



A REVIEW STUDY ON BIOLOGICAL ILL EFFECTS AND HEALTH HAZARDS OF AFLATOXINS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author US designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SG managed the analyses of the study. Author MG managed the literature searches. All authors read and approved the final manuscript.

Received: 01 November 2020

Accepted: 04 January 2021

Published: 28 January 2021

Review Article

ABSTRACT

Aflatoxins are a group of secondary metabolites produced mainly by *Aspergillus flavus* and also by *Aspergillus parasiticus*, that are acutely toxic to warm blooded animals including human beings. Aflatoxin producing species of *Aspergillus* are quite common and widespread in nature. They can colonise and contaminate grains before harvest or during storage. Host crops are particularly susceptible to infection by *Aspergillus* following prolonged exposure to a high humidity environment. Other favourable conditions include high moisture content and high temperature. The aflatoxins can be found on a wide range of commodities including cereals, pulses, oilseeds, spices and dry fruits. In India, aflatoxins have been reported from a variety of edible substances such as cereals, oil seeds, spices, vegetables, dry fruits, pulses, areca nut, coconut and fast foods.

The aflatoxins became the focus of intense investigations when outbreaks of disease called "Turkey X' disease" occurred in Poultry during 1960 in U.K. Since the beginning of mycotoxin research the aflatoxin problem has been an economic burden for the food crops, livestock and poultry industry. The aflatoxins are acutely toxic being carcinogenic, mutagenic and teratogenic to rat and other experimental animals, so there has always been a concern about contamination of food with aflatoxins and threat to both human and animal health. Primarily aflatoxin B₁, B₂, G₁, G₂ and M₁ are of interest. The aflatoxin M₁ is hydroxylated metabolite of B₁ that is the most frequently occurring aflatoxin. Further aflatoxin B₁, is one of the most potent hepato- carcinogens known, hence levels of aflatoxins in the diet are an important consideration for human health. It is therefore, essential to devise suitable cheap and safe control measures to minimize the deleterious effects of these mycotoxins. The aim of this review study was to explore the biological ill effects and health hazards due to these aflatoxins.

Keywords: Aflatoxins; carcinogenic; mutagenic; teratogenic; health hazards.

1. INTRODUCTION

Aflatoxins are highly toxic mycotoxins that are secondary metabolites derived from polyketides produced by fungal species such as *Aspergillus flavus*,

A. parasiticus, and *A. Nomius* [1,2]. These fungi growing on food and feed which, when consumed have some undesirable effect on the animals consuming them. These effects can range from vomiting, feed refusal, and weight loss, various types

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of tumours and in some cases death [3,4]. These aflatoxins can lead to serious threats to human and animal health by causing various complications such as hepatotoxicity, teratogenicity, and Carcinogenicity [5,6].

The toxic and therapeutic effects of mycotoxins produced by *Claviceps purpurea*, causal agent of ergot of rye have been known since biblical times. This disease was also known as 'St. Anthony's fire or Holy Fire' in Europe during the feudal days and its periodic outbreaks resulted in thousands of deaths [7]. One of the well-documented examples of mycotoxicoses is a disease called Alimentary Toxic Aleukia (ATA). The disease was recorded in Eastern Siberia, Western Siberia, Amur region and Orenburg district of USSR from 1931 to 1934 [8]. In the years 1942 to 1947, a serious outbreak of this disease again occurred in Orenburg district of Russia and more than 10 per cent of the population was affected and heavy mortalities were recorded [9]. It was demonstrated that *F. sporotrichioides* and *F. poae* were principal agents of alimentary toxic aleukia [10].

In 1960, more than a lakh young turkeys on poultry farms in South and East of England died in the course of a few months from an apparently new disease that was termed "Turkey X disease" [11]. The factor in these outbreaks was Brazilian groundnut meal in the rations. This peanut meal was found to be heavily infected by the light green mould colonies. The toxin producing fungus was then identified as *Aspergillus flavus* Link ex Fries and the toxin was given the name Aflatoxins [12]. Aflatoxins were isolated in crystalline form and observed that this fungal metabolite was photosensitive in hydroxylic solvents [13]. Isolation and characterization of four closely related aflatoxins was first reported by Hartley et al in 1963 [14].

14 or more types of aflatoxin are produced by the *Aspergillus* species in nature, but four aflatoxins B₁, B₂, G₁ and G₂ are particularly dangerous to humans and animals as they have been found in contaminated nuts, grains and their derived products. Where the "B" and "G" refer to the blue and green fluorescent colours produced under UV light on thin layer chromatography plates [15,16,17].

