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Review Article

Toxopathological And Histopathological Effects Of Aflatoxins .

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Abstract: *Aspergillus flavus and Aspergillus parasiticus produce aflatoxins, as secondary metabolites, making them one of the primary sources of food contamination, especially in humid and hot weather conditions that promote fungal growth. The four main types of aflatoxins are B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2 ,that found in various foods. Additionally, there are other variants such as Aflatoxin M1 (AFM1) and M2 (AFM2) in milk, which are metabolites of AFB1 and AFB2. Mycotoxins generated by certain fungus, was harmful effects on animal health,. These dangerous substances frequently contaminate agricultural products including grains, nuts, and feeds. Aflatoxins are well known for their strong hepatotoxicity, which causes a variety of negative consequences on animals, such as stunted development, weakened immune systems, and reproductive problems, this highlights the necessity for rigorous monitoring of feed and foods sources, along with effective management measures, to mitigate aflatoxin exposure and its adverse effects on animal health. It is important to note that plants sources that are known for higher aflatoxin production should be avoided. Therefore, it is recommended to monitor meals provided to animals as fodder and to prevent exposure to these chemicals by implementing effective agricultural pest control measures , This review gives a concise review of the alarming consequences of aflatoxin exposure on animals, highlighting the need to comprehend and mitigate these effects in order to protect the health and productivity of animal populations.*

.**Keywords:** *Aflatoxins, Histopathological, Embryos, kidney, liver, Immune system. Mycotoxins*

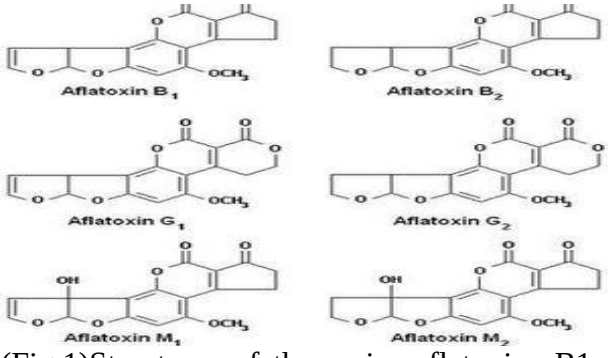
1.Introduction

Mycotoxins are secondary metabolites that have negative effects on human and animal health that contaminated several food products [1]. The Food and Agriculture Organization of the United Nations (FAO) estimates that mycotoxins contaminated around 25% of agricultural raw materials globally, causing health concerns and economic losses [2]. Mycotoxins are low-molecular-weight molecules that have the potential to induce both

acute and chronic illness [3], In many species, they can cause harmful physiological alterations [4]. Mycotoxins may be divided into five major families, including trichothecenes, zearalenone, ochratoxins, fumonisins, and aflatoxins [5]. According to Gong *et al*., [6] aflatoxins are one of the most dangerous mycotoxins, that have a potential to cause cancer [7].. Mycotoxins provide serious health risks to both people and animals which found in the soil, crops, and foods, as well as in every other area of the environment [8],. Aflatoxins (AFTs) are heat resistant and can be eliminated at temperatures of 280°C and also soluble in methanol and other organic solvents but insoluble in water [9]. Long-term AFT exposure can impair the immune system and cause various health issues, including death, in reality, AFTs have a 25% mortality rate [10]. Numerous illnesses, including cancer, liver damage, and immunological suppression also result in children's growth retardation. The four aflatoxins G2, G1, B2, and B1 are the most toxic among the 18 mycotoxins, *Aspergillus parasiticus* generates G2, G1, B2, and B1 whereas *Aspergillus flavus* produces B2 and B1 [11, 12], AFB1 is the most poisonous of the aflatoxins , and it is found in a wide range of foods, including nuts, rice, and maize [13]. AFB1 has been classified according to (WHO) as one of the most carcinogenic natural chemicals . It has been shown to induce liver damage, birth abnormalities, and immunological suppression in people and animals [14]. As a result, the International Agency for Research on Cancer has categorized AFB1 as a Group 1 carcinogen. Different species may experience inflammation , intestinal and liver cell damage as a result of exposure to AFB1-contaminated substances. [15,16] . Additionally, it enhances programmed cell death and decreases lymphocyte activity [17]. AFB1 can enter the human food chain via contaminated meats, eggs, and milk [18]. AFB1 has also been linked to the development of cancer in the liver, stomach, lungs, kidneys, colon, rectum, breast, and gallbladder [19]. In fact, AFT exposure is linked to 4.6-28.2% of Hepatocellular Carcinoma (HCC) cases [20].

-Type of Aflatoxins

Aspergillus flavus and *Aspergillus parasiticus* produce aflatoxins as secondary metabolites among food contamination, especially when it's humid and hot outside, which encourage the growth of fungi [21]. Aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2) are the four primary forms of aflatoxins that present in a variety of meals and may also be further variants, such as milk's, Aflatoxin M1 (AFM1) and M2 (AFM2), which are also metabolites of AFB1 and AFB2. Aflatoxin B1 (AFB1) and M1 (AFM1) are both considered human carcinogens by the International Agency for Research on Cancer as in figure 1 [20].



(Fig:1)Structures of the major aflatoxins B1 , B2 , G1 , G2 , M1 , M2 , B2A and G2A [20].

Aflatoxins B1 and B2

Aflatoxins B1 and B2 are harmful secondary metabolites generated by fungus such as *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius, Aspergillus bombycis, Aspergillus arachidicola, Aspergillus minisclerotigenes, Aspergillus ochraceoroseus, and Aspergillus rambell*ii. The most powerful cancer-causing kind is aflatoxin B1 (AFB1), Its ill consequences are felt by both people and animals. Because of changes in biological transformation, the sensitivity and toxicity of AFB1 vary greatly between species, Certain species, such as turkeys, rodents, pigs, lambs, and dogs, are especially vulnerable to AFB1. Other species, such as monkeys, mice,

and chickens, demonstrate resistance., the LD50 values for Aflatoxin B1 vary depending on the species and gender, ranging from 9 to 60 milligrams of AFB1 per kilogram of body weight [22, 23]. Aflatoxin B2 (AFB2) is a light blue toxic secondary metabolite produced by the same species as AFB1, including *A. arachidicola, A. flavus, A. minisclerotigenes, A. nomius, and A. parasiticus*. This metabolite can be synthesized through the interaction between quinone and 2,3-dihydrofuran [24].

-Aflatoxins G1

Common soil fungal species include *A. parasiticus, A. nominus, A. bombyccis, A. arachidicola, and A. flavus* generate aflatoxins G1 (AFG1) and G2 (AFG2). AFG1 has been connected to liver cancer and toxicity in both humans and animals. Contrarily, AFG2 displays significantly less activity [25,26].

-Aflatoxins M1 and M2

Aflatoxins M1 (AFM1) and M2 (AFM2) are AFB1 and AFB2 hydroxylated derivatives, respectively. They are formed by the fungus *A. flavus and A. parasiticus*. AFB1 and AFB2 enter the human or animal body and are processed by cytochrome P450 enzymes to form a reactive epoxide intermediate, which can then be converted to hydroxylated metabolites, resulting in the less harmful aflatoxins, AFM1 and AFM2. In the case of animals consuming AFB1-contaminated feed, 0.5% to 5% of the ingested toxin is converted to AFM1 in the liver. Milk, cheese, and other dairy products contain AFM1 and AFM2 residues [27,28].

-Aflatoxycol

The initial documentation of natural food contamination with Aflatoxicol (AFL) was recorded Righetti *et al*., [29] AFL, a derivative of AFB1, was identified as a result of the specific reduction of AFB1's cyclopentanone carbonyl. AFL manifests in two geometric isomers: AFL1/AFL-A/Ro and AFL2/AFL-B. The disparity between these isomers lies in the arrangement of the hydroxyl group within the cyclopentane ring. Fungi such as *Tetrahymena pyriformis, Trichoderma viride, Dactylium dendroides, Streptococcus lactis, Absidia repens, Mucor griseocyanus, Aspergillus niger, Mucor ambiguus, and Tetrahymena Rhizopiguus* are responsible for the enzymatic bioreduction that leads to the production of both forms of AFL. Despite being notably less toxic than AFB1 by a factor of 18, AFL has demonstrated remarkable carcinogenic and mutagenic properties [30,31].

