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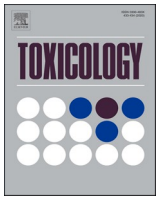

Aflatoxin B_1-induced hepatotoxicity through mitochondrial dysfunction, oxidative stress, and inflammation as central pathological mechanisms: A review of experimental evidence

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ABSTRACT

Aflatoxin B_1 (AFB_1) is a class of mycotoxin known to contaminate agricultural products, animal feed and animal food products, subsequently causing detrimental effects on human and animal health. AFB_1 is the most common and potent aflatoxin found in food and contributes significantly to liver injury as well as the development of hepatocellular carcinoma. Although the liver is a primary target organ for AFB_1 toxicity and biotransformation, underlying mechanisms implicated in liver injuries induced by these mycotoxins remain to be fully elucidated for therapeutic purposes. This review aims to dissect the complexities of the pathophysiological and molecular mechanisms implicated in hepatotoxicity induced by AFB_1, including mitochondrial dysfunction, oxidative stress and hepatic inflammation. Mechanistically, AFB_1 disrupt mitochondrial bioenergetics and membrane potential, promotes mitochondrial cholesterol trafficking and induces mitophagy. Moreover, mitochondrial dysfunction may lead to hepatic oxidative stress as a consequence of uncontrolled production of reactive oxygen species and defects in the antioxidant defense system. Retrieved experimental evidence also showed that AFB_1 may lead to hepatic inflammation through gut microbiota dysbiosis, the release of DAMPs and cytokines, and immune cell recruitment. Overall, these mechanisms could be utilized as potential targets to extrapolate treatment for liver injury caused by AFB_1.

1. Introduction

Aflatoxin B1 (AFB_1) is the most common and potent aflatoxin that poses a serious threat to public health security and the economy due to its life-threatening adverse toxicity in humans and livestock (Omotayo, 2019). In general, aflatoxins are a type of mycotoxin mainly produced by *Aspergillus parasiticus* and *Aspergillus flavus* as secondary metabolites, and mycotoxins at large are responsible for about 60 %–80 % of global

Abbreviations: Acox1, acyl-CoA oxidase 1; AFB_1, aflatoxin B1; AFBO, aflatoxin B_1-8,9-epoxide; Apob, Apolipoprotein B; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATP, adenosine triphosphate; BMP2, bone morphogenetic protein 2; CAT, catalase; Cebpα, CCAAT-enhancer binding protein alpha; CCL20, chemokine C-C motif chemokine ligand 20; COX-2, cyclooxygenase-2; CYP450, cytochrome P450; Cyt-c, cytochrome c; DAMPs, damage-associated molecular patterns; Drp1, dynamin-related protein 1; ETC, electron transport chain; FAD, flavin adenine dinucleotide; FATP, fatty acid transport protein; FAS, fatty acid synthase; FB_1, fumonisin B_1; FGF-15, fibroblast growth factor 15; FXR, farnesoid X receptor; GGT, gamma glutamyl transferase; GPx, glutathione peroxide; GSH, glutathione; HCC, hepatocellular carcinoma; HSCs, hepatic stellate cells; HMGB1, high mobility group box 1; ILs, interleukins; ILCs, innate lymphoid cells; LDs, lipid droplets; LPS, lipopolysaccharide; MDA, malondialdehyde; MCP-1, monocyte chemoattractant protein-1; Mtp, microsomal triglyceride transfer protein; MFN2, mitofusin; MMP, mitochondrial membrane potential; mito-p53, mitochondrial p53; mTORC1, rapamycin complex 1; NAD, nicotinamide; NLRP3, NOD-like receptor protein 3; NO, nitric oxide; NF-κB, nuclear factor kappa B; Nrf1, nuclear respiratory factor 1; PAMPs, pathogen-associated molecular patterns; PGC-1α, proliferator-activated receptor γ coactivator-1α; PLIN2, perilipin 2; PPARγ, peroxisome proliferator-activated receptor gamma; SOD1, superoxide dismutase 1; Srebf1, sterol responsive element binding proteins; STAT3, signal transducer and activator of transcription 3; TAFLD, toxicant-associated fatty liver disease; Tfam, mitochondrial transcription factor A; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha; TR, thioredoxin; Rab7a, Ras-associated protein 7a; ROS, reactive oxygen species; VDAC1, voltage-dependent anion channel protein 1.

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food crop contamination (Eskola, 2020). The latter could be detected in animal feed, Chinese herbal medicine, and agricultural food products such as grains and cow's milk, which form an integral part of the human diet (Girolami, 2022). Amongst its detrimental effects, AFB_1 has the strongest mutagenic and carcinogenic properties, hence it has been classified as a Group I carcinogen, and its metabolite aflatoxin M_1 is classified as a Group IIB carcinogen (Martínez, 2023). It is well-known that exposure to AFB_1 could lead to various toxic effects, including hepatotoxicity, nephrotoxicity, neurotoxicity, intestinal toxicity, spleen toxicity, and cardiotoxicity in different experimental models (Sharma Pooja et al., 2015; Rotimi, 2017; Yilmaz, 2018; Huang, 2020; Zhang, 2022; Rajaura, 2024). It has been reported that aflatoxins affect multiple body organs and tissues, including the liver, kidneys, lungs, brain, testes, and many endocrine and exocrine organs, which in turn cause various acute and chronic diseases (Bbosa, 2013). However, the pathophysiological mechanisms associated with these toxic repercussions are not clear.

In the liver, AFB_1 exposure induces hepatotoxicity, a condition that can cause hepatitis, cirrhosis, and liver immunological damage, as well as the manifestation of human hepatocellular carcinoma (Yu, 2012; Zhou, 2019). Beyond its key role in regulating various metabolic processes, such as bile production, lipid metabolism, and glucose homeostasis, the liver is a well-known detoxification organ (Kieffer et al., 2016). Cumulative experimental evidence has demonstrated that AFB_1 toxicity disrupts mitochondrial function and structure, which subsequently leads to the onset of oxidative stress and hepatic inflammation in the liver (Xu, 2021; Chen, 2023; Ye, 2023). For example, one study reported that AFB_1 induced hepatocyte pyroptosis, a form of programmed cell death, by activating Kupffer cells, which in turn promotes liver inflammation via cyclooxygenase-2 (COX-2) dephosphorylation in HepaRG cells and C57BL/6 mice (Zhang, 2019). Pyroptotic hepatocytes released inflammatory cytokine interleukin (IL)-1β and damage-associated molecular pattern molecules (DAMPs) to stimulate signal transducer and activator of transcription 3 (STAT3)-mediated pro-inflammatory signalling of Kupffer cells, which causes the amplification of NOD-like receptor protein 3 (NLRP3)-dependent liver inflammation (Zhang, 2019). Recently, AFB_1 was shown to display hepatoxicity by enhancing hepatic inflammatory response indicated by an increase in the expression levels of cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and high mobility group box 1 (HMGB1) protein in mice (Lv, 2024). On the other hand, AFB_1 partially induces liver injury by causing lesions in the mitochondria, the "powerhouse of the cell", inhibiting mitochondrial biogenesis and impairing the function of this organelle in Kunming mice (Xu, 2021). While mitochondrial homeostasis requires a balance between mitochondrial biogenesis, fission and fusion, and mitophagy, AFB_1 affect mitochondrial dynamics and function by suppressing mitochondrial membrane fusion proteins such as mitofusin 2 (MFN2), and elevating dynamin-related protein 1 (Drp1) and Mito-p53 during the development of liver injury (Che, 2023). Altogether, hepatic oxidative stress, inflammation and mitochondrial dysfunction are emerging as one of the main drivers of AFB_1-induced liver toxicity.

To date, understanding the precise pathological mechanisms underlying AFB_1-induced hepatotoxicity remains crucial for therapeutic purposes or detoxification directed to the adverse outcomes of these toxins in humans and animals. With a few exceptions, the mechanisms by which mycotoxins cause hepatic injury are still elusive; thus, this review aims to provide insights into AFB_1-induced liver toxicity mediated by mitochondrial dysfunction, oxidative stress and inflammation as key pathological processes. The literature search was performed in major research databases including PubMed, Google Scholar and ScienceDirect using the following keywords "aflatoxin B_1", "hepatotoxicity", "mitochondrial dysfunction", "oxidative stress", "inflammation", and their relevant medical subject headings.

