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RESEARCH ARTICLE

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Effects of Vernonia amygdalina Extract on the Modulation of Liver Antioxidant Enzymes, Cytokines, Adipokines, DNA Biomarkers, and Growth in Aflatoxin B1-Exposed Broiler Chickens

Olugbenga David Oloruntola[a], Samuel Adebowale Adeyeye[b], Fehintoluwa Stellamaris Oluwaniyi[a], Olumuyiwa Joseph Olarotimi[a], Deborah Adebukola Oloruntola[c], Emmanuel Kehinde Asaniyan[d], Ojurereoluwa Adebimpe Ayodele[a], Olufemi Emmanuel Adeniji[a].

[a] Animal Science Department, Adekunle Ajasin University, Akungba Akoko, Nigeria.
[b] Department of Animal Production and Health, The Federal College of Agriculture, Akure, Nigeria.
[c] Department of Medical Laboratory Science, University of Medical Sciences, Ondo City, Nigeria.
[d] Department of Animal Production and Health, Olusegun Agagu University of Science and Technology, Okiti Pupa, Nigeria.

ABSTRACT

Aflatoxicosis has emerged as a notable factor that hampers the well-being and productivity of broilers in recent times. This study examined *V. amygdalina* leaf extract's protective effects against aflatoxin B1-induced hepatoxicity and growth suppression in broiler chickens. 240 Cobb500 day-old chicks were divided into 4 groups: CONT (uncontaminated diet), AFTB1 (0.5 ppm aflatoxin B1 contamination), VE1AF (0.5 ppm aflatoxin B1 + 1 g *V. amygdalina* leaf powder/liter of water), and VE2AF (0.5 ppm aflatoxin B1 + 2 g *V. amygdalina* leaf powder/liter of water) in a completely randomized design. Liver catalase, glutathione peroxidase, and superoxide dismutase levels exhibited a marked reduction in the AFTB1 group compared to CONT and VE2AF groups ($p < 0.05$). Malondialdehyde levels, indicative of lipid peroxidation, were markedly increased in the AFTB1 group compared to all other groups ($p < 0.05$). The levels of pro-inflammatory cytokines (TNF-α, IL-6, and IFN-γ) exhibited a marked increase in the AFTB1 group compared to CONT and VE2AF groups ($p < 0.05$). Additionally, anti-inflammatory cytokines, adipokines, and oxidative DNA damage biomarkers varied significantly among treatment groups ($p < 0.05$). Both 1 g and 2 g of *V. amygdalina* leaf powder per liter of water effectively countered the detrimental effects of aflatoxin B1 on liver health in broiler chickens.

Keywords

Aflatoxicosis, Hepatoxicity mitigation, Phytochemicals, Poultry

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Abbreviations

AFB1: Aflatoxin B1
DNA: Deoxyribonucleic Acid
TNF-α: Tumor necrosis factor α
IL-6: Interleukin 6
IFN-γ: Interferon Gamma
IL-1β: Interleukin 1 Beta

Corresponding author: Olugbenga David Oloruntola
Email: olugbenga.oloruntola@aaua.edu.ng
Tel: +23(480)3584-1626

Introduction

Aflatoxicosis has recently been identified as a significant factor limiting the well-being and physiological condition of broilers [1]. Aflatoxins, which are secondary metabolic intermediates or by-products produced by certain subtypes or strains of Aspergillus fungi, pose substantial health risks to both human and animal populations globally [2]. Among these mycotoxins, aflatoxin B1 (AFB1) emerges as one of the most potent and prevalent contaminants in agricultural commodities, particularly in regions characterized by warm and humid climates such as the tropics [3]. Chronic exposure to AFB1 through contaminated feedstuffs has been linked to various adverse effects on animal health, with the liver serving as the primary target organ due to its pivotal role in detoxification and metabolism [4].

Aflatoxicosis, arising from exposure to aflatoxins, primarily manifests as hepatic damage, culminating in hepatotoxicity and the potential development of hepatocellular carcinoma and growth depression [1]. In poultry production, broiler chickens exhibit heightened susceptibility to aflatoxicosis owing to their elevated metabolic rate and rapid growth, rendering them particularly vulnerable to feed-borne mycotoxin contamination [5].