-Aflatoxin Q1

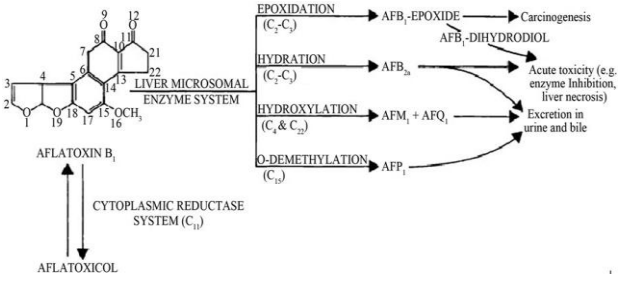
Aflatoxin Q1 (AFQ1) stands as a monohydroxylated derivative derived from AFB1, representing a significant metabolite of AFB1. Inceptionally, AFQ1 was uncovered within rhesus monkeys subjected to AFB1 exposure, as documented by Yourtee *et al*., [32]. An additional investigation postulated that AFQ1 could potentially function as a principal detoxification metabolite within the biotransformation process of the original fungal toxins. Notably, AFQ1 exhibits a toxicity level approximately 18 times lower and a mutagenic potential around 80 times less than that of AFB1 [26].

-Aflatoxin metabolism

Metabolic processing of aflatoxin takes place after consuming contaminated food. The liver is the primary and major target organ for aflatoxins, which are absorbed in the intestines. In the early stages of biotransformation in mammals, cytochrome P450 enzymes, especially CYP1A2 and CYP3A4, are essential [33], These enzymes can produce compounds that bind to DNA and have genetic consequences. Their protein expression levels are increased. AFB1-8,9-epoxide is produced during the initial step of aflatoxin conjugation in the liver, or it can degrade into the less dangerous form AFM1 [34], Conjugation reactions, notably involving glucuronic acid and sulfates, are involved in the second phase of food metabolism. Toxic epoxide metabolites generated in the first phase can be removed by epoxide hydrolase hydrolysis or reduction to less toxic metabolites such as AFM1 and AFQ1. These metabolites are eliminated in urine after being released into bile [35]. AFB1

can undergo a reversible reduction process in liver cells via the cytoplasmic reduction system, resulting in the creation of Aflatoxicol (AFL). AFL can subsequently be converted back to AFB1, which finishes its metabolism by producing a number of metabolites such as Aflatoxin P1 (AFP1) and Aflatoxin Q1 (AFQ1) [36]. AFB1-8,9-epoxide, the reactive intermediate, may be epoxidized to produce AFB1-exo-8,9-epoxide (AFBO) this chemical may attach to bigger molecules in proteins, DNA, and ribonucleic acid (RNA) to create the harmful compounds that are caused by aflatoxin. When Aflatoxin B1 (AFB1) binds to guanine within DNA molecules, it gives rise to AFB1-N7-Guanine (AFGuan), a chemical complex that results in the substitution of guanine with thymine at the third codon of the host DNA at position 249[37],. This specific mutation holds responsibility for the impairment of the p53 gene function, a pivotal driver in the development of liver cancer, [,an alternate metabolic pathway involving AFB1 leads to the creation of Aflatoxin M1 (AFM1), which can further undergo a process of epoxidation, resulting in its transformation into AFM1-epoxide. Similar to AFBO, AFM1-epoxide exhibits the capacity to form binding interactions with large molecules [38].

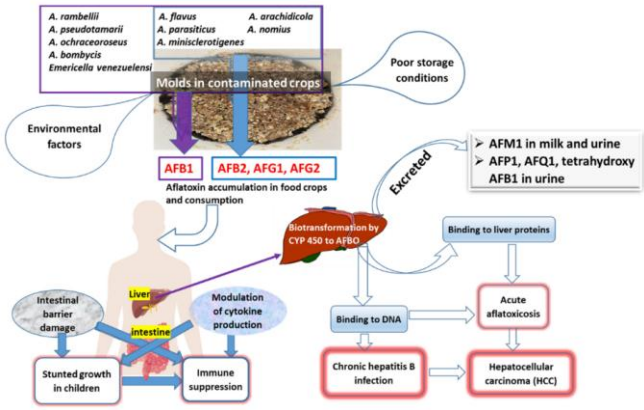
Both AFB1 and AFM1 are categorized as primary carcinogens due to their direct involvement in cancer formation, whereas AFM2 is recognized as a secondary carcinogen, as classified by the International Agency for Research on Cancer as in figure 2,[39].



(Fig:2) Metabolism of aflatoxin in liver [40].

-Effects of aflatoxins on animals

Animals are fed contaminated feed, aflatoxin can be identified in milk, eggs, and meat products Aflatoxin poisoning affects both domestic animals and people, with a higher frequency in underdeveloped nations than in wealthy countries. [41], . In animals, aflatoxin fungal poisoning causes liver damage, decreased milk and egg production, and increased susceptibility to dangerous bacteria such as Salmonella owing to immunological suppression [40], In humans, mycotoxin exposure causes gastrointestinal problems, anemia, and decreased fertility [42]. Rabbits are among the most sensitive animals to the toxic effects of this pollutant, followed by ducks, turkeys, and chickens, which are very sensitive, followed by fish and pigs, which are less sensitive, then cows and sheep, which are the most resistant, and there is also a difference between and there is also a difference between the sexes, where female birds produce AFBO more than Males, and the production of turkeys and ducks is more than chickens and quails and young animals are more sensitive to this pollutant than older animals as in figure 3,[43].



(Fig:3) Sources, exposure, and pathogenesis pathway of aflatoxins [44].

-Effect on poultry

Acute liver poisoning, necrosis, cancer, mutations, blood problems, and immunological suppression are all serious effects of aflatoxins on chickens , Chickens are the most vulnerable of the poultry species to even very low doses of AFB1 toxicity, followed by ducks, turkeys, and geese in the hierarchy of sensitivity [45]. Most poultry species are immunological suppressed

by aflatoxins, which also cause programmed cell death in the thymus gland, a reduction in bursa formation, and a decrease in T-cell immune response According to research, young chicks fed these mycotoxins had smaller and paler livers, the formation of nodules, gallbladder congestion, and enlargement [23].. Trebak *et al*., (46) discovered indications of tiredness and decreased appetite in exposed hens. Other symptoms include a reduction of development and weight [47]. The influence of aflatoxins on chicken health is diverse, and it can cause serious health problems as well as economic losses in the poultry sector. According to a different research, high levels of aflatoxins in chicken led to shrinkage of the spleen, fatty degeneration, tissue fibrosis, inflammation of the kidney tubules, and interstitial nephritis. These poisons also cause broiler hens to develop hemorrhagic lesions in their stomach, heart, intestines, lungs, and muscles, which may cause fatal blood coagulation disturbances [48].

Aflatoxin poisoning reduces egg production and quality in layer hens. Human health hazards are also posed by residual AFB1 in chicken bodies [49]. Moldy feed, particularly feed contaminated with AFB1, such as peanuts and moldy grains, has been shown to inhibit the immune system . AFB1 has also been shown to cause programmed cell death and tissue damage in a variety of organs [50].

In addition, AFB1 poisoning can decrease body weight, cause liver and renal illnesses, raise mortality rates, and have an impact on young poultry's feed consumption [51]. The adverse effects of aflatoxins on poultry health highlight the importance of implementing measures to minimize their presence in feed and prevent potential health risks, so it's imperative to develop efficient detoxification strategies to mitigate AFB1-induced inflammation and immune suppression in poultry [52]. To far, several methods, including physical, chemical, and biological ones, have been found to reduce the toxicity of AFB1.. Physical detoxification methods (adsorption, heating, and irradiation) and chemical detoxification methods (ammoniation, solvent extraction, and oxidation) both have drawbacks, including food losses, high equipment costs, and low efficacy . Biological strategies were found to be more successful than other ways in detoxifying fungal toxins [53].

-Impact on ruminants

Ruminant animals typically exhibit greater resilience to fungal toxins owing to the presence of rumen bacteria that possess the capability to break down these toxins. Nonetheless, aflatoxins undergo partial degradation by the rumen flora, resulting in the emergence of a secondary toxic and carcinogenic metabolite termed aflatoxicol. In the context of cattle, sheep, goats, and deer, the consumption of aflatoxins can lead to complications related to reproductive health, immune suppression, and diminished production of milk, meat, and wool,sustained intake of feed containing aflatoxin levels in the range of 600 micrograms/kg can prompt a decline in overall livestock health, [54]. Within the range of 100 to 1000 micrograms/kg of aflatoxins in the livestock diet, noticeable effects include inhibition of growth and an increase in liver and kidney weights. Particularly in dairy cows, exposure to these concentrations results in reduced milk production and compromised reproductive efficiency [55].

-Influence on other species

Aquatic animals may consume food that has been contaminated with AFB1. To find out whether AFB1 causes cancer in different fish species such salmon, tilapia, and Nile fish, a study was carried out. It was discovered that the harmful consequences of these fungi toxins, such as weight loss, immunological suppression, liver, kidney, and muscle damage, were the same in fish and other animals [56]. According to research, AFB1 in contaminated feed at concentrations between 1 to 58.4 micrograms/kg can cause jaundice, depression, loss of appetite, and even death in horses.

