

Original Research Article

Antioxidative, hematopoietic and hepatoprotective effects of *Moringa oleifera* seed powder in broilers

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Abstract

Purpose: To evaluate the impact of *Moringa oleifera* seed powder (MOSP) on oxidative stress markers, hematological parameters, and hepatic enzymes of broilers.

Methods: Thirty-two broilers were divided into four groups using a completely randomized design (CRD). Group I served as control, while groups II, III, and IV received 100, 200, and 300 mg/kg body weight of MOSP, respectively. The treatment was administered orally daily for eight weeks. After treatment, blood samples were assessed for oxidative stress enzymes assay (catalase (CAT); superoxide dismutase (SOD); glutathione peroxidase (GPx); glutathione S-transferase (GST); malondialdehyde (MDA) and nitric oxide (NO)), haematological analysis (white blood cell (WBC) count, red cell distribution width (RDW), hemoglobin (HGB) concentration, and hematocrit (HCT) levels, red blood cell (RBC), platelet (PLT), eosinophil, and basophil counts) and liver function test (aspartate aminotransferase (ALT), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)).

Results: The MOSP significantly ($p < 0.05$) increased the activities of antioxidant enzymes (CAT, SOD, GPx and GST), and reduced oxidative stress markers (MDA and NO) when compared to control. A significant increase was observed in WBC, RDW, HGB and HCT concentrations in MOLP-treated animals. No significant difference was observed in RBC, PLT, eosinophil and basophil counts across all groups ($p > 0.05$). Liver enzyme activities decreased significantly in MOLP-treated animals ($p < 0.05$), indicating improved liver health.

Conclusion: The substantial antioxidative, hematological, and hepatoprotective effects of MOSP suggest that MOSP could be utilized as a functional feed supplement or a natural alternative to synthetic antioxidants and growth enhancers in poultry, with broader implications for promoting health and productivity in livestock.

Keywords: *Moringa oleifera*, Oxidative stress, Liver function, Hematology, Broiler

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INTRODUCTION

The poultry sector in developing nations faces numerous constraints, with the rising feed cost being one of the most significant. Elevated costs of protein and energy resources have

exacerbated this problem, leading to a continuous struggle for cost-effective feed solutions [1]. Responding to this, poultry rearers have increasingly turned their attention to non-conventional feed sources, emphasizing protein substitutes as viable alternatives [2]. Among

these, leguminous multipurpose trees and shrubs have gained prominence due to their potential to provide proteins, vitamins, and minerals for poultry nutrition. Common examples of plant-based feed ingredients include seed meals derived from *Leucaena leucocephala*, *Gliricidia sepium*, *Sesbania sesban*, and *Manihot esculenta* [3].

Moringa oleifera, also called the "miracle tree," is a drought-tolerant plant capable of thriving in regions with annual rainfall as low as 250 mm [4]. Notable phytochemicals in *M. oleifera* include phenolics, flavonoids, alkaloids, tannins, triterpenoids, sterols, and isothiocyanates [5]. Additionally, it contains unique compounds such as moringyne [6]. These compounds have demonstrated various bioactivities, including anthelmintic, antiviral, and antibacterial properties [7]. Through its diverse phytochemical profile, *M. oleifera* has proven to be a valuable resource in addressing various health and agricultural challenges, positioning it as a promising focus for ongoing and future studies [7]. Studies have shown that *M. oleifera* possesses nutritional properties comparable to conventional protein sources like soybean and rapeseed meal [8]. Beyond its use in animal nutrition, it has been widely utilized in human diets to combat malnutrition, particularly in resource-limited settings where its leaves are consumed to enhance nutrient intake in vulnerable populations [8]. In poultry production, oxidative stress and compromised liver function are key challenges that affect productivity and health. Oxidative stress arises from the disproportion between reactive oxygen species (ROS) production and the antioxidant defence of the body, which gives rise to cell damage [9]. This condition is particularly relevant in broiler chickens, often subjected to high metabolic demands and environmental stressors. Antioxidant-rich feed supplements, such as *M. oleifera* seeds, mitigate oxidative damage by enhancing the antioxidant capacity of the birds [10]. The liver is integral to metabolism, detoxification, and nutrient assimilation, making liver function enzymes essential biomarkers for evaluating the health of broiler chickens [10]. Also, hematological indices, which include red blood cell count (RBC), white blood cell (WBC) counts and hemoglobin levels (HGB), provide valuable insights into the physiological and immune status of poultry [11].

