Niger. J. Physiol. Sci. 40 (June 2025): 203 – 212
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full-length Research Article

Persea americana bark extract Modulates N^{w}–nitro-L-arginine methyl ester (L-NAME)-induced hypertension through NF-ƙβ/NRF2/KIM-1/CTnI signaling pathways in Wistar rats

***Adejumobi O.A.[1], Oriaku V.[1], Gbadegoye J.O.[7], Afolabi J.M.[7], Ogunpolu B.S.[8], Ajani T.F.[1], Omotosho O.O.[1], Ohore O.G.[3], Oyagbemi A.A.[2], Adedapo A.A.[4], Yakubu M.A.[5], Ashafa A.O.T.[6], Nottidge H.O.[1] and Omobowale T.O.[1]**

[1]*Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan.*
[2]*Department of Veterinary Physiology and Biochemistry, University of Ibadan, Nigeria.*
[3]*Department of Veterinary Pathology, University of Ibadan, Nigeria.*
[4]*Toxicology and Assessment Section, Michigan Department of Health and Human Services, Lansing, MI, USA*
[5]*Department of Environmental and Interdisciplinary Studies, Center for Cardiovascular Diseases, COPHS, Texas State University, Houston, TX, U.S.A.*
[6]*Deparment of Plant Science, University of Free State, Qwaqwa Campus, South Africa.*
[7]*Department of Physiology, University of Tennessee Health Sciences Centre, USA.*
[8]*Department of Cardiovascular Pharmacology, Kent State University, USA.*

Summary: Hypertension is one of the major risk factors for cardiovascular diseases. It has become a significant public health concern in both developed and developing countries. In this study we evaluated the ameliorative effect of methanol bark extract of *Persea americana* (PA) on L-NAME-induced hypertension and its attendant cardiac and renal complications. Sixty rats were divided into six groups. Group A was the negative control and received distilled water throughout the study. Group B received a daily repetitive dose of L-NAME alone at 40 mg/kg for 21 days. Groups C, D, and E received a daily repetitive dose of L-NAME at 40 mg/kg and extract at 100 mg/kg, 200 mg/kg and 400 mg/kg, respectively, for 21 days. Group F received a daily repetitive dose of L-NAME at 40 mg/kg and lisinopril at 10 mg/kg for 21 days. The results showed that L-NAME significantly elevated blood pressure, markers of renal damage, oxidative stress, and expression of KIM-1, NRF2, NF-KB and CTnI, while it decreased both enzymatic and non-enzymatic antioxidant parameters. The extract and lisinopril, however, ameliorated these effects in the rat model. These findings showed that the bark extract of PA may play a role in reducing oxidative stress, inflammation, cardio-renal organ damage and blood pressure levels in hypertension, possibly through free radical scavenging, antioxidant system potentiation, NF-ƙβ/NRF2/KIM-1/CTnI signaling pathways.

Keywords: *Persea americana* bark extract, hypertension, oxidative stress, antioxidant, L-NAME, lisinopril

**Author for correspondence: muyenko@yahoo.com, Tel: +234-8033728003*

Manuscript received: October 2024; Accepted: February 2025

DOI: https://doi.org/10.54548/njps.v40i1.24

INTRODUCTION

Hypertension accounts for about 15 percent of health loss in adults and little decreases in the prevalence of hypertension can facilitate great health gains in blood pressure values (Abreu *et al*., 2018). It is characterized by elevations in blood pressure with attendant organ damage in the heart, kidneys, and the eye (Carasin *et al*., 2007). The pathogenesis of hypertension is multifactorial, and beyond reducing blood pressure values, an ideal antihypertensive should also ameliorate the complications of hypertension seen in several other target organs such as the heart, brain and the kidneys (Dais *et al*., 2018). This, however, is not targeted in current drug strategies.

It has been shown that a prerequisite of hypertension is constriction of renal blood vessels and this is facilitated by discrepancies in the levels of renal vasoconstrictors and vasodilators favoring vasoconstrictors (Johnson et al., 2018). The kidney plays a huge role in the development of hypertension. Ultimately, hypertension leads to cardiac damage and elevated blood pressure, particularly systolic blood pressure, is a significant factor contributing to myocardial infarction (Messerli *et al*., 2017).

Persia americana (avocado pear) is widely found in America, Africa, and the tropics. The leaf is simple, finely toothed, glossy, and green in colour. The fruit has a bell shape with colour mostly green or brown. Apart from the nutritional value of *Persia americana,* extracts from the leaf

and seed of the plant have been found to be of good medicinal value (Ojewole *et al*., 2007).

Lisinopril, an angiotensin converting enzyme (ACE) inhibitor acts by reducing the synthesis of angiotensin-2 by inhibiting the action of ACE. ACE Inhibitors have been shown to prevent the onset of nephropathy in hypertension (NHFA, 2016).

In this study we evaluated the ability of *Persea americana* to reduce blood pressure and ameliorate the consequent renal and cardiac damage and probable mechanisms of action.

MATERIALS AND METHODS

Animals: Sixty male Wistar rats were used in this study. The rats were randomly selected and acclimatized to the feed and the environment for about three weeks. They were provided with standard diet rat feed and water *ad libitum*. They were kept in spacious cages in a well-ventilated house under natural light conditions; 12 hours light, 12 hours dark daily for the period of acclimatisation. Animals used for this experiment were handle in accordance to the guidelines, rules and regulations of handling experimental animals and ethical approval was given by the University of Ibadan Animal Care and Research Ethic Committee with the approval number UI-ACUREC/17/0118.

Experimental design: The rats were divided into six groups of ten rats each. A daily repetitive dose of L-NAME (at a dosage of 40 mg/kg), lisinopril (at a dosage of 10 mg/kg) and extract was given for 21days to each rat according to the group/experimental demand. A (Control), B (L- NAME alone), C (L-NAME + 100 mg of extract), D (L-NAME + 200 mg of extract), E (L-NAME + 400 mg of extract), F (L-NAME + Lisinopril at 10 mg/kg).

Blood pressure measurements: Blood pressure parameters, including systolic, diastolic, and mean arterial blood pressures, were determined non-invasively in conscious animals by tail plethysmography using an automated blood pressure monitor (CODA 4.1, Kent Scientific Corporation, Connecticut, USA). The average of at least nine readings, taken in the quiescent state, following acclimatization, was recorded per animal (Omobowale *et al*., 2019)

Serum preparation: Approximately three milliliters of blood were collected by retro–orbital venous puncture using plain capillary tubes into plain bottles and allowed to clot. The clotted blood was then centrifuged at 4, 000 revolutions per minute (rpm) for 10 minutes. Clear serum was separated with Pasteur pipette into another plain tube and then stored at 4^{o}C until they were analysed.

Renal and cardiac homogenate preparation: The organs excised were rinsed and homogenized using 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% KCl. The homogenates were subjected to cold centrifugation at 4^{o}C using a speed of 10, 000 for 15 minutes. The post mitochondrial fractions (PMFs) obtained from cardiac and renal homogenates were used for biochemical assays.

