

# Metabolic Pathways and Pathological Outcomes of Aflatoxins: A Review

Aashma Dahal,<sup>1</sup> Ashish Lamichhane,<sup>2</sup> Alina Karna<sup>3</sup>

<sup>1</sup>Department of Public Health, Madan Bhandari Academy of Health Sciences, Hetauda, Nepal;

<sup>2</sup>Feed the Future, Innovation Lab for Nutrition/Helen Keller International, Kathmandu, Nepal;

<sup>3</sup>Department of Lab Medicine, Madan Bhandari Academy of Health Sciences, Hetauda, Nepal.

## ABSTRACT

Aflatoxin is a secondary fungal metabolite that contaminates foods, mostly staple diets like maize, peanuts, chillies, and even rice. These foods are also a major constituent of weaning food for infants in Asia and Sub-Saharan Africa. The fungal metabolite contaminates food during production, harvest, storage and processing. The contamination is largely promoted by genotypes of crops, soil conditions, temperate regions, and insect activity. Once ingested into the body, aflatoxins get metabolized into different hydroxylated derivatives such as AFb1, AfM1, AFP1, aflatoxicol and Aflatoxin B1. AFB1 is the most carcinogenic and potent of the known metabolites and they have been categorized as Group I carcinogenic agent by the International Agency for Research on Cancer. The toxic metabolites of aflatoxins have been found in blood samples, breast milk and also have been shown to traverse the placental route. Through various metabolic pathways aflatoxins are responsible for different types of pathological outcomes like gut enteropathy, anemia, stunting, and other immunological disorders. Moreover, socioeconomic determinants have indirectly shown to be strong predictors of aflatoxins exposure and thus its related pathological outcomes. Since we have a very limited number of researches about aflatoxins, this review altogether puts forward what is known about the toxin and its harmful metabolites.

**Keywords:** Aflatoxins; aflatoxinB1; carcinogens; fungal toxins.

## INTRODUCTION

Aflatoxin is a secondary fungal metabolite that contaminates foods, mostly staple diets like maize, peanuts, rice etc.<sup>1</sup> Animal products like milk and eggs from poultry are also at risk of fungal contamination of aflatoxins. The fungal action could contaminate the food during production, harvest, storage, and processing. The invasion of the toxin is also largely promoted by genotypes of crops, soil conditions, and insect activity.<sup>2</sup> The USFDA considers Aflatoxins to be an unavoidable contaminant of food.<sup>3</sup> Its toxicity is called aflatoxicosis and worldwide 4.5 billion

people are chronically exposed to large amounts of the fungal toxin.

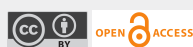
Aflatoxins have been categorized as Group I carcinogenic agent by the International Agency for Research on Cancer (IARC) which means that the agent has sufficient evidence of causing cancer to humans on repeated exposures. There are four major aflatoxins, aflatoxin B1 (AFB1), AFB2, AFG1, and AFG2, and AFB1 is the most carcinogenic and potent of the known metabolites.

The study of aflatoxin is a less emphasized area in nutrition toxicology. Though new, this is an important area for more research as food toxins like Aflac toxins have huge direct and direct implications on human health. In this regard, this review is useful to put more light on what is already known about the toxic metabolite to make people wary about the harmful effects.

### Correspondence

Aashma Dahal

Email: aashma.dahal9090@gmail.com



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### Aflatoxins Metabolism

Aflatoxins once ingested into the body, get metabolized into different hydroxylated derivatives such as AFb1, AfM1, AFP1, aflatoxicol, and Aflatoxin B1 in the liver. AFB1 is the most potent and thus harmful of all the metabolites, the latter are less toxic than the parent compound. The carcinogenicity of aflatoxin results from its conversion to aflatoxin 8, 9 oxides by the hepatic enzyme cytochrome 450.<sup>5</sup> These metabolites are then secreted into different body fluids like urine and breastmilk. Their hydroxyl-metabolites aflatoxin M1 (AFM1) and M2 (AFM2) can be presented in milk and milk products when lactating cattle or other animals ingest contaminated feed.<sup>6</sup>

According to a study carried out in 19 human liver samples, the rate of metabolism of aflatoxins is highly correlated with the level of cytochrome P450 enzymes in the microsomes. Therefore, it can be hypothesized that differing levels of the enzyme is the cause for inter-individual variation in metabolism of the toxin.<sup>5</sup> The expression of a protein in the P45011C gene family is also correlated with

