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Mediative Effects of Acalypha Wilkesiana Leaf Powder on The Cellular, Brain, And Breast Muscle Oxidative Stress Biomarkers Status of Cobb 500 Broiler Chickens Fed Aflatoxin B1-Contaminated Diets

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Abstract

This study evaluated the potential of Acalypha wilkesiana leaf powder (AWLP) to mitigate the toxic effects of Aflatoxin B_1 (AFB_1) in broiler chickens. A 3×3 factorial design was used to assess the interaction between three levels of AFB1 (0, 0.25, and 0.5 mg/kg) and three levels of AWLP (0, 250, and 500 mg/kg) in the diets of Cobb 500 broilers. One hundred- and eighty-nine-day-old chicks were randomly assigned to nine dietary treatments over a 6-week feeding trial. Oxidative stress biomarkers and cellular stress markers were evaluated. AFB_1 exposure significantly induced oxidative damage in brain and muscle tissues, as reflected by elevated lipid peroxidation and reduced antioxidant enzyme activities (catalase and glutathione peroxidase). AWLP supplementation at 250 mg/kg reduced lipid peroxidation, and significant interaction effects were observed for several parameters, including NF-κB, HSP70, and oxidative stress markers, confirming AWLP's dose-dependent modulatory role. Although AWLP demonstrated antioxidant and hepatoprotective benefits, it was insufficient as a standalone remedy for AFB_1 toxicity. These findings suggest that AWLP at moderate levels may serve as a functional phytogenic additive in poultry feed, particularly in regions prone to aflatoxin contamination, but further integration with other detoxifiers is recommended.

Keywords: Acalypha wilkesiana, Aflatoxin B1, broiler, oxidative stress, phytogenic additives, cellular biomarkers, poultry health.

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1. Introduction

Aflatoxins are among the most important mycotoxins affecting food and feed safety in tropical and subtropical regions, where hot and humid conditions favour Aspergillus growth (Mgbeahuruike, 2016; Balendres *et al*., 2019). Aflatoxin B_1 (AFB_1) is the most toxic aflatoxin and is well known for causing liver injury, immune suppression and carcinogenic effects (Habeeb, 2018; WHO, 2018). Even at low levels in poultry feed, AFB_1 stunts growth, reduces carcass quality and

weakens immune responses (Iqbal *et al*., 2014; Umoh *et al*., 2011). AFB_1 is also a strong pro-oxidant: In the liver it is converted to reactive metabolites that produce reactive oxygen species, heighten lipid peroxidation and weaken key antioxidant defenses (e.g., SOD, CAT, GPx), so oxidative injury to lipids, proteins and DNA is a central feature of aflatoxicosis in broilers (Rotruck *et al*., 1973; Misra and Fridovich, 1972; Aebi, 1974). Consequently, oxidative damage to lipids, proteins and DNA plays a major role in aflatoxicosis in broiler chickens.

In addition to classical oxidative effects, AFB_1 exposure also triggers cellular stress and inflammatory signalling pathways associated with tissue damage. In addition to oxidative damage, AFB_1 activates cellular stress and inflammatory pathways, for example, HSP70 rises as a protective chaperone while NF-κB drives pro-inflammatory and apoptotic signalling (Hou *et al*., 2022; Zuo *et al*., 2013). Evaluating several oxidative and cellular stress markers together gives a fuller picture of tissue responses, especially in organs such as the brain and breast muscle that affect welfare and meat quality but are less commonly examined than the liver. Such integrated evaluations are particularly important for organs like the brain and breast muscle, which influence behaviour, welfare and meat quality, yet remain less frequently studied compared to the liver.

Because chemical binders and synthetic detoxifiers can be costly or not very efficient, there has been a rise in the quest for natural phytogenic additions that have antioxidant and protective qualities (Yang et al., 2015; Ogwuegbu and Mthiyane, 2024). Phytogenic compounds—including polyphenols, flavonoids, tannins and terpenoids, can influence oxidative and inflammatory pathways, scavenge free radicals and support endogenous antioxidant enzyme activities. Several medicinal plants have proven the potential to ameliorate mycotoxin-induced biochemical abnormalities in chickens (Nazarizadeh et al., 2019; Achilonu et al., 2018). These findings encourage the assessment of available tropical medicinal herbs as economical supplements for decreasing aflatoxin-induced oxidative stress in broilers.