Young horses had skeletal and cardiac anomalies, bile duct enlargement, liver damage, kidney damage, and eventually died [53], in dogs that consumed food contaminated with AFB1, they experienced liver inflammation, severe depression, loss of appetite, weakness, and the presence of both AFB1 and AFB2 in the study conducted by Gazzotti *et al*., [57] resulted in a high contamination rate of 88% in dog food. Moreover, it was observed that the presence of AFB1, AFB2, AFG1, and AFG2 in dog food led to the development of breast tumors in females and testicular tumors in males [58].

-Effect on Fetuses and Newborns

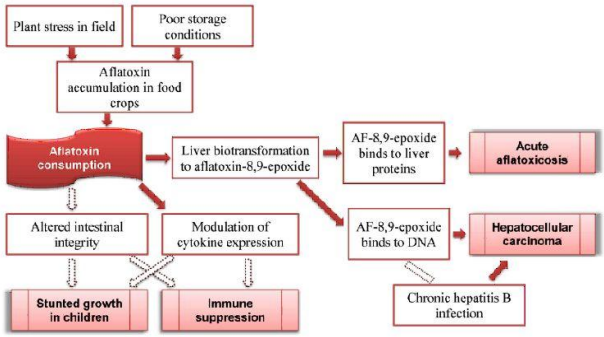
Aflatoxin B1 (AFB1), the most significant, is a secondary fungal metabolite renowned for its genotoxic and mutagenic toxicity. As a potent activator of carcinogenesis, AFB1 causes bone abnormalities, visceral malformations, organ damage, behavioral and reproductive alterations, and low birth weight. Furthermore, prenatal life has a greater mutagenic sensitivity to AFB1 than adult life, indicating that prenatal exposure raises the chance of tumor formation. According to human research, exposure to this fungus toxin during pregnancy is associated with decreased birth weight and head circumference [59]. According to the World Health Organization, [59], diet has a significant role in determining the future health of the child. Several studies have shown that aflatoxin exposure can happen in utero through placental transfer . Fetal development restriction and low birth weight have been associated to high levels of exposure in the womb According to several research, epigenetic modifications brought on by fetal aflatoxin exposure may explain this. DNA methylation levels in cord blood cells from babies 2 to 8 months old were affected by prenatal aflatoxin exposure. They discovered that exposure to these toxins changed these methylation patterns, which are associated to growth and immunity. Studies also show that anemia may follow aflatoxin exposure in people , Anemia can cause pro-inflammatory cytokines to be released and insulin-like growth factor hormones to be depleted, both of which can be toxic and impair placental and fetal development [60]. It can also cause a reduction in head size. Indeed, it has been demonstrated that aflatoxin exposure during pregnancy can result in fetal mortality [60]. Many harmful effects are associated with prenatal exposure to AFB1, such as decreased birth weight, stillbirth, skeletal deformities, impact on fertility, immune impairment, behavioral changes, and predisposition to tumor development ,AFB1 specifically weakens memory, induces oxidative stress, increases lipid peroxidation, and reduces antioxidant enzyme levels . Exposure to AFB1 during pregnancy can delay reflex responses and learning ability, as well as affect movement and behavior [61,62].

Pig oocyte development in animal fetuses is impacted by AFB1 through epigenetic changes, oxidative stress, and programmed cell death. By causing oxidative stress, DNA damage, and programmed cell death, it also affects the early development of any fetus [62]. Additionally, it can change both the first and second stages of metabolic processing in pregnant mice, altering AFB1's biological efficacy and so causing additional liver damage, Mice's birth weight is decreased and their hormone and lipid levels are disturbed when they are exposed to AFB1 before birth. It also affects the levels of methylation of p53 and the growth regulator H19 in the liver and serum. These pathological changes may increase the risk of developing hepatocellular carcinoma (HCC) in fetuses [63],sexually mature animals exposed to AFB1 can have an effect on gamete generation, quality, and maturation. AFB1 also impairs fetal development, offering a long-term health risk to both pregnant women and their children. Oxidative stress, DNA damage and repair, apoptosis, and epigenetic change all contribute to AFB1's reproductive toxicity. Aflatoxin exposure reduces birth weight and the number of offspring in both rats and rabbits [64,65],. According to studies, after 6 hours after

fertilization, zebrafish embryos exposed to these fungal toxins at a concentration of 15–75 nanograms/mL exhibit notable neurobehavioral abnormalities. Due to considerable programmed cell death, AFB1 exposure in zebrafish embryos prevents the formation of the liver during the embryonic stage. Inhibiting the immune system is another effect [66]. Due to the extensive use of AFB1-contaminated foods in underdeveloped nations, aflatoxins can cause preterm births, miscarriages, and low birth weights [67]. AFB1 was injected into chicken eggs at levels of 10 and 100 nanograms/egg via the chorioallantoic membrane of embryos after 96 hours of incubation in order to better understand the effects of aflatoxins on chicken embryos and particular organs. The fetal death rate in embryos receiving 0.00 and 9.83 nanograms/egg AFB1 was 25.00% and 66.67%, respectively, according to the research. In one study, embryos given 2.19 nanograms/egg AFB1 died at a rate of 16.67%. Similarly, in the AF 100 group, embryos receiving more than 90 nanograms/egg AFB1 had 100% fetal death. When compared to the control group, all treatment groups exhibited considerably greater relative weights of the liver and kidneys,Hepatic lipidosis and cellular necrosis displayed a notably higher occurrence in the AF-100 group in comparison to the AF-10 group. Within the kidney, there was a marked elevation in congestion and tubular necrosis in the AF-100 group as opposed to the AF-10 group. Microscopic examination revealed pronounced alterations in both the liver and kidneys of hatchlings originating from high-dose eggs in contrast to those from low-dose eggs, underscoring a dose-dependent pattern of response.In summary, the administration of fungal extracts containing aflatoxins leads to an escalation in fetal mortality rates and embryo toxicity [68].

--Effect of aflatoxins on organs

Aflatoxin-containing substances are mutagenic, carcinogenic, and immunosuppressive. They also cause cancer. They can cause mortality in both people and animals and disrupt a number of metabolic processes [69], Additionally, they cause a variety of pathological harm to the heart, uterus, liver, kidneys, spleen, and other organs, AFB1 also promotes the production of TNF, IL6, IL1, iNOS, COX2, NLRP3, and caspases 1, 3, and 11 , This causes harm to the liver and kidneys as in figure 4,[70].



(Fig:4) Aflatoxin disease pathways in humans [71].

1- The kidneys

Because the kidneys are the major excretory channel for toxins, they are highly vulnerable to them and have a high renal toxicity [72]. AF-treated rats' kidneys display structural abnormalities, congestion, dilatation of renal tubules, infiltration of inflammatory cells, Bowman's space edema, and renal tubule enlargement, Another study found that rats given varied dosages of AFs for 8 days over a 6-week period had variable degrees of tubular atrophy in kidney cells [73].

Research has pointed to AFs stimulating renal damage in rats by inhibiting antioxidant efficiency and promoting lipid peroxidation, Metabolites derived from aflatoxins have been identified to impose significant toxicity on renal nephrons, even prior to their elimination through urine [74]. Moreover, specific investigations have indicated that aflatoxin B1 triggers substantial renal impairment across diverse animal models by inducing oxidative stress within this organ [74] , Other research has revealed that it leads to increased levels of urea, creatinine, and uric acid in the blood [73]. Pathological histological studies on kidneys

across different animal models have demonstrated that aflatoxin exposure results in tubular atrophy, intraluminal hemorrhage, glomerular damage, and tubular epithelial necrosis [65, 75].

Other studies have shown severe cases of cortical necrosis caused by aflatoxins and aflatoxins also negatively impact renal function According to research on birds, the renal cortex occasionally exhibits histological necrosis[65, [74].. Aflatoxin B1 was also shown to produce tubular necrosis and degeneration in fish , Additionally, research has shown that rats exposed to aflatoxin have hyaline casts in their renal tubules [76], According to research, the toxicity of aflatoxins B1, B2, G1, and G2 causes the production of hyaline casts in both male and female rats. Furthermore, Zamir-Nasta *et al.*, [77] discovered that exposure on the fifteenth day caused histological alterations, such as dilatation of blood vessels and proliferation of focal cells in the glomeruli. In another investigation, pregnant pigs exposed to aflatoxin B1 on day 35 had tissue bleeding and renal tubule atrophy [77].

AFB1 is taken up by the kidneys, and its accumulation within kidney tissues triggers an elevation in p21 regulation through MYC, PLK1, and PLD1 pathways. This ultimately leads to a halt in the cell cycle progression at the S phase and contributes to kidney damage. In the case of HEK293 cells treated with AFB1, there was a notable increase in programmed cell death and DNA fragmentation, which correlated with the upregulation of p53, Bax, and caspases [78],furthermore, AFB1 and AFM1 exhibit a synergistic effect in intensifying oxidative stress and the pathways involved in programmed cell death within renal cells. This is achieved through the modulation of L-proline expression [78].

