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Bitter leaf extract modulates antioxidant enzymes, immunoglobulins, organ weights, meat oxidation, and hepatic stress markers in broiler chickens exposed to aflatoxin B1

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ABSTRACT

Background: This study examined the effects of bitter leaf extract on the oxidative enzymes, stress markers, immunoglobulins, organ weights, meat oxidation, and liver health in broiler chickens exposed to aflatoxin-B1. **Methods**: 240 day-old mixed-sex Cobb-500 broiler chickens were randomly assigned to the following treatment groups: CONT (Control); BE0AF (0.5 mg/kg aflatoxin-B1); BE1AF (0.5 mg/kg aflatoxin B1 + 1 g bitter leaf powder/ L H2O) or BE2AF (0.5mg/kg aflatoxin-B1 + 2 g bitter leaf powder/ L H2O), each with 60 chickens (6 replicates of 10 chickens). **Findings:** BE0AF significantly ($P<0.05$) lowered the serum superoxide dismutase (SOD) and higher malondialdehyde (MDA) levels compared to untreated chickens. BE2AF resulted in similar MDA levels compared to chickens in the BE1AF and CONT groups. BE0AF group showed higher ($P<0.05$) lactate dehydrogenase (LDH) and lower catalase and glutathione peroxidase (GPx) levels than other groups. Immunoglobulin G levels were notably ($P<0.05$) lower in the BE0AF group, while immunoglobulins M and A were significantly ($P<0.05$) lower compared to the BE3AF and BE2AF groups. Liver and spleen weights were higher in the aflatoxin group, and meat catalase was lower ($P<0.05$) in BE0AF. Lipid and protein oxidation were higher ($P<0.05$) in BE0AF compared to CONT. Furthermore, liver HSP70, NF-κB, and LDH levels were higher ($P<0.05$) in BE0AF. **Conclusion**: Oral administration of bitter leaf extract of 1-2g/ L H2O bitter leaf powder mitigated oxidative stress and protected against aflatoxin-induced damage by improving various physiological markers in broiler chickens. **Novelty/Originality of this article:** This study was to assess the impact of bitter leaf aqueous extract on organ weights, immunoglobulin levels, serum biomarkers, meat oxidation, hepatic oxidative enzymes, and nuclear factor kappa B in broiler chickens exposed to AFB_1.

KEYWORDS: hepatoxicity; mycotoxin; oxidative stress; phytochemicals; poultry products.

1. Introduction

Poultry farming plays a pivotal role in ensuring global food security by providing affordable, high-quality animal protein in the form of meat and eggs. The rapid growth rate, high feed efficiency, and relatively low production costs of broiler chickens have positioned poultry as one of the most important livestock sectors worldwide. In developing and tropical countries, poultry production is particularly significant for bridging the protein gap, improving livelihoods, and supporting rural and peri-urban economies. However, despite these advantages, the sustainability, productivity, and profitability of the poultry industry

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are increasingly threatened by multiple stressors, including climatic challenges, disease pressure, and feed-related constraints.

Among feed-related challenges, feed-borne contaminants remain a major obstacle to optimal poultry production. Of particular concern are mycotoxins, toxic secondary metabolites produced by filamentous fungi that commonly contaminate cereal grains and oilseed meals used in poultry diets. Mycotoxin contamination is difficult to eliminate completely due to the widespread occurrence of fungal spores, inadequate storage conditions, and fluctuating environmental factors. Consequently, mycotoxins continue to pose serious risks to animal health, production efficiency, and food safety across the global poultry value chain (Oloruntola, 2024).

Aflatoxin B_1 (AFB_1), a highly toxic secondary metabolite produced predominantly by Aspergillus flavus and Aspergillus parasiticus, is considered the most potent and prevalent mycotoxin contaminating poultry feeds, especially in tropical and subtropical regions where high temperature and humidity favour fungal proliferation (Fouad et al., 2019). Climatic conditions in these regions, characterized by prolonged rainy seasons, high ambient temperature, and inadequate post-harvest drying and storage infrastructure, create an ideal environment for aflatoxin contamination. Owing to the heavy reliance on cereals and oilseed meals in poultry diets, broiler chickens are particularly vulnerable to chronic exposure to AFB_1, even at subclinical levels that may not immediately manifest overt clinical signs (Amminikutty et al., 2023).

AFB_1 contamination poses serious challenges not only to animal health and welfare but also to meat quality, food safety, and public health. In broiler chickens, AFB_1 exposure has been widely associated with immunosuppression, oxidative stress, metabolic dysregulation, impaired growth performance, and increased susceptibility to infectious diseases (Fouad et al., 2019). These adverse effects are often dose- and duration-dependent, with chronic low-level exposure producing insidious yet economically significant consequences. Such physiological disturbances translate into reduced productivity, inferior carcass characteristics, and compromised meat quality, resulting in substantial economic losses for the poultry industry. Furthermore, the carryover of aflatoxin residues or its metabolites into edible tissues represents a potential health risk to consumers, emphasizing the need for effective mitigation strategies to protect both animal and human health.

One of the principal mechanisms underlying AFB_1 toxicity is the induction of oxidative stress. Following ingestion, AFB_1 is bioactivated in the liver by cytochrome P450 enzymes to the highly reactive aflatoxin B_1-8,9-epoxide, which can bind covalently to cellular macromolecules such as DNA, proteins, and lipids. This bioactivation process leads to excessive generation of reactive oxygen species (ROS), overwhelming the endogenous antioxidant defence systems of broiler chickens (Jobe et al., 2023). When the balance between pro-oxidants and antioxidants is disrupted, oxidative stress ensues, initiating a cascade of cellular and molecular damage.

Elevated ROS production promotes lipid peroxidation, protein oxidation, and membrane destabilization across multiple tissues, including the liver, immune organs, blood, and skeletal muscles. Lipid peroxidation products such as malondialdehyde serve as biomarkers of oxidative damage and are indicative of compromised cellular integrity. Protein oxidation alters enzyme activity and structural proteins, while membrane damage disrupts nutrient transport and cellular signaling. Collectively, these alterations impair physiological homeostasis and exacerbate the toxic effects of AFB_1 in broiler chickens.

The deleterious impact of AFB_1-induced oxidative stress on meat quality is of particular concern. Muscle tissues rich in polyunsaturated fatty acids are highly susceptible to oxidative degradation. Lipid peroxidation in broiler meat accelerates oxidative rancidity, disrupts cellular membrane integrity, and compromises protein functionality, ultimately resulting in meat discolouration, off-flavour development, texture deterioration, and reduced shelf life during processing and storage (Chen et al., 2022). These oxidative changes negatively affect consumer acceptance and market value of poultry products, particularly in markets that demand high-quality, minimally processed meat.

Moreover, oxidative protein damage can impair water-holding capacity and tenderness, further diminishing meat quality attributes. Such changes are not only detrimental to sensory characteristics but also influence technological properties important for meat processing. Consequently, strategies aimed at reducing oxidative stress in broiler chickens have become a central focus in efforts to improve meat quality and extend shelf life, particularly under conditions of dietary or environmental stress.