2. An overview of aflatoxin B_1's natural source, chemical nature and its role in cytotoxicity

2.1. Natural source, chemistry, hepatic metabolism, and hepatotoxicity of aflatoxin B_1

Aflatoxins were first discovered in the early 1960s from a highly toxic peanut meal said to be responsible for causing hitherto unknown turkey "X" disease, which killed a total of 100,000 turkey poults in England (Pickova, 2021). Because these toxic chemicals were identified from *Aspergillus flavus*, the name of the toxin was termed "aflatoxin" (Pickova, 2021). Currently, there are four major aflatoxins, AFB_1, AFB_2, AFG_1, and AFG_2, as well as their toxic metabolites AFM_1 and AFM_2, whose contamination of foods and feeds poses serious global health concerns (Pickova, 2021). Aflatoxins share similar chemical structures, forming a unique group of highly oxygenated, naturally occurring heterocyclic compounds. Amongst these toxins, AFB_1 a tetrahydrocyclopenta-[c]furo[3′,2′:4,5]furo[2,3-*h*]chromene skeleton with oxygen functionality at positions 1, 4 and 11 (CID186907, Fig. 1) is the most widespread and highly toxic with adverse implications on food security and human health (Rushing and Selim, 2019). Recently reviewed evidence has demonstrated that AFB_1 induces toxic effects in the liver through impaired mitochondrial antioxidant defence system and oxidative stress (Jobe, 2023). In line with oxidative stress, pathological processes such as mitochondrial dysfunction and hepatic inflammation play central roles in the development of hepatotoxicity (Jobe, 2023).

In general, hepatotoxicity is a liver injury characterized by oxidative stress, mitochondrial damage or dysfunction, hepatitis, and hepatic apoptosis and necrosis (Mihajlovic and Vinken, 2022), which are caused by many xenobiotics, including fungal toxins, dietary supplements, drugs, and herbal products (Andrade, 2019). Hepatotoxicity also includes liver steatosis, termed toxicant-associated fatty liver disease (TAFLD), which could be used to describe fatty liver diseases caused by toxicants other than alcohol (Rajak, 2022). AFB_1 is a fungal toxin that has been reported to cause both acute hepatotoxicity and hepatocellular carcinoma in humans and laboratory animals (Roebuck and Maxuitenko, 1993). It has been reported that during AFB_1-induced liver injury, the levels of liver enzymes, including alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) are elevated as a response to hepatic stress and damage (Luyendyk, 2002; Altyar, 2023). Upon delivered to the liver, AFB_1 is bioactivated by cytochrome P450 (CYP450), a group of enzymes abundant in the liver and related with the bioactivation and metabolism of different kinds of xenobiotics and endogenous compounds, into the highly reactive exo-AFB_1-8,9-epoxide (AFBO) (Zhang, 2016; Wang, 2022). As monooxygenase, they bind molecular oxygen to their heme iron and insert oxygen into the AFB_1 molecule to form epoxidized AFBO, hydroxylated AFM_1 and AFQ_1. AFB_1 metabolites of varying carcinogenic potential, including CYP3A4, which plays an important role in biotransforming AFB_1 to the toxic product AFB_1-8,9-epoxide in the human liver, and CYP2A13 has significant activity in metabolizing AFB_1 to AFB_1-8,9-epoxide and AFM_1-8,9-epoxide in the human lung (Dohnal et al., 2014). The latter epoxide of AFB_1-8,9-epoxide could conjugate with glutathione S-transferase (GST), while the CYP450 family of CYP2A6, CYP3A37, CYP1A5, and CYP1A1 are responsible for bioactivation of AFB_1 (Dohnal et al., 2014; Wang, 2024). Of note, CYP450 enzymes bioactivate AFB_1 to an electrophilic, highly reactive and unstable metabolite that can react with cellular macromolecules such as DNA and proteins leading to genotoxicity and cytotoxicity, respectively (Diaz et al., 2010).

2.2. Cytotoxicity and genotoxicity of aflatoxin B_1

Aryl hydrocarbon receptor (AHR), an emerging crucial mediator of AFB_1-induced toxicity, is a receptor protein that binds and mediates the

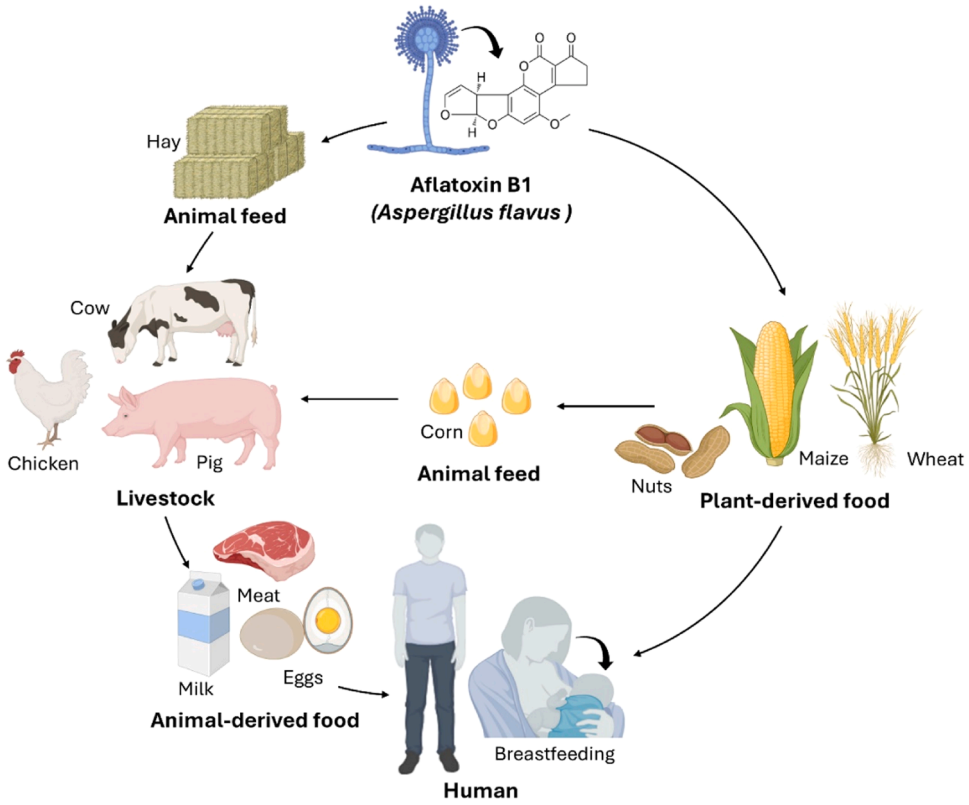

Fig. 1. Aflatoxin B_1 chemical structure, and its route of contamination for humans. Briefly, AFB_1, a mycotoxin produced by *Aspergillus flavus,* is a common contaminant from plant-derived food and animal feeds consumed by both humans and animals, respectively. However, AFB_1 is not fully metabolize in animals are unable to fully metabolize AFB_1 following the ingestion of feed contaminated with AFB_1. Thus, animal- and plant-derived food products are the major source of AFB_1 contamination for humans, which leads to toxicity, organ damage and carcinogenesis.

actions of both endogenous and exogenous ligands, such as microbial metabolites, toxins, and environmental contaminants (Ames, 2018; Zhu, 2021). It acts as a nuclear transcription factor that regulates the expression of several genes involved in phases I and II of the metabolism, immune response, cell cycle, differentiation, apoptosis and carcinogenesis, and it is mainly expressed in various barrier positions in the body, including the liver (Zhu, 2021; Mary, 2015; Heo, 2023). Upon activation, AHR forms a heterodimer with the AHR-nuclear translocator protein and is translocated to the nucleus, where it interacts with specific sequences of the DNA, such as dioxin-responsive elements (Rowlands and Gustafsson, 1997).

Previous work has revealed that AHR mediates the actions of some non-genotoxic carcinogens that are metabolized through the CYPP450-related metabolic pathway (Zhu, 2021). For instance, AFB_1 may generate DNA adducts via the process catalysed by the CYP450 oxidase family, including CYP1A2, CYP3A4, and CYP2A6 (Jiang, 2018). This occurs when AFB_1 binds with DNA covalently to form AFB_1-N7-guanine, a key adduct responsible for the genotoxicity of AFB_1. The AFB_1 metabolite, AFB_1-8,9-epoxide, reacts spontaneously with biological amines in proteins and nucleic acids by covalently binding to the N7 position on guanine, forming the adduct AFB_1-N7-guanine (Akash, 2021), which induces pro-inflammatory and pro-proliferative state and promotes further tissue damage (Rushing and Selim, 2019). The major AFB_1-DNA adducts are identified as 8,9-dihydro-8-(N7-guanyl)-9-hydroxyaflatoxin B_1 (AFB_1-N7-Gua) and AFB_1-formamidopyrimidine (AFB_1-FAP) (Wen, 1997). Adducts represent an integration of exposure, absorption, distribution, metabolism, DNA repair, and cell turnover (Szabó, 2024). Moreover, AFB_1-8, 9-epoxide (AFB_1-E) intermediates mainly interact with the guanine bases in the DNA of the liver cells and lead to the promotion of the mutational effect at the codon 249 in exon 7 of the p53 tumor suppressor gene (Hamid, 2013). Ultimately, this mutation provokes the development of hepatocellular carcinoma (HCC). Although the most widely accepted mechanism in AFB_1-induced carcinogenicity is the mutational effect of DNA adducts, forming AFB_1-mediated reactive oxygen species (ROS) emerges as another potential mechanism of DNA damage (Hamid, 2013; Engin and Engin, 2019).

