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The Clinically Silent Pandemic: A Review of Aflatoxin B1 Exposure and its Role in Hepatocellular Carcinoma and Immune Suppression

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Abstract

Aflatoxin B1 (AFB1) exposure represents a pervasive yet underrecognized global health threat, contributing significantly to the burden of hepatocellular carcinoma (HCC) and widespread immune dysfunction, necessitating a comprehensive review to consolidate current knowledge and guide public health action. This review provides evidence on the molecular mechanisms of AFB1-induced hepatocarcinogenesis, including deoxyribonucleic acid (DNA) adduct formation, p53 mutations, and endoplasmic reticulum stress, as well as its immunosuppressive effects via oxidative stress, cytokine dysregulation, and impaired immune cell function. It further examines the synergistic interactions between AFB1 and other risk factors such as hepatitis viral infections and explores potential therapeutic targets and intervention strategies derived from recent preclinical and clinical studies. Future research should prioritize standardized biomarker assessments, longitudinal human studies, and the development of integrated intervention frameworks that combine dietary safety measures, vaccination programs, and novel therapeutics to mitigate the multifaceted health impacts of AFB1 exposure globally.

Keywords: Aflatoxin B1, Hepatocellular carcinoma, Immune suppression, Liver cancer, Mycotoxin, p53 mutation, Therapeutic targets, Toxicity

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Introduction

AFB1 exposure represents a significant yet often underrecognized public health concern, particularly due to its role in the development of HCC and its capacity to induce immune suppression [1-6]. The literature underscores the multifaceted impact of AFB1 on human health, emphasizing its carcinogenic potential, mechanisms of immune modulation, and the synergistic effects with other risk factors such as hepatitis virus infections [7-11]. The biological properties of AFB1 are central to understanding its carcinogenicity. As detailed by Chałaśkiewicz et al. [10], AFB1 and its metabolite M1 are implicated in cancer development through their ability to form DNA adducts, leading to mutations. Specifically, AFB1 exposure has been associated with mutations in the p53 tumor suppressor gene, which is a critical event in hepatocarcinogenesis. The analysis of AFB1-lysine adducts in human populations provides a biomarker for assessing exposure levels, highlighting the public health relevance of monitoring AFB1 in food commodities [11].

Epidemiological studies have demonstrated a clear link between AFB1 exposure and increased risk of HCC, especially in regions with high dietary contamination [12-16]. For instance, in Africa, the synergistic effect of AFB1 and hepatitis B virus (HBV) infection significantly elevates HCC risk [17]. The interaction between these two factors appears to amplify carcinogenic processes, possibly through mechanisms involving DNA damage and immune suppression. The meta-analysis cited in Balan et al. [18] further supports this, indicating that reducing AFB1 exposure could lead to a substantial decrease (approximately 23%) in HCC incidence in high-risk areas. This underscores the importance of food safety interventions and exposure mitigation strategies. Beyond its carcinogenic effects, AFB1 exerts profound influence on the immune system. The degree of immune suppression associated with AFB1 exposure is a critical aspect of its pathogenic profile. As discussed by Kuhn and Ghannoum [19], immune suppression not only facilitates persistent infections but also impairs the body's ability to surveil and eliminate emerging tumor cells. The clinical implications of this immune modulation are significant, as they may contribute to the progression and aggressiveness of HCC. The immune suppression induced by AFB1 may involve alterations in cytokine production, lymphocyte function, and overall immune responsiveness, although the precise mechanisms require further elucidation.

At the cellular level, endoplasmic reticulum stress and the unfolded protein response are implicated in AFB1-induced hepatocarcinogenesis [20-24]. The literature highlights that endoplasmic reticulum stress plays a dual role: initially protective but ultimately contributing to tumor progression when dysregulated [25]. AFB1-induced DNA damage and oxidative stress can trigger endoplasmic reticulum stress pathways, which may support tumor cell survival by preventing apoptosis. This adaptive response enables malignant hepatocytes to proliferate despite genotoxic insults, thereby facilitating tumor development. The unfolded protein response's role in supporting tumor cell metabolism and proliferation underscores its potential as a therapeutic target in AFB1-related HCC. The molecular interplay between AFB1 exposure, endoplasmic reticulum stress, and metabolic dysregulation further complicates the pathogenesis of HCC. As outlined by Luna-Marco et al. [25], the unfolded protein response can promote tumor cell survival by modulating cellular metabolism and resisting apoptosis. This adaptive mechanism may be exploited by hepatocytes under chronic AFB1 exposure, leading to malignant transformation. The integration of these pathways suggests that AFB1 not only causes direct DNA damage but also creates a cellular environment conducive to tumor growth through metabolic reprogramming and stress response pathways. The public health implications of AFB1 exposure are compounded by its prevalence in various food commodities, including nuts, spices, and processed foods [18, 26]. The widespread contamination underscores the need for effective monitoring and regulation to prevent long-term health consequences. The evidence suggests that interventions aimed at reducing dietary AFB1 levels could significantly decrease HCC incidence, especially in high-risk populations. The meta-analytical data indicates a 23% reduction in HCC with lowered AFB1 exposure emphasizes the potential impact of such measures.

In addition to its carcinogenic effects, AFB1's role in immune suppression may facilitate persistent infections with hepatitis viruses, particularly HBV and hepatitis C virus (HCV), which are well-established risk factors for HCC [17]. The interaction between viral infections and AFB1 exposure appears to be synergistic, with each factor exacerbating the other's carcinogenic potential [8, 27-29]. This synergy complicates disease management and highlights the importance of integrated approaches that address both viral infections and environmental toxins.

