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Peristrophe roxburghiana leaf extracts exhibited anti-hypertensive and anti-lipidemic properties in L-NAME hypertensive rats

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ABSTRACT

Aims: Hypertension is a global disease that has been combating the world health for ages. *Peristrophe roxburghiana* (PR) is used in traditional medicine to treat hypertension and other ailments. The present study examined phytochemical constituents, antioxidant activities and GC–MS analysis of extracts of PR leaf and also evaluated their anti-hypertensive and anti-lipidemic effects in NG-nitro-L-arginine methyl ester (L-NAME) hypertensive rats.

Methods: Wistar rats were grouped into two groups: control and hypertensive. Hypertension was induced in the hypertensive group by oral gavage of 60 mg/kg b.w of L-NAME for 3 weeks. After induction, the hypertensive group was randomly sub-grouped into hypertensive, hypertensive treated and hypertensive untreated groups. These were orally gavaged respectively with 60 mg/kg b.w of L-NAME, 60 mg/kg b.w/day of L-NAME + 200 mg/kg b.w of different extracts of PR (aqueous, ethanolic and methanolic extracts) and 60 mg/kg b.w of L-NAME + 20 mg/kg b.w ramipril for 3 weeks. The blood pressure was measured by tail-cuff method at the third and sixth weeks.

Key findings: The results showed that the extracts of PR significantly decrease blood pressure, pro-atherogenic lipids and atherogenic ratios in L-NAME hypertensive rats. White blood cells count, neutrophil count and creatinine level were also effectively decreased by the extracts. Furthermore, the extracts increase serum nitric oxide (NO) level, anti-atherogenic lipid, glutathione level, lymphocyte and platelet count in the rats.

Significance: Extracts of PR leaf decrease blood pressure and increase NO level in L-NAME hypertensive rats and also corrected the hyperlipidemia and inflammatory response arising from the reduction in NO bioavailability.

1. Introduction

Hypertension is a disease that has been with us for ages and affects every human race [1]. Hypertension is a cardiovascular risk factor connected to several complications which consequently lead to death. Hypertensive state is associated with narrowing, weakening and thickening of vascular wall, which consequently result in atherosclerosis, coronary artery disease, retinopathy, renal disorder, brain infarction with hemorrhage, stroke and finally death [1]. Dyslipidemia is another risk factor associated with the cardiovascular system. It has been implicated in the pathogenesis of several diseases [2]. Elevated cholesterol level is a persistent cardiovascular risk factor resulting in coronary artery disease, atherosclerosis, ischemic heart disease and stroke. High serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) are cardiovascular disease risk factors, but elevated level of high-density lipoprotein cholesterol (HDL-C) in the serum prevents the occurrence of cardiovascular diseases. High serum triglyceride (TG) has also been linked to high risk of developing cardiovascular diseases [2].

Malfunctioning in any of the several endogenous blood pressure regulating machineries or mechanisms results in hypertension. Reduction in production of endothelium-vasorelaxing factors such as nitric oxide (NO) and prostacyclin or elevation of endothelium- vasoconstricting factor production, or both result in hypertension [3]. NO is the principal endothelium-vasorelaxation factor that regulates vascular resistance and a decrease its bioavailability plays a central role in the development of hypertension [4] and many disease conditions linked with hypertension [5]. NO has been reported to be the most important factor responsible for the anti-atherosclerotic properties of the endothelium [6]. Besides, nitric oxide synthase (NOS) inhibition has been

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shown to accelerate the development of atherosclerosis in animal models [7] and increase lipolysis [8]. NO seems to be involved in lipid metabolism. The blockade of NOS by administration of NG-nitro-L-arginine methyl ester (L-NAME) increased TC, LDL-C and TG levels, and decreased HDL-C concentration. Conversely, NO donors decrease TG, TC, and increase HDL-C levels [9].

Peristrophe roxburghiana (PR) belongs to the Acanthaceae family and genus *Peristrophe*. It has been found in several countries including China, Cambodia, India, Malaysia, Vietnam [10] and Nigeria. Trinh and co-workers [11] using spectral analysis reported that the key component of the colour of aqueous *Peristrophe roxburghiana* leaf extract is peristrophine. Phytochemicals such as coumarin, β-sitosterol, 1-octadecanol, allantoin, oleanolic acid and cyanidin have been isolated from the leaf extracts [10]. Its common names are: Malapudak (Malaysia); Noja (Javanese), Magenta plant or Lá cẩm (Vietnamese) and Iyip Christ (Ibibio). In conventional medicine, PR is used for the treatment of lung heat cough, mouth and tongue sores, boil, tuberculosis, acute bronchitis, inflammation, hepatitis, diabetes, poisonous snake bites and as treatment for hypertension in some regions of China [12]. In Malaysia, its fresh leaves are ground and used as treatment for skin diseases, painful sprain and to soothe swollen parts [10]. In Nigeria, specifically Akwa-Ibom State, its leaf is boiled and used as blood tonic, and for general wellbeing. There is paucity of scientific studies on the beneficial effects of PR and some of those documented are written in language not readable to all. Anti-hypertensive effect of PR was reported in two-kidneys, two-clip and two-kidneys one-clip hypertensive rats [13,14]. Its extract has been shown to effectively decrease hemorrheological parameters such as plasma viscosity, packed cell volume, fibrinogen level, whole blood viscosity and thrombus formation [15]. The aim of the present study was to examine the phytochemicals, antioxidant capacity and GC–MS analysis of three different extracts of *Peristrophe roxburghiana* leaf and also evaluate their anti-hypertensive and anti-lipidemic effects in L-NAME hypertensive rats.

2. Materials and methods

2.1. Drugs

L-NAME was obtained from Santa Cruz Biotechnology (Finnell Street, Dallas, Texas, USA.), ramipril was obtained from Sigma (St. Louis, MO, USA).

2.2. Collection and identification of plant

Leaves of *Peristrophe roxburghiana* were collected from its natural habitat in Ikono local government Akwa-Ibom, State, Nigeria. It was identified and authenticated at the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, University of Uyo, Nigeria, and assigned an authentication number - UUHO43.

2.3. Preparation and extraction of Peristrophe roxburghiana leaf

Peristrophe roxburghiana leaves were first rinsed with clean water after which they were rinsed in distilled water. The leaves were air-dried at room temperature and pulverized. Cold extraction of PR leaves was done by macerating 500 g of the pulverized leaves in distilled water (5 L), ethanol (5 L) and methanol (5 L) respectively for a period of three days with constant stirring. Filtration was done using muslin cloth, followed by Whatman's filter paper. The filtrates were concentrated by rotary evaporator at 35 °C. The aqueous extract was freeze dried after being concentrated. The final yields were 64.8 g of aqueous extract, 19.4 g of ethanolic extract and 31.2 g of methanolic extract given a percentage yield of 13.0%, 3.9% and 6.3% respectively. The extracts were appropriately labeled and stored in the refrigerator at 4 °C until needed.

2.4. Antioxidant capacity of Peristrophe roxburghiana leaf extracts

2.4.1. Diphenyl-1-picrylhydrazyl hydrate (DPPH) assay

The ability of the extracts to scavenge free radicals was examined using DPPH as described by Brand-Williams and coworker [16]. DPPH is reduced in the presence of antioxidant compound resulting into a colour change from deep violet to light yellow. The absorbance was measured at 517 nm.

2.4.2. Anti-lipid peroxidation activity

The ability of the extracts to inhibit lipid peroxidation was measured by thiobarbituric acid reaction method as described by Yoshiuki et al. [17] and Masao et al. [18] using ascorbic acid as a positive control. The absorbance was measured spectrophotometrically at 532 nm.

2.4.3. Determination of total antioxidant capacity (TAC)

The extracts total antioxidant capacity was measured as described by Prieto et al. [19]. It was determined by the ability of the extract to reduce molybdenum VI to molybdenum V and this results in the formation of a green phosphate/molybdenum V complex, intensity measured at 695 nm.

2.5. Phytochemical screening of extracts of Peristrophe roxburghiana leaf

The determination of the phytochemical constituents of the extracts of *Peristrophe roxburghiana* leaf was carried out according to the methods described by Harborne [20].

- The presence of alkaloids in the extract was determined by the use of Mayer's test, Wagner's test, Dragendroff's test and Hager's test.
- The presence of glycosides in the extract was assayed by modified Borntrager's test and Legal's test.
- The presence of saponins was assayed by froth test and foam test
- The presence of phytosterols was assayed by Salkowski's test and Libermann Burchard's test.
- The presence of phenols was assayed by ferric chloride test.
- The presence of tannins was assayed by Gelatin test.
- The presence of flavonoids was assayed by alkaline reagent test and lead acetate test.
- The presence of terpenoid was assayed by *Salkowski test*
- The presence of resins was assayed by acetic anhydride reagent test
- The presence of phlobatannins was assayed by precipitate test.

2.6. Gas chromatography-mass spectroscopy (GC–MS) analysis of bioactive compounds in extracts of Peristrophe roxburghiana leaf

The aqueous, ethanolic and methanolic extracts of PR leaf were subjected to GC–MS analysis. 2 g of the aqueous, ethanolic and methanol extracts of *Peristrophe roxburghiana* leaf were dissolved in 4 mL of absolute methanol, ethanol and methanol respectively. These were then centrifuged for 10 min at 5000g; 0.05 mL of the volatile supernatant was transferred to Varian 450 Gas Chromatograph coupled to a Varian 240 − MS ion − trap mass spectrometer (VF-5 MS Column) with injector and oven temperature at 250 °C and 200 °C. The heating rate of GC–MS was programmed at 100 °C/min to 250 °C at 10 °C/min. The injection was performed in the split ratio of 200 and the volume was 10.0 μL. The flow of carrier gases was constant at 1.0 mL/min during the run of volatile MSC (0.50 mL) solution [21].

The mass spectra of the components in the extracts were determined between 50 and 55 min [22]. Their names, molecular formula, molecular weight and their structures were determined. The relative percentage quantity of each component present in the extracts was determined by comparing the average peak area of each component to the total areas. The identification of the chemical compounds present in the extracts were determined by comparing the retention time and mass

spectra data in the national institute of standards and technology (NIST) MS search 2.0 library [22].

2.7. Acute toxicity study

Modified Lorke's method [23] was used to determine the median lethal dose (LD_{50}) of the extracts using seven weeks old male Wistar rats (36 rats) with weight range of 80–100 g. The animals were grouped into nine and varying doses of 300, 3000 and 5000 mg/kg were orally gavaged to the animals respectively. The animals were placed under observation for 24 h for mortality and 14 days for any sign of toxicity such as circulatory changes, a change in fur and eyes, tremor, locomotors activity, lethargy, salivation, behavioral pattern, diarrhea and body weight depreciation.

2.8. Experimental animals

Thirty-seven male Wistar rats, nine to ten weeks old with weight between 130 and 150 g were used for this study. They were obtained from the central animal house, University of Ibadan, Nigeria. The animals were housed under standard laboratory conditions, fed with normal rat chow and were given access to clean water *ad libitum*. The rats were allowed to acclimatize for three weeks before the experiment commence. The experimental protocol and the handling of the animals were in line with the Animal Ethics Committee of the University of Ibadan guidelines for the care and use of laboratory animals.

2.8.1. Induction of hypertension

The hypertension induction was done by blocking the production of NO with L-NAME. A dosage of 60 mg/kg b.w/day of L-NAME was orally gavaged to the animals for three weeks. The systolic and diastolic pressure was measured at the onset, third and sixth weeks of the study while conscious by occlusion tail-cuff technique (CODA, Kent Scientific, USA).

2.8.2. Study design

The rats were randomly divided into two groups:

- Control group ($n = 5$)
- Hypertensive group ($n = 32$).

The rats in the control group were orally gavaged with 0.2 mL of distilled water while the rats in the hypertensive group were orally gavaged with 60 mg/kg b.w of L-NAME daily. At the third week, the blood pressure of all the rats was measure to certify that hypertension has been induced. After the induction of hypertension, two rats were randomly selected from the hypertensive rats and were sacrificed. The remaining hypertensive rats were randomly sub-grouped into six ($n = 5$):

- Hypertensive group (H): received 60 mg/kg b.w/day of L-NAME after the induction of hypertension.
- Hypertensive aqueous extract of PR group (HA): received 60 mg/kg b.w/day of L-NAME plus 200 mg/kg b.w/day of aqueous extract of PR after the induction of hypertension
- Hypertensive ethanolic extract of PR group (HE): received 60 mg/kg b.w/day of L-NAME plus 200 mg/kg b.w/day of ethanolic extract of PR after the induction of hypertension
- Hypertensive methanolic extract of PR group (HM): received 60 mg/kg b.w/day of L-NAME plus 200 mg/kg b.w/day of methanolic extract of PR after the induction of hypertension
- Hypertensive ramipril group (HR): received 60 mg/kg b.w/day of L-NAME plus 20 mg/kg b.w/day ramipril after the induction of hypertension
- Hypertensive recovery group (R): was not treated after the induction of hypertension

Administration was done orally using the oral administration cannular for another three weeks.