Furthermore, the combination of AFB1 exposure and a low-protein diet resulted in decreased weight gain, exacerbated kidney dysfunction, and heightened oxidative stress in mice [79], The impact of AFB1 exposure on oxidative stress was observed across multiple organs—liver, kidney, and spleen—of carp, with Nrf2 regulation playing a significant role. Interestingly, reactive oxygen species (ROS) appeared to emerge earlier in the kidney when compared to the liver and spleen],thus, it becomes evident that the renal toxicity induced by AFB1 is primarily manifested through oxidative stress mechanisms that involve p21, L-proline, Nrf2, and other associated genes. Furthermore, AFB1's harmful effects on the kidneys can synergize with those of other mycotoxins [80], Aflatoxin G1 also induces various renal pathologies, including extensive necrosis in the renal cortex, the presence of hyaline casts, glomerular atrophy, enlargement of glomerular capillaries and cells, expansion of urinary spaces, and cellular swelling of tubules. In both mice and rats, AFB1, B1, B2, G1, and G2 were found to inflict similar damages [76].

Other research studies have indicated that exposure to aflatoxin G1 is associated with the occurrence of tubular necrosis, tubular dilation, and swelling of tubular cells [75]. Furthermore, heightened levels of urea and creatinine in the bloodstream have been observed, indicating compromised kidney function and disruptions in protein metabolism [73].

In the case of mice treated with a mixture of aflatoxins B1, B2, G1, and G2, there was a notable elevation in the concentrations of urea and creatinine in the blood. Simultaneously, levels of sodium, potassium, and other biochemical indicators relevant to kidney function displayed a significant decrease [76]. Another study involving piglets fed a diet contaminated with a mixture of aflatoxins highlighted a decrease in blood levels of sodium and potassium. This outcome suggests that toxins play a clear role in inhibiting the activity of the sodium-potassium pump, which operates in conjunction with ATPase [34].

Aflatoxin G1 also exerts adverse effects on renal tissues in mice by inducing alterations in plasma concentrations of urea, creatinine, sodium, and potassium. Both Aflatoxins B1

and G1 have demonstrated the capability to induce liver and kidney tumors in mice. Of particular note, exposure to aflatoxin G1 led to a significant increase in creatinine levels in mice. Moreover, there was a notable reduction in sodium and potassium levels in the bloodstream, accompanied by observable damages within the renal cortex and tubules, The research study concluded that aflatoxin G1 elicits detrimental impacts on kidney tissues by diminishing the expression of aquaporin-1 and prompting changes in urea, creatinine, sodium, and potassium concentrations. This highlights the pathological tissue toxicity associated with aflatoxin G1 [77]. Consequently, it can be inferred that kidney damage stemming from aflatoxin G1 contributes to disruptions in electrolyte balance and compromises the excretion of waste products such as urea and creatinine.

2- The liver

The liver is particularly vulnerable to the detrimental effects of aflatoxins, which give rise to a range of issues including inflammation, necrosis, fatty liver disease, fibrosis, and the development of liver tumors. Aflatoxins also have the potential to trigger acute liver failure, a condition that can prove fatal in mice, the liver emerges as the primary target for AFB1, with its capacity to induce liver damage increasing in tandem with the dose administered[53],. The mechanisms behind this damage encompass factors like the promotion of fat accumulation, the initiation of fatty acid oxidation , and the shortening of telomeres [81] ,Recent research findings have revealed that AFB1 contributes to the generation of reactive oxygen species (ROS) within liver cells, consequently leading to oxidative stress, inflammatory responses, and liver damage [82].

In addition, continuous exposure to AFB1 alters the metabolism of lipids and fatty acids and causes liver cells to undergo programmed cell death. By upregulating cyclooxygenase-2 (COX2), it stimulates Kupffer cells and intensifies the inflammatory response in the liver [82]. According to Rotimi *et al*., [83], prolonged exposure to AFB1 also impairs lipid and fatty acid metabolism. These findings highlight the liver's great vulnerability to aflatoxins, which can alter normal metabolic processes and cause a variety of pathological outcomes. AFB1 also significantly impairs mitochondrial activity and lowers antioxidant defenses in mouse livers . Similarly, AFB1-exposed animals showed mitochondrial malfunction and higher rates of mitochondria-dependent programmed cell death in the liver [84]. Primary hepatocytes treated with varied doses of AFB1 demonstrated mitochondrial malfunction, oxidative stress, and mitochondria-dependent programmed cell death mediated by reactive oxygen species (ROS) in another investigation [85].

AFB1 is a substantial risk factor for primary liver cancer, according to epidemiological research []. Furthermore, there is evidence that AFB1 can promote mutations in the p53 gene and cause liver cancer when combined with chronic hepatitis B virus (HBV) infection [85]. In 2012, liver cancer was ranked sixth in the world, with up to 83% of occurrences happening in underdeveloped countries [86]. The most common kinds of cancer have been identified in people infected with the hepatitis B (HBV) and hepatitis C (HCV) viruses, which are common in Asian and African nations, Studies have highlighted that individuals exposed to aflatoxins and concurrently infected with viral hepatitis are at an elevated risk of developing liver cancer [86]. Aflatoxins are also associated with other forms of liver damage, including liver fibrosis and hepatomegaly [16].

Numerous investigations have underscored the liver as the primary organ affected by aflatoxins. For example, Gong *et al.*, [2012] conducted a study demonstrating that AFB1 can incite liver damage and even contribute to the development of liver cancer by promoting oxidative stress, inflammation, and mitochondrial dysfunction. These effects are facilitated through the engagement of pathways

involving key factors such as P53, ROS, COX2, NRF2, and other signaling cascades. In a broader context, AFB1 is accountable for both acute and chronic liver failure [87]. The metabolic and toxic consequences attributed to AFB1 and AFM1 are chiefly observed within the liver [88]. Previous investigations have unveiled a specific interaction between AFB1, AFM1, and hepatitis B virus (HBV), resulting in a noteworthy 12-fold escalation in the risk of liver cancer [89] ,furthermore, AFB1 exposure in mice has been proven to amplify the production of reactive oxygen species (ROS) and the release of pro-inflammatory cytokines like TNFα within liver cells [89]. These cytokines significantly contribute to the progression of liver cancer Additionally, liver telocytes have been identified as contributors to the function of hepatic stellate cells, and the impairment of these small cells can disrupt the regulation of hepatic stellate cells, leading to dysfunction [90]. In a study involving rats treated with AFB1, alterations in the morphology of Telocytes located in the perivascular space of the liver were observed [90]. Another study conducted by [90]] emphasized the pivotal role of these cells in the context of liver fibrosis. Additionally, a study by [72] revealed varying degrees of periductal fibrosis and bile duct enlargement in groups treated with AFB1, The histopathological repercussions of aflatoxin exposure on the liver encompassed nuclear enlargement, necrosis, and the infiltration of inflammatory cells, [72], Similar outcomes were documented in mice exposed to AFB1. [74] reported necrosis and hydropic degeneration occurring in the livers of rats injected with AFB1 within the sinusoids. unveiled severe liver damage when rats were administered AFs orally. The detrimental impact of AFs was closely linked to compromised antioxidant defense mechanisms and an escalated production of lipid peroxidation (LPO) in the liver [91],these adverse effects can be attributed to the formation of reactive epoxide AFs within the liver, subsequently leading to ensuing damage [92].

3- The Brain

Exposure to aflatoxins can cause neuronal degeneration, destruction, vascular congestion, bleeding, and necrosis in brain cells [73], the degree of necrotic alterations in the brain was dose-dependent [72], AFs increase oxidative stress and produce free radicals (ROS), which lead to brain damage by causing degenerative alterations in the cerebral cortex and hippocampus, eating AFB1 once a week for eight weeks dramatically reduces brain function in animals [93].

Long-term exposure to AFB1 might allow it to breach the blood-brain barrier, leading to neurotoxic effects and even chronic neuronal degeneration, similar to what is observed in Alzheimer's disease , AFB1 inhibits the proliferation of astrocytes in humans, causing cell cycle arrest and mitochondria-dependent programmed cell death, Environmental exposure to AFB1 has the potential to trigger neuroinflammatory responses through the activation of microglial cells, thereby increasing vulnerability to neurodegenerative diseases [94],the neurotoxic consequences of AFB1 exposure have been demonstrated in zebrafish embryos through nuclear magnetic resonance (NMR) imaging [95],this exposure resulted in a decrease in embryo survival rates by inhibiting the growth of neural crest cells [94],furthermore, AFB1-treated neuroblastoma tumor cells (specifically, the IMR-32 cell line) exhibited a series of effects including the accumulation of reactive oxygen species (ROS), DNA damage, cell cycle arrest at the S phase, and programmed cell death [96]. The impact of AFB1 exposure extends to human microglial cell lines (CHME5) and dendritic cells derived from human monocyte-derived dendritic cells (MDDCs), influencing the expression of crucial functional genes and ultimately leading to heightened programmed cell death [96,97].