Nigeria's poultry industry is grappling with numerous challenges, chief among them being the escalating feed cost due to high prices for protein and energy sources, which has significantly strained poultry production. Thus,

there is a need to explore non-conventional feed sources as alternatives [12]. Plant derivatives are valuable resources, offering vital nutrients such as proteins, vitamins, and minerals [13]. Despite the advances made with conventional plant-based feed ingredients such as soybean and rapeseed meal, there has been a pressing need for more cost-effective, sustainable, and nutrient-rich alternatives. Therefore, the objective of this study was to examine the effects of MOSP on oxidative stress markers, liver function enzymes, and hematological parameters in broilers.

EXPERIMENTAL

Study location and animals

The study was conducted at the Animal House, Department of Genetics and Biotechnology, University of Calabar, Nigeria, between July and September 2024. Thirty-two broilers were sourced from New Hope Agricultural and Technology Ltd., Calabar, and housed in a controlled, animal-friendly environment. They were fed standard broiler starter (21 % crude protein) and finisher (20 % crude protein) diets from Top Feed Nigeria Ltd., with continuous access to feed and water. Ethical standards following Helsinki protocols for care of experimental animals were observed [14], including regular monitoring for distress or disease. No adverse effects or abnormal behaviors were observed throughout the treatment period.

Preparation of MOSP

Seeds of *M. oleifera* were purchased from Watt Market, Calabar, Nigeria. The seed was dried under the shade for five days and then milled to a fine powder using an electric blender (HBM-508G) from Hanabishi Electric Corporation Ltd, Philippines. The powder was stored in airtight containers and used for the study.

Design and procedure

The 32 broilers were divided into four treatment groups using a completely randomized design with 8 birds in each group. Group I served as control, group II received 100 mg/kg body weight (bw) of MOSP, group III received 200 mg/kg bw of MOSP, and Group IV received 300 mg/kg bw of MOSP. The MOSP was incorporated into the birds' feed, and treatment lasted for eight weeks. The MOSP was administered via feed incorporation based on the individual body weight of the animals. Following an overnight fasting period to enhance feed intake, the calculated dose of MOSP was thoroughly mixed

into a small, pre-measured portion of the daily feed ration. Animals were observed closely to ensure complete consumption of MOSP-supplemented feed prior to the provision of the remaining standard feed for the day. This approach ensured precise delivery and minimized variability in individual intake. After the duration of treatment, blood samples were obtained for oxidative stress markers assay, liver function enzyme estimations, and hematological analysis.

Ethical approval

Ethical approval was obtained from the Ethical Committee, Faculty of Biological Sciences, University of Calabar, Nigeria (approval no. BIOSC24-10) while adhering strictly to the ARRIVE guidelines for animal experimentation (<https://arriveguidelines.org>).

Determination of oxidative stress markers

Antioxidant enzymes

Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) were measured using Sunlong ELISA kits following manufacturer's protocols. MDA levels, indicating lipid peroxidation, were assessed via a TBA-based photometric assay at 532 nm [15]. Nitric oxide metabolites (NO_2^- and NO_3^-) were quantified with a spectrophotometer (Jenway 6405, Essex, England) at 540 nm using the Griess assay [16].

Determination of hematological parameters

Blood samples were collected from each bird through a venous collection method into ethylenediamine tetra-acetic acid (EDTA) sample bottles to prevent coagulation. Hematological parameters were estimated using a fully automated hematology analyzer (ABX Micros 60) from Sysmex Corporation International Company, Japan.

Determination of liver function enzymes

Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) were determined using the method described by [17].

Statistical analysis

Data analysis was done using one-way ANOVA and post hoc Tukey test at ($p < 0.05$). The software used was Statistical Package for Social Sciences (SPSS) version 27 (IBM, New York,

USA). All results obtained were presented as mean $\pm$ standard error of the mean (SEM) with 8 replicates.