2.9. Measurement of blood pressure

Blood pressure was measured non-invasively by occlusion tail-cuff method in conscious rats using a volume pressure recording sensor (CODA, Kent Scientific, USA). Prior to the commencement of the experiment the rats were trained to get accustomed to the blood pressure set during the acclimatization period. The animals were put in a restrainer placed on a thermostatically regulated warming plate with mini-cuffs fix around the tail to detect the artery pulsations. The rats were allowed to get accustomed to the cuffs for 10 to 15 min before measurement commenced. The measurement was done thrice per session and the average was calculated [24]. The blood pressure of the rats was measured at the onset, third week and sixth week of the study.

2.10. Collection of blood sample

At the third week of the study, the rats were anaesthetized with ketamin/xylazin (2:1) and blood sample was obtained by ocular puncture into a plain bottle to assess for nitric oxide, lipid profile, malondialdehyde, antioxidant enzymes, urea and creatinine levels. After which two of the animals were sacrifice by cervical dislocation and heart, aorta and kidney were harvested for histological examination. At the end of the experiment, same method was employed to obtain blood sample into a plain bottle to evaluate for nitric oxide, lipid profile, malondialdehyde, antioxidant enzymes, urea and creatinine levels, and EDTA bottle for hematological assessment before the rats were sacrificed. The rats were euthanized and the aorta, kidney and heart were harvested and placed in 10% formalin for histological examination. The blood samples were centrifuged at 2500 rpm for 10 min, and the serum was isolated and stored at −20 °C for the determination of nitric oxide, lipid profile, malondialdehyde, antioxidant enzymes, urea and creatinine levels.

2.11. Measurement of serum nitric oxide

Nitrite is a major oxidation product of nitric oxide, so serum nitrite level was measured in order to determine nitric oxide concentration [25]. Nitrite concentration was measured spectrophotometrically at 548 nm.

2.12. Estimation of serum lipid profile

The assessment of serum lipid profile was carried out according to the methods described by Ojiako et al. [26]. Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were measured using commercial kits (Fortress Diagnostics Limited, Antrim, UK).

2.12.1. Estimation of low-density lipoprotein cholesterol (LDL-C)

The concentration of LDL-C was calculated by the formula of Friedewald et al. [27]:

$$LDL - C = TC–HDL - C–(TAG/5)$$

2.12.2. Estimation of very low density lipoprotein cholesterol (VLDL-C)

VLDL-C was estimated based on the formula according to Crook [28]:

$$VLDL - C = TG/5 \text{ in mg/dl}$$

2.12.3. Determination of atherogenic ratios

The atherogenic index of plasma (AI) was estimated by the formula described by Suanarunsawat et al. [29]:

log (TG/HDL − C)

Atherogenic coefficient (AC) was estimated as follows:

(TC − HDL − C)/HDL − C

Cardiac risk ratio (CRR) was estimated as follows:

TC/HDL − C

2.13. Estimation of hematological parameters

Red blood cell count (RBC), haemoglobin concentration (Hb conc.), packed cell volume (PCV), white blood cell count (WBC), differential white blood cell count and red blood cell indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were measured by automated hematological analyzer (Mindray BC-2800, China).

2.14. Estimation of malondialdehyde (MDA) and antioxidant enzyme activities

The degree of lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) concentration as described by Ohkawa et al. [30]. The degree of lipid peroxidation is determined by the level of malondialdehyde a byproduct from the breakdown of polyunsaturated fatty acids. The reaction malondialdehyde with thiobarbituric acid gives a pink colour and the absorbance is measured spectrophotometrically at 535 nm.

Superoxide dismutase (SOD) activity was determined in accordance to the method described by Misra and Fridovich [31] using superoxide dismutase assay kit (R&D Systems, Inc., Minneapolis, USA) following the manufacturer's instructions. Catalase (CAT) activity was estimated as described by Aebi [32] using CAT assay kit (Sigma, St. Louis, MO, USA) adhering to the instructions of the manufacturer. Glutathione (GSH) was determined by estimating the level of reduced GSH as described by Beutler et al. [33]. The method is based on the resulting colour change when reduced GSH react with 5′,5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent). The product of the reaction was then measured spectrophotometrically at 412 nm. The absorbance at 412 nm is proportional to the level of reduced GSH.

2.15. Estimation of serum creatinine and urea level

The concentration of creatinine is estimated by the method described by Jaffe-Slot [34] using an assay kit (Fortress Diagnostics Limited, Antrim, UK). The principle is based on the reaction of creatinine with alkaline solution of sodium picrate to form orange coloured complex. The intensity was measured at 500 nm. Urea level in the serum was determined by colorimetric method as described by Donald et al. [35] using an assay kit (Fortress Diagnostics Limited, Antrim, UK). The principle is based on the ability of urease to hydrolyze urea to ammonia and carbon dioxide. The ammonia reacts with 2-oxoglutarate and NADH in reaction activated by glutamate dehydrogenase to form glutamate and NAD^+. The intensity was measured at 540 nm.

2.16. Histological examination of heart, aorta and kidney

The histological assessment of the rats' heart, aorta and kidney was examined by hematoxylin and eosin (H & E) staining techniques as previously described [36]. The tissues were sectioned, placed on slides and stained with H & E. The photomicrographs were taken at 400 magnification.

2.17. Statistical analysis

Data were presented as mean ± SEM and were analyzed by analysis of variance (ANOVA) using GraphPad prism 7.01 (GraphPad software, Inc., USA). After ANOVA, post-hoc comparison was done using Turkey's test. $P < 0.05$ was accepted as statistically significant.

Table 1
Phytochemical screening of extracts of *Peristrophe roxburghiana* leaf.

Phytochemicals	Aqueous extract	Ethanolic extract	Methanolic extract
Tannins	+ +	+ +	+
Glycosides	−	−	−
Resins	+ +	+ +	+ +
Saponins	−	−	−
phlobatannins	−	+ + +	+ + +
Flavonoids	+ +	+ +	+ +
Sterols	−	+	+ +
Phenols	+	+ + +	+ + +
Alkaloids	−	+	+ +
Terpenoid	+ +	+	+

(−): Negative test. (+): Weak positive test. (+ +): Positive test. (+ + +): Strongly positive test.

3. Results

3.1. Phytochemical screening of extracts Peristrophe roxburghiana leaf

The phytochemical screening of methanolic and ethanolic extracts of PR leaf showed the presence of tannins, resins, phlobatannins, flavonoids, sterols, phenols, alkaloids, and terpenoids, while in aqueous extract, phlobatannin, alkaloid and sterols were absent. Tannin was strongly expressed in aqueous and ethanolic extracts than methanolic extract. Terpenoid was more in the aqueous extract than ethanolic extract and methanolic extract. Phenols content was strongly expressed in ethanolic and methanolic extracts than aqueous extract, while methanolic extract has more sterol and alkanoid than ethanolic extract (Table 1).

3.2. Antioxidant activities of extracts Peristrophe roxburghiana leaf

The antioxidant activities of extracts of PR leaf were evaluated by assessing their 50% inhibitory capacity on MDA and DPPH as well as their TAC. The 50% Inhibitory capacity (IC_{50}) of methanolic extract on MDA was significantly lower than ethanolic and aqueous extract ($p < 0.05$), while that of DPPH was significantly lower in both methanolic and ethanolic extracts than aqueous extract ($p < 0.05$). The TAC was significantly higher in methanolic and ethanolic extracts than aqueous extract ($p < 0.05$) (Table 2).

3.3. GC–MS report of chemical compounds in extracts of Peristrophe roxburghiana leaf

Tables 3a, 3b, 3c show the chemical compounds identified in aqueous, ethanolic and methanolic extracts of PR according to their elusion order VF-5 MS capillary column. Total of eighteen, seven and fifteen compounds were identified in the aqueous, ethanolic and methanolic extracts of PR respectively. The most abundant compounds identified in

Table 2
Antioxidant activities of extracts of *Peristrophe roxburghiana* leaf.

Variables	Aqueous extract	Ethanolic extract	Methanolic extract
MDA (IC_{50})	29.9 ± 1.07[a,b]	15.2 ± 1.06[b]	9.6 ± 0.41
DPPH (IC_{50})	9.91 ± 1.18[a,b]	1.34 ± 0.04	1.20 ± 0.02
TAC	87.3 ± 0.82[a,b]	225 ± 1.95[b]	281 ± 1.09

Data are presented as mean ± SEM. MDA: malondialdehyde, DPPH: 2,2-diphenyl-1-picrylhydrazyl and TAC: total antioxidant capacity.
[a] $p < 0.05$ compared to ethanolic extract.
[b] $p < 0.05$ compared to methanolic extract.

Table 3a
GC–MS report of chemical compounds in aqueous extract of *Peristrophe roxburghiana* leaf.

S/N	Retention time (min)	Quantity (%)	Compound name	Chemical formula	Molecular weight (g/mol)
1	5.587	1.605	Indole-2-one,2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl	$C_{11}H_{13}NO_3$	207
2	14.639	1.485	2,2,4-Trichloro-1,3-cyclopentenedione	$C_5HCl_3O_2$	199
3	18.042	1.325	Silicic acid diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296
4	19.743	1.386	Acetic acid, mercapto-methyl ester	$C_3H_6O_2S$	106
5	21.945	1.285	n-Decanoic acid	$C_{10}H_{20}O_2$	172
6	22.758	1.654	Benzo [h] quinoline 2 4-dimethyl-	$C_{15}H_{13}N$	207
7	23.008	1.327	(R, R) tartaric acid	$C_4H_6O_6$	150
8	23.784	1.584	7,9-Dimethyl-7H-5,6,7,9,11a-pentaaza-benzo[a] fluorene-8,10-dione	$C_{21}H_{17}N_5O_2$	371
9	25.873	1.255	Quinoline, 2-(1-methyl-1H-imidazol-4-yl)	$C_{13}H_{11}N_3$	209
10	27.012	1.248	1,2-Benzisothiazol-3-amine	$C_7H_6N_2S$	150
11	27.343	41.381	Cycloheptasiloxane, tetradecamethyl	$C_{14}H_{42}O_7Si_7$	519
12	28.232	1.913	2-Ethylacridine	$C_{15}H_{13}N$	207
13	29.458	1.282	3-Phenylfurazan	$C_8H_6N_2O$	146
14	31.910	1.715	4-Nitro-2-methylaniline, N-*tert*-butyldimethylsilyl	$C_{13}H_{22}N_2O_2Si$	266
15	32.110	21.035	Cyclooctasiloxane hexadecamethyl	$C_{16}H_{48}O_8Si_8$	593
16	34.406	1.259	Benzaldehyde, 2-hydroxy-5-nitro	$C_7H_5NO_4$	167
17	35.075	9.444	Indole, 3-(4-nitrophenylamino)	$C_{14}H_{11}N_3O_2$	253
18	36.113	7.818	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	667

aqueous extract were cycloheptasiloxane, tetradecamethyl (41.381%), cyclooctasiloxane hexadecamethyl (21.035%), indole, 3-(4-nitrophenylamino) (9.44%) and cyclononasiloxane, octadecamethyl (7.818%). Ethanolic extract has crinan-1alpha-ol (58.824%), 2-amino-alpha-[2-chlorophenyl]cinnamic acid (14.548%), chrysene, 11-butyl-1,2,3,4-tetrahydro (13.051%), cycloheptasiloxane tetradecamethyl (4.668%) and caryophyllene (4.369%) as most abundant. The prevailing compounds in methalonic extract are cycloheptasiloxane tetradecamethyl (45.22%), cyclooctasiloxane hexadecamethyl (23.26%), cyclononasiloxane, octadecamethyl (9.87%) and indole, 3-(4-nitrophenylamino) (3.42%). The least compounds identified were: **aqueous extract**: 2-ethylacridine (1.913%), 4-nitro-2-methylaniline, n-*tert*-butyldimethylsilyl (1.715%), benzo [h] quinoline 2,4-dimethyl (1.654%), indole-2-one,2,3-dihydro-n-hydroxy-4-methoxy-3,3-dimethyl (1.605%), 7,9-dimethyl-7h-5,6,7,9,11a- pentaaza-benzo[a] fluorene-8,10-dione (1.584%), 2,2,4-trichloro-1,3-cyclopentenedione (1.485), acetic acid, mercapto-methyl ester (1.386%), silicic acid diethyl bis(trimethylsilyl) ester (1.325%), n-decanoic acid (1.285%), 3-phenylfurazan (1.282%), benzaldehyde, 2-hydroxy-5-nitro (1.259%), quinoline, 2-(1-methyl-1h-imidazol-4-yl) (1.255%) and 1,2-benzisothiazol-3-amine (1.248%); **ethanolic extract**: gamma- elemene (2.551%) and benzoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester; **methanolic extract**: methyl (7-hydroxy-1h-benzimidazol-2-yl) carbamate (2.39%), 1h-indole, 5-methyl-2-phenyl (2.05%), 2-pyridinamine, n-(4,5-dihydro-5-methyl-2-thiazolyl)-3-methyl (1.78%), 3-methyl-2-phenylindole (1.77%), trans-4-(2-(5-nitro-2-furyl) vinyl)-2-quinolinamine (1.67%), methyl sec-butyl disulphide (1.53%), arsenous acid, tris (trimethylsilyl) ester (1.43%), 1,10-phenanthroline, 2,9-dimethyl (1.41%), anthracene, 9,10-dihydro-9,9,10-trimethyl (1.40%), pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl] (1.40%) and beta.-[3-[n-aziridyl] propyl] amino propionitrile (1.38%).