Acalypha wilkesiana is a common ornamental and medicinal shrub rich in phenolic compounds and flavonoids that exhibit antioxidant and anti-inflammatory effects (Omage and Azeke, 2014). Its extracts and leaf powders have demonstrated antimicrobial and metabolic benefits, making AWLP a practical, locally available phytogenic candidate for reducing aflatoxin-related oxidative damage in poultry.

Although herbal feed additives are increasingly investigated, there are few studies on tissue-specific antioxidant responses to AFB_1 when diets are supplemented with *A. wilkesiana*. In particular, information on oxidative biomarkers in the brain and breast muscle under AFB_1 challenge is limited. These gaps highlight the need to evaluate not only antioxidant enzymes but also cellular stress biomarkers such as HSP70 and NF-κB to understand how phytogenic additives influence systemic and tissue-level responses.

2. Materials and Methods

2.1. Study Area

The study was conducted at the Teaching and Research Farm of the Federal University of Technology, Akure (FUTA). It is located between 70°15'0" North and 50°12'0" East. The weather in Akure is similar to that in Southwest Nigeria, where the southwest monsoon winds from the ocean bring rain, and the dry Northwest winds from the Sahara Desert provide dry air. The rainy season in the study area typically spans April through October, about seven months in duration. The temperature stays between 21 °C and 29 °C all year, and the humidity is considerable. The amount of rain that falls each year ranges from 1,150 mm to 2,000 mm (Ogunrayi et al., 2016).

2.2. Collection and preparation of Acalypha wilkesiana leaf powder (AWLP)

Fresh leaves of the copperleaf, *Acalypha wilkesiana* hoffmannii (Green), were collected from their mother plants in Akure, Nigeria. Proper identification of the cultivars was sought out at the Herbarium of the Crop, Soil and Pest Department, FUTA. The leaves were washed, air-dried under shade and milled to a fine powder and stored in a cool, dry environment until use.

We employed established methods to determine the proximate composition, antioxidant capabilities, and phytochemical components of copper leaf powder (AWLP). The proximate chemical composition was ascertained using the methodology outlined by AOAC (2010). The determination of phenol (Ignat et al., 2013), flavonoids (Bohm and Kocipal-Abyazan, 1994), saponin (Brunner, 1984), terpenoids (Sofowora, 1993), 2, 2-diphenyl-1-picrylhydrazine hydrate (Gyamfi et al., 1999), and ferric-reducing antioxidant property (Pulido et al., 2002) was conducted.

2.3. Aflatoxin production and quantification

The aflatoxin was produced from a pure culture of *Aspergillus flavus* (NRRL 3251). The pure culture was maintained on potato dextrose agar for the production of Aflatoxin (AF), which was produced by contaminating grit maize with Aspergillus flavus within 7 days at 25 °C before harvesting the mould spores. The spore suspension was thereafter used for the production of aflatoxin on maize. 500 grams of maize grits were placed into autoclavable polypropylene bags, which were heated to 121 °C and subjected to a pressure of 120 kPa for 60 minutes. The autoclaved grit maize was then inoculated with *a suspension of A. flavus spores and incubated for seven days at 28 °C*. The grit maize was dried in an oven at 70 °C after the fungal development and was milled into powder. Thin layer chromatography (AOAC, 2010) was used to measure the content of AFB_1 and other typical Aspergillus mycotoxins in the maize, such as A. niger, A. terreus, A. nidulans, and *A. fumigatus* (Sugui *et al*., 2015).

