International Journal of Clinical & Biomedical Research
Vol 9, Issue 4, 2023.

Sumathi Publications
Review Article

Aflatoxins: An Innocent Cause of Lethality in Humans

Mohammad Salim[1], Shafikun Nisha[1], Mohammad Shahid Masroor[2], Shagufta Parween[3]

1. Sanjay Gandhi Smriti Govt. Autonomous P.G. College, Sidhi, A.P.S. Univ., Bhopal (M.P.), India
2. People's College of Dental Sciences & Research Center, People's Univ., Bhopal (M.P.), India
3. All India Institute of Medical Sciences (AIMS), Bhopal (M.P.) India

Abstract

According to Food and Agriculture Organization, United Nations, approximately one-fourth of the world's food crops are continuously being contaminated comprehensively by mycotoxins. More than 300 types of mycotoxins are produced by one-fourth of the cereal crops infected The foodstuffs containing aflatoxins are indiscriminately being consumed by people developing various ailments, diseases and even cancer innocently. Aflatoxins (Afs) have been the most widely studied mycotoxin worldwide. The disease caused by the ingestion of aflatoxins is collectively called aflatoxicosis. They are mostly found in peanuts, mouldy maize grains, rice and perishable dairy products. There are four major types of aflatoxins (AFB1, AFB2, AFG1 and AFG2) found in nature mainly produced by the *Aspergillus flavus, A. parasiticus and A. nomius*. Aflatoxin B1 has always been a potent carcinogen classified by the WHO in Group "A" as causing cancer in humans. It induces mutation in the p53 gene to develop hepatocellular carcinoma (HCC) in humans. This is the third leading cause of cancer deaths worldwide. Keeping view in mind the present paper is an attempt to review the research done so far in the field of aflatoxins in humans. The review focuses on occurrence, epidemiology, historical glimpses, chemical nature and the types of aflatoxins, detection and detoxification, diagnosis, clinical symptoms and the treatment of aflatoxicosis in humans.

Keywords: Aflatoxins, Occurrence, Detection, Detoxification, Aflatoxicosis, AFB1, AFB2, AFG1 , AFG2, Hepatocellular Carcinoma.

Introduction

Mycotoxins are produced by mould fungi grown on a variety of foodstuffs. They cause various ailments, diseases and even cancer in humans when these contaminated foods are ingested innocently. Similarly, due to some unavoidable circumstances, these contaminated foods are deliberately being consumed by society without knowing their ill effects and diseases caused by them in future (AFB_1, AFB_2, AFG_1 and AFG_2) [1,2].

Mycotoxins are the toxic secondary metabolites usually released by the saprophytic filamentous moulds like *Aspergillus*, *Penicillium* and *Fusarium* easily grown on cereals, foodstuffs and foods of animal origin. They are naturally cultured while the infected crops are either growing during harvest or post-harvest storage conditions. Unfortunately, more than 300 types of mycotoxins are being produced by one-fourth of the cereal crops infected with fungi globally. These mycotoxins, with their different modes of action, are either cytotoxic, genotoxic, mutagenic or carcinogenic. The mycotoxin appears not

Correspondence: Dr. Mohammad Salim, Sanjay Gandhi Smriti Govt. Autonomous P.G. College, Sidhi, A.P.S. Univ., Bhopal (M.P.), India. Email id: msaleem195195@gmail.com

eISSN: 2395-0471
pISSN: 2521-0394

only as yet to define properly but are also quite challenging to classify them. They can be classified either as hepatotoxic, nephrotoxic, neurotoxic or immunotoxic. Similarly, depending on the definitions used, though the mycotoxins are of fungal origin, not all toxic substances released by the fungi are called mycotoxins [3-6].

Further, these mycotoxins cause immune suppression, toxic aleukia, leukaemia , impaired ribosomal functions, lymphoma, astrocytomas, mesothelioma, bronchial, uterine and renal cell carcinoma, demyelination, seizures, headache, muscular and neural disorders. They can also inhibit protein, RNA and DNA synthesis. These mycotoxins virtually destroyed the entire organ and systems of the body. They cause chromosomal aberrations, abnormal sister chromatids exchange and mismatched and displaced DNA synthesis [7-12].

One of the most important naturally occurring mycotoxins is aflatoxin, which develops hepatocellular carcinoma in humans (IARC, 1987) [13]. These aflatoxins are aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2, aflatoxin M_1 and aflatoxin M_2. While fatality caused by acute aflatoxin poisoning is as multiple organ failure, chronic aflatoxin poisoning causes cancer development of the same organ in future via the abnormalities occurring in the cellular genome. Aflatoxin B_1 mutated the p53 gene inhibiting the glucose and glycogen breakdown. It develops uncontrolled proliferation of cells and cancer in humans [14-17].

The present paper deals with the study of structure, types and biosynthesis, historical glimpse, occurrence, distribution and epidemiology of aflatoxins with their disease-causing abilities including cancer, diagnosis, clinical symptoms, detection, detoxification and treatment of aflatoxicosis in humans in the light of recent researches done so far in the field of medical microbiology and toxicology.

Historical Glimpse

In London, nearly 1 lac Turkey birds died suddenly due to the ingestion of Brazilian peanut meal contaminated with *Aspergillus flavus*. The people for the first time in 1960 knew about the horror of mycotoxins globally. Further, the term mycotoxin was coined in 1962. Similarly, the name aflatoxin is given by virtue of its origin from *Aspergillus flavus* as “afla” [14,18]. Finally, aflatoxin was separated by thin-layer chromatography (TLC). It was administered in one-day-old ducklings; within 24 hours symptoms of the same turkey “X” disease appeared with the damaged liver

Similarly, the same effects were also observed in rats, chickens, pigs and other animals as well. These aflatoxins have also been detected in the mother’s milk and urine [5,19]. While these aflatoxins are usually found in agriculture and dairy products infected by *A. flavus* and *A. parasiticus*; they are mostly found in peanuts, oilseeds, rice, meat, cheese, bread, and butter [1, 2, 6, 12, 20].