3.4. Acute toxicity and lethality study

The acute toxicity and lethality study of aqueous, ethanolic and methanolic extracts of PR was carried out on male Wistar rats. The study recorded no mortality after 24 h and 14 days of observation. There was also absence of behavioral changes and toxicity symptoms in the rats administered with varying doses of the extracts (Table 4).

3.5. Systolic blood pressure (SBP)

At the third week of study, SBP increased significantly in the hypertensive group compared to the control group ($p < 0.05$). After three weeks of treatment, SBP of the hypertensive recovery group, hypertensive methanolic, hypertensive ethanolic, hypertensive aqueous and hypertensive ramipril groups decreased significantly compared to the hypertensive group and SBP recorded at the third week ($p < 0.05$). There was a significant increase in SBP in the hypertensive recovery group, hypertensive methanolic, hypertensive ethanolic and hypertensive ramipril compared to the control group ($p < 0.05$), however, the hypertensive group treated with aqueous extract recorded a SBP not significantly different from the control. SBP increased progressively in the hypertensive group, as a significant increase was observed at sixth of L-NAME administration compared to the third week SBP ($p < 0.05$). SBP was significantly lower in the hypertensive group treated with aqueous extract of PR compared to the hypertensive groups treated with ethanolic extract of PR, methanolic extract of PR and ramipril ($p < 0.05$). It was also lower that than of the hypertensive recovery group ($p < 0.05$) (Fig. 1).

3.6. Diastolic blood pressure (DBP)

DBP increased significantly at the third week of study in the

Table 3b
GC–MS report of chemical compounds in ethanolic extract of *Peristrophe roxburghiana* leaf.

S/N	Retention time (min)	Quantity (%)	Compound name	Chemical formula	Molecular weight (g/mol)
1	24.284	4.369	Caryophyllene	$C_{15}H_{24}$	204
2	26.668	2.551	Gamma-Elemene	$C_{15}H_{24}$	204
3	27.343	4.668	Cycloheptasiloxane tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	519
4	32.103	1.989	Benzoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{13}H_{22}O_3Si_2$	282
5	39.604	58.824	Crinan-1alpha-ol	$C_{16}H_{19}NO_3$	273
6	40.304	14.548	2-Amino-alpha-[2-chlorophenyl]cinnamic acid	$C_{15}H_{12}ClNO_2$	273
7	40.454	13.051	Chrysene, 11-butyl-1,2,3,4-tetrahydro	$C_{22}H_{24}$	288

Table 3c
GC–MS report of chemical compounds in methanolic extract of *Peristrophe roxburghiana* leaf.

S/N	Retention time (min)	Quantity (%)	Compound name	Chemical formula	Molecular weight (g/mol)
1	13.388	1.78	2-Pyridinamine, *N*-(4, 5-dihydro-5-methyl-2-thiazolyl)-3-methyl	$C_{10}H_{13}N_3S$	207
2	17.529	1.43	Arsenous acid, tris (trimethylsilyl) ester	$C_9H_{27}AsO_3Si_3$	342
3	25.410	1.67	Trans-4-(2-(5-Nitro-2-furyl) vinyl)-2-quinolinamine	$C_{15}H_{11}N_3O_3$	281
4	26.593	2.39	Methyl (7-hydroxy-1H-benzimidazol-2-yl) carbamate	$C_9H_9N_3O_3$	207
5	27.137	1.53	Methyl sec-butyl disulphide	$C_5H_{12}S_2$	136
6	27.174	1.41	1,10-Phenanthroline, 2,9-dimethyl-	$C_{14}H_{12}N_2$	208
7	27.362	45.22	Cycloheptasiloxane tetradecamethyl	$C_{14}H_{42}O_7Si_7$	519
8	28.294	1.38	Beta.-[3-[N-Aziridyl] propyl] amino propionitrile	$C_8H_{15}N_3$	153
9	28.563	1.77	3-Methyl-2-phenylindole	$C_{15}H_{13}$	207
10	31.553	1.40	Anthracene, 9, 10-dihydro-9, 9, 10-trimethyl-	$C_{17}H_{18}$	222
11	32.129	23.26	Cyclooctasiloxane hexadecamethyl	$C_{16}H_{48}O_8Si_8$	593
12	32.942	1.40	Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl]-	$C_{12}H_{14}$	207
13	35.100	3.42	Indole, 3-(4-nitrophenylamino)	$C_{14}H_{11}N_3O_2$	253
14	35.419	2.05	1H-Indole, 5-methyl-2-phenyl	$C_{15}H_{13}N$	207
15	36.101	9.87	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	667

hypertensive group compared to the control group ($p < 0.05$). After three weeks of treatment, DBP of the hypertensive recovery group, hypertensive methanolic, hypertensive ethanolic, hypertensive aqueous and hypertensive ramipril groups decreased significantly compared to the hypertensive group and DBP recorded at the third week ($p < 0.05$). There was a significant increase in DBP of the hypertensive treated groups compared to the control group ($p < 0.05$), but there was no significant difference in the hypertensive aqueous and recovery groups DBP compared to the control. DBP increased progressively in the hypertensive group, as a significant increase was observed at sixth week of L-NAME administration compared to the third week DBP ($p < 0.05$). DBP was significantly lower in the hypertensive group treated with aqueous extract of PR compared to the hypertensive ramipril group ($p < 0.05$) (Fig. 2).

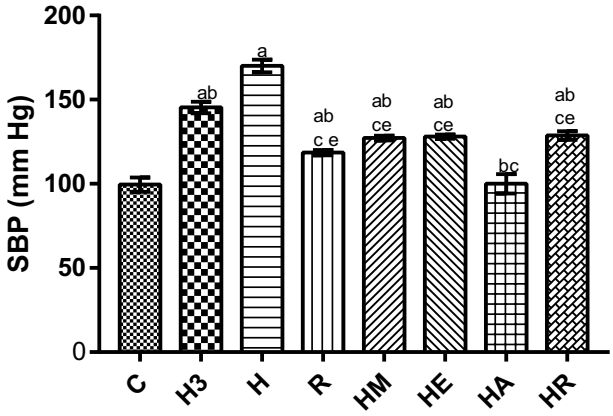

Fig. 1. Systolic blood pressure (SBP).
Data are presented as mean ± SEM, $n = 5$, **a** = $p < 0.05$ compared to C, b = p < 0.05 compared to H, **c** = p < 0.05 compared to H3, **e** = p < 0.05 compared to HA. **C**–control group, **H3–**hypertension induced (SBP recorded in the hypertensive rats at the third week), **H**–hypertensive group (received 60 mg/kg b.w of L-NAME after hypertension was induced), **R** – hypertensive recovery group (was not treated after hypertension was induced), **HM**–hypertensive methanolic group (received 60 mg/kg b.w of L-NAME + 200 mg/kg b.w of methanolic extract of PR after hypertension was induced), **HE**–hypertensive ethanolic group (received 60 mg/kg b.w of L-NAME + 200 mg/kg b.w of ethanolic extract of PR after hypertension was induced), **HA**–hypertensive aqueous group (received 60 mg/kg b.w of L-NAME + 200 mg/kg b.w of aqueous extract of PR after hypertension was induced) and **HR**- hypertensive ramipril group (received 60 mg/kg b.w of L-NAME + 20 mg/kg b.w of ramipril after hypertension was induced).

3.7. Mean arterial pressure (MAP)

At the third week of the study, MAP increased significantly in the hypertensive group compared to the control group ($p < 0.05$). After three weeks of treatment, the MAP of the hypertensive recovery group, hypertensive methanolic, hypertensive ethanolic, hypertensive aqueous and hypertensive ramipril groups decreased significantly compared to the hypertensive group and MAP recorded at the third week ($p < 0.05$). There was a significant increase in the MAP of the hypertensive treated groups and recovery group compared to the control group ($p < 0.05$), however MAP of the hypertensive group treated with the aqueous extract of PR was not significantly different from that of the control. MAP increased progressively in the hypertensive group, as a significant increase was observed at the sixth week of L-NAME administration compared to the third week MAP ($p < 0.05$). MAP was significantly lower in the hypertensive aqueous group compared to the hypertensive ethanolic, methanolic and ramipril groups ($p < 0.05$) (Fig. 3).

3.8. Serum nitric oxide (NO)

Serum NO significantly decreased at the third week of study compared to the control ($p < 0.05$). At end of the experiment, NO level

Table 4
Acute toxicity and lethality study of extracts of *Peristrophe roxburghiana* leaf in male Wistar rats.

Dosage (n = 3)		Aqueous extract	Methanolic extract	Ethanolic extract
300 mg/kg b.w.	Number of mortality after 24 h	Nil	Nil	Nil
	Behavioral changes and toxicity symptoms after 14 Days	Nil	Nil	Nil
3000 mg/kg b.w.	Number of mortality after 24 h	Nil	Nil	Nil
	Behavioral changes and toxicity symptoms after 14 Days	Nil	Nil	Nil
5000 mg/kg b.w.	Number of mortality after 24 h	Nil	Nil	Nil
	Behavioral changes and toxicity symptoms after 14 Days	Nil	Nil	Nil

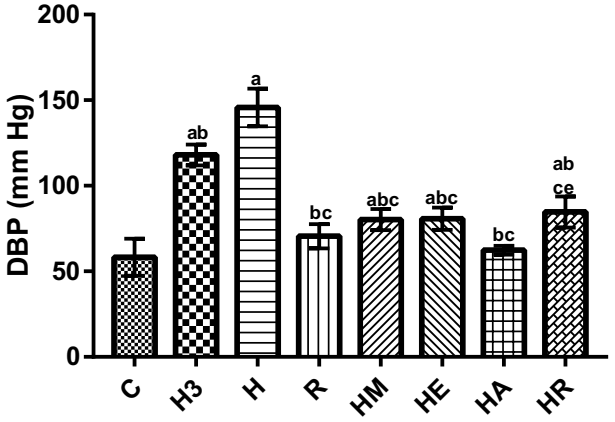

Fig. 2. Diastolic blood pressure (**DBP**). Data are presented as mean ± SEM, $n = 5$, **a** = $p < 0.05$ compared to C, b = $p < 0.05$ compared to H, **c** = $p < 0.05$ compared to H3, **e** = $p < 0.05$ compared to HA. **C**–control group, **H3–** hypertension induced (DBP recorded in the hypertensive rats at the third week), **H**–hypertensive group (received 60 mg/kg b.w of L-NAME after hypertension was induced), **R**–hypertensive recovery group (was not treated after hypertension was induced), **HM**–hypertensive methanolic group (received 60 mg/kg b.w of L-NAME +200 mg/kg b.w of methanolic extract of PR after hypertension was induced), **HE**–hypertensive ethanolic group (received 60 mg/kg b.w of L-NAME +200 mg/kg b.w of ethanolic extract of PR after hypertension was induced), **HA**–hypertensive aqueous group (received 60 mg/kg b.w of L-NAME +200 mg/kg b.w of aqueous extract of PR after hypertension was induced) and **HR**- hypertensive ramipril group (received 60 mg/kg b.w of L-NAME +20 mg/kg b.w of ramipril after hypertension was induced).