Beyond its effects on muscle tissues, AFB_1 exerts pronounced hepatotoxic effects in broiler chickens. The liver, as the primary organ responsible for detoxification and metabolism, is particularly vulnerable to AFB_1-induced injury. Hepatic damage is often reflected by altered serum biochemical markers, including elevated levels of lactate dehydrogenase (LDH), alanine aminotransferase, and other cytosolic enzymes, indicating compromised membrane integrity and hepatocellular leakage. Prolonged exposure can also result in hepatomegaly, fatty liver changes, and impaired metabolic function.

Additionally, AFB_1 exposure has been linked to increased expression of stress-related proteins such as heat shock protein 70 (HSP70) and the activation of nuclear factor kappa B (NF-κB), a key transcription factor regulating inflammatory and immune responses (Jobe et al., 2023). Activation of NF-κB promotes the transcription of pro-inflammatory cytokines, thereby exacerbating inflammatory damage in hepatic and extrahepatic tissues. Persistent inflammation further aggravates oxidative stress, creating a vicious cycle that accelerates tissue injury and functional decline.

AFB_1-induced immunosuppression further compounds its toxicity in broiler chickens. The toxin impairs both humoral and cellular immune responses by disrupting lymphoid organ development, reducing immunoglobulin synthesis, and altering cytokine production. Decreased levels of immunoglobulins weaken the birds' defence against pathogens, predisposing them to secondary infections and increased mortality. Such immunological impairments not only affect bird health and welfare but also compromise vaccination efficacy and disease control programmes.

These immune alterations are often accompanied by changes in immune organ weights, such as the bursa of Fabricius, thymus, and spleen, reflecting impaired immune development and function. The cumulative effects of immunosuppression, oxidative stress, and metabolic disruption underscore the multifaceted nature of AFB_1 toxicity and highlight the need for integrated mitigation strategies.

Given the significant health risks associated with AFB_1 exposure and the economic implications for the poultry industry, the development of effective preventive and mitigation strategies is imperative. Conventional approaches, such as chemical detoxification, adsorbents, and strict feed quality control, have shown variable efficacy and may raise concerns related to cost, nutrient binding, or environmental safety. In some cases, these methods may reduce feed efficiency or interfere with the bioavailability of essential nutrients. Consequently, there has been growing interest in natural, sustainable alternatives capable of alleviating AFB_1-induced toxicity without adverse side effects. Such alternatives are increasingly favoured due to consumer demand for antibiotic-free and environmentally friendly poultry production systems.

In recent years, phytogenic feed additives and phytochemicals have emerged as promising candidates for mitigating mycotoxin-induced damage in poultry production systems (Oloruntola, 2024). Phytogens, derived from herbs, spices, and medicinal plants, contain diverse bioactive compounds, including polyphenols, flavonoids, terpenoids, saponins, and alkaloids, which exhibit potent antioxidant, anti-inflammatory, hepatoprotective, and immunomodulatory properties. These compounds exert their protective effects through multiple mechanisms, such as scavenging free radicals, enhancing endogenous antioxidant enzyme activity, modulating detoxification pathways, and regulating inflammatory signalling cascades (Surai et al., 2019).

In the liver, phytogenic compounds have been shown to attenuate AFB_1-induced oxidative stress by upregulating antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, while simultaneously reducing lipid peroxidation and inflammatory responses (Machado et al., 2023). Furthermore, phytochemicals can

modulate key cellular signalling pathways, including NF-κB and mitogen-activated protein kinase pathways, thereby suppressing pro-inflammatory cytokine expression and limiting tissue damage. In the immune system, phytogens enhance immunoglobulin production, improve immune cell function, and restore immune homeostasis disrupted by mycotoxin exposure (Machado et al., 2023; Oloruntola, 2024).

Phytogenic supplementation has also demonstrated efficacy in improving meat oxidative stability in broiler chickens exposed to oxidative stressors. By reducing ROS accumulation and lipid peroxidation in muscle tissues, phytochemicals help preserve meat colour, flavour, and shelf life, ultimately delivering safer and higher-quality poultry products to consumers. These attributes position phytogens as viable natural alternatives to synthetic antioxidants and chemical detoxifying agents.

Among the various phytogenic candidates, bitter leaf (*Vernonia amygdalina*) has attracted considerable attention due to its rich phytochemical profile and wide use in traditional medicine. Bitter leaf extract is well known for its antioxidant, anti-inflammatory, hepatoprotective, and immunomodulatory properties (Ugbogu et al., 2021). Bioactive compounds identified in *Vernonia amygdalina* include phenolic acids, flavonoids, sesquiterpene lactones, alkaloids, and saponins, all of which contribute to its biological activities. These compounds have been reported to enhance antioxidant defence, modulate immune responses, and protect against toxin-induced organ damage in experimental models.

Despite the established pharmacological potential of bitter leaf extract, empirical evidence regarding its efficacy in mitigating AFB_1-induced toxicity in broiler chickens remains limited. In particular, there is a paucity of studies examining its influence on immunoglobulin responses, serum biochemical alterations, hepatic oxidative enzymes, NF-κB activation, and meat oxidative stability in the context of aflatoxin exposure. Addressing these gaps is essential for elucidating the mechanistic basis of bitter leaf-mediated protection and for validating its practical application as a natural feed or water additive in poultry production systems.

Therefore, the present study was designed to evaluate the effects of bitter leaf aqueous extract on organ weights, immunoglobulin levels, serum biomarkers, meat oxidative stability, hepatic oxidative enzymes, and nuclear factor kappa B activation in broiler chickens exposed to aflatoxin B_1. By integrating biochemical, immunological, and oxidative stress indices, this study aims to provide mechanistic insights into the protective potential of bitter leaf extract against AFB_1-induced toxicity and to contribute to the development of sustainable, plant-based strategies for improving poultry health, product quality, and food safety.

2. Methods

2.1 Preparation of bitter leaf aqueous extract

This study was conducted at the Avian Experimental Unit of the University Teaching and Research Farm. The feeding trial was carried out during the dry season, between December 2023 and January 2024. Fresh bitter leaves were harvested from the Crop Production Unit of the AAUA farm and thoroughly washed under running tap water to remove adhering dirt and debris. The leaves were sliced into smaller portions, air-dried at ambient room temperature for seven days under shade to prevent nutrient degradation, milled into fine powder using an electric grinder, and stored in airtight containers under refrigeration until extraction.

2.2 Aflatoxin B1, composition of experimental diet, and preparation of aqueous extract

Aspergillus flavus was cultured on sterilized maize grits to produce aflatoxin B_1 (AFB_1), following established mycotoxin production protocols. After sufficient fungal growth and toxin production, the contaminated maize substrate was processed, and the concentration

of AFB_1 was quantified to confirm the desired contamination level, as previously described by Oloruntola et al. (2024). The quantified AFB_1-contaminated maize was subsequently incorporated into experimental diets to achieve the targeted aflatoxin concentration.

