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Full length Research Article

Effect of *Moringa oleifera* Feed Inclusion on Nω-nitro-L-arginine methyl ester (L-NAME)-induced Hypertension in a Rat Model

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Summary: *Moringa oleifera* (MO) has been recognized for its numerous beneficial properties. This study aimed to evaluate the potential antihypertensive effects of MO seeds in rats subjected to Nω-nitro-L-arginine methyl ester (L-NAME) exposure. Fifty male Wistar rats were randomly divided into five groups of 10 rats each for the experiment. Group A served as the control, received normal saline only, Group B received L-NAME (40 mg/kg) only, Group C received L-NAME (40 mg/kg) + 10% MO feed, Group D received L-NAME (40 mg/kg) + 20% MO feed, and Group E received L-NAME (40 mg/kg) + Lisinopril (10 mg/kg). Treatment was daily and covered a period of 5 weeks. Blood pressure and electrocardiographic measurements were obtained using a non-invasive tail cuff blood pressure device and a 6/7 lead computer ECG equipment, respectively. Heart and kidney tissues were analyzed for oxidative stress parameters, and immunohistochemistry and histopathology of the heart and kidney were conducted using standard methods. L-NAME treatment led to a significant increase in diastolic and systolic values compared to the control group. Serum nitric oxide concentration significantly decreased in rats that received L-NAME alone, while co-treatment with MO and Lisinopril showed a significant increase in nitric oxide levels. Co-treatment with MO and Lisinopril significantly reduced malondialdehyde (MDA) concentrations in the cardiac and renal tissues, whereas L-NAME alone caused a significant increase in MDA concentration. The expressions of cardiac and renal caspase-3 significantly increased in L-NAME alone treated rats, while co-treatments with MO and Lisinopril significantly reduced the expressions of caspase-3. In conclusion, co-treatment with MO effectively reduced arterial pressure and indices of hypertension in rats, mitigated the oxidative stress and apoptosis induced by L-NAME. Therefore, the inclusion of MO seeds in hypertension management may serve as an effective remedy.

Keywords*: Moringa oliefera, L-NAME, Hypertension, Oxidative Stress, Nitric oxide*

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INTRODUCTION

Hypertension poses significant health burden and has profound implications for critical organs such as the kidney and the heart (Aydogdu *et al*., 2019; Panthiya *et al*., 2022). Epidemiological studies have indicated an increase trend in the occurrence of hypertension globally (Li *et al*., 2020). Effective control and prevention of hypertension will largely reduce the occurrence of cardiovascular and kidney diseases (Aydogdu *et al*., 2019). A common underlying factor in the pathophysiology of hypertension is inhibition of endothelial nitric oxide synthase (NOS) activity, which leads to reduction in nitric oxide (NO) production and consequently hypertension (Boe *et al*., 2013; Panthiya *et al*., 2022).

Nω-nitro-L-arginine methyl ester (L-NAME) is a nitric oxide synthase inhibitor which has been used widely to induce hypertension in rat models. Previous research has elucidated that the reduction of NO due to the actions of L-NAME can potentially lead to the senescence of endothelial cells (Silva *et al*., 2017). Production of NO has shown to decrease with increase in age (Leo *et al*., 2015). Long term inhibition of NOS in rats by L-NAME has been reported to result in increased vascular expression of plasminogen activator inhibitor-1 (Boe *et al*., 2013). However, the effects of long term use of L-NAME does not only mediate

inhibition of endothelial NOS, but has also been reported to increased oxidative stress (Chia *et al*., 2021). Chronic L-NAME treatment in rats has been deployed as a chemical method for the induction of nitric oxide deficiency - inducing endothelial malfunction (Leo *et al*., 2015). L-NAME administration has been found to increase arterial blood pressure, cause vascular dysfunction, and alterations of vascular tissues after many weeks of continuous administration (Panthiya *et al*., 2022). Liver injury has also been reported after chronic exposure of rats to L-NAME, with increased serum concentrations of cholesterol, triglyceride, alanine transaminase and aspartate transaminase activity (Li *et al*., 2020). Nitric oxide acts in parts by stimulating soluble guanylyl cyclase (sGC). The sGC coverts guanosine triphosphate to cyclic guanosine monophosphate which in turn activates cyclic guanosine monophosphate dependent protein kinase, leading to relaxation of vascular smooth muscle (Leo *et al*., 2015). A number of NO signaling pathways in the smooth muscle contribute to increase development of vascular diseases (Ma *et al*., 2023). Increase in oxidative stress, reduction of NO concentration, and down-regulation of endothelial NOS in vascular tissues are associated with the toxic effects of L-NAME in Wistar rats (Panthiya *et al*., 2022). Oxidative stress has been associated with the pathophysiology of hypertension (Aremu *et al*., 2019).

Moringa oleifera (MO) is a plant widely distributed in tropical and subtropical countries (Ghasi *et al*., 2000). It has been used as a very rich source of protein in animals feed (Tutubalang *et al*., 2022). MO has been studied for its high quality of antioxidants, including, polyphenols, polysaccharides, alkaloids and nutritional benefits (Abdel-Raheem and Hassan, 2021). It has been reported to be abundant in secondary metabolites, vitamins, and carotenoids that are of good benefits and could cause improvement in animals' performance (Lu *et al*., 2016; Emam *et al*., 2022). It has hypocholesterolemic effects in obese individuals (Ghasi *et al*., 2000). The use of MO in animals' diet has attracted immense research works due to its numerous biological and pharmacological actions (Sultana *et al*., 2021; El-Kassas *et al*., 2022). MO has been demonstrated to significantly increase the concentration and activities of antioxidant enzymes and pro-inflammatory cytokines (Leo *et al*., 2015; El-Kassas *et al*., 2022). The carotenoids and vitamin E present in MO have been demonstrated to support diverse physiological functions of epithelial tissues, visceral organs, mucosal epithelial secretions, and cellular immunity by shielding cells from harmful free radicals (Zaneb *et al*., 2017; Mansour *et al*., 2018; Tutubalang *et al*., 2022). In addition, MO has been sobserved for its antimicrobial and immunomodulatory attributes in poultry (Zaneb *et al*., 2017; Moreno-Mondoza *et al*., 2021). Considering the reported escalating cases of hypertension globally and the proven health benefits of MO, this study was designed to investigate the impact of MO seed-inclusion in feed on L-NAME-induced hypertension in male Wistar rats.

MATERIALS AND METHODS

Preparation of MO feed inclusion: Dried seeds of MO were obtained and ground into powder. Thereafter, they were formulated into 10% and 20% feed content in a commercial feed mill and pelleted.