Overall, literature collectively underscores AFB1 as a critical environmental carcinogen with a dual role in promoting HCC and impairing immune function. Its ability to induce DNA mutations, particularly in the p53 gene, coupled with its capacity to modulate cellular stress responses and immune pathways, positions AFB1 as a key contributor to the 'silent pandemic' of liver cancer. Addressing AFB1 exposure through food safety measures, public health interventions, and targeted therapies could substantially reduce the burden of HCC worldwide, especially in regions where exposure is endemic. The complex interplay between AFB1, immune suppression, and viral infections necessitates a comprehensive approach to mitigate its impact on global health.

The Link Between AFB1 and HCC

AFB1 is a potent carcinogen that has been extensively linked to the development of HCC, particularly in regions with high exposure to this toxin. The carcinogenic potential of AFB1 is primarily attributed to its ability to form DNA adducts, leading to mutations in critical genes such as p53, which are crucial in the pathogenesis of HCC [30-33]. The interaction between AFB1 and other risk factors, such as chronic HBV infection, further exacerbates the risk of HCC, highlighting the complex interplay of genetic and environmental factors in liver carcinogenesis. The following sections delve into the mechanisms and interactions that underpin the link between AFB1 and HCC.

Molecular mechanisms of AFB1-induced HCC

AFB1's carcinogenic effects are largely attributed to its metabolism in the liver, where it is converted into a reactive epoxide intermediate [34]. This epoxide can bind to DNA, forming AFB1-DNA adducts, particularly at guanine residues [34, 35]. These adducts can lead to mutations in critical genes, such as TP53, a tumor suppressor gene [6]. Specifically, AFB1 exposure is associated with a specific hotspot mutation in TP53, which is considered a molecular fingerprint of aflatoxin exposure [6]. This mutation compromises p53's tumor suppressor functions, increasing the risk of HCC development [6]. Furthermore, AFB1 toxicity and subsequent development of HCC is mediated by the aryl hydrocarbon receptor [36]. Aryl hydrocarbon receptor is a ligand-binding transcription factor regulating cell metabolism, differentiation, and immunity. Studies have shown that aryl hydrocarbon receptor deficient cells tolerated high concentrations of AFB1 and that aryl hydrocarbon receptor expression was elevated in primary tumor sections obtained from AFB1-HCC patients [36].

AFB1 is metabolized by cytochrome P450 enzymes into AFB1-exo-8,9-epoxide, a highly reactive compound that forms adduct with DNA, leading to mutations, particularly in the p53 gene [23, 37]. The formation of AFB1-DNA adducts has been observed in both high and low exposure areas, suggesting that even low-dose, chronic exposure can contribute to liver cancer development [35]. AFB1 exposure has been shown to upregulate oncogenes such as H19 and early region 2 binding factors, promoting cell growth and invasion in HCC cells [38]. Genetic polymorphisms, such as those in the X-ray repair cross complementing 1 gene, have been associated with increased susceptibility to AFB1-induced HCC, highlighting the role of genetic predisposition in modulating risk [37]. AFB1 exposure affects various molecular pathways, including those involving kinases and transcription factors, which are crucial in cell cycle regulation and tumor progression [39, 40]. Proteomic analyses have identified alterations in detoxification and anti-apoptosis pathways in HCC tumors associated with AFB1 and HBV, suggesting complex molecular interactions in carcinogenesis [41].

Synergistic effects with other risk factors

The risk of HCC is significantly elevated when AFB1 exposure occurs in conjunction with other risk factors, such as chronic HBV infection [6, 7]. HBV X protein can functionally inactivate wild-type p53, synergistically disrupting the p53 pathway with AFB1-induced mutations [6]. This interaction underscores the importance of addressing both AFB1 exposure and HBV infection in HCC prevention strategies [6]. Other risk factors such as HCV infection, alcohol consumption, nonalcoholic steatohepatitis, liver cirrhosis, obesity, and diabetes can also lead to HCC (Table 1) [42].

- HCV infection also shows a synergistic effect with AFB1, as evidenced by increased tumor incidence in HCV-transgenic mice exposed to AFB1. This is attributed to enhanced inflammatory responses and altered lipid metabolism [43]. Both HBV and HCV infections generate reactive oxygen and nitrogen species, which increase oxidative stress. This stress facilitates the integration of AFB1 metabolites into DNA, promoting mutations and impairing DNA repair mechanisms.

Table 1: Synergistic risk factors for AFB1-induced HCC.

Risk factor	Type of interaction with AFB1	Proposed mechanism
HBV	Strong synergism	HBV X protein inactivates p53; AFB1 causes p53 mutation; combined profound disruption of p53 pathway
HCV	Synergism	HCV induces oxidative stress and inflammation, facilitating AFB1-DNA adduct formation and impairing repair
Alcohol consumption	Additive/synergistic	Alcohol induces CYP2E1, potentially increasing AFB1 activation; shared oxidative stress pathways
Diabetes/obesity	Additive	Underlying metabolic dysfunction (e.g., non-alcoholic steatohepatitis) creates a pro-inflammatory liver environment
Genetic polymorphisms	Modifies susceptibility	Variations in detoxification genes (e.g., GST M1-null, epoxide hydrolase) reduce ability to neutralize AFB1-epoxide

- Alcohol consumption and diabetes have been identified as additional risk factors that interact positively with AFB1 exposure, further elevating the risk of HCC. The synergism index for AFB1 with alcohol and diabetes was found to be significant in logistic regression analyses [44].