Standard basal diets were formulated to meet or exceed the nutrient requirements of broiler chickens during the starter (1–21 days) and finisher (22–42 days) phases, in accordance with recommended nutritional guidelines. The ingredient composition and calculated nutrient contents of the diets are presented in Table 1. For each production phase, the prepared diets were divided into four equal portions, labelled portions 1, 2, 3, and 4, to facilitate dietary treatments.

Table 1. Composition of the experimental diets

Ingredients (%)	Broiler Starter Phase (1-3 weeks)	Broiler Grower Phase (4-6 weeks)
Maize	50.36	58.36
Maize bran	3.00	0.00
Rice bran	0.00	3.02
Fish meal	3.00	3.00
Soybean meal	38.00	30.00
Bone meal	3.00	3.00
Premix	0.31	0.31
Limestone	0.49	0.47
Salt	0.31	0.31
Lysine	0.24	0.24
Methionine	0.29	0.29
Soy oil	1.00	1.00
Composition (%)		
Metabolizable energy (Kcal/kg)	2910	3053
Available phosphorus	0.59	0.38
Calcium	0.96	0.74
Crude fibre	3.52	3.58
Crude fat	4.23	2.38
Crude protein	22.00	19.00
Methionine	0.49	0.45
Lysine	1.28	1.07

In line with the protocol described by Olarotimi et al. (2023), portion 1 served as the uncontaminated control diet and was not treated with aflatoxin. Portions 2, 3, and 4 were deliberately contaminated with AFB_1 at a concentration of 0.5 mg/kg feed by thoroughly mixing the quantified AFB_1-contaminated maize grits into the basal diet to ensure uniform distribution of the toxin. Adequate mixing was carried out to minimize variation in aflatoxin exposure among birds within each treatment group.

Bitter leaf aqueous extract was prepared daily to ensure freshness and bioactivity of the phytochemicals. Specifically, one gram (1 g) of bitter leaf powder was soaked in one litre of warm water at approximately 70 °C for 12 hours to facilitate efficient extraction of water-soluble bioactive compounds. The mixture was then filtered using a clean muslin cloth to remove solid residues, yielding a clear bitter leaf aqueous extract designated as BE1 (1 g/L).

The filtrate was collected and stored in sterile containers prior to administration. A higher concentration extract (2 g/L) was prepared using the same extraction procedure and designated as BE2. Every day, a litre of warm water (70°C) was used to soak one gram (1g) of bitter leaf powder for 12 hours. Bitter leaf aqueous extract (BE1), containing 1g/litre, was obtained by filtering the preparation using a muslin cloth to remove debris from the filtrate. The filtrate was then stored in sterile containers. Bitter leaf aqueous extract was also made at higher concentration (2g/litre) using the same process, which was assigned BE2, as its name.

2.3 Experimental chickens and treatments

Two hundred and forty (240) one-day-old, mixed-sex Cobb 500 broiler chicks were used in this study. Upon arrival, the chicks were weighed, wing-tagged for identification, and allowed a brief acclimatization period before the commencement of the feeding trial. The birds were housed in a deep-litter experimental pen under standard hygienic and biosecurity conditions. Feed and clean drinking water were provided ad libitum throughout the experimental period.

The chicks were randomly allocated to four experimental treatment groups, each comprising sixty (60) birds (N = 60 per treatment), following a completely randomized design to minimize bias. Randomization was performed to ensure uniform distribution of body weight and sex across treatment groups at the start of the experiment. The four dietary and water-based treatments were designated as CONT, BE0AF, BE1AF, and BE2AF.

Birds in treatment groups 2, 3, and 4 (BE0AF, BE1AF, and BE2AF) were exposed to dietary aflatoxin B_1 contamination at a concentration of 0.5 mg/kg of feed, while birds in the first treatment group (CONT) received an uncontaminated basal diet. In addition to aflatoxin exposure, birds in treatment groups 3 and 4 were orally administered bitter leaf aqueous extract via drinking water at concentrations of 1 g/litre (BE1AF) and 2 g/litre (BE2AF), respectively. Birds in the CONT and BE0AF groups received plain drinking water without bitter leaf supplementation. The bitter leaf aqueous extract was prepared fresh daily and offered ad libitum to ensure consistent intake.

The treatment structure was summarized as follows: CONT, no aflatoxin B_1 contamination and no administration of bitter leaf aqueous extract; BE0AF, 0.5 mg/kg aflatoxin B_1 contamination without bitter leaf extract; BE1AF, 0.5 mg/kg aflatoxin B_1 contamination combined with 1 g bitter leaf powder per litre of drinking water; and BE2AF, 0.5 mg/kg aflatoxin B_1 contamination combined with 2 g bitter leaf powder per litre of drinking water (Vipin et al., 2017).

Throughout the study, environmental conditions within the experimental pen were closely monitored and managed to support optimal bird performance and welfare. The brooding temperature was maintained at 31 ± 2°C during the first seven days of the feeding period. Thereafter, the temperature was gradually reduced by approximately 2°C per week from day 8 to day 27 to accommodate the birds' developing thermoregulatory capacity. From day 28 to day 42, the broiler chickens were allowed to experience ambient environmental temperatures typical of the production setting. Lighting in the experimental pen was regulated using an artificial illumination programme, providing 18 hours of light and 6 hours of darkness per 24-hour cycle. This lighting schedule was consistently maintained until three days prior to the termination of the experiment, in accordance with standard broiler management practices.

2.4 Collecting blood, liver and meat samples, and statistical data analyses

Eighteen On the forty-second day of the experiment, eighteen (18) broiler chickens were randomly selected from each treatment group, with one bird chosen per replication to ensure adequate representation and to minimize sampling bias. Prior to sampling, feed was withdrawn overnight (approximately 12 hours) to reduce the influence of recent feed intake on blood metabolites and biochemical parameters, while access to drinking water was maintained to prevent dehydration. The selected birds were humanely sacrificed in accordance with standard ethical and institutional animal welfare guidelines, and samples were collected immediately to prevent post-mortem biochemical alterations.

Blood samples (9 mL per bird) were collected aseptically from the jugular vein using sterile syringes and transferred into plain, sterile test tubes without anticoagulants for serum separation. The blood samples were placed in a slanted position on laboratory racks and allowed to clot undisturbed at room temperature for approximately 15 minutes. Thereafter, the clotted blood samples were centrifuged at 2,500 rpm for 10 minutes to separate the serum. The resulting clear supernatant serum was carefully aspirated using

sterile pipettes and transferred into labelled cryovials. Serum samples were immediately stored at −20 °C until further biochemical and immunological analyses were performed.