Occurrence, Distribution and Epidemiology

Aflatoxins are a group of toxins produced by the *Aspergillus* mould fungi abundantly found in moist and humid conditions; they can easily contaminate the crops growing in fields at harvest or during the handling of the same plants. Farmers are usually exposed during the handling of crop plants. Similarly, other people are also affected by ingesting contaminated plant products and consuming the infected meat or dairy products. Further, various environmental factors including climatic changes influence the growth of *Aspergillus* in nature [21-24].

Aflatoxicosis is the poisoning of aflatoxins in humans. Chronic poisoning with aflatoxins developed hepatocellular carcinoma (HCC) in humans. HCC is the third leading cause of cancer deaths worldwide. This is quite surprising that approximately 83% of deaths from the intoxication of aflatoxins occur only in southeast Asia and sub-Saharan Africa [9,16,25,26]. This is due to the chronic infections of hepatitis B and C viruses in association with exposure to aflatoxins intoxication in that region. This has already been reported that the individuals already suffering from hepatitis viruses if exposed to aflatoxins in future are 30X more at risk of developing HCC than the individuals exposed to aflatoxins alone. Unfortunately, both HBV infection and aflatoxin exposure are very common in poor countries like India, Taiwan, Uganda and Kenya [27].

Several outbreaks of aflatoxin poisoning took place globally. There were a couple of outbreaks of aflatoxin poisoning that happened in northwest and western India in 1974. These outbreaks were due to the consumption of mouldy grains of maize. Aflatoxin B1 in large concentration was detected in persons who died of the same cause [28,29]. Similarly, Kenya faces aflatoxin exposure in 1981 and 2014 [30].

Chemistry, Biosynthesis and Types of Aflatoxins

Chemically, the aflatoxins are Di furanocoumarin derivatives produced by the polyketide’s pathways. They are produced by many strains of *Aspergillus* as *A. flavus*, *A. parasiticus*, *A. ochraceoroseus, A. bombycis, A. nomius* and *A. pseudotamari*. Further, based on their fluorescence under UV light and relative mobility in thin-layer chromatography (TLC), there are four major types of aflatoxins named as aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and

aflatoxin G_2 (AFG_2). Similarly, aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM_2) were first isolated from milk. However, there are well over 20 aflatoxins found in nature. These aflatoxins are now famous for their hepatoxic, carcinogenic, teratogenic and immunosuppressive effects. Although the fungus A. flavus is either grey-green or yellow-green, its aflatoxin is colourless, odourless, and tasteless. In addition, aflatoxin B and G gave blue and yellow-green colours under UV light respectively. Aflatoxin B_1 has been the most potent and widely studied aflatoxin produced by *A. flavus* (Figure 1) [2,31-33]. Several genes and their enzymes are involved in the biosynthesis of aflatoxins [34-36].

These aflatoxins contain naturally occurring highly oxygenated heterocyclic compounds having closely related molecular formulae as under:

1. Aflatoxin B_1 (C_{17} H_{12} O_6)
2. Aflatoxin B_2 (C_{17} H_{14} O_6)
3. Aflatoxin G_1 (C_{17} H_{12} O_7)
4. Aflatoxin G_2 (C_{17} H_{14} O_7)
5. Aflatoxin M_1 (4-hydroxy aflatoxin B_1)
6. Aflatoxin M_2 (4-dihydroxy aflatoxin B_2)

The toxicity level of different aflatoxins is graded as AFB_1>AFG_1>AFB_2>AFG_2. It means that AFB_1 is rather more toxic than any other aflatoxins found in nature [37].

Diagnosis and Clinical Symptoms of Aflatoxicosis

Aflatoxin intoxication is diagnosed simply by the testing of urine. This is also carried out with the help of nasal secretions, sputum, blood or tissue biopsy. The clinical manifestations of acute aflatoxicosis are as under:

Difficult digestion, nausea and vomiting, anorexia, loss of appetite, abdominal and colic pain with or without diarrhoea, oral and skin burning, red-coloured rashes on the body, sore throat, running nose, coughing, sneezing and watery eyes, sinus infection and fever, icterus, jaundice with lethargy and fatigue, feeling agitated and depressed, Convulsion, ataxia, loss of muscle control, coordination, balance and speech, difficult walking, swallowing and eye movements, blurring in eyes, seizures, bloody faeces, elevated liver function test, difficult breathing with increased heart and respiratory rate. [9, 12, 38]. The clinical symptoms of chronic aflatoxicosis have some additional life-threatening symptoms in addition to the symptoms of acute aflatoxicosis. Some of these lethal symptoms of chronic aflatoxin poisoning are as under:

1. Peripheral limb, pulmonary and cerebral oedema
2. Impaired immunity with serious infections
3. Stunted growth
4. Hepatomegaly, fatty degeneration of the liver, liver cirrhosis, hepatocellular carcinoma and liver failure
5. Multiple organs failure
6. Finally, coma and death [17, 39]

Moreover, as the symptoms differ individually, one should not quickly be linked with mould exposures unless the aflatoxin poisoning is fully established.