- Genetic factors, such as variations in detoxification enzymes like epoxide hydrolase and glutathione S-transferase M1, can influence susceptibility to AFB1-induced carcinogenesis. Individuals with certain genotypes are at higher risk when co-exposed to HBV and AFB1 [45].

Evidence from human studies

Epidemiological studies have consistently demonstrated a strong association between AFB1 exposure and HCC incidence, particularly in high-exposure regions [6, 7]. A case-only study in New York City found that a significant proportion of HCC patients had detectable AFB1-albumin adducts, suggesting that environmental exposure to aflatoxins may contribute to the high incidence of HCC in the region [46]. Another study showed the presence of AFB1-DNA adducts in HCC tissues even in low-exposure areas, indicating that chronic, low-dose AFB1 consumption may accumulate in the liver and potentially contribute to liver cancer [35].

In Mexico, a study found that 85.5% of serum samples from a representative population contained AFB1, with a median level of 0.117 pg/μL. The presence of the aflatoxin signature mutation in the TP53 gene was detected in 6% of HCC cases, indicating a significant role of AFB1 in HCC etiology in the region [47]. In southern and eastern Mexico, the prevalence of AFB1 exposure is high, with detection rates of 91.9% in adults. The exposure levels are notably higher in rural areas and among individuals of lower socioeconomic status, with Veracruz showing the highest median AFB1-lys levels compared to other regions like Yucatán [48]. In India, AFB1 was detected in 58.1% of liver biopsies from HCC patients, with a significant association between AFB1 presence and tumor tissue, suggesting a strong link between AFB1 exposure and HCC development [49]. In Guatemala, 100% of participants in a study had detectable levels of AFB1-albumin adducts, with higher exposure levels in rural, low-income populations. This exposure is comparable to levels associated with increased liver cancer risk in Asia and Africa [50].

In summary, while the link between AFB1 and HCC is well-established, it is important to consider the broader context of liver carcinogenesis, which involves multiple risk factors and complex interactions. Other factors such as HCV infection, metabolic syndrome, and genetic predispositions also play significant roles in HCC development. Therefore, a comprehensive approach that addresses all potential risk factors is essential for effective prevention and management of HCC.

AFB1 and Immune Suppression

AFB1 is a potent mycotoxin known for its immunosuppressive effects in both humans and animals. It exerts its immunotoxic effects through various mechanisms, including oxidative stress, apoptosis, and modulation of cytokine production. These effects can lead to increased susceptibility to infections and other health complications. The following sections detail the mechanisms and impacts of AFB1-induced immune suppression.

Immunotoxic effects of AFB1

In addition to its direct carcinogenic effects, AFB1 can significantly impair the immune system, further promote tumor development and increase susceptibility to infections [51, 52]. AFB1 exhibits both immunosuppressive and immunostimulatory effects, depending on factors such as dose, duration of exposure, and the presence of other immune stimuli [51]. High doses and long-term exposure tend to induce immunosuppression [51]. AFB1 interferes with vitamin D receptor signaling, which is essential for immune regulation. This interference affects the transcriptional activation of vitamin D receptor and disrupts the formation of protein complexes necessary for vitamin D-mediated immune responses [53]. AFB1 suppresses the type I interferon signaling pathway, which is crucial for the innate immune response. This suppression weakens the body's first line of defense against pathogens and increases susceptibility to infections and cancer [54].

In broiler chickens, AFB1 exposure led to dose-dependent immune suppression, with activated charcoal showing some protective effects at lower AFB1 concentrations [55]. In ducklings, AFB1 reduced growth performance and altered immune-related gene expression, further demonstrating its immunosuppressive effects [56]. In mice, AFB1 exposure resulted in liver damage and altered cytokine production, leading to immune suppression. Lycopene supplementation was found to alleviate these effects by reducing oxidative stress and apoptosis in the spleen [57, 58].

Mechanisms of immunosuppression

AFB1's immunosuppressive mechanisms include oxidative stress, apoptosis and autophagy of immune cells, as well as inhibition of immunity-related signaling pathways [51]. It can also affect the activity of specific immune cells and induce immune suppression, resulting in reduced host resistance to pathogens [52]. Recent studies have also highlighted the role of the nod-like receptor protein 3 (NLRP3) inflammasome in mycotoxin-induced toxicological mechanisms [59]. Activation of the NLRP3 inflammasome is linked to tissue damage and inflammation induced by AFB1 exposure [59]. AFB1 exposure leads to increased production of reactive oxygen species and oxidative stress, which are critical in mediating its immunotoxic effects. This oxidative stress is associated with the activation of inflammatory pathways, as evidenced by increased expression of pro-inflammatory cytokines such as tumor necrosis factor-α and C-X-C motif chemokine ligand 2 in macrophages [60]. The oxidative stress induced by AFB1 affects the mitochondrial respiratory chain, leading to further activation of inflammatory signaling pathways [60].

AFB1 induces apoptosis in immune cells, such as leukocytes, through mechanisms involving ATP depletion and caspase activation. This process is observed in neutrophils, lymphocytes, and monocytes, where AFB1 exposure results in significant ATP depletion and increased caspase-3/7 activity [61]. The activation of apoptosis pathways contributes to the reduction in immune cell viability and function, thereby compromising the immune response [61]. AFB1 modulates cytokine production, leading to altered immune responses. It suppresses the production of key cytokines involved in immune regulation, such as interleukin-4 (IL-4) and interferon-gamma, which are crucial for T cell function [62]. The suppression of type I interferon signaling by AFB1 further weakens the innate immune response, predisposing individuals to infections and cancer [54]. AFB1 exposure results in DNA damage and alterations in gene expression, mediated by changes in DNA methylation. The Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway, which is crucial for immune signaling, is activated in response to AFB1-induced DNA damage, contributing to immunotoxicity [63]. The involvement of DNA methyltransferases in mediating these epigenetic changes highlights the role of epigenetic regulation in AFB1-induced immunosuppression [63].

