Sahel Journal of Life Sciences FUDMA (SAJOLS)
December 2023 Vol. 1(1): 237-251
ISSN: 3027-0456 (Print)
ISSN: xxxx-xxxx (Online)
DOI: *https://doi.org/10.33003/sajols-2023-0101-026*

Research Article

Synergistic effect of oral zinc and dietary *M. oleifera* Leaf Supplementation on Diabetes-induced Oxidative Stress and Inflammation

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Received: 3rd December, 2023 **Accepted**: 13th December, 2023 **Published**: 31st December, 2023

ABSTRACT

Individual interventions with zinc or *M. oleifera* leaf powder have shown some promise in managing diabetes-induced oxidative stress and inflammation but the effect of their combination has not been reported. This study examined the synergistic effect of oral zinc and dietary *M. oleifera* leaf supplementation on diabetes-induced oxidative stress and inflammation. Seven groups of 56 rats were used in the experiment: (1) normal rats + normal diet, (2) normal rats + 3% *M. oleifera* leaf-supplemented diet, (3) non-treated diabetic rats, (4)diabetic rats + 3% *M. oleifera* leaf-supplemented diet (5) diabetic rats + 200 mg/kg metformin, (6) diabetic rats + 100 mg/kg oral zinc sulfate, and (7) diabetic rats + 3% *M. oleifera* leaf-supplemented diet + 100 mg/kg oral zinc sulfate. ELISA assay kits were used for antioxidant and anti-inflammatory markers respectively. The results suggest that when all treated diabetic groups were compared with non-treated diabetic control at $p<0.05$, groups 5, and 7 showed a significant increase in superoxide dismutase activity. Group 4 showed a significant increase in reduced glutathione. No significant changes were observed in catalase and the total antioxidant capacity. MDA decreased significantly in group 7. There was a significant reduction in interleukin-6 in groups 6 and 7. Group 7 showed significantly reduced C-reactive protein. Tumour necrosis factor-α reduced significantly in group 6. Overall, the combined supplementation with zinc and *M. oleifera* outperformed the individual treatments with either zinc or *M. oleifera* in ameliorating some biomarkers of diabetes-induced oxidative stress and inflammation, indicating a possible synergistic effect of their combination.

Keywords: Antioxidant; Anti-inflammatory; Diabetes Mellitus; *Moringa oleifera*; Zinc

Citation: Idoko, A. S., Muntari, A., Yusuf, A. P., Kabir, S., Abdulrahman, B., Lawal, N. and Ganiyu, A. I. (2023). Synergistic effect of oral zinc and dietary *M. oleifera* Leaf Supplementation on Diabetes-induced Oxidative Stress and Inflammation. *Sahel Journal of Life Sciences FUDMA*, 1(1): 237-251. DOI: *https://doi.org/10.33003/sajols-2023-0101-026*

INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disease that leads to the damage of pancreatic β-cells, in response to inflammatory signals, β-cells engage adaptive mechanisms where the endoplasmic reticulum (ER) and mitochondria act in concert to restore cellular homeostasis (Vig *et al.,* 2021). There is compelling data to show an increased prevalence of diabetes mellitus in Africa. The estimated prevalence of diabetes in Africa is 5% to 7% in urban areas and 1% in rural areas of sub-Saharan Africa

(Ogbera & Ekpebegh, 2014). By 2030 diabetes is predicted to become the seventh leading cause of death in the world and total deaths from diabetes are projected to rise by more than 50% in the next 10 years (Male *et al.,* 2017). Despite the rising prevalence of diabetes mellitus in Nigeria, population-based studies are scarce. This situation is worse in rural areas where the people are typically poor, not very educated, and lack good hospitals (Pisoschi & Pop, 2015).

High levels of inflammatory cytokines, activation of leukocytes, and increased tissue fibrosis are a result of diabetes (Male *et al*., 2017). Accumulation of reactive oxygen species (ROS) leads to oxidative stress, which results in the increased damage of β-cells of the pancreas and biomolecules (Male *et al.,* 2017). ROS are produced by aerobic metabolism, electron transport activity (which releases unpaired electrons), by-products of normal enzymatic reactions, as well as during inflammatory response, stress, and human activities (Male *et al.*, 2017). The enzymes localized in the different subcellular compartments and comprising the antioxidant machinery include Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), Monodehydroascorbate reductase (MDHAR), Dehydroascorbate reductase (DHAR), Glutathione Reductase (GR), and Glutathione Peroxidase (GPX). These enzymes ameliorate oxidative stress by converting ROS to harmless moleccules such as water, which can be easily excreated from the body (Das & Roychoudhury, 2014; Lobo *et al*., 2010).

Treatment of DM in Nigeria has always included the administration of insulin and oral hypoglycemic agents in conjunction with dietary counseling and lifestyle modification (Ogbera & Ekpebegh, 2014). The search for herbal plants that have the potential for prevention and treatment is certainly very much needed. Evidence shows that *Moringa Oleifera* (*M.* oleifera) can reduce fasting sugar levels. *M. oleifera* extract also plays a role in the treatment of insulin resistance (Charles *et al.,* 2022). *M. oleifera* has rich antioxidant content and diverse therapeutic abilities. It can prevent the occurrence and complications of diabetic-induced kidney injury through its protective effect on the oxidative status and inflammatory cytokines in the kidneys of diabetic rodents (Putri *et al*., 2023; Omodanisi *et al*., 2017).

Zinc (Zn) is essential for numerous aspects of cellular metabolism. Everyday intake of Zn is essential to sustain its steady state since the body lacks a dedicated Zn storage system (Rink & Gabriel, 2000). Several studies show that zinc supplementation improves glucose handling in diabetes patients (Khan *et al.*, 2013). There is evidence that in STZ-induced diabetic models, serum and tissue zinc levels are significantly lower due to increased use and removal, which results in worsened oxidative stress and inflammations; zinc supplementation was able to restore normal levels of zinc in tissues and ameliorate the associated metabolic derangements (Barman & Srinivasan, 2022; Martins *et al*., 2022; Valera *et al*., 2015). Considering these facts, zinc supplementation could be a promising therapeutic approach for the treatment of diabetes-induced oxidative stress and inflammation.