Detection of Aflatoxins

After proper sampling, the samples are screened for the detection of aflatoxins with the help of various techniques such as thin-layer chromatography (TLC), liquid chromatography (LC), high-pressure liquid chromatography (HPLC) and some immunochemical methods such as radioimmunoassay (RIA), Enzyme-linked immunosorbent assay (ELISA) and immunoaffinity

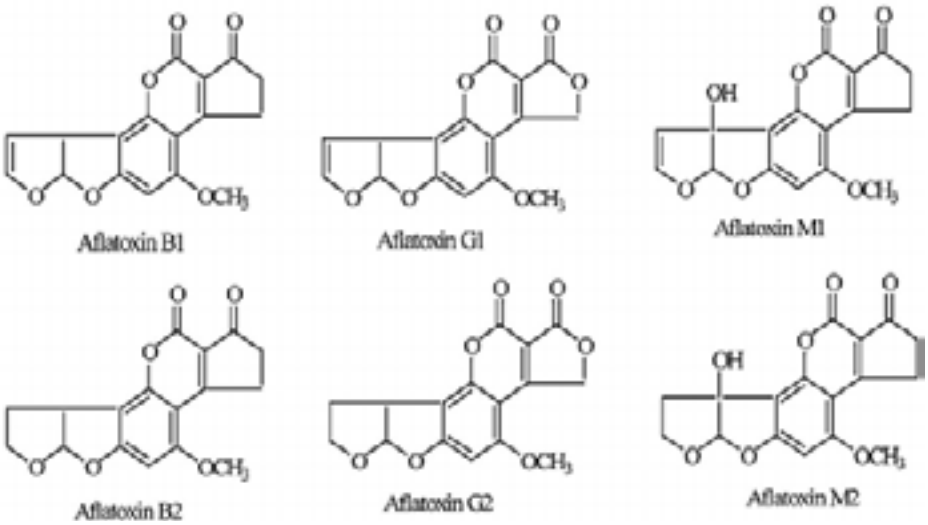


Figure 1. Skeletal formulae of different aflatoxins

column assay (ICA). Finally, the confirming tests are performed for the identity of aflatoxins by mass spectrophotometry (MS). Similarly, human exposure to aflatoxins is measured by the analysis of aflatoxin DNA adducts and albumin adducts as the basis for the cause of genotoxicity in people. Urine samples were also collected to establish the aflatoxin exposure for the detection of 2,3, dihydroxy-2-(N7- guanyl)-3- hydroxy aflatoxin B1 (AFB-gual) in urine [37,39-41].

Aflatoxins Causing Diseases and Cancer in Human

The symptoms of aflatoxin exposure appeared either by the inhalation and ingestion of spores or through direct skin contact. The main route of entry of aflatoxin absorption is the GI tract. On the contrary, the aflatoxin is excreted usually from bile, urine and milk. Similarly, the depth of disease intensity depends upon the dose and duration of exposure, age, sex and nutrition[10].

Aflatoxins are produced by Aspergillus in soil, crops, hay and perishable plant parts mainly during the process of harvesting, storage and processing of food materials. Its toxicity developed nausea, vomiting, convulsions and abdominal pain. Chronic infection causes hepatocellular carcinoma in humans [17,27,42].

Some of the diseases caused by aflatoxins in humans and animals are as under:

• *A. flavus* is an opportunistic fungus in humans and animals causing aspergillosis of the lungs, especially in immunocompromised individuals. The mould spores are thus colonized inside the lung cavities. The fungi might cause a lump combined with white blood corpuscles and blood clots [43].

• Aflatoxins are found in blood and semen [8]. It has also been reported that a higher amount of aflatoxin ingestion developed infertility in men [44].

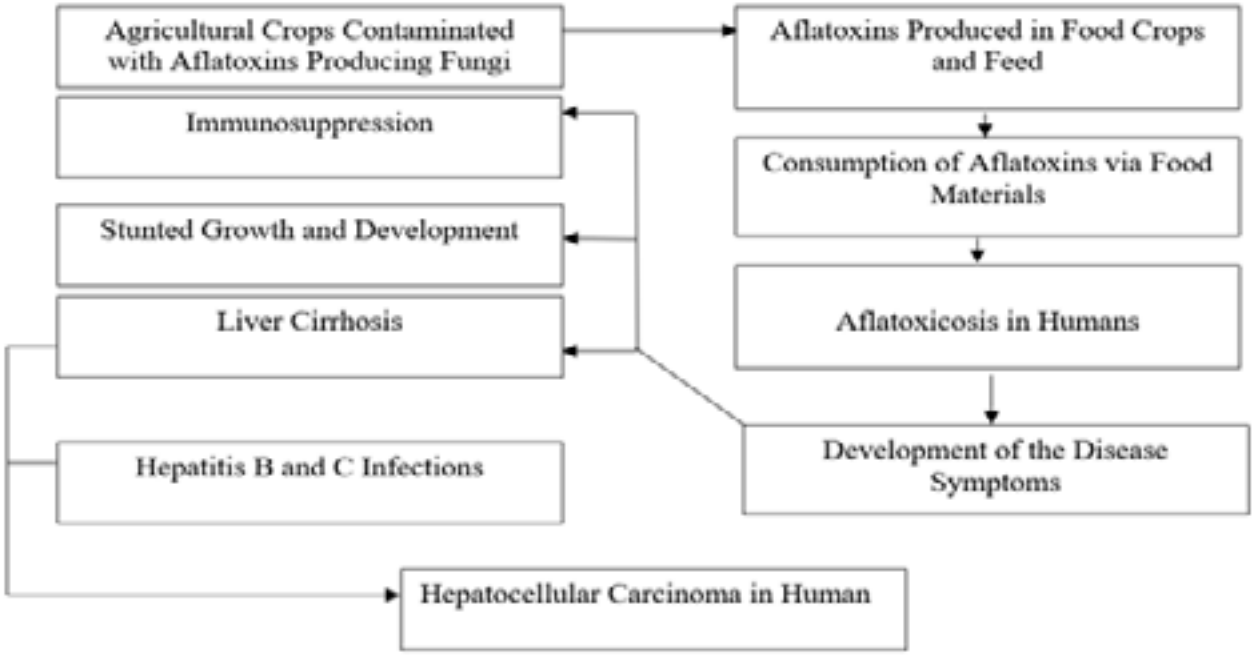
• Aflatoxin induces pre-term births in humans and animals [45].