2.4. Experimental design and diets

The experiment was conducted using a 3 × 3 factorial design in a completely randomised design. There were three levels of inclusion of aflatoxin and 3 levels of inclusion of the leaf powder. The maximum permitted level for AFB_1 for poultry feed ingredients ranges from 0.02 to 0.1mg/kg (Magnoli *et al*., 2019). In a study carried out by Oloruntola *et al*. (2022) using *Justicia carnea* at inclusion levels of 250mg and 500mg to ameliorate the possible effect of aflatoxin contamination responsible for depressed performance and compromised immunity of birds, 0.5mg/kg of AFB_1 level of contamination was reported to have an adverse effect on the tested birds. A basal diet (Table 1) was formulated to meet the NRC (1994) requirement for broiler chickens. Thereafter, this diet was divided into equal portions and labelled diets 1 to 9 and described as follows:

Diet 1: Control (No AFB_1 contamination, no AWLP supplementation)

Diet 2: AFB_1 (0.25mg AFB_1/Kg feed)

Diet 3: AFB_1 (0.5mg AFB_1/kg feed)

Diet 4: AWLP (250mg AWLP/kg feed)

Diet 5: AWLP (500mg AWLP/kg feed)

Diet 6: AFB_1 + AWLP (0.25mg AFB_1 + 250mg/kg feed)

Diet 7: AFB_1 + AWLP (0.5mg AFB_1 + 250mg AWLP/kg feed)

Diet 8: AFB_1 + AWLP (0.25mg AFB_1 +500mg AWLP/kg feed)

Diet 9: AFB_1 + AWLP (0.5mg AFB_1 + 500mg AWLP/kg feed)

2.5. Birds, housing and management

In a completely random design (CRD), one hundred and eighty-nine (189) day-old Cobb 500 broiler chicks were randomly assigned to nine different food treatments (3 replicates; 7 birds/replicate; 21 birds/treatment). Nine birds lived in their pen, which had a floor coated in wood shavings as litter. The feeding experiment lasted six weeks, with three weeks for the Starter phase and three weeks for the Finisher phase. During the trial, animals were provided continuous lighting (23 h/day) and had ad libitum access to feed and clean water. All experimental procedures were approved by the institutional ethics committee (CERAD, APH/16/6148) and complied with ARRIVE guidelines.

Table 1: Composition of the experimental diets

Ingredients (Kg/100kg)	Starter feed (%)	Finisher diet (%)
Maize	52.35	59.35
Rice bran	7.00	0.00
Maize bran	0.00	6.00
Soybean meal	30.00	24.00
Fishmeal	3.00	3.00
Soy oil	3.00	3.00
Limestone	0.50	0.50
Bone meal	3.00	3.00
Salt	0.30	0.30
Premix	0.30	0.30
Methionine	0.30	0.30
Lysine	0.25	0.25
Total	100.00	100.00
Nutrient composition (g/kg)		
Crude protein	22.18	20.03
Metabolizable energy (Kcal/kg)	3018.00	3108.10
Calcium	1.03	1.04
Crude fibre	3.52	3.58
Crude fat	4.23	2.38
Available Phosphorus	0.48	0.43

2.6. Blood sample collection, slaughtering procedure, carcass traits, and internal organ evaluation

On the 42nd day of the experiment, two birds from each replicate were randomly chosen, tagged, weighed, shocked, and killed by severing their necks with a sharp, clean, and dry stainless knife. We found the serum antioxidant enzymes glutathione peroxidase, superoxide dismutase, and catalase, as described by Rotruck et al. (1973), Misra and Fridovich (1972), and Aebi (1974).

2.7. Serum Hormonal Biomarkers Assays

Leptin, Heat Shock Protein 70, Deoxyribonucleic Acid Damage Biomarker and Nuclear Factor Kappa B-cells levels were analyzed using commercially available Enzyme-linked Immunosorbent Assay (ELISA Kit).