AFB1metabolism and mutagenicity. The toxicity, however varies largely with the dose of the exposure, duration of exposure, and interspecies differences and is reduced when AFBO, the toxic metabolite combines with guanyltransferase.<sup>6</sup>

### Biomarkers of Aflatoxins

Aflatoxin exposure and its presence in the body can be predicted by several biomarkers. Its metabolites can be traced in blood and other body fluids like breast milk and urine. For example, aflatoxin M1 is found in breast milk which makes breastfed infants susceptible to exposure to the toxin. Initially, aflatoxins combine with blood albumin to form aflatoxin albumin (ALF-AB) adduct. The toxic metabolites of aflatoxins also combine with lysine to form aflatoxin-lysine adduct in the blood serum which are detected in various blood serum samples. Some of the studies have marked the increase of several immunological markers like cytokines and macrophages in the blood of infected individuals. Research papers on cancerogenic studies have used aflatoxin DNA adducts as their biomarkers for aflatoxin studies.<sup>6</sup>

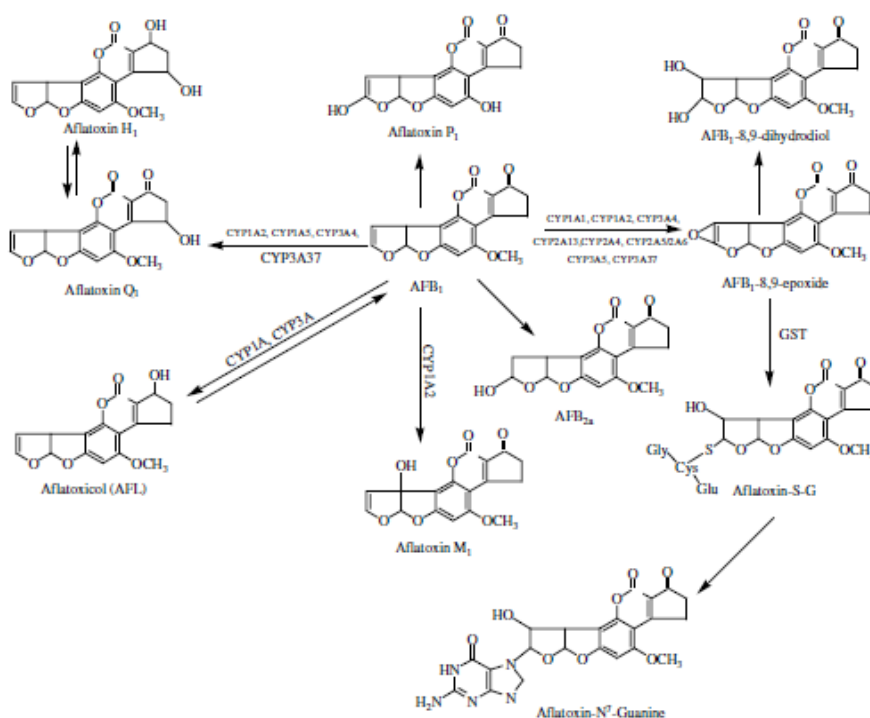


Figure 1: Major metabolites of aflatoxins(adapted from Dohnal et.al, 2014)<sup>7</sup>

## Socioeconomic Status and Aflatoxins

A study by Leroy et.al reported that all the predictors of poverty which included lack of income for Household expenditure, food security, and severe hunger, lack of capacity to procure and use inorganic fertilizers were associated with consumption of mouldy food which put them at high risk of aflatoxin exposure. Further, improper sanitation and drinking water facilities leave the children from such poor households prone to further co-morbidities like diarrhea and other infections. Therefore, it can be concluded that nutritional outcomes of aflatoxins exposure in infected children could be partial because of their socio-economic conditions especially poverty.<sup>8</sup>

On the other hand, in Benin and Togo, there were marked differences in between aflatoxin levels of children based on the agro-ecological zones they lived in. The level of aflatoxins exposure was higher in agroecological regions where the consumption frequency of maize was high. This could be because of the varying temperatures, soil, and harvesting practices in those agroecological zones.<sup>5</sup>