RESULTS

Oxidative stress markers

The administration of MOSP significantly influenced oxidative stress and hematological markers in broilers. Antioxidant enzymes: CAT, SOD, GPx, and GST increased significantly in MOSP-treated animals when compared with control group (Table 1). Conversely, MDA and NO levels were significantly decreased in MOSP-treated broilers compared to control group (Table 1).

Hematological parameters

Hematological analysis revealed increased white blood cell (WBC) count and red cell distribution width (RDW), while haemoglobin (HGB) and haematocrit (HCT) levels peaked in Group III compared to control animals (Table 2). Differential leukocyte count showed elevated neutrophil, lymphocyte, and monocyte percentages in treated groups compared with control. Red blood cell (RBC) and platelet (PLT) counts, eosinophil, and basophil levels showed no significant changes (Table 2).

Liver function enzymes

Liver enzymes (AST, ALT, ALP) activities decreased significantly with higher MOSP doses when compared with control (Figures 1 - 3), suggesting improved liver function.

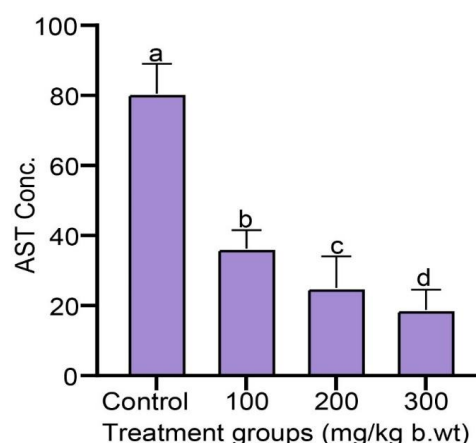


Figure 1: Effect of MOSP on aspartate aminotransferase (AST) activity (IU/L) in broiler chicken. Values are expressed as mean $\pm$ SEM ($n = 8$). Values with distinct superscript letters differ significantly at the 95 % significance level

Table 1: Oxidative stress response of broiler chicken treated with MOSP

Parameter	Group I	Group II	Group III	Group IV
CAT (pg/mL)	2.54 ± 0.61 ^a	24.13 ± 1.88 ^b	23.29 ± 0.94 ^b	34.58 ± 3.86 ^c
SOD (pg/mL)	16.85 ± 3.52 ^a	619.05 ± 5.46 ^c	568.25 ± 56.90 ^b	700.8 ± 5.72 ^d
GPx (IU/mL)	2.35 ± 0.78 ^a	13.45 ± 4.04 ^b	80.31 ± 3.12 ^d	74.24 ± 13.90 ^c
GST (IU/mL)	1.33 ± 0.38 ^a	41.96 ± 9.12 ^b	41.98 ± 2.01 ^b	107.20 ± 22.69 ^c
MDA (nmol/L)	0.95 ± 0.05 ^a	0.93 ± 0.03 ^a	0.88 ± 0.18 ^b	0.45 ± 0.30 ^c
NO (nmol/L)	65.75 ± 2.25 ^a	40.75 ± 1.88 ^b	27.50 ± 1.44 ^c	18.45 ± 0.84 ^d

Note: Values are expressed as mean ± SEM (n=8). Values with distinct superscript letters differ significantly at a 95 % significance level

Table 2: Effect of MOSP on haematological parameters in broiler chicken

Parameter	Group I	Group II	Group III	Group IV
WBC (x10 ⁶ /μL)	68.68±1.39 ^a	94.17±11.16 ^b	105.18±6.41 ^c	364.02±25.19 ^d
RBC (x10 ⁶ /μL)	2.06±0.35 ^a	2.17±0.22 ^a	2.35±0.27 ^a	2.31±0.28 ^a
PLT count (x10 ³ /μL)	3.50±0.87 ^a	3.75±0.48 ^a	3.50±0.87 ^a	4.00±0.71 ^a
RDW (%)	10.63±0.09 ^a	11.20±0.13 ^b	11.15±0.04 ^b	11.40±0.04 ^b
HGB (g/dL)	6.55±0.82 ^a	11.90±1.68 ^b	14.48±0.76 ^c	11.75±0.12 ^b
HCT (%)	21.75±0.46 ^a	29.51±4.27 ^b	37.68±1.96 ^c	31.49±0.21 ^b
Neutrophils (%)	40.27±2.87 ^a	42.62±4.09 ^b	45.25±1.74 ^b	47.85±0.76 ^c
Lymphocytes (%)	18.10±1.31 ^a	27.65±3.55 ^b	34.65±2.69 ^c	40.28±0.43 ^b
Monocytes (%)	33.20±7.27 ^a	34.10±8.59 ^b	33.98±4.75 ^b	42.90±5.41 ^c
Eosinophils (%)	0.78±0.48 ^a	0.65±0.05 ^a	0.85±0.05 ^a	0.82±0.08 ^a
Basophils (%)	0.05±0.03 ^a	0.05±0.03 ^a	0.05±0.03 ^a	0.05±0.01 ^a