Additionally, aflatoxin M₁ (AFM₁) and M₂ (AFM₂) are derived from aflatoxin B types through different metabolic processes and expressed in animals. These can be found in milk in areas of high aflatoxin exposure. Subsequently humans may be exposed to this aflatoxin through milk and milk products, including breast milk, especially in areas where the poorest quality grain is used for animal feed that

contaminated with aflatoxin. This toxic substance was having the blue violet fluorescence with an Rf value much lower than that of aflatoxin B₁ and consequently designated as 'milk toxins' [18,19,20]. A compound was found from the culture of *Aspergillus parasiticus* identical to aflatoxin B₃ and was named as parasiticol. Other derivatives of aflatoxin include aflatoxicol, aflatoxin H, aflatoxin P, and Q [21,22,23].

The biological ill effects and health hazards of aflatoxins show great deal of variation. Aflatoxins and particularly aflatoxin B₁ are acutely toxic to a wide variety of living organisms including laboratory and domestic animals, cells in culture, micro-organisms and plants [24,25,26]. Toxicity of aflatoxin to duckling and chick embryo is used as test for biological confirmation of aflatoxins [27]. The focus of this review study was to find out different concepts of biological ill effects and health hazards of Aflatoxins as their hepatotoxic, carcinogenic, mutagenic and teratogenic effects.

2. CARCINOGENIC EFFECTS

The aflatoxins were the first mycotoxin to be extensively studied for their carcinogenic effect and are now recognized as potent hepatocarcinogens [28]. The acute toxicity and carcinogenicity of the aflatoxin to rats have been studied extensively since the recognition of turkey X' disease among farm animals. Prior to the isolation of aflatoxin, Lancaster et al. [29] showed that peanut meal toxic to poultry has also induced hepatic carcinomas in rats. There were several other reports regarding the high incidence of hepatomas in rats fed a diet containing aflatoxin [30,31].

Aflatoxins are carcinogenic to mice, fish, rats, marmosets, ducks, tree shrews and monkeys. Aflatoxin B₁ that is highly toxic among mycotoxins primarily causes hepatocellular carcinoma and cholangia carcinoma in the liver. Aflatoxin B₁ displays the highest oral carcinogenicity in a wide range of animals. Wogan and Newberne [32] reported that purified aflatoxins B₁ at levels of 15 ppb added to a semi synthetic diet induced liver cell carcinomas in 25/25 fischer rats surviving for 68 weeks, (male) to 80 weeks (female). The dosage corresponded to an intake of about 0.2 µg/day/rats and total amount of aflatoxin B₁ for carcinoma induction can be estimated at <100 µg. Seven years later, They also reported that tumours were induced in 21/22 male fischer rat fed 1 ppb, aflatoxins B₁ under conditions essentially the same as in the earlier experiment. Aflatoxins produce mainly cancer of liver, Kidney and colon. Carnaghan et al. [33] found that oral LD₅₀ for one day old duckling for the four aflatoxins are B₁ 0.36 mg/kg, B₂ 1.7 mg/kg,

G₁ 0.78 mg/kg and G₂ 3.5 mg/kg, when measured after 7 days. For the rats LD₅₀ of aflatoxin B₁ is 7.2 mg/kg orally and 62 mg/kg intraperitoneally for the male and 7.9 mg/kg orally and 13.2 mg/kg intraperitoneally for female [34]. Carnaghan [35] observed that hepatic tumours developed in 8 out of 11 duck in 14 months when fed aflatoxin B₁ at dietary level of 30 µg/kg.

Halver [36] demonstrated that 96% of trout developed hepatoma when fed on a diet containing 20 ppb of aflatoxin for 20 months. However, hepatoma could be induced at a level of 0.05 ppb but 0.1 ppb was capable of inducing 10% tumour incidence. For the dog aflatoxins B₁ at a dose of 1 mg/kg killed 2/3 of the test animals after 14 days [37]. Oettle [38] suggested that aflatoxin have a role in the etiology of liver cancer in man. In at least three parts of the world (Thailand, Philippines and East Africa) good epidemiological evidence of liver cancer and exposure to aflatoxins has been reported. However, Enomoto and Saito [39] reported that aflatoxins may be responsible for liver cancer in men and animals. Survey conducted in Uganda [40,41], Thailand [42], Kenya [43] and Mozambique [44] have shown that incidence of primary liver cancer in human population was related with the level of aflatoxin in ration ingested. The acute and chronic effects of aflatoxin in human beings can only be estimated by observing suspected cases of aflatoxicosis and by investigating the diet associated with such cases [45,46].