To mitigate the adverse effects of aflatoxicosis in broiler chickens, researchers have explored various strategies, among them, the utilization of dietary supplements with potential ameliorative properties [6]. One such promising candidate is the extract obtained from the leaves of *Vernonia amygdalina* using water, a plant indigenous to tropical regions renowned for its pharmacological attributes, including antioxidant and hepatoprotective effects [7]. Despite its traditional application and promising biological activities, the efficacy of bitter leaf extract as an ameliorative agent against aflatoxicosis in poultry remains largely unexplored, particularly concerning AFB1-induced hepatotoxicity.

Therefore, the current investigation aimed to assess *Vernonia amygdalina* leaf aqueous extract as a novel ameliorative agent for hepatoxicity in AFB1-exposed broiler chickens by evaluating its impact on liver function and biochemical markers of hepatic injury and growth.

Abbreviations - cont'd

8-OHdG: 8-hydroxy-2' –deoxyguanosine (ng/ml)
HPLC: High-performance liquid chromatography
CONT: No aflatoxin B1 contamination; no administration of Vernonia amygdalina extract.
AFB1: 0.5 ppm aflatoxin B1 contamination.
VE1AF: 0.5 ppm aflatoxin B1 contamination + 1 g Vernonia amygdalina leaf powder per liter of water.
VE2AF: 0.5 ppm aflatoxin B1 contamination + 2 g Vernonia amygdalina leaf powder per liter of water.
CAT: Catalase
SOD: Superoxide dismutase
GPx: Glutathione peroxidase

Results

Antioxidant enzymes and malondialdehyde in the liver

A significant decrease in catalase levels was observed in the liver of the AFTB1 treatment group compared to the CONT, VE1AF, and VE2AF groups ($p < 0.05$). Likewise, the concentration of liver glutathione peroxidase and superoxide dismutase in the broiler chickens exposed to AFTB1 treatment significantly decreased ($p < 0.05$) compared to the CONT and VE2AF. Conversely, the quantity of these enzymes in the CONT, VE1AF, and VE2AF exhibited no significant difference ($p > 0.05$) (Table 1). Moreover, the liver malondialdehyde levels in the birds subjected to AFTB1 were notably reduced ($p < 0.05$) compared to the other treatment groups.

Inflammatory cytokines in the liver

The concentrations of tumor necrosis factor α, interleukin 6, and interferon gamma in the liver were notably elevated (p <0.05) in the AFTB1 group compared to both the CONT and VE2AF groups (Table 2). Furthermore, the concentrations of these cytokines in the AFTB1 treatment group exhibited no significant difference ($p > 0.05$) compared to those in the VE1AF group. The presence of interleukin 1 Beta in the liver displayed a trend ($p = 0.09$) toward being influenced by the treatment.

Anti-inflammatory cytokine, adipokine, and oxidative DNA damage biomarkers in liver

Table 3 displays the levels of anti-inflammatory cytokines, adipokines, and oxidative DNA damage biomarkers in the livers of broilers exposed to Aflatoxin B1 and administered *Vernonia amygdalina* aqueous leaf extract. The liver adiponectin concentration of birds from the AFTB1 group was notably reduced ($p < 0.05$) compared to those in the CONT, VE1AF, and VE2AF groups. In a similar vein, liver tissue leptin levels of the AFTB1 group were significantly decreased ($p < 0.05$) compared to the CONT group. In contrast, leptin levels in the VE1AF and VE2AF showed no significant difference ($p < 0.05$) compared to the CONT. Moreover, the liver 8-hydroxy-2'-deoxyguanosine levels of AFTB1 were elevated ($p < 0.05$) compared to those in the CONT, while the levels in the VE1AF and VE2AF groups showed no significant difference ($p < 0.05$) compared to the CONT group.

Table 1.
Antioxidant enzymes and malondialdehyde in the liver of broiler chickens exposed to Aflatoxin B1 and administered *Vernonia amygdalina* aqueous leaf extract

Parameters	CONT	AFTB1	VE1AF	VE2AF	SEM	P value
Catalase (ng/mg)	18.52a	12.88b	17.56a	17.57a	0.78	0.01
Glutathione peroxidase (ng/mg)	18.51a	14.09b	16.53ab	18.39a	0.66	0.02
Superoxide dismutase (ng/mg)	68.42a	53.10b	59.57ab	70.23a	2.52	0.02
Malondialdehyde (u/mg)	2.58b	3.73a	2.47b	2.60b	0.19	0.03

Within the same row, differing superscript letters indicate significant differences ($p < 0.05$). CONT: Control; AFTB1: 0.5 ppm aflatoxin contamination; VE1AF: 0.5 ppm aflatoxin B1 contamination + 1 g *Vernonia amygdalina* leaf powder/liter of water; VE2AF: 0.5 ppm aflatoxin B_1 contamination + 2 g *Vernonia amygdalina* leaf powder/liter of water.