• It poses a threat to a developing baby *in utero* and is transferred from mother to infant via milk [10, 46, 47]. Aflatoxin causes delayed development of the embryo and stunted growth in children [32, 48].

• Aflatoxin affects almost all major organs of the body like liver [15,16], Kidney [49], lungs [43], brain [50], testes [51], epididymis [52], Skeletal muscles [53], endocrine and exocrine glands [38,53].

The disease caused by the ingestion of aflatoxins is collectively known as aflatoxicosis. Though the aflatoxins are usually excreted by the urine, liquid bile and through milk, they are quite toxic while inside the body. While the higher degree of aflatoxicosis results in death, their chronicity mainly developed immune suppression and cancer in humans. Aflatoxin B1 usually develops hepatocellular carcinoma in humans [33]. It is more easily developed in those already having hepatitis B infection in the past. The disease is further characterized by nausea and vomiting, stomach pain, oedema and aspergillosis in the lungs, convulsions, fatty liver, kidney failure, heart anomaly, cerebral oedema, coma and death [1, 17, 38] (Flowchart 1).

Aflatoxin's cancer-causing abilities are due to the production of DNA adducts. It causes



Flowchart 1. Aflatoxin causes diseases and cancer in human

hepatocellular carcinoma in humans. Aflatoxin causes p53 gene mutation. This is a tumour suppressor gene found in humans protecting from cancer. This is mutated in such a way that transversion of amino acid took place at codon 249 developing HCC in humans [15, 54, 55]. Aflatoxins interfere with the mechanism of host protein biosynthesis. They interact with the nucleosides to form adducts with DNA, RNA and proteins. It inhibits RNA synthesis. Disordered protein biosynthesis and the lack of proteins developed necrosis and damage to the liver, kidney, heart and skeletal muscles [4, 38, 49, 56, 57]. Further, in addition to chromosomal strand breaks, unmatched DNA synthesis and chromosomal structural mutations, aflatoxins also produce mutagenic substances by hepatic cytochrome p450 to cause cancer in human .The aflatoxigenic effect in the liver is mainly due to lipid peroxidation and oxidative damage to DNA. Hepatic aflatoxicosis resulted in proteinuria, ketonuria, glycosuria and hematuria. Chronic liver failure leads to the accumulation of ammonia and fatty acids causing the damage of brain in individuals due to hyperammonemia crossing the blood-brain barrier [4, 42, 58].

The increased synthesis of glutamate neurotransmitters causing cytotoxicity in the brain cells developed hepatic encephalopathy. Most specifically pediatric encephalopathy with the fatty degeneration of viscera (Reye's syndrome) gives the symptoms as the loss of balance, coordination and orientation, sudden memory loss with insomnia. Aflatoxins also cause brain tumours such as glioma, meningioma and schwannoma [11, 38, 59].

Detoxification of Aflatoxins

Aflatoxin mitigation is a process which we use to reduce the aflatoxin concentration in foodstuffs. The process also reduces the health risk from the occurrence of aflatoxins in foodstuffs. Several physical, chemical and biological methods are employed for the detoxification of aflatoxins. While physical methods include separation, thermal inactivation, irradiation, extraction, adsorption and light treatment chemical methods are specifically employed as the use of several chemicals to degrade and inactivate the aflatoxins. The dietary chemicals and chemo sorbents are also being applied. Ammoniation and treatment with sodium bisulfite are the approaches for the same purposes [60-62]. Further, the sunlight treatment for drying the pepper (Capsicum annum) is also found effective in reducing the levels of the same mycotoxin [63, 64]. Similarly, heat is also effective. Since the fungus *Aspergillus parasiticus* has low resistance to heat, the treatment given to them can reduce the level of aflatoxins from peanuts, walnuts, hazelnuts and pistachios if roasted. Though the aflatoxins are thermostable they can be removed partially if the nuts are roasted at 150^{o}C for 90 minutes. However, mycotoxins cannot completely be destroyed under normal cooking temperature s [1]. An antioxidant ethoxyquin has been reported as chemopreventive and anticarcinogenic for aflatoxin B1 in humans [65]. Ozone therapy for food commodities like peanuts has also been found effective in reducing the level of aflatoxins [66,67]. Similarly, detoxification of aflatoxins from contaminated poultry feeds by bentonite, activated charcoal and Fuller's earth have also been tried [68].

Currently, microbiological post-harvest intervention with the use of several probiotic and non-probiotic bacteria and yeasts has been tried. Some of them are *Bifidobacteria, Lactobacilli, Streptococcus thermophilus, Bacillus subtilis, Bacillus licheniformis, Escherichia coli, Enterobacter spp., Saccharomyces* and *Candia spp.* [61,69,70]. With the use of these microorganisms, it has been observed that the level of aflatoxins is reduced significantly. The yeast supplementation has also reduced the level of aflatoxins in milk. The degradation of aflatoxins with the use of these probiotic bacteria and yeast has proved to be an eco-friendly measure of detoxification [71-73].