Impact on immune cell function

AFB1 influences immune cell polarization, particularly in macrophages, through pathways involving aryl hydrocarbon receptor (AHR)/toll-like receptor 4 crosstalk and mitochondrial oxidative stress. This leads to a pro-inflammatory M1-like phenotype, exacerbating immune dysfunction [64]. Metabolic reprogramming, such as alterations in glycolysis and ferroptosis pathways, further contributes to immune cell dysfunction and immunosuppression [65]. AFB1 exposure can modulate the tumor microenvironment, influencing the activity of immune cells such as macrophages [66, 67]. AFB1 can promote M2-like macrophage polarization via IL-6 expression in HCC [67]. M2 macrophages are associated with immunosuppression and tumor progression [66, 67]. CD4+ T cells also play a critical role in the development of HCC and nonalcoholic steatohepatitis [68]. AFB1 exposure results in ATP depletion and caspase activation in leukocytes, leading to apoptosis and necrosis of immune cells such as neutrophils, lymphocytes, and monocytes. This cellular damage reduces the overall immune competence [61]. In chickens, AFB1 exposure results in significant changes in gene expression in immune organs, affecting immune and metabolic pathways. This includes upregulation and downregulation of numerous genes and miRNAs associated with immune responses [69]. In lymphocytes, AFB1 exposure to rats leads to a decrease in CD8(+) T cells and natural killer cells, along with altered cytokine expression, indicating immunosuppression without overt tissue damage [70]. In macrophages, AFB1 exposure decreases cell viability and phagocytic activity, while increasing inflammatory responses through oxidative stress pathways [60]. The mycotoxin also disrupts the gut microbiota, leading to translocation of microbial metabolites to the spleen, which triggers pyroptosis, a form of programmed cell death, further compromising immune function [71].

Immune checkpoints and HCC

The dysregulation of the immune system in HCC has led to the exploration of immunotherapy as a treatment strategy [72, 73]. Immune checkpoint inhibitors, such as anti-programmed cell death protein 1 (anti-PD-1) and anti-cytotoxic T-lymphocyte-associated protein 4 antibodies, have shown therapeutic effects in various cancers, including HCC [36, 72]. T cell immunoglobulin mucin-3, a new immune checkpoint molecule, also plays an important role in the development of HCC and is a promising target for immunotherapy [72]. Aryl hydrocarbon receptor also upregulates anti-programmed death-ligand 1 (anti-PD-L1), a clinically relevant immune regulator and anti-PD-L1 therapy exhibited greater efficacy in HCC xenografts derived from cells with ectopic expression of aryl hydrocarbon receptor [36].

AFB1 forms adducts with DNA, leading to mutations and activation of DNA checkpoint controls. This activation is part of the body's response to DNA damage, which can lead to oncogene-induced senescence, a barrier to tumorigenesis. However, AFB1 downregulates ARID3A and ARID3B proteins, overcoming this senescence and promoting hepatic tumors [74]. The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway is activated by AFB1, which is crucial for cell survival and proliferation, further contributing to tumorigenesis [74, 75]. AFB1 inhibits the type I interferon response pathway, which is essential for antiviral defense and tumor suppression. This inhibition occurs through the suppression of key signaling proteins such as JAK1, STAT1, and 2'-5'-oligoadenylate synthetase 3, thereby increasing the risk of HCC [76]. The suppression of the type I interferon pathway by AFB1 suggests a novel mechanism by which AFB1 may induce HCC, highlighting the importance of immune checkpoints in cancer prevention [76].

In summary, while AFB1 is a significant immunosuppressive agent, the extent of its effects can vary based on factors such as dosage, exposure duration, and the species affected. Additionally, the combination of AFB1 with other mycotoxins can exacerbate its immunotoxic effects, highlighting the need for comprehensive management strategies [52, 77]. Further research into the mechanisms of AFB1-induced immunosuppression and effective mitigation strategies is essential to protect both human and animal health.

Potential Therapeutic Targets

Given the complex interplay between AFB1, HCC, and the immune system, identifying potential therapeutic targets is crucial [67, 78]. Research has identified several molecular targets and pathways that could be exploited for therapeutic interventions (Table 2).

Table 2: Summary of potential therapeutic interventions against AFB1-induced HCC.

Intervention category	Example(s)	Proposed mechanism of action
Pharmacological agents	CDDO-Im, PDE5 inhibitors (Sildenafil)	Induces detoxification of enzymes, enhances antioxidant defense, and modulates cell survival pathways
Natural compounds/phytochemicals	BER NPs, lycopene, tridham, kalpaamruthaa	Antioxidants, anti-inflammatory, normalizing metabolic enzyme alterations, promote tumor regression
Immunotherapy	Anti-PD-1/PD-L1, anti-IL-6	Reverses AFB1-induced immune checkpoint upregulation and M2 macrophage polarization, enhances T-cell activity
Targeted molecular therapy	AHR modulators, BUB1B inhibitors	Blocks specific pathways critical for AFB1 toxicity (AHR) or cell cycle progression in AFB1-transformed cells (BUB1B)
Nutritional and public health	Chlorophyllin, NAC, improved storage	Binds AFB1 in gastrointestinal tract (chlorophyllin), precursor for glutathione (NAC), reduces primary exposure (storage)

- Targeting IL-6: IL-6 is a key cytokine involved in AFB1-mediated M2 macrophage polarization [67]. Targeting IL-6 in combination with PD1 antibody therapy has shown promising results in reducing tumor growth and enhancing CD8+ T cell infiltration in preclinical models [67].