Despite the sustainable evidence supporting the antidiabetic activity of *M. oleifera* extract as well as the antidiabetic potential of zinc supplementations that are currently being unveiled, studies investigating the possible synergistic roles of zinc and *M. oleifera* supplementation in diabetic models are under-reported in the existing literature. This study, therefore, aims to investigate the possible effect of zinc and *M. oleifera* supplementation on diabetes-induced oxidative stress and inflammation.

MATERIALS AND METHODS

Plant sample collection and preparation

Fresh leaves of *M. oleifera* were purchased from commercial producers and were identified in the Biological Sciences Department of Federal University Dutsin-Ma, Katsina State, Nigeria with the voucher number (FUDMA/PSB/00044). Samples were shade-dried, ground to powder, and stored in sterilized empty jars for further analysis.

Diet formulation, zinc content, and proximate composition of the formulated diets

A total of 30 g of the leaf powder was mixed with 970 g of the formulated diet to form the 3% *M. oleifera* leaf-supplemented diet (Oyeleye *et al*., 2022). Zinc analysis of the formulated diets was conducted by atomic absorption spectroscopy as described by (Jajda *et al*., 2015). Proximate analysis of the formulated diets was conducted following the standard protocol of AOAC as described by Silva *et al.* (2020). The dietary composition of the normal and *M. oleifera* leaf-supplemented diets is shown in Table 1.

Table 1. Dietary Composition of the Formulated Diets

Ingredients	Normal Diet (g/kg)	*M. oleifera* Diet (g/kg)
Cornstarch	462	432
Soybean Meal	330	330
Soybean oil	100	100
Rice husk	50	50
Vitamin premix	50	50
D-methionine	4	4
Salt	4	4
Moringa leaves powder	-	30

Animal Ethics

The study was conducted following the guidelines of the Committee for the Care and Use of Laboratory Animals of Federal University Dutsin-Ma, Katsina State, Nigeria.

Animal Housing

A total of 56 adult male Wistar rats (6-8 weeks old) weighing 130-180 g were purchased from the animal house of Ahmadu Bello University, Zaria. A total of 56 rats were selected at random and were used for the study. Rats were kept in the Federal University Dutsin-Ma animal house on a normal chow and water *ad libitum* at room temperature, and natural light: dark cycles. Rats were allowed to acclimatize to the new environment for fourteen days, after which the experiment commenced.

Pharmacological Agents

The Zinc sulfate ($ZnSO_4$) and metformin tablets used in this research were products of Emzor Pharmaceutical Industries Limited, Nigeria, and Hovid Healthcare Company, Malaysia respectively. A100 mg and 1 g of zinc and metformin tablets were dissolved in 40 ml of distilled water to make standard working solutions of 2.5 and 25 mg/ml respectively. Appropriate volumes of the solutions equivalent to doses of 100 and 200 mg/kg body weight of zinc and metformin respectively were administered to each rat daily by oral gavage.

Induction of type 1 diabetes mellitus (T1DM)

To induce T1DM, rats were fasted for 6-8 hours and subjected to intraperitoneal injections of streptozotocin (STZ) 65 mg/kg in a 0.1 M citrate buffer, pH 4.5 (Furman, 2021; Motyl & McCabe, 2009). Rats were provided with 5% dextrose water as their drinking water for 48 hours. Two non-diabetic control groups (groups 1 and 2) of five normal rats each, were injected with 1 ml of citrate buffer intraperitoneally. To confirm diabetes, STZ-treated rats fasted for 6-8 hours on an experimental day 3 (between 7 a.m. and 1 to 3 p.m.), fasting blood glucose (FBG) was checked, and rats with FBG > 250 mg/dl were considered diabetic (Furman, 2021; Oboh *et al*., 2018).

Experimental Design

A total of fifty-six (56) rats were randomized into seven (7) groups of eight (8) rats each. Group 1 was normal rats on a normal diet (NNO). Group 2 was normal rats on a 3% *M. oleifera* leaf-supplemented diet (NMO). Group 3 was diabetic rats on a normal diet (DNO). Group 4 was diabetic rats fed a 3% *M. oleifera* leaf-supplemented diet only. Group 5 was diabetic rats administered 200 mg/kg of Metformin only (an antidiabetic-reference drug). Group 6 was diabetic rats administered 100 mg/kg zinc sulfate only. Group 7 was diabetic rats administered 100 mg/kg zinc sulfate and fed a 3% *M. oleifera* leaf-supplemented diet. After six (6) weeks of treatment, rats fasted for 6-8 hours overnight. Rats were anesthetized with chloroform. For biochemical analyses, blood samples were collected directly from the abdominal aorta into plain bottles. Serum was extracted from the samples by centrifuging them for 15 minutes at 3400 revolutions per minute. The serum was then collected into plain containers and stored in a refrigerator at -20 ^{0}C for future use.

Biochemical Analysis

Antioxidant Enzymes and MDA

Indices of the cellular antioxidant system including catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), and total antioxidant capacity (TAC) as well the marker of lipid peroxidation malondialdehyde (MDA) were assayed using their respective *ELISA* kits from Shanghai Coon Koon Biotech Co., Ltd, following the manufacturer's instructions.

Inflammatory markers

Inflammatory markers including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) were assayed using their respective ELISA kits from Wuhan Fine Biotech Co., Ltd. Wuhan, China, following the manufacturer's instructions.

Data Analysis

All data were presented as means ± standard error of the means (SEM) of n=3 biologically-independent experiments. One-way analysis of variance (ANOVA) was conducted, and Tukey's post hoc test was performed to compare the mean differences among the seven (7) treatments and control groups. All statistical analyses and graphical illustrations were performed using the GraphPad Prism software, version 10.0.2 at ($p<0.05$).

RESULTS

Proximate composition and dietary zinc content

As shown in Table 1, the moisture, carbohydrates, crude fat, and crude protein contents decreased significantly, whereas the ash and crude fiber contents decreased significantly in the *M. oleifera* leaf-supplemented diet compared to the normal diet. The *M. oleifera* diet also had a significantly higher quantity of zinc than the normal diet (Table 2).

Confirmation of Type 1 Diabetes

At the onset of the experiment (day 0), the initial mean fasting blood sugar (FBS) for all the groups was at the baseline (<90 mg/dl). At experimental day 2 (48 hours after induction), there was a spike in the mean blood glucose levels (>300mg/dl) in all the diabetic groups, while the normal controls maintained the baseline blood glucose level (<90 mg/dl) (Figure 1).