Serum antioxidant enzyme activities were determined using established spectrophotometric methods. Superoxide dismutase (SOD) activity was measured according to the procedure described by Gao et al. (2011), while malondialdehyde (MDA), an index of lipid peroxidation, was quantified using the method of D'souza et al. (2012). Catalase (CAT) activity was assessed following the protocol outlined by Holovska et al. (2003), and glutathione peroxidase (GPX) activity was determined as previously described by Payne & Southern (2005). Serum lactate dehydrogenase (LDH), a marker of tissue damage and membrane integrity, was analyzed using commercially available diagnostic kits, following the manufacturers' instructions. In addition, serum immunoglobulin concentrations, including immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA), were quantified using enzyme-linked immunosorbent assay (ELISA) kits obtained from Fortress Diagnostics Limited (United Kingdom). All assays were conducted in duplicate to improve analytical accuracy and reliability.

Breast muscle samples were excised from the sacrificed birds prior to dressing to avoid contamination and structural alterations associated with processing. The collected breast meat samples were aerobically packaged in air-permeable polyethylene bags and stored in a deep freezer at −18 °C for a period of 20 days to simulate frozen storage conditions commonly encountered in poultry meat preservation. After the storage period, the oxidative stability of the meat samples was evaluated.

Catalase activity in the breast meat was assessed by monitoring the decomposition of hydrogen peroxide (H_2O_2), characterized by a decrease in absorbance at 240 nm, according to the method described by Muhlisin et al. (2016). Lipid oxidation in the meat samples was determined using the thiobarbituric acid (TBA) assay, which measures thiobarbituric acid reactive substances (TBARS), as described by Tokur et al. (2006). Protein oxidation was evaluated following the procedure outlined by Souza et al. (2013), which quantifies protein carbonyl content as an indicator of oxidative protein damage. Cholesterol concentration in the meat samples was determined spectrophotometrically using commercial assay kits obtained from Asan Pharm. Co., Ltd. (Seoul, Korea), in accordance with the manufacturer's guidelines.

Following meat sample collection, the slaughtered birds were carefully dissected to obtain internal organs. The heart, lungs, liver, spleen, pancreas, and proventriculus plus gizzard were excised, freed of adhering connective tissues, and gently blotted dry using paper towels. Each organ was weighed individually using a sensitive digital scale. Relative organ weights were calculated and expressed as a percentage of the live body weight of each bird, providing insight into organ hypertrophy or atrophy associated with aflatoxin exposure and bitter leaf extract supplementation.

For hepatic biochemical and stress marker analyses, liver tissues were collected immediately after slaughter. Liver samples (three per replication) were rinsed in ice-cold phosphate-buffered saline to remove blood residues and minimize enzymatic degradation. Thereafter, 20% (w/v) of each liver sample was homogenized in 0.15 M potassium chloride (KCl) solution at 4 °C using a chilled homogenizer to preserve enzyme activity and protein integrity. The resulting homogenates were centrifuged at 12,000 rpm for 45 minutes at temperatures ranging between 0 and 4 °C to obtain clear supernatants.

The supernatants were subsequently used for the determination of hepatic stress and inflammatory markers. Heat shock protein 70 (HSP70) content was analyzed as an indicator of cellular stress response. In addition, the concentration of nuclear factor kappa B-p65 (NF-κB p65), a key transcription factor involved in inflammatory and immune signaling, was determined using a human NF-κB p65 ELISA kit obtained from Elabscience (USA), following the manufacturer's protocol. Liver tissue lactate dehydrogenase (LDH) levels were also quantified using a human LDH sandwich ELISA kit sourced from LSBio (Shirley, MA, USA), providing further insight into hepatic cellular integrity and damage.

All biochemical and ELISA analyses were performed according to the respective manufacturers' instructions, and absorbance readings were obtained using a calibrated

microplate reader or spectrophotometer, as appropriate. Furthermore, this study data were analysed based on ANOVA, using SPSS software, version 20. Subsequently, we used the Duncan multiple range test to determine whether there were significant differences among the means of the treatment groups.

3. Results and Discussion

Table 2 presents the effects of aflatoxin B_1 (AFB_1) exposure and bitter leaf aqueous extract supplementation on key oxidative stress biomarkers in the serum of broiler chickens. Birds exposed to AFB_1 without phytogenic intervention (BE0AF) exhibited a pronounced oxidative imbalance, evidenced by significantly reduced superoxide dismutase (SOD) activity and elevated malondialdehyde (MDA) and lactate dehydrogenase (LDH) concentrations compared with the control (CONT) and extract-treated groups. These findings indicate that AFB_1 exposure disrupts the endogenous antioxidant defense system and promotes systemic oxidative stress, thereby compromising cellular and tissue integrity. In contrast, supplementation with bitter leaf extract (BE1AF and BE2AF) restored antioxidant enzyme activities and suppressed lipid peroxidation to levels comparable with the control group, highlighting its potent antioxidative and cytoprotective potential.

Table 2. Serum oxidative stress biomarkers in broilers exposed to AFB_1 and treated with aqueous bitter leaf extract.

Parameters	CONT	BE0AF	BE1AF	BE2AF	SEM	P value
Superoxide dismutase (u/ml)	4.12^{a}	2.35^{b}	3.91^{a}	3.99^{a}	0.23	0.01
Malondialdehyde (mmol/g)	36.06^{c}	58.17^{a}	46.25^{b}	38.52^{bc}	2.80	0.01
Lactate dehydrogenase (lu/l)	53.32^{b}	71.96^{a}	60.70^{b}	56.65^{b}	2.43	0.02
Catalase (ng/mg)	50.01^{a}	35.61^{c}	41.87^{ab}	47.82^{a}	2.25	0.04
Glutathione peroxidase (ml/mg)	50.01^{a}	35.60^{c}	41.87^{ab}	47.80^{a}	3.90	0.01

Means within a row in Table 2, Table 3, Table 4, and Table 5., with different letters are significantly different ($P<0.05$). No aflatoxin B1 contamination; the BE0AF is 0.5 mg/kg aflatoxin B1, BE1AF is 0.5mg/kg aflatoxin B1+ 1 g/l—bitter leaf plant leaf aqueous extract. BE1AF: 0.5mg/kg aflatoxin B1+ 2 g/l bitter leaf plant leaf aqueous extract. Then, SEM has meaning as standard error of mean. Serum SOD activity serves as a frontline enzymatic defense against oxidative stress by catalyzing the dismutation of superoxide radicals ($O_2^{-}\bullet$) into hydrogen peroxide (H_2O_2), which is subsequently detoxified by catalase (CAT) and glutathione peroxidase (GPx). The markedly reduced SOD activity in the BE0AF group confirms that AFB_1 exposure overwhelms endogenous antioxidant systems, leading to impaired redox homeostasis. This finding aligns with established evidence that AFB_1 metabolism generates excessive reactive oxygen species (ROS) during cytochrome P450–mediated bioactivation, resulting in oxidative modification and functional inactivation of antioxidant enzymes (Jobe et al., 2023). Oxidative damage to enzyme active sites, protein carbonylation, and alterations of metal cofactors have all been implicated in AFB_1-induced antioxidant depletion. Furthermore, chronic or subclinical AFB_1 exposure may lead to progressive decline in enzyme activity over time, thereby exacerbating oxidative stress and predisposing tissues to cumulative damage. These observations are consistent with prior reports in poultry and mammalian models demonstrating that AFB_1-induced ROS production directly impairs the activity of enzymatic antioxidants, resulting in redox imbalance and enhanced susceptibility to lipid and protein oxidation (Fouad et al., 2019; Jobe et al., 2023).