2.8. Brain lipid oxidation, antioxidant enzymes, and antioxidant status of the meat

After slaughtering the birds, three were chosen from each treatment group (one bird per replication) to measure total protein and antioxidant levels in the brain, as well as lipid oxidation, antioxidant enzyme levels, and cholesterol levels in the meat. Using a high-speed homogenizer, the whole brains of broiler chickens were

taken out and mixed with cold saline 0.9% in a 1:10 (w/v) ratio. The homogenate samples were spun at 2000 revolutions per minute for 20 minutes. Then, they were divided into 1.0 mL portions and stored at -18 °C until needed. We will also determine the activity levels of catalase and glutathione peroxidase.

After the birds were dressed, a piece of breast flesh was cut from each one, placed in an oxygen-permeable bag, and frozen for 20 days at -18 °C. After that, the thiobarbituric acid (TBA) test was used to determine the extent of oxidation in the meat's lipids. We also measured the activities of glutathione peroxidase and catalase.

2.9. Statistical analysis

Each treatment had three replicates of seven birds each (n = 21 per treatment). Data were analyzed using one-way ANOVA under the General Linear Model (GLM) procedure of SPSS version 20. Means ± SEM are presented; means separation by Duncan's multiple range test; significance set at $P < 0.05$.

3. Results

3.1. Cellular stress biomarkers

Table 2 below shows the cellular stress biomarker status of Cobb 500 broiler chickens exposed to AFB_1-contaminated diets in response to AWLP. Overall diet significantly affected most cellular stress biomarkers ($P < 0.05$), while leptin showed no diet effect. Heat shock protein 70 (HSP70) displayed a clear treatment response: the highest mean was observed in birds on Diet 6 (AFB_1 + 250 mg/kg AWLP), while the lowest value occurred in birds fed 500 mg/kg AWLP without AFB_1 (Diet 5). The DNA damage marker (Deoxy) was comparatively elevated in the control (Diet 1) and reduced in several AWLP- and AFB_1-combination groups. NF-κB concentrations varied by treatment, with Diet 8 showing the highest values and Diet 2 the lowest. Analysis of AWLP main effects showed significant differences between inclusion levels for HSP70 (0 and 250 mg/kg > 500 mg/kg). Significant AWLP × AFB_1 interactions (interaction $P \leq 0.02$) for most cellular markers indicate that AWLP effects depend on both dose and AFB_1 presence.

Table 2: cellular stress biomarker status of Cobb 500 broiler chickens exposed to AFB_1-contaminated diets in response to AWLP

Diet	AWLP	AFB_1	HSP 70	Leptin	Deoxy	NF-$_K$B
1			3.00^{ab}	176.00	2.51^{a}	16.34^{a}
2			2.88^{abc}	172.50	2.14^{bc}	12.03^{c}
3			2.61^{bcd}	168.00	2.09^{bc}	13.11^{abc}
4			2.36^{cd}	171.50	1.97^{c}	15.59^{ab}
5			2.22^{d}	170.50	1.89^{c}	14.91^{abc}
6			3.20^{a}	165.00	2.01^{c}	12.28^{c}
7			2.99^{ab}	173.50	2.06^{bc}	14.56^{abc}
8			2.54^{bcd}	172.00	2.36^{ab}	16.44^{a}
9			2.73^{abcd}	165.50	2.08^{bc}	13.59^{abc}
SEM			0.07	1.10	0.04	0.39
P Value			0.01	0.27	0.01	0.02
	0		2.83^{a}	172.16	2.25^{a}	13.82
	250		2.85^{a}	170.00	2.01^{c}	14.14
	500		2.49^{b}	169.33	2.11^{b}	14.98
	SEM		0.09	1.80	0.05	0.52
	P Value		0.03	0.52	0.02	0.29
		0	2.53^{b}	172.66	2.12	15.61^{a}
		250	2.87^{a}	169.83	2.17	13.58^{b}
		500	2.78^{a}	169.00	2.08	13.75^{b}
		SEM	0.09	1.80	0.05	0.52
		P Value	0.05	0.34	0.49	0.02
Interaction *P Value*			0.02	0.16	0.01	0.02

Means with different superscripts in the same column are significantly (P<0.05) different; AWLP: *Acalypha wilkesiana* Leaf Powder; AFB_1: Aflatoxin B_1; HSP 70: Heat Shock Protein 70; Deoxy: Deoxyribonucleic Acid Damage Biomarkers; NF-KB: Nuclear Factor kappa-B cell; SEM: Standard error of the means.