Synergistic effect of zinc and *Moringa oleifera* supplementation on antioxidant makers

Superoxide dismutase (SOD)

Analysis of superoxide dismutase (SOD) in the serum revealed significantly ($p<0.05$) reduced activity of the enzyme in DNO compared to normal controls (NNO and NMO) (Figure 2). Similarly, treatments with metformin and the combined supplementation with zinc and *M. oleifera*-diet both resulted in significant ($p<0.05$) increase in SOD levels in the DME and DZM groups compared to DNO.

Total antioxidant capacity (TAC)

Analysis of the serum total antioxidant capacity showed that TAC reduced drastically in DNO compared to the controls (NNO and NMO) as well as the treated diabetic groups (DMO, DME, DZN, and DZM) (Figure 3). However, the reduction was only significant when DNO was compared to NMO ($p<0.0$) (Figure 3).

Catalase (CAT)

Serum catalase activity was analyzed and the results were presented in Figure 4. The results indicated that DNO had the lowest catalase activity among all the control and treated diabetic groups. The reduction in catalase activity was only significant in NNO compared to DNO at ($p<0.05$).

Table 2. Proximate composition and dietary zinc content

Parameter	Normal Diet	*M. Oleifera* Diet
Ash (%)	2.13 ± 0.05 [a]	3.85 ± 0.05 [b]
Moisture (%)	8.82 ± 0.08 [a]	7.65 ± 0.06 [b]
Crude Protein (%)	9.18 ± 0.02 [a]	8.81 ± 0.02 [b]
Crude Fibre (%)	2.41 ± 0.02 [a]	3.88 ± 0.02 [b]
Crude Fat (%)	2.60 ± 0.02 [a]	2.30 ± 0.02 [b]
Carbohydrate (%)	75.00 ± 0.45[a]	73.85 ± 0.35 [b]
Zinc (µg/kg)	63.97 ± 0.37[a]	168.07 ± 1.02 [b]

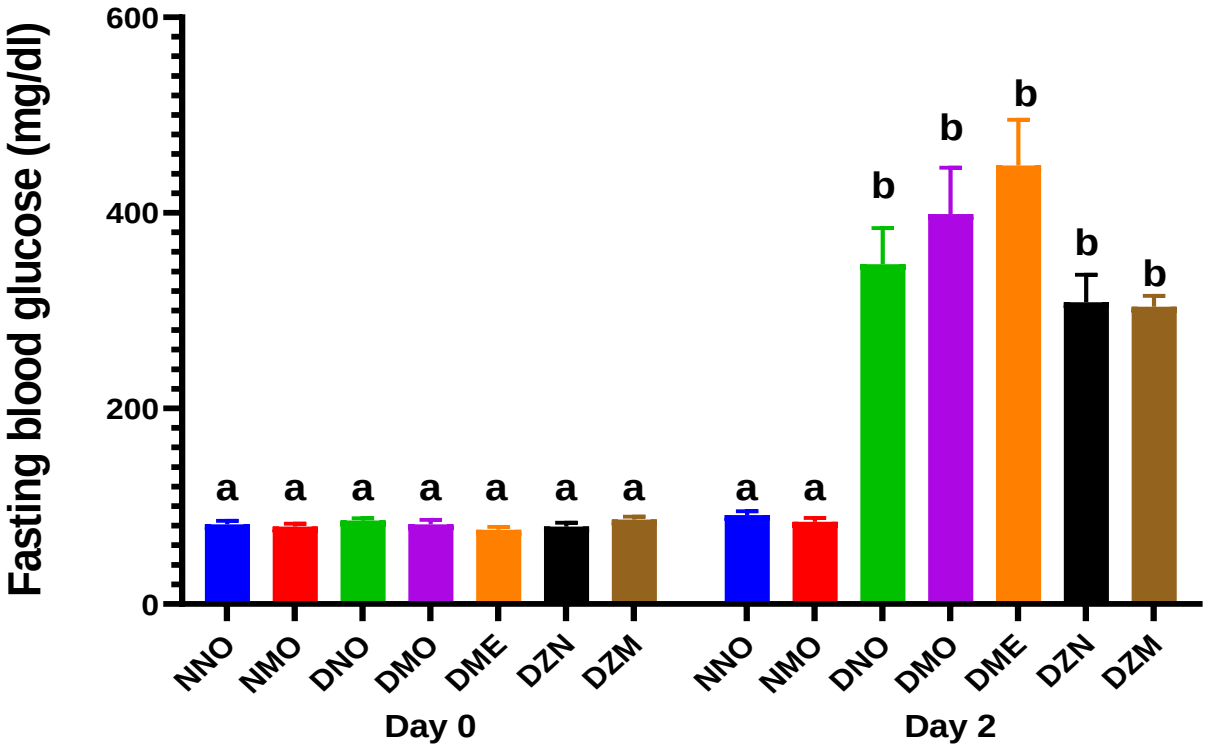

Figure 1: Blood glucose levels before and after induction of diabetes

Each bar represents the mean of n = 8 biologically independent rats. "b" indicates groups that are significantly higher than NNO and NMO at *p<0.05.* "a" indicates groups that are not significantly different from NNO or NMO at *p<0.05*. NNO: Normal rats + normal diet, NMO: Normal rats + *M. oleifera* diet, DNO: Diabetic rats + normal diet, DMO: Diabetic rats + *M.oleifera* diet, DME: Diabetic rats + metformin, DZN: Diabetic rats + zinc sulfate + DZM: Diabetic rats + zinc sulfate + *M.oleifera* diet

