatoxinas foram descobertas na década de 1960, após uma investigação acerca da morte de centenas de aves na Inglaterra, que foram alimentadas com ração proveniente do Brasil e da África. Desde então, foi constatado que essas substâncias possuem, além de uma alta toxicidade, um grande poder mutagênico, carcinogênico e teratogênico (KEW, 2013). A aflatoxina B1 (AFB1) foi descrita como o agente carcinogênico natural mais potente conhecido (MURRAY; ROSENTHAL; PFALLER, 2017).

**Palavras-chave:** Carcinoma hepatocelular, Exposição, Aflatoxinas.

## 1 INTRODUCTION

Aflatoxins are toxic substances produced by microscopic fungi of the genus Aspergillus, having as main representatives A. flavus, A. parasiticus and A. nomius (TRABULSI et al., 2015). According to Oliveira and Germano (1997), this group of chemical compounds has 17 representatives, being the most relevant for the health area the types B1, B2, G1 and G2. Trabulsi et al. (2015) state that aflatoxins were discovered in the 1960s, after an investigation into the deaths of hundreds of birds in England, which were fed feed from Brazil and Africa. Since then, it has been found that these substances have, in addition to a

high toxicity, a great mutagenic, carcinogenic, and teratogenic power (KEW, 2013). Aflatoxin B1 (AFB1) has been described as the most potent natural carcinogen known (MURRAY; ROSENTHAL; Pfaller, 2017).

According to Oliveira and Germano (1997) and Magnussen and Parsi (2013), various foods such as corn, rice, beans, wheat, nuts, spices, and dried fruits may be susceptible to the presence of aflatoxins. The Food and Agriculture Organization of the United Nations estimates that about 25% of the crops in the world are contaminated by these mycotoxins, offering risks of accumulation in the body of animals and, consequently, being also present in meat, milk, and eggs (MARIN et al., 2013). In addition, Marin et al. (2013) indicate that these substances, because they have great chemical stability, are very resistant to food preparation processes. Consequently, these fungal metabolites are the main factor of contamination of non-infectious origin in food products and it is estimated that around 4.5 to 5.5 billion people are at risk of exposure to them (KEW, 2013). Factors such as droughts and pests in plantations, in addition to inadequate storage of grains due to lack of access to information and appropriate technologies, favor the growth of these fungi, a fact that occurs mainly in developing countries (MAGNUSSEN; PARSI, 2013).

There are two types of exposure to these compounds: acute, which is related to ingestion of high doses and can cause symptoms such as nausea, vomiting, jaundice, bleeding, lethargy, seizures, coma, acute liver injury and even death; and chronic, which is related to long-term intake of smaller amounts and can cause teratogenesis, prematurity and miscarriages during pregnancy, growth delays and child development, liver cirrhosis, hormonal problems, immunosuppression and carcinogenesis, especially in relation to the development of hepatocellular carcinoma (HCC) (MARIN et al., 2013; DHAKAL; HASHMI; SBAR, 2022). Data on the health impact of aflatoxins state that, worldwide, 4.6% to 28.2% of HCC cases are caused by these fungal metabolites (DHAKAL; HASHMI; SBAR, 2022).

Due to the great harms caused by the presence of these mycotoxins in various food sources, it is necessary to identify and quantify them in food before ingestion, since, in humans, it becomes more difficult to detect it due to its rapid metabolization (TRABULSI et al., 2015). Thus, in agreement with Dhakal, Hashmi and Sbar (2022), identification in food and feed can be done by thin-layer chromatography, high-performance liquid, liquid mass spectroscopy and ELISA; whereas, in men, it can be done by measuring the concentrations of AFB1-lysine, AFB-guanine adduct in the urine within 24 hours and the level of AFB-albumin adduct in serum.

Primary malignant tumors of the liver constitute the 6th type of cancer with the highest occurrence and the 2nd most common cause of cancer-related death in the world, with hepatocellular carcinoma being one of the main representatives of this group with a percentage of representativeness equivalent to 85 to 90% of cases (LLOVET et al., 2021).

According to Dani (2011) and Yang et al. (2019), the disease in question has an incidence of 500 thousand to 1 million cases/year, being more common in developing countries, mainly in the countries of

the regions of sub-Saharan Africa and East Asia, which concentrate about 85% of all cases of HCC in the world and, most of them have scarce assistance, technological and informational resources. In addition, McGlynn, Petrick and El-Serag (2020) argue that this disease has a poor prognosis, which explains the incidence and mortality have very approximate values.