Experimental Animals: Fifty male Wistar rats, weighing approximately 90-150 g each were obtained from the animal house of Faculty of Veterinary Medicine of University of Ibadan. They were kept in plastic cages and allowed to acclimatize for 4 weeks after which their weights were >170-250 g in the Faculty of Veterinary Medicine animal house before the experiment. The animals were maintained on pelletized growers' feed and tap water ad libitum. The animals were divided into five groups of ten each and kept under natural photoperiod of about 12 h light and 12 h darkness daily.

Experimental Design: The animals were randomly divided into five (5) groups of ten (10) animals per group and assigned as follows: Group A: Normal saline only (orally); Group B: L-NAME only (orally); Group C: L-NAME+ 10% *Moringa oleifera* inclusion in feed; Group D: L-NAME + 20% *Moringa oleifera* inclusion in feed; Group E: L-NAME + 10 mg/kg bw Lisinopril (orally).

Induction of Hypertension and Blood Pressure Measurement: Hypertension was induced through oral administration of L-NAME (40 mg/kg/day) for 5 weeks. A non-invasive tail cuff blood pressure system (CODA™ tail-cuff blood pressure system, Connecticut, USA) was used to measure rats' blood pressure. The blood pressure of rats was measured on day 36 after the commencement of L-NAME administration. The systolic and diastolic blood pressures were measured after drug administration. Blood pressure was measured, three readings were recorded per group and the mean value was used as the blood pressure measurement.

Electrocardiography: The electrocardiographic evaluation of the rats was done using a 6/7 lead computer ECG machine, EDAN VE-1010 as earlier described (Omóbòwálé *et al*., 2018).

Blood collection: Blood was collected on day 36 of experiment through the retro-orbital sinus using capillary tubes into heparinised tubes (Parasuraman *et al*., 2010). Blood samples were allowed to stand for 20 mins to allow coagulation and thereafter centrifuged at 10,000rpm for 10 mins. Sera were stored at -20^{o}C until analysed.

Animal Sacrifice: All rats were sacrificed on day 36 of the experiment by quick cervical dislocation (Aguwa *et al*., 2020). The hearts and kidneys were harvested, rinsed briefly in normal saline and blotted with filter paper before being kept at -20^{o}C until biochemical analysis. The samples for histology were preserved in 10% neutral buffered formalin for 7 days (until tissues were well fixed) and thereafter subjected to histological processing.

Evaluation of Biochemical Assays: Tissue levels of nitric oxide were quantified indirectly by measuring the total nitrite, as described by Olaleye *et al*. (2007). Malondialdehyde concentration was determined by measuring the thiobarbituric acid reactive substances (TBAR) produced during lipid peroxidation. This procedure was carried out using the method of Varshney and Kale

(1990). The values were expressed as µmol/mg/protein. Hydrogen peroxide levels in the cardiac and renal tissues were determined by the method of Wolff (1994). The values were expressed as µ/mg protein.

The cardiac and renal reduced glutathione (GSH) were estimated by the method of Jollow *et al*. (1974).

Glutathione S-transferase (GST) was estimated by the method of Habig *et al*. (1974). Glutathione peroxidase (GPx) activity in tissues was measured according to the method of Buetler *et al*. (1963).

Histology and immunohistochemistry Methods

Histology: Histological studies on the cardiac and renal tissues stained with Haematoxylin and Eosin (H&E) stain were carried out as described by Avwioro (2002).

Immunohistochemistry: Immunohistochemistry procedure was done as described by Oyagbemi *et al*. (2021) with slight modification using 2-step plus Poly-HRP Anti Mouse/Rabbit IgG Detection System with DAB solution (Catalog number: E-IR-R217 from Elabscience Biotechnology®, China). The cardiac and renal samples were fixed with 10% paraformaldehyde, embedded in paraffin and sectioned at a thickness of 5µm. The slides were subsequently dewaxed in xylene (100%) solution for 2minutes and afterward, hydration was carried out in different concentrations of ethanol (100%, 90%, 80% and 70%) for 2 minutes each. The hydrated tissue sections were rinsed and put in a phosphate buffered saline (PBS) tank for 5 minutes. The antigen retrieval was performed with citrate buffer (pH 6.0), in a microwave oven. Endogenous peroxide (H2O2) block was carried out following manufacturer's instructions on the kit (E-AB-15447). Drops of H2O2 were added to cover the sections and incubated in a humidifying chamber at room temperature for 10 min. The slides were rinsed afterwards and put back in the PBS tank for 5 minutes. Goat serum (E-1R-R217A) was added onto the slides to prevent non-specific binding and incubated in a humidifying chamber at room temperature for 30 minutes. Thereafter, the tissues were probed with Synthetic peptide of human HTR1A (E-AB15447:1:100) as primary antibodies for the cardiac and renal tissues. They were then incubated for 2 hours at room temperature. Following incubation, the tissue slides were rinsed with PBS and a secondary antibody labelled (E-1R-R217B) was added and the slides were incubated in a humidifying chamber at room temperature for 20 min. The slides were subsequently rinsed and immersed in PBS tank for 5 minutes. Finally, a few drops of the substrate diaminobenzidine (DAB) (50 µL of DAB concentrate (E-1R-R217D) + 1mL DAB solution (E-1R-R217E)) were added at room temperature for 10 seconds in the dark. The reaction was terminated with deionised water and slides were immersed in haematoxylin for 3 seconds before rinsing with PBS. The slides were placed in 70%, 80%, 90% and 100% of ethanol and then xylene (100%) for 2 minutes each. Slides were removed, allowed to dry and a DPX mountant was applied. Sections were observed with a light microscope (Leica LAS-EZ®) using Leica software application suite version 3.4 equipped with a digital camera.

Statistics Analyses: The results were expressed as mean ± standard deviation. One-way ANOVA was used to analyse the differences among them. Comparisons between the groups were done using the Student's t-test. Data were analysed using GraphPad Prism for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com.). Values of $P < 0.05$ were considered significant.

RESULTS

Blood pressure and Nitric oxide (NO): Figure 1 shows the result of treatments on blood pressure of the rats. A significant ($P < 0.05$) increase was observed in systolic and diastolic in L-NAME treated group compared to the control group. The concentration of NO rose significantly ($P < 0.05$) in rats co-treated with 20% MO group compared with the NO concentration obtained in rats treated with L-NAME alone.