Biochemical analysis

Renal and Cardiac Makers of Oxidative Stress: Hydrogen peroxide generation was determined according to the method of Wolff 1994. The reaction mixture was subsequently incubated at room temperature for 30 minutes. The mixtures were read at absorbance at 560 nm and H_2O_2 generated was extrapolated from H_2O_2 standard curve. The Malondialdehyde (MDA) content as an index of lipid peroxidation was quantified in the PMFs of cardiac and renal tissue according to the method Varshney and Kale, 1990. The absorbance was measured against a blank of distilled water at 532 nm. Lipid peroxidation was calculated with a molar extinction coefficient of 1.56×10^5/M/cm. Protein carbonyl (PCO) contents in the renal and cardiac tissues were measured using the method of Reznick and Packer, 1994. The absorbance of the sample was measured at 370 nm. The carbonyl content was calculated based on the molar extinction coefficient of $4 \quad 1 \quad 1$ DNPH ($2.2\ 10$ cm M) and expressed as nmoles/mg protein while vitamin C contents were measured as earlier described by Jacques-Silva *et al.* (2001).

Renal and Cardiac Antioxidants: The Superoxide dismutase (SOD) assay was carried out by the method of Misra and Fridovich, (1972), with slight modification by Omobowale *et al.* (2014). The increase in absorbance at 480 nm was monitored every 30 s for 150 s. The one unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome. Reduced glutathione (GSH) was estimated by the method of Jollow and Mitchell, (1974). Catalase (CAT) activity was determined according to the method of Sinha, 1972. One unit of CAT activity represents the amount of enzyme required to decompose 1 µmol of H_2O_2/min. Glutathione peroxidase (GPx) activity was also measured according to Beutler *et al*. 1963. Glutathione-S-transferase (GST) was estimated by the method of Habig *et al.* (1974) using 1-chloro-2, 4-dinitrobenzene as substrate. The protein and non-protein thiol contents were determined as described by Ellman, (1954). Protein concentration was determined by the Biuret method of Gornal *et al.*, 1949 , using bovine serum albumin (BSA) as standard.

Determination of Serum Markers of Oxidative Stress.: The serum nitric oxide concentrations were measured spectrophotometrically at 548 nm according to the method of Olaleye *et al*. (2007). The serum myeloperoxidase (MPO) activity was determined according to the method of Xia and Zweier, 1997. The advanced oxidation protein product (AOPP) contents were determined as described by Kayali *et al.* (2006). Briefly, 0.4 ml of cardiac and renal PMFs were treated with 0.8 ml phosphate buffer (0.1 M; pH 7.4). The absorbance of the reaction mixture was immediately recorded at 340 nm wavelength. The content of AOPP for each sample was calculated using the extinction coefficient of 261 cm^{-1} mM^{-1} and the results were expressed as µmoles/mg protein. The activity of xanthine oxidase was determined according to method of Akaike *et al.* (1990).

Serum Markers of Renal and Liver Damage: The blood urea nitrogen (BUN), creatinine, ALT, AST and ALT were

determined using Randox kits following the manufacturer's instructions.

Histopathology: Small pieces of kidney and heart were fixed in 10% formalin, embedded in paraffin wax, and sections of 5-6 mm in thickness were made and thereafter stained with hematoxylin and eosin for histopathological examination according to the methods as previously described by Drury *et al*. (1996). Thereafter, the sections were examined with light microscopy.

Immunohistochemical staining for kidney injury molecule I (Kim-1), nuclear factor kappa beta (NF-κB), nuclear factor erythroid 2-related factor 2 (Nrf2) and **cardiac troponin I (CTnI) expressions :** Immunohistochemistry procedures were conducted as previously documented (Oyagbemi *et al*., 2017). For the assessment of kidney injury molecule I (Kim-1), nuclear factor kappa beta (NF-κB), cardiac troponin I (CTnI), and the suppression of nuclear factor erythroid 2-related factor 2 (Nrf2) expression in both kidney and heart tissues, fixed specimens were embedded in paraffin and sliced into 5 µm thick sections. The regions displaying positive immunoreactivity for Kim-1, NF-κB, Nrf2, and CTnI in anti-rabbit staining were observed, starting from a low magnification on each section and subsequently at 400× magnification using a photomicroscope (Olympus) and a digital camera (Toupcam®, Touptek Photonics, Zhejiang, China).

Statistical Analysis : Data obtained were analyzed with One-way ANOVA with Dunnett's post-test at a 95% confidence limit. All values are expressed as mean ±S.D. The test of significance between two groups was estimated by Student's t test.

RESULTS

Percentage differences in the body weight of hypertensive rats: The percentage (%) weight changes across the groups were as follows: Groups A, C, and D experienced weight gains of 1.4%, 0.57%, and 21.3%, respectively. In contrast, groups B, E, and F incurred weight losses of 9.12%, 12.1%, and 11.1%, respectively.

Organ weight: There are no significant differences in the weights of the heart, kidney, and liver across the groups (Table 2).

Blood pressure: Rats treated with L-NAME only had a significant ($P<0.05$) increase in systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MAP) values when compared with the control, extract and lisinopril treated groups (Table 3).

Electrocardiograph of hypertensive rats treated with *Persea americana* bark extract: Heart rate of group C was significantly higher when compared with Groups B, E and F. The PR interval of group B rats was significantly ($p<0.05$) higher than that of group A. There was no significant difference ($P>0.05$) in the QRS complex across all the group. Group B showed a statistically significant ($P<0.05$) increase in the QT interval compared to groups A, C, D, E, and F. Similarly, the QTc value in group B rats was markedly greater than that reported in group A (Table 4).

Haematological and Serum Biochemical Parameters: Treatment with L-NAME only caused significant ($P<0.05$) decreases in packed cell volume, haemoglobin, red blood cell values when compared with the control, extract and lisinopril-treated groups. These parameters were, however, restored in extract-treated groups (Table 5).

Table 1:
Effect of *Persea americana* bark extract on body weight of Wistar rats

Groups	Initial weight (g)	Final weight (g)	(%) weight gain/loss
(A) Control	198.82 ± 4.11^{b}	201.67 ± 6.07^{a}	1.4%
(B) L-NAME	188.90 ± 4.39^{bc}	171.67 ± 5.89^{b}	-9.12%
(C) L-NAME + PABE A (100 mg/kg)	169.75 ± 3.68^{de}	170.71 ± 4.55^{bc}	0.57%
(D) L-NAME + PABE B (200 mg/kg)	167.17 ± 3.10^{e}	202.78 ± 5.66^{a}	21.3%
(E) L-NAME + PABE C (400 mg/kg)	179.54 ± 2.36^{cd}	157.78 ± 5.90^{c}	-12.1%
(F) L-NAME + Lisinopril (10 mg/kg)	212.43 ± 4.81^{a}	188.89 ± 7.44^{ab}	-11.1%

PABE- Persea americana bark extract. L-NAME- N^{w}–nitro-L-arginine methyl ester at 40 mg/kg body weight
Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE A (100 mg/kg), Group D (L-NAME+ PABE B (200 mg/kg), Group E (L-NAME + PABE C (400 mg/kg). F (L-NAME + Lisinopril (10 mg/kg). The alphabet superscripts indicate significant differences across groups at P<0.05.