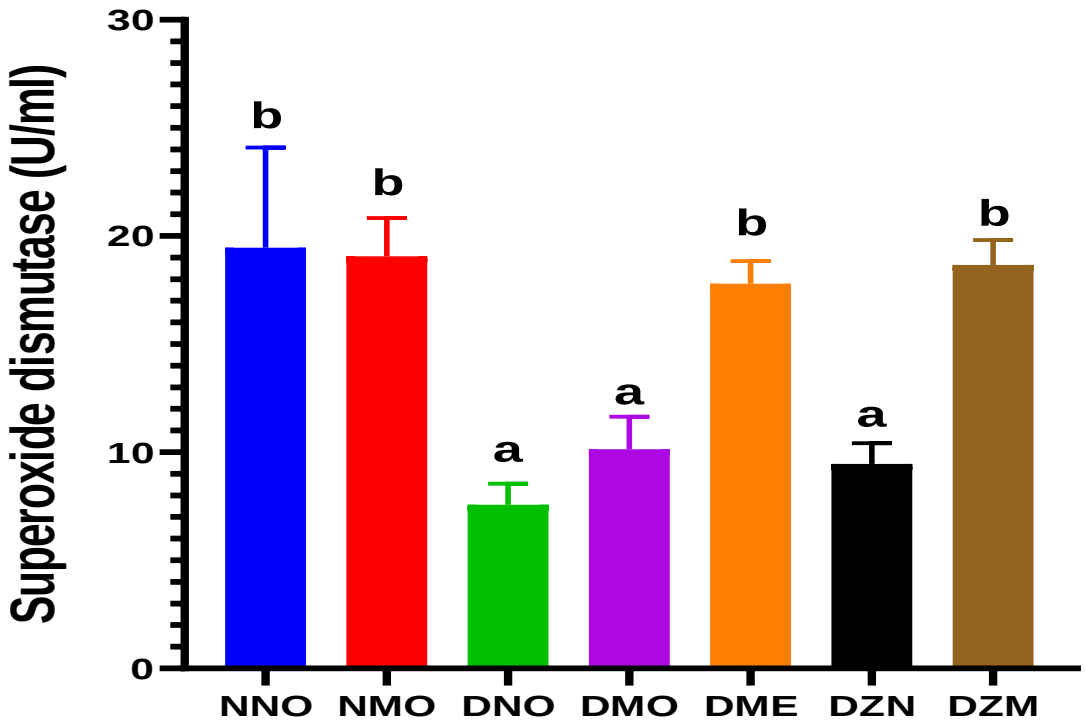

Figure 2: Effect of oral zinc and dietary *M. oleifera* leaf supplementation on fasting serum superoxide dismutase levels of STZ diabetic rat.

Each bar and its error bar represent the mean ± SEM of n = 3 biologically independent experiments. "b" indicates groups that are significantly higher than DNO at $p<0.05$. "a" indicates groups that are not significantly different from DNO at $p<0.05$. NNO: normal rats + normal diet, NMO: normal rats + 3% *M. oleifera* leaf-supplemented diet, DNO: diabetic rats + normal diet, DMO: diabetic rats + 3% *M. oleifera* leaf-supplemented diet, DME: diabetic rats + 200 mg/kg metformin, DZN: diabetic rats + 100 mg/kg zinc sulfate, DZM: diabetic rats + 100 mg/kg zinc sulfate + 3% *M. oleifera* leaf-supplemented diet.

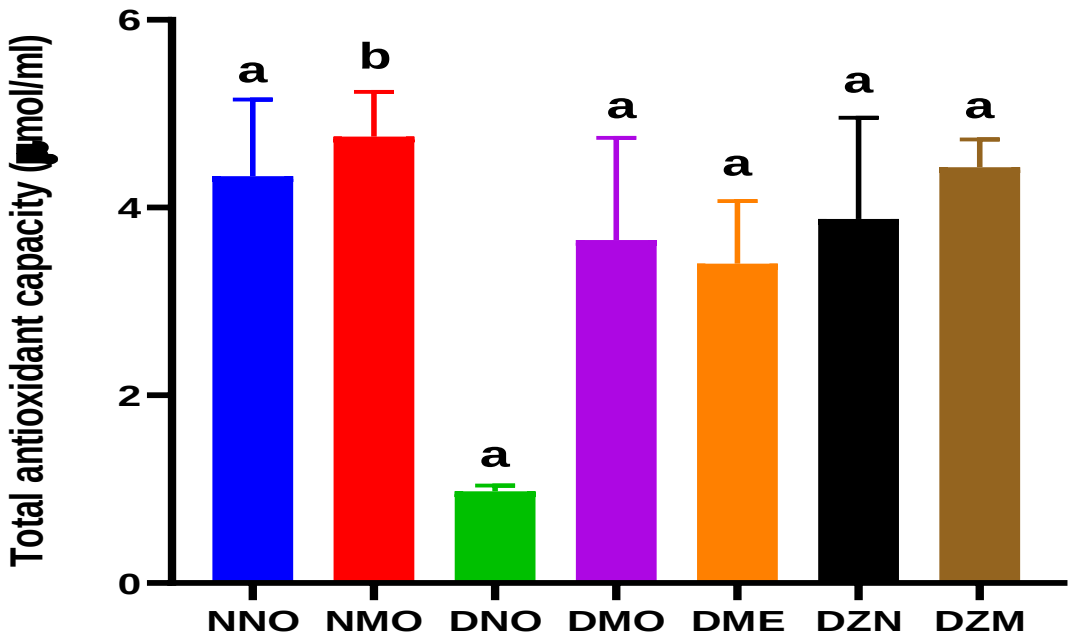

Figure 3: Effect of oral zinc and dietary *M. oleifera* leaf supplementation on fasting serum total antioxidant levels of STZ diabetic rat.

Each bar and its error bar represent the mean ± SEM of n = 3 biologically independent experiments. "b" indicates groups that are significantly higher than DNO at *p<0.05.* "a" indicates groups that are not significantly different from DNO at *p<0.05* . NNO: normal rats + normal diet, NMO: normal rats + 3% *M. oleifera* leaf-supplemented diet, DNO: diabetic rats + normal diet, DMO: diabetic rats + 3% *M. oleifera* leaf-supplemented diet, DME: diabetic rats + 200 mg/kg metformin, DZN: diabetic rats + 100 mg/kg zinc sulfate, DZM: diabetic rats + 100 mg/kg zinc sulfate + 3% *M. oleifera* leaf-supplemented diet.