Cukrova et. al. [47] studied the early effect of aflatoxin B₁ administered, in vivo on the growth of bone marrow CFU-GM and the production of cytokinesis in rats. In this case the granulopoiesis toxicity of aflatoxin B₁ (AFB₁) showed an impact of this mycotoxin on the production of some humoral regulatory factors dealing with the Granulocytic development pathway. In dose of AFB₁ studied represented approximately 115 of LD₅₀ for young male rats. An early suppressive effect of AFB₁ towards CFU-GM was transient in treated animals.

3. HEPATOTOXIC EFFECTS

Aflatoxins are known to induce both nonspecific liver injury such as fatty and pale livers, moderate to extensive necrosis and hemorrhage together with specific lesions. The distribution of lesions, however, varies in different species depending upon the dose, quality and route of aflatoxin involved. Lesion pattern may be periportal, centrilobular, midzonal or diffuse necrosis. Biliary proliferation is very rapid, reaches a peak after 3 days and then regress. Aflatoxin B₁ is the most documented and studied hepatotoxic mycotoxin. The hepatic tissues of liver can absorb toxic substances from the blood stream and thus remove

them from circulation. The susceptibility of animal to aflatoxin B₁ varies but in all cases the primary causes of illness and death results from liver damage. The liver damage has been demonstrated both in field outbreaks of aflatoxicosis and in Laboratory administrated conditioned with poultry pigs, cattle and dogs. The hepatic lesions induced by aflatoxin B₁ in the ducklings have become the basis of a bioassay and the toxicity values are expressed on the degree of biliary proliferation in case of sub lethal doses [48,49].

Tulpule et al. [50] observed, liver lesions in rhesus monkeys similar to those produced by aflatoxin in the ducklings. Feeding pure aflatoxin 0.5-1.0 mg/kg body weight/day (60% B₁, 40% G₁) produced typical histopathological changes in liver generally associated with aflatoxin damage. When a total dose of 10-15 mg/kg body weight/day was administered, all the test animals died within a period of 28 days. In further studies on the aflatoxin effects on rhesus monkeys [51], the observations were made on the animals treated with 1.0 mg/kg body weight/day. It was noted that the fat content of the liver was 34% by net weight and organ was enlarged. Madhavan and Rao [52] have observed that the day old ducklings fed 10-40 µg day for 2-6 days developed hepatic interaction. An intense congestion of the vessels of the liver following toxic action, most likely is responsible for this disturbance. However, liver infarctions were not seen in monkeys, guinea pig or rats dosed with aflatoxin.

Kalengayi et al. [53] examined the sequential histochemical change in rat liver after single dose of aflatoxin B₁ intoxication. Liver injury and hepatic GH-resistance by AFB₁ exposure causes growth impairment in this mammalian rat model. This model could guide interventions in at risk and affected children [54]. Organic dust exposure may cause elevation in AFB₁/Alb and liver enzymes of exposed workers, and gene polymorphism plays an important role in susceptibility to hepatic parenchymal cell injury [55].

4. MUTAGENIC EFFECTS

Aflatoxin B₁ is the most potent mutagen out of the aflatoxins and a strong parallel exists between the ability of the aflatoxins to be mutagenic or carcinogenic. Microsomal activation is an absolute chromosomal aberrations and DNA breakage in plant and animal cells. Legator and Withrow [56] reported that 0.01 microgram of aflatoxin B₁ inhibited mitotic division in human embryonic lung cells. The synthesis of DNA in human embryonic cells was inhibited using 0.05 0.1 microgram/ml of aflatoxin B₁. In the same case, giant cells have also appeared so as to show abnormal morphological pattern.

Biological effects of aflatoxins on *Neurospora crassa* have been extensively studied by many workers; the mutagenic activities of aflatoxin B₁ and G₁ require metabolic activation which can be carried out by the vegetative culture of *N. crassa*. Aflatoxin B₁ and G₁ cause similar spectra of genetic alterations, which include base pair substitutions, frameshift mutations, multilocus deletions and other type of genetic alterations. Therefore, it can be suggested that aflatoxin B₁ is a potent mutagen while aflatoxin G₁ is a moderate mutagen in *N. Crassa* [57,58].