Table 2:
Effect of *Persia americana* bark extract on organ weight of Wistar rats

Treatment	Mean ± Standard error				
	Body weight (mg)	Heart (mg)	Kidney (mg)	Liver (mg)	
Control	201.67 ± 6.07^{a}	0.68 ± 0.10^{a}	0.80 ± 0.10a	6.07	±0.74a
L-NAME	171.67 ± 5.89^{b}	0.60 ± 0.04^{a}	0.86 ± 0.02a	5.61	± 0.23a
L-NAME + PABE A (100 mg/kg)	170.71 ± 4.55^{bc}	0.59 ± 0.11^{a}	0.74 ± 0.09^{a}	4.41	$\pm 0.63^{a}$
L-NAME + PABE B (200 mg/kg)	202.78 ± 5.66^{a}	0.57 ± 0.07^{a}	0.89 ± 0.09^{a}	5.12	$\pm 0.63^{a}$
L-NAME + PABE C (400 mg/kg)	157.78 ± 5.90^{c}	0.53 ± 0.03^{a}	0.81 ± 0.08^{a}	5.17	$\pm 0.44^{a}$
L-NAME + Lisinopril 10 mg/kg	188.89 ± 7.44^{ab}	0.61 ± 0.07^{a}	0.88 ± 0.06^{a}	5.57 ± 0.45^{a}	

PABE- *Persea americana* bark extract. L-NAME- N^{w}–nitro-L-arginine methyl ester at 40 mg/kg body weight
Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE A (100 mg/kg), Group D (L-NAME+ PABE B (200 mg/kg), Group E (L-NAME + PABE C (400 mg/kg). F (L-NAME + Lisinopril 10 mg/kg). The alphabet superscripts indicate significant differences across groups at P<0.05.

Table 3:
Effect of *Persea americana* bark extract on Systolic, Diastolic, Mean Arterial Blood Pressure of Wistar rats

Treatment	Mean ± Standard error		
	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Mean Arterial Pressure (MAP) (mmHg)
Control	124.67 ± 6.89	79.67 ± 9.33	94.50 ± 6.25
L-NAME	230.00 ± 3.51^{a}	181.67 ± 13.28^{a}	197.33 ± 9.84^{a}
L-NAME + PABE A (100 mg/kg)	160.33 ± 17.95^{b}	131.33 ± 14.25^{b}	140.67 ± 15.25^{b}
L-NAME + PABE B (200 mg/kg)	130.33 ± 10.91^{b}	91.33 ± 17.84^{c}	104.67 ± 15.17^{c}
L-NAME + PABE C (400 mg/kg)	149.67 ± 10.68^{b}	113.67 ± 9.49^{bc}	125.67 ± 9.62^{bc}
L-NAME + Lisinopril 10 mg/kg	126.33 ± 9.82^{b}	82.33 ± 6.64^{c}	96.67 ± 7.79^{c}

PABE- Persea americana bark extract. L-NAME- N^{w}–nitro-L-arginine methyl ester at 40 mg/kg body weight
Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE 100 mg/kg), Group D (L-NAME+ PABE 200 mg/kg), Group E (L-NAME + PABE 400 mg/kg). F (L-NAME + Lisinopril 10 mg/kg). The alphabet superscripts indicate significant differences across groups at P<0.05.

Table 4:
Effect of *Persea americana* bark extract on ECG parameters of Wistar rats.

Groups	HEART RATE	P (ms)	PR (ms)	QRS (ms)	QT (ms)	QTc (ms)	Ra (mV)
A	198.67±27.94	19.33±5.03	48.33±8.96	10.00±3.46	29.33±3.79	52.33±9.50	0.28±0.11
B	181.00± 19.98	24.67±3.51	51.00±3.61^{a}	15.67±2.08	88.33±3.73^{a}	141.33±15.59^{a}	0.40±0.09
C	247.67±2.08^{b}	19.00±1.00	27.33±1.53^{ab}	15.67±4.16^{b}	76.33±1.53^{a}	138.00±2.65^{a}	0.33±0.09
D	197.00±1.00	31.00±2.65	48.00±1.00^{ac}	13.00±1.00	67.67±11.06^{abc}	115.33±24.17^{a}	0.27±0.04
E	177.33±40.28^{c}	23.00±9.85	43.33±1.53^{ac}	13.33±1.52	47.67±2.52^{abcd}	89.33±2.08^{bc}	0.26±0.06
F	182.33±3.06^{c}	21.33±1.53	36.00±1.00^{abd}	8.33±1.33^{b}	53.00±2.65^{bcd}	87.67±2.31^{bc}	0.33±0.16

PABE- Persea americana bark extract. L-NAME- N^{w}–nitro-L-arginine methyl ester at 40 mg/kg body weight
Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE A 100 mg/kg), Group D (L-NAME+ PABE B 200 mg/kg), and Group E (L-NAME + PABE C 400 mg/kg). F (L-NAME + Lisinopril 10 mg/kg). The alphabet superscripts indicate significant differences across groups at P<0.05.

Table 5:
Effect of *Persea americana* bark extract on red blood cell indices of Wistar rats

Groups	Mean ± Standard error			
	PCV (%)	Hb	RBC (10^{6}cells/µL)	Platelet (10^{3}cells/µL)
A	40.40 ± 2.60^{a}	13.78 ± 1.23^{a}	6.69 ± 0.57^{a}	139400.00 ± 13151.43^{a}
B	36.75 ± 3.12^{a}	12.13 ± 1.07^{a}	5.94 ± 0.62^{a}	132250.00 ± 9294.94^{a}
C	41.25 ± 2.53^{a}	13.60 ± 0.84^{a}	6.90 ± 0.65^{a}	127250.00 ± 16779.82^{a}
D	41.20 ± 3.07^{a}	13.74 ± 0.99^{a}	6.92 ± 0.62^{a}	188800.00 ± 41921.83^{a}
E	37.00 ± 4.25^{a}	12.76 ± 1.68^{a}	6.37 ± 0.71^{a}	131200.00 ± 9774.46^{a}
F	36.86 ± 2.04^{a}	12.17± 0.73^{a}	6.17 ± 0.36^{a}	132600.00 ± 22174.80^{a}

PABE- Persea americana bark extract. L-NAME- N^{w}–nitro-L-arginine methyl ester at 40 mg/kg body weight
Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE A 100 mg/kg), Group D (L-NAME+ PABE B 200 mg/kg), Group E (L-NAME + PABE C 400 mg/kg). F (L-NAME + Lisinopril 10 mg/kg). The alphabet superscripts indicate significant differences across groups at P<0.05.

Table 6:
Effect of *Persea americana* bark extract on White Blood Cell (WBC) Indices of Wistar rats in murine model of hypertension

Groups	WBC (10^{3}cells/µL)	Lymphocyte (10^{3}cells/µL)	Neutrophil (10^{3}cells/µL)	Monocyte (10^{3}cells/µL)	Eosinophil (10^{3}cells/µL)
A	4800.00 ± 1028.47^{a}	66.00 ± 1.76^{a}	31.00 ± 1.92^{a}	2.00 ± 0.45^{a}	1.00 ± 0.32^{a}
B	4437.50 ± 798.80^{a}	68.00 ± 1.78^{a}	27.75 ± 2.84^{a}	2.25 ± 0.48^{a}	2.00 ± 0.71^{a}
C	4675.00 ± 1478.53^{a}	67.00 ± 1.96^{a}	29.75 ± 2.14^{a}	1.50 ± 0.29^{a}	1.75 ± 0.75^{a}
D	3960.00 ± 341.47^{a}	65.20 ± 2.40^{a}	28.80 ± 2.04^{a}	2.00 ± 0.32^{a}	2.00 ± 0.32^{a}
E	4580.00 ± 363.52^{a}	69.00 ± 1.82^{a}	27.20 ± 1.59^{a}	1.80 ± 0.37^{a}	2.00 ± 0.32^{a}
F	4342.86 ± 330.84^{a}	66.57 ± 2.44^{a}	29.00 ± 1.88^{a}	1.86 ± 0.34^{a}	1.71 ± 0.29^{a}

PABE- Persea americana back extract. L-NAME- N^{w}–nitro-L-arginine methyl ester at 40 mg/kg body weight
Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE A 100 mg/kg), Group D (L-NAME+ PABE B 200 mg/kg), Group E (L-NAME + PABE C 400 mg/kg). F (L-NAME + Lisinopril 10 mg/kg). The alphabet superscripts indicate significant differences across groups at P<0.05

Across the treatment groups, the leucocytes parameters including; White blood cell count, Lymphocyte, Neutrophils, Monocyte and Eosinophil had no statistically significant changes (Table 6).