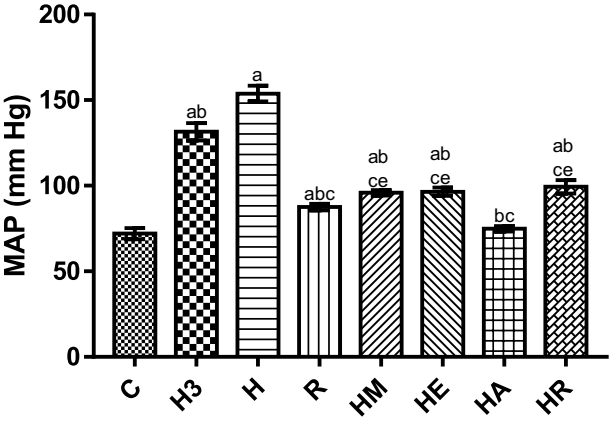

Fig. 3. Mean arterial pressure (MAP).
Data are presented as mean ± SEM, n = 5, **a** = $p < 0.05$ compared to C, b = $p < 0.05$ compared to H, **c** = $p < 0.05$ compared to H3, **e** = $p < 0.05$ compared to HA. **C**–control group, **H3–**hypertension induced (MAP recorded in the hypertensive rats at the third week), **H**–hypertensive group (received 60 mg/kg b.w of L-NAME after hypertension was induced), **R–**hypertensive recovery group (was not treated after hypertension was induced), **HM**–hypertensive methanolic group (received 60 mg/kg b.w of L-NAME +200 mg/kg b.w of methanolic extract of PR after hypertension was induced), **HE**–hypertensive ethanolic group (received 60 mg/kg b.w of L-NAME +200 mg/kg b.w of ethanolic extract of PR after hypertension was induced), **HA**–hypertensive aqueous group (received 60 mg/kg b.w of L-NAME +200 mg/kg b.w of aqueous extract of PR after hypertension was induced) and **HR**–hypertensive ramipril group (received 60 mg/kg b.w of L-NAME +20 mg/kg b.w of ramipril after hypertension was induced).

decreased significantly in the hypertensive group, hypertensive aqueous group and hypertensive ramipril group compared to the control ($p < 0.05$). Whereas, in the hypertensive groups treated with methanolic, ethanolic and aqueous extract of PR NO significantly increased when compared to hypertensive group ($p < 0.05$). The increase

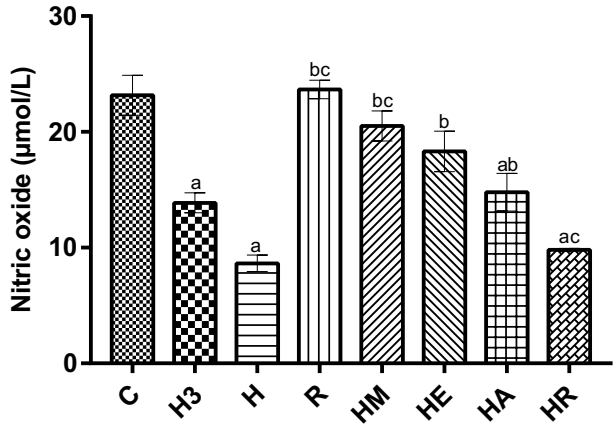

Fig. 4. Nitric oxide (NO) level.
Data are presented as mean ± SEM, n = 5, **a** = $p < 0.05$ compared to C, **b** = $p < 0.05$ compared to H, **c** = $p < 0.05$ compared to H3. **C**–control group, **H3–** hypertension induced (NO recorded in the hypertensive rats at the third week), **H**–hypertensive group (received 60 mg/kg b.w of L-NAME after hypertension was induced), **R**–hypertensive recovery group (was not treated after hypertension was induced), **HM**–hypertensive methanolic group (received 60 mg/kg b.w of L-NAME +200 mg/kg b.w of methanolic extract of PR after hypertension was induced), **HE**–hypertensive ethanolic group (received 60 mg/kg b.w of L-NAME +200 mg/kg b.w of ethanolic extract of PR after hypertension was induced), **HA**–hypertensive aqueous group (received 60 mg/kg b.w of L-NAME +200 mg/kg b.w of aqueous extract of PR after hypertension was induced) and **HR**–hypertensive ramipril group (received 60 mg/kg b.w of L-NAME +20 mg/kg b.w of ramipril after hypertension was induced).

observed in the methanolic extract treated hypertensive group was significant compared to the third week NO level ($p < 0.05$). There was a significant rise in NO level in the recovery group compared to the hypertensive group ($p < 0.05$). The fall in NO level in the hypertensive ramipril group was also significant compared to NO level recorded at the third week of the study ($p < 0.05$) (Fig. 4).

3.9. Serum lipid profile

At the establishment of hypertension and in the hypertensive group, there was a significant increase in TG, TC and LDL-C levels compared to the control ($p < 0.05$). After three weeks of treatment, TG and TC levels of the hypertensive aqueous group, hypertensive ethanolic group and hypertensive ramipril group decreased significantly compared to the hypertensive group as well as at the establishment of hypertension ($p < 0.05$), but TG level of the hypertensive ethanolic group with TG and TC levels of the hypertensive ramipril group was not significantly different compared to TG and TC recorded at the third week of the study. TG level in the hypertensive methanolic group increased significantly compared to the control, third week TG and hypertensive group ($p < 0.05$). TC level of the recovery group and hypertensive methanolic group increased significantly compared to control ($p < 0.05$) but decreased significantly when compared to the hypertensive group ($p < 0.05$). TC increased progressively in the hypertensive group, as a significant increase was observed at the sixth week of L-NAME administration compared to the third week TC ($p < 0.05$). LDL-C of the hypertensive methanolic group, hypertensive ethanolic group, hypertensive aqueous group, hypertensive ramipril group and the hypertensive recovery group decreased significantly when compared to the hypertensive group ($p < 0.05$). There was no significant difference VLDL-C in all the tested groups compared to the control. A significant decrease was observed HDL-C level at third week of study in hypertensive group compared to control ($p < 0.05$). At the end of the experiment, HDL-C level in the recovery group, hypertensive methanolic group, hypertensive ethanolic group, hypertensive aqueous

Table 5
Serum lipid profile.

Variables	C	H3	H	R	HM	HE	HA	HR
TG (mg/dl)	25.30 ± 0.51	30.25 ± 0.30[a]	32.40 ± 0.96[a]	24.80 ± 1.01[b,c]	37.70 ± 1.70[a,b,c]	26.50 ± 2.19[b]	21.70 ± 0.76[b,c]	26.50 ± 1.35[b]
TC (mg/dl)	89.90 ± 0.63	107.00 ± 1.70[a,b]	131.00 ± 0.95[a]	117.00 ± 3.04[a,b]	118.00 ± 4.29[a,b]	85.30 ± 0.98[b,c]	88.90 ± 1.56[b,c]	93.60 ± 5.22[b]
LDL-C (mg/dl)	51.70 ± 1.92	80.50 ± 0.70[a]	109.00 ± 1.55[a]	84.50 ± 0.88[a,b]	85.80 ± 1.82[a,b]	56.40 ± 1.73[b]	55.30 ± 1.81[b,c]	46.30 ± 2.55[b,c]
VLDL-C (mg/dl)	5.05 ± 0.37	6.05 ± 0.39	6.21 ± 0.39	4.97 ± 0.55	7.54 ± 1.18	5.30 ± 0.65	4.35 ± 0.32	5.30 ± 0.40
HDL-C (mg/dl)	30.80 ± 3.20	22.77 ± 2.11[a,b]	14.78 ± 0.33[a]	29.70 ± 0.45[b,c]	33.60 ± 2.21[b,c]	33.80 ± 1.39[b,c]	29.40 ± 0.85[b]	31.60 ± 0.80[b,c]

Data are presented as mean ± SEM, n = 5. **C**–control group, **H3**–hypertension induced (lipid profile recorded in the hypertensive rats at the third week), **H**–hypertensive group (received 60 mg/kg b.w. of L-NAME after hypertension was induced), **R**–hypertensive recovery group (was not treated after hypertension was induced), **HM**–hypertensive methanolic group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of methanolic extract of PR after hypertension was induced), **HE**–hypertensive ethanolic group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of ethanolic extract of PR after hypertension was induced), **HA**–hypertensive aqueous group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of aqueous extract of PR after hypertension was induced) and **HR**–hypertensive ramipril group (received 60 mg/kg b.w. of L-NAME + 20 mg/kg b.w. of ramipril after hypertension was induced).
[a] $p < 0.05$ compared to C.
[b] $p < 0.05$ compared to H.
[c] $p < 0.05$ compared to H3.

group and hypertensive ramipril increased significantly compared to the hypertensive group ($p < 0.05$). The increase observed in the hypertensive recovery group, hypertensive methanolic group, hypertensive ethanolic group and hypertensive ramipril was significant compared to HDL-C level recorded at the third week ($p < 0.05$). There was a significant decrease in HDL-C level in the hypertensive group at the end of the experiment compared to the control and HDL-C observed at the third week of the study ($p < 0.05$) (Table 5).

3.10. Atherogenic indices

There was no significant difference observed in CRR at the third week of study compared to control. However, a significant increase in AI and AC was observed at the third week compared to the control ($p < 0.05$). AC, AI and CRR of the hypertensive group increased significantly compared to the control group at the end of the study ($p < 0.05$). In the hypertensive treated groups, AC, AI and CRR decreased significantly compared to the hypertensive group ($p < 0.05$). The decrease in AI observed in the ethanolic, aqueous and ramipril treated hypertensive groups were significant with that observed at third week of study. In the recovery group, AC, AI and CRR significantly decreased compared to the hypertensive group ($p < 0.05$) (Table 6).

3.11. Serum and tissue oxidative parameters

In the **serum**, there was no significant difference in MDA level, SOD and CAT activities in all the tested groups compared to the control. However, the level of GSH increased significantly in the hypertensive methanolic group and hypertensive aqueous group compared to the control, hypertensive group as well as that recorded at the third week of the study ($p < 0.05$) (Fig. 5). In the **aorta**, there was no significant change in MDA and CAT levels in all the tested groups compared to the control. However, there was a significant decrease in SOD activity in the ethanolic extracted treated hypertensive rats compared to the control and hypertensive groups ($p < 0.05$) and a significant increase was observed in GSH level in the hypertensive aqueous group compared to the control ($p < 0.05$) (Table 7). In the **heart**, there was no significant change in MDA, GSH and CAT levels in all the tested groups compared to the control, however a significant decrease was observed in SOD activity in the methanolic, ethanolic and ramipril treated hypertensive groups compared to the hypertensive group ($p < 0.05$), while the hypertensive group treated with aqueous extract recorded a significant increase in SOD activities compared to the control and hypertensive groups ($p < 0.05$) (Table 8). In the **kidney**, there was no significant change in MDA, SOD, CAT and GSH levels in all the tested groups compared to the control (Table 9).

3.12. Creatinine and urea levels

Serum creatinine level was not significantly difference at the third week of the study compared to the control. However, at the end of the experiment, creatinine level increased significantly in the hypertensive group, hypertensive methanolic and hypertensive ramipril groups compared to control ($p < 0.05$). In the hypertensive aqueous, hypertensive ethanolic and recovery groups creatinine level decreased significantly compared to the hypertensive group ($p < 0.05$). There was no significant difference in serum urea level in all the tested groups compared to the control (Fig. 6).

3.13. White blood cells (WBC) count and differential WBC count

There was a significant increase WBC count in the hypertensive

Table 6
Atherogenic indices.