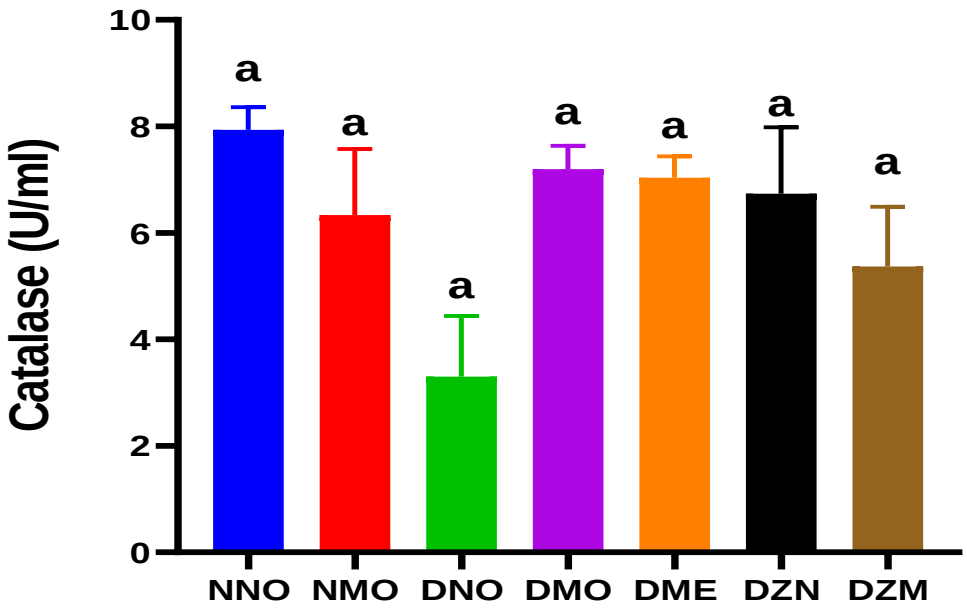

Figure 4: Effect of oral zinc and dietary *M. oleifera* leaf supplementation on fasting serum catalase levels.

Each bar and its error bar represent the mean ± SEM of n = 3 biologically independent experiments. "b" indicates groups that are significantly higher than DNO at *p<0.05.* "a" indicates groups that are not significantly different from DNO at *p<0.05* . Groups without alphabet superscripts are not significant. NNO: normal rats + normal diet, NMO: normal rats + 3% *M. oleifera* leaf-supplemented diet, DNO: diabetic rats + normal diet, DMO: diabetic rats + 3% *M. oleifera* leaf-supplemented diet, DME: diabetic rats + 200 mg/kg metformin, DZN: diabetic rats + 100 mg/kg zinc sulfate, DZM: diabetic rats + 100 mg/kg zinc sulfate + 3% *M. oleifera* leaf-supplemented diet.

Malondialdehyde (MDA)

Analysis of serum malondialdehyde (MDA) levels showed significantly high MDA levels in the DNO group compared to all the diabetic and control groups. DMO presented with the highest serum MDA among the treated diabetic groups, while DZM had the lowest value among them. There was a slight but statistically insignificant increase in MDA levels in NNO compared to NMO. Statistically, the observed increase in serum MDA was only significant in DNO compared to NNO (*p<0.05*), NMO (*p<0.01*), and DZM (*p<0.01*) (Figure 5).

Reduce glutathione (GSH)

The serum levels of reduced glutathione (GSH) showed significantly low levels in the DNO group compared to all the diabetic and control groups, while NNO had the highest serum GSH levels compared to NMO in the control group. DMO had the highest serum GSH among the treated diabetic groups and the increase was significant (*p<0.01*) compared to DNO, while DME had the lowest value among them. There was a slight but statistically insignificant increase in GSH levels in NNO compared to NMO (Figure 6).

Synergistic effect of zinc and *Moringa oleifera* supplementation on anti-inflammatory makers

Tumor necrosis factor alpha (TNFα)

Analysis of the serum levels of Tumor Necrosis Factor Alpha (TNFα) showed significantly (*p<0.05*) higher TNF-α levels in the DNO group compared to control groups, while NNO and NMO had similar serum TNF-α levels. TNF-α decreased in all the treated diabetic groups compared to DNO but the reduction was only significant in DZN compared to DNO (*p<0.05*)(Figure 7).

Interleukin 6 (IL-6)

Analysis of serum interleukin-6 (IL-6) revealed elevated IL-6 levels in DNO compared to the normal controls (Figure 8). However, serum IL-6 levels were reduced in all the treatment groups compared to DNO although the reduction was only significant (*p<0.05*) in DZN and DZM (Figure 8).

C-reactive protein (CRP)

The results of the C-reactive protein (CRP) assay revealed significantly higher serum levels of the protein in DNO and DMO compared to the normal controls, where *p<0.01* in DNO versus NNO, *p<0.05* in DMO versus NNO, and *p<0.05* in DNO versus NMO (Figure 9). Among the treated diabetic groups, DZM showed a significant (*p<0.05*) reduction in CRP compared to DNO (Figure 9).

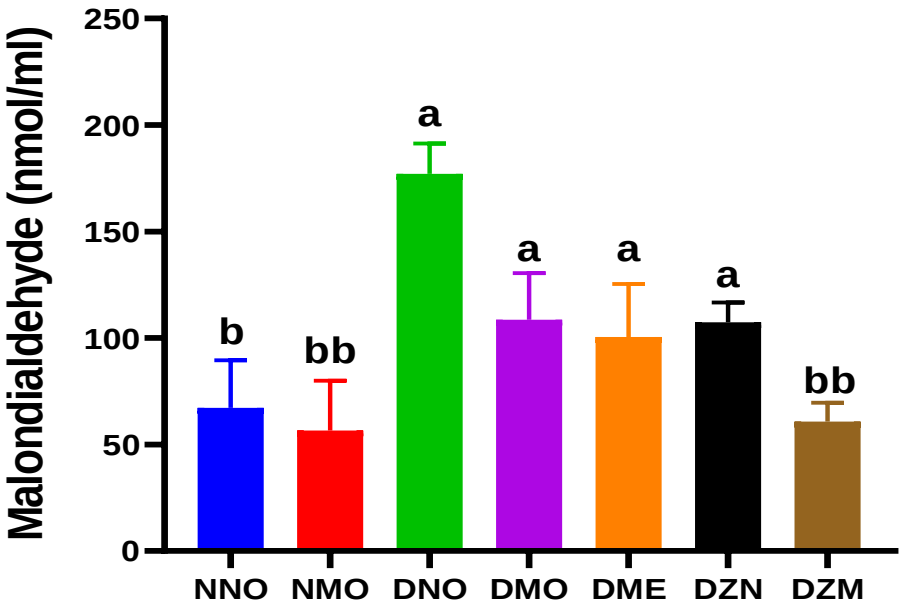

Figure 5: Effect of Oral Zinc and Dietary *M. oleifera* leaf supplementation on fasting serum malondialdehyde levels of STZ-induced diabetic rats.