Furthermore, a significant ($p<0.05$) increases in hepatocellular leakage enzymes (AST and ALP) and also, markers of renal damage such as BUN, creatinine were recorded in the group treated with L-NAME alone when compared with the controls (Table 7).

Oxidants and antioxidant status of hypertensive rats treated with *Persea americana* bark extract: Non-enzymatic antioxidant indicators for the heart and kidneys, non-protein thiol, protein thiol, and reduced glutathione were all significantly decreased by L-NAME, whereas these markers were significantly increased in the extract and lisinopril-treated groups (Table 8).

Superoxide dismutase, glutathione peroxidase, and glutathione-s-transferase levels in the heart and kidneys were significantly decreased in the hypertensive group, whereas they were significantly improved in the extract and lisinopril-treated groups (Table 9).

Markers of oxidative stress such as malondialdehyde, hydrogen peroxide, and protein carbonyl levels were significantly elevated in both the heart and the kidneys after L-NAME administration. Groups given extract and lisinopril demonstrated noticeably lower levels (Table 10).

When compared to the control, extract, and lisinopril-treated groups, L-NAME significantly reduced serum nitric oxide while significantly raising serum myeloperoxidase and advanced oxidative protein products (Table 11).

Immunohistochemistry: When compared to the control, extract, and lisinopril treatment groups, the L-NAME alone group displayed the highest levels of CTnI and NF-kB in heart tissue (Figures 1 and 2), as well as the highest expression of NRF-2 in the heart and kidney tissue (Figures 3). In the kidney and cardiac tissues, L-NAME alone also reveals the highest expression of KIM-1 and NF- kB (Figure 4).

Table 7:
Effect of *Persea americana* bark extract on serum biochemical parameters of Wistar rats in murine model of hypertension.

Groups	ALP (U/L)	ALT (U/L)	AST (U/L)	BUN mg/dL	CREATININE (mg/dL)	HDL (mmol/L)
A	93.5±9.15	27.50±1.00	38.00±2.26	15.48±0.88	0.65±0.06	20.58±2.41
B	112.67±11.75^{a}	30.00±1.00^{a}	48.00±1.00^{a}	16.73±0.61^{a}	0.73±0.06	25.40±0.92^{a}
C	95.00±6.56^{b}	31.00±1.00^{a}	47.33±1.53^{a}	18.13±0.38^{ab}	0.83±0.15^{a}	24.73±1.07^{a}
D	110.25±5.32^{ac}	32.00±1.41^{ab}	46.25±2.36^{a}	17.17±0.77	1.00±0.16^{ab}	23.13±1.91
E	117.75±4.03^{ac}	32.75±1.26^{ab}	46.75±1.71^{a}	16.98±0.39^{a}	0.90±0.18^{a}	22.33±1.66^{b}
F	95.67±9.45^{de}	29.00±1.00	40.67±1.15^{b}	17.23±0.93^{a}	0.70±0.10	21.93±1.33^{b}

PABE- Persea americana back extract. L-NAME- N^{w}–nitro-L-arginine methyl ester at 40 mg/kg body weight
Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE A 100 mg/kg), Group D (L-NAME+ PABE B 200 mg/kg), Group E (L-NAME + PABE C 400 mg/kg). F (L-NAME + Lisinopril 10 mg/kg). The alphabet superscripts indicate significant differences across groups at P<0.05.

Table 8:
Effect of *Persea americana* bark extract on cardiac and renal non-enzymatic antioxidant system of Wistar rats in murine model of hypertension.

Groups (mg/kg)	PT heart	PT kidney	NPT heart	NPT kidney	GSH heart	GSH kidney
A	35.81±7.0	53.6±17.27	20.94± 2.06	20.69 ± 2.49	64.13±24.66	93.12±17.82
B	30.11±5.0^{a}	35.52±2.93^{a}	13.32±5.89^{a}	13.46±2.714^{a}	51.82±13.2^{a}	59.35±20.20^{a}
C	34.59±10.62	46.69±6.98	15.32±4.91	18.50±8.37	54.21±13.2	94.64±14.81^{b}
D	35.58±13.83	41.90±9.06^{c}	15.45±4.80	16.49±4.54	73.72±11.53^{bc}	94.29±19.99^{b}
E	39.4±11.19^{b}	45.34±16.72	16.65±3.74	16.03±4.34	57.88±13.35	76.82±22.71
F	36.99±9.57^{b}	41.88±8.88^{ab}	16.07±3.16	16.74±6.14	74.21±15.09^{bc}	87.14±13.06^{b}

PABE- Persea americana back extract. L-NAME- N^{w}–nitro-L-arginine methyl ester at 40 mg/kg body weight
Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE A 100 mg/kg), Group D (L-NAME+ PABE B 200 mg/kg), Group E (L-NAME + PABE C 400 mg/kg). F (L-NAME + Lisinopril 10 mg/kg). The alphabet superscripts indicate significant differences across groups at P<0.05.GSH (reduced glutathione; µmol /mg protein), non-protein thiol (µmol /mg protein), protein thiol (µmol /mg protein).

Table 9:
Effect of *Persea americana* bark extract on cardiac and renal enzymatic antioxidant system of rats in murine model of hypertension

GROUPS	SOD heart	SOD kidney	GPX heart	GPX kidney	GST heart	GST kidney
A	16.92±4.20	21.57±4.5	93.53±15.8	116.4±35.03	0.11±0.05	0.09±0.05
B	12.7±3.27^{a*}	13.09±4.47	72.13±10.3^{a}	93.95±22.97^{a}	0.03±0.01^{a}	0.03±0.02^{a}
C	16.03±5.44	20.06±8.40	96.69±21.57^{b}	112±52.97	0.09±0.01^{b}	0.08±0.06^{b}
D	15.12±6.01	16.19±0.85	95.77±25.40^{b}	101.5±5.51	0.10±0.02^{b}	0.08±0.04^{b}
E	17.96±3.72^{b}	16.17±5.92	109.8±30.84^{b}	111.5±27.46^{b}	0.28±0.^{09a,b,c,d}	0.06±0.04
F	16.47±3.60^{b}	18.26±7.24	89.49±17.71^{b}	104.9±7.625	0.12±0.02^{be}	0.09±0.03^{b}

PABE- Persea americana back extract. L-NAME- N^{w}–nitro-L-arginine methyl ester at 40 mg/kg body weight
Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE A 100 mg/kg), Group D (L-NAME+ PABE B 200 mg/kg), Group E (L-NAME + PABE C 400 mg/kg). F (L-NAME + Lisinopril 10 mg/kg). The alphabet superscripts indicate significant differences across groups at P<0.05 .SOD (superoxide dismutase; units/mg protein), GST (glutathione-S-transferase; mmole1-chloro-2,4-dinitrobenzene-GSH complex formed/min/mg protein), GPx (glutathione peroxidase; units/mg protein)