Consequently, AFB1 has the capacity to impede the growth of neuronal cells, intensify

programmed cell death processes, disturb neural equilibrium, and elevate susceptibility to neurodegenerative diseases [76].

4- Immunological effects

Using ELISA, researchers discovered substantial changes in malondialdehyde (MDA), superoxide dismutase (SOD), and total antioxidant capacity (T-AOC) levels in mice serum treated with AFB1 , MDA levels were noticeably greater, but SOD and T-AOC levels were noticeably reduced. Proline is a metabolite of AFB1 and AFM1, and its content was shown to be greatly lowered in mice treated with these AFs [78]. MDA is a peroxidation product created by free radicals that measures the degree of oxidative damage [98]. According to research, proline metabolism affects oxidative stress in a variety of living creatures [89],AFB1 therapy decreased RAW264.7 macrophage cell survival in mice in a dose- and time-dependent manner by boosting the generation of ROS and malondialdehyde (MDA) and lowering GSH levels. These alterations were connected to the regulation of the mRNAs for NOS2, TNF, and CXCL2, as well as the decrease in CD86 regulation. The mitochondrial respiratory chain is also damaged by oxidative stress brought on by AFB1 in macrophages, which activates inflammatory response pathways [99]. Moreover, AFB1 demonstrated a capacity to diminish the functionality of 3D4/21 cells, prompting programmed cell death, the release of pro-inflammatory cytokines, DNA damage, and the induction of oxidative stress. Additionally, treatment of 3D4/21 cells with AFB1 led to heightened levels of DNA methyltransferases DNMT1 and DNMT3a, consequently triggering the activation of the JAK2/STAT3 signaling pathway. This activation was mitigated by inhibiting p-JAK2 and p-STAT3 through the blockade of DNMT1 and DNMT3a, thereby alleviating the immune toxicity induced by AFB1 [100], when AFB1 was combined with ochratoxin A (OTA), this compound mixture exhibited concentration-dependent effects such as an increase in TNFα and IL6 production in the cells, a reduction in lactate dehydrogenase release, and a suppression of the macrophage index. Additionally, this combined treatment led to a significant decrease in GSH production, an increase in ROS levels, an enhancement of IκBα degradation, NF-κB phosphorylation, and the translocation of NF-κB into the nucleus. Consequently, AFB1 and OTA acted synergistically to intensify immune toxicity by activating the NF-κB signaling pathway [101]. According to research, even modest dosages of AFB1 have a deleterious influence on immunological organs in animals, reduce antibody titers, and cause lymphoid tissue destruction. Monogastric animals are more vulnerable to AFB1-induced immunological toxicity, notably poultry and pigs. AFB1 exposure promoted programmed cell death in thymocytes via mitochondrial signaling pathways and death receptors, as well as DNA damage [23], AFB1 promotes tissue damage, cell cycle arrest, and programmed cell death in the Fabricius bursa of chicks, resulting in immune system dysfunction [102].

Ethical Clearance

Through a review of the literature, this study explored the toxicological and histopathological effects of aflatoxins on the health of animals in general.

4.Conclusion

Aflatoxins predominantly affect the liver, interfering with cellular functions and causing hepatotoxicity, As a result of the liver injury, metabolic activities are impaired, resulting in decreased nutrient consumption, growth retardation, and feed efficiency. Also the immune system weakens, making animals more prone to illnesses and reducing the effectiveness of vaccines. Infertility, embryonic death, and decreased milk production in dairy cows. Aflatoxin exposure has also been associated to an increase in animal cancer notably hepatocellular carcinoma , This review emphasizes the requirement for stringent feed and food source monitoring, as well as efficient management measures, in order to reduce

aflatoxin exposure and its adverse effects on animal health , and It should be mentioned that the plant sources that produce the most aflatoxins are recommended, and recommending monitoring of foods given to animals as fodder , Control agricultural pests to avoid the risk of exposure to these toxins.

References

1-Eskola M, Kos G, Elliott CT, Hajslova J, Mayar S, Krska R. (2020). Worldwide contamination of food-crops with mycotoxins: validity of the widely cited 'FAO estimate' of 25. Critical Reviews in Food Science and Nutrition 60:2773–2789 DOI 10.1080/10408398.2019.1658570.

2- FAO—Food and Agriculture Organization of the United Nations (2013) Mycotoxins. Food safety and quality. http://www.fao.org/food/food-safet y-quality/a-z-index/mycotoxins/en/. Accessed 22 Jan 2013.

3-Alvarado AM, Zamora-Sanabria R, and Granados-Chinchilla F . (2017).A Focus on Aflatoxins in Feedstuffs: Levels of Contamination, Prevalence, Control Strategies, and Impacts on Animal Health,Chapter 6 ,PP;116 – 152 . http://dx.doi.org/10.5772/intechopen.69468.

4- Wang J, Mukhtar H, Ma L, Pang Q, Wang X. (2018a). VHH Antibodies: reagents for mycotoxin detection in food products. Sensors 18:485 DOI 10.3390/s18020485.

5- Arroyo-Manzanares, N.; Huertas-Pérez, J.F.; García-Campaña, A.M.; Gámiz-Gracia, L.(2015). Aflatoxins in animal feeds: A straightforward and cost-effective analytical method. Food Control . 54, 74–78. https://doi.org/10.1016/j.foodcont.2015.01.027.

6- Gong, Y. Y., Watson, S., & Routledge, M. N. (2016). Aflatoxin Exposure and Associated Human Health Effects, a Review of Epidemiological Studies. Food safety (Tokyo, Japan), 4(1), 14–27. https://doi.org/10.14252/foodsafetyfscj.2015026.

7- Luo, J., Vogel, R. F., and Niessen, L. (2014). Rapid detection of aflatoxin producing fungi in food by real-time quantitative loop-mediated isothermal amplification. Food microbiology, 44, 142–148. https://doi.org/10.1016/j.fm.2014.06.004.

8- Moeinian K, Yaghmaeian K, Ghorbani R. (2014).[Aflatoxin M1 concentration in raw milk produced in the cities of Semnan Province-Iran (Persian)]. Koomesh.; 15(2):176-81. http:// koomeshjournal.semums.ac.ir/article-1-1942-en.html.

9- Li C., Liu X., Wu J., Ji X., and Xu Q. (2022). Research progress in toxicological effects and mechanism of aflatoxin B_1 toxin. PeerJ, 10, e13850. https:// doi. Org /10. 7717/peerj.13850.

10- Al-Zoreky, N. S., and Saleh, F. A. (2019). Limited survey on aflatoxin contamination in rice. Saudi journal of biological sciences, 26(2), 225–231. https://doi.org/10.1016/j.sjbs.2017.05.010.

11. Mohammed Ali, S., Ali Mohammed, A., & Romil Mutib, H. (2018). The inhibitory effect of Pleurotus ostreatus on the growth of *Aspergillus niger* and the production of Ochratoxin A. and isolated from apples. *Al-Kufa University Journal for Biology*, *10*(1). Retrieved from https://journal.uokufa.edu.iq/index.php/ajb/article/view/8201

12- Jalili C, Zamir-Nasta T, Ghahari L, Kakeh Baraei S.(2019). Relationship between synthesis of P21 and Cyclin D1 proteins and histomorphometric parameters of mice testicular tissue in presence of Aflatoxin G1 (Persian)]. Koomesh.; 21(1):195-200. http://koomeshjournal.semums.ac.ir/article-1-4467-en.html

13-Negash, D. A . (2018).Review of Aflatoxin: Occurrence, Prevention, and Gaps in Both Food and Feed Safety. J. Appl. Microbiol. Res., 1, 1–35.

14- Meissonnier, G. M., Pinton, P., Laffitte, J., Cossalter, A. M., Gong, Y. Y., Wild, C. P., Bertin, G., Galtier, P., & Oswald, I. P. (2008). Immunotoxicity of aflatoxin B1: impairment of the cell-mediated response to vaccine antigen and modulation of cytokine

expression. Toxicology and applied pharmacology, 231(2), 142–149. https://doi.org/10.1016/j.taap.2008.04.004

15- International Agency for Research on Cancer. IARC.(2012). monographs on the evaluation of carcinogenic risks to humans; vol 56, Chemical agents and related occupations. IARC, Lyon; 100F: 1–599.

16- Monson MS, Settlage RE, McMahon KW, Mendoza KM, Rawal S, El-Nezami HS, Coulombe RA, Reed KM. (2014). Response of the hepatic transcriptome to aflatoxin B1 in domestic turkey (Meleagris gallopavo). PLOS ONE 9:e100930 DOI 10.1371/journal.pone.0100930.