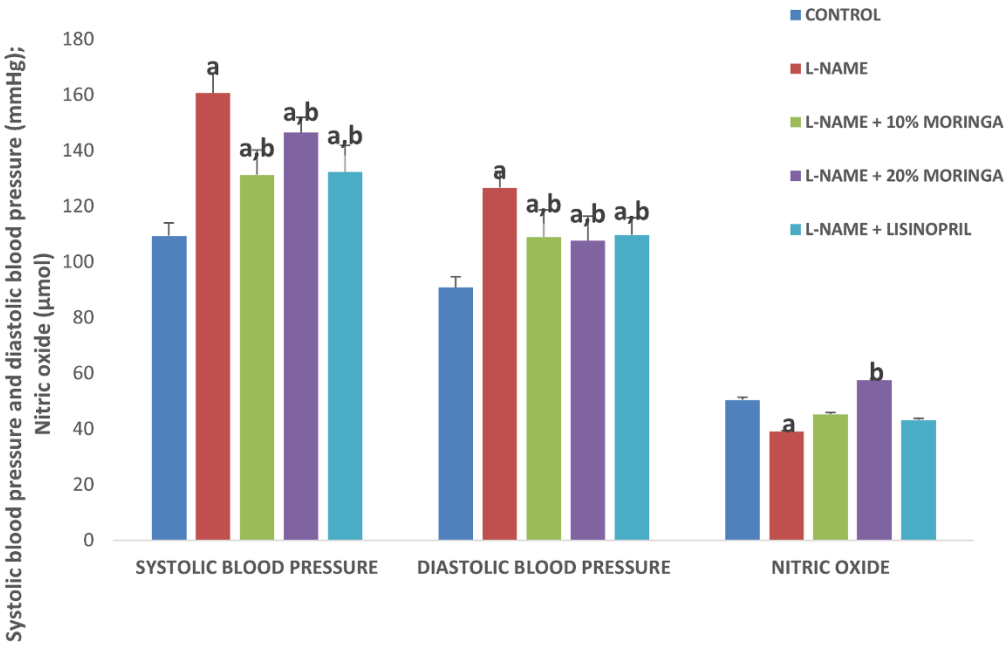

Figure 1: Effect of *Moringa oleifera* on systolic blood pressure, diastolic blood pressure and serum nitric oxide (NO) level in the experimental rats treated with L-NAME. Values are presented as mean ±standard deviation. *Superscript (a) indicates significant difference at P < 0.05 compared with control (Group A) while superscript (b) Indicates significant difference at P < 0.05 compared with Group B.*

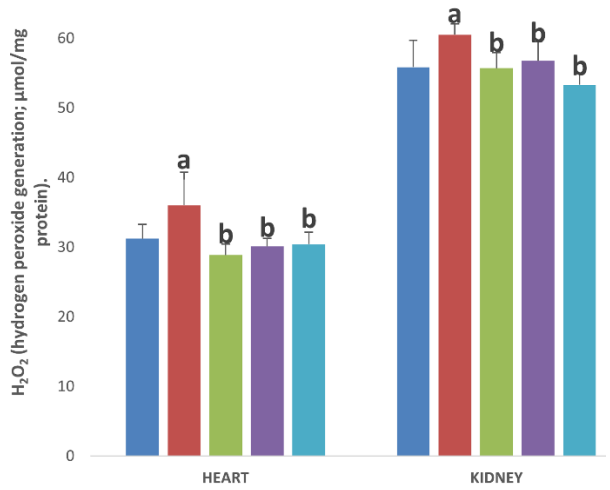

Figure 2:
The effect of *Moringa oleifera* on hydrogen peroxide generation (H_2O_2) activity in the cardiac and renal tissues of L-NAME treated Wistar rats. Values are presented as mean ± standard deviation.
Superscript (a) indicates significant difference at P < 0.05 compared with control (Group A) while superscript (b) Indicates significant difference at P < 0.05 compared with Group B.

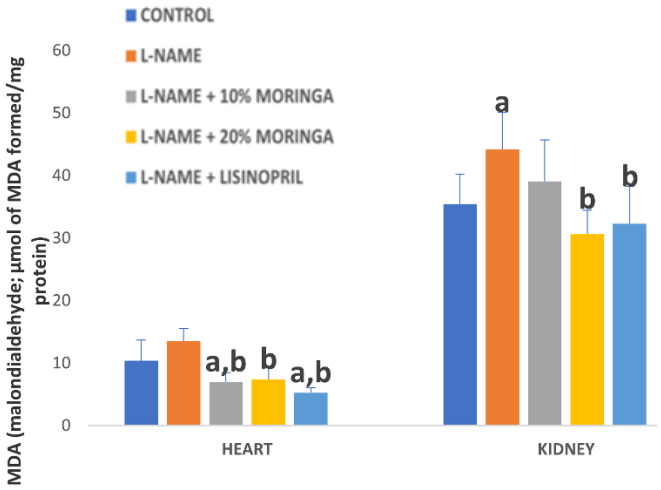

Figure 3:
The effect of *Moringa oleifera* on malondialdehyde (MDA) activity in the cardiac and renal tissues of L-NAME treated Wistar rats. Values are presented as mean ± standard deviation.
Superscript (a) indicates significant difference at P < 0.05 compared with control (Group A) while superscript (b) Indicates significant difference at P < 0.05 compared with Group B.

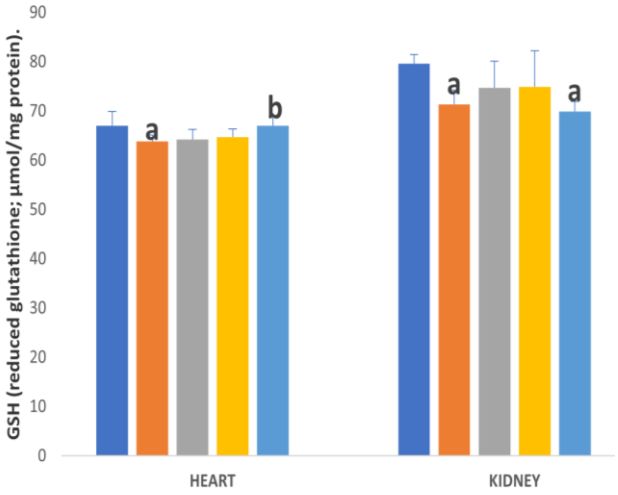

Figure 4:
The effect of *Moringa oleifera* on reduced glutathione (GSH) activity in the cardiac and renal tissues of L-NAME treated Wistar rats. Values are presented as mean ± standard deviation.
Superscript (a) indicates significant difference at P < 0.05 compared with control (Group A) while superscript (b) Indicates significant difference at P < 0.05 compared with Group B.

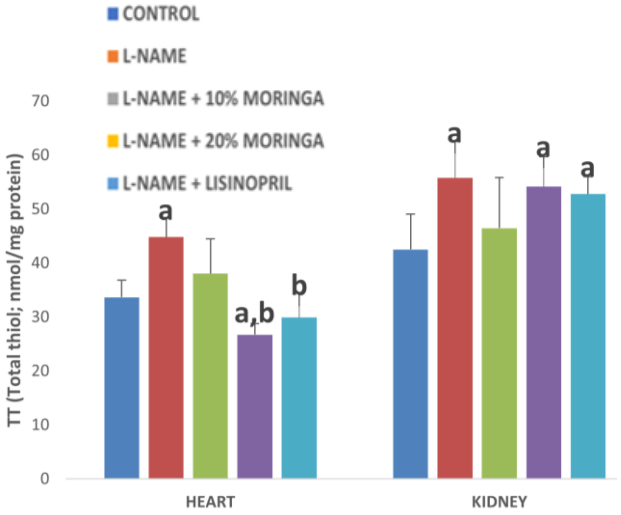

Figure 5:
The effect of *Moringa oleifera* on total protein thiol (TPT) activity in the cardiac and renal tissues of L-NAME treated Wistar rats. Values are presented as mean ± standard deviation.
Superscript (a) indicates significant difference at P < 0.05 compared with control (Group A) while superscript (b) Indicates significant difference at P < 0.05 compared with Group B

Hydrogen peroxide (H_2O_2): The concentration of H_2O_2 deceased significantly (P < 0.05) in the L-NAME-treated group when compared with the MO co-treated groups (Figure 3).