Table 10:
Effect of *Persea americana* bark extract on cardiac and renal markers of oxidative stress of rats in murine model of hypertension

Groups	MDA heart	MDA kidney	H_2O_2 heart	H_2O_2 kidney	PC heart	PC kidney
A	0.57±0.22	0.49±0.22	82.39±19.36	121.2±28.9[a]	960±187.5	918.6±478.7
B	0.87±0.25[a]	1.20±0.12	110.1±13.9[a]	153.30± 17.66[a]	1252±475.4[a]	1709±559.3[a]
C	0.54±0.27[b]	0.47±0.19	100.5.6±10. `91[a]	109.3±13.68[b]	1027±418.1	1363±356.7
D	0.53±0.14[b]	0.60±0.19	97.30±15.22	117.5±10.51[b]	901.5±356.50	832.8±137.70[ab]
E	0.6±0.11[b]	0.34±0.12	99.08±10.99	114.2±13.79[b]	1029±122[b]	1043±381.40[b]
F	0.42±0.103[b]	0.50±0.07	91.04±15.73[b]	108.4 ±9.77[b]	940.1±185.6[b]	821.9±308.9[bc]

PABE- *Persea americana* back extract. L-NAME- N^w–nitro-L-arginine methyl ester at 40 mg/kg body weight
Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE A 100 mg/kg), Group D (L-NAME+ PABE B 200 mg/kg), Group E (L-NAME + PABE C 400 mg/kg). F (L-NAME + Lisinopril 10 mg/kg). The alphabet superscripts indicate significant differences across groups at P<0.05.MDA (malondialdehyde; µmol /mg protein), H_2O_2 (hydrogen peroxide; µmol /mg protein), PC (protein carbonyl: µmol /mg protein).

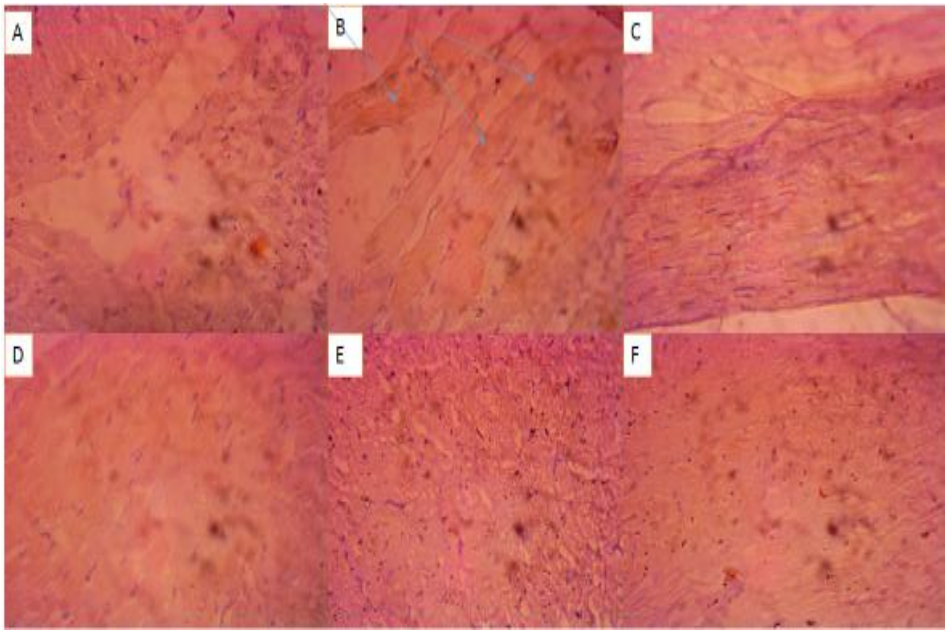

Plate 1
Immunohistochemistry of NF-kB in heart tissue in rats. PABE- Persea americana back extract. L-NAME- Nw–nitro-L-arginine methyl ester at 40 mg/kg body weight. Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE A 100 mg/kg), Group D (L-NAME+ PABE B 200 mg/kg), Group E (L-NAME + PABE C 400 mg/kg). F (L-NAME + Lisinopril 10 mg/kg). The slides were counterstained with high-definition hematoxylin and viewed with ×100 objectives.

Plate 1
Immunohistochemistry of NF-kB in heart tissue in rats. PABE- *Persea americana* back extract. L-NAME- Nw–nitro-L-arginine methyl ester at 40 mg/kg body weight. Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE A 100 mg/kg), Group D (L-NAME + PABE B 200 mg/kg), Group E (L-NAME + PABE C 400 mg/kg). F (L-NAME + Lisinopril 10 mg/kg). The slides were counterstained with high-definition hematoxylin and viewed with ×100 objectives.

Table 11:
Effect of *Persea americana* bark extract on serum markers of inflammation and oxidative stress of Wistar rats in a murine model of hypertension

Treatment groups	NO	MPO	AOPP
A	0.44±0.51	17.73±4.05	44.37±14.57
B	0.32±0.08	26.54±4.19[a]	69.88±29.60[a]
C	0.46±0.093[b]	25.7±2.90[a]	53.43±15.24
D	0.39±0.04[b]	24.29±7.67[a]	47.97±14.28[b]
E	0.42±0.15	19.73±3.99[b]	65.36±18.54[a]
F	0.46±0.15[b]	22.53±6.31[a]	49.3±8.17[b]

PABE- *Persea americana* back extract. L-NAME- N^w–nitro-L-arginine methyl ester at 40 mg/kg body weight
Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE A 100 mg/kg), Group D (L-NAME+ PABE B 200 mg/kg), Group E (L-NAME + PABE C 400 mg/kg). F (L-NAME + Lisinopril 10 mg/kg). The alphabet superscripts indicate significant differences across groups at P<0.05. NO (nitric oxide; units/mg protein), MPO (myeloperoxidase; µmole/L), AOPP (advanced oxidative protein product; units/mg protein).

DISCUSSION

Hypertension is a major risk factor for renal and cardiac disease and a decrease in blood pressure significantly lowers the risk of attending cardiovascular events (Hermann *et al*., 2006). *Persea americana* significantly lowered SBP, DBP and MAP values and corrected the resultant cardiac arrhythmia in this study to values comparable to lisinopril, an ACE inhibitor.

Several studies have elucidated the importance of nitric oxide in the pathogenesis of hypertension as it plays a major role in the regulation of blood pressure. The impairment of nitric oxide bioavailability is an important part of hypertension (Hermann *et al*., 2006). Some antihypertensives act by exploiting the effect of nitric oxide on vascular smooth muscle, they elicit the release of nitric oxide and thus facilitate vasodilation (Adefegha and Oboh, 2016). In this study, we reported significant decrease in nitric oxide bioavailability in the L-NAME group whereas the groups treated with *Persea americana* and lisinopril exhibited significant increases in nitric oxide bioavailability. Thus, suggesting that *Persea americana* acts by facilitating nitric oxide bioavailability.