Table 2.
Pro-inflammatory cytokines in the liver of broiler chickens exposed to Aflatoxin B1 and administered *Vernonia amygdalina* aqueous leaf extract.

Parameters	CONT	AFTB1	VE1AF	VE2AF	SEM	P value
Tumor necrosis factor α (pg/ml)	6.97b	9.11a	8.08ab	6.95b	0.33	0.02
Interleukin 6 (pg/ml)	9.03b	11.39a	10.26ab	9.53b	0.35	0.04
Interleukin 1 Beta (pg/ml)	11.79	15.16	13.56	12.11	0.55	0.09
Interferon Gamma (pg/ml)	1.31b	1.56a	1.45ab	1.36b	0.03	0.04

Within the same row, differing superscript letters indicate significant differences ($p < 0.05$). CONT: Control; AFTB1: 0.5 ppm aflatoxin contamination; VE1AF: 0.5 ppm aflatoxin B1 contamination + 1 g *Vernonia amygdalina* leaf powder/liter of water; VE2AF: 0.5 ppm aflatoxin B1 contamination + 2 g *Vernonia amygdalina* leaf powder/liter of water.

Table 3.
Anti-inflammatory cytokine, adipokine, and oxidative DNA damage biomarkers in the liver of broiler chickens exposed to Aflatoxin B1 administered *Vernonia amygdalina* aqueous leaf extract.

Parameters	CONT	AFTB1	VE1AF	VE2AF	SEM	P value
Adiponectin (ng/ml)	12.45a	8.66b	11.45a	11.70a	0.53	0.03
Leptin (ng/ml)	1.55a	1.29b	1.44ab	1.52ab	0.03	0.04
8-hydroxy-2' –deoxyguanosine (ng/ml)	12.40b	16.32a	14.24ab	12.70b	0.59	0.04

Within the same row, differing superscript letters indicate significant differences ($P < 0.05$). CONT: Control; AFTB1: 0.5 ppm aflatoxin contamination; VE1AF: 0.5 ppm aflatoxin B1 contamination + 1 g *Vernonia amygdalina* leaf powder/liter of water; VE2AF: 0.5 ppm aflatoxin B1 contamination + 2 g *Vernonia amygdalina* leaf powder/liter of water.

The Relative Growth Rate

The Relative Growth Rate (RGR) of broiler chickens exposed to Aflatoxin B1 (AFB1) contamination and treated with *Vernonia amygdalina* aqueous leaf extract showed variation among the different experimental groups (Figure 1). Broilers in the CONT had a similar ($p < 0.05$) RGR to the VE1AF (0.5 ppm AFB1 + 1 g/L *V. amygdalina*) and VE2AF 0.5 ppm AFB1 + 2 g/L *V. amygdalina*). The broiler chickens in the AFTB1 group (0.5 ppm AFB1 contamination without supplementation) had the lowest RGR compared to those in CONT, VE1AF, and VE2AF.

Discussion

Catalase is an important antioxidant enzyme responsible for catalyzing the breaking down of hydrogen peroxide into oxygen and water, thereby shielding cells from oxidative harm. The notable decrease observed in liver catalase presence within the AFTB1