3.2. Brain oxidative stress biomarkers

Table 3 below illustrates how AWLP mitigates the brain oxidative stress biomarkers of Cobb 500 broiler chickens fed diets contaminated with AFB_1. Diet influenced most brain oxidative biomarkers ($P < 0.05$), except ACE and SOD which were largely stable across treatments. Brain lipid peroxidation reached its highest level with Diet 6 and its lowest with Diet 4, indicating certain AWLP–AFB_1 combinations increased membrane oxidation. CAT and GPx activities varied by treatment, CAT was highest in Diet 7 and lowest in Diet 9, while GPx peaked in Diet 4 and was reduced in Diets 1 and 8. Although AWLP or AFB_1 main effects alone were generally not consistent across brain markers, several AWLP×AFB_1 interactions reached significance (interaction $P \leq 0.05$), suggesting

that the presence of AFB_1 modified how AWLP influenced brain antioxidant enzymes.

Table 3: Mitigating Aflatoxin B_1 Toxicity in Cobb 500 broiler chickens with the Influence of AWLP Supplementation on brain oxidative stress biomarkers

Diet	AWLP	AFB_1	LIPIDOX	ACE	SOD	CAT	GPx
1			93.22^{abc}	0.68	76.59	80.17^{d}	14.23^{b}
2			86.55^{bc}	0.73	80.52	90.92^{abc}	16.48^{ab}
3			91.14^{abc}	0.72	83.06	89.90^{abc}	16.60^{a}
4			85.41^{c}	0.66	81.32	85.04^{cd}	16.85^{a}
5			91.51^{abc}	0.74	85.36	91.46^{ab}	16.31^{ab}
6			99.00^{a}	0.67	88.34	85.56^{bcd}	16.71^{a}
7			91.60^{abc}	0.73	80.85	92.89^{a}	14.82^{ab}
8			87.05^{bc}	0.73	81.07	85.11^{cd}	14.22^{b}
9			94.69^{bc}	0.68	85.55	80.22^{d}	15.26^{ab}
SEM			1.09	0.01	0.90	1.01	0.27
P Value			0.04	0.59	0.06	0.01	0.05
	0		90.30	0.71	80.05	86.99	15.77
	250		92.00	0.69	83.50	87.83	16.12
	500		91.08	0.72	83.99	85.60	15.26
	SEM		1.56	0.02	1.32	1.06	0.40
	P Value		0.74	0.57	0.10	0.35	0.32
		0	90.04	0.69	81.09	85.55	15.80
		250	90.86	0.71	83.31	87.19	15.80
		500	92.47	0.71	83.15	87.67	15.56
		SEM	1.56	0.02	1.32	1.06	0.40
		P Value	0.54	0.77	0.43	0.36	0.88
Interaction *P Value*			0.01	0.33	0.05	0.01	0.01

^{abc} = Means with different superscripts in the same column are significantly (P<0.05) different; AWLP: *Acalypha wilkesiana* Leaf Powder; AFB_1: Aflatoxin B_1; LIPIDOX: Lipid Peroxidation; ACE: Angiotensin-Converting Enzyme; SOD: Superoxide Dismutase; CAT: catalase; GPx: Glutathione Peroxidase; SEM: Standard error of the mean.

3.3. Breast muscle oxidative stress biomarkers

The protective potential of AWLP Leaf Powder on breast muscle oxidative stress biomarkers in AFB_1-exposed Cobb 500 broiler chickens are shown in Table 4 below. Breast muscle markers were also diet-sensitive ($P < 0.05$) with SOD as the notable exception. Breast LIPIDOX reached its maximum in Diet 2 and its minimum in Diet 6. ACE and CAT activities were significantly influenced by dietary treatment, ACE highest in Diet 3 and CAT highest in Diet 2. GPx was highest in Diet 4 and lowest in Diet 6. AWLP main effects were significant for ACE

and CAT ($P \leq 0.02$), and AFB1 had a significant main effect on GPx ($P < 0.05$). Interaction effects (AWLP×AFB1) were significant for LIPIDOX, ACE, CAT and GPx (interaction $P \leq 0.04$), indicating dose- and toxin-dependent responses in muscle oxidative status.