In contrast, the restoration of SOD activity in BE1AF and BE2AF chickens indicates that bitter leaf extract effectively counteracted AFB_1-induced oxidative suppression. This effect is likely mediated by polyphenolic compounds and flavonoids present in *Vernonia amygdalina*, which exhibit strong superoxide scavenging capacity and may also induce antioxidant gene expression via nuclear factor erythroid 2–related factor 2 (Nrf2) signaling. Activation of Nrf2 enhances transcription of antioxidant enzymes, including SOD, CAT, and

GPx, thereby reinforcing cellular defense mechanisms and stabilizing redox balance (Forni et al., 2019). Additionally, the bitter leaf extract may provide a reservoir of exogenous antioxidants capable of directly neutralizing ROS, preventing enzyme inactivation, and reducing oxidative pressure on cellular defense systems. The dose-dependent effects observed between BE1AF and BE2AF suggest that higher concentrations of bitter leaf extract may provide augmented scavenging activity, further mitigating the toxic impacts of AFB_1 exposure.

The elevated serum MDA concentration observed in the BE0AF group provides further confirmation of severe oxidative stress and membrane lipid peroxidation induced by AFB_1. MDA is a stable end-product of polyunsaturated fatty acid oxidation and is widely used as an index of lipid peroxidation severity. The bioactivation of AFB_1 to its epoxide metabolite initiates free radical chain reactions that destabilize membrane phospholipids, resulting in loss of membrane integrity and increased permeability. These effects compromise cellular function across multiple tissues, including hepatocytes, immune cells, erythrocytes, and skeletal muscle fibers. The resultant accumulation of lipid peroxidation products not only disrupts membrane fluidity and ion homeostasis but may also impair intracellular signaling pathways, energy metabolism, and immune cell function. Moreover, MDA and other aldehyde products generated during lipid peroxidation can form adducts with proteins and nucleic acids, further exacerbating cellular injury and contributing to systemic oxidative stress (Chen et al., 2022; Li et al., 2022).

Notably, MDA levels in BE1AF and BE2AF chickens were statistically similar to those of the control group, demonstrating that bitter leaf extract effectively suppressed lipid peroxidation. This protection can be attributed to the extract's ability to neutralize ROS, chelate transition metals involved in Fenton reactions, and stabilize membrane lipids. Phytochemicals such as flavonoids, phenolic acids, and sesquiterpene lactones interrupt lipid peroxidation chain reactions by donating hydrogen atoms to lipid radicals, thereby preventing propagation of oxidative damage (Fratta & Cominacimi, 2023). The observed reduction in MDA accumulation further suggests that bitter leaf extract preserves membrane integrity, reduces secondary oxidative stress signals, and prevents activation of downstream pro-apoptotic and inflammatory pathways, which are often triggered by oxidative lipid metabolites.

Serum LDH concentration, a marker of cellular membrane integrity and tissue injury, was significantly elevated in the BE0AF group. LDH leakage into the bloodstream reflects hepatocellular damage and necrosis resulting from oxidative injury. AFB_1-induced lipid peroxidation compromises plasma membrane stability, facilitating efflux of intracellular enzymes. The marked reduction in LDH levels observed in BE1AF and BE2AF groups underscores the hepatoprotective effect of bitter leaf extract. By preserving membrane integrity and limiting oxidative injury, the extract prevented enzyme leakage and maintained cellular viability. These findings support previous reports that phytogenic antioxidants can mitigate cytotoxicity induced by mycotoxins by stabilizing membranes, scavenging ROS, and modulating stress-responsive signaling pathways (Machado et al., 2023; Oloruntola, 2024).

Similarly, the activities of CAT and GPx were significantly depressed in the BE0AF group, reflecting exhaustion of hydrogen peroxide detoxification pathways under sustained oxidative pressure. CAT and GPx play complementary roles in neutralizing H_2O_2, thereby preventing formation of hydroxyl radicals through Fenton chemistry. Their depletion signifies profound oxidative insult and impaired antioxidant recycling. The normalization of CAT and GPx activities in extract-treated groups confirms that bitter leaf phytochemicals supported antioxidant regeneration, preserved enzyme function, and prevented redox collapse. This suggests that bitter leaf extract not only provides direct radical scavenging activity but may also enhance endogenous enzymatic defenses by modulating gene expression and stabilizing enzyme cofactors. Such dual action is critical in alleviating the cumulative oxidative burden induced by chronic aflatoxin exposure.

Collectively, these findings demonstrate that AFB_1 induces a systemic oxidative crisis in broiler chickens, characterized by antioxidant enzyme depletion, lipid peroxidation, and

cellular injury. Bitter leaf aqueous extract effectively countered these effects by restoring antioxidant capacity and stabilizing cellular membranes. The extract's bioactive compounds appear to act through multiple mechanisms, including direct free radical scavenging, metal ion chelation, enhancement of Nrf2-mediated antioxidant gene expression, and maintenance of membrane integrity. These results underscore the potential of *Vernonia amygdalina* as a natural feed additive for mitigating aflatoxin-induced oxidative stress in poultry and improving overall health and productivity (Farombi & Owoeye, 2011). The protective effects observed at both 1 g/L and 2 g/L inclusion levels suggest a dose-responsive efficacy, supporting its practical application in broiler production systems challenged by feed-borne mycotoxins.

Furthermore, the study highlights the importance of integrating phytogenic strategies in poultry nutrition, particularly in tropical and subtropical regions where AFB_1 contamination is prevalent. By enhancing endogenous antioxidant defenses and limiting oxidative damage, bitter leaf extract can improve resilience to environmental and dietary stressors, thereby safeguarding animal welfare, product quality, and food safety. Future research could explore the molecular pathways underlying these protective effects, including detailed profiling of Nrf2 target genes, downstream antioxidant enzymes, and cross-talk with inflammatory signaling pathways such as NF-κB. Such investigations would further elucidate the mechanistic basis of bitter leaf-mediated cytoprotection and support its broader adoption in sustainable poultry production systems.

Table 3 summarises the effects of AFB_1 exposure and bitter leaf extract supplementation on serum immunoglobulin concentrations in broiler chickens. Chickens in the BE0AF group exhibited significantly reduced IgG, IgM, and IgA levels compared with the control (CONT) and extract-treated groups, confirming the immunosuppressive nature of AFB_1. Conversely, bitter leaf supplementation restored immunoglobulin concentrations to near-control levels, indicating potent immunorestorative activity. The observed immunosuppression in BE0AF chickens underscores the profound negative impact of AFB_1 on humoral immunity and highlights the vulnerability of broiler chickens to feed-borne mycotoxins, particularly in intensive production systems.