Malondialdehyde (MDA): Figure 3 showed the concentration of MDA in the heart and kidney tissues. There was significant (P < 0.05) increase in the concentration of MDA in rats treated with L-NAME compared to the level obtained in the groups co-treated with MO seeds.

Antioxidant parameters: The level of GSH in cardiac and renal tissues is shown in Figure 4. The level of GSH significantly (P < 0.05) reduced in the rats treated with L-NAME alone when compared with the co-treatment groups. The value of TPT was significantly higher (P < 0.05) when compared with the co-treatment groups showed in Figure 5. The concentration of non-protein thiol (NPT) increased significantly (P < 0.05) in L-NAME compared with the value obtained in co-treatment groups (Figure 6). The glutathione S-transferase (GST) activity in the co-treatment groups increased significantly (P < 0.05) when compared with L-NAME group (Figure 7). Figure 8 showed the activity of glutathione peroxidase (GPx) in the heart and kidney tissues. The activity of GPx obtained in MO seed feed inclusion treatment groups was higher (P < 0.05) than

that obtained in L-NAME alone group. Vitamin C concentration was found to reduce significantly ($P < 0.05$) in L-NAME alone treated group compared with co-treatment groups (Figure 9). Protein carbonyl level increased significantly in L-NAME alone treated rats when compared with the co-treatment groups (Figure 10).

Histology: The photomicrograph of the kidney of the rats (Plates 1) showed mild haemorragic lesion and thrombosis in hypertensive rats, while the hypertensive rats treated with MO had mild haemorrhagic lesion, mild tubular inflammation, glomerular infilteration. The group treated with lisinophil had slight perivascular inflammation, mild thrombosis and glonerulonephrosis. In the heart tissue (plate 2), photomicrograph revealed moderate thrombosis and perivascular inflammation in hypertensive rats. These abnormalities were not found in the photomicrograph of the groups treated with MO exract and lisinophl.

Immunohistochemistry Immunohistochemistry showed a higher expression of immune positive C – Reactive Proteins (CRP) on the heart of the hypertensive rats when compared to that of the control. However, there was lower expression of the CRP in heart tissue of rats treated with MO and lisinopril when compared to the heart tissue of the hypertentive rats that were not treated. There was higher expression of extracellular regulated kinase (ERK) in the kidney tissues of the hypertensive rats when compared to the control. A high expression of ERK was also observed the group treated with 10% MO. Whereas a lower expression of MO was observed by the groups treated with 20% MO and lisinopril.

Electrocardiogram: When compared with the controls, no statistically significant ($p<0.05$) differences were observed between the L-NAME-only treated groups and the others.

DISCUSSION

The objective of the current investigation was to assess the antihypertensive efficacy of Moringa oleifera. Hypertension was generated in rats with the oral administration of L-NAME at a dose of 40mg/kg. This phenomenon can be attributed to its capacity to inhibit NOS activity, resulting in a subsequent decrease in NO levels within the circulatory system (Gardina *et al*., 2010; Krol and Kepinska, 2020). The L-NAME treated group exhibited a notable reduction in serum NO levels compared to the control group. This decrease can be attributed to the down-regulation of endothelial nitric oxide synthase expression in hypertensive rats, as previously reported by Zhou and Frohlich (2007) and Panthiya *et al*. (2022). Rats which were fed MO seed inclusion in their diet, in conjunction with L-NAME, exhibited a statistically significant ($p<0.05$) elevation in NO levels as compared to the groups that received L-NAME alone. The observed phenomenon can likely be attributed to the antioxidant properties of the MO, which consequently mitigated the oxidative damage generated by L-NAME.

In this study, oxidative stress markers were significantly increased in L-NAME group compared with groups treated with MO and Lisinopril. The MDA is a product of lipid peroxidation and the contents of tissue MDA is reported to be a reliable marker of lipid peroxidation (Ayala *et al*., 2014). The administration of L-NAME alone exhibited a significant increase in the MDA contents, which is an indication of oxidative stress-induced cardiac and renal damage. However, co-treatment with MO caused a significant reduction in this MDA contents implying that MO impact an antioxidant activity, which involves chelating of ions and scavenging of ROS, thus protecting the cardiac and renal tissues from lipid peroxidation. These present results are in agreement with those of several authors who reported the association of oxidative stress and lipid peroxidation (Bergin *et al*., 2021; Sabanna and Ratan, 2021; Cordiano *et al*., 2023).

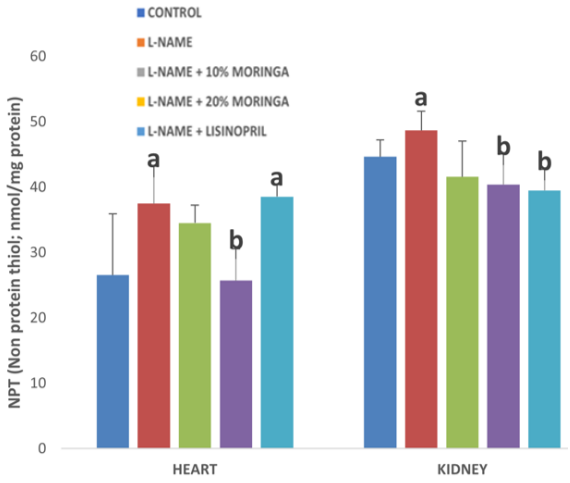

Figure 6:
The effect of *Moringa oleifera* on non-protein thiol (NPT) activity in the cardiac and renal tissues of L-NAME treated Wistar rats. Values are presented as mean ± standard deviation.
Superscript (a) indicates significant difference at $P < 0.05$ compared with control (Group A) while superscript (b) Indicates significant difference at $P < 0.05$ compared with Group B.