Additionally, in the field of hepatic toxicology, there is growing evidence suggesting that altered miRNA expression plays a role in xenobiotic metabolism, DNA damage response, and hepatotoxicity induced by chemicals such as AFB_1 (Liu, 2015). Therefore, miRNAs are being considered as potential biomarkers for early-stage HCC (Liu, 2015). miRNAs are endogenous, non-coding small RNAs that regulate gene expression post-transcriptionally by degrading target mRNA or inhibiting translation, and changes in miRNA expression may occur early in essential cellular processes and could be related to specific factors such as AFB1 (Balasubramanian, 2020; Valencia-Quintana, 2014; Zhu, 2015). Specific miRNAs such as miR-27a, miR-27b, miR-122, miR-148, miR-155, miR-192, miR-214, miR-221, miR-429, and miR-500 have been reported as potential markers for HCC due to aflatoxin exposure (Kew, 2013; Ferreira, 2019). However, their prognostic significance and association with HCC induction require future validation, and miRNA biomarkers based on toxicological studies will be crucial for identifying stages in the development of health effects from environmental agents, including AFB1 (Kew, 2013; Ferreira, 2019). Using miRNA biomarkers supported by toxicological studies will be crucial for identifying stages in the development of health effects from environmental agents, including AFB_1 (Valencia-Quintana, 2014).

3. Putative mechanisms of aflatoxin B1-induced hepatotoxicity based on in vitro and in vivo experimental evidence

In general, the mechanistic concept of hepatotoxicity or liver injury-

caused drugs could be explained by a 3-step model based on pathophysiological changes exerted by the initial injury, including (i) direct cell stress, (ii) direct mitochondrial impairment or inhibition, and (iii) specific immune reactions (Russmann et al., 2009). Initial injuries lead to events that cause mitochondrial permeability transition, which subsequently results in necrosis or apoptosis depending on the depletion of adenosine triphosphate (ATP) (Russmann et al., 2009). In the context of toxicants-induced liver injury, cumulative body experimental evidence indicates hepatic mitochondrial dysfunction, oxidative stress, and inflammation are underlying mechanisms in aflatoxin B_1-induced hepatotoxicity, as discussed in the following subsections (Tables 1, 2, and 3).

3.1. *Aflatoxin B1-induced liver injury by disrupting mitochondrial bioenergetics and membrane potential, as well as promoting mitochondrial cholesterol trafficking and mitophagy*

It is well accepted that the mitochondrion, a powerhouse of the cell responsible for generating ATP, plays a central role in events leading to apoptotic and/or necrotic cell death during drug-induced hepatotoxicity (Vendemiale, 1996). For example, drugs and their reactive metabolites uncouple or inhibit the mitochondrial respiratory chain, causing ATP depletion and excessive ROS production, which subsequently impair β-oxidation, leading to steatosis and mitochondrial permeability transition (Russmann et al., 2009). The opening of the mitochondrial permeability transition pore is closely linked to hepatic apoptosis and necrosis (Lee and Farrell, 2001). Similar mechanisms have been implicated in hepatoxicity induced by aflatoxin B_1 (Fig. 2), as demonstrated in many *in vitro* and *in vivo* studies summarized in Table 1.

Preliminary studies uncovered that AFB_1 (7 mg/kg) impaired hepatic mitochondrial function by altering respiratory enzymes and energy-linked functions in Wistar rats when exposed for up to 3 days (Pasupathy et al., 1999; Sajan et al., 1995; Sajan et al., 1996; Toskulkao and Glinsukon, 1990). A study by Toskulkao and Glinsukon, (1990) reported that AFB_1 decreased hepatic mitochondrial respiratory enzyme NADH-cytochrome c (Cyt-c) reductase activity and the increased lipid peroxide level of lysosomes might lead to a decrease in hepatic ATP content. From these preceding observations, Sajan and colleagues (Sajan et al., 1996) found that the hepatic mitochondrial respiratory rates and ATPase activities returned to normal levels in Wistar rats acutely exposed to AFB_1 for 3 days, suggesting the recovery of mitochondrial functions from toxic effects. Thus, an elegant study by Rotimi and co-workers (Rotimi, 2019) assessed the time course effect of 1 mg/kg AFB_1 exposure in Wistar rats for 1,4 and 7 day(s). It was found that after 7 days of exposure, AFB_1 disrupted hepatic mitochondrial lipids and induced mitochondrial oxidative stress in part by increasing cholesterol and upregulating nuclear respiratory factor 1 (Nrf-1) expression, respectfully (Rotimi, 2019). These outcomes revealed that even though recovery is achieved in a single dose and acute exposure to AFB_1, continued exposure might bring a sustained mitochondrial dysfunction that could lead to adverse liver injury.

In different mice models, chronic exposure to various doses of AFB_1 for up to 30 days revealed the role of lysosome-to-mitochondrial cholesterol trafficking, mitophagy, mitochondrial membrane potential (MMP), and/or mitochondria-dependent necroptosis in AFB_1-induced liver toxicity/lipotoxicity (Xu, 2021; Che, 2023; Ren et al., 2020; Lin, 2023). A study by Ren and colleagues (Ren et al., 2020) reported that the administration of 0.6 mg/kg AFB_1 every 2 days for 1 month induced mitophagy, disrupted the normal mitochondrial lipid metabolism, and caused steatosis by increasing cyclooxygenase-2 (COX-2) gene expression in C57BL/6 mice. Mechanisms implicated in hepatic lipotoxicity induced by AFB_1 were recently elucidated through both *in vitro* and *in vivo* assessment (Che, 2023; Lin, 2023). Exposing C57BL/6 mice AFB_1 at 12.5 mg/kg for 21 days induced hepatic lipotoxicity, including abnormal lipid droplets (LDs) growth, mitochondria-LDs contacts increase, lipophagy disruption, and lipid accumulation via mitochondrial

Table 1
Summary of studies reporting on the role of mitochondrial dysfunction in AFB_1-induced hepatotoxicity.

Author, year	Experimental model	Toxic dose and duration	Main findings
Toskulkao and Glinsukon, 1990 (Toskulkao and Glinsukon, 1990)	Wistar rats	2.0 mg/kg for 3 days	Decrease hepatic mitochondrial respiratory enzyme NADH-Cyt-c reductase activities and the increase in lipid peroxide level of lysosomes, which in turn decreased ATP content
Sajan et al., 1995 (Sajan et al., 1995)	Albino Wistar rats	7 mg/kg for 3 days	Impaired mitochondrial respiratory function by decreasing succinoxidase and Cyt oxidase activities, as well as decreased the content of intramitochondrial Cyt a3 and Cyt b
Sajan et al., 1996 (Sajan et al., 1996)	Albino Wistar rats	7 mg/kg for 3 days	Impaired mitochondrial respiratory activity by decreasing oxygen consumption of mitochondria via inhibition of state 3 respiration rate and adenosine diphosphate phosphorylation rate, which were accompanied by several NAD and FAD-linked oxidizing substrates
Pasupathy et al., 1999 (Pasupathy et al., 1999)	Albino Wistar rats	7 mg/kg for 1 day	Induced the turnover of mitochondrial phosphoinositides as marked by activation of phosphatidylinositol cycle, increased diacylglycerol, inositol 1,4,5-trisphosphate
Liu et al., 2014 (Liu et al., 2014)	HepG2 cells	0.1, 7.5, 17 μM for 24 h	Induced apoptosis and disrupted MMP by increasing gene expression of Bax, Caspase−3, and p53 and decreasing Bcl−2 expression
Rotimi et al., 2019 (Rotimi, 2019)	Albino Wistar rats	1 mg/kg for 1, 4, and 7 day (s)	Disrupted hepatic mitochondrial lipids by increasing mitochondrial cholesterol and triacylglycerols
Ren et al., 2020 (Ren et al., 2020)	HepG2 cells	25 μM for 48 h	Elevated lipid droplets and disrupted normal mitochondrial lipid metabolism via COX−2 and mitophagy pathway
	C57BL/6 mice	0.6 mg/kg for 30 days	Induced mitophagy and steatosis by disrupting the normal mitochondrial lipid metabolism including increased alanine aminotransferase, AST, total cholesterol, and total triglyceride levels, which occur due to COX−2-induced mitophagy
Zhang et al., 2020 (Zhang, 2020)	Human liver LO2 cells	20 and 60 μg/mL for 24 h	Induced apoptosis and cell cycle S phase arrest, reduced MMP, and increased reactive oxygen species generation, as well as the DNA methylation level by regulating the gonadotropin-releasing hormone receptor pathway, the Wnt signaling pathway, and the transforming growth factor-beta signaling pathway