- Inhibition of BuB1 mitotic checkpoint serine/threonine kinase (BUB1B): BUB1B is a spindle assembly checkpoint regulator involved in AFB1-induced hepatocarcinogenesis [78]. Targeting BUB1B may disrupt cell proliferation and cell cycle dynamics, offering a potential therapeutic avenue [78].

- Modulation of aryl hydrocarbon receptor: Since aryl hydrocarbon receptor mediates the AFB1 toxicity associated with HCC, modulation of aryl hydrocarbon receptor presents as a therapeutic option for the treatment of AFB1-associated HCCs [36].

- DNA adducts: AFB1-DNA adducts are significant in the carcinogenic process, affecting the efficacy of post-operative adjuvant transarterial chemoembolization treatment. High levels of AFB1-DNA adduct are associated with poorer survival outcomes, but post-operative adjuvant transarterial chemoembolization shows improved efficacy in patients with high AFB1-DNA adducts levels, suggesting a potential therapeutic target [79].

- Signaling pathways: The PI3K/Akt pathway is implicated in AFB1-induced lipid metabolism disorders and apoptosis, contributing to hepatotoxicity and HCC development. Targeting this pathway could mitigate AFB1's harmful effects and offer a therapeutic avenue [75]. Other pathways, such as the cell cycle, hypoxia-inducible factor-1 (HIF-1), and Ras-associated protein-1 (Rap1), are also involved in AFB1-induced hepatocarcinogenesis, with paeoniflorigenone showing potential in modulating these pathways [80].

- p53 and Ras mutations: AFB1 is known to cause specific mutations in the p53 tumor suppressor gene, particularly a G to T transversion at codon 249, which is prevalent in regions with high AFB1 exposure. Additionally, mutations in the Ras oncogenes are observed in experimental models of AFB1-induced HCC, although less frequently in humans [81]. These mutations represent potential targets for therapeutic intervention.

- Protein targets: Proteomic analyses have identified proteins such as aldehyde dehydrogenase 1 family member A1, keratin 18, and tissue factor pathway inhibitor 2 as potential therapeutic targets in HCC, with implications for drug development [82].

While these findings highlight promising therapeutic targets for AFB1 and HCC, it is essential to consider the broader context of HCC treatment. The synergistic effects of AFB1 and HBV in HCC development underscore the need for comprehensive treatment strategies that address both risk factors simultaneously [23]. Additionally, the role of microRNAs, such as miR-429, in modulating HCC progression and prognosis suggests another layer of complexity and potential therapeutic intervention [83]. These insights collectively emphasize the multifaceted nature of HCC treatment and the need for continued research to refine and expand therapeutic options.

Clinical Studies

The therapeutic effects for AFB1-induced HCC have been explored through various interventions, including pharmacological agents, natural compounds, and traditional medicines. These studies highlight the potential of different treatments to mitigate the carcinogenic effects of AFB1, primarily by targeting oxidative stress, inflammation, and molecular pathways involved in cancer progression.

A study by Chhonker et al. [84] aimed to evaluate the anticancer properties of phosphodiesterase type 5 (PDE5) inhibitors, specifically tadalafil and sildenafil, against AFB1-induced HCC in rats. Rats were divided into five groups, with most groups receiving 5% alcohol for three weeks, followed by two successive doses of AFB1 (1 mg/kg body weight (bw), intraperitoneal injection). After 6 weeks of AFB1 treatment, PDE5 inhibitors (tadalafil and sildenafil, 10 mg/kg bw) were administered along with drinking water for two weeks. AFB1-induced HCC decreased the mRNA expression and activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase, catalase, glutathione reductase, and glutathione S-transferase (GST), and reduced glutathione content, while simultaneously increasing lipid peroxidation. Post-treatment with PDE5 inhibitors (tadalafil and sildenafil) restored these parameters towards normal levels. Sildenafil was found to be more effective than tadalafil in restoring these parameters. The results suggest that PDE5 inhibitors may act as anticancer agents by preventing the development and progression of HCC through the modulation of key parameters within the antioxidant pathway.

A study by Liang et al. [80] identified 34 shared targets as potential candidate genes for paeoniflorigenone in combating liver cancer induced by AFB1. Pathway analysis revealed that these identified genes are involved in several key biological pathways, including the cell cycle, HIF-1, and Rap1 pathways. A risk assessment model was developed using least absolute shrinkage and selection operator regression, which demonstrated an association between the identified genes and the tumor immune microenvironment. Furthermore, the genes included in this risk model were found to be linked to the immune response in liver cancer. Molecular docking studies indicated that PA interacts with its targets primarily through hydrogen bonding and hydrophobic interactions. These findings offer insights into the possible mechanisms of paeoniflorigenone in the treatment of liver cancer and provide a predictive model for assessing the risk level of individuals with liver cancer. This has significant implications for therapeutic strategies in managing liver cancer patients. In summary, the research successfully identified key genetic targets and pathways influenced by paeoniflorigenone in AFB1-induced liver cancer, developed a predictive risk model linked to the immune microenvironment, and elucidated the molecular interactions of paeoniflorigenone with its targets, thereby contributing valuable insights for future therapeutic approaches.