Aflatoxin may also affect the biosynthesis of R.N.A. by preventing the transcription of DNA by RNA polymerase [59,60]. The activity of nuclear and cytoplasmic RNA is also inhibited by aflatoxins [61,62,63]. Sashidhar et al. [64] studied the effect of dietary aflatoxins on nicotinamide nucleotide synthesis in liver cells. The molecular basis of AFB₁ induced mutagenesis in hepatic cellular carcinoma (HCC) was that the lesion was highly mutagenic, yielding replication error frequencies of 97%, with the predominant base substitution being a G to T transversion [65].

5. TERATOGENIC EFFECTS

Discovery of the embryocidal and teratogenic effects of toxins produced by fungi that may be found on foods of humans and domestic animals indicate possible health hazards beyond toxicity or carcinogenicity in adults. Prenatal effects have been documented with experimental animals, which suggest that aflatoxin B₁ is teratogenic.

Le Breton et al. [66] reported that intraperitoneal injection of aflatoxin B₁ to female rats resulted in the production of hemorrhage at the uteroplacental junction and fetal death. On repeated smaller dose, retardation of fetal growth was seen. Butler and Wigglesworth [67] failed to find hemorrhage or fetal death after oral dosage but confirmed the retardation of fetal growth when the aflatoxin was related to the severity of the maternal liver lesion. They also found that nonpregnant female rats were more susceptible to aflatoxin than pregnant females. With hamsters, a single intraperitoneal injection of aflatoxin B₁ at 4 µg/kg body weight/day was administered on 8th day of pregnancy, caused a high proportion of malformed and dead or reabsorbed foetuses [68,69].

Aflatoxin is a potent teratogen because of its ability to bind DNA and subsequently inhibit protein synthesis. Skeletal anomalies have reported in the offspring of animals treated with aflatoxin during pregnancy [70].

6. EFFECTS ON HUMAN BEINGS

Human mycotoxicosis are aflatoxicosis, ergotism, ATA, and yellow rice disease. The acute and chronic effects of aflatoxins in human beings can only be estimated by observing suspected cases of aflatoxicosis and by investigating the diet associated with such cases. The Kenyan and Thailand studies indicated a good co-relation between the level of aflatoxin ingested and the incidence of primary liver cancer [42,43].

Enwonwu [71] also suggested that aflatoxin, malnutrition and HBV (Hepatitis B Virus) frequently coexist with hepatocellular carcinomas, and aflatoxin could be contributing to the suppression of the immune deficiency against HBV. Therefore, both HBV and AF were jointly contributing to the development of hepatocellular carcinoma. Groopman et al. [72] and Wang et al [73] have reported more than 50 hepatocellular carcinoma patients in Taiwan. The detection rate was slightly lower in man (69%) than in woman (75%) and younger patients had a significantly higher rate (83%) than did older ones (58%). The results suggest that aflatoxin B₁ may be involved in the pathogenesis of hepatocellular carcinoma in Taiwan.

Harrison et al. [74] have reported aflatoxin exposure in the United Kingdom and suggested that it constitute a cancer risk in human beings. Unsal et al. [75] examined Mozambican type of hepatocellular carcinoma in man, which was characterized by a high incidence of P53 mutations related to aflatoxins.

Dietary exposure to aflatoxins is among the major HCC risk factors. Aflatoxin B₁, which is a genotoxic hepatocarcinogen, which presumptively causes cancer by inducing DNA adducts leading to genetic changes in target liver cells in humans [76]. Aflatoxin exposure during pregnancy directly or indirectly cause maternal anemia and adverse birth outcomes like low birthweight and impair fetal growth [77].

7. CONCLUSION

Consumption of aflatoxin contaminated foods is a common problem in both humans and animals worldwide. They cause deleterious effects on the various body organs and body systems including the development of cancers especially the liver cancer mainly due to AFB₁ exposure. These aflatoxins can lead to serious ill effects and health hazards to human and animal by causing various complications such as hepatotoxicity, teratogenicity, mutagenicity and carcinogenicity. During the pandemic to check rapid transmission of infection of COVID-19, Global

disaster in 2020, countries had to lockdown in world therefore socio-economic life of human population was affected adversely [78]. Difficulties in management of food and grain storage godowns during lockdown period as well as adversely affected economic life of humans may also increase the consumption of aflatoxin contaminated foods. Therefore, safe control measures to minimize the deleterious effects of these aflatoxins should be done strictly.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

Authors express their sincere thanks to Dr. Surendra Singh and Prof. D. K. Hazra for guiding and providing help to procure materials.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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