Each bar and its error bar represent the mean ± SEM of n = 3 biologically independent experiments. "b and bb" indicate groups that are significantly lower than DNO at *p<0.05* and *p<0.01* respectively. "a" indicates groups that are not significantly different from DNO at *p<0.05*. NNO: normal rats + normal diet, NMO: normal rats + 3% *M. oleifera* leaf-supplemented diet, DNO: diabetic rats + normal diet, DMO: diabetic rats + 3% *M. oleifera* leaf-supplemented diet, DME: diabetic rats + 200 mg/kg metformin, DZN: diabetic rats + 100 mg/kg zinc sulfate, DZM: diabetic rats + 100 mg/kg zinc sulfate + 3% *M. oleifera* leaf-supplemented diet.

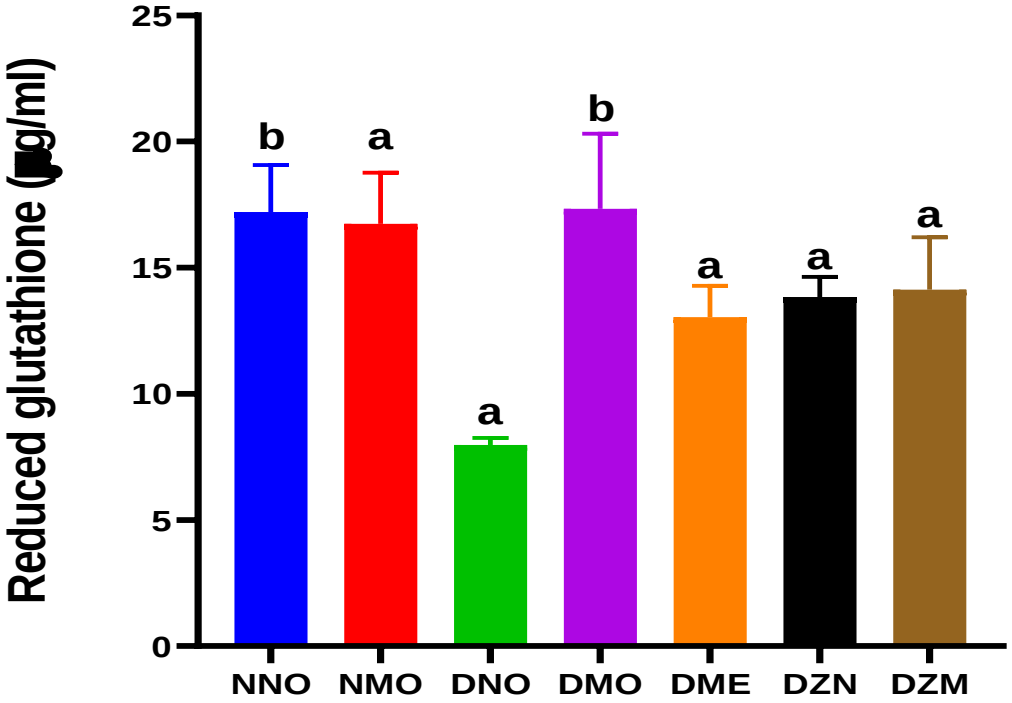

Figure 6: Effect of oral zinc and dietary *M. oleifera* leaf supplementation on fasting serum reduced glutathione levels of STZ-induced diabetic rats.

Each bar and its error bar represent the mean ± SEM of n = 3 biologically independent experiments"b" indicates groups that are significantly higher than DNO at *p<0.05.* "a" indicates groups that are not significantly different from DNO at *p<0.05*. NNO: normal rats + normal diet, NMO: normal rats + 3% *M. oleifera* leaf-supplemented diet, DNO: diabetic rats + normal diet, DMO: diabetic rats + 3% *M. oleifera* leaf-supplemented diet, DME: diabetic rats + 200 mg/kg metformin, DZN: diabetic rats + 100 mg/kg zinc sulfate, DZM: diabetic rats + 100 mg/kg zinc sulfate + 3% *M. oleifera* leaf-supplemented diet.

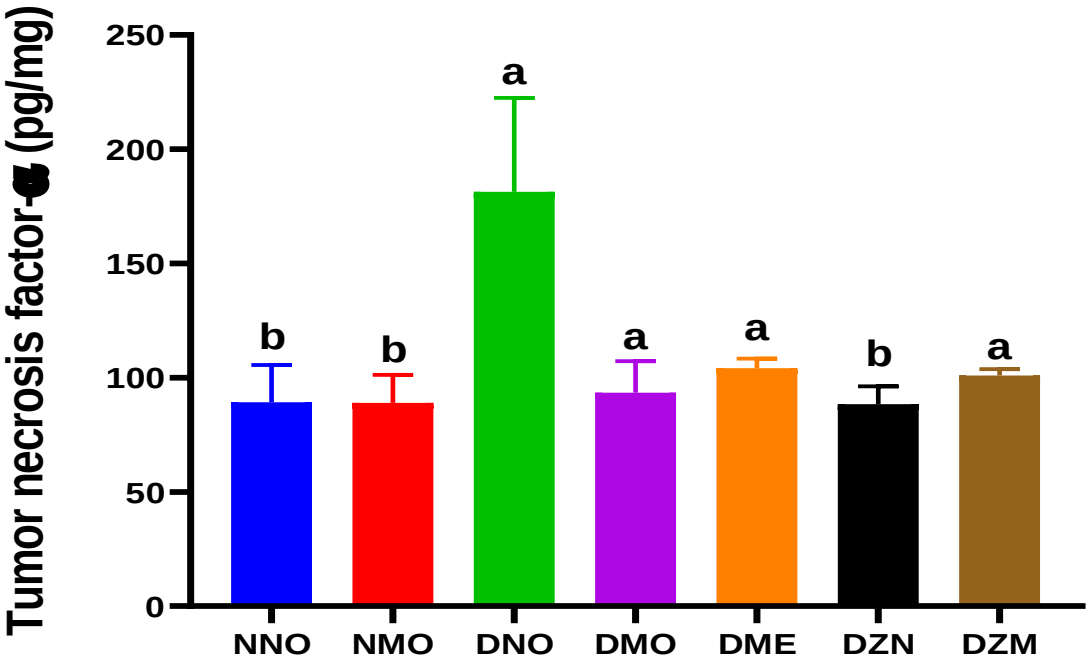

Figure 7: Effect of oral zinc and dietary *M. oleifera* leaf supplementation on fasting serum tumor necrosis factor alpha (TNF-α) levels.