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Table 1 (*continued*)

Author, year	Experimental model	Toxic dose and duration	Main findings
Xu et al., 2021 (Xu, 2021)	Kunming mice	0.75 and 1.5 mg/kg for 30 days	Induced mitochondrial dysfunction by damaging mitochondria structure, activating apoptosis, decreased MMP, as well as increased cytoplasmic Cyt-c protein expression, Bax, p53, caspase−3/9 protein. Elevated pyruvate content and inhibited tricarboxylic acid cycle rate-limiting enzymes and complexes I-V activities, disturbed complexes I-V subunits, and reduced ATP. Altered expression of mitochondrial biogenic genes PGC−1α, Nrf1, and Tfam
Chen et al., 2022 (Chen, 2022)	HepG2 cells	15 and 25 μg/mL for 24 h	Impaired mitochondrial bioenergetics by decreasing basal respiration, maximal respiration, and ATP production, as well as disrupting MMP and increasing ROS production
Chen et al., 2023a (Chen, 2023)	HepG2 cells	0.5, 2, and 8 μg/mL for 24 h	Impaired glycolytic activity and mitochondrial bioenergetics by disrupting MMP, decreasing the basal respiration, maximal respiration, ATP production, and proton leak and increasing the spare respiratory capacity, increasing intracellular ROS generation. Moreover, genes encoding for complexes I, II, III, and IV were downregulated while p53 signalling pathway genes were differential expressed
Che et al., 2023 (Che, 2023)	HepG2 and HepaRG	1.25 μM for 48 h	Induced abnormal lipid droplets, mitochondria-lipid droplets, lipophagy disruption, and lipid accumulation by promoting mito-p53 and lipid droplets-associated protein PLIN2 interaction-mediated mitochondria-lipid droplets contacts
	C57BL/6 mice	2.5 mg/kg for 21 days	Induced steatosis through mito-p53 and PLIN2 interaction mediated by three organelles-mitochondria, lipid droplets, and lysosomal networks.
Lin et al., 2023 (Lin, 2023)	HepaRG and HepG2 cells	1.6 μM for 36 h	Induced cholesterol trafficking disorder-mediated hepatotoxicity via Rab7a - mTORC1 signalling regulated cholesterol-related lysosomal membrane permeabilization and mitochondria-dependent necroptosis

Abbreviations: ATP, adenosine triphosphate; Cyt-c, cytochrome c; COX-2, cyclooxygenase-2; FAD, flavin adenine dinucleotide; MMP, mitochondrial membrane potential; mTORC1, rapamycin complex 1; mito-p53, mitochondrial p53; NAD, nicotinamide; Nrf1, nuclear respiratory factor 1; PGC-1α, proliferator-activated receptor γ coactivator-1α; PLIN2, perilipin 2, Rab7a, Ras-associated protein 7a; Tfam, mitochondrial transcription factor A.

Table 2
Summary of studies reporting on the role of oxidative stress in AFB_1-induced hepatotoxicity.

Author, year	Experimental model	Toxic dose and duration	Main findings
Adedara et al., 2010 (Adedara, 2010)	Albino Wistar rats	9 mg/kg for 7 days	Elevated lipid peroxidation and decreased both non-enzymatic antioxidant GSH level and enzymatic antioxidant, including CAT and GST activities, while SOD remained unaffected. This was accompanied by increased markers of liver injury AST, ALT, GGT, and ALP
Singh et al., 2015 (Singh et al., 2015)	Albino charle's foster rats	1 mg/kg for 2 days	Elevated levels of ROS, with the decline of SOD1, CAT, GPx, and total GST, which contributed to hepatocellular carcinoma
Qin et al., 2016 (Qin, 2016)	SB rats	1 mg/kg for 4 weeks	Induced hepatocellular carcinoma, in part by decreasing SOD1, GPx1 and GPx2
Rotimi et al., 2016 (Rotimi, 2016)	Albino rats	0.04 mg/kg for 56 days	Induced oxidative injury as observed by increased lipid peroxidation and alterations in the activity of antioxidant enzymes, including GST, SOD, CAT, and GPx
Rotimi et al., 2019 (Rotimi, 2019)	Albino Wistar rats	1 mg/kg for 1, 4, and 7 day(s)	Induced liver injury and mitochondrial oxidative/nitrosative stress as marked by an increased biomarker of liver function ALP, which was accompanied by increased GSH on day 1 while biomarkers of oxidative stress MDA, NO, GST, and TR were increased on day 7
Akinrinde et al., 2020a (Akinrinde et al., 2020)	Wistar rats	2.5 and 5 mg/kg for 1 day	Induced hepatic oxidative damage by increasing hydrogen peroxide and MDA, while decreasing antioxidants GSH, GPx, SOD, and total thiols
Tadee et al., 2020 (Tadee et al., 2020)	HepG2 cells	1.25, 2.5 and 5 μM for 24 h	Induced genotoxicity and oxidative stress by increasing ROS level and decreasing GSH content with slightly reduction in GPx, and SOD activity
Zhang et al., 2020 (Zhang, 2020)	LO−2 cells	60.05 μM for 24 h	Induced apoptosis and cell cycle S phase arrest reduced mitochondrial membrane potential, and increased ROS generation, as well as the DNA methylation level. The latter played a role via regulating the gonadotropin-releasing hormone receptor pathway, the Wnt signaling pathway, and the TGF-β signaling pathway
Xu et al., 2021 (Xu, 2021)	Kunming mice	0, 0.375, 0.75 and 1.5 mg/kg for 30 days	Damaged liver microstructure, including intrahepatic hemorrhage, hepatic cord derangement, bile duct hyperplasia and hepatocellular karyopyknosis or hypochromatosis with

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Table 2 (*continued*)

Author, year	Experimental model	Toxic dose and duration	Main findings
			elevated the levels of AST, ALT, ALP and LDH. Moreover, there was severe damage to the mitochondrial ultrastructure of liver with increased apoptosis and ROS content including MDA and hydrogen peroxide while total antioxidant capacity and CAT activity were decreased.
Yan et al., 2022 (Yan, 2022)	Wistar rats	1 mg/kg and 2 mg/kg for 7 days	Induced liver damage as determined by increased ALT, AST, ALP and TBIL levels, which were accompanied by altered hepatic antioxidative status and induced lipid peroxidation as marked by increased MDA content and decreased both SOD and GPx activity
Owumi et al., 2023 (Owumi, 2023)	Wistar rats	0.070 mg/kg for 28 days	Damaged hepatic cytoarchitecture and increase the markers of liver injury including ALT, AST, ALP and GGT. This was accompanied by decreased antioxidants SOD, CAT, GPx, GSH and GST activities while increased ROS and nitrogen species, 8-hydroxy−2′-deoxyguanosine, xanthine oxidase, and myeloperoxidase levels

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; GPx, glutathione peroxide; GSH, glutathione glutathione-S-transferase; GST glutathione-S-transferase; GGT, gamma glutamyl transferase; MDA, malondialdehyde; NO, nitric oxide; ROS, reactive oxygen species; SOD1, superoxide dismutase 1; TGF-β, Transforming growth factor-beta; TR, thioredoxin reductases.

p53 (mito-p53) and perilipin 2 (PLIN2) interaction-mediated mitochondria-lipid droplet contacts (Che, 2023). The AFB_1-stimulated inter-organelle lipid trafficking mediated hepatic lipotoxicity and hepatic steatosis were also evaluated using HepG2 and HepaRG liver cell lines. After 48 h of exposure, 5 μM AFB_1 elevated lipid droplets in HepG2 cells via COX-2 and mitophagy pathway (Ren et al., 2020). AFB_1 (1.25 μM) promoted the mito-p53 and LDs-associated protein PLIN2 interaction-mediated mitochondria-LDs contacts, which led to lipid accumulation in HepG2 and HepaRG cells after 48 h exposure (Che, 2023). It was further uncovered that cholesterol trafficking disorder-mediated hepatic lipotoxicity was regulated by ras-associated protein 7a (Rab7a)- rapamycin complex 1 (mTORC1) signaling in HepG2 and HepaRG cells exposed to AFB_1 at 1.6 μM 36 h exposure (Lin, 2023). This suggested that lysosomal Rab7a-mTORC1 signaling could be a novel mechanism to determine AFB_1-induced hepatic lipotoxicity caused by cholesterol trafficking, which is regulated via lysosomal Niemann-Pick type C1 protein and mitochondrial translocator protein from the lysosome to mitochondria.