17-Theumer MG, Lopez AG, Masih DT, Chulze SN, Rubinstein HR. (2003). Immunobiological effects of AFB1 and AFB1-FB1 mixture in experimental subchronic mycotoxicoses in rats. Toxicology 186:159–170 DOI 10.1016/s0300-483x(02)00603-0.

18- Kumar P, Mahato DK, Kamle M, Mohanta TK, Kang SG. (2017). Aflatoxins: a global concern for food safety, human health and their management. Frontiers in Microbiology 7:2170 DOI 10.3389/fmicb.2016.02170.

19-Eom SY, Yim DH, Zhang Y, Yun JK, Moon SI, Yun HY, Song YJ, Youn SJ, Hyun T, Park JS, Kim BS, Lee JY, Kim YD, Kim H. (2013). Dietary aflatoxin B1 intake, genetic polymorphisms of CYP1A2, CYP2E1, EPHX1, GSTM1, and GSTT1, and gastric cancer risk in Korean. Cancer Causes Control 24:1963–1972 DOI 10.1007/s10552-013-0272-3.

20-Kucukcakan B, Hayrulai-Musliu Z. (2015). Challenging role of dietary Aflatoxin B1 exposure and hepatitis B infection on risk of hepatocellular carcinoma. Open Access Macedonian Journal of Medical Sciences 3:363–369 DOI 10.3889/oamjms.2015.032.

21- Wild, C. P., and Gong, Y. Y. (2010). Mycotoxins and human disease: a largely ignored global health issue. Carcinogenesis, 31(1), 71–82. https://doi.org/10.1093/carcin/bgp264

22- Rawal, S., Kim, J. E., and Coulombe, R., Jr (2010). Aflatoxin B1 in poultry: toxicology, metabolism and prevention. Research in veterinary science, 89(3), 325–331. https://doi.org/10.1016/j.rvsc.2010.04.011

23- Ishikawa, A. T., Hirooka, E. Y., Alvares E Silva, P. L., Bracarense, A. P. F. R. L., Flaiban, K. K. M. D. C., Akagi, C. Y., Kawamura, O., Costa, M. C. D., and Itano, E. N. (2017). Impact of a Single Oral Acute Dose of Aflatoxin B_1 on Liver Function/Cytokines and the Lymphoproliferative Response in C57Bl/6 Mice. Toxins, 9(11), 374. https://doi.org/10.3390/toxins9110374.

24- Zhou, G.; Corey, E.J. (2005).Short, enantioselective total synthesis of aflatoxin B2 using an asymmetric [3+2]-cycloaddition step. J. Am. Chem. Soc., 127, 11958–11959. https://doi.org/10.1021/ja054503m.

25- Bennett, J. W., and Klich, M. (2003). Mycotoxins. Clinical microbiology reviews, 16(3), 497–516. https://doi.org/10.1128/CMR.16.3.497-516.2003.

26. razzaq majjed, T., Adel, M., & Al-Amer, A. (2023). Detection of Candida species isolated from immunocompromised patients in AL-Najaf province: Detection of Candida species isolated from immunocompromised patients in AL-Najaf province. *Al-Kufa University Journal for Biology*, *15*(2), 49–54. https://doi.org/10.36320/ajb/v15.i2.11882.

27- Puga-Torres, B., Salazar, D., Cachiguango, M., Cisneros, G., and Gómez-Bravo, C. (2020). Determination of Aflatoxin M1 in Raw Milk from Different Provinces of Ecuador. Toxins, 12(8), 498. MDPI AG. Retrieved from http://dx.doi.org/10.3390/toxins12080498

28- Carvajal, M., Rojo, F., Méndez, I., and Bolaños, A. (2003). Aflatoxin B1 and its interconverting metabolite aflatoxicol in milk: the situation in Mexico. Food additives and contaminants, 20(11), 1077–1086. https://doi.org/10.1080/0265203031000159447 8.

29- Karabulut, S., Paytakov, G., & Leszczynski, J. (2014). Reduction of aflatoxin B1 to aflatoxicol: a comprehensive DFT study provides clues to its toxicity. Journal of the science of food and agriculture, 94(15), 3134–3140. https://doi.org/10.1002/jsfa.6663

30-Antonissen, G., Devreese, M., De Baere, S., Martel, A., Van Immerseel, F., & Croubels, S. (2017). Impact of Fusarium mycotoxins on hepatic and intestinal mRNA expression of cytochrome

31- Carvajal-Moreno, Magda. (2015). Metabolic Changes of Aflatoxin B1 to become an Active Carcinogen and the Control of this Toxin. Immunome Research. 11. 10.4172/1745-7580.10000104.

32- Diaz, G. J., Murcia, H. W., Cepeda, S. M., and Boermans, H. J. (2010). The role of selected cytochrome P450 enzymes on the bioactivation of aflatoxin B1 by duck liver microsomes. Avian pathology : journal of the W.V.P.A, 39(4), 279–285. https://doi.org/10.1080/03079457.2010.495109 .

33- Dhakal A, Hashmi MF, Sbar E. (2023). Aflatoxin Toxicity. [Updated 2023 Feb 19]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557781/.

34- Mitchell, N. J., Kumi, J., Johnson, N. M., Dotse, E., Marroquin-Cardona, A., Wang, J. S., Jolly, P. E., Ankrah, N. A., & Phillips, T. D. (2013). Reduction in the urinary aflatoxin M1 biomarker as an early indicator of the efficacy of dietary interventions to reduce exposure to aflatoxins. Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals, 18(5), 391–398. https://doi.org/10.3109/1354750X.2013.798031.

35- Wild, C. P., & Turner, P. C. (2002). The toxicology of aflatoxins as a basis for public health decisions. Mutagenesis, 17(6), 471–481. https://doi.org/10.1093/mutage/17.6.471.

36- Bujons, J., Hsieh, D. P., Kado, N. Y., & Messeguer, A. (1995). Aflatoxin M1 8,9-epoxide: preparation and mutagenic activity. Chemical research in toxicology, 8(3), 328–332. https://doi.org/10.1021/tx00045a002.

37- IARC.(1993). Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 56, Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins; IARC: Lyon, France, 1993; pp. 1–599. Available online: https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-HazardsTo-Humans/Some-Naturally-Occurring-Substances-Food-Items-And-Constituents-Heterocyclic-Aromatic-Amines-AndMycotoxins-1993 (accessed on 1 November 2021).

38-Dhanasekaran, D., Shanmugapriya, S., Thajuddin, N., & Panneerselvam, A. (2011). Aflatoxins and Aflatoxicosis in Human and Animals. InTech. doi: 10.5772/22717.

39- Dawit G., Barbara S., Tegegne A., Hanson J.& Grace D. Aflatoxin contamination of milk and dairy feeds in the Greater Addis Ababa milk shed, Ethiopia. Food Control. vol. 59, pp. 773 779,2016. https://doi.org/10.1016/j.foodcont.2015.06.060.

40- Nurul Adilah, Z., and Mohd Redzwan, S. (2017). Effect of dietary macronutrients on aflatoxicosis: a mini-review. Journal of the science of food and agriculture, 97(8), 2277–2281. https://doi.org/10.1002/jsfa.8234

41- Lozano, M. C., and Diaz, G. J. (2006). Microsomal and cytosolic biotransformation of aflatoxin B1 in four poultry species. British poultry science, 47(6), 734–741. https://doi.org/10.1080/00071660601084390

42- Abrehame, S., Manoj, V. R., Hailu, M., Chen, Y.-Y., Lin, Y.-C., and Chen, Y.-P. (2023). Aflatoxins: Source, Detection, Clinical Features and Prevention. Processes, 11(1), 204. MDPI AG. Retrieved from http://dx.doi.org/10.3390/pr11010204