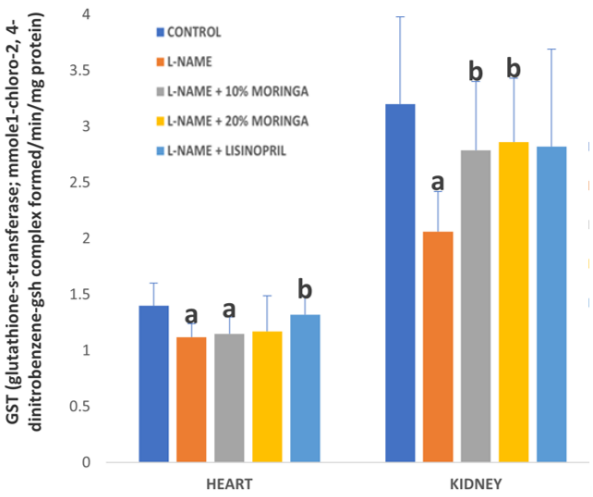

Figure 7:
The effect of *Moringa oleifera* on glutathione S-transferase (GST) activity in the cardiac and renal tissues of L-NAME treated Wister rats. Values are presented as mean ± standard deviation.
Superscript (a) indicates significant difference at $P < 0.05$ compared with control (Group A) while superscript (b) Indicates significant difference at $P < 0.05$ compared with Group B.

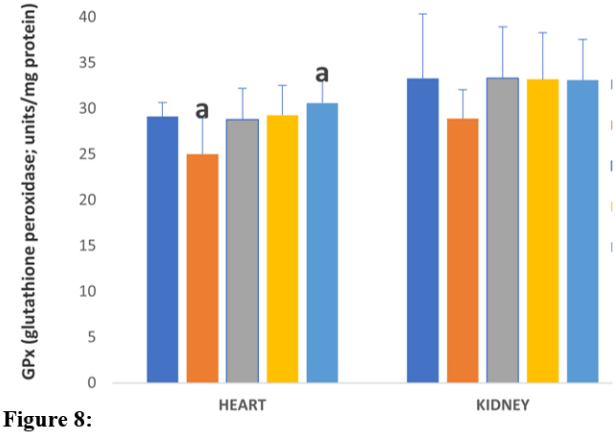

Figure 8:
The effect of *Moringa oleifera* on glutathione peroxidase (GPx) activity in the cardiac and renal tissues of L-NAME treated Wistar rats. Values are presented as mean ± standard deviation.
Superscript (a) indicates significant difference at P < 0.05 compared with control (Group A) while superscript (b) Indicates significant difference at P < 0.05 compared with Group B

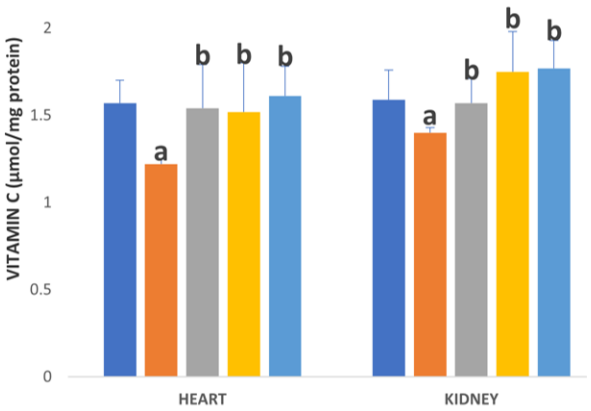

Figure 9:
The effect of *Moringa oleifera* on Vitamin C levels in the cardiac and renal tissues of L-NAME treated Wistar rats. Values are presented as mean ± standard deviation.
Superscript (a) indicates significant difference at P < 0.05 compared with control (Group A) while superscript (b) Indicates significant difference at P < 0.05 compared with Group B

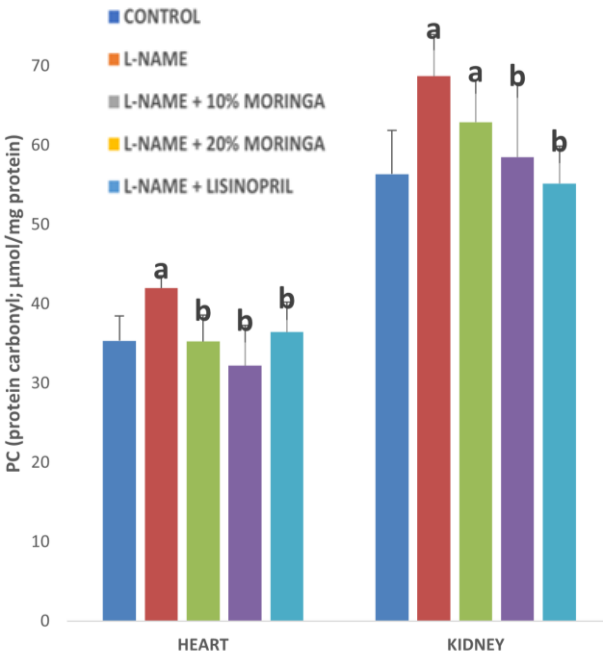

Figure 10:
The effect of *Moringa oleifera* on protein carbonyl (PC) activity in the cardiac and renal tissues of L-NAME treated Wistar rats. Values are presented as mean ± standard deviation.
Superscript (a) indicates significant difference at P < 0.05 compared with control (Group A) while superscript (b) Indicates significant difference at P < 0.05 compared with Group B.

Treatment with L-NAME produced a significant increase in the H2O2 level in both cardiac and renal tissues, which might be due to its ability to raise levels of oxidative stress markers as reported by Pal *et al*., 2023. Co-treatment with *Moringa oleifera* and Lisinopril however, caused a significant reduction in the H2O2 levels, indicating the ability of MO to counteract the deleterious effects of free radical generation by the L-NAME.

Our results showed that GPx was significantly decreased following L-NAME treatment. This however corresponds to previous work by Efosa *et al* (2023) who reported a decrease in GPx activity following administration of L-NAME in Wistar rats; and in agreement with the findings that oxidative stress led to decreased activities of antioxidative enzymes. The finding in this study could be as a result of an adaptive response of the GPx antioxidant system against a sudden and aggressive attack by free radicals produced following administration of L-NAME. Co-treatment with MO caused a significant increase in the enzyme activity compared to the L-NAME alone and this could probably be due to the antioxidant protective ability of the MO that involves quenching of the free radicals generated by L-NAME treatment, thereby ameliorating L-NAME-induced oxidative damage in cardiac and renal tissues.

In this present study, GST activity was significantly inhibited in the cardiac and renal tissues in L-NAME treated rats. This is in contrast with the results obtained by (Gogebakan *et al*., 2012) which showed a significant increase in GST in L-NAME treated rats. However, co-treatment with MO and Lisinopril counteracts the inhibition of GST against free radical generation and oxidative stress. The significant increase in the activity of GST in MO and Lisinopril treatment groups implied that MO ameliorates L-NAME induced free radical generation due to its antioxidant and free radical scavenging properties.