## Aflatoxins and Complementary Feeding

As previously mentioned, aflatoxins are known to contaminate staple food like rice, maize, peanuts which also form the major ingredients of weaning foods for infants in Sub-Saharan Africa and Asia. Therefore, children and mainly children who have started weaning foods are more vulnerable to aflatoxicosis in such areas. Since they eat such diet on a routine basis, it could expose them more to aflatoxins and cause growth problems as a consequence.<sup>5</sup>

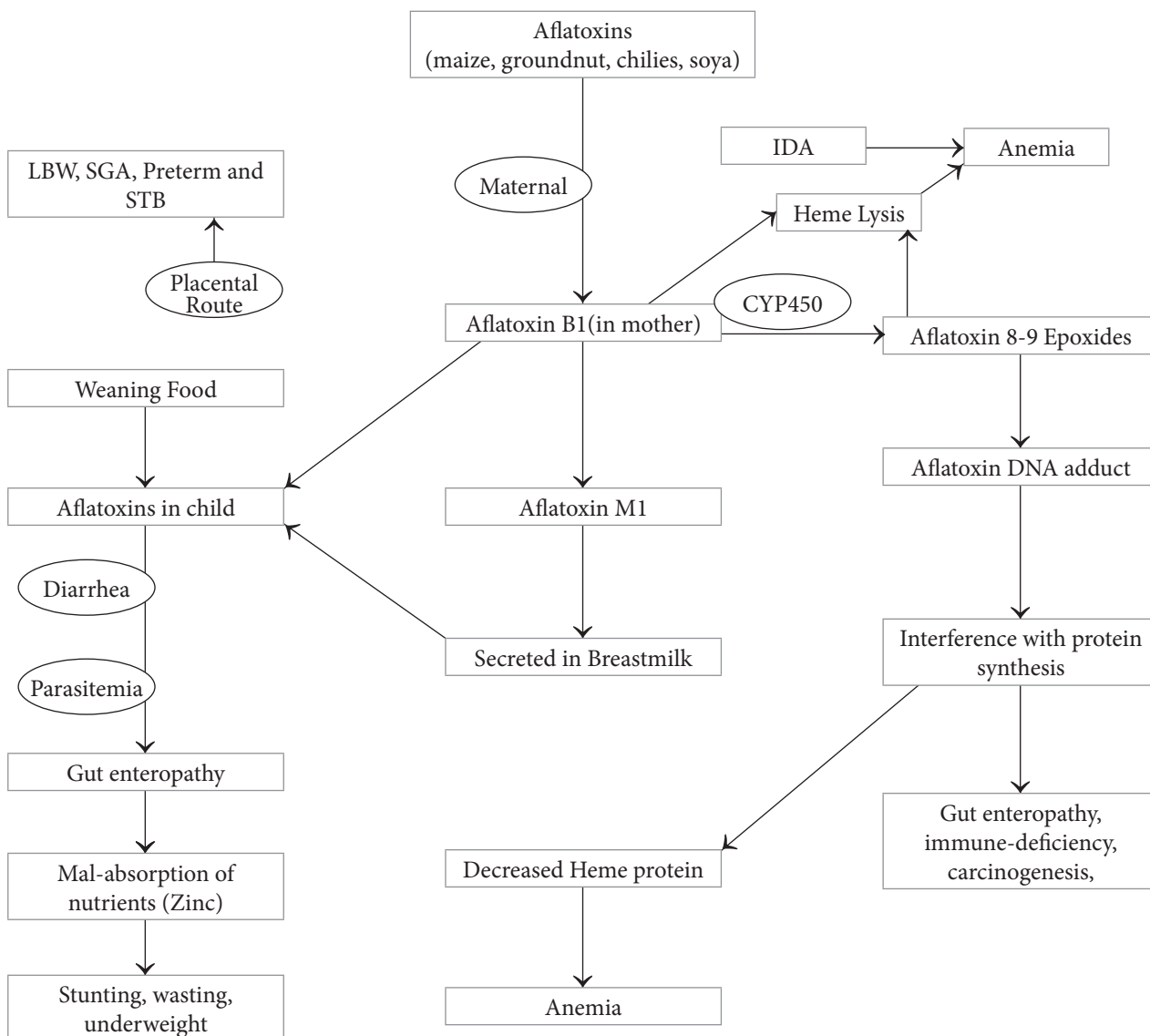
A cross-sectional study carried out by Gong et.al in Benin and Togo included a cross-section of 479 children aged 9 months to 5 years, 1.8 fold difference was found in between the aflatoxin adduct levels in between weaned and breastfed children. There was also a linear trend of increment of aflatoxin levels with age which greatly supports the hypothesis that