Each bar and its error bar represent the mean ± SEM of n = 3 biologically independent experiments. "b" indicates groups that are significantly lower than DNO at *p<0.05.* "a" indicates groups that are not significantly different from DNO at *p<0.05* . NNO: normal rats + normal diet, NMO: normal rats + 3% *M. oleifera* leaf-supplemented diet, DNO: diabetic rats + normal diet, DMO: diabetic rats + 3% *M. oleifera* leaf-supplemented diet, DME: diabetic rats + 200 mg/kg metformin, DZN: diabetic rats + 100 mg/kg zinc sulfate, DZM: diabetic rats + 100 mg/kg zinc sulfate + 3% *M. oleifera* leaf-supplemented diet.

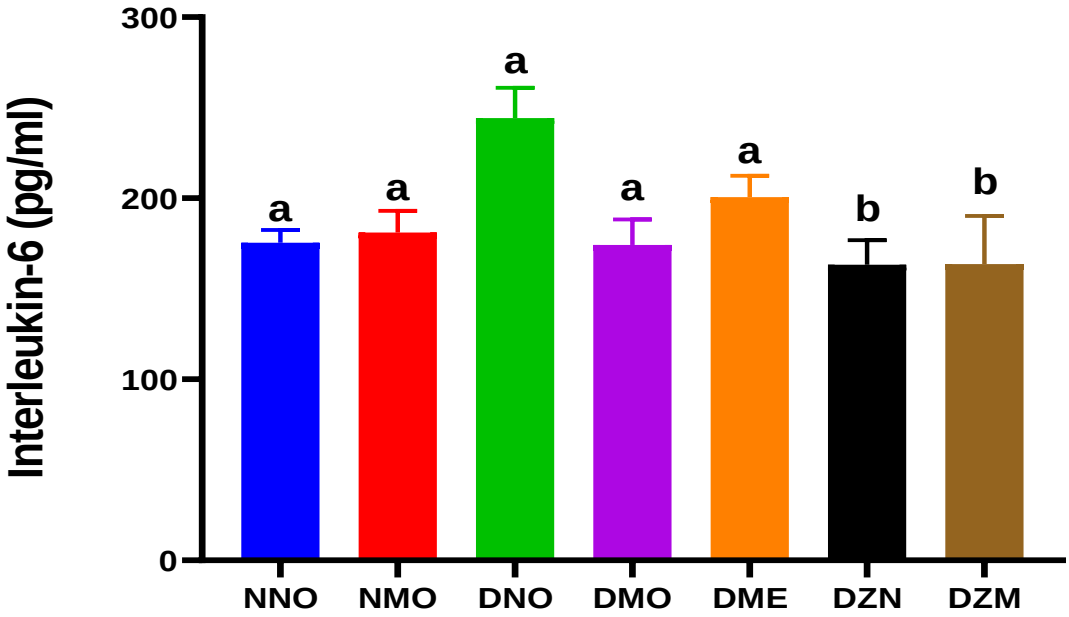

Figure 8: Effect of oral zinc and dietary *M. oleifera* leaf supplementation on fasting serum Interleukin 6 (IL-6) levels of STZ diabetic rats.

Each bar and its error bar represent the mean ± SEM of n = 3 biologically independent experiments. "b" indicates groups that are significantly lower than DNO at $p<0.05$. "a" indicates groups that are not significantly different from DNO at $p<0.05$ NNO: normal rats + normal diet, NMO: normal rats + 3% *M. oleifera* leaf-supplemented diet, DNO: diabetic rats + normal diet, DMO: diabetic rats + 3% *M. oleifera* leaf-supplemented diet, DME: diabetic rats + 200 mg/kg metformin, DZN: diabetic rats + 100 mg/kg zinc sulfate, DZM: diabetic rats + 100 mg/kg zinc sulfate + 3% *M. oleifera* leaf-supplemented diet.

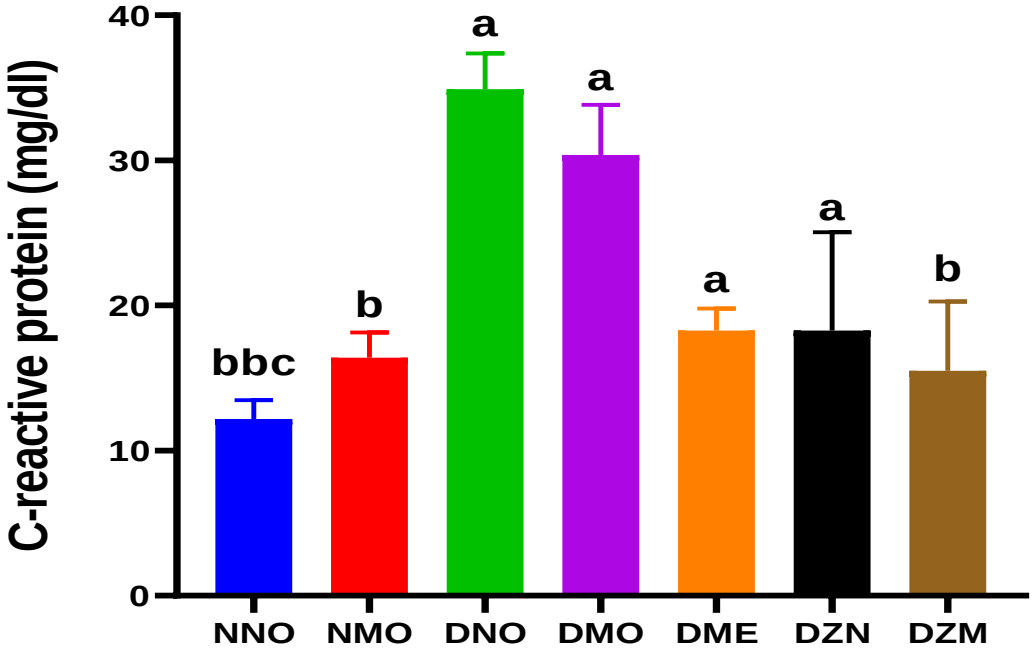

Figure 9: Effect of Oral Zinc and Dietary *M. oleifera* leaf supplementation on fasting serum C-reactive protein (CRP) of STZ diabetic rats.