Variables	C	H3	H	R	HM	HE	HA	HR
AI	0.06 ± 0.06	0.17 ± 0.06[a]	0.35 ± 0.03[a]	−0.08 ± 0.04[b]	0.03 ± 0.05[b]	−0.08 ± 0.08[b,c]	−0.10 ± 0.06[b,c]	−0.02 ± 0.065[b,c]
AC	2.87 ± 0.39	4.23 ± 025[a]	5.56 ± 0.46[a]	3.26 ± 0.40[b]	2.91 ± 0.41[b]	2.81 ± 0.62[b]	2.01 ± 0.26[b,c]	2.13 ± 0.27[b,c]
CRR	3.87 ± 0.67	5.23 ± 0.25	7.30 ± 0.82[a]	4.26 ± 0.40[b]	3.91 ± 0.41[b]	3.81 ± 0.62[b]	3.13 ± 0.27[b]	3.01 ± 0.26[b,c]

Data are presented as mean ± SEM, $n = 5$, **C**–control group, **H3**–hypertension induced (atherogenic indices recorded in the hypertensive rats at the third week), **H**–hypertensive group (received 60 mg/kg b.w. of L-NAME after hypertension was induced), **R**–hypertensive recovery group (was not treated after hypertension was induced), **HM**–hypertensive methanolic group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of methanolic extract of PR after hypertension was induced), **HE**–hypertensive ethanolic group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of ethanolic extract of PR after hypertension was induced), **HA**–hypertensive aqueous group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of aqueous extract of PR after hypertension was induced) and **HR**–hypertensive ramipril group (received 60 mg/kg b.w. of L-NAME + 20 mg/kg b.w. of ramipril after hypertension was induced).
[a] $p < 0.05$ compared to C.
[b] $p < 0.05$ compared to H.
[c] $p < 0.05$ compared to H3.

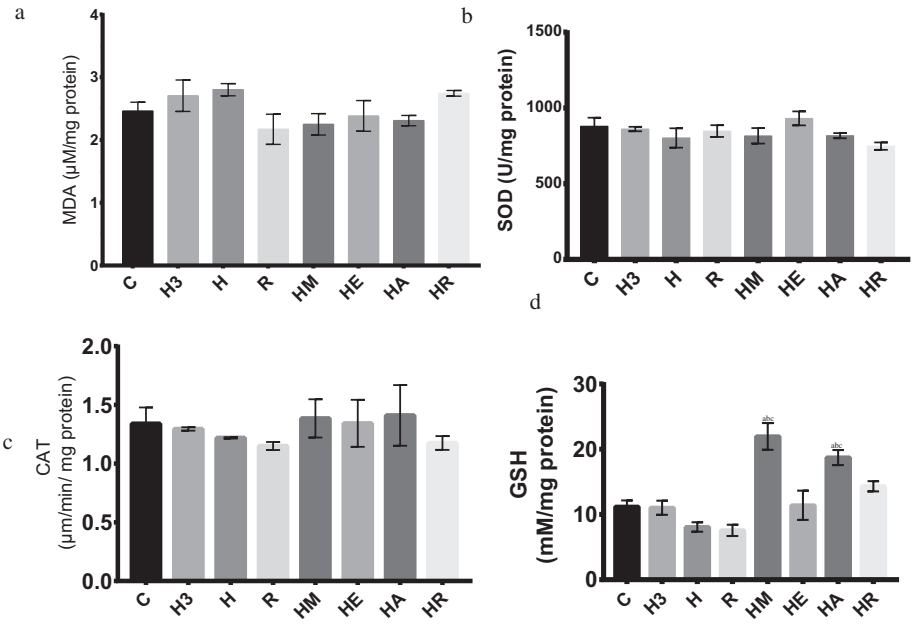

Fig. 5. Serum oxidative parameters.
Data are presented as mean ± SEM, n = 5, **a** = $p < 0.05$ compared to C, **b** = $p < 0.05$ compared to H, **c** = $p < 0.05$ compared to H3. **C**–control group, **H3**–hypertension induced (oxidative parameters recorded in the hypertensive rats at the third week), **H**–hypertensive group (received 60 mg/kg b.w of L-NAME after hypertension was induced), **R**–hypertensive recovery group (was not treated after hypertension was induced), **HM**–hypertensive methanolic group (received 60 mg/kg b.w of L-NAME + 200 mg/kg b.w of methanolic extract of PR after hypertension was induced), **HE**–hypertensive ethanolic group (received 60 mg/kg b.w of L-NAME + 200 mg/kg b.w of ethanolic extract of PR after hypertension was induced), **HA**–hypertensive aqueous group (received 60 mg/kg b.w of L-NAME + 200 mg/kg b.w of aqueous extract of PR after hypertension was induced) and **HR**–hypertensive ramipril group (received 60 mg/kg b.w of L-NAME + 20 mg/kg b.w of ramipril after hypertension was induced). **a**–MDA level, **b** – SOD activity, **c**–CAT activity and **d** – GSH level.

group and hypertensive ramipril group compared to control ($p < 0.05$). WBC count in the hypertensive methanolic, hypertensive ethanolic, hypertensive aqueous groups as well as the recovery group decreased significantly compared to the hypertensive group ($p < 0.05$). The differential WBC count showed an increase in neutrophil level in the hypertensive group, recovery group and hypertensive ethanolic group, and a decrease in lymphocyte level in these groups compared to control ($p < 0.05$). In the hypertensive methanolic group, hypertensive aqueous group and hypertensive ramipril group there was a significant decreased in neutrophil level and a significant increase in lymphocyte level compared to the hypertensive group ($p < 0.05$). There was no significant difference in eosinophil and monocyte levels in all the tested groups compared to the control, except for the hypertensive aqueous group which recorded an increase in monocyte level compared to the control ($p < 0.05$) (Table 10).

3.14. Red blood cells (RBC) count, RBC indices and platelet count

There was no significant difference in RBC count, PCV, Hb concentration, RBC indices and platelet count in all the tested groups compared to the control. However, there was a significant increase in platelet count in the hypertensive recovery group and hypertensive methanolic group compared to the control and the hypertensive group ($p < 0.05$) (Table 11).

3.15. Histology of the heart

The histological examination of the heart showed mild infiltration of inflammatory cells in the heart at the third week of the study. The histology of the hypertensive group showed mild infiltration of inflammatory cells. The histological examination of the heart of the methanolic extract treated hypertensive group, ethanolic extract treated hypertensive group, ramipril treated hypertensive group and hypertensive recovery group showed mild infiltration of inflammatory cells. The control and hypertensive aqueous group showed normal heart architecture (Fig. 7).

3.16. Histology of the aorta

The histological examination of the aorta showed relatively rough surface of tunica intima at the third week of the study. The histology of the hypertensive group showed marked focal thickening of the tunica intima, while the histological examination of the aorta of the methanolic extract treated hypertensive group and ramipril treated

Table 7
Aorta oxidative parameters.

Variables	C	H	R	HM	HE	HA	HR
MDA (µM/mg protein)	6.63 ± 1.22	4.10 ± 0.83	2.94 ± 1.30	3.82 ± 1.07	5.72 ± 3.02	4.10 ± 1.00	3.51 ± 0.82
SOD (U/mg protein)	1425.79 ± 369	1494.00 ± 26.80	776.00 ± 38.40	1637.00 ± 253	594.00 ± 113[a,b]	1175.00 ± 142	698.50 ± 247
CAT (µm/min/mg protein)	1.12 ± 0.01	1.00 ± 0.09	1.03 ± 0.05	1.02 ± 0.05	1.07 ± 0.04	1.03 ± 0.06	1.01 ± 0.10
GSH (mM/mg protein)	0.20 ± 0.06	0.28 ± 0.00	0.17 ± 0.01	0.22 ± 0.05	0.14 ± 0.03	1.48 ± 0.71[a]	1.08 ± 0.31

Data are presented as mean ± SEM, n = 5, C- control group, **H**–hypertensive group (received 60 mg/kg b.w. of L-NAME after hypertension was induced), **R**–hypertensive recovery group (was not treated after hypertension was induced), **HM**–hypertensive methanolic group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of methanolic extract of PR after hypertension was induced), **HE**–hypertensive ethanolic group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of ethanolic extract of PR after hypertension was induced), **HA**–hypertensive aqueous group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of aqueous extract of PR after hypertension was induced) and **HR**–hypertensive ramipril group (received 60 mg/kg b.w of L-NAME + 20 mg/kg b.w of ramipril after hypertension was induced).
[a] $p < 0.05$ compared to C.
[b] $p < 0.05$ compared to H.

Table 8
Heart oxidative parameters.

Variables	Variables	C	H	R	HM	HE	HA	HR
MDA (μM/mg protein)	MDA (μmol)	12.9 ± 1.19	14.51 ± 1.76	13.7 ± 0.95	18.10 ± 2.85	14.40 ± 3.78	10.10 ± 2.05	13.50 ± 1.34
SOD (U/mg protein)	SOD (U/L)	900.17 ± 56.40	1031.04 ± 93.00	939.95 ± 99.80	508.04 ± 69.00[b]	885.01 ± 32.9[b]	1476.76 ± 76.20[a,b]	600.46 ± 93.80[b]
CAT (μm/min/mg protein)	CAT (μm/min/ml)	1.12 ± 0.03	1.02 ± 0.08	1.01 ± 0.12	0.85 ± 0.15	1.08 ± 0.09	1.10 ± 0.10	1.07 ± 0.09
GSH (mM/mg protein)	GSH (mmol/L)	2.60 ± 0.35	3.12 ± 0.54	1.52 ± 0.31	2.00 ± 0.19	1.90 ± 0.29	1.42 ± 0.32	2.20 ± 0.30

Data are presented as mean ± SEM, n = 5, **C**–control group, **H**–hypertensive group (received 60 mg/kg b.w of L-NAME after hypertension was induced), **R**–hypertensive recovery group (was not treated after hypertension was induced), **HM**–hypertensive methanolic group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of methanolic extract of PR after hypertension was induced), **HE**–hypertensive ethanolic group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of ethanolic extract of PR after hypertension was induced), **HA**–hypertensive aqueous group (received 60 mg/kg b.w of L-NAME + 200 mg/kg b.w. of aqueous extract of PR after hypertension was induced) and **HR**–hypertensive ramipril group (received 60 mg/kg b.w. of L-NAME + 20 mg/kg b.w of ramipril after hypertension was induced).

[a] $p < 0.05$ compared to C.
[b] $p < 0.05$ compared to H.

hypertensive group showed invasion of the tunica media connective tissue by groups of large epithelioid cells. The histology of the aorta of the control, hypertensive recovery, hypertensive ethanolic and hypertensive aqueous groups show normal aorta architecture (Fig. 8).

3.17. Histology of the kidney

The histological examination of the kidney showed focal areas of lymphoid aggregates at the establishment of hypertension, in the methanolic extract treated hypertensive group and ethanolic extract treated hypertensive group. The histology of the hypertensive group and ramipril treated hypertensive group showed mild infiltration of inflammatory cells. The histology of the control and hypertensive aqueous group showed normal kidney architecture (Fig. 9).

4. Discussion

Varieties of chemical compounds in medicinal plants provide limitless opportunities for the discovery of new therapeutic agents. Plants have been used as medicines for ages and the efficacy of some of these therapeutic plants have been documented in literature. Plants contain a large number of bioactive compounds that have been shown to be beneficial and have biological activities like anti-inflammatory, antihypertensive, anticancer, antimicrobial, antioxidant and wound healing property. The present study investigated the phytochemical constituents, antioxidant activities and GC–MS analysis of aqueous, ethanolic and methanolic extracts of PR and also assesses their antihypertensive and anti-lipidemic effects in L-NAME hypertensive rats.

The phytochemical screening of the aqueous, ethanolic and methanolic extracts of PR leaf showed the presence of tannins, resins, flavonoids, phenol and terpenoid in all the extracts, while phlobatannins, sterols and alkaloids were present in the ethanolic and methanolic extracts. The methanolic and ethanolic extracts showed strong presence of phenol, while terpenoid presence was stronger in the aqueous extract. The antioxidant activities of the aqueous, ethanolic and methanolic extracts of PR leaf were evaluated by assessing their antilipid peroxidation activity, their ability to scavenge DPPH radicals and by measuring their total antioxidant capacity showed that the methanolic and ethanolic extracts have a low inhibitory capacity for MDA and DPPH as well as has high antioxidant capacity. The high level of antioxidant activities recorded in the methanolic and ethanolic extracts might be due to the high phenolic compounds. Phenols are well known antioxidant. They have been reported to have a direct antioxidant property as well as act indirectly to activate endogenous antioxidant system [37].