43- Oğuz, H., Keçeci, T., Birdane, Y. O., Onder, F., and Kurtoğlu, V. (2000). Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. Research in veterinary science, 69(1), 89–93. https://doi.org/10.1053/rvsc.2000.0395
44- Trebak, F., Alaoui, A., Alexandre, D., El Ouezzani, S., Anouar, Y., Chartrel, N., and Magoul, R. (2015). Impact of aflatoxin B1 on hypothalamic neuropeptides regulating feeding behavior. Neurotoxicology, 49, 165–173. https://doi.org/10.1016/j.neuro.2015.06.008
45- Liu, Y. L., Meng, G. Q., Wang, H. R., Zhu, H. L., Hou, Y. Q., Wang, W. J., and Ding, B. Y. (2011). Effect of three mycotoxin adsorbents on growth performance, nutrient retention and meat quality in broilers fed on mould-contaminated feed. British poultry science, 52(2), 255–263. https://doi.org/10.1080/00071668.2011.559453
.
46- Allocati, N., Masulli, M., Di Ilio, C., & Federici, L. (2018). Glutathione transferases: substrates, inihibitors and pro-drugs in cancer and neurodegenerative diseases. Oncogenesis, 7(1), 81–15. https://doi.org/10.1038/s41389-017-0025-3.
47- Diaz, G. J., Murcia, H. W., Cepeda, S. M., and Boermans, H. J. (2010). The role of selected cytochrome P450 enzymes on the bioactivation of aflatoxin B1 by duck liver microsomes. Avian pathology : journal of the W.V.P.A, 39(4), 279–285. https://doi.org/10.1080/03079457.2010.495109
.
48- Buzala M, Słomka A, Janicki B, Ponczek MB, Żekanowska E. (2017). Review: The mechanism of blood coagulation, its disorders and measurement in poultry. Livestock Science;195:1-8. DOI: 10.1016/j.livsci.2016.11.009.
49-Valchev I, Marutsova V, Zarkov I, Ganchev A, Nikolov Y. (2017).Effects of aflatoxin B1 alone or co-administered with Mycotox NG on performance and humoral immunity of turkey broilers. Bulgarian Journal of Veterinary Medicine ;20(1):38-50. DOI: 10.15547/ bjvm.1019.
50-Liu T, Ma Q, Zhao L, Jia R, Zhang J, Ji C, Wang X (2016) Protective efects of sporoderm-broken spores of ganderma lucidum on growth perfor- mance, antioxidant capacity and immune function of broiler chickens exposed to low level of afatoxin B1. Toxins 8:278. https://doi.org/10.3390/ toxins8100278.
51-Saleemi MK, Ashraf K, Gul ST, Naseem MN, Sajid MS, Mohsin M, He C, Zubair M, Khrar A (2019) Toxicopathological efects of feeding afatoxins B1 in broil- ers and its ameliosration with indigenous mycotoxin binder. Ecotoxicol Environ Saf 187:109712. https://doi.org/10.1016/j.ecoenv.2019.109712.
52-Guo, H. W., Chang, J., Wang, P., Yin, Q. Q., Liu, C. Q., Xu, X. X., Dang, X. W., Hu, X. F., and Wang, Q. L. (2021). Effects of compound probiotics and aflatoxin-degradation enzyme on alleviating aflatoxin-induced cytotoxicity in chicken embryo primary intestinal epithelium, liver and kidney cells. AMB Express, 11(1), 35. https://doi.org/10.1186/s13568-021-01196-7.
53-Gregorio M, Neef D, Jager A, Corassin C, Carão Á, Albuquerque R, Azevedo A, Oliveira C (2014) Mineral adsorbents for prevention of mycotoxins in animal feeds. Toxin Rev 33:125–135. https://doi.org/10.3109/15569 543.2014.905604.
54-Zain ME. (2011).Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society;15(2):129-144. https://doi.org/10.1016/j.jscs.2010.06.006
55-Gallo A, Giuberti G, Frisvad JC, Bertuzzi T, Nielsen KF. (2015).Review on mycotoxin issues in ruminants: Occurrence in forages, effects of mycotoxin ingestion on health status and animal performance and practical strategies to counteract their negative effects. Toxins;7(8):3057-3111. DOI: 10.3390/toxins7083057.
56- Farabi SMV, Yousefian M, Hajimoradloo A. (2006). Aflatoxicosis in juvenile Huso huso fed a contaminated diet. Journal of Applied

Ichthyology ;22(s1):234-237. DOI: 10.1111/j.1439-0426.2007.00958.x
57. Dawit G., Barbara S., Tegegne A., Hanson J.& Grace D. Aflatoxin contamination of milk and dairy feeds in the Greater Addis Ababa milk shed, Ethiopia. Food Control. vol. 59, pp. 773 779,2016. https://doi.org/10.1016/j.foodcont.2015.06.060.
58- Frehse MS, Martins MIM, Ono EYS, Bracarense APFRL, Bissoqui LY, Teixeira EMK, Santos NJR, Freire RL. (2015).Aflatoxins ingestion and canine mammary tumors: There is an association?. Food and Chemical Toxicology;84:74-78. DOI: 10.1016/j.fct.2015.08.004.
59 Silva, J.V.B ., Oliveira, C.A.F.; Ramalho, L.N.Z.(2021). Effects of Prenatal Exposure to Aflatoxin B1: A Review. Molecules , 26, 7312. https://doi.org/10.3390/ molecules26237312.
60- Turner, P. C., Collinson, A. C., Cheung, Y. B., Gong, Y., Hall, A. J., Prentice, A. M., & Wild, C. P. (2007). Aflatoxin exposure in utero causes growth faltering in Gambian infants. International journal of epidemiology, 36(5), 1119–1125. https://doi.org/10.1093/ije/dym122
61-Benkerroum N. (2020). Chronic and Acute Toxicities of Aflatoxins: Mechanisms of Action. International journal of environmental research and public health, 17(2), 423. https://doi.org/10.3390/ijerph17020423.
62- Liu J, Wang QC, Han J, Xiong B, Sun SC. (2015a). Aflatoxin B1 is toxic to porcine oocyte maturation. Mutagenesis 30:527–535 DOI 10.1093/mutage/gev015.
63- Sriwattanapong K, Slocum SL, Chawanthayatham S, Fedeles BI, Egner PA, Groopman JD, Satayavivad J, Croy RG, Essigmann JM. (2017). Editor's highlight: pregnancy alters aflatoxin b1 metabolism and increases DNA damage in mouse liver. Toxicological Sciences 160:173–179 DOI 10.1093/toxsci/kfx171.
64- Abdulrazzaq, Y.; Padmanabhan, R.; Salim, M.; Kochyil, J.; Shafiullah, M. (2011).Teratogenic effects of aflatoxin B1 in mice exposed in early and late gestation. Pediatr. Res, 70, 405. [CrossRef]
65.Liu, Y. L., Meng, G. Q., Wang, H. R., Zhu, H. L., Hou, Y. Q., Wang, W. J., and Ding, B. Y. (2011). Effect of three mycotoxin adsorbents on growth performance, nutrient retention and meat quality in broilers fed on mould-contaminated feed. British poultry science, 52(2), 255–263. https://doi.org/10.1080/00071668.2011.559453
66- Wu, T. S., Cheng, Y. C., Chen, P. J., Huang, Y. T., Yu, F. Y., and Liu, B. H. (2019). Exposure to aflatoxin B_1 interferes with locomotion and neural development in zebrafish embryos and larvae. Chemosphere, 217, 905–913. https://doi.org/10.1016/j.chemosphere.2018.11.058
67 Lawn, J. E., Blencowe, H., Waiswa, P., Amouzou, A., Mathers, C., Hogan, D., Flenady, V., Frøen, J. F., Qureshi, Z. U., Calderwood, C., Shiekh, S., Jassir, F. B., You, D., McClure, E. M., Mathai, M., Cousens, S., Lancet Ending Preventable Stillbirths Series study group, and Lancet Stillbirth Epidemiology investigator group (2016). Stillbirths: rates, risk factors, and acceleration towards 2030. Lancet (London, England), 387(10018), 587–603. https://doi.org/10.1016/S0140-6736(15)00837-5
68- Saleemi MK, MZ Khan, A Khan, ZU Hassan, WA Khan, S Rafique, Z Fatima and A Sultan, (2015). Embryotoxic and histopathological investigations of in-ovo inoculation of aflatoxigenic fungal extracts in chicken embryos. Pak Vet J, 35(4): 403-408.
69-Wangikar, P. B., Dwivedi, P., Sinha, N., Sharma, A. K., and Telang, A. G. (2005). Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin B1 with special reference to microscopic effects. Toxicology, 215(1-2), 37–47. https://doi.org/10.1016/j.tox.2005.06.022.
70-Iqbal M, Abbas M, Adil A, Nazir and I. Ahmad.(2019). Aflatoxins biosynthesis, toxicity and intervention strategies: A review.