The thiols are organic compounds that contain sulphydryl group which constitute the major portion of the total body antioxidants which play an important role in the defense against reactive oxygen species. Both intracellular and extracellular thiols are components of total thiol. In this present study, the levels of both protein and non-protein thiol increased significantly in the L-NAME treatment group when compared with the control group. This is not in consonance with previous studies that decreased levels of thiols which reported in some diseases of the kidney, cardiovascular system and several other organs in the body (Prakash *et al*., 2009). Co-treatment with MO and Lisinopril caused a significant decrease in the total thiol level and this could be as a result of the antioxidant property of MO.

Blood pressure measurement in this study showed a significant increase in the systolic and diastolic pressure in L-NAME treatment group when compared with the control group. Also, the systolic, diastolic and mean arterial

pressure decreased significantly in the MO and Lisinopril treated groups when compared with L-NAME treated group indicating that MO has the ability to reverse the inhibition of NOS by L-NAME, thereby, increasing the level of nitric oxide in the circulation.

The findings showed that ascorbic acid activity is reduced in the L-NAME treated group which inhibits the reduction and neutralisation of ROS such as hydrogen peroxide whereas MO and Lisinopril treated groups caused an increase compared to the L-NAME group and thus promotes free radical formation and however, will also reduce metal ions that generate free radicals through the Fenton reaction (Stohs and Bagchi, 1995). There is also an increased PC of L-NAME treated group which in turn will aid generation of free radicals and increase metal ions whereas MO and Lisinopril treated group has reduced PC activity compared to the L-NAME treated group only.

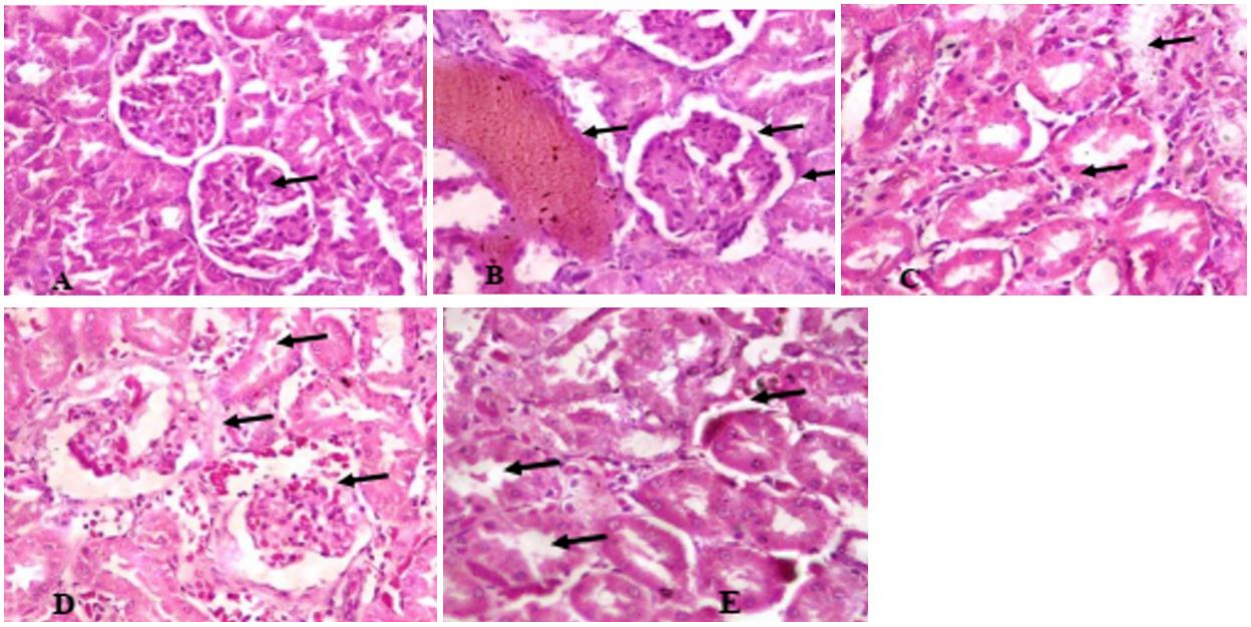

Plate 1:
Photomicrograph showing kidney of rats (Group A) - Control: Shows mild haemorrhagic lesion; (Group B) administered with L-NAME 40 mg/kg) shows mild haemorrhagic lesion and thrombosis (black arrow); (Group C: 10% *Moringa oleifera* + L-NAME 40 mg/kg) shows mild glomerular congestion and mild haemorrhagic lesion, (Group D: 20% *Moringa oleifera* + L-NAME 40 mg/kg shows mild haemorrhagic lesion, glomerular inflammation, and mild peritubular inflammation; Group E (10 mg/kg lisinopril + L-NAME 40 mg/kg) shows slight peritubular inflammation, mild thrombosis and glomerulosclerosis. Plates are stained with H and E stains and viewed with X 100 objectives.

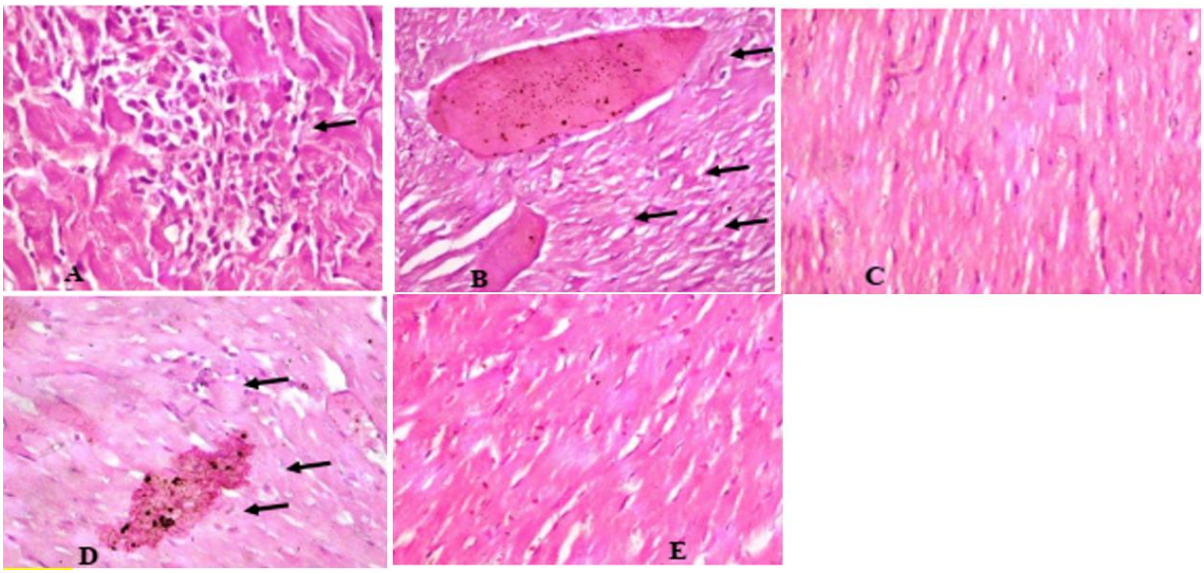

Plate 2:
Photomicrograph showing heart of rats (Group A)-Control: moderate infiltration of inflammatory cells (thin arrow) to the myocardium; (Group B) administered with (L-NAME)-40 mg/kg) shows moderate thrombosis (black arrow) and mild perivascular inflammation (black arrow); (Group C: 10% *Moringa oleifera* + L-NAME 40 mg/kg) shows normal architecture and cellularity of myocytes. No visible lesion seen. (Group D: 20% *Moringa oleifera* + L-NAME 40 mg/kg shows moderate thrombosis; Group E (10 mg/kg lisinopril + L-NAME 40 mg/kg) shows normal architecture and cellularity of myocytes. No visible lesion seen. Plates are stained with H and E stains and viewed with X 100 objectives