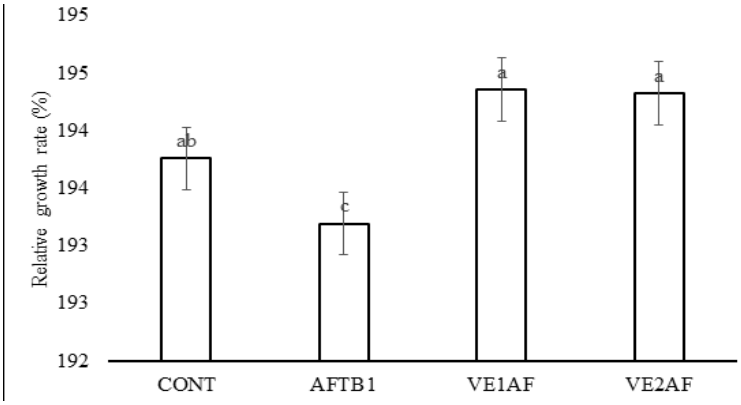

Figure 1.
Relative growth rate of broiler chickens exposed to Aflatoxin B1 and administered *Vernonia amygdalina* aqueous leaf extract.
CONT: Control; AFTB1: 0.5 ppm aflatoxin contamination; VE1AF: 0.5 ppm aflatoxin B1 contamination + 1 g V*ernonia amygdalina* leaf powder/litre of water; VE2AF: 0.5 ppm aflatoxin B1 contamination + 2 g *Vernonia amygdalina* leaf powder/litre of water.

group, as opposed to both the CONT and Vernonia amygdalina-treated groups, implies that exposure to AFB1 could potentially compromise antioxidant defense mechanisms, likely as a result of heightened oxidative stress. AFB1 is known to stimulate the reactive oxygen species (ROS) production within the liver [1, 2], which can overwhelm the antioxidant capacity of catalase, leading to its depletion [8]. However, the administration of Vernonia amygdalina aqueous leaf extract may help alleviate this oxidative stress by providing additional antioxidant compounds that can scavenge ROS and support the activity of catalase [9]. Glutathione peroxidase and superoxide dismutase play crucial roles as enzymes with antioxidant properties that scavenge reactive ROS and shield cells from oxidative harm. The significant decrease in liver glutathione peroxidase and liver superoxide dismutase levels in the AFTB1 compared to the control (CONT) and VE2AF groups suggests a compromised antioxidant defense system in response to AFB1 exposure. This could be attributed to the depletion of antioxidant reserves or inhibition of enzyme activity by AFB1-induced oxidative stress. Interestingly, the resemblance in enzyme levels between the AFTB1 and VE1AF groups suggests that administration of Vernonia amygdalina aqueous leaf extract, particularly at the lower concentration, may partially mitigate the decrease in glutathione peroxidase and superoxide dismutase levels induced by AFB1 [10]. Vernonia amygdalina is known to contain various active compounds including flavonoids, alkaloids, and phenolic compounds, which possess potent antioxidant properties. These compounds may enhance the antioxidant capacity of the liver and help replenish the levels of glutathione peroxidase and superoxide dismutase in the presence of AFB1-induced oxidative stress [7]. These bioactive compounds present in Vernonia amygdalina leaf extract could scavenge free radicals and reactive oxygen species generated by aflatoxin B1 exposure, and could also trigger the production or activation of antioxidant enzymes like catalase, glutathione peroxidase, and superoxide dismutase in the liver mediated through stimulation of transcription factors such as nuclear factor erythroid 2-related factor 2 (Nrf2), which governs the expression of antioxidant enzymes and other protective genes within the cell [11; 12].

Malondialdehyde (MDA) is a marker of lipid peroxidation and oxidative damage. The unexpected decrease in liver MDA levels in the AFTB1 compared to the other groups is intriguing. One possible explanation could be the activation of compensatory antioxidant mechanisms in response to AFB1-induced oxidative stress [13]. It is plausible that the administration of Vernonia amygdalina aqueous leaf extract, particularly at higher concentrations, may stimulate the synthesis or enhance the activity of antioxidant enzymes and other defense mechanisms, leading to the reduction of lipid peroxidation and MDA formation in the liver [14]. Additionally, Vernonia amygdalina contains bioactive substances including sesquiterpene lactones and flavonoids, which have been documented to exhibit both anti-inflammatory and antioxidant characteristics. These compounds may directly scavenge free radicals and inhibit lipid peroxidation, contributing to the observed decrease in liver MDA levels [15].