Last but not the least, while some operations for the management of mycotoxins are in practice to reduce the level of aflatoxins, different awareness programmes should be framed and conducted to make people aware of the harmful effects of mycotoxins.

Treatment of Aflatoxicosis

Chronic ingestion and inhalation of aflatoxins are lethal. There is no antidote for aflatoxins. Similarly, no specific treatment is reported. However, most of the disease symptoms are disappeared as soon as the toxicity is removed from the body. Only symptomatic and supportive therapies are to be given. Sometimes, a very weird situation is developed when a medical practitioner treats the patient as a case of simple poisoning. However, there are some reports of *Lactobacillus* effectively binding dietary mycotoxins [74]. A large case series of successful treatments of patients exposed to mould and mycotoxins is given by [31]. Finally, as the growth of fungi producing mycotoxins in crops and food commodities has always been an unavoidable problem leading to chronic mycotoxicoses resulting in death, several prophylactic measures are being employed for the prevention of these aflatoxins. It has been reported that a small dose of chlorophyll or chlorophyllin might reverse the effects of aflatoxin poisoning [75].

CONCLUSION

The paper discusses the toxicity of aflatoxins in humans. This is a general phenomenon that plant products and food commodities are infected by fungi . But, currently, it has now become a global problem as nearly 25% of the world's food crops are continuously being contaminated comprehensively by mycotoxins (FAO, UN). These mycotoxins are often produced by mould fungi The contaminated foodstuffs containing mycotoxins if ingested developed various ailments, diseases and even cancer in humans. In the recent past , there has been an increasing trend in researching the carcinogenicity of mycotoxins in humans. Aflatoxins are now probably existing the best known researched and extensively studied mycotoxins of the world. AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, and AFM_2 are released mainly by the *Aspergillus flavus, A. parasiticus* and *A. nomius*. Aflatoxin B_1 is a potent carcinogen causing mutation developing specific AGG to AGT amino acid transversion at codon 249 of the p53 gene. It develops hepatobiliary carcinoma in humans. It also causes breast cancer in humans and animals via point mutations and DNA strand breaks

Further, this is an epidemiologically and experimentally proved fact that chronic low-level exposure to aflatoxin B_1 produces hepatocellular carcinoma in future. Sometimes Kwashiorkor and Reye's syndrome patients marked by the fatty degeneration of viscera and liver were also detected with pediatric aflatoxicosis. Currently, various preventive measures have been taken to avoid contamination and the availability of aflatoxins in foodstuffs.

References

1. Bhat R, Rai RV, Karim AA. Mycotoxins in food and feed: present status and future concerns. Compr Rev Food Sci Food Saf. 2010;9(1):57-81. doi: 10.1111/j.1541-4337.2009.00094.x, PMID 33467806.
2. Pickova D, Ostry V, Toman J, Malir F. Aflatoxins: history, significant milestones, recent data on their toxicity and ways to mitigation. Toxins. 2021;13(6):399-421. doi: 10.3390/toxins13060399, PMID 34205163.
3. Verma RJ, Raval PJ. Cytotoxicity of aflatoxin on red blood corpuscles. Bull Environ Contam Toxicol . 1991;47(3):428-32. doi: 10.1007/BF01702206, PMID 1768960.
4. Verma RJ. Aflatoxin cause DNA damage. Int J Hum Genet. 2004;4(4):231-6. doi: 10.1080/09723757.2004.11885899.
5. Bennett JW, Klich M. Mycotoxins. Clin Microbiol Rev. 2003;16(3):497-516. doi: 10.1128/CMR.16.3.497-516.2003.
6. Rushing BR, Selim MI. Aflatoxin b1: a review on metabolism, toxicity, occurrence in food, occupational exposure and detoxification methods. Food Chem Toxicol. 2019;124:81-100. doi: 10.1016/j.fct.2018.11.047, PMID 30468841
7. Oyelami OA, Maxwell SM, Adelusola KA, Aladekoma TA, Oyelese AO. Aflatoxins in the autopsy brain tissue of children in Nigeria. Mycopathologia. 1995;132(1):35-8. doi: 10.1007/BF01138602.
8. Uriah N, Ibeh IN, Oluwafemi F. A study on the impact of aflatoxin on human reproduction. Afr J Reprod Health. 2001;5(1):106-10. doi: 10.2307/3583204.
9. Kirk GD, Bah E, Montesano R. Molecular epidemiology of human liver cancer: insights into etiology, pathogenesis and prevention from the Gambia, West Africa. Carcinogenesis. 2006;27(10):2070-82. doi: 10.1093/carcin/bgl060, PMID 16679307.
10. Turner PC, Collinson AC, Cheung YB, Gong YY, Hall AJ, Prentice AM et al. Aflatoxin exposure in utero causes growth faltering in Gambian infants. Int J Epidemiol. 2007;36(5):1119-25. doi: 10.1093/ije/dym122, PMID 17576701.
11. Murray PR, Rosenthal KS, Pfaller MA, editors. Mycotoxins and mycotoxicoses. In: Medical microbiology. 6th ed. New York: Elsevier; 2009. P. 211-6.
12. Wild CP, Gong YY. Mycotoxins and human disease: A largely ignored global health issue. Carcinogenesis. 2010;31(1):71-82. doi: 10.1093/carcin/bgp264, PMID 19875698.
13. I.A.R.C. Monographs on the evaluation of carcinogenic risks to humans: Overall evaluations of carcinogenicity. An updating of IARC of IARC monographs; IARC Press: Lyon, France,1987; 1: ISBN 978-92-832-1411-3.
14. Forgacs J. Mycotoxicosis: the neglected diseases. Food stuffs. 1962;34:124-34.
15. Deng ZL, Ma Y. Aflatoxin sufferer and p53 gene mutation in hepatocellular carcinoma. World J Gastroenterol. 1998;4(1):28-9. doi: 10.3748/wjg.v4.i1.28, PMID 11819223.
16. Kimanya ME, Routledge MN, Mpolya E, Ezekiel CN, Shirima CP, Gong YY. Estimating the risk of aflatoxin-induced liver cancer in Tanzania based on biomarker data. PLOS ONE. 2021;16(3):e0247281. doi: 10.1371/journal.pone.0247281, PMID 33705417.
17. Dhakal A, Evelyn S. Aflatoxin toxicity. OBH-Interfaith Medical Center. [Updated 2021, Nov.23]. In:Statpearls [Internet]. Treasure Island (FL): Stat Pearls Publishing; 2022.