The genomic somatic changes associated with epigenetic modifications, in accumulation, contribute to this disease manifesting itself, with exposure to triggering agents being a factor that defines the path of this progression (LLOVET et al., 2021). Zaterka and Eisig (2016) state that hepatocellular carcinoma presents several risk factors, such as age over 50 years, male sex, hemochromatosis, obesity, diabetes mellitus, nonalcoholic fatty disease, liver cirrhosis, hepatitis B and C and exposure to aflatoxins. In addition, it was observed that exposure to aflatoxins B1 increases the possibility of the onset of the disease by 4 times, but when associated with infection by the hepatitis B virus there is an increase to 60 times, confirming the idea that both can act synergistically, potentiating the damage and, consequently, the chance of developing malignancy (ZATERKA, EISIG, 2016).

It should be emphasized that this disease has several preventable factors, as cited by Dani (2011). Decreasing exposure to aflatoxins to undetectable serum levels would contribute to a decrease of about 23% of HCC cases in Africa and Asia, avoiding about 72,800 to 98,800 new cases per year (KEW, 2013).

## 2 GOAL

This literature review seeks to elucidate the pathophysiological mechanism of hepatocellular carcinoma resulting from the consumption of food contaminated by aflatoxins.

## 3 METHODOLOGY

This study consists of a literature review. For the data search, the digital platforms PubMed and SciELO were used. For the selection of articles, we used as inclusion criteria: academic articles of greater impact around study from 1995 to 2022, in English and Portuguese, with the descriptors "aflatoxins" and "hepatocarcinoma" and combinations between them. The exclusion criteria were articles that were not within the chosen time, in languages other than English and Portuguese, that were not relevant and that did not address aflatoxins, hepatocarcinoma and the relationship between both. In addition to the digital databases, gastroenterology books, such as Dani (2011) and Zaterka and Eisig (2016), and microbiology, such as Trabulsi et al. (2015) were used. In the end, 11 scientific articles and 3 books were obtained.

## 4 DEVELOPMENT

AFB1 is initially absorbed in the gastrointestinal tract and then biotransformed in the liver by the action of enzymes belonging to the cytochrome P-450 superfamily, and may undergo three possible

reactions: hydroxylation, O-demethylation or epoxidation (OLIVEIRA; GERMANO, 1997). The first two reactions involve, respectively, the formation of aflatoxins M1, Q1, B2a and P1, which, because they have a hydroxyl group in their structure, can be associated with glucuronic acid or sulfates, which explains their great water solubility and, thus, the ability to be excreted in urine, bile and feces, participating in the process of detoxication of aflatoxin (OLIVEIRA; GERMANO, 1997). In turn, according to Oliveira and Germano (1997) and McLean and Dutton (1995), during epoxidation, AFB1 originates AFB1-epoxide or 8,9-oxide of AFB1, which can pass into the detoxification pathway when they are conjugated with reduced glutathione through glutathione-S-transferases, in addition to also being able to bind to DNA, RNA or proteins, resulting in the formation of adducts, which represent the primary lesion caused by aflatoxins, altering both the conformation of nucleic acids and their protein products.

Based on this, these DNA adducts are formed by binding AFB1-epoxide to guanine DNA molecule at position N7 at the level of codon 249 of the p53 suppressor gene; after, a depuration occurs and these adducts can be conjugated and excreted in the urine, while in the DNA, the apurin sites are occupied by adenines and the transversion of guanine to thymines occurs, giving rise to mutations that justify the changes in the structure and biological activity of AFB1 on the DNA, as well as the carcinogenic effects related to the development of hepatocellular carcinoma (OLIVEIRA; German, 1997; MCLEAN; Dutton, 1995).

In addition to the carcinogenic contribution resulting from changes in the TP53 tumor suppressor gene, this molecular interaction can also lead to the activation of proto-oncogenes, such as N-RAS, K-RAS, c-Ha-RAS and c-MYC (MURRAY; ROSENTHAL; Pfaller, 2017).

## 5 FINAL CONSIDERATIONS

It is concluded that the aflatoxins produced by the fungus Aspergillus are related to the pathophysiology of hepatocellular carcinoma.

These toxins are present in much of the world's crops and in many foods that are poorly packaged, such as peanuts, soybeans, corn, rice and beans. This presence also impacts on livestock since these crops are used in the preparation of animal feed. The chemical stability of AFB1 to processing in food preparation, in addition to these factors, implies a great risk of contamination and ingestion of these substances.