Tumor Necrosis Factor α (TNF-α), Interleukin 6 (IL-6), and Interferon Gamma (IFN-γ) are cytokines known for their pro-inflammatory effects that play crucial roles in facilitating inflammatory reactions [16]. The notably elevated levels of these cytokines in the livers of broilers in the AFTB1, in comparison to the CONT and VE2AF groups, suggest a robust inflammatory reaction triggered by Aflatoxin B1 contamination. Aflatoxin B1 is known to trigger inflammation in the liver by activating NF-κB and other inflammatory signaling pathways [17]. The observed elevation in TNF-α, IL-6, and IFN-γ presence corroborates previous findings linking Aflatoxin B1 exposure to hepatic inflammation. Administration of Vernonia amygdalina aqueous leaf extract appeared to counteract the rise in pro-inflammatory cytokine levels caused by Aflatoxin B1 contamination [6]. The concentrations of TNF-α, IL-6, and IFN-γ in the CONT were similar to those in the VE1AF group, suggesting a potential safeguarding impact of Ver-

nonia amygdalina against inflammation triggered by Aflatoxin B1. Vernonia amygdalina contains various bioactive compounds, such as flavonoids, alkaloids, and phenolic compounds, known for their anti-inflammatory properties. These compounds may modulate inflammatory signaling pathways and attenuate the production of pro-inflammatory cytokines in response to AFB1 exposure [7].

While not reaching statistical significance ($p = 0.09$), there was an observed trend indicating a potential impact of treatment on liver Interleukin 1 Beta (IL-1β) levels. Interleukin 1 Beta is another crucial pro-inflammatory cytokine known for its involvement in regulating immune responses and inflammatory pathways. The observed trend suggests a potential influence of AFB1 contamination and Vernonia amygdalina administration on IL-1β levels, warranting further investigation to elucidate its significance.

Adiponectin is known for its anti-inflammatory and insulin-sensitizing properties [18]. The significant decrease in liver adiponectin levels in the AFTB1 treatment group suggests a disruption in adiponectin signaling induced by AFB1 contamination. This disruption could contribute to metabolic dysfunction and inflammation in the liver [19]. Vernonia amygdalina is rich in bioactive compounds, which have demonstrated anti-inflammatory properties. These compounds may help restore adiponectin levels by modulating inflammatory pathways and improving insulin sensitivity [20]. Additionally, Vernonia amygdalina has been reported to enhance glucose metabolism and insulin sensitivity in various animal models [21], further supporting its potential to counteract the effects of Aflatoxin B1-induced adiponectin dysregulation.

Leptin plays a vital function in regulating energy equilibrium and metabolism [22]. A notable decrease in liver leptin levels in the AFTB1 treatment group suggests a disruption in leptin signaling induced by AFB1 contamination. Leptin resistance and decreased leptin levels are commonly observed in liver diseases, including those induced by aflatoxicosis [23]. Vernonia amygdalina contains bioactive compounds like flavonoids and sesquiterpene lactones, which have demonstrated the ability to regulate leptin signaling and enhance leptin sensitivity. [24]. These compounds may help restore leptin levels and mitigate the adverse effects of AFB1 on energy metabolism and lipid homeostasis in the liver.

8-Hydroxydeoxyguanosine is a biomarker for oxidative DNA damage, reflecting elevated hepatic oxidative stress [25]. The significant increase in liver 8-OHdG levels in the AFTB1 group indicates oxidative DNA damage induced by AFB1 contamination. Vernonia amygdalina is abundant in antioxidant compounds like phenolic acids, flavonoids, and tannins, which exhibit strong capabilities in scavenging free radicals. These compounds may help mitigate oxidative stress and prevent DNA damage by neutralizing reactive oxygen species (ROS) generated by AFB1 exposure [26]. Moreover, Vernonia amygdalina has been shown to boost the activity of endogenous antioxidant enzymes like superoxide dismutase and catalase [27], thereby reinforcing its protective effects against AFB1-induced oxidative DNA damage. Vernonia amygdalina leaf extract comprises a diverse array of bioactive compounds encompassing alkaloids, flavonoids, phenolics, and terpenoids, which jointly contribute to its potential to prevent hepatic DNA damage in broilers exposed to aflatoxin B1 by scavenging free radicals, inhibiting lipid peroxidation, and reducing inflammation, thereby mitigating oxidative stress [17]. Additionally, some compounds may chelate metal ions involved in reactive oxygen species generation, further reducing oxidative damage. These bioactive compounds may also directly interact with DNA, stabilizing its structure and preventing damage [28]. Together, these mechanisms help protect DNA molecules in the liver from the harmful effects of aflatoxin B1 exposure, making Vernonia amygdalina leaf extract a promising candidate for preventing hepatotoxicity and carcinogenesis in broiler chickens.