Other elegant studies have further demonstrated that AFB_1-induced mitochondrial dysfunction involves inhibition of mitochondrial biogenesis, disruption of the mitochondrial electron transport chain (ETC) and MMP, and excessive ROS production as the central components of apoptotic and necrotic cell death linked to liver injury (Xu,

Table 3
Summary of studies reporting on the role of inflammation in AFB_1-induced hepatotoxicity.

Author, year	Experimental model	Toxic dose and duration	Main findings
Jeannot et al., 2012 (Jeannot, 2012)	Hepatitis virus C transgenic mice	6 mg/kg (single dose) and monitored for 1 month	Induced tumorous or pre-tumorous lesions and steatohepatitis including increased inflammation, hepatic triglyceride content, and altered lipid as marked by increased lesions comprised of small lobular inflammatory foci and altered genes regulating lipid catabolism (PPARγ, Acox1), lipoprotein secretion (Mtp, Apob), lipid uptake (Fat, Fatp) and lipogenesis (Srebf1, Cebpα, Fas)
Corcuera et al., 2015 (Corcuera, 2015)	Fisher 344 rats	0.25 mg/kg for 1 day	Induced hepatic necrosis and increased focal inflammation associated with hemorrhages and leucocytic and lymphocytic infiltration and inflammation of the parenchyma and portal spaces, as well as increased in ALT and AST
Akinrinmade et al., 2016 (Akinrinmade et al., 2016)	Wistar rats	2.5 mg/kg for 1 day	Induced thrombosis, vascular congestion, and peri-portal inflammatory cell infiltrations by elevating pro-inflammation cytokines IL−1β and TNF-α levels
Zhang et al., 2019(Zhang, 2019)	HepaRG cells	1 μM for 24 h	Induced hepatic pyroptosis and hepatic inflammation through dephosphorylation of COX−2, NLRP3 inflammasome activation, caspase 1 cleavage, and IL−1β release
	C57BL/6 mice	1 mg/kg for 28 days	Induced liver inflammatory injury and hepatic pyroptotic cell death as marked by NLRP3 inflammasome activation, recruitment of Kupffer cells, the release of DAMPs, and upregulated expressions of COX−2 and caspase 1, as well as elevated pro-inflammatory cytokines, including IL−1β, IL−18, IL−6, and TNF-α
Niu et al., 2021 (Niu, 2021)	Hepatitis B virus X transgenic mice	7 mg/kg for 14 months	Induced liver tumor incidence by up-regulating oncogenic KRAS to enhance IL−11 receptor subunit alpha−1-mediated inflammation
Liu et al., 2021 (Liu, 2021)	BALB/c mice	0.2 mg/kg for 28 days	Induced apoptosis by increasing Bax and activated (cleaved) caspase−3 and increased the expression

(*continued on next page*)

Table 3 (*continued*)

Author, year	Experimental model	Toxic dose and duration	Main findings
			of proinflammatory factors IFN-γ, TNF-α, IL−2, and IL−4 and anti-inflammatory factor IL−10. These effects were attributed to AFB_1 degradation products
Vornoli et al., 2022 (Vornoli, 2022)	Sprague-Dawley rats	0.47 mg/kg for 14 days	Induced steatohepatitis intraparenchymal inflammation, hepatocyte necrosis, foci, fibrosis, hepatocyte hypertrophy, hepatocellular adenomas, and hyperplasia of the hepatocytes according to histological analysis
Liu et al., 2023 (Liu, 2023)	C57BL/6 J mice	0.75 mg/kg for 14 days	Induced liver swelling and necrosis, severe diffuse bile duct hyperplasia, and inflammatory infiltration, by disrupting gut microbiota via inhibition of FXR/FGF−15 signaling. Moreover, gene expression of MCP−1, IL−6 and TNF-α were upregulated
Ye et al., 2023 (Ye, 2023)	Balb/c mice	0.025 mg/kg for 28 days	Induced hepatic inflammation by altering the gut microbiota, which releases LPS to promote liver pyroptosis, and activate TLR−4, NLRP3, caspase−1, gasdermin D, IL−1β, and IL−18
Zhang et al., 2024b (Zhang, 2024)	C57BL/6 mice	0.75 mg/kg for 28 days (co-exposed to polystyrene nano-plastics)	Increased systemic and hepatic inflammation, and induced fibrosis as marked by elevated inflammatory factors TNF-α, IL−6, and IL−1β, and increased inflammatory cell infiltration, accompanied by increased ALT and AST levels, and gut microbiota dysbiosis
Wu et al., 2024 (Wu, 2024)	HHL−16 cells	1, 5, 10, 20, 50, and 100 μg/mL for 24 h	Induced chronic liver inflammation and disrupted cell growth pathways as marked by increased gene expression of BMP2, IL−6, and CCL20

Abbreviations: Acox1, acyl-CoA oxidase 1; Apob, Apolipoprotein B; BMP2, bone morphogenetic protein 2; CCL20, chemokine C-C motif chemokine ligand 20; COX-2, cyclooxygenase-2; Cebpα, CCAAT-enhancer binding protein alpha; DAMPs, damage-associated molecular patterns; FATP, fatty acid transport protein; FAS, fatty acid synthase; FGF-15, fibroblast growth factor 15; FXR, farnesoid X receptor; IFN-γ, interferon gamma; ILs, interleukins; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; Mtp, microsomal triglyceride transfer protein; NLRP3, NOD-like receptor protein 3; PPARγ, peroxisome proliferator-activated receptor gamma; Srebf1, sterol responsive element binding proteins; TNF-α, tumor necrosis factor-alpha; TLR-4, toll-like receptor.

2021; Liu et al., 2014; Zhang, 2020; Chen, 2022; Chen, 2023). A study by Xu and co-workers (Xu, 2021) showed that 0.375, 0.75, and 1.5 mg/kg AFB_1 caused damage to mitochondria structure, decreased MMP, and increased expression of cytoplasmic Cyt-c, and apoptosis-related genes Bax, p53, and Caspase-3/9 in the liver of Kunming mice after 30 days of exposure. Moreover, the activity and gene expression of ETC complexes I, II, III, and IV subunits were suppressed, accompanied by reduced ATP content (Xu, 2021). AFB_1 impaired expression of genes/factors regulating mitochondrial function and biogenesis, such as voltage-dependent anion channel protein 1 (VDAC1), peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), Nrf1, and mitochondrial transcription factor A (Tfam) (Xu, 2021). HepG2 cells exposed to 16.9 μM dose of AFB_1 for 24 h presented with impaired integrity of MMP, induced G0/G1 or S phase arrest, which were accompanied by increased expression of apoptosis-related proteins of Bax, Caspase-3, and p53 and decreased expression of Bcl-2 protein (Liu et al., 2014). Similar outcomes were observed when LO2 cells were exposed to AFB_1 for 24 h, which displayed apoptosis and cell cycle S phase arrest, reduced MMP, and increased ROS generation, as well as the DNA methylation level (Zhang, 2020). However, it was revealed that DNA methylation mechanisms were regulated via the gonadotropin-releasing hormone receptor pathway and the TGF-beta signaling pathways (Zhang, 2020). In comparative studies of AFB_1 with another dominant mycotoxin fumonisin B_1 (FB_1), it was found that both toxins could disrupt the ETC to induce mitochondrial dysfunction (Chen, 2022; Chen, 2023). Of note, AFB_1 and FB_1 co-exposure exerted a potent inhibitory effect due to greater inhibition of all mitochondrial respiration parameters, increased ROS production, and disrupted MMP in HepG2 cells (Chen, 2022; Chen, 2023). Chen et al. (Chen, 2022) reported that AFB_1 decreased the oxygen consumption rate, disrupted the MMP, blocked ATP production, and induced ROS production at 25 and 15 μg/mL in HepG2 cells after 24 h exposure. In recent evidence, it was further discovered that AFB_1 impaired mitochondrial bioenergetics in HepG2 cells was strongly associated with downregulation of the genes encoding for complexes I, II, III, and IV, and differential expression of the genes regulating the p53 signaling pathway, a major orchestrator of mitochondrial apoptosis (Chen, 2023).