It was observed that, through the reactions of the organism to the excretion of this substance, AFB1-epoxide is formed, which binds to the genetic material and thus leads to the inactivation of the TP53 suppressor gene, in addition to activating proto-oncogenic genes such as N-RAS, K-RAS, c-Ha-RAS and c-MYC.

## REFERENCES

DANI, R.. Gastroenterologia essencial. 4ª. ed. Rio de Janeiro (RJ): Editora Guanabara Koogan, 2011

DHAKAL, A.; HASHMI, M. F.; SBAR, E.. Aflatoxin Toxicity. 1ª ed. Treasure Island (FL): StatPearls Publishing, 2022

JACKSON, P. E.; GROOPMAN, J. D.. Aflatoxin and liver cancer. Best Practice & Research Clinical Gastroenterology, [S.L.], v. 13, n. 4, p. 545-555, dez. 1999. Elsevier BV. http://dx.doi.org/10.1053/bega.1999.004

KEW, M.. Aflatoxins as a Cause of Hepatocellular Carcinoma. Journal of Gastrointestinal and Liver Diseases, v. 22, n. 3, p. 305-310, 1 set. 2013

LLOVET, J. M.; KELLEY, R. K.; VILLANUEVA, A.; SINGAL, A. G.; PIKARSKI, E.; ROAYAIE, S.; LENCIONI, R.; KOIKE, K.; ZUCMAN-ROSSI, J.; FINN, R. S.. Hepatocellular carcinoma. Nature Reviews Disease Primers, [S.L.], v. 7, n. 1, p. 1-52, 21 jan. 2021. Springer Science and Business Media LLC. https://doi.org/10.1038/s41572-020-00240-3

MAGNUSSEN, A.; PARSI, M. A.. Aflatoxins, hepatocellular carcinoma and public health. World Journal Of Gastroenterology, [S.L.], v. 19, n. 10, p. 1508, 2013. Baishideng Publishing Group Inc.. http://dx.doi.org/10.3748/wjg.v19.i10.1508

MARIN, S.; RAMOS, A. J.; CANO-SANCHO, G.; SANCHIS, V.. Mycotoxins: occurrence, toxicology, and exposure assessment. Food And Chemical Toxicology, [S.L.], v. 60, p. 218-237, out. 2013. Elsevier BV. http://dx.doi.org/10.1016/j.fct.2013.07.047

MCGLYNN, K. A.; PETRICK, J. L.; EL-SERAG, H. B.. Epidemiology of Hepatocellular Carcinoma. Hepatology, [S.L.], v. 73, n. 1, p. 4-13, 24 nov. 2020. Ovid Technologies (Wolters Kluwer Health). http://dx.doi.org/10.1002/hep.31288

MCLEAN, M.; DUTTON, M. F.. Cellular interactions and metabolism of aflatoxin: an update. Pharmacology & Therapeutics, [S.L.], v. 65, n. 2, p. 163-192, jan. 1995. Elsevier BV. http://dx.doi.org/10.1016/0163-7258(94)00054-7

MURRAY, P. R.; ROSENTHAL, K. S.; PFALLER, M. A.. Microbiologia Médica. 8ª ed. Rio de Janeiro: Elsevier, 2017

OLIVEIRA, C. A. F. de; GERMANO, P. M. L.. Aflatoxinas: conceitos sobre mecanismos de toxicidade e seu envolvimento na etiologia do câncer hepático celular. Revista de Saúde Pública, [S.L.], v. 31, n. 4, p. 417-424, ago. 1997. FapUNIFESP (SciELO). http://dx.doi.org/10.1590/s0034-89101997000400011

TRABULSI, L. R.; ALBERTHUM, F.. Microbiologia. 6ª ed. São Paulo: Atheneu, 2015

YANG, J. D.; HAINAUT, P.; GORES, G. J.; AMADOU, A.; PLYMOTH, A.; ROBERTS, L. R.. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nature Reviews Gastroenterology & Hepatology, [S.L.], v. 16, n. 10, p. 589-604, 22 ago. 2019. Springer Science and Business Media LLC. http://dx.doi.org/10.1038/s41575-019-0186-y

ZATERKA, S.; EISIG, J. N.. Tratado de gastroenterologia: da graduação à pós-graduação. 2ª ed. São Paulo: Editora Atheneu, 2016