Table 4: The protective potential of AWLP Leaf Powder on breast muscle oxidative stress biomarkers in AFB_1-exposed Cobb 500 broiler chickens

Diet	AWLP	AFB_1	LIPIDOX	ACE	SOD	CAT	GPx
1			102.50^{bc}	49.92^{cd}	79.60	21.32^{a}	61.57^{abc}
2			107.40^{a}	51.68^{b}	69.07	21.73^{a}	60.32^{bc}
3			106.20^{ab}	52.93^{a}	70.18	20.53^{ab}	62.30^{ab}
4			105.00^{abc}	51.71^{b}	75.66	18.41^{bc}	66.01^{a}
5			101.80^{c}	50.45^{c}	75.05	21.43^{a}	59.17^{bc}
6			101.60^{c}	49.07^{d}	75.85	21.03^{ab}	52.98^{d}
7			103.80^{abc}	49.04^{d}	74.95	18.21^{bc}	58.81^{bc}
8			104.30^{abc}	50.54^{c}	79.37	17.26^{c}	56.70^{cd}
9			104.45^{abc}	50.07^{cd}	74.71	18.93^{abc}	63.62^{ab}
SEM			0.50	0.25	0.93	0.39	0.82
P Value			0.05	0.01	0.08	0.01	0.01
	0		105.36	51.51^{a}	72.95	21.19^{a}	61.39
	250		103.46	49.94^{b}	75.48	19.21^{b}	59.26
	500		103.51	50.35^{a}	76.38	19.20^{b}	59.83
	SEM		0.72	0.20	1.38	0.50	0.86
	P Value		0.13	0.01	0.22	0.02	0.22
		0	103.10	50.69	76.77	20.39	62.25^{a}
		250	104.43	50.43	74.76	20.00	56.66^{b}
		500	104.81	50.68	73.28	19.22	61.57^{a}
		SEM	0.72	0.20	1.38	0.50	0.86
		P Value	0.23	0.60	0.23	0.27	0.01
Interaction *P Value*			0.04	0.01	0.06	0.02	0.01

^{abc} = Means with different superscripts in the same column are significantly ($P<0.05$) different; AWLP: *Acalypha wilkesiana* Leaf Powder; AFB_1: Aflatoxin B_1; LIPIDOX: Lipid Peroxidation; ACE: Angiotensin-Converting Enzyme; SOD: Superoxide Dismutase; CAT: catalase; GPx: Glutathione Peroxidase; SEM: Standard error of the means.

4. Discussion

4.1. Cellular stress biomarkers status

Changes in HSP70 and NF-κB across treatments indicate altered cellular stress signalling and inflammatory tone following AFB_1 exposure. The pronounced HSP70 elevation in Diet 6 (AFB_1 + 250 mg/kg AWLP) likely reflects an adaptive stress response to combined toxin and phytochemical stimuli, whereas the lower HSP70 in

birds receiving 500 mg/kg AWLP without AFB1 suggests that AWLP alone, at this higher inclusion, may reduce baseline cellular stress signalling. These findings align with documented aflatoxin-induced activation of heat-shock proteins and inflammatory pathways (Monson *et al*., 2015; El Golli-Bennour and Bacha, 2011). The reduction in the Deoxy marker in several AWLP-treated groups may indicate modulated DNA damage signalling or enhanced engagement of repair pathways under certain AWLP×AFB_1 regimes (Shahba *et al*., 2021). Collectively, the cellular results suggest AWLP can alter stress-sensing pathways, but protection is not uniform across doses or in the presence of substantial AFB_1 challenge (Martínez-Uña *et al*., 2020; Oloruntola *et al*., 2024).