3.2. Aflatoxin B_1 -induced liver injury by promoting hepatic oxidative stress via the increased production of reactive oxygen species and alteration in antioxidant markers

Oxidative stress represents another underlining mechanism for AFB_1-induced liver injury that is characterized by an imbalance between the antioxidant defense system and reactive oxygen species (ROS), which causes the liver's hydropic degeneration, fatty vacuolar degeneration, and bile duct proliferation and eventually leads to tumorigenesis (Rickenbacher, 2014; Xu, 2019). As briefly discussed above, mitochondria are prominent targets for the hepatotoxicity of many drugs and the dysfunction of these vital organelles results in intracellular oxidant stress with excessive ROS production (Jaeschke, 2002). Although the literature has been increasingly highlighting the role of oxidative stress in the pathogenesis of various AFB_1-induced toxicity, including renal injury, testicular damage, and dermal toxicity (Jobe, 2023; Dlamini, 2021; Wu, 2023; Huang, 2019), mechanisms of AFB_1-induced liver injury linked to oxidative Stress-related pathways remains a main subject of investigations.

As summarized in Table 2, many studies have uncovered that exposure to AFB_1 causes hepatic oxidative stress by elevating reactive species such as ROS and malondialdehyde (MDA) while decreasing enzymatic and non-enzymatic antioxidants, including glutathione (GSH) content, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST) (Fig. 3). Exposure to a high dose (9 mg/kg) of AFB_1 for 7 consecutive days caused liver injury in Albino Wistar rats as marked by elevated AST, ALT, GGT and ALP, which were associated with a decrease in GSH level, CAT and GST

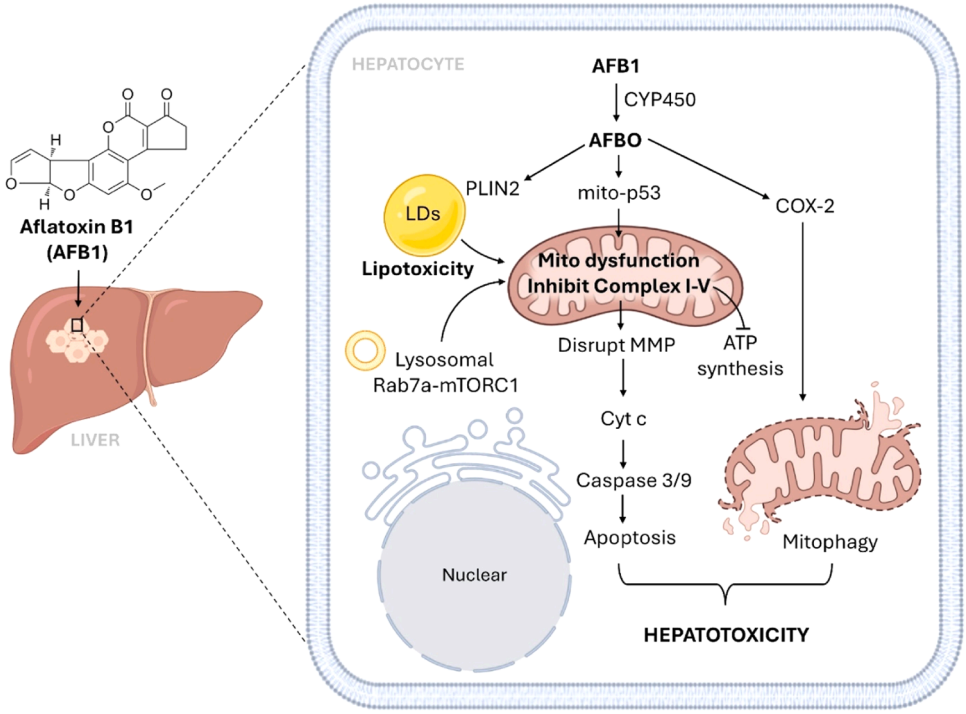

Fig. 2. Diagram illustrating the mechanism of aflatoxin B_1 (AFB_1)-induced liver injury mediated by mitochondrial dysfunction. **Abbreviations:** ATP, adenosine triphosphate; Cyt-c, cytochrome c; COX-2, cyclooxygenase-2; LDs, lipid droplets; MMP, mitochondrial membrane potential; mTORC1, mito-p53, mitochondrial p53; rapamycin complex 1; PLIN2, perilipin 2, Rab7a, Ras-associated protein 7a.

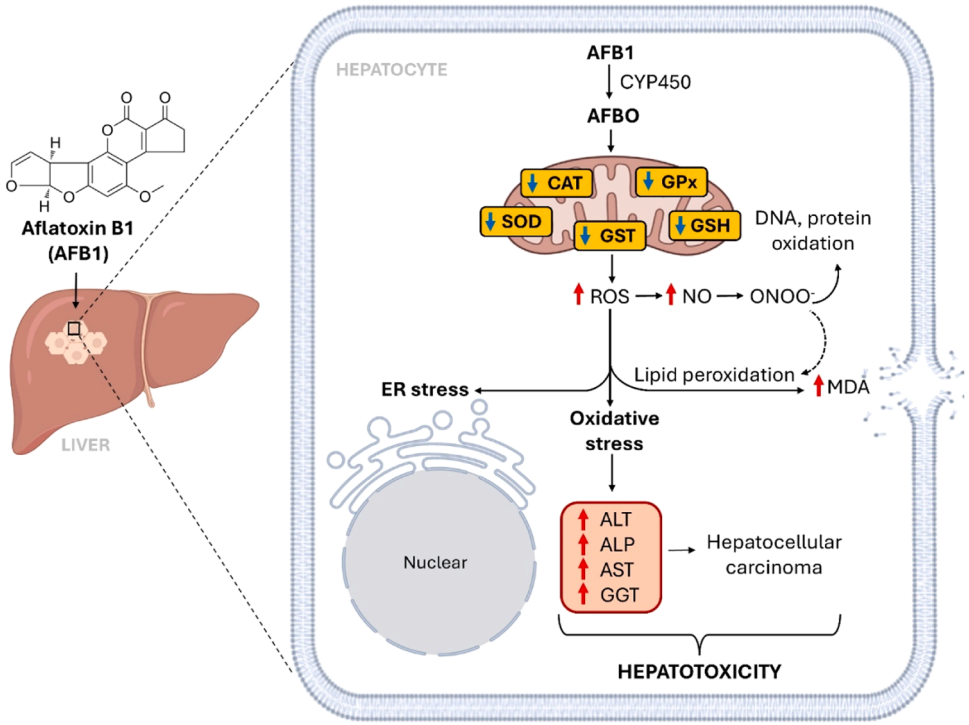

Fig. 3. Diagram illustrating the mechanism of aflatoxin B_1 (AFB_1)-induced liver injury mediated by oxidative stress. **Abbreviations:** ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; DNA, deoxyribose nucleic acid; ER, endoplasmic reticulum; GPx, glutathione peroxide; GSH, glutathione glutathione-S-transferase; GST glutathione-S-transferase; GGT, gamma-glutamyl transferase; MDA, malondialdehyde; NO, nitric oxide; ONOO, peroxynitrite; ROS, reactive oxygen species; SOD1, superoxide dismutase.

activities, and increased lipid peroxidation (Adedara, 2010) Other studies have demonstrated that even lower dose (1 mg/kg) of AFB_1 induced hepatocellular carcinoma in part by elevating ROS production and suppressing antioxidants SOD1, CAT, GPx, and total GST after acute (2 days) and chronic (4 weeks) exposure in rat models (Singh et al., 2015; Qin, 2016). Interestingly, 0.04 mg/kg AFB_1 was able to induce

hepatic oxidative damage after 56 days in albino rats, and this was exacerbated by protein malnutrition (Rotimi, 2016). To elucidate the time-course effects of AFB_1, albino Wistar rats were exposed to 1 mg/kg AFB_1 for 1, 4, and 7 days (Rotimi, 2019). It was uncovered that AFB1-induced liver injury was mediated by mitochondrial oxidative/-nitrosative stress with elevated ALP and increased GSH on day 1, while biomarkers of oxidative stress MDA, NO, GST, and TR were increased on day 7 (Rotimi, 2019).