the weaned food must be the source of aflatoxin exposure in young children.<sup>5</sup> Furthermore, lower AF-alb levels were associated with breastmilk than weaning foods which reflects lower levels of aflatoxins exposure from breast milk than weaning and family foods. In addition, the majority of aflatoxins in milk are in the form of the less toxic and carcinogenic AFM1 hydroxylated metabolite rather than the highly toxic AFB1 predominantly found in foods.<sup>5</sup>

A birth cohort study done in 1600 mothers and their newborn's pairs between July 2015 and July 2017, (n = 1675) in Banke district, of Province 5, Nepal examined factors like mother's age, food consumption, and body mass index, as well as, seasonality and wealth quintiles with AFM1 levels in different body fluid samples of lactating mothers. Approximately 94% of breast milk samples had detectable levels of AFM1 (geometric mean 0.78 ng/L). The consumption of particular foods such as yogurt, milk, hydrogenated oil, and ripe pumpkin was positively associated with breast milk AFM1 levels (p-value <0.05), while consumption of legumes was associated with lower breast milk AFM1 levels (p-value <0.05). Seasonal influences were seen with breast milk collected in the pre-winter and winter seasons exhibiting significantly higher AFM1 levels compared to the spring (p-value <0.05).<sup>9</sup>

## Aflatoxins and their Pathological Outcomes

Based on the findings of a four-year study conducted in Cameroon, there was a significant relationship between kwashiorkor and marasmus and the AFB1 levels in children when compared with children in the control group. Since only the samples of urine of the children were being tested for aflatoxin levels a temporal relationship could not be established. In the same study, samples of different food products like eggs, cow's milk, breast milk, etc were tested positive for aflatoxin levels. Therefore, food contaminated food products could be a major source of aflatoxin exposure to the children in the area.<sup>1</sup>



**Figure 2: Flowchart of aflatoxins and its pathological outcomes.**

Another cross-sectional study was done in Kumasi Ghana, in pregnant women, to find out the association between the effects of aflatoxin exposure levels on birth outcomes: birth-weight, stillbirths, SGA, and preterm births. In this particular study, low birth weight was significantly associated with a level of aflatoxin exposure of the mother and the odds of giving both to a low birth weight baby was twice in women with higher aflatoxin-b1 lysine adducts levels as compared to women with low levels of Alf-b1 lysine adducts. However, due to the limitation of the study design, it failed to show any temporal relationship.<sup>2</sup>

In a cohort study carried out in 472 Gambian children aged 6 to 9 years to study immune modifications in relation to aflatoxin exposure, there was a weak association of aflatoxin exposure with wasting but not with stunting and underweight.<sup>2</sup> In a study done in Benin and Togo in children from 9 months to five years, dose-response relation was seen between aflatoxin exposure and the degree of stunting and underweight in children.

Gong et al, 2002 showed that stunting and weight for age was inversely related to aflatoxin levels in the Gambia. In one of the studies carried out in Benin and Togo, there was a strong association

between increased AF-alb levels and stunting in children (HAZ; trend test:  $F = 15.19$ ,  $P = 0.0001$ ,  $r^2 = 0.3766$ ).<sup>5</sup>

Turner et al. investigated the relationship between aflatoxin exposure in-utero and growth faltering in Gambian children, it pointed out that HA scores decreased about one-fifth of SD for every increase in one unit of log of average maternal Af-alb.<sup>8</sup> In the same study, the prevalence of exposure of cord blood with aflatoxin was high (48.5 percent), however it was lower than the aflatoxins levels in maternal samples. Therefore, it suggested that the placental route could be one of the routes of aflatoxin exposure of newborns which subsequently leads to growth faltering in their later life.<sup>2</sup>

The study in Ghana by Shuaib et.al looked at the association between anemia and aflatoxin B1 biomarker levels in 755 pregnant women. It was found that both the crude and adjusted associations of AF-ALB with anemia status indicated that a linear trend was present. Also, when aflatoxins were assessed as ordinal variables in the logistic model, the odds of anemia increased 21% (OR, 1.21; with each quartile of AL-ALB).