18. Nesbitt BF, O'kelly J, Sargeant K, Sheridan ANN. Aspergillus flavus and Turkey X disease: toxic metabolites of Aspergilllus flavus. Nature. 1962;195:1062-3. doi: 10.1038/1951062a0, PMID 14479064.

19. Veldman A, Mejis JAC, Borggreve GJ, Heeres JJ. Carryover of aflatoxin from cows' food to milk. Anim Prod. 1992;55:163-8.

20. Benkerroum N. Aflatoxins: producing molds, structure health issues and incidence in Southeast Asian and Sub-Saharan African countries. Int J Environ Res Public Health. 2020;17(4):1215. doi: 10.3390/ijerph17041215, PMID 32070028.

21. Park DL. Effect of processing on aflatoxin. Adv Exp Med Biol. 2002;504:173-9. doi: 10.1007/978-1-4615-0629-4_17, PMID 11922084.

22. Reiter E, Zentek J, Razzazi E. Review on sample preparation strategies and methods used for the analysis of aflatoxins in food and feed. Mol Nutr Food Res. 2009;53(4):508-24. doi: 10.1002/mnfr.200800145, PMID 19360755.

23. Medina A, Rodriguez A, Magan N. Effect of climate change on Aspergillus flavus and aflatoxin B1 production. Front Microbiol. 2014;5:348. doi: 10.3389/fmicb.2014.00348, PMID 25101060.

24. Kumar P, Mahato DK, Kamle M, Mohanta TK, Kang SG. Aflatoxins: a Global Concern for Food Safety, Human Health and Their Management. Front Microbiol;07. doi: 10.3389/fmicb.2016.02170'

25. Shephard GS. Aflatoxin and food safety: recent African perspectives. J Toxicol Toxin Rev. 2003;22(2-3):267-86. doi: 10.1081/TXR-120024094.

26. Kew MC. Epidemiology of hepatocellular carcinoma in sub-Saharan Africa. Ann Hepatol. 2013;12(2):173-82. doi: 10.1016/S1665-2681(19)31354-7, PMID 23396727.

27. Liu Y, WU F. Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. Environ Health Perspect. 2010;118(6):818-24. doi: 10.1289/ehp.0901388, PMID 20172840.

28. Krishnamachari KA, Bhat RV, Nagarajan V, Tilak TB. Investigations into an outbreak of hepatitis in parts of western India. Indian J Med Res. 1975;63(7):1036-49. PMID 1213790.

29. Reddy BN, Raghavender CR. Outbreaks of Aflatoxicoses in India. AJFAND;7(16):1-15. doi: 10.18697/ajfand.16.2750'

30. Ngindu A, Johnson BK, Kenya PR, Ngira JA, Ocheng DM, Nandwa H Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. Lancet. 1982;1(8285):1346-8. doi: 10.1016/s0140-6736(82)92411-4. PMID 6123648'

31. Rea WJ. A Large Case-series of Successful Treatment of Patients Exposed to Mold and Mycotoxin. Clin Ther. 2018;40(6):889-93. doi: 10.1016/j.clinthera.2018.05.003. PMID 29861191

32. Gong YY, Watson S, Routledge MN. Aflatoxin exposure and associated human health effect; a review of epidemiological studies. Food Saf (Tokyo). 2016;4(1):14-27. doi: 10.14252/foodsafetyfscj.2015026, PMID 32231900.

33. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. doi: 10.3322/caac.21492, PMID 30207593.

34. Chang PK, Cary JW, Bhatnagar D, Bennett JW, Linz JE. Cloning of the Asperigillus Parasiticus apa-2 gene associated with the regulation of aflatoxin biosynthesis. Appl Environ Microbiol. 1993;59:3237-3279.

35. Payne GA, Nystrom GJ, Bhatnagar D, Cleveland TE, Woloshuk CP. Cloning of the afl-2 gene involved in aflatoxin biosynthesis from Aspergillus flavus. Appl Environ Microbiol. 1993;59(1):156-62. doi: 10.1128/aem.59.1.156-162.1993, PMID 8439147.

36. Mahanti N, Bhatnagar D, Cary JW, Joubran J, Linz JE. Structure and function of fas-1A, a gene encoding a putative fatty acid synthetase directly involved in aflatoxin biosynthesis in Aspergillus parasiticus. Appl Environ Microbiol. 1996;62(1):191-5. doi: 10.1128/aem.62.1.191-195.1996, PMID 8572694.