Moreover, AFB_1 triggered liver injury by increasing ROS content in all AFB_1-treated Kunming mice, as well as MDA and H_2O_2 in the MG and HG were significantly increased while CAT activity and SOD activities were significantly decreased (Xu, 2021). Also, in Wistar rats, AFB_1 at 2.5 mg/kg and 5 mg/kg caused significant increases in hydrogen peroxide (H_2O_2) and Malondialdehyde (MDA) concentrations as well as a dose-dependent increase in the activity of glutathione S-transferase (GST) (Akinrinde et al., 2020). Additionally, the liver from AFB_1-treated rats demonstrated a significantly enhanced level of ROS corresponding with the declined immunostaining for SOD-1, an enzyme of the anti-oxidant pathway, in the FAH regions and decreased activity of the other antioxidant enzymes, including GPx1 (Singh et al., 2015). Similar observations were reported *invitro* in LO-2 cells exposed to 60.05 μM of AFB_1 and HepG2 cells exposed to 1.25, 2.5 and 5 μM for 24 h, in which AFB_1 exposure induced hepatoxicity indicated by increased ROS generation, apoptosis and cell cycle S phase arrest, reduced mitochondrial membrane potential (ΔΨm), as well as alterations of GSH content, GPx, and SOD activity (Zhang, 2020; Tadee et al., 2020).

3.3. Aflatoxin B_1- induced liver injury by promoting hepatic inflammation via gut microbiota dysbiosis, the release of DAMPs and cytokines, and immune cell activation

Since the liver is enriched with a large number of innate immune cells, including Kupffer cells, innate lymphoid cells (ILCs) like natural killer cells, and ILC-like unconventional T cells, it acts as the frontline immune organ that is constantly exposed to the gut-derived pathogens and toxins (Yang Zhou, 2023). In response to a specific insult that causes hepatocellular damage and compromises the liver microenvironment, damaged and dying liver cells release danger-associated molecular patterns (DAMPs), or pathogen-associated molecular patterns (PAMPs) in the case of infection (Yang Zhou, 2023; Kou, 2022). These hepatocyte stress and injury signals lead to the cascades of pro-inflammatory response, including activation or recruitment of immune cells. The release of inflammatory mediators, such as TGF-β, TNF-α, IL-1β, IL-6, and MCP-1, activates hepatic stellate cells (HSCs), which in turn, exacerbate hepatic inflammation to a chronic state and promote excessive extracellular matrix deposition, which ultimately leads to fibrosis and organ failure (Hammerich and Tacke, 2023; Paik, 2006). It has been increasingly demonstrated that AFB_1 exerts its toxic effects in the liver as a consequence of hepatic inflammation (Fig. 4); this evidence is summarized in Table 3. Briefly, AFB_1 induced inflammatory liver injury and altered lipid metabolism in part by disrupting gut microbiota, elevating inflammatory mediators and immune cell activation, and inhibiting cell growth pathways (Ye, 2023; Liu, 2023; Zhang, 2024). Consistently, AFB_1 exposure aggravated inflammation of the liver, and this was associated with hepatic fibrosis, necrosis, pyroptosis (Zhang, 2019; Corcuera, 2015; Liu, 2021; Akinrinmade et al., 2016; Wu, 2024), as well as adverse liver complications such as steatosis, steatohepatitis, and hepatocellular carcinoma (Jeannot, 2012; Niu, 2021; Vornoli, 2022).

Although AFB_1 is well-recognized as a potent hepatic carcinogenic agent that can work synergistically with the hepatitis B virus to induce hepatocellular carcinoma, the underlying molecular mechanisms remain elusive (Rushing and Selim, 2019). A study by Jeannot and colleagues (Jeannot, 2012) showed that a single dose of 6 mg/kg AFB_1 caused tumors (adenomas or carcinomas), preneoplastic lesions (hyperplasia or foci), and steatohepatitis in part by activating inflammatory response and altering lipid metabolism in hepatitis virus C transgenic mice after 12 months. To further explore the implication of hepatic inflammation on the synergistic effects of AFB_1 and Hepatitis B

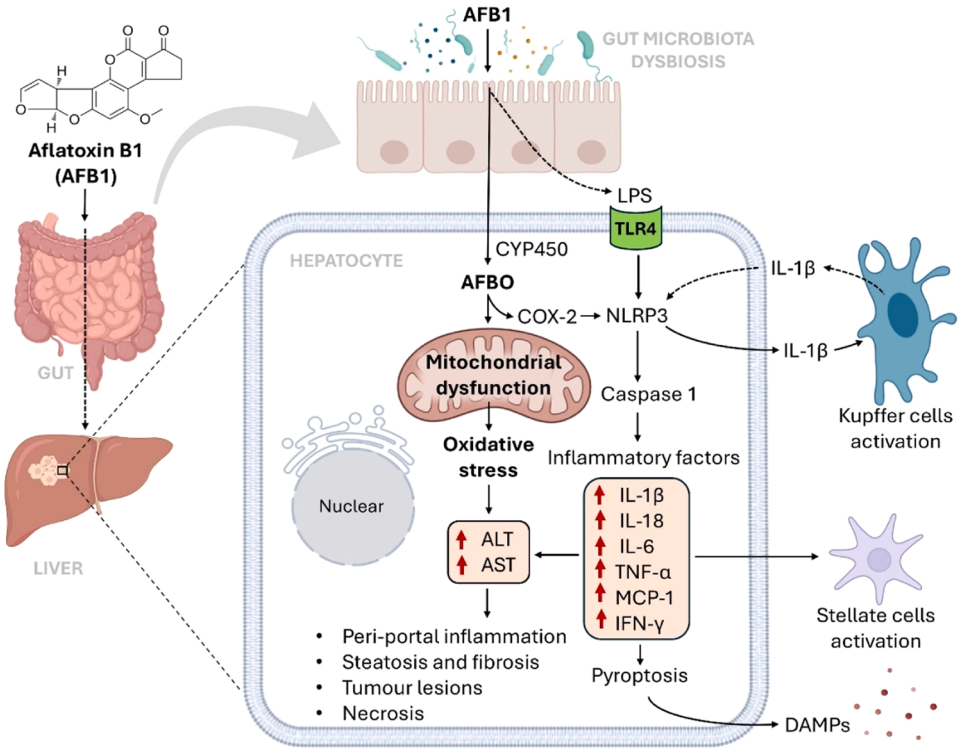

Fig. 4. Diagram illustrating the mechanism of aflatoxin B_1 (AFB_1)-induced liver injury mediated by hepatic inflammation. **Abbreviations:** COX-2, cyclooxygenase-2; DAMPs, damage-associated molecular patterns; ILs, interleukins; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; IFN-γ, interferon gamma; NLRP3, NOD-like receptor protein 3; TNF-α, tumor necrosis factor-alpha; TLR-4, Toll-like receptor 4.

Virus-induced hepatocarcinogenesis, Niu and co-workers (Niu, 2021) exposed hepatitis B virus X transgenic mice to 7 mg/kg AFB_1 for 14 months and found that it developed liver tumor by up-regulating oncogenic KRAS to trigger IL-11:IL-11 receptor subunit alpha-1 -mediated inflammation. Histological analysis of the liver from Sprague-Dawley rats administered with low doses of 0.47 mg/kg AFB_1 for 14 days showed the emergence of steatosis, steatohepatitis, and hepatocellular adenomas, which were characterized by the increased inflammatory foci, cystic dilation of biliary ducts, and hepatocellular hyperplasia and hypertrophy (Vornoli, 2022).

From the view of the above concise discussion, it is evident that AFB_1-related liver injuries, such as liver steatosis, steatohepatitis, hepatic fibrosis, and hepatocellular adenomas, occur as a consequence of hepatic immunotoxicity. Several studies have demonstrated that AFB_1-induces the inflammation of the liver by promoting inflammatory infiltration and cytokines release as well as the activation of Kupfer cells, which ultimately leads to necrotic and pyroptotic cell death (Zhang, 2019; Akinrinmade et al., 2016; Wu, 2024). For instance, Zhang and co-workers (Zhang, 2019) found that AFB_1 promotes liver inflammatory injury by activating Kupfer cells via the release of IL-1β mediated by COX-2 dephosphorylation and NLRP3-inflammasome activation, which subsequently promotes DAMPs release from pyroptotic hepatocytes. *In vitro* and *in vivo* experiments demonstrated an increased pyroptosis and inflammatory response, DAMPs release, and increased expressions of COX-2 and caspase 1, and IL-1β in human liver HepaRG cells exposed to AFB_1 at 1 μM for 24 h, as well as in C57BL/6 mice administered with 1 mg/kg of these toxins for 4 weeks (Zhang, 2019). In rats, 0.25 mg/kg AFB_1 induced hepatic necrosis, focal inflammation, and increased ALT and AST as makers of liver damage after 1-day exposure (Corcuera, 2015), while 2.5 mg/kg dose induced thrombosis, vascular congestion, and peri-portal inflammatory cell infiltrations indicated in part by increasing IL-1β and TNF-α levels (Akinrinmade et al., 2016). Of note, there was an increase in the hepatoxicity of AFB_1 by increasing the inflammatory response and disrupting cell growth pathways in HHL-16 cells after 24 h exposure (Wu, 2024).