Furthermore, the study also highlighted that there appeared to be a stronger association between anemia and aflatoxin in the absence of iron deficiency: OR and 95% CI in the absence of IDA is 2.02 (1.19–3.41), whereas it is 0.95(0.34–2.64) in the presence of Iron Deficiency Anemia.<sup>3</sup> It has thus been hypothesized that IDA could play a protective role in iron-deficient individuals by depressing the conversion of aflatoxin to toxic metabolites. Further, the study fails to prove any hypothesis of causality because of its inability to measure plasma ferritin, plasma transferrin saturation, and serum soluble transferrin receptor. It explains there are many confounders like Vitamin B12 levels, vitamin A levels, and other nutritional deficiencies which play as confounders in the analysis of etiology of anemia which need to be controlled to identify the causative pathways of aflatoxins and anemia.

Previously, Studies with mammals (cattle, guinea pigs, and rabbits) have shown that aflatoxins cause hemolysis of RBCs and may interact with RNA and DNA to cause a depression of hemopoiesis. The study concluded that it would not be unreasonable to extrapolate similar findings in humans.<sup>3</sup>

Different groups of mycotoxins like aflatoxins, fumonisins have their routes for causing gut enteropathy. Studies have highlighted the fact that gut enteropathy is one of the many causes of which lead to stunting in the long run. The impaired gut function and permeability lower absorptive functions of the gut, hence causing less absorption of nutrients which might lead to stunting.<sup>11</sup>

Aflatoxins cause inhibition in protein pathways by the formation of epoxides which combine with DNA instantly because of their very short half-life leading to a mutagenic effect in DNA and RNA. Inhibition of protein synthesis could lead to physical alteration in the intestine and mal-absorption of nutrients. Altered protein synthesis leads to reduced expression of SGLT1, GLUT5, and L-serine transporters leading to glucose-galactose malabsorption and impaired reabsorption of water in the colon which may cause diarrhea, which could affect intestinal permeability as well as the uptake of key nutrients such as copper and zinc.

FUM causes inhibition of ceramide synthase that affects sphingolipid metabolism, which compromises the cellular wall and may also lead to increased intestinal permeability directly or by inhibiting regeneration of the epithelial barrier. AF, aflatoxin; DON, deoxynivalenol; FUM, fumonisin; GLUT 5, fructose transporter; IGF, insulin-like growth factor; IGF-ALS, insulin-like growth factor acid-labile subunit. Thus, gut enteropathy could be the intermediate pathway in aflatoxin ingestion and nutritional outcomes.<sup>10</sup>

### Immunological Outcomes

Turner et.al conducted a study in 6 to 9 years in Gambian children where several immunological

tests like cell-mediated immunity, vaccine response, IgA was tested against aflatoxin adduct levels, the results showed that aflatoxin exposure was strongly associated ( $p = 0.006$ ) with reduced sIgA levels when fitted to a regression model. sIgA (serum Immunoglobulin A) binds to the bacterial and viral surface of the antigens and thus provide a barrier for infections. The paper, therefore, argues that aflatoxins exposure could result in increased susceptibility to infectious diseases reducing the levels of IgA levels. It also adds that it could be also be linked to the epoxide formation and alteration of the protein synthesis pathway which leads to reduced sIgA levels in the exposed children.

Also, there was no relation between cell-mediated immunity tests, vaccine response and other immunological tests, and aflatoxin biomarker levels.<sup>2</sup>

### Research Recommendations

1. A cross-sectional study is not enough to assess temporality in between nutritional outcomes and the aflatoxin biomarker levels. Therefore, more cohort studies should be carried out to overcome

the limitations of the study design.

2. Strategies for developing interventions to reduce aflatoxin exposure during the weaning period have to be developed.
3. Interventions targeting better harvesting practices, soil testing, and improvement in storage practices should be carried out.
4. Women visiting antenatal clinics must be told about the hazards of eating unwholesome foods.

### SUMMARY

Aflatoxin is a secondary fungal metabolite that contaminates our staple diets like maize and rice. It gets metabolized into different hydroxylated derivatives such as AFb1, AfM1, AFP1, aflatoxicol in the liver resulting in several harmful pathological outcomes. International Agency for Research on Cancer has listed aflatoxins in Group I carcinogens.

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