Vernonia amygdalina leaf extract demonstrates potential as a protective agent against aflatoxin-induced hepatoxicity in broiler chickens. Supplementation with the extract effectively modulates antioxidant enzyme activity, pro-inflammatory cytokine levels, and oxidative DNA damage biomarkers, mitigating liver damage associated with aflatoxin exposure. However, further research is needed to optimize dosage regimens and evaluate long-term effects in commercial poultry production. Incorporating Vernonia amygdalina supplementation may offer a promising strategy to enhance liver health and welfare in broiler production systems.

Broilers in the AFTB1 group exhibited the lowest RGR, which aligns with previous studies demonstrating that aflatoxins negatively impact growth performance in poultry [5]. Aflatoxin B1, the most toxic aflatoxin, exerts its adverse effects primarily through hepatic dysfunction, oxidative stress, and immunosuppression [2]. Upon ingestion, AFB1 undergoes bioactivation in the liver via cytochrome P450 enzymes, leading to the formation of the reactive metabolite Aflatoxin B1-8,9-epoxide (AFBO), which forms adducts with DNA and proteins, thereby impairing cellular function and protein synthesis [29]. Consequently, broilers exposed to AFB1 suffer from reduced feed intake, poor nutrient absorption, hepatic damage, and suppressed immune respons-

es, all of which contribute to growth retardation [2, 30]. The significant reduction in RGR observed in the AFTB1 group compared to the CONT group confirms the growth-depressing effect of aflatoxin B1 contamination [2, 30]. A key observation in this study was the restorative effect of *V. amygdalina* supplementation. Broilers in the VE1AF (0.5 ppm AFB1 + 1 g/L *V. amygdalina*) and VE2AF (0.5 ppm AFB1 + 2 g/L *V. amygdalina*) groups exhibited improved RGR compared to the AFTB1 group, suggesting that *V. amygdalina* may serve as a natural detoxifier against aflatoxin-induced growth suppression. The ameliorative effect of *V. amygdalina* can be attributed to its rich phytochemical profile, which includes flavonoids, alkaloids, phenols, saponins, tannins, and terpenoids [6, 31]. The observation that the VE2AF group had the highest relative growth rate (RGR) indicates that a higher dosage (2 g/L) of *V. amygdalina* offered a more significant protective effect compared to the lower dosage (1 g/L). This dose-dependent response aligns with previous research showing that greater concentrations of *V. amygdalina* extract lead to improved antioxidant capacity and metabolic function in animals exposed to toxins [6].

Materials & Methods

Ethical Approval and Experimental Site

The protocol for animal care and use received approval from the Animal Welfare and Use Committee in the Department of Animal Science at Adekunle Ajasin University, located in Akungba Akoko, Nigeria [2]. The research was conducted at the Avian Experimental Unit located within the Teaching and Research Farm at Adekunle Ajasin University, Akungba Akoko, Nigeria [2]. The feeding trial took place during the dry season, specifically between December 2023 and January 2024.

Preparation of Vernonia amygdalina Leaf powder

Fresh Vernonia amygdalina leaves were harvested from their parent plants, within the premises of the Crop Production Unit of Adekunle Ajasin University Teaching and Research Farm, Akungba Akoko, Nigeria. The leaves were washed with clean water, drained, sliced into smaller segments, and laid out on polythene sheets to air-dry under shade for a period of 7 days. Following drying, the leaves were finely ground into Vernonia amygdalina leaf powder using an electric blender, and subsequently stored in an airtight container until required for use.

Aflatoxin B1, Experimental Diet Composition, and Aqueous Extract Preparation

Aflatoxin B1 (AFB1) was produced by growing Aspergillus flavus on coarse maize meal and quantified for AFB1 levels utilizing a high-performance liquid chromatography (HPLC) system, comprising a model 600 pump, model 470 scanning fluorescence detector, 717 autosampler, and in-line degasser [32]. Standard diets for the starter (1-21 days) and finisher (22-42 days) stages of broiler production were formulated [30, 33] and are presented in Table 4. Each phase's diets were divided into four equivalent shares (named treatments 1 to 4). The first portion remained uncontaminated with aflatoxin B1, while the remaining portions (2, 3, and 4) were contaminated with aflatoxin B1 to a level of 0.5 ppm, following the procedures described by Olarotimi et al. [6].