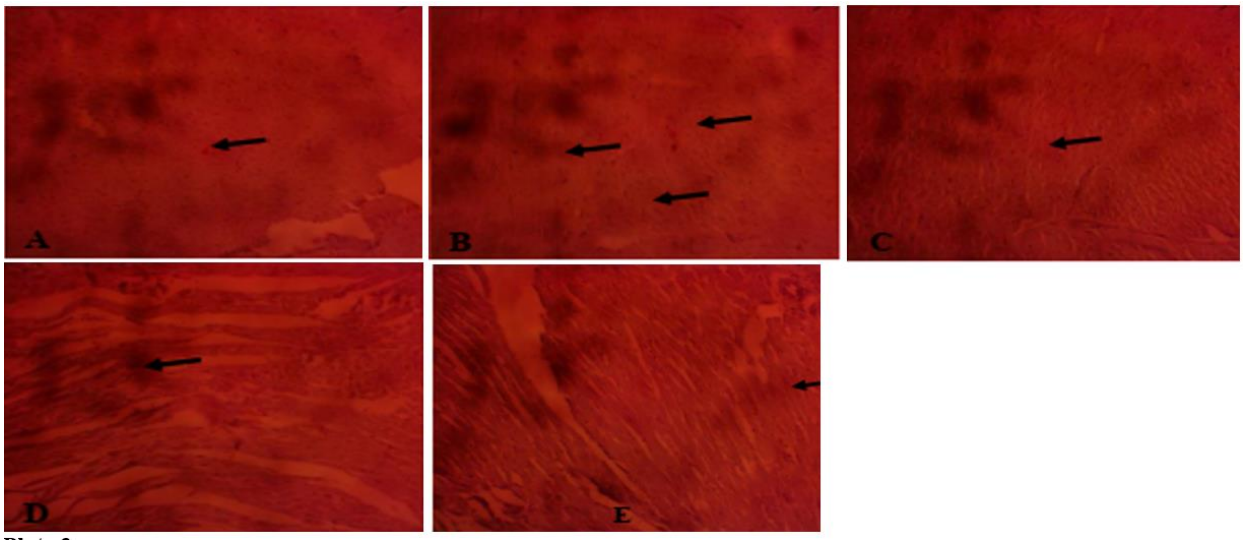

Plate 3:
Immunohistochemistry of C-reactive proteins (CRP) in the heart of rats. A – Control: There were lower immune-positive expressions of CRP. Group B L-NAME 40 mg/kg shows higher immune-positive expression of CRP when compared to the control; Group C: 10% *Moringa oleifera* + L-NAME 40 mg/kg) shows lower expressions of CRP when compared with the L-NAME only treated group (black arrow) and (Group D) administered 20% *Moringa oleifera* + L-NAME 40 mg/kg also shows lower expressions of CRP when compared with the L-NAME only treated group (black arrow) and Group E (10 mg/kg lisinopril + L-NAME 40 mg/kg shows lower expressions of CRP compared with L-NAME alone. The slides were counterstained with high-definition hematoxylin and viewed x 100 objectives.