4.2. Brain oxidative stress biomarkers

Neural tissue demonstrated sensitivity to AFB_1, showing increased lipid peroxidation in several AFB1-containing diets and shifts in CAT and GPx activity. Because SOD values remained largely unchanged, the disturbance appears focused on hydrogen-peroxide handling (CAT) and GPx-dependent pathways rather than superoxide dismutation (Surai *et al*., 2019). The ability of moderate AWLP inclusion (250 mg/kg) to partially restore CAT or GPx in some treatments supports a protective, enzyme-supportive role for AWLP phytochemicals, consistent with phytogenic antioxidant action reported in similar contexts (Abdelli *et al*., 2021). However, brain markers did not fully normalize, implying that AWLP alone at these doses offers only limited neuroprotection; more potent agents, combination treatments, or earlier intervention may be necessary for full protection (Wang *et al*., 2023; Manafi *et al*., 2014).

4.3. Breast muscle oxidative stress biomarkers

Muscle tissue exhibited marked LIPIDOX increases and variable antioxidant enzyme responses across diets, confirming that AFB1 compromises muscle redox balance and may therefore affect meat quality and tissue integrity (Mavrommatis *et al*., 2021; Reddy *et al*., 2015). At 250 mg/kg, AWLP helped improve GPx activity and reduce lipid peroxidation for some diets, but increasing the dose to 500 mg/kg did not consistently increase protection and in some cases resembled the AFB_1-only profile. This non-linear response may reflect hormesis or a metabolic strain from high phytochemical intake that overwhelms detoxification or redox systems (Upadhyay and Dixit, 2015). The observed effects on ACE and CAT further point to systemic influences of AWLP on oxidative and possibly vascular parameters; these changes merit follow-up with functional measures and histopathology.

4.4. Study limitations

AFB_1 quantification relied on thin-layer chromatography; more sensitive chromatographic methods (HPLC/LC-MS) would improve precision. Biomarker assessments were limited to selected enzymes and oxidative markers; broader panels (e.g., cytokine profiling, histopathology scoring using blinded assessment) would strengthen mechanistic inference. Only two inclusion rates of AWLP were tested; finer dose-response studies would help define an optimal inclusion. The trial used laboratory-produced contaminated maize; naturally contaminated feeds and field conditions may produce different responses.

5. Conclusion

This study revealed that dietary inclusion of *Acalypha wilkesiana* leaf powder (AWLP) at 250 mg/kg moderately alleviated the toxic effects of AFB_1 in broiler chickens. AWLP increased growth performance, antioxidant enzyme activity, and serum protein levels while lowering lipid peroxidation and stress indicators. However, 500 mg/kg inclusion was less effective, potentially due to phytochemical overload. Though AWLP alone demonstrated therapeutic characteristics, it could not totally mitigate AFB_1 toxicity when paired. These findings indicate AWLP's position as a phytogenic supplement with antioxidant and protective properties, but its use should be refined and maybe coupled with other detoxifiers for enhanced efficacy.

6. Declarations

6.1. Ethical Approval

The Centre for Research and Development (CERAD) of the FUTA, reviewed and approved this study under APH/16/6148. Notably, all animal experimentation was conducted in accordance with applicable laws, regulations, and guidelines, prioritizing animal welfare and minimizing any potential harm.

6.2. ARRIVE guidelines

The experimentation was conducted in accordance with applicable laws and ARRIVE guidelines.

6.3. Competing interests

The authors declare that they have no competing interests.

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8. Author's Contribution

Conceptualization, original draft writing, reviewing, and editing: Adesolasi Bridget ADEOLA, Clement Oluwafemi OSOWE, Ramon Abiodun BAMIGBOYE, Olufemi Adesanya ADU. Formal analysis, investigations, funding acquisition, Resources, data curation, and supervision: Adesolasi Bridget ADEOLA, Aderonke Perpetua AJAMA, Gbenga Emmanuel ONIBI, Olufemi Adesanya ADU.

9. Funding

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10. Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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