One gram (1g) of *Vernonia amygdalina* leaf powder was immersed in one liter of warm water (70°C) for 24 hours, and this procedure was repeated daily. The preparation was subsequently filtered using a muslin cloth to segregate debris from the filtrate, resulting in the production of 1g/liter *Vernonia amygdalina* aqueous extract (VE1), which was stored in clean containers. The same procedure was employed to produce 2g/liter *Vernonia amygdalina* aqueous extract (VE2).

Experimental Birds and Treatments

The present investigation was conducted using 240 one-day-old chicks (mixed sex) from the Cobb 500 broiler breed. The day-old broiler chicks were randomly allocated into four distinct experimental treatment groups, with each group comprising 60 birds (6 replicates per treatment, with 10 birds per replicate). Birds in the first treatment group (CONT) were fed an uncontaminated diet, while those in the remaining treatment groups (AFB1, VE1AF, and VE2AF) were exposed to 0.5 ppm of aflatoxin B1 in their feed. Additionally, birds in treatment groups 3 and 4 were orally administered VE1 and VE2, respectively. The treatment details are summarized as follows:

- CONT: No aflatoxin B1 contamination; no administration of Vernonia amygdalina extract.
- AFB1: 0.5 ppm aflatoxin B1 contamination.
- VE1AF: 0.5 ppm aflatoxin B1 contamination + 1 g *Vernonia amygdalina* leaf powder per liter of water.
- VE2AF: 0.5 ppm aflatoxin B1 contamination + 2 g *Vernonia amygdalina* leaf powder per liter of water.

The ambient conditions within the experimental enclosure were meticulously regulated throughout the observation period. For the initial 7 days of the feeding experiment, the temperature in the experimental enclosure was upheld at 31 ± 2°C. Subsequently, from day 8 to day 27 of the study, the ambient temperature was gradually decreased by 2°C per week. During the final phase of the rearing period, from day 28 to day 42, the broiler chickens were subjected to the natural ambient temperature of their environment.

Additionally, at the beginning of the feeding trial, the illumination in the experimental enclosure followed a daily pattern comprising six hours of darkness over the course of each 24-hour period. The illumination regimen remained consistent for up to 3 days before culling.

Sample collection and analysis

On the 42nd day of the trial, eighteen birds were chosen randomly from each treatment (one bird per replicate) and euthanized. Following euthanasia, the carcasses underwent spray-washing and were subsequently cooled for 30 minutes at 2°C. The livers of the selected broiler chickens (three per replicate) were then excised for hepatotoxicity studies immediately after slaughter. The liver tissue was collected using a sharp, sterile knife and rinsed with a cold phosphate-buffered solution to eliminate any blood or debris.

Liver tissue homogenates were prepared by homogenizing 20% liver samples from each broiler chicken at 4°C in 0.15 M KCl solution. Subsequently, the homogenates underwent centrifugation at 12,000 rpm for 45 minutes at 0 to 4°C to obtain the supernatant, as described by Venkatanarayana et al. [33]. The supernatants were subjected to a variety of analyses as outlined hereafter:

Malondialdehyde (MDA), glutathione peroxidase (GPx), catalase, and superoxide dismutase (SOD) levels were quantified according to methods previously described by Jimoh [34]. To measure SOD activity, 2.1 ml of 50 mM buffer, 0.02 ml of enzyme source, and 0.86 ml of distilled water were combined to create a reaction mixture. Adding 0.02 ml of 10 mM pyrogallol initiated the reaction, and the change in absorbance was monitored at 420 nm. In a conventional assay setup

with three milliliters, one unit of SOD activity is equivalent to the quantity of enzyme required to reduce the auto-oxidation of pyrogallol by fifty percent. SOD's specific activity is measured in units per minute per protein nanogram. The test setup consisted of 1.0 ml of 0.059 M H_2O_2 and 1.9 ml of 0.05 M buffer at pH 7.0 to assess catalase activity. The enzyme source was added in an amount of 0.1 ml to initiate the reaction. For five minutes, the absorbance drop was observed at 240 nm at one-minute intervals. The amount of H_2O_2 that is broken down into nanomoles per minute per nanogram of protein is the catalase activity. A reaction mixture containing 0.5 ml of 0.4 M buffer at pH 7.0, 0.2 ml of enzyme source, 0.2 ml of 2 mM GSH, and 0.1 ml of 0.2 mM H_2O_2 was created to assess glutathione peroxidase activity. After that, this combination was incubated for ten minutes at room temperature. Additionally, a control tube was created with all the reagents removed, save for the enzyme source. After adding 0.5 ml of 10% TCA and centrifuging for five minutes at 4000 rpm, the reaction was stopped. Next, the amount of glutathione (GSH) in 0.5 milliliters of the supernatant was calculated. Per milligrams of protein, glutathione peroxidase activity is reported as micrograms of GSH used per minute. Preparing a reaction mixture with a total volume of 3.0 ml, comprising 1.0 ml of serum and 1.0 ml of TCA (0.67%), was necessary for the lipid peroxidation assay. After that, each test tube spent 45 minutes submerged in boiling water. The tubes were then placed in a cold bath and centrifuged for ten minutes at 2500 rpm. A measurement of the optical density of the supernatant at 532 nm was used to calculate the amount of malondialdehyde (MDA) generated in each sample.