Note: Values are expressed as mean ± SEM (n = 8). Values with distinct superscript letters differ significantly at the 95 % significance level

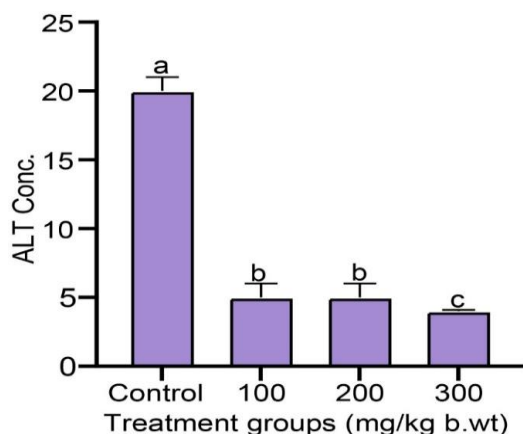


Figure 2: Effect of MOSP on alanine aminotransferase (ALT) activity (IU/L) in broiler chicken. **Note:** Values are expressed as mean ± SEM (n = 8). Values with distinct superscript letters differ significantly at the 95 % significance level

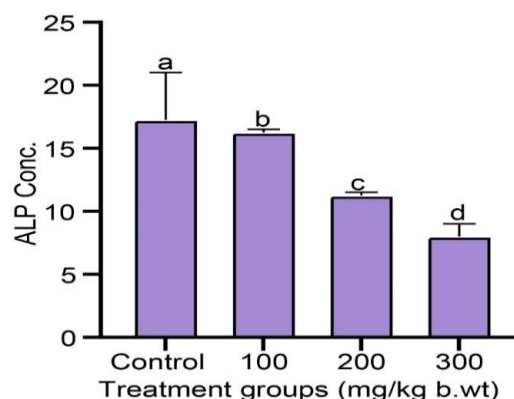


Figure 3: Effect of MOSP on alkaline phosphatase (ALP) activity (IU/L) in broiler chicken. **Note:** Values are expressed as mean ± SEM (n=8). Values with distinct superscript letters differ significantly at the 95 % significance level

DISCUSSION

The initiation of oxidative stress is a vital cellular mechanism, resulting in cellular and tissue damage across different organisms [18]. The results of this study indicated that MOSP significantly enhanced the activity of CAT, SOD, GPx and GST in comparison to control group. These findings align with previous studies, which reported that Moringa seed extract increased the activities of CAT, SOD and GPx in the liver and kidney [8,9].

Similarly, a significant enhancement of antioxidant activity in diabetic rats treated with Moringa leaf extract has been reported in other studies [19]. These results suggest that MOSP strengthens the antioxidant defence system, reduces oxidative stress and protects cellular components from damage. The significant elevation in the activity of CAT in MOSP-treated groups indicates enhanced capacity to mitigate oxidative stress by reducing H₂O₂ accumulation [19].

A previous study reports similar results, attributing the antioxidant properties of Moringa to its high polyphenol content, which supports the upregulation of enzymes like CAT [20]. Likewise, SOD activity was markedly increased in MOSP-treated animals. This enhancement of SOD activity suggests improved ability to neutralize ROS, and it is consistent with the findings of Oguntibeju *et al* [19], who associated the increase in SOD levels with the presence of flavonoids and phenolic acids in Moringa. Additionally, GPx activity was significantly elevated, indicating protection against lipid peroxidation. These findings align with the report that glutathione peroxidase activity is essential for protecting polyunsaturated membrane lipids by inhibiting the initiation of free radical damage [19]. Glutathione peroxidase neutralizes reactive oxygen species, thereby preventing the cascade of lipid peroxidation. A suggested mechanism involves disrupting the chain reactions that would otherwise propagate free radical attacks on membrane lipids, effectively preserving the structural and functional integrity of the cell membranes [5]. Similarly, the significant rise in GST activity observed in this study suggests that MOSP facilitates the detoxification of harmful substances by enhancing their conjugation with glutathione.