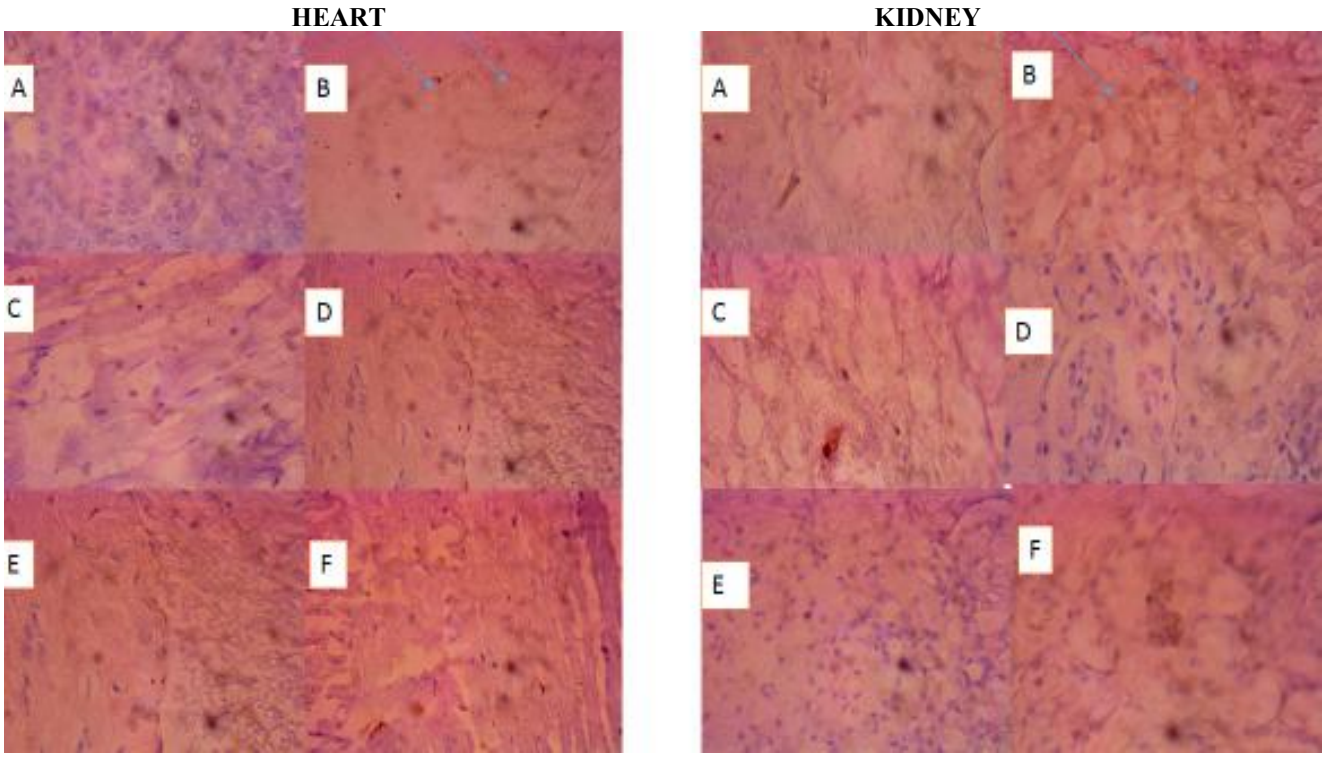

Plate 2:
Immunohistochemistry of Nrf-2 in heart and kidney tissues of rats.
PABE- *Persea americana* bark extract. L-NAME- N^{w}–nitro-L-arginine methyl ester at 40 mg/kg body weight. Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE A 100 mg/kg), Group D (L-NAME+ PABE B 200 mg/kg), Group E (L-NAME + PABE C 400 mg/kg). F (L-NAME + Lisinopril 10 mg/kg). The slides were counterstained with high definition hematoxylin and viewed ×100 objectives.

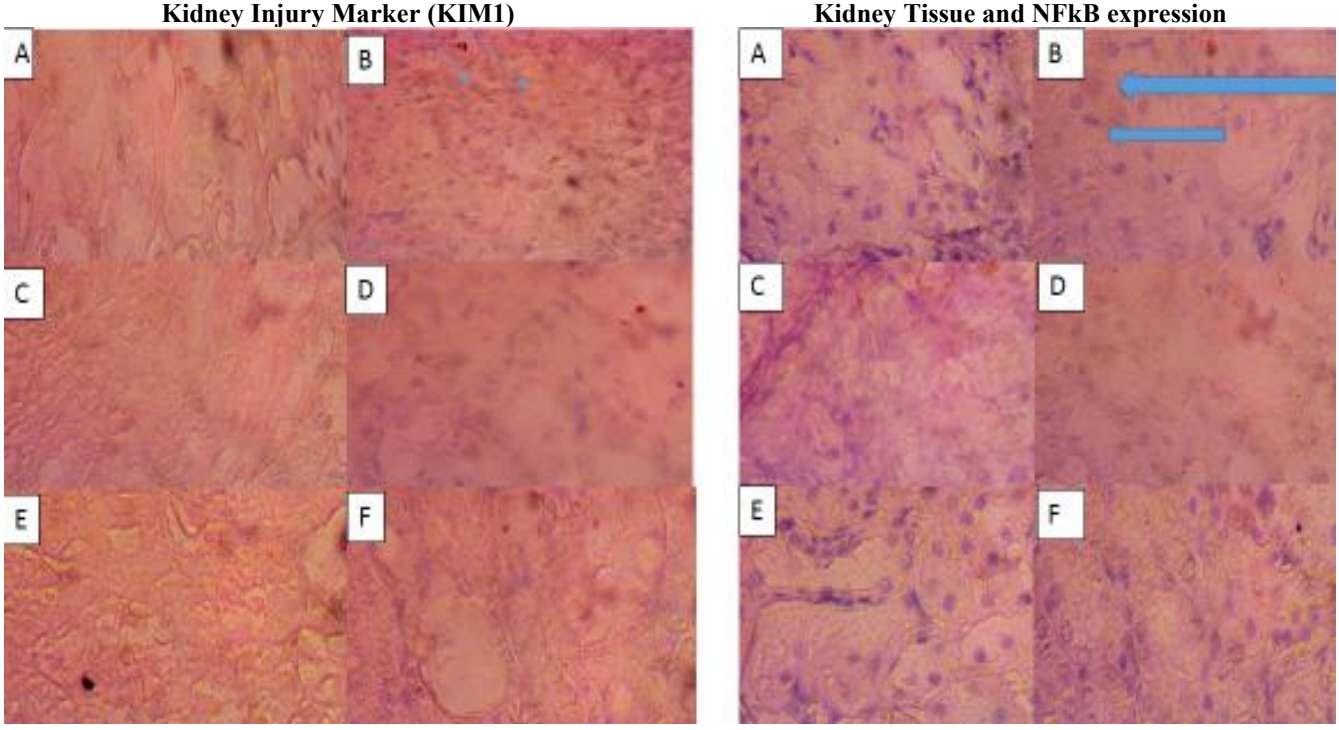

Plate 3:
Immunohistochemistry of Kim-1 and NFkB in kidney tissue of rats.
PABE- *Persea americana* back extract. L-NAME- N^{w}–nitro-L-arginine methyl ester at 40 mg/kg body weight. Values presented as mean ± SEM. Group A (Control), Group B (L-NAME), Group C (L-NAME + PABE A 100 mg/kg), Group D (L-NAME+ PABE B 200 mg/kg), Group E (L-NAME + PABE C 400 mg/kg). F (L-NAME + Lisinopril 10 mg/kg). The slides were counterstained with high-definition hematoxylin and viewed ×100 objectives.