Table 3. Serum immunoglobulins in broilers exposed to AFB_1 and supplemented with aqueous bitter leaf extract

Parameters	CONT	BE0AF	BE1AF	BE2AF	SEM	P value
Immunoglobulin G (ng/dl)	7.88^{a}	5.85^{b}	6.92^{a}	7.67^{a}	0.27	0.01
Immunoglobulin M (ng/dl)	0.69^{a}	0.37^{b}	0.53^{ab}	0.61^{a}	0.04	0.03
Immunoglobulin A (ng/dl)	0.65^{a}	0.27^{b}	0.48^{ab}	0.61^{a}	0.05	0.02

AFB_1-induced immunosuppression arises from multiple converging mechanisms, including oxidative damage to immune cells, impaired protein synthesis, and dysregulation of cytokine signalling. The liver plays a central role in immunoglobulin synthesis by providing essential amino acids, energy substrates, and metabolic support; thus, hepatic dysfunction directly compromises humoral immune responses. Chronic exposure to AFB_1 promotes ROS generation and oxidative stress, which in turn induces apoptosis of lymphocytes, reduces B-cell proliferation, and impairs plasma cell function (Fouad et al., 2019). These processes collectively limit the biosynthesis and secretion of IgG, IgM, and IgA, leaving the host immunocompromised and more susceptible to infections. In addition, AFB_1 can interfere with antigen presentation by dendritic cells, impairing T-helper cell activation and subsequent B-cell maturation, further contributing to the observed decline in antibody levels.

The marked reduction in IgG levels in BE0AF chickens is particularly significant, as IgG is the predominant immunoglobulin responsible for long-term systemic immunity and pathogen neutralization. Depletion of IgM and IgA further compromises primary immune responses and mucosal immunity, respectively, rendering birds highly susceptible to both systemic and enteric infections. IgM serves as the first line of defense during initial pathogen exposure, while IgA provides critical protection at mucosal surfaces, including the

gastrointestinal tract, which is especially relevant in broilers exposed to feed-borne toxins. These findings corroborate earlier studies reporting that AFB_1 interferes with γ-globulin synthesis, reduces antigen-specific antibody titers, and disrupts lymphoid organ architecture, thereby compromising overall immune competence.

The restoration of immunoglobulin concentrations in BE1AF and BE2AF groups suggests that bitter leaf extract mitigated AFB_1-induced immunotoxicity through multiple protective pathways. The antioxidant phytochemicals present in *Vernonia amygdalina*, including flavonoids, phenolic acids, and sesquiterpene lactones, reduce oxidative damage to immune cells, preserving their viability, proliferation, and biosynthetic capacity. By neutralizing ROS and maintaining redox homeostasis, bitter leaf extract protects B lymphocytes and plasma cells from apoptosis, thereby supporting sustained antibody production. Moreover, certain flavonoids and alkaloids in the extract modulate inflammatory signalling by inhibiting NF-κB and MAPK pathways, reducing pro-inflammatory cytokine release, and preventing cytokine-mediated immune suppression (Lee et al., 2019; Zhong et al., 2022). This dual action—antioxidant protection and anti-inflammatory regulation—ensures that immune cells remain functional and responsive even under mycotoxin challenge.

Importantly, immunoglobulin synthesis is an energy- and protein-dependent process, requiring adequate availability of amino acids, cofactors, and optimal metabolic support. By preserving liver integrity and reducing oxidative stress, bitter leaf extract indirectly supports immune competence, as the liver remains capable of supplying substrates and regulatory signals essential for antibody production. The observed normalization of IgG, IgM, and IgA levels in extract-treated groups indicates that bitter leaf not only exerts direct antioxidant and anti-inflammatory effects on immune cells but also enhances systemic metabolic and hepatic homeostasis.

Furthermore, the dose-dependent effect observed between BE1AF and BE2AF, with slightly higher immunoglobulin levels in the BE2AF group, suggests that increasing concentrations of bitter leaf extract may confer stronger immunoprotective benefits. This finding aligns with previous research showing that higher doses of phytogenic compounds can augment both humoral and cellular immune responses in poultry under oxidative or toxic stress (Oloruntola, 2024; Machado et al., 2023). Collectively, these results highlight the multifaceted role of bitter leaf extract in counteracting AFB_1-induced immunosuppression by combining antioxidant, anti-inflammatory, hepatoprotective, and immunomodulatory mechanisms, ultimately preserving the birds' capacity to respond effectively to pathogens.

These findings have practical significance for poultry production in regions where aflatoxin contamination is prevalent. By improving humoral immunity, bitter leaf extract not only enhances disease resistance but also reduces the likelihood of secondary infections and associated production losses. Incorporation of such natural phytogenic feed additives represents a sustainable and effective strategy to mitigate aflatoxin-induced immunotoxicity, improve bird welfare, and support optimal performance and product quality in commercial broiler operations.

Hepatomegaly observed in BE0AF chickens reflects the liver's central role in AFB_1 metabolism and detoxification. The bioactivation of AFB_1 by cytochrome P450 enzymes generates reactive epoxide metabolites, which impose substantial oxidative and metabolic stress on hepatocytes. This stress results in cellular swelling, inflammatory infiltration, and compensatory hypertrophy as the liver attempts to detoxify and repair itself. Elevated liver weight thus serves as a sensitive indicator of hepatic stress and mycotoxin-induced tissue injury. Similarly, splenomegaly likely represents a stress-induced immune response, as the spleen attempts to compensate for impaired systemic immunity by enhancing lymphocyte proliferation and immune cell recruitment (Machado et al., 2023). Increased spleen weight may also reflect inflammatory activation, congestion, and expansion of white pulp in response to oxidative and antigenic challenges posed by AFB_1.

Table 4. Relative internal organ weight (% slaughter weight) of broilers exposed to AFB_1 and aqueous bitter leaf extract

Parameters	CONT	BE0AF	BE1AF	BE2AF	SEM	P value
Heart	0.38	0.33	0.34	0.35	0.01	0.37
Lung	0.34	0.45	0.38	0.35	0.02	0.08
Liver	1.64^{b}	2.20^{a}	1.97^{ab}	1.66^{b}	0.09	0.04
Spleen	0.07^{b}	0.16^{a}	0.08^{b}	0.09^{b}	0.01	0.01
Pancreas	0.14	0.16	0.17	0.15	0.01	0.45
Proventriculus and gizzard	1.43	1.41	1.44	1.49	0.03	0.81

Table 4 presents the relative organ weights of broiler chickens subjected to AFB_1 exposure and bitter leaf extract supplementation. The liver and spleen relative weights were significantly increased in the BE0AF group compared with the control and BE2AF groups, indicating organ hypertrophy and inflammatory stress. In contrast, organ weights in BE1AF and BE2AF groups were comparable to those of control birds, suggesting that bitter leaf extract effectively prevented pathological organ enlargement. The observed alterations in organ weights reflect the systemic impact of AFB_1 toxicity and the protective influence of phytogenic supplementation.