A study by Ravinayagam et al. [85] investigated the effects of tridham on AFB1-induced HCC in male Wistar rats, focusing on oxidative stress markers and tumor regression. Rats administered with AFB1 (Group II) showed elevated levels of lipid peroxides, thiobarbituric acid substances, and protein carbonyls compared to control animals (Group I). These are indicators of oxidative tissue damage. Concurrently, group II animals exhibited reduced levels of both enzymatic and non-enzymatic antioxidants when compared to the control group. Histological observations confirmed the successful induction of tumors in the group II animals. Oral administration of tridham (300 mg/kg bw/day) for 45 days to HCC-bearing animals (Group III) significantly restored the levels of antioxidants that were depleted by AFB1 (Figure 1). The administration of tridham also led to a significant reduction in tissue damage in group III animals. Histological examination of group III animals revealed a complete regression of the tumors, indicating tridham's potent anticarcinogenic effect. These results collectively highlight tridham's significant

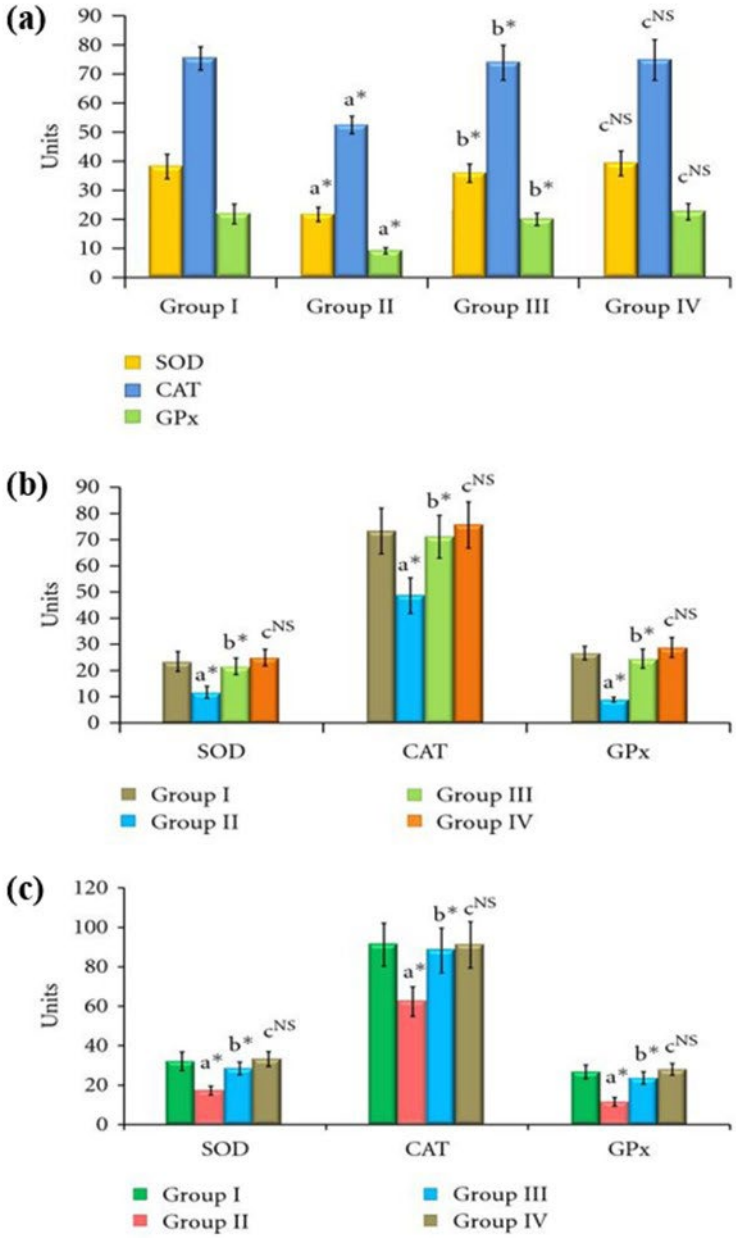

Figure 1: Effect of tridham on enzymic antioxidants in **(a)** serum, **(b)** liver, and **(c)** kidney of control and experimental animals [85].

antioxidant properties, which contribute to its therapeutic efficacy in managing AFB1-induced HCC in rats. The study suggests that tridham can mitigate oxidative stress and promote tumor regression in this experimental model.

A study by Kanchana et al. [86] investigated the potential anticancer effect of kalpaamruthaa against AFB1-induced HCC in rats. In HCC-induced rats, an overall increase in glycolytic enzymes was observed, accompanied by a reduction in gluconeogenic enzymes, mitochondrial Krebs cycle enzymes, and respiratory chain enzymes. These altered enzyme activities were effectively counteracted by supplementation with kalpaamruthaa. Supplementation with kalpaamruthaa also prevented bw loss in HCC-induced rats by enhancing the host's energy metabolism. Histological studies of liver sections supported the biochemical findings, further confirming the anticancer effect of kalpaamruthaa against AFB1-induced HCC. In summary, the results reveal that kalpaamruthaa possesses a potential anticancer effect against AFB1-induced HCC in rats, demonstrated through its ability to normalize altered enzyme activities, prevent bw loss, and supported by histological evidence.