Chemistry International 5(3), 168-191, https://ssrn.com/abstract=3407341 or http://dx.doi.org/10.2139/ssrn.3407341 .
71- Wu F. Khlangwiset P. (2010). Health economic impacts and cost-effectiveness of aflatoxin reduction strategies in Africa: Case studies in biocontrol and postharvest interventions. Food Additives & Contaminants 27 496 509.
72- Kanchana K, Hedhayathullahkhan HB, Vedagiri A, Palanivelu S, Panchanatham S. (2013).Pharmacological effect of Kalpaamruthaa on renal and cardiac damage induced by ingestion of aflatoxin B1 studied in Wistar rats. Biomed Prev Nutr; 3(3):233-40. [DOI:10.1016/j.bionut.2012.10.004].
73- Hammoud, G.M., Abd El-Shafea, Y.M., Salem, A.A. and Nail, N.S. (2018). Effect of grape seedsextract on attenuation of aflatoxins toxicity in rats. IJGHC. Sec. B; 7 (2): 204-223.
74- Darwish, H.R., Omara, E.A., Abdel-Aziz, K.B., Farag, I.M., Nada, S.A. and Tawfek, N.S. (2011). Saccharomyces cerevisiae modulates aflatoxininduced toxicity in male Albino mice. Report andOpinion.3:(12), 32-43.
75- Abdulmajeed NA. (2011).Therapeutic ability of some plant extracts on aflatoxin B1 induced renal and cardiac damage. Arab J Chem ; 4(1):1-10. [DOI:10.1016/j.arabjc.2010.06.005].
76- Fahmy A. (2017).Histological and Immunohistochemical study on the possible protective effects of curcumin and garlic against aflatoxinB1 induced toxicity on the renal cortex of adult male guinea pig. J Am Sci.; 13(3):115-124. http://www.jofamericanscience.org/journals/am-sci/am130317/12_31827jas130317_115_124.pdf.
77- Shivasharanappa GY, Mundas S, Rao D G, Tikare V, Shridhar N B. (2013). Histopathological changes in pigs exposed to aflatoxin B1 during pregnancy. Indian J Anima Res; 47(5):386-91. https://arccjournals.com/journal/indian-journal-of-animal-research/ARCC162.
78- Huang B, Mu P, Chen X, Tang S, Ye W, Zhu W, Deng Y. (2019). Aflatoxin B1 induces S phase arrest by upregulating the expression of p21 via MYC, PLK1 and PLD1. Biochemical Pharmacology 166:108–119 DOI 10.1016/j.bcp.2019.05.008.
.
79- Rotimi OA, Rotimi SO, Oluwafemi F, Ademuyiwa O, Balogun EA. (2018). Oxidative stress in extrahepatic tissues of rats co-exposed to Aflatoxin B1 and low protein diet. Toxicological Research 34:211–220 DOI 10.5487/TR.2018.34.3.211.
80- Kövesi B, Pelyhe C, Zándoki E, Mézes M, Balogh K. (2020). Combined effects of aflatoxin B1 and deoxynivalenol on the expression of glutathione redox system regulatory genes in common carp. Journal of Animal Physiology and Animal Nutrition 104:1531–1539 DOI 10.1111/jpn.13343.
81-Lu X, Hu B, Shao L, Tian Y, Jin T, Jin Y, Ji S, Fan X. (2013). Integrated analysis of transcriptomics and metabonomics profiles in aflatoxin B1-induced hepatotoxicity in rat. Food and Chemical Toxicology 55:444–455 DOI 10.1016/j.fct.2013.01.020.
82- Singh KB, Maurya BK, Trigun SK. (2015). Activation of oxidative stress and inflammatory factors could account for histopathological progression of aflatoxin-B1 induced hepatocarcinogenesis in rat. Molecular and Cellular Biochemistry 401:185–196.
83- Rotimi OA, Rotimi SO, Goodrich JM, Adelani IB, Agbonihale E, Talabi G. (2019). Time-course effects of acute aflatoxin B1 exposure on hepatic mitochondrial lipids and oxidative stress in rats. Frontiers in Pharmacology 10:467 DOI 10.3389/fphar.2019.00467.
84- Liu Y, Wang W. (2016). Aflatoxin B1 impairs mitochondrial functions, activates ROS generation, induces apoptosis and involves Nrf2 signal pathway in primary broiler hepatocytes. Animal Science Journal 87:1490–1500 DOI 10.1111/asj.12550.
85- Kucukcakan B, Hayrulai-Musliu Z. (2015). Challenging role of dietary Aflatoxin B1

exposure and hepatitis B infection on risk of hepatocellular carcinoma. Open Access Macedonian Journal of Medical Sciences 3:363–369 DOI 10.3889/oamjms.2015.032.

86- Bosetti, C., Turati, F., and La Vecchia, C. (2014). Hepatocellular carcinoma epidemiology. Best practice & research. Clinical gastroenterology, 28(5), 753–770. https://doi.org/10.1016/j.bpg.2014.08.007

87- Tchana, A. Moundipa, P. and Tchouanguep, F. M. (2010). "Afatoxin contamination in food and body fuids in relation to malnutrition and cancer status in Cameroon," International Journal of Environmental Research and Public Health, vol. 7, no. 1, pp. 178–188.

.

88- Cui, X. Muhammad, I. Li R. et al. (2017)."Development of a UPLCFLD Method for Detection of Afatoxin B1 and M1 in Animal Tissue to Study the Efect of Curcumin on Mycotoxin Clearance Rates," Frontiers in Pharmacology, vol. 8, p. 650.

89- Sun, Z. Chen, T. Torgeirsson, S. et al., "Dramatic reduction of liver cancer incidence in young adults: 28 Year followup of etiological interventions in an endemic area of China," Carcinogenesis, vol. 34, no. 8, pp. 1800–1805, 2013.

90- Qian, G.; Wang, F.; Tang, L.; Massey, M.E.; Mitchell, N.J.; Su, J.; Williams, J.H.; Phillips, T.D.; Wang, J.-S. (2013).Integrative toxicopathological evaluation of aflatoxin B1 exposure in F344 rats. Toxicol. Pathol., 41, 1093–1105. [CrossRef].

91- Sivanesan, D. and Begum, V.H. (2014). Antioxidant potential of Aegle marmelos leaves against Aflatoxin B1 induced liver toxicity in rats. Chem. Sci. Trans. 3(2): 791-795.

92-Guarisco, J.A., Hall, J.O., Roger, A., Coulombe, J.R., Panter, K.E., Wierenga, T.L. and Pfister,J.A., (2007). Butylatedhydroxytoluene reduces aflatoxin B1 bioavailability and hepatic adductformation in turkeys. Poisonous Plants: Global Research and Solutions, pp. 197–202.

93-Mohamed, S.S., Mohamed, S.R., Abou-Arab, A.A., Naguib, K.M., Helmy, M.H. and Owiss, N.A. (2014). Comparison study on native olive waste extract and its nano-particles effect on oxidative stress induced by aflatoxin B1 in rat brain. Int. J. Curr. Microbiol. App. Sci. 3(4): 141-152.

94- Alsayyah A, ElMazoudy R, Al-Namshan M, Al-Jafary M, Alaqeel N. (2019). Chronic neurodegeneration by aflatoxin B1 depends on alterations of brain enzyme activity and immunoexpression of astrocyte in male rats. Ecotoxicology and Environmental Safety 182:109407 DOI 10.1016/j.ecoenv.2019.109407.

.

95-Zuberi Z, Eeza MNH, Matysik J, Berry JP, Alia A. (2019). NMR-based metabolic profiles of intact zebrafish embryos exposed to Aflatoxin B1 recapitulates hepatotoxicity and supports possible neurotoxicity. Toxins 11(5):258 DOI 10.3390/toxins11050258.

96- Huang B, Chen Q, Wang L, Gao X, Zhu W, Mu P, Deng Y. (2020). Aflatoxin B1 induces neurotoxicity through reactive oxygen species generation, DNA damage, apoptosis, and S-phase cell cycle arrest. International Journal of Molecular Sciences 21:6517 DOI 10.3390/ijms21186517.

97- Mehrzad J, Bahari A, Bassami MR, Mahmoudi M, Dehghani H. (2018b). Immunobiologically relevant level of aflatoxin B1 alters transcription of key functional immune genes, phagocytosis and survival of human dendritic cells. Immunology Letters 197:44–52 DOI 10.1016/j.imlet.2018.03.008.

98Liu, S. Li, G. and Dai, L. (2010)."Efect of Ligustrazine on MDA, SOD and T-AOC in Erythrocyte Membrane in Patients with Hemoglobin H Disease," Journal of Clinical Experimental Medicine, vol. 11, 2010.

99- Ma J, Liu Y, Guo Y, Ma Q, Ji C, Zhao L. (2021). Transcriptional profiling of Aflatoxin B1- induced oxidative stress and inflammatory

response in macrophages. Toxins 13:401 DOI 10.3390/toxins13060401.
100- Zhou X, Gan F, Hou L, Liu Z, Su J, Lin Z, Le G, Huang K. (2019). Aflatoxin B1 induces immunotoxicity through the DNA methyltransferase-mediated JAK2/STAT3 pathway in 3D4/21 cells. Journal of Agricultural and Food Chemistry 67:3772–3780 DOI 10.1021/acs.jafc.8b07309.
101- Hou L, Gan F, Zhou X, Zhou Y, Qian G, Liu Z, Huang K. (2018). Immunotoxicity of ochratoxin A and aflatoxin B1 in combination is associated with the nuclear factor kappa B signaling pathway in 3D4/21cells. Chemosphere 199:718–727 DOI 10.1016/j.chemosphere.2018.02.009.
102 Hu P, Zuo Z, Li H, Wang F, Peng X, Fang J, Cui H, Gao C, Song H, Zhou Y, Chen Z. (2018). The molecular mechanism of cell cycle arrest in the Bursa of Fabricius in chick exposed to Aflatoxin B 1. Scientific Reports 8:1770 DOI 10.1038/s41598-018-20164-z.
.

1.