The normalization of liver and spleen weights in bitter leaf–treated birds underscores the extract's capacity to mitigate organ stress and inflammation (Tokofai et al., 2021). Bioactive compounds in *Vernonia amygdalina*, including flavonoids, sesquiterpene lactones, and phenolic acids, exert hepatoprotective and anti-inflammatory effects by scavenging reactive oxygen species, stabilizing cell membranes, and suppressing pro-inflammatory mediators. By alleviating oxidative damage and maintaining tissue architecture, bitter leaf extract prevents compensatory hypertrophy and pathological enlargement. These findings confirm that supplementation with bitter leaf preserves physiological organ development and function under mycotoxin challenge, supporting both systemic health and metabolic efficiency. Furthermore, the observed dose-dependent effects, with BE2AF showing organ weights closest to control levels, suggest that higher concentrations of the extract provide enhanced protection, reinforcing its practical relevance as a natural dietary intervention in AFB_1-contaminated poultry production systems.

Table 5 illustrates the effects of AFB_1 exposure and bitter leaf extract on meat oxidative status in broiler chickens. Chickens in the BE0AF group exhibited significantly reduced meat catalase (CAT) activity and elevated lipid and protein oxidation compared with all other groups. Conversely, BE1AF and BE2AF birds displayed oxidative indices comparable to the control group, indicating effective protection of skeletal muscle tissues against aflatoxin-induced oxidative damage. These results highlight the systemic impact of AFB_1 toxicity on both circulating and peripheral tissues, including postmortem meat, which is highly relevant for meat quality and shelf-life outcomes.

Table 5. Meat oxidative status and cholesterol in AFB_1-exposed broilers with aqueous bitter leaf extract

Parameters	CONT	BE0AF	BE1AF	BE2AF	SEM	P value
Catalase (kU)	51.36^{a}	26.33^{b}	43.23^{ab}	47.63^{a}	3.67	0.04
Lipid oxidation (umole/l)	0.04^{c}	0.08^{a}	0.05^{bc}	0.06^{bc}	0.01	0.01
Protein oxidation (nmol/mgprotein)	78.56^{c}	113.97^{a}	96.91^{bc}	97.04^{bc}	3.97	0.01
Cholesterol (mg/dl)	9.45	6.31	8.13	7.67	0.49	0.14

AFB_1-induced oxidative stress extends beyond systemic circulation to skeletal muscle tissues, compromising postmortem meat quality. Reduced CAT activity in BE0AF meat indicates depletion of antioxidant defences, leading to accumulation of hydrogen peroxide (H_2O_2) and subsequent propagation of reactive oxygen species (ROS). The elevated lipid peroxidation destabilises muscle membranes, while increased protein oxidation modifies myofibrillar structure, impairing water-holding capacity, tenderness, and flavour stability (Chen et al., 2023). These oxidative modifications contribute to early onset of rancidity,

undesirable colour changes, and compromised sensory properties, which are key factors affecting consumer acceptance and commercial value of poultry meat.

The elevated lipid and protein oxidation observed in BE0AF chickens also implies higher susceptibility of meat to oxidative spoilage during storage and processing. The presence of reactive aldehydes and oxidised amino acids may further promote cross-linking of muscle proteins and loss of functional properties, thereby reducing overall meat quality. Such oxidative deterioration underscores the need for interventions that can maintain both muscle antioxidant capacity and postmortem tissue stability.

In contrast, bitter leaf supplementation preserved meat antioxidant capacity and suppressed oxidative deterioration. Phytochemicals present in *Vernonia amygdalina* likely accumulated in muscle tissues, providing localized antioxidant protection and enhancing endogenous enzyme activity. By stabilising mitochondrial function, scavenging ROS, and reducing oxidative chain reactions, the extract maintained muscle redox balance, preserved membrane integrity, and limited protein carbonylation. Consequently, meat from BE1AF and BE2AF birds retained CAT activity and exhibited lower levels of lipid and protein oxidation, supporting improved quality, longer shelf life, and greater safety for consumers. These findings demonstrate that bitter leaf extract is an effective natural feed additive capable of mitigating AFB_1-induced oxidative stress in poultry, with direct implications for meat production and postharvest quality management.

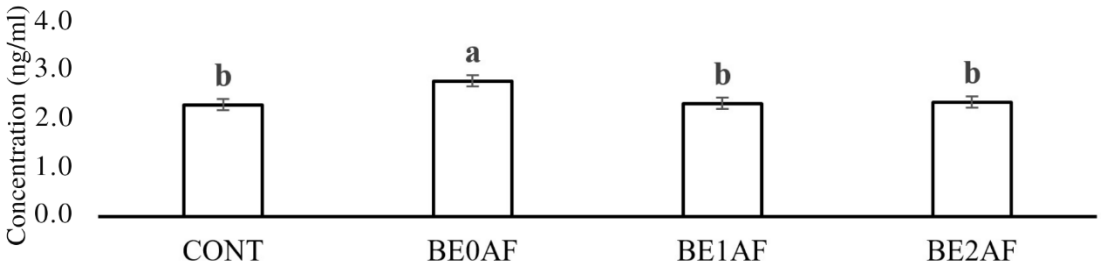

Fig. 1. The Heat shock protein 70 concentration in liver of aflatoxin B_1-exposed broiler chickens administered aqueous bitter leaf extract.

Furthemore, in Figure 1, Figure 2, and Figure 3 hava notes that no aflatoxin B1 contamination; the BE0AF is 0.5 mg/kg aflatoxin B1, BE1AF is 0.5mg/kg aflatoxin B1+ 1 g/l—bitter leaf plant leaf aqueous extract. BE1AF: 0.5mg/kg aflatoxin B1+ 2 g/l bitter leaf plant leaf aqueous extract. Figure 1 depicts hepatic HSP70 levels across treatment groups. HSP70 expression was significantly elevated in BE0AF chickens, reflecting the activation of cellular stress responses under AFB_1 exposure. HSP70 functions as a molecular chaperone, protecting proteins from misfolding, aggregation, and degradation during oxidative and toxic stress (Kócsó et al., 2021). The upregulation of HSP70 in BE0AF birds is indicative of the liver mounting an adaptive response to counteract protein denaturation, oxidative damage, and cytotoxicity induced by AFB_1 metabolites.

While increased HSP70 expression represents an adaptive protective response, sustained elevation also signifies chronic cellular distress, ongoing proteotoxic stress, and a high metabolic burden on hepatocytes. Persistent activation of HSP70 can reflect prolonged exposure to reactive oxygen species (ROS), impaired protein folding machinery, and overwhelmed endogenous defense systems. In contrast, the markedly reduced HSP70 levels observed in BE1AF and BE2AF groups suggest that bitter leaf extract effectively alleviated cellular stress by lowering ROS accumulation, stabilising protein conformation, and preventing proteotoxic damage. The cytoprotective effect of the extract likely involves both direct antioxidant activity of flavonoids and phenolic compounds, as well as indirect modulation of stress-responsive signalling pathways. These findings highlight the extract's role in maintaining hepatic protein homeostasis and cellular viability under mycotoxin challenge.