A study by Khedr et al. [87] aimed to evaluate the therapeutic effect of berberine (BER) nanoparticles (NPs) against AFB1-induced hepatotoxicity, comparing it to free BER. Both bovine serum albumin (BSA) NPs and BER-BSA NPs were successfully synthesized using the desolvation method and observed to have spherical, regular, and uniform shapes via transmission electron microscopy imaging. The BER encapsulation efficiency was found to be 78.5%. The formed BER-BSA NPs showed a loading capacity of 7.71% and a synthesis yield of 92.6%. AFB1 administration (200 µg/kg/day for 5 consecutive days)

successfully induced aflatoxicosis in rats. AFB1 led to several detrimental changes, including an increase in pro-oxidant markers, a decrease in antioxidant systems, stimulation of inflammatory enzymes, inhibition of anti-inflammatory markers, a decrease in tumor suppressor enzymes, an increase in oncogenes, increased glycolytic activity, prevention of cell death, and promotion of cell growth. Treatment with BER-NPs (10 mg/kg) was significantly more efficient than treatment with free BER (100 mg/kg) in counteracting the effects of aflatoxicosis. Most of the biochemical markers and hepatic architecture that were negatively affected by AFB1 were normalized in the BER-BSA NP-treated group, whereas this normalization was not observed in the BER-treated group. In conclusion, the research demonstrated that BER-BSA NPs are a more effective therapeutic approach than free BER in reversing AFB1-induced liver hyperplasia due to their enhanced bioavailability and targeted delivery.

A study by Aborehab and Waly [88] investigated the potential hepatoprotective effects of *Nigella Sativa*, *Panax Ginseng*, and *Cupressus Sempervirens* in a rat model of AFB-1 induced HCC. Herbal treatments significantly reduced levels of IL-6, high-sensitivity C-reactive protein, and malondialdehyde. These markers are indicative of inflammation and oxidative stress. The treatments also led to a significant increase in SOD and nuclear factor erythroid 2-related factor 2. SOD is a crucial antioxidant enzyme, while nuclear factor erythroid 2-related factor 2 is a master regulator of antioxidant responses (Figure 2). Histopathological examinations revealed an improvement in the liver tissue of the treated groups. This suggests that the herbal extracts helped mitigate the cellular damage caused by AFB-1. The study confirmed a beneficial hepatoprotective effect of the orally administered herbal extracts in the AFB-1 induced HCC rat model. This protective action is putatively mediated through the modulation of inflammatory cytokines and the amelioration of oxidative stress. In conclusion, the study demonstrated that *N. Sativa*, *P. Ginseng*, and *C. Sempervirens* extracts exhibit significant hepatoprotective properties by reducing inflammation and oxidative stress, as evidenced by changes in biochemical markers and improved liver histology.

A study by Johnson et al. [89] investigated the chemoprotective efficacy of the synthetic oleanane triterpenoid 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im) against