37. Jaimez J, Fente CA, Vazquez BI, Franco CM, Cepeda A, Mahuzier G et al. Application of the assay of aflatoxins by liquid chromatography with fluorescence detection in food analysis. J Chromatogr A. 2000;882(1-2):1-10. doi: 10.1016/s0021-9673(00)00212-0, PMID 10895926.

38. Bbosa GS, Kitya D, Lubega A, Kyegomble DB. Review of the biological and health effects of aflatoxins on body organs and body systems. In: Razzaghi-Abyaneh M, editor (Rijeka:in tech). Aflatoxins-recent advance and future prospects.2013; 239-265.

39. Piermarini S, Micheli L, Ammida NHS, Palleschi G, Moscone D. Electrochemical immunosensor array using a 96-well

screen-printed microplate for aflatoxin B1 detection. Biosensors and Bioelectronics. 2007;22(7):1434-40. doi: 10.1016/j.bios.2006.06.029'

40. Fallah AA, Rahnama M, Jafari T, Saei-Dehkordi SS. Seasonal variation of aflatoxin M1 contamination in industrial and traditional Iranian dairy products. Food Control. 2011;22(10):1653-6. doi: 10.1016/j.foodcont.2011.03.024.

41. Andrade PD, Gomes da Silva JLG, Caldas ED. Simultaneous analysis of aflatoxins B1, B2, G1, G2, M1 and ochratoxin A in breast milk by high-performance liquid chromatography/fluorescence after liquid-liquid extraction with low temperature (LLE-LTP). J Chromatogr A. 2013;1304:61-8. doi: 10.1016/j.chroma.2013.06.049, PMID 23871563.

42. Magnussen A, Parsi MA. Aflatoxins, hepatocellular carcinoma and public health. World J Gastroenterol. 2013;19(10):1508-12. doi: 10.3748/wjg.v19.i10.1508, PMID 23539499.

43. Fossses VM and Waymack JR. Aspergillosis. [Updated 2022, Jan 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022.

44. Gupta RC. Aflatoxins, ochratoxins and citrinins. Reprod Dev Toxicol. 2011;55:753-61.

45. De Vries HR, Maxwell SM, Hendrickse RG. Fetal and neonatal exposure to aflatoxins Acta Paediatrica exposure to aflatoxins. Acta Paedicatrica Scand. 1989;78:373-8.

46. Van Egmond HP. Mycotoxins. Int Dairy Fed Special Issue. 1991;101:131-5.

47. Gromadzka K, Pankiewicz J, Beszterda M, Paczkowska M, Nowakowska B, Kocyłowski R. The Presence of Mycotoxins in Human Amniotic Fluid. Toxins (Basel). 2021;13(6). doi: 10.3390/toxins13060409. PMID 34207874'

48. Shirima CP, Kimanya ME, Routledge MN, Srey C, Kinabo JL, Humpf HU et al. A prospective study of growth and biomarkers of exposure to aflatoxin and fumonisin during early childhood in Tanzania. Environ Health Perspect. 2015;123(2):173-8. doi: 10.1289/ehp.1408097, PMID 25325363.

49. Massey TE, Stewart RK, Daniels JM, Liu L. Biochemical and molecular aspects of mammalian susceptibility to aflatoxin b1 carcinogenicity. Proc Soc Exp Biol Med. 1995;208(3):213-27. doi: 10.3181/00379727-208-43852a, PMID 7878060.

50. Qureshi H, Hamid SS, Ali SS, Anwar J, Siddiqui AA, Khan NA. Cytotoxic effects of aflatoxin B1 on human brain microvascular endothelial cells of the blood-brain barrier. Med Mycol. 2015;53(4):409-16. doi: 10.1093/mmy/myv010, PMID 25851265.

51. Faisal K, Periasamy VS, Sahabudeen S, Radha A, Anandhi R, Akbarsha MA. Spermatotoxic effect of aflatoxin B1 in rats. Reproduction. 2008;135(3):303-10. doi: 10.1530/REP-07-0367, PMID 18299423.

52. Agnes VF, Akbarsha MA. Pale Vacuolated epithelial cells in the epididymis of aflatoxin-treated mice. Reproduction. 2001;122(4):629-41. doi: 10.1530/rep.0.1220629, PMID 11570970.

53. Storvik M, Huuskonen P, Kyllönen T, Lehtonen S, El-Nezami H, Auriola S et al. Aflatoxin B1- a potential endocrine disruptor-up regulates CYP19A1 in JEG-3 cells. Toxicol Lett. 2011;202(3):161-7. doi: 10.1016/j.toxlet.2011.01.028, PMID 21296134.

54. Soini Y, Chia SC, Benett WP, Wang JS, Bergasa NV. An aflatoxin-associated mutational hotspot at codon 249 in the p53 tumor suppressor gene occurs in hepatocellular carcinomas from Mexico. Carcinogenesis. 1996; 17: 1007-1012.

55. Hsu IC, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature. 1991;350(6317):427-8. doi: 10.1038/350427a0, PMID 1849234.

56. Caceres I, Khoury AA, Khoury RE, Lorber S, Oswald IP, Khoury AE et al. Aflatoxin biosynthesis and genetic regulation: a review. Toxins (Basel). 2020;12(3):150-77. doi: 10.3390/toxins12030150, PMID 32121226.