On the other hand, the levels of MDA and NO were significantly reduced in MOSP-treated animals compared to control animals. The reduction in MDA level suggests that MOSP effectively inhibits lipid peroxidation. This finding is consistent with earlier studies, which attributed the effect to the potency of the active components of MOSP on cellular antioxidant activity, possibly through inducing enzyme activity via allosteric regulation or gene expression induction [19,20]. Furthermore, the significant reduction in NO levels observed in this study highlights the potential of MOSP to regulate NO production and prevent oxidative stress and inflammation.

This study also revealed the effect of MOSP on the hematological health of broilers, underscoring its role in boosting immunity, growth, and productivity. MOSP significantly enhanced key parameters such as WBC, HGB levels, HCT, and immune cell populations like neutrophils, lymphocytes, and monocytes, and this observation is in line with the findings of [21]. These changes indicate improved immune function and oxygen transport capability, primarily attributed to the rich phytochemicals, iron content, and antioxidant properties of MOSP [5]. The findings of this study agree with existing literature that highlights the hematopoietic and

immunomodulatory effects of Moringa seed, particularly its ability to protect red blood cells from oxidative damage and promote hemoglobin synthesis [22]. While no significant effects were noted on parameters like eosinophils, basophils, and platelet counts, a numerical increase in these parameters in MOSP-treated broilers compared to control group suggests selective modulation of immune responses, possibly due to dosage, supplementation duration, or baseline health conditions, thereby underscoring the potential of MOSP as a natural supplement for enhancing hematological health and immune resilience in poultry, with implications for its broader use in promoting animal productivity and welfare.

This study further demonstrated that MOSP exhibited hepatoprotective properties by significantly reducing the activities of key liver enzymes (AST, ALT and ALP) in treated animals compared to controls, which is corroborated by [23]. These enzymes are essential markers of liver health, with elevated levels typically indicating liver damage or dysfunction [23]. The reduction in AST levels highlights the role of MOSP in protecting liver cells from damage, consistent with a previous study that attributed this effect to the antioxidant properties of MOSP phytochemicals, such as flavonoids and polyphenols, which neutralize oxidative stress [5]. Similarly, the observed decrease in ALT activity in MOSP-treated animals when compared to control suggests a protective effect on liver function, possibly through enhanced regeneration and reduced inflammation [23]. Results obtained in this study also revealed a substantial drop in the activity of ALP in MOSP-fed animals in contrast to control, which suggests the role of MOSP in promoting bile flow and preventing bile duct damage [24]. These effects are linked to the substantial antioxidant composition of MOSP, particularly quercetin and kaempferol, which reduce oxidative stress and inflammation [6].

CONCLUSION

The findings of this study reveal a MOSP-induced significant upregulation of key antioxidant enzymes and concomitant reduction in lipid peroxidation markers, as well as enhancement in hematological parameters of broiler chickens. Moreover, the hepatoprotective effects of MOSP were evident from the significant reductions in liver enzyme activities. MOSP demonstrates substantial antioxidative, hematological, and hepatoprotective effects, highlighting its potential as a natural supplement for enhancing animal health and productivity.

DECLARATIONS

Acknowledgement/Funding

None.

Conflict of interest

No conflict of interest is associated with this study.

Ethical approval

Ethical approval was obtained from the Ethical Committee, Faculty of Biological Sciences, University of Calabar, Nigeria (approval no. BIOSC24-10).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Contribution of authors

We declare that this work was done by the authors named in this article, and the authors will bear all liabilities for claims relating to the content of this article. Ukam Uno and Utip Ekaluo designed the study and conducted the experiments. Reagan Agbor and Ekerette Ekerette analyzed and interpreted the data, while Ekei Ikpeme, Idara Esua and Uduak Edem prepared the manuscript, with contributions from all the coauthors.

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