Each bar and its error bar represent the mean ± SEM of n = 3 biologically independent experiments. "b and bb" indicate groups that are significantly lower than DNO at *p<0.05* and *p<0.01* respectively. "c" indicates groups that are significantly lower than DMO at *p<0.05*. "a" indicates groups that are not significantly different from DNO or DMO at *p<0.05*. NNO: normal rats + normal diet, NMO: normal rats + 3% *M. oleifera* leaf-supplemented diet, DNO: diabetic rats + normal diet, DMO: diabetic rats + 3% *M. oleifera* leaf-supplemented diet, DME: diabetic rats + 200 mg/kg metformin, DZN: diabetic rats + 100 mg/kg zinc sulfate, DZM: diabetic rats + 100 mg/kg zinc sulfate + 3% *M. oleifera* leaf-supplemented diet.

DISCUSSION

The present study was designed to investigate the synergistic effect of oral zinc and dietary *M. oleifera* co-supplementation on diabetes-induced oxidative stress and inflammation in Wistar rats. The findings indicated a strong cooperative effect of the combined treatment in improving the mentioned factors when compared to separate treatments. This could potentially serve as the basis for creating a powerful dietary supplement for managing diabetes.

Chronic low-grade inflammation characterized by the elevation of proinflammatory cytokines in the blood as well as oxidative stress caused by reduced cellular antioxidant defense, are the major risk factors for the progression of diabetes mellitus and its complications (Forman & Zhang, 2021; Pickering *et al*., 2018; Kloubert & Rink, 2015). In this study, elevation of proinflammatory cytokines (IL-6, TNF-α, and CRP) was observed in the non-treated diabetic control. Moreover, there was a decrease in the serum level of antioxidant enzymes such as reduced glutathione, superoxide dismutase, and catalase in the non-treated diabetic control. However, there were significant improvements in the antioxidant and anti-inflammatory responses in the treatment groups particularly in the zinc and *M. oleifera* combined group, indicating a possible synergistic effect of the combination.

A previous study suggests that zinc deficiency might promote oxidative stress in diabetic animals (Barman & Srinivasan, 2022). Antioxidant enzyme activities, such as superoxide dismutase (SOD) and catalase (CAT), were improved in response to zinc supplementation in streptozotocin-induced diabetic rats (Martins *et al*., 2022). In addition, diabetic individuals who received supplements of *M. oleifer*a leaf extract had lower levels of oxidative stress

indicators such as malondialdehyde (MDA) and higher levels of endogenous antioxidants like reduced glutathione (GSH) (Saini *et al*., 2016). In this study, a decrease in serum MDA was also observed in all treated diabetic groups, particularly in the zinc and *M. oleifera* combined group, indicating a possible synergy of the combination.

The present study also looks at the inflammatory makers where the serum levels of IL-6, CRP, and TNF-α show elevated levels in diabetic control which is consistent with the fact that these makers were set to increase in a diabetic condition (Bae *et al.*, 2019). IL-6, TNF-α, and CRP levels have been shown to decrease with zinc supplementation in normal human subjects with zinc deficiency (Foster & Samman, 2012). The study is consistent with this work that shows improvement in inflammatory markers in diabetic rats supplemented with zinc.

Studies have also looked into the anti-inflammatory abilities of *M. oleifera*. The plant contains bioactive substances, including quercetin and kaempferol, which reduce inflammation by preventing the synthesis of pro-inflammatory cytokines like TNF-α and IL-6 (Vergara-Jimenez *et al*., 2017). Similarly, IL-6, TNF-α, and CRP were also found to reduce significantly in diabetic rats treated with *M. oleifera* extracts (Mthiyane *et al*., 2022). This result is not consistent with the current study because although there were reductions in IL-6 and TNF-α in the *M. oleifera*-treated group, the reductions were insignificant compared to the diabetic controls. This is perhaps because, in this study, the amount of *M. oleifera* in the diet (only 3%) is not sufficient enough to significantly counteract diabetes-induced inflammation. Moreover, this may be supported by the fact that the combined treatment showed a similar response to the zinc-only treatment group in ameliorating diabetes-induced inflammation, which implies that the *M. oleifera* diet might have a much lower effect on the inflammatory markers than zinc supplementation.

CONCLUSION

The study's findings indicate a promising combined effect of oral zinc and dietary *Moringa oleifera* in streptozotocin-induced diabetic rats. This co-supplementation led to significant improvements in various antioxidant markers like SOD and TAC, as well as a reduction in MDA levels compared to individual treatments. However, the combined treatment may not have a substantial synergistic effect on diabetes-induced inflammation because no significant disparities were observed in the levels of serum inflammatory markers between the combined treatment and the individual interventions especially when the combined treatment is compared with zinc-only treated groups. Overall, it is concluded that combined supplementation with oral zinc and dietary *M. oleifera* leaf powder acts synergistically against diabetes-induced oxidative stress with little or no synergistic effect on diabetes-induced inflammation.

Author Contributions

A. Muntari and A. I. Ganiyu: Conceptualization; A. Muntari and A.P. Yusuf: Formal analysis; A. Muntari, A.P. Yusuf, and S. Kabir: Investigation; A. Muntari, A.P. Yusuf, and S. Kabir: Funding; A. Muntari and A. I. Ganiyu: Methodology; A. I. Ganiyu: Project administration; A. S. Idoko: Supervision; B. Abdulrahman and N. Lawal: Validation; A. Muntari: Writing - original draft; A.P. Yusuf, A. I. Ganiyu, B. Abdulrahman, N. Lawal, and A. S. Idoko: Writing - review & editing. All authors have read and agreed to the final version of this manuscript.

Funding

No agencies, funding organizations, or any external sources of funds were involved in the funding of this research.

Acknowledgments

The authors wish to acknowledge the Department of Biochemistry and Molecular Biology, Federal University Dutsin-Ma for providing the enabling environment and most of the equipment used in conducting this research.

Conflicts of Interest

The authors have no competing interest to declare.

Data Availability

The corresponding author, [A.I.G], will provide the data that support the study's findings upon request.

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