Utilizing a Rat IL-6 ELISA kit from Elabscience Biotechnology Inc., USA, interleukin 6 (IL-6) levels were determined. The levels of tumor necrosis factor-alpha (TNF-α) were measured with an ELISA kit from Elabscience Biotechnology Inc. in the United States. Using the Quantikine Human IFN-γ Immunoassay from R&D Systems, Minneapolis, MN, USA, interferon-gamma (IFN-γ) levels were ascertained. The Human IL-1 ELISA MAX Deluxe kit from BioLegend, San Diego, CA, USA, was used to measure the quantities of interleukin-1 beta (IL-1β). The lipHsp70 ELISA technique, as reported by Breuninger et al. [35], was utilized to measure the amounts of heat shock protein (HSP 70) present in the liver. An ELISA kit from Millipore, USA, was used to measure the levels of serum adiponectin. The method outlined by Zhang et al. [36] was used to assess the amounts of 8-hydroxy-2'-deoxyguanosine (8-OHdG). Utilizing the Human Leptin ELISA kit from Abcam, Shanghai, China, the concentration of leptin was ascertained. The Human NFKB-p65 ELISA Kit from Elabscience, USA, was used to measure the levels of Nuclear Factor Kappa B-p65 (NFKB-p65).

At the beginning of the feeding trial (Day 1) and at the conclusion of the study (Day 42), the body weights of the broiler chicks were measured and documented. The Relative Growth Rate (RGR) was computed based on the formula previously outlined by Oloruntola et al. [2]:

RGR = [(wt2 - wt1) / ((wt1 + wt2) / 2)] * 100

Where Wt_1 represents the initial body weight of the broiler chicks before the commencement of the experiment, and Wt_2 corresponds to their final weight at the end of the study.

Table 4.
Components of the test diets

Ingredients (%)	Starter Phase (1- 28 days)	Finisher phase (28 – 42 days)
Maize	51.35	56.35
Maize bran	3.00	5.92
Rice bran	0.00	2.00
Fish meal	3.00	2.90
Soybean meal	37.00	26.95
Bone meal	3.00	3.10
Premix	0.31	0.31
Limestone	0.49	0.50
Salt	0.31	0.32
Lysine	0.24	0.25
Methionine	0.30	0.30
Soy oil	1.00	1.10
Composition (%)		
Metabolizable energy (Kcal/kg)	2920	3051
Available phosphorus	0.58	0.36
Calcium	0.94	0.74
Crude fibre	3.52	3.57
Crude fat	4.23	2.38
Crude protein	22.00	19.00
Methionine	0.48	0.45
Lysine	1.25	1.04

Statistical data analysis

SPSS v.20 software was used to do an analysis of variance (ANOVA) on the data. The Duncan multiple range test, which was included in the same program, was then used to see if the treatment means differed significantly from one another.

Authors' Contributions

O.D.O., S.A.A., and F.S.O. conceived and planned the experiments. F.O.S., D.A.O., and E.K.A. produced the aflatoxin B1 cultured maize. O.J.O., A.B.F., O.E.A., and O.A.A. contributed to the preparation of samples for analysis. O.D.O., F.O.S., D.A.O., and E.K.A. contributed to the laboratory analysis of samples. All authors carried out the experiments and contributed to the interpretation of the results. O.D.O. took the lead in writing the manuscript, while E.K.A. revised the first draft. All authors reviewed the final manuscript.

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Conflict of interest

The authors declare that there is no conflict of the interest

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