GC–MS analysis of aqueous, ethanolic and methanolic extracts of PR leaf showed that the aqueous, ethanolic and methanolic extracts contain numerous polar and non-polar bioactive compounds like aromatic compounds, fatty acid, organic acid, terpenes and diverse types of esters. The three extracts have cycloheptasiloxane tetradecamethyl which is highest in the methanolic extract. Cyclooctasiloxane hexadecamethyl and cyclononasiloxane octadecamethyl were also identified in the

Table 9
Kidney oxidative parameters.

Variables	Variables	C	H	R	HM	HE	HA	HR
MDA (μM/mg protein)	MDA (μmol)	18.30 ± 1.88	24.11 ± 3.92	18.70 ± 2.99	15.43 ± 1.40	23.47 ± 6.16	19.11 ± 3.65	24.22 ± 3.22
SOD (U/mg protein)	SOD (U/L)	2190 ± 162	1873 ± 411	1647 ± 111	2776.06 ± 630	1729 ± 180	1549 ± 148	2144 ± 251
CAT (μm/min/mg protein)	CAT (μm/min/ml)	1.10 ± 0.02	1.10 ± 0.01	1.10 ± 0.02	1.02 ± 0.05	1.00 ± 0.03	1.11 ± 0.01	0.99 ± 0.06
GSH (mM/mg protein)	GSH (mmol/L)	1.24 ± 0.33	2.05 ± 0.23	1.04 ± 0.42	0.66 ± 0.22	0.57 ± 0.18	0.79 ± 0.28	1.01 ± 0.45

Data are presented as mean ± SEM, $n = 5$. **C**–control group, **H**–hypertensive group (received 60 mg/kg b.w. of L-NAME after hypertension was induced), **R**–hypertensive recovery group (was not treated after hypertension was induced), **HM**–hypertensive methanolic group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of methanolic extract of PR after hypertension was induced), **HE**–hypertensive ethanolic group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of ethanolic extract of PR after hypertension was induced), **HA**–hypertensive aqueous group (received 60 mg/kg b.w of L-NAME + 200 mg/kg b.w. of aqueous extract of PR after hypertension was induced) and **HR**- hypertensive ramipril group (received 60 mg/kg b.w. of L-NAME + 20 mg/kg b.w. of ramipril after hypertension was induced).

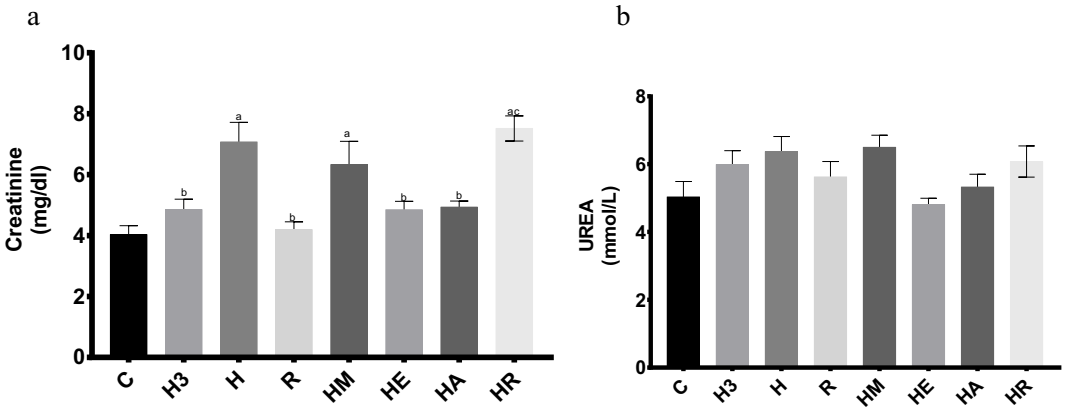

Fig. 6. Creatinine and urea levels.
Data are presented as mean ± SEM, n = 5, **a** = $p < 0.05$ compared to C, **b** = $p < 0.05$ compared to H, **c** = $p < 0.05$ compared to H3. **C**–control group, **H3–**hypertension induced (creatinine and urea levels recorded in the hypertensive rats at the third week), **H**–hypertensive group (received 60 mg/kg b.w. of L-NAME after hypertension was induced), **R**–hypertensive recovery group (was not treated after hypertension was induced), **HM**–hypertensive methanolic group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of methanolic extract of PR after hypertension was induced), **HE**–hypertensive ethanolic group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of ethanolic extract of PR after hypertension was induced), **HA**–hypertensive aqueous group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of aqueous extract of PR after hypertension was induced) and **HR**–hypertensive ramipril group (received 60 mg/kg b.w. of L-NAME + 20 mg/kg b.w. of ramipril after hypertension was induced). **a**–creatinine level and **b**– urea level.

Table 10
White blood cells (WBC) count and differential WBC count.

Variables	C	H	R	HM	HE	HA	HR
WBC (/mm^3)	17,220 ± 471	27,133.33 ± 1398[a]	17,340 ± 1496[b]	14,675 ± 269[b]	19,200 ± 1468[b]	15,000 ± 1514[b]	25,960 ± 2403[a]
Differential WBC							
Neutrophil (%)	50.20 ± 0.49	57.67 ± 0.68[a]	56.80 ± 1.24[a]	50.00 ± 0.68[b]	54.25 ± 0.81[a]	51.75 ± 1.21[b]	49.80 ± 1.07[b]
Eosinophil (%)	1.00 ± 0.32	1.67 ± 0.25	1.4 ± 0.4	0.75 ± 0.45	1.50 ± 0.25	1.25 ± 0.37	0.40 ± 0.25
Lymphocyte (%)	46.6 ± 0.75	36.40 ± 1.21[a]	37.40 ± 1.50[a]	44.60 ± 2.11[b]	39.80 ± 0.583[a]	41.75 ± 0.894[b]	48.40 ± 0.812[b]
Monocyte (%)	2.20 ± 0.37	5.00 ± 0.58	4.40 ± 1.03	2.75 ± 1.11	3.75 ± 0.75	5.40 ± 0.60[a]	1.40 ± 0.25

Data are presented as mean ± SEM, $n = 5$. **C**–control group, **H**–hypertensive group (received 60 mg/kg b.w of L-NAME after hypertension was induced), **R–**hypertensive recovery group (was not treated after hypertension was induced), **HM**–hypertensive methanolic group (received 60 mg/kg b.w of L-NAME + 200 mg/kg b.w of methanolic extract of PR after hypertension was induced), **HE**–hypertensive ethanolic group (received 60 mg/kg b.w of L-NAME + 200 mg/kg b.w of ethanolic extract of PR after hypertension was induced), **HA**–hypertensive aqueous group (received 60 mg/kg b.w of L-NAME + 200 mg/kg b.w. of aqueous extract of PR after hypertension was induced) and **HR**–hypertensive ramipril group (received 60 mg/kg b.w of L-NAME + 20 mg/kg b.w of ramipril after hypertension was induced).
[a] $p < 0.05$ compared to C.
[b] $p < 0.05$ compared to H.

Table 11
Red blood cells (RBC) count, RBC indices and platelet count.

Variables	C	H	R	HM	HE	HA	HR
RBC (10^{12}/L)	8.75 ± 0.26	8.78 ± 0.33	7.85 ± 0.41	8.70 ± 0.33	9.16 ± 0.31	9.06 ± 0.28	7.72 ± 0.23
PCV (%)	52.34 ± 1.64	51.16 ± 1.76	49.04 ± 3.17	52.04 ± 2.21	55.46 ± 2.53	54.20 ± 1.54	43.32 ± 1.31
Hb conc. (g/dL)	15.06 ± 0.46	14.44 ± 0.63	13.16 ± 0.73	14.52 ± 0.50	15.28 ± 0.79	15.40 ± 0.37	13.02 ± 0.30
MCV (fL)	59.76 ± 0.35	58.44 ± 0.98	62.40 ± 1.05	59.86 ± 0.93	60.54 ± 0.91	60.02 ± 1.79	56.24 ± 1.02
MCH (pg)	17.12 ± 0.18	16.40 ± 0.28	16.70 ± 0.19	16.66 ± 0.11	16.60 ± 0.39	16.98 ± 0.40	16.84 ± 0.39
MCHC (g/dL)	28.74 ± 0.15	28.16 ± 0.31	26.84 ± 0.26	27.88 ± 0.33	27.48 ± 0.25	28.36 ± 0.20	30.04 ± 0.32
Platelet ($\times 10^9$/L)	814.20 ± 31	766.20 ± 22	964.00 ± 38[a,b]	993.60 ± 45[a,b]	909.00 ± 40	803.00 ± 13	865.00 ± 27

Data are presented as mean ± SEM, n = 5, **C - control groupH**–hypertensive group (received 60 mg/kg b.w of L-NAME after hypertension was induced), **R**–hypertensive recovery group (was not treated after hypertension was induced), **HM**–hypertensive methanolic group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w. of methanolic extract of PR after hypertension was induced), **HE**–hypertensive ethanolic group (received 60 mg/kg b.w. of L-NAME + 200 mg/kg b.w of ethanolic extract of PR after hypertension was induced), **HA**–hypertensive aqueous group (received 60 mg/kg b.w of L-NAME + 200 mg/kg b.w of aqueous extract of PR after hypertension was induced) and **HR**–hypertensive ramipril group (received 60 mg/kg b.w. of L-NAME + 20 mg/kg b.w. of ramipril after hypertension was induced).
[a] $p < 0.05$ compared to C.
[b] $p < 0.05$ compared to H.

aqueous and methanolic extracts. Cycloheptasiloxane tetradecamethyl, cyclooctasiloxane hexadecamethyl and cyclononasiloxane octadecamethyl are cyclomethicones, which belong to the methyl siloxanes family. Cyclomethicones are widely used for biomedical and cosmetic purposes [38]. Decanoic acid and tartaric acid were identified in the aqueous extract. Tartaric acid is an organic acid that have been found in many fruits specifically grapes and bananas [39]. GS-MC analysis of ethanolic extract showed the presence of caryophyllene and elemenes.

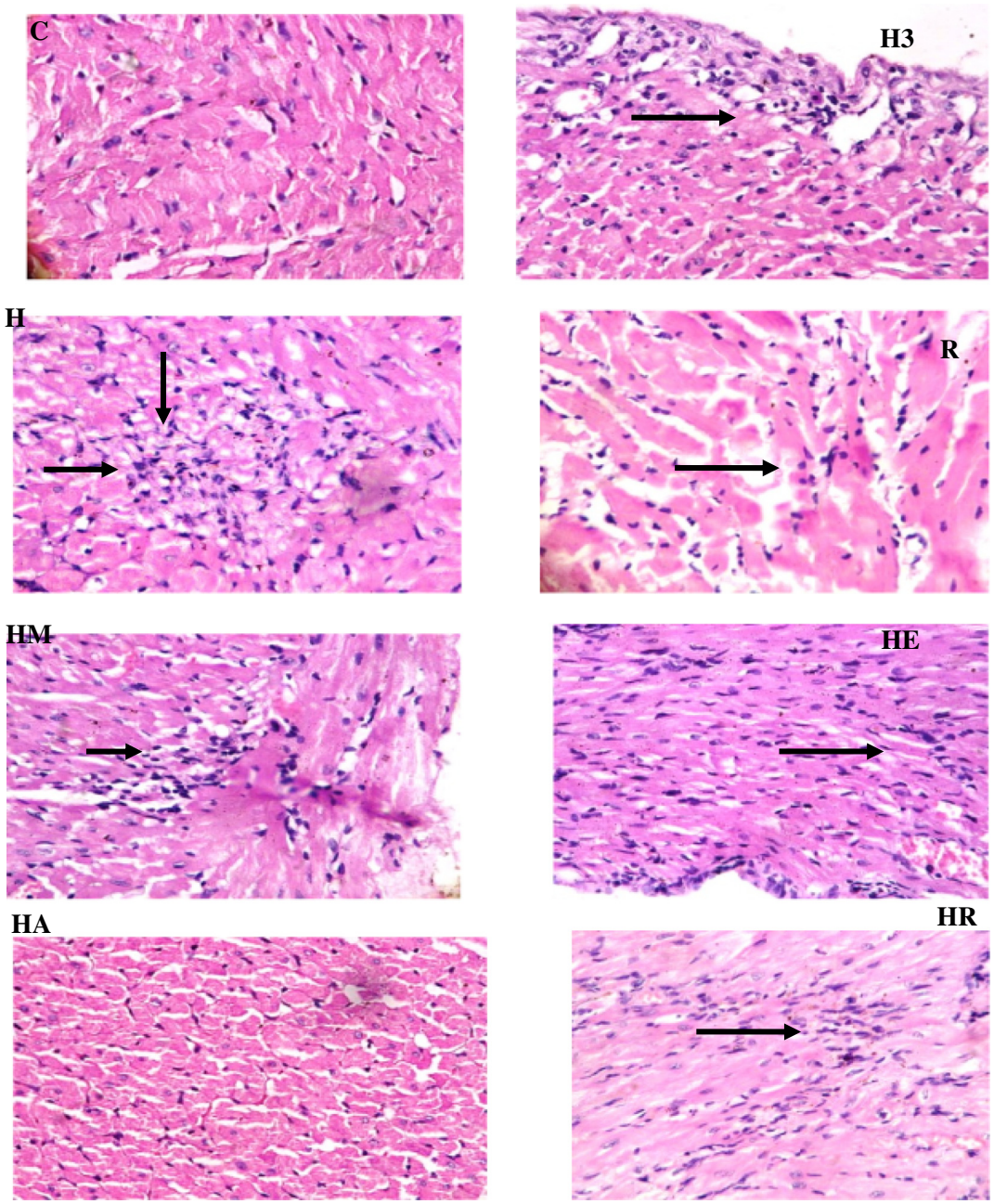

Fig. 7. Photomicrographs of left ventricle of the heart (Mag. ×400, H&E Staining).
Black arrow–shows infiltration of inflammatory cells. **C**–control group, **H3**–hypertension induced (3 weeks), **H**–hypertensive group, **R**–hypertensive recovery group, **HM**–hypertensive methanolic group, **HE**–hypertensive ethanolic group, **HA**–hypertensive aqueous group and **HR**–hypertensive ramipril group.