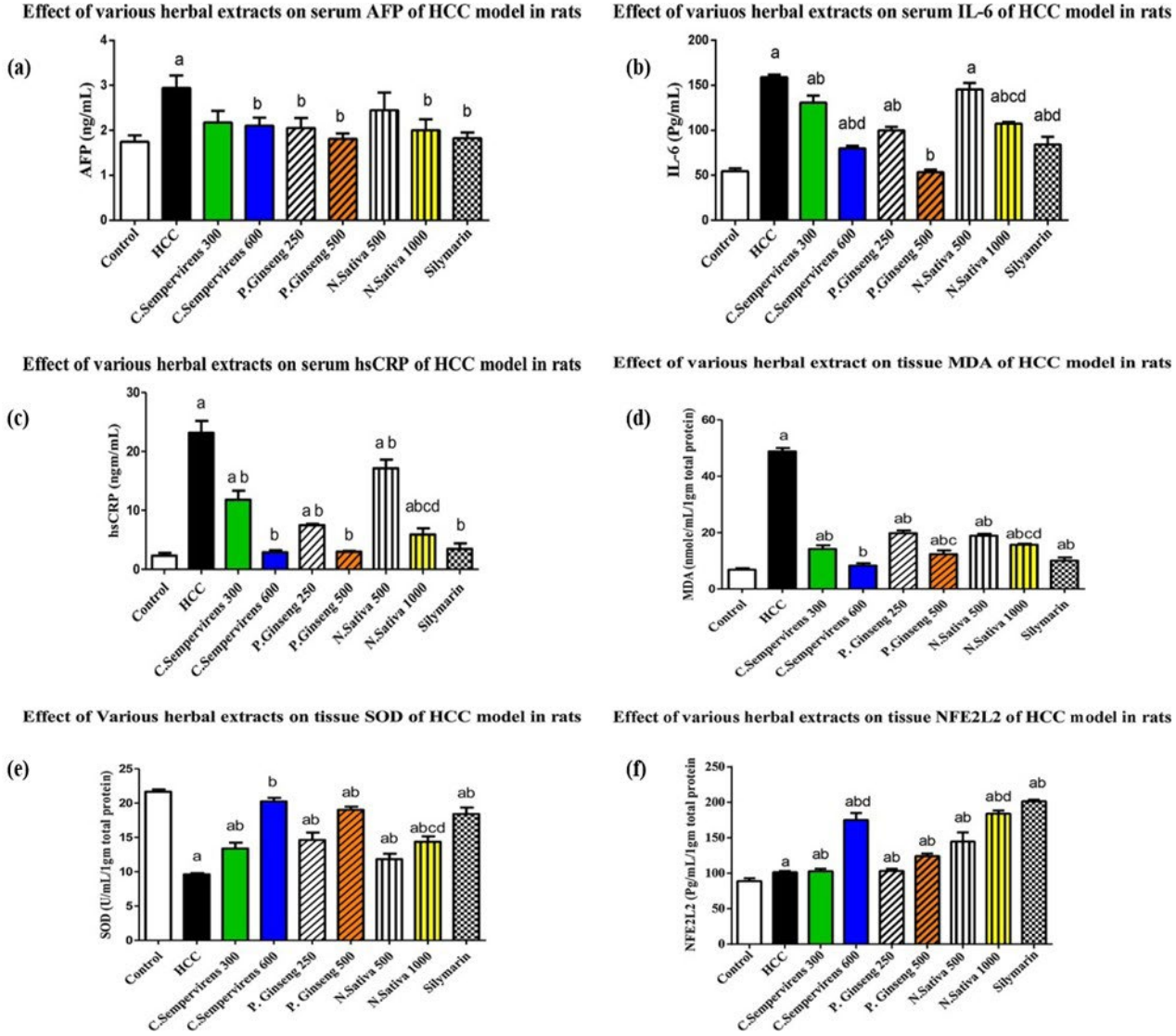

Figure 2: Serum level of **(a)** alpha-fetoprotein (ng/mL), **(b)** IL-6 (Pg/mL), and **(c)** high-sensitivity C-reactive protein (ng/mL) and tissue level of **(d)** malondialdehyde (nmole/mL/1 gm total protein), **(e)** SOD (U/mL/1 gm total protein), and **(f)** transcription factor Nrf2 (U/mL/1 gm total protein) in the experimental groups [88].

AFB1-induced HCC in F344 rats. The key results highlight CDDO-Im's remarkable ability to prevent liver cancer and modulate AFB1 metabolism and genotoxicity. CDDO-Im treatment led to complete protection against AFB1-induced liver cancer, with 0 out of 20 rats developing cancer. This is in stark contrast to the AFB1-only group, which showed a 96% incidence of liver cancer (22 out of 23 rats). The integrated level of urinary AFB1-N(7)-guanine, a marker of DNA adducts, was significantly reduced by 66% with CDDO-Im treatment. Conversely, aflatoxin-N-acetylcysteine (NAC), a detoxication product, was consistently elevated by 300% after the initial AFB1 dose in CDDO-Im-treated rats. This suggests an enhanced detoxification pathway. In AFB1-treated rats, the hepatic burden of GST-P-positive foci, which are preneoplastic lesions, substantially increased from 0% to 13.8% over four weeks. However, these foci were largely absent when CDDO-Im intervention was applied. The characteristic toxicogenomic RNA expression signature induced by AFB1 was not observed in rats treated with both AFB1 and CDDO-Im. This indicates that CDDO-Im effectively mitigated the gene expression changes typically associated with AFB1 toxicity. In summary, CDDO-Im demonstrated complete protection against AFB1-induced liver cancer, significantly reduced DNA adduct formation, enhanced detoxification, suppressed preneoplastic lesions, and abrogated the toxicogenomic signature of AFB1. These findings suggest a concept of a threshold for DNA damage in cancer development, as CDDO-Im was effective even with a significant aflatoxin adduct burden.

While these studies demonstrate promising anticarcinogenic effects against AFB1-induced HCC, it is important to consider the complexity of AFB1's carcinogenic mechanisms. The inhibition of the type I interferon pathway and the dysregulation of lipid metabolism are significant factors in AFB1-induced carcinogenesis, suggesting that a multifaceted approach targeting various pathways may be necessary for effective prevention and treatment of HCC.

Conclusion

The literature on AFB1 exposure underscores its status as a pervasive public health challenge, especially in developing countries where staple foods such as maize, ground nuts, and cereals are frequently contaminated. Dietary intake remains the primary exposure route, with additional significant contributions from environmental and occupational settings, particularly in agricultural and feed-processing sectors. Early-life exposures via maternal transfer, breastfeeding, and introduction of contaminated weaning foods contribute to the vulnerability of infants and young children, with exposure levels influenced by seasonal, socioeconomic, and geographic factors. Biomarker-based assessments, including aflatoxin-albumin adducts and urinary metabolites, provide robust quantitative measures, yet variations in methodologies warrant standardization to improve cross-study comparability and exposure characterization.

Biochemically, AFB1 undergoes metabolic activation primarily through cytochrome P450 enzymes producing reactive epoxide intermediates that form DNA adducts, notably targeting the TP53 tumor suppressor gene. This genotoxicity is central to the initiation and progression of HCC. Epigenetic changes, including DNA methylation alterations observed in early-life exposures, expand the understanding of AFB1's impact beyond direct mutagenesis, implicating immune modulation and growth-related pathways. Oxidative stress and inflammatory responses further mediate carcinogenesis and immune suppression, although detailed mechanistic insights in human populations remain limited. The synergistic interactions between AFB1 exposure and HBV infection notably amplify HCC risk, emphasizing the complexity of gene-environment interplay in disease etiology.

Population-level evidence consistently associates chronic AFB1 exposure with severe health outcomes, such as impaired child growth, immune dysfunction, and cognitive deficits, particularly in high-risk groups like children in sub-Saharan Africa and South Asia. These adverse effects intertwine with confounding factors including malnutrition, infections, and poverty, complicating attribution and necessitating multifaceted study designs. Immune suppression is evident across different populations, including among immunocompromised individuals, highlighting a broad spectrum of vulnerability. Occupational exposures, though studied less, reveal elevated cancer risks, underscoring the need for expanded surveillance and workplace interventions.

Comparative analyses reveal that co-exposures to other mycotoxins, such as fumonisins and ochratoxin A, may exert additive or synergistic effects on growth impairment and carcinogenesis. However, integrated risk assessments and mechanistic studies on these interactions are sparse, representing a critical knowledge gap. Intervention strategies focusing on agricultural biocontrol, improved food handling, regulatory enforcement, and public health measures, including HBV vaccination, show promise but face implementation challenges in resource-poor settings. The literature calls for standardized exposure assessment protocols, longitudinal and mechanistic research, and integrated intervention frameworks to effectively mitigate the multifaceted health risks posed by AFB1 globally.

Acknowledgments

None.

Conflict of Interest

None.

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