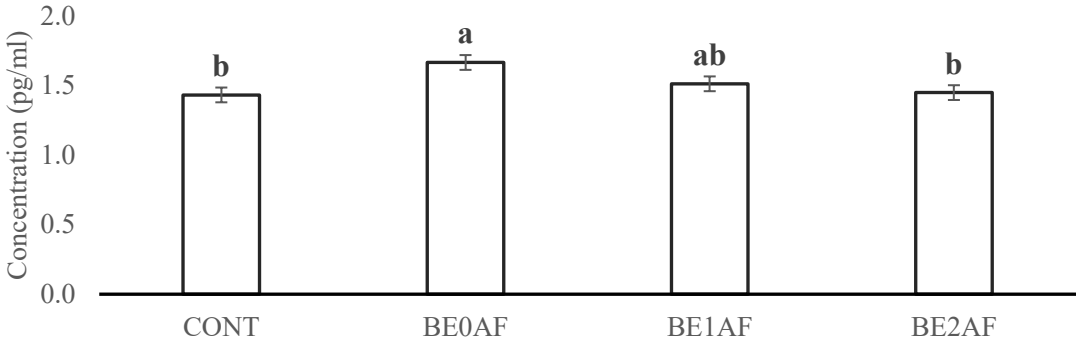

Fig. 2. The nuclear factor kappa in liver of aflatoxin B_1-exposed broiler chickens administered aqueous bitter leaf extract.

Figure 2 shows hepatic NF-κB levels. Chickens in the BE0AF group exhibited significantly elevated NF-κB expression, indicating activation of inflammatory signalling cascades. NF-κB is a key transcription factor regulating the expression of pro-inflammatory cytokines, chemokines, and adhesion molecules, and its activation is a hallmark of AFB_1-induced hepatotoxicity (Liu et al., 2017). The elevated NF-κB levels in BE0AF chickens reflect both ROS-mediated oxidative stress and AFB_1-driven hepatocellular injury, which together trigger inflammatory amplification loops and contribute to progressive tissue damage.

The normalisation of NF-κB levels in BE1AF and BE2AF groups confirms that bitter leaf extract effectively suppressed inflammatory signalling in the liver. This suppression is likely mediated through inhibition of ROS-mediated NF-κB activation, stabilization of IκB proteins, and attenuation of upstream pro-inflammatory cytokine release. By mitigating chronic inflammation, the extract prevented excessive hepatocellular apoptosis and necrosis, thereby preserving liver function. These results suggest that bitter leaf's anti-inflammatory properties, combined with its antioxidant capacity, can interrupt the feed-forward cycle between oxidative stress and inflammatory signalling, which is central to AFB_1 hepatotoxicity.

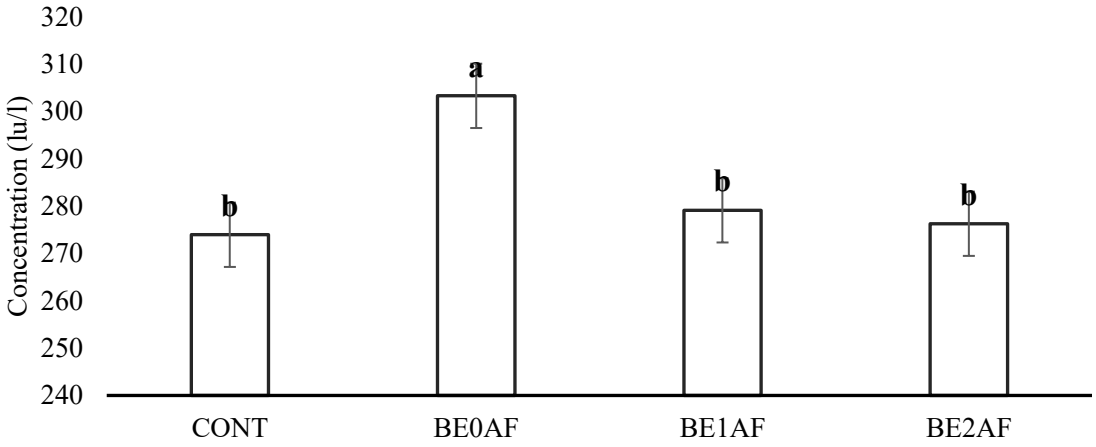

Fig. 3. The Lactate dehydrogenase in liver of Aflatoxin B1-exposed broiler chickens administered aqueous bitter leaf extract.

Figure 3 illustrates hepatic lactate dehydrogenase (LDH) levels. Elevated LDH activity in BE0AF chickens confirms hepatocellular membrane disruption and enzyme leakage induced by AFB_1 toxicity (Hua et al., 2021). LDH leakage into the bloodstream or tissue extracellular space is a sensitive indicator of membrane damage and hepatocyte injury, reflecting oxidative peroxidation of membrane lipids and compromised cellular integrity.

In contrast, LDH levels in extract-treated birds (BE1AF and BE2AF) were comparable to those of control chickens, indicating preserved hepatocellular membrane integrity and

reduced cellular leakage. This hepatoprotective effect is attributed to the multifaceted bioactivity of bitter leaf extract, including antioxidant scavenging of ROS, anti-inflammatory modulation of cytokine signalling, and detoxifying effects that limit AFB_1 bioactivation and promote phase II conjugation pathways (Edo et al., 2023). By stabilising membrane lipids, preventing oxidative disruption, and supporting enzymatic repair mechanisms, the extract maintained cellular homeostasis and reduced the extent of hepatocellular damage.

Collectively, the combined observations of HSP70, NF-κB, and LDH indicate that AFB_1 exposure induces a severe hepatotoxic stress response characterized by protein misfolding, chronic inflammation, and membrane disruption. Bitter leaf extract supplementation effectively counteracted these deleterious effects, demonstrating strong cytoprotective, anti-inflammatory, and antioxidant properties. The dose-dependent trend, with BE2AF showing responses closest to control levels, further suggests that higher concentrations of bitter leaf extract provide enhanced hepatoprotection, highlighting its potential as a natural therapeutic intervention in poultry exposed to aflatoxin-contaminated feed.

4. Conclusions

Aflatoxin B1 exposure (0.5 mg/kg) caused oxidative stress, immune suppression, and liver injury in broiler chickens, but supplementation with aqueous bitter leaf extract mitigated these effects by enhancing antioxidant defence, preserving immunoglobulin levels, and protecting hepatic function. Between the two supplementation levels, 2 g/L bitter leaf extract provided the most effective protection. Supplementation of 2 g/L aqueous bitter leaf extract in drinking water is recommended for optimal protection of broiler chickens against aflatoxin B1-induced toxicity.

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Author Contribution
The author was solely responsible for all aspects of this study, including conceptualization, methodology, data analysis, and manuscript preparation.

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Ethical Review Board Statement
The animal care and treatment protocol of this study was reviewed and approved by the Animal Care and Use Committee, Adekunle Ajasin University, Akungba Akoko, Nigeria (Approval Code: AAUA/ANS/RES/2024/026).

Informed Consent Statement
Not available.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest
The author declares no conflict of interest.

Declaration of Generative AI Use
Not available.

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