Caryophyllene and elemenes are sesquiterpene. Caryophyllene is a constituent of many essential oils, specifically oil of cloves, hops, rosemary, copaiba and cannabis. It has been reported to be beneficial in the treatment of colitis, osteoarthritis, diabetes, cerebral ischemia, anxiety as well as depression and liver fibrosis [40]. There is scarcity of reports on phytochemical and biochemical constituents of PR. However, compounds such as coumarin, β-sitosterol, 1-octadecanol, allantoin and oleanolic acid have been reportedly isolated from PR [41], but these were not identified in the present study.

Acute toxicity results showed that oral treatment of the rats with different doses up to 5000 mg/kg of extracts of PR did not cause any sign of toxicity nor death in male Wistar rats. This observation suggests that the lethal dose (LD_{50}) of extracts of PR could be > 5000 mg/kg. Therefore, aqueous, ethanolic and methanolic extracts of PR may be considered to have wide safety margin.

The inhibition of NOS resulted in increased SBP, DBP and MAP in agreement with previous studies [42,43]. The induction of hypertension with the use of non-selective NOS inhibitors is a well-known animal model of hypertension and has been affirmed by several studies [43–45]. The administration of L-NAME in the present study showed a time dependent effect on blood pressure. The blood pressure recorded at the sixth week of the study was elevated than that recorded at the third week of the study. Studies have reported that L-NAME has a dose and time dependent effect on blood pressure [46,47]. In addition, in the hypertensive group that neither received L-NAME nor the treatments after hypertension induction recorded a decrease in blood pressure after three weeks. However, the blood pressure observed was significantly higher than that of the control. This demonstrated that the inhibitory effect of L-NAME on NOS might be reversible; studies have shown that the inhibitory effect of non-selective NOS inhibitors is reversible

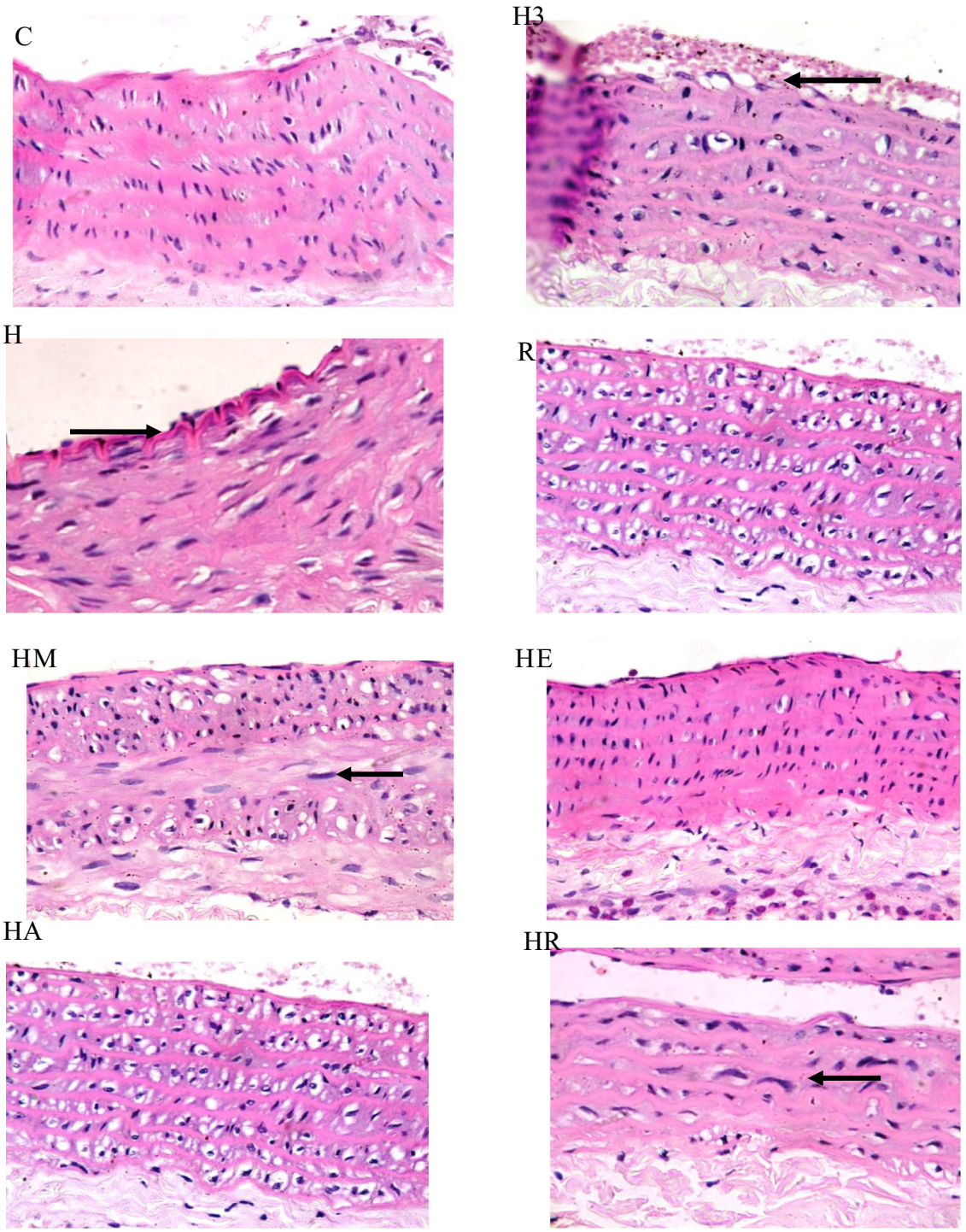

Fig. 8. Photomicrographs of the thoracic aortic sections (Mag. X 400, H&E Staining)
C shows normal aorta architecture**, H3** shows relatively rough surface of tunica intima (Black arrow), **H** shows marked focal thickening of the tunica intima (Black arrow), **R** shows normal aorta architecture, **HM** shows invasion of the tunica media connective tissue by groups of large epithelioid cells (Black arrow), **HE** and **HA** show normal aorta architecture and **HR** shows invasion of the tunica media connective tissue by groups of large epithelioid cells (Black arrow). **C**–control group, **H3**–hypertension induced (3 weeks), **H**–hypertensive group, **R**–recovery group, **HM**- hypertensive methanolic group, **HE**- hypertensive ethanolic group, **HA**- hypertensive aqueous group and **HR**–hypertensive ramipril group.

[48,49].

The methanolic, ethanolic and aqueous extracts of PR cause a reduction in SBP, DBP and MAP. There are paucity of data on the effect of PR on blood pressure, however Zhuang, et al. [13] reported its anti-hypertensive effect in two-kidneys, two-clip hypertensive rats, and Cheng et al. [14] reported its antihypertensive in renal hypertensive and hyperlipidemic rats. The ability of methanolic, ethanolic and aqueous extract of PR leaf to reduce blood pressure might be due to its ability to inhibit RAS. Cheng et al. [14] reported that the administration of PR extract cause a decrease in angiotensin II level. The result of the present study showed that the aqueous extract of PR showed the highest anti-hypertensive effect. The GC–MS analysis of the extracts showed that each extract has some compounds peculiar to it and has more of some compounds than the other extracts, thus it can be implied that the

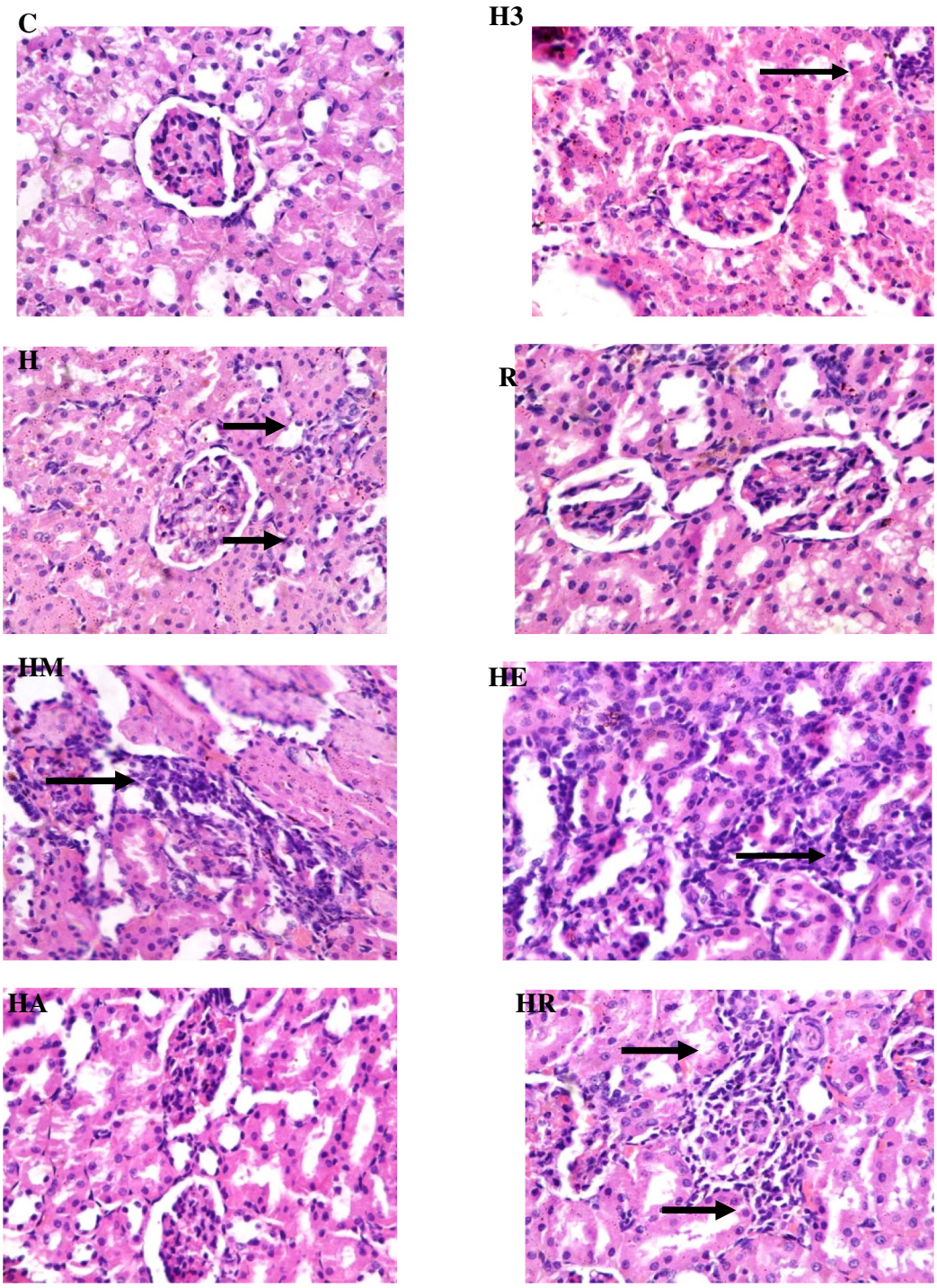

Fig. 9. Photomicrographs of Kidney sections. (Mag. × 400, H&E Staining)
C shows normal kidney, **H3** shows focal areas of lymphoid aggregates (Black arrow), **H** shows architectural anarchy and mild infiltration of inflammatory cells (Black arrows), **R** shows normal kidney, **HM** and **HE** shows focal areas of lymphoid aggregates (Black arrow), **HA** shows normal kidney and **HR** shows infiltration of inflammatory cells (Black arrow). **C**–control group, **H3**–hypertension induced (3 weeks), **H**–hypertensive group, **R**–recovery group, **HM**–hypertensive methanolic group, **HE**–hypertensive ethanolic group, **HA**–hypertensive aqueous group and **HR**–hypertensive ramipril group.

high anti-hypertensive effect of the aqueous extract might be due to one or more of these bioactive compound(s).