Hepatocellular leakage enzymes such as AST and ALP were significantly increased in the L-NAME alone group, these enzymes are mostly associated with the liver parenchyma, and elevate serum levels are usually seen in acute hepatic injury (Kannan *et al*., 2013). The increase seen in the L-NAME alone group suggests hepatic damage as a complication of hypertension.

BUN and creatinine are traditional markers of renal damage, with creatinine showing more specificity than BUN (Onuigbo *et al*., 2017). These markers were significantly increased in the L-NAME alone group confirming that kidney damage is a complication of persistent elevations in arterial blood pressure. *Persea americana* however, caused a reduction in the levels of both BUN and creatinine to levels comparable with Lisinopril, a known antihypertensive.

Oxidative stress, which is defined as an imbalance in the levels of oxidants and antioxidants favoring oxidants, has been implicated in the advent of hypertension, where it causes damage to macromolecules such as DNA, protein and lipids and it is also exacerbated by insufficient antioxidants in the body (Wu and David, 2015). Renal and cardiac dysfunction in hypertension is associated with oxidative damage.

In the kidney, oxidative stress promotes vasoconstriction, increases sodium retention and increases vascular resistance, therefore worsening hypertension (Duni *et al*., 2018). Cardiac diseases are also associated with oxidative stress (Jin *et al*., 2017)

In this present study, we reported significant elevations in various markers of oxidative stress, such as advanced oxidative protein products, malondialdehyde, protein carbonyl, hydrogen peroxide in the renal and cardiac homogenate of the L-NAME alone group. Treatment with *Persea americana* however brought their values to levels comparable with the control and lisinopril treated groups.

Several antioxidants such as superoxide dismutase, catalase, reduced glutathione, glutathione peroxidase acts as the first line of defense against oxidative stress-induced damage (Nguyen *et al*., 2007). It is thus widely accepted that consuming food with high concentrations of these antioxidants can be useful in the prevention and treatment of various cardiovascular and renal diseases (Smith *et al*. 2016). SOD acts by removing superoxide radical thus preventing the deleterious effects of superoxide radical (Kabel, 2014), the glutathione system including reduced glutathione, glutathione peroxidase, glutathione transferase functions in detoxification metabolism. Reduced glutathione functions in maintaining the redox system of the cell (Kabel, 2014). In this study, L-NAME significantly elicited a depletion in levels of these enzymatic and non-enzymatic antioxidants in the renal and cardiac homogenates, however, treatment with *Persea americana* and lisinopril brought on significant increases in their levels, confirming the antioxidant effect of *Persea americana* .

Inflammation goes hand in hand with oxidative stress, and both act in an interrelated way in hypertension (Duni *et al*., 2018). In this study, myeloperoxidase, a marker of inflammation was significantly elevated in the L-NAME alone group confirming the role of inflammation in the pathogenesis of hypertension, *Persea americana* however significantly reduced its levels in the treated groups.

Nuclear erythroid-2 like factor-2 (Nrf2) is a transcription factor that controls the expression of antioxidants in the body by activating the Antioxidant Response Elements (ARE), this activity of Nrf2 is only turned on when the body is assailed with oxidants (Smith *et al*. 2016). Therefore, enhancing the activity of Nrf2 will help to downregulate the effects of reactive oxygen species (Rajappa *et al*., 2017). In this study, we reported decreased expression of Nrf2 in the heart and kidney tissue of the L-NAME alone group, this finding is supported by the decreased levels of both enzymatic and non-enzymatic antioxidants in the renal and cardiac homogenate of the L-NAME alone group. *Persea americana* and Lisinopril however brought on an increased in Nrf2 expression in the treated groups, this is also supported by the increased levels of antioxidants in the renal and cardiac homogenate of the treated groups.

NF-kB modulates cell growth, cell survival, development processes, immune and inflammatory responses as well as apoptosis and its activation has been linked to a number of cancers as it has been shown to trigger the activation of reactive oxygen species (Kusano and Bucalen, 2011). Inflammatory signals from the tissue also serves as an activator of NF-kB and it has been implicated in the pathogenesis of several diseases including hypertension, cardiomyopathy and diabetes (Kusano and Bucalen, 2011). Its downregulation in the kidney and heart tissue of the extract treated groups thus suggests that *Persea americana* possesses anti-inflammatory and anti-apoptotic effects.

Kidney Injury Molecule 1 (Kim1) is a specific biomarker for tubular injury, it is usually undetectable in normal kidneys but markedly increased in kidney injury thus making it a sensitive and specific biomarker for renal damage (Huo *et al*., 2010). In this study, KIM-1 was highly expressed in the L-NAME alone group whereas the extract and lisinopril treated groups showed decreased expression, this thus confirms the ability of *Persea americana* to protect against hypertension induced renal damage.

Cardiac troponins are sensitive and specific markers of myocardial injury although they do not give any information regarding the mechanism of injury. They have redefined how acute myocardial infarction is diagnosed in humans (Weito and May, 2008). Troponins are regulatory proteins that are part of the contractile apparatus of skeletal and cardiac muscle tissue. They are not present in smooth muscle tissue; Elevated cardiac troponin levels therefore indicate myocardial damage (Wells and Meg, 2008). We reported increased expression of CTnI levels in the heart tissue of the L-NAME alone group whereas *Persea americana* and Lisinopril treated groups showed lesser expressions.

Our findings support the assertion that oxidative stress is involved in the pathogenesis of hypertension and the target organ complications. It also justifies the use of *Persea americana* bark extract in folkloric medicine. A desirable effect of an antihypertensive is that in addition to reducing blood pressure, it should also ameliorate hypertensive cardiac and kidney damage, this makes *Persea americana* an excellent antihypertensive drug candidate.

The findings of this study suggest an important role for *Persea americana* bark extract in the management of hypertension and its complications.

Funding: This work was partly funded by a TetFund Academic Staff Training and Development grant and partly by the researchers.

REFERENCES

Abreu, I., Sousa, D.P. and Pinto, F.J. (2018) Cardiovascular disease and high blood pressure trend analyses from 2002 to 2016 after the implementation of a salt reduction strategy. BMC Public Health. 18: 1–9.

Adefegha, S. A. and Oboh, G. (2016). Antioxidant and inhibitory properties of Clerodendrum volubile leaf extracts on key enzymes relevant to non-insulin dependent diabetes mellitus and hypertension. Journal of Taibah University for Science. *10*(4): 521-533. http://dx.doi.org/10.1016/j.jtusci.2015.10.008.

Akaike, T., Ando, M., Oda, T., *et al*. (1990) Dependence on O_2-generation by xanthine oxidase of pathogenesis of influenza virus infection in mice. J. Lab. Clin. Med. 85**:** 739–745.

Beutler, E., Duron, O., Kelly, B.M. (1963). Improved method for the determination of blood glutathione. .J. Lab. Clin. Med. 61: 882-888.

Cavasin, M. A., Liao, T. D., Yang, X. P., Yang, J. J., and Carretero, O. A. (2007). Decreased endogenous levels of Ac-SDKP promote organ fibrosis. Hypertension. 50(1): 130-136. doi: 10.1161/HYPERTENSIONAHA.106.084103

Dai, X., Hua, L., Chen, Y., Wang, J., Li, J., Wu, F., and Liang, C. (2018). Mechanisms in hypertension and target organ damage: Is the role of the thymus key? International Journal of Molecular Medicine. 42(1): 3-12.