Recent studies found that AFB_1 induces hepatic inflammation and liver injury by disturbing gut microbiota (Ye, 2023; Liu, 2023; Zhang, 2024). A study by Ye and colleagues (Ye, 2023) demonstrated that AFB_1 altered gut microbiota composition, caused colonic barrier dysfunction, and promoted liver pyroptosis to perpetuate inflammation in the liver of Balb/c mice exposed to 0.025 mg/kg of toxins for 28 days. AFB_1 promoted gut microbiota dysbiosis and reduced fecal bile salt by inhibiting intestinal farnesoid X receptor (FXR)/fibroblast growth factor 15 (FGF-15) signaling to induce inflammatory infiltration, swelling, and necrosis in the liver of C57BL/6 J mice exposed to 0.75 mg/kg of these toxins for 14 days (Liu, 2023). At a similar dose, it was further uncovered that AFB_1-induced hepatic inflammation and fibrosis by promoting gut microbial dysbiosis and altering fecal metabolome via the over-activation of the Toll-like receptor (TLR)4/myeloid differentiation primary response 88 (MyD88)/nuclear factor (NF)-κB signaling pathway in C57BL/6 mice after 28 days, and this was aggravated by co-exposure with polystyrene nano-plastics (Zhang, 2024).

4. Epidemiological evidence uncovers the toxic effects of aflatoxin B1 exposure on humans

It is well-acknowledged that dietary exposure to aflatoxins is a serious public health concern that affects mostly people living in sub-Saharan Africa and Southeast Asia, where dietary staple food crops such as groundnuts and maize are often highly contaminated by these toxins because of hot and humid climates (Gong et al., 2016). Several epidemiological studies have assessed aflatoxin exposure and its association with adverse health effects in the high-risk population (Mathew and Pingle, 2024; Turner, 2012; Groopman, 1994). To achieve this, several biomarkers for aflatoxin exposure have been identified. For example, AFM_1 and aflatoxin-N7-guanine adducts found in breast milk and urine reflect the previous day's exposure (Groopman, 1994; Islam, 2021), whereas the plasma aflatoxin-albumin adduct (AF-alb) with a half-life of approximately 2–3 months allows the measurement of chronic exposure (Turner, 2008). The latter has been used in several epidemiological studies to measure exposure period and resultant health effects in many populations (Turner, 2000; Xu et al., 2018; Shirima, 2013). AF-alb biomarker has revealed high aflatoxin exposure in both children and adults from East African countries, such as Uganda and Tanzania (Shirima, 2013; Asiki, 2014). In North and South Africa, where the climate is drier, the prevalence of aflatoxin exposure is reported to be lower than in East and West Africa (Gong et al., 2016).

Several clinical studies have reported that AFB_1 exposure to humans is associated with a high risk of morbidity and mortality, which are mediated by aflatoxicosis (Gong et al., 2016; Azziz-Baumgartner, 2005; Ali, 2019). For example, serum aflatoxin B_1–lysine adduct was correlated with aflatoxicosis such as acute liver failure and jaundice that lead to death in the Kenyan population (Azziz-Baumgartner, 2005; Ali, 2019). Thus, plasm AFB_1 albumin adducts may be utilised as a diagnostic tool for acute aflatoxicosis and monitoring interventions to alleviate aflatoxin exposure. Chronic exposure to aflatoxins is known to cause HCC, and other chronic toxic health effects include impaired child growth and immune suppression (Gong et al., 2016). Although the direct impact of AFB_1 on human health is still poorly understood, recent biomarker developments provide opportunities for future research. To prevent aflatoxicosis, long-term and culturally appropriate strategies should be developed.

5. Summary and future perspectives

Mechanistically, experimental evidence indicates that AFB_1 causes liver injuries by disrupting mitochondrial function and structure, leading to oxidative stress, marked by uncontrolled ROS production and an impaired antioxidant defense system, as shown in Fig. 5. Moreover, hepatic oxidative stress concurrently occurs with hepatic inflammation via gut microbiota dysbiosis, the release of DAMPs and cytokines, and immune cell activation. Apart from the mechanisms discussed above, toxic metabolites of AFB_1 such as AFBO may also form adducts with DNA and other critical macromolecules, leading to genotoxicity. In recent toxicological research, AHR has emerged as a key mediator of AFB_1's toxic effects by regulating phases I and II metabolisms, immune response, cell growth and development, apoptosis and carcinogenesis. However, these toxicological mechanisms should be further elucidated using different experimental models. Future research should formulate therapeutic or detoxifying agents to protect and soothe AFB_1-induced hepatotoxicity by targeting mitochondrial dysfunction, oxidative stress and hepatic inflammation. Furthermore, prospective clinical studies are warranted to monitor the long-term impact of AFB_1 exposure in humans.

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CRediT authorship contribution statement

Zibele Ndlovu: Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Tsholofelo P. Moloi:** Writing – review

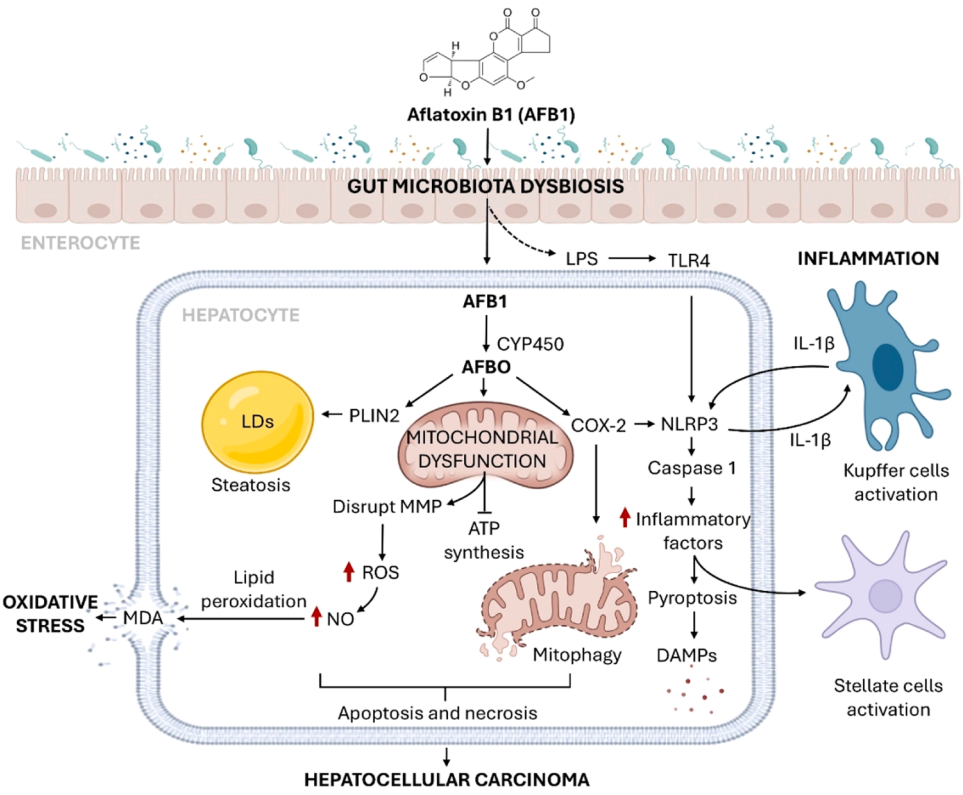

Fig. 5. Summary of the potential mechanism of aflatoxin B_1 (AFB_1)-induced liver injury implicating mitochondrial dysfunction, oxidative stress and inflammation as key pathological processes. **Abbreviations:** AFBO, aflatoxin B_1-8,9-epoxide; ATP, adenosine triphosphate; Cyt-c, cytochrome c; COX-2, cyclooxygenase-2; CYP450, cytochrome P450; DAMPs, damage-associated molecular patterns; IL-1β, interleukin 1 beta; LDs, lipid droplets; LPS, lipopolysaccharide; MMP, mitochondrial membrane potential; MDA, malondialdehyde; NO, nitric oxide; NLRP3, NOD-like receptor protein 3; PLIN2, perilipin 2; ROS, reactive oxygen species; TLR-4, Toll-like receptor 4.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

No data was used for the research described in the article.

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