Consistent with documented data [45,50], serum NO significantly decreased in the hypertensive group both at the third week of the study when hypertension was induced and at the end of the study. Interestingly, the fall in NO recorded at the establishment of hypertension was restored to normal level after three weeks in the hypertensive recovery group which was not administered with L-NAME or the interventions. This affirmed that L-NAME effect on NOS is reversible.

Hypertensive groups treated with methanolic, ethanolic, and aqueous extracts of PR recorded an increase in serum NO level. This is similar to findings of Zhuang, et al. [13] and Cheng et al. [14] who both reported an increase in NO level in renovascular hypertensive rats. The question arising from this finding is the pathway that stimulated the increase in NO level observed in the hypertensive rats treated with PR extracts, since NOS pathway essential for the production of NO was

inhibited in this study. However, it is possible that the extracts of PR have a strong effect on NOS which is more powerful than the influence of L-NAME or they might have cause the release of NO through a NOS independent pathway. Literature has documented several nitrodilators [51]. Nitrodilators are exogenous compounds which when administered into the body cause the production of NO independent of endothelial NOS [52].

WBC count was significantly high in the hypertensive group and ramipril treated hypertensive group. This was expected in the hypertensive group, NO blockage has been reported to initiate inflammation due to upregulation of leukocyte and endothelial adhesion molecules [53,54]. Zeliha et al. [55] also reported a significant increase in WBC counts in animals administered with L-NAME. Surprisingly, ramipril treated hypertensive rats recorded a high WBC count, however, the differential WBC shows a higher level of lymphocytes and lower level of neutrophils.

In all hypertensive group treated with PR extracts, low level of WBC count were recorded. The fall in WBC count suggest that PR extracts have the ability to correct the inflammatory response initiated by L-NAME administration. Interestingly, the differential white blood cells count also indicated a higher lymphocytes and lower neutrophil level in methanolic and aqueous PR extracts treated hypertensive groups. Neutrophil-to-lymphocyte ratio is regarded as a marker of the body's immune response to offending agents. It is also regarded as a rapid and simple parameter indicative of systemic inflammation and stress. High neutrophil-to-lymphocyte ratio gives an indication of an impaired cell-mediated immunity associated with systemic inflammation [56]. Anti-inflammatory activity has been reported in some compounds isolated from PR leaf [57]. The anti-inflammatory effect observed in this study might due the presence of flavonoid and terpenoid. Flavonoid has been reported to have an anti-inflammatory effect [58]. Terpenoid has also been reported to have an anti-inflammatory and immunomodulatory properties [59,60].

Peristrophe roxburghiana is used as blood tonic in Akwa-ibom State, thus, it was hypothesized that PR extracts will increase RBC count, surprisingly, none of PR extracts used in this study increase RBC count. This suggests that PR does not play a role in RBC production. Conversely, PR extract might have a positive effect on RBC count in anemic condition. Platelet level was actually higher in the recovery group and methanolic extract treated hypertensive rats. NO has been reported to inhibit platelet aggregation [61,62]. Thus, a reduction in NO level might cause platelet to aggregate and in turn the aggregation of platelet might reduce their count. A fall in platelet count has been reported in patients with anticoagulant-dependent (AD) pseudo-thrombocytopenia, a spurious phenomenon due to anticoagulant-induced aggregation of platelets [63]. The high platelet count observed in the methanolic extract treated hypertensive rats, might be due to the ability of this extract to inhibit the aggregation of platelets. As high platelet count was also observed in the recovery group which did not receive any form of treatment after hypertension induction, and a marginal decrease was observed in the hypertensive group. Anti-thrombotic activity of PR extract has been reported in literature [64].

The inhibition of nitric oxide synthase resulted in high TC, TG, LDL-C and a low level of HDL-C. Similarly, a study reported an increase in TC, LDL-C, VLDL-C, and TG levels, and decreased HDL-C concentration in rats [65]. Reduction in nitric oxide bioavailability has been associated with increased lipolysis [8]. Increased lipolysis activity was reported to be associated with elevated TG level and decrease HDL-C concentration [66].

Interestingly, low TG, TC, LDL-C and high HDL-C was observed in the hypertensive group treated with aqueous extract and ethanolic extract of PR. Methanolic extract treated hypertensive group recorded an increase TG, but a decrease in TC and LDL-C. Moreover, a strong increase in HDL-C was recorded. On the positive side, all the extracts caused an increase in HDL-C, which have been reported as protective cholesterol and a reduction in its concentration is an independent predictor of cardiovascular diseases [67]. High serum NO was observed in all PR extracts treated hypertensive groups, thus, the beneficial effect of PR extracts on lipid metabolism might be due to their ability to increase NO, which in turn modulate lipid metabolism. It is also possible that the extracts acted directly on liver or *via* other pathways independent of NO. Study has reported that the administration of NO donors decreases TG and TC, and increases HDL-C [9]. The effect of PR on blood lipid level reported in this study is consistent with study of Cheng and colleagues [14], who reported that PR has a hypolipidemic effect.

The cholesterol lowering effect of ethanolic and methanolic extracts might be due to the sterol content in the extracts as shown in the phytochemical screening. Plant sterols have been reported to reduce blood cholesterol by limiting the absorption of cholesterol in the intestine and consequently enhancing its elimination [68]. However, the phytochemical screening showed that the aqueous extract lack sterol, thus the lipid lowering effect recorded in the hypertensive aqueous group might be due to another substance(s).

Atherogenic ratios reflect the relationship between pro-atherogenic lipoprotein and anti- atherogenic lipoprotein particles [69]. Atherogenic ratios serve as indicators for metabolic disturbances such as hyperlipidemia, atherosclerosis, high blood pressure and cardiovascular diseases [70]. They give a clearer picture of blood cholesterol concentration that shift towards atherogenic hyperlipidemia. Intriguingly, atherogenic indices were significantly low in the entire hypertensive treated groups. Atherogenic indices of the methanolic extract treated hypertensive rats proved that although the extract increases TG, it has an anti-atherogenic activity. In summation, the extracts of PR used in this study have anti-atherogenic ability which might be a useful therapy in cardiovascular disease. The anti-atherogenic effect observed in PR extracts treated hypertensive rats might be due to the presence of flavonoids. Flavonoid has been reported to have antioxidants ability and also have the ability to prevent oxidation of LDL-C, a process involved in atherogenesis [71]. Flavonoid compounds have also been reported to inhibit the lipase activity [72].

MDA level is commonly known as a marker of oxidative stress and the antioxidant status in chronic diseases. It has been hypothesized that oxidative stress is a key player in the pathogenesis of hypertension [73]. Numerous studies have documented that oxidative stress is increased in patients with hypertension. It has been shown that serum lipid peroxides or reactive oxygen species released from isolated vessels are increased in essential hypertensive patients or hypertensive animal models [74]. Surprisingly, in contrast the study observed no significant change in MDA level in the serum and the tissues in the entire studied groups. Furthermore, the activity of CAT in the serum and the tissues was not significant. This may imply that the stress initiated by the administration of L-NAME did not overwhelm the antioxidant systems. Oxidative stress has been reported to occur when the production of free radicals overwhelms antioxidant systems [75]. However, the study by Talas et al. [76] reported a significant increase in MDA level and a decrease in CAT activity in animals that received L-NAME for 15 days. On the other hand, the present study observed a significant increase in serum GSH level in the hypertensive groups treated with aqueous and methanolic extracts of PR, and its level also increase in the aorta of the hypertensive groups treated with aqueous. GSH itself is not an enzyme, but a substrate for the antioxidant reaction catalyzed by glutathione peroxidise in the glutathione system. Glutathione system comprises the enzymes that synthesize glutathione within a cell as well as dedicated enzymes (glutathione peroxidase and glutathione reductase) that use GSH as the means to exert antioxidant effects [77]. The high GSH observed in the study may imply that the aqueous and methanolic extracts of PR stimulated the synthesis of GSH to enhance the antioxidant power of the glutathione system to curtail free radicals. Literature has reported that, in the absence of adequate GSH concentrations, numerous oxidative and nitrosative reactive intermediates persist, including superoxide, hydroxide, peroxide and peroxynitrite radicals, which all can

lead to modification of cellular macromolecules [77,78].

Creatinine is a waste product of creatine and phosphocreatine and urea is a normal waste product that comes from the breakdown of protein [79]. Serum creatinine is regarded as the window into the state of the function and structure of the kidney. An elevated serum creatinine signifies kidney injury.

High serum creatinine was observed in the hypertensive, hypertensive methanolic and hypertensive rampril groups. Similarly, NO blockade had reportedly caused renal lesions, which are characterized by glomerulosclerosis, interstitial fibrosis, and microvascular lesions [80]. Chronic inhibition of NOS by L-NAME causes progressive renal injury with interstitial macrophage infiltration [81]. L-NAME has also been demonstrated to worsen cisplatin-induced nephrotoxicity whereas L-arginine was found to ameliorate the nephrotoxic effect of cisplatin. The protective effect of L-arginine was demonstrated by a reduction in serum creatinine and blood urea nitrogen concentrations [82]. The hypertensive groups treated with aqueous and ethanolic PR extracts recorded a significant decrease in creatinine level. This indicated that aqueous and ethanolic PR extracts might have a beneficial effect on kidney functions.

Study has reported that L-NAME administration resulted in microvascular structural changes with thickening of the media and luminal narrowing [83]. In consistent, the histological examination of aortic tissue showed a relatively rough surface of tunica intima in the hypertensive group sacrificed at the third week of the study. Furthermore, a marked focal thickening of the tunica intima was seen in the hypertensive group at the end of the study. Histological findings of the PR treated groups showed an invasion of the tunica media connective tissue by groups of large epithelioid cells in the methanolic extract treated hypertensive group, while ethanolic and aqueous extract treated hypertensive groups showed a normal tunica intima and tunica media. This implies that the aqueous and ethanolic extracts of PR might have a protective effect on the aorta.

L-NAME has been reported to cause injury to the heart and kidney [84,85]. Similarly, the histological examination of the heart and kidney of the hypertensive group showed infiltration of inflammatory cells in the tissues. Histological examination of cardiac tissue showed a mild infiltration of inflammatory cells in the methanolic extract treated hypertensive group and ethanolic extract treated hypertensive group while in aqueous extract treated hypertensive group showed a normal architecture of the heart. This implies that the aqueous extract might have cardio-protective ability. Histological examination of renal tissue showed focal areas of lymphoid aggregates in the methanolic extract treated hypertensive group and ethanolic extract treated hypertensive group while aqueous extract treated groups showed normal glomeruli, bowman capsule and tubules. The histological report of the aqueous extract of PR is consistent with the creatinine report; this therefore signifies that the aqueous extract of PR might indeed have a renoprotective effect.

5. Conclusion

In all, the extracts of PR leaf used in this study showed good potentials that can be effectively used in the management of high blood pressure and dyslipidemia. However, further studies are still needed to assess their mechanisms of action and the various bioactive constituents identified by GC–MS analysis in order to identify the specific compound responsible for these effects.

Author's contributions

- Esther Oluwasola Aluko designed the work and drafted the manuscript.
- Olumuyiwa Abiola Adejumobi coordinated and measured the blood pressure of the animals
- Adesoji Adedipe Fasanmade revised and approved the final version of the manuscript.

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Declaration of competing interest

Authors declare no conflict of interest.

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