Drury, R.A., Wallington, E.A., Cancerson, R. (1996) Carlton's Histopathological Techniques, 4th ed. Oxford, London, New York: Oxford University Press. 1-10

Duni, A.V., Liakopoulos, K.R., and Evangelia, D. (2018) Chronic Kidney Disease and Disproportionally Increased Cardiovascular Damage : Does Oxidative Stress Explain the Burden. Oxid Med Cell Longev. 2018: 1–18.

Ellman, G.L. (1959) Tissue sulfhydryl groups. Arch. Biochem. Biophys. 8: 70–77.

Gornal, A.G., Bardawill, J.C., David, M.M. (1949) Determination of serum proteins by means of biuret reaction. J. Biol. Chem. 177: 751–766.

Habig, W.H., Pabst, MJ., Jakoby, WB. (1974) Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 25**:** 7130-7139.

Hermann, M., Andreas, F. and Thomas, F.L. (2006) Nitric Oxide in Hypertension." The Journal of Clinical Hypertension. 8 (12 Suppl 4): 17–29. http://doi.wiley.com/10.1111/j.1524-6175.2006.06032.x.

Huo, W., Zhang, K., Nie, Z., Li, Q., and Jin, F. (2010). Kidney injury molecule-1 (KIM-1): a novel kidney-specific injury molecule playing potential double-edged functions in kidney injury. Transplantat. Reviews. 24(3): 143-146. http://dx.doi.org/10.1016/j.trre.2010.02.002.

Jacques-Silva, M. C., Nogueira, C. W., Broch, L. C., Flores, E. M., and Rocha, J. B. T. (2001). Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice. *Pharmacology & toxicology*. *88*(3): 119-125.

Jin, L. I., Piao, Z. H., Sun, S., Liu, B., Kim, G. R., Seok, Y. M., ... and Jeong, M. H. (2017). Gallic acid reduces blood pressure and attenuates oxidative stress and cardiac hypertrophy in spontaneously hypertensive rats. Scientific Reports. *7*(1): 15607 http://dx.doi.org/10.1038/s41598-017-15925-1.

Johnson, R.J., Miguel, A.L., Gabriela, S., and Bernardo, R. (2018). The Discovery of Hypertension : Evolving Views on the Role of the Kidneys, and Current Hot Topics." Am J Physiol Renal Physiol. 308(3): 1–23.

Jollow, D.J., Mitchell, J.R. (1974) Zampaglione Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacol. 11: 151-169.

Kabel, A M. (2014) Free Radicals and Antioxidants: Role of Enzymes and Nutrition." World Journal of Nutrition and Health. 2(3): 35–38. http://pubs.sciepub.com/jnh/2/3/2/index.html.

Kannan, N., Kunnathur, M,S., and Chandrasekaran G. (2013) Protective Effect of Acacia Nilotica (L.) against Acetaminophen-Induced Hepatocellular Damage in Wistar Rats." Advances in Phar.macological Sciences.

Kayali, R., Cakatay, U., Akcay, T., Altug, T. (2006) Effect of alpha-lipoic acid supplementation on markers of protein oxidation in post-mitotic tissues of ageing rat. Cell. Biochem. Funct. 4: 79–85.

Kusano, C. and Bucalen, F. (2011) The biology of nuclear factor kappa beta (NFKB) in health and pathology." Journal of Arts, Science & Commerce. 2(3): 53–59.

Messerli, F.H., Stefano, F.R., and Sripal, B. (2017) The Transition From Hypertension to Heart Failure." Heart Failure. 5(8): 543–51.

Misra, H.P., Fridovich, I. (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247**:** 3170-3175.

National Heart Foundation of Australia. (2016) Guideline for the diagnosis and management of hypertension in adults. 1-10.

Nguyen T, Paul N, Cecil B. The Nrf2-Antioxidant Response Element Signaling Pathway and Its Activation by Oxidative Stress. J. Biol. Chem. 2009: 284(20): 13291–95.

Ojewole, J.A,, Kamadyaapa, D.R., Gondwe, M.M., *et al*. (2007) Cardiovascular effects of *Persea americana* Mill (Lauraceae) (avocado) aqueous leaf extract in experimental animals. Cardiovasc J Afr. 18: 69-76.

Olaleye, S.B., Adaramoye, O.A., Erigbali, P.P., *et al*. (2007) Lead exposure increases oxidative stress in the gastric mucosa of HCl/ethanol-exposed rats. *World. J. Gastroenterol.* 13**:** 5121-5126.

Omobowale, T,O,, Oyagbemi, A.A., Akinrinde, A.S. *et al*. (2014). Failure of recovery from lead induced hepatoxicity and disruption of erythrocyte antioxidant defence system in Wistar rats. Environmental toxicology and pharmacology. 37(3): 1202–1211.

Omóbòwálé, T,O., Oyagbemi, A.A, Ogunpolu, B.S., *et al*. (2019) Antihypertensive Effect of Polyphenol-Rich Fraction of *Azadirachta indica* on Nω-Nitro-L-Arginine Methyl Ester-Induced Hypertension and Cardiorenal Dysfunction. Drug Research. 69(1):12–22. https://doi.org/10.1055/a-0635-0638.

Onuigbo M., Nneoma A. and Mohan S. (2017) Acute Kidney Injury in Heart Failure Revisited—The Ameliorating Impact of 'Decongestive Diuresis' on Renal Dysfunction in Type 1 Acute Cardiorenal Syndrome: Accelerated Rising Pro B Naturetic Peptide Is a Predictor of Good Renal Prognosis. Journal of Clinical Medicine. 6(9): 82. http://www.mdpi.com/2077-0383/6/9/82.

Oyagbemi A.A., Omobowale T.O., Asenuga E.R., Afolabi J.M., Adejumobi O.A., Adedapo A.A. and Yakubu M.A.

(2017) Effect of arsenic acid withdrawal on hepatotoxicity and disrupt of erythrocyte antioxidant defence system. Toxicity report. 4: 521-529

Rajappa, R., Venugopalreddy, B., Subbarao, V.M. (2017) Naturally Occurring Nrf2 Activators in the Management of Diabetes." Nutri Food Sci Int J. 2(4): 1-8.

Reznick, A. Z., & Packer, L. (1994). Oxidative damage to proteins: spectrophotometric method for carbonyl assay. In Methods in enzymology (Vol. 233, pp. 357-363). Academic press

Varshney, R., and Kale, R.K. (1990) Effect of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. Int. J. Radiat. Biol**.** 58**:** 733-743.

Wells, S.M., and Meg, S. (2008) Cardiac Troponins." Journal of Veterinary Emergency and Critical Care. 18 (3): 235–45.

Wolff, S.F. (1994) Ferrous ion oxidation in the presence of ferric ion indicator xylenol orange for measurement of hydrogen peroxides. Methods Enzymol.: 233**:** 182-189.

Wu, J., and David G.H. (2015) Oxidative Stress and Hypertension." In Blood Pressure and Arterial Wall Mechanics in Cardiovascular Diseases. 175922015.

Xia, Y., Zweier, J.L. (1997) Measurement of myeloperoxidase in leukocyte-containing tissues. Anal. Biochem. 245**:** 93–96.