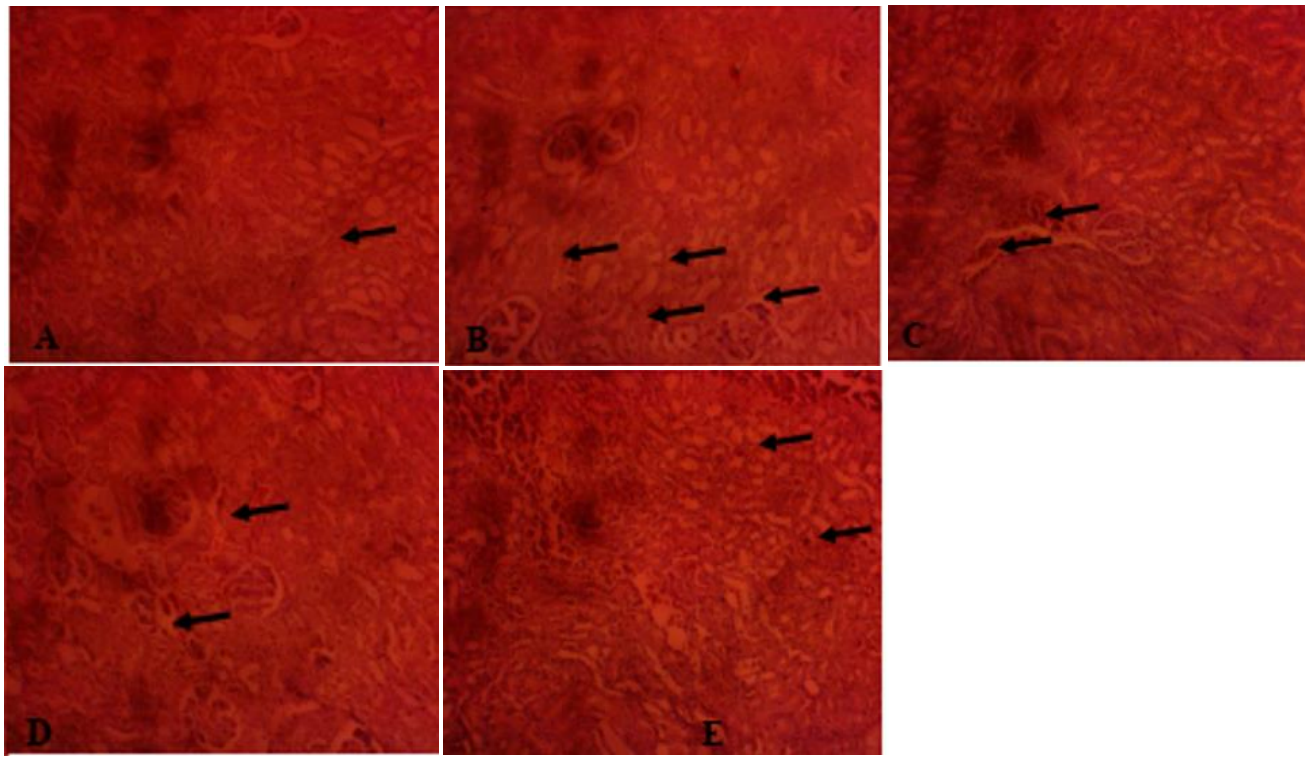

Plate 4:
Immunohistochemistry of extracellular regulated kinase (ERK) in the kidney of rats. A – Control: There was lower immune-positive expression of ERK. Group B L-NAME 40 mg/kg) shows higher immune-positive higher expression of ERK when compared to the control; Group C: 10% *Moringa oleifera* + L-NAME 40 mg/kg) shows higher expressions of ERK when compared with the L-NAME only treated group (black arrow) and (Group D) administered 20% *Moringa oleifera* + L-NAME 40 mg/kg also shows higher expressions of ERK when compared with the L-NAME only treated group (black arrow) and Group E (10 mg/kg lisinopril + L-NAME 40 mg/kg shows higher expressions of ERK compared with L-NAME alone. The slides were counterstained with high-definition hematoxylin and viewed x 100 objectives.

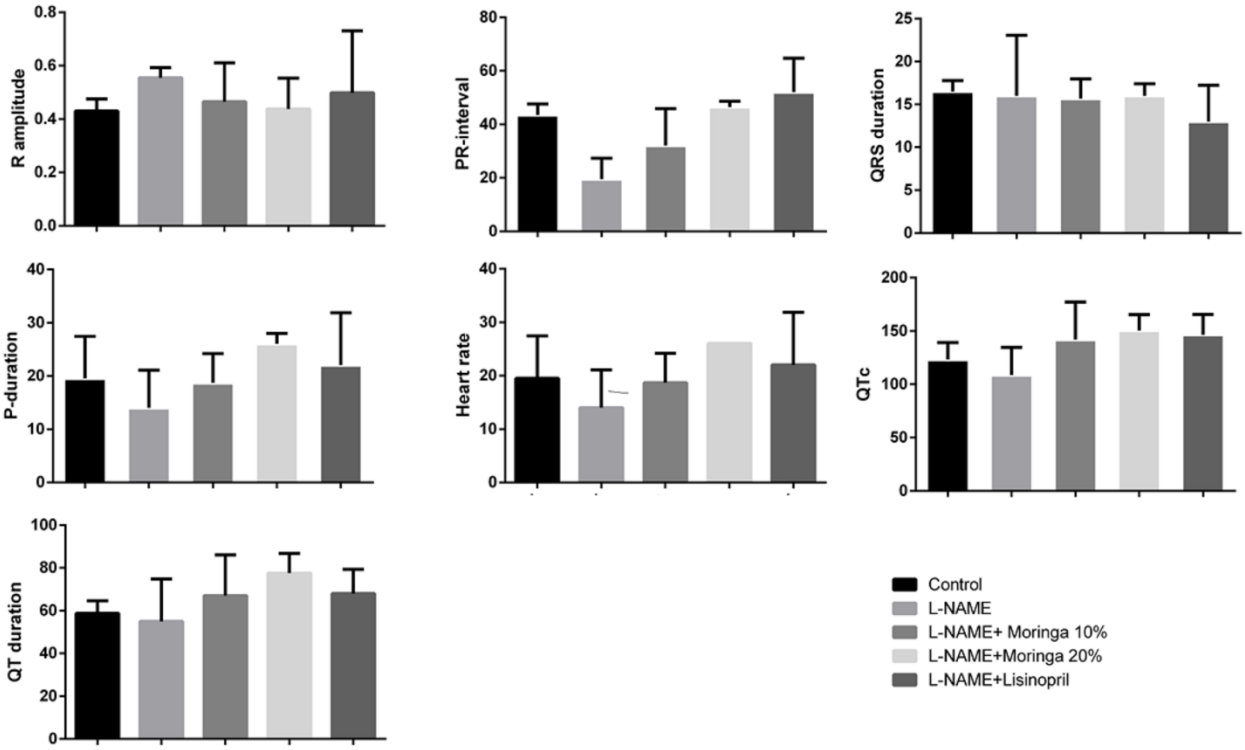

Figure. 11
Effect of *Moringa oleifera* seed inclusion and lisinopril on the electrocardiogram in L-Name-induced hypertension

The immunohistochemistry results revealed a higher expression of cardiac and renal Caspase-3 in L-NAME treated rats, thus indicative of apoptosis. Caspase-3 is an enzyme that plays a central role in the execution-phase of apoptosis following its activation by Caspase 8, 9 and 10. Caspases are actually inactive pro-enzymes that undergo proteolytic cleavage to produce two subunits, a large and small, that dimerizes to form the active enzyme (Redza-Dutordoir and Averill-Bates, 2016). Caspase-3 is activated in apoptotic cell both by extrinsic (death ligand) and intrinsic (mitochondrial) pathways (Rodriquez-Gonzalez and Gutierrez-Kobeh, 2024).

As an executioner caspase, the caspase-3 zymogen has virtually no activity until it is cleaved by an initiator caspase after apoptotic signaling events have occurred (Waters *et al*., 2009). One of such apoptotic signaling event is the introduction of granzyme B (an enzyme shown to be involved in inducing inflammation), which can activate initiator caspases into cells targeted for apoptosis by killer T cells (Metkar *et al*., 2003; Dalken *et al*., 2006). This extrinsic activation triggers the hallmark caspase cascade that characterizes the apoptotic pathway, in which caspase-3 plays a dominant role (Rodriquez-Gonzalez and Gutierrez-Kobeh, 2024). In intrinsic activation, cytochrome c released from the mitochondria, works in combination with caspase-9, apoptosis-activating factor 1 (Apaf-1), and ATP to process procaspase-3 (Garrido *et al*., 2006; Brentnall *et al*., 2013). These molecules along with some other regulatory proteins activate caspase-3 in vivo (Brentnall *et al*., 2013). This current study shows that L-NAME administration elicited an increased expression of Caspase-3, indicating an apoptotic process associated with the L-NAME-induced hypertension; whereas co-treatment with MO and Lisinopril caused downregulation in the L-NAME induced apoptosis, characterized by a marked decrease in the Caspase-3 expression, suggesting anti-apoptotic property of MO against the L-NAME induced apoptosis and hypertension.

In conclusion, the results of this study indicate that *Moringa oleifera* has the potential for antihypertensive therapy as our study revealed. Co-treatment with Moringa oleifera, alongside of L-NAME, effectively maintained the systolic and diastolic pressure to that of normotensive animals. The findings confirmed the anecdote that *Moringa oleifera* seed possess antihypertensive properties, hence can be used for the management of hypertension with less side effects.

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