57. Khan R, Ghazali FM, Mahyudin NA, Samsudin NIP. Aflatoxin Biosynthesis, Genetic Regulation, Toxicity, and Control Strategies: a Review. JoF;7(8). doi: 10.3390/jof7080606'

58. Johnson WW, Ueng YF, Widersten M, Mannervik B, Hayes JD, Sherratt PJ et al. Conjugation of highly reactive aflatoxin b1 exo-8,9-epoxide catalyzed by rat and human glutathione transferases: estimation of kinetic parameters. Biochemistry. 1997;36(11):3056-60. doi: 10.1021/bi962537o, PMID 9115980.

59. Dvorakova I. Aflatoxin and encephalopathy with fully degeneration of viscera (Reye). Annals Nutr Aliment. 1977;31:977-89.

60. Galvano F, Piva A, Ritieni A, Galvano G. Dietary strategies to counteract the effects of mycotoxins: a review. J Food Prot. 2001;64(1):120-31. doi: 10.4315/0362-028x-

64.1.120, PMID 11198434.

61. Jard G, Liboz T, Mathieu F, Guyonvarc'h A, Lebrihi A. Review of mycotoxin reduction in food and feed: from prevention in the field to detoxification by absorption or transformation. Food Addit Coutam Part Chem Anal Control Expo Risk Assess. 2011;28(11):1590-609. doi: 10.1080/19440049.2011.595377.

62. Karlovsky P, Suman M, Berthiller F, De Meester J, Eisenbrand G, Perrin I et al. Impact of food processing and detoxification treatments on mycotoxin contamination. Mycotoxin Res. 2016;32(4):179-205. doi: 10.1007/s12550-016-0257-7, PMID 27554261.

63. 63. Adegoke GO, Allamu AE, Akingbala JO, Akanni AO. Influence of sundrying on the chemical composition, aflatoxin content and fungal counts of two pepper varieties-Capsicum annum and Capsicum frutescens. Plant Foods Hum Nutr. 1996;49(2):113-7. doi: 10.1007/BF01091967, PMID 8811723.

64. Ham H, Kim S, Kim MH, Lee S, Hong SK, Ryu JG et al.. Mycobiota of ground red pepper and their aflatoxigenic potential. J Microbiol. 2016;54(12):832-7. doi: 10.1007/s12275-016-6480-2. PMID 27888464'

65. Bammler TK, Slone DH, Eaton DL. Effects of dietary oltripraz and ethoxyquin on aflatoxin B1 biotransformation in nonhuman primates. Toxicol Sci. 2000;54(1):30-41. doi: 10.1093/toxsci/54.1.30, PMID 10746929.

66. de Alencar ER, Faroni LR, Soares Nde F, da Silva WA, Carvalho MC. Efficacy of ozone as a fungicidal and detoxifying agent of aflatoxins in peanuts. J Sci Food Agric. 2012;92(4):899-905. doi: 10.1002/jsfa.4668. PMID 22095762'

67. Chen R, Ma F, Li PW, Zhang W, Ding XX, Zhang Q et al. Effect of ozone on aflatoxins detoxification and nutritional quality of peanuts. Food Chem. 2014;146:284-8. doi: 10.1016/j.foodchem.2013.09.059, PMID 24176344.

68. Anthony CM, Tochukwu EE, Obasi CC, Vincent CS, Maghus K. Detoxification of alflaatoxin-contaminated poultry feeds by 3 adsorbants, bentonite, activated charcoal and fuller's earth. J Applpoult Reseearch. 2018;27(4):461-71.

69. Afshar P, Shokrzadeh M, Raeisi SN, Ghorbani-HasanSaraei A, Nasiraii LR. Aflatoxins biodetoxification strategies based on probiotic bacteria'. Toxicon. 2020;178:50-8. doi: 10.1016/j.toxicon.2020.02.007, PMID 32250747.

70. Muhiadin BJ, Saari N, Meor Hussin AS. Review on the biological detoxification of mycotoxins using lactic acid bacteria to enhance the sustainability of foods supply. Molecules. 2020;25(11):2655. doi: 10.3390/molecules25112655, PMID 32517380.

71. Perczak A, Goliński P, Bryła M, Waśkiewicz A. The efficiency of lactic acid bacteria against pathogenic fungi and mycotoxins. Arh Hig Rada Toksikol. 2018;69(1):32-45. doi: 10.2478/aiht-2018-69-3051, PMID 29604200.

72. Intanoo M, Kongkeitkajorn MB, Suriyasathaporn W, Phasuk Y, Bernard JK, Pattarajinda V. Effect of Supplemental Kluyveromyces marxianus and Pichia kudriavzevii on Aflatoxin M1 Excretion in Milk of Lactating Dairy Cows. Animals;10(4). doi: 10.3390/ani10040709'

73. Peles F, Sipos P, Kovács S, Győri Z, Pócsi I, Pusztahelyi T. Biological control and mitigation of aflatoxin contamination in commodities. Toxins. 2021;13(2):104. doi: 10.3390/toxins13020104, PMID 33535580

74. El-Nezami H, Kankaanpää PE, Salminen S, Ahokas JT. Physicochemical alterations enhance the ability of dairy strains of lactic acid bacteria to remove aflatoxin from contaminated media. J Food Prot. 1998;61(4):466-8. doi: 10.4315/0362-028x-61.4.466, PMID 9709211.

75. Jubert C, Mata J, Bench G, Dashwood R, Pereira C, Tracewell W et al. Effects of chlorophyll and chlorophyllin on low-dose aflatoxin B (l) pharmacokinetics in human volunteers. Cancer Prev Res (Phila). 2009;2(12):1015-22. doi: 10.1158/1940-6207.CAPR-09-0099, PMID 19952359.