Original Research Article

Reversal of Hypertension and Amelioration of Oxidative Stress by *Persea americana* and *Allium sativum* in Experimentally-Induced Hypertensive Wistar Rats

ABSTRACT

Aim: This study evaluated the antihypertensive and antioxidant effects of hexane, ethyl acetate and methanol extracts of *P. americana* and *A. sativum* in experimentally-induced hypertensive Wistar rats.
Study design: Experimental Research
Place and Duration of Study: Department of Veterinary Pharmacology and Toxicology, University of Ibadan (Animal House), between May 2019 and December 2019.
Methodology: The experiment was carried out in 85 rats randomly divided into 17 groups. Group 1 were normotensive rats while hypertension was induced in groups 2-16 by unilateral nephrectomy and inclusion of NaCl (1%) in drinking water. Group 17 had abdominal incision without nephrectomy (sham). Treatment groups were administered extracts at 20mg/kg or 50mg/kg dose or standard antihypertensives; lisinopril or hydrochlorothiazide.
Results: Results of the experiment showed treatment of hypertensive rats with 50mg/kg of *A. sativum* hexane and *P. americana* methanol extract caused the most significant decrease in blood pressure compared to normotensive rats. Various extracts of these two plants elevated antioxidants levels (GPx, GST, GSH and SOD) in the brain, heart, kidney and liver significantly while H_2O_2 and MDA were significantly decreased compared to untreated hypertensive rats. NO, an important enzyme for normal endothelial function was also restored in the extract-treated rat, as a deficiency contributes greatly to the development of hypertension.
Conclusion: The study concluded that *P. americana* and *A. sativum* do not only lower blood pressure. The plants also inhibited generation of free radicals by enhancing the antioxidant system and mopped up generated free radicals demonstrated by decline in H_2O_2 and MDA levels. These plants have been shown in this study to contain potential drug candidates which can be proposed for treatment of hypertension.

Keywords: [Antihypertensive, antioxidant, Persea americana, *Allium sativum }*

1. INTRODUCTION

Hypertension is a cardiovascular pathology clinically manifested as elevations in systolic and diastolic blood pressure (>130/90mmHg) and commonly called high blood pressure [1]. It is a major non-communicable disease prevalent in adults of every race [2]. High blood pressure has been implicated as a predisposing factor for diabetic, cardiovascular, renal and neurological events [3]. Reactive oxygen species (ROS) play an essential role in controlling biological processes that occur within the endothelium, both in normotensive and hypertensive conditions [4,5,6]. The pathogenesis of hypertension has been closely linked to oxidative stress, demonstrated as elevated ROS generation, nitric oxide (NO) diminution and reduced antioxidants [7].

In clinical or experimentally-induced hypertension, a decline in enzymatic antioxidant activity occurs, implying reduced antioxidant defenses involved in hypertension [8,9). Recent treatment focused on inhibition of ROS production and or enhancement of antioxidant system of hypertensive patients. Rodrigo *et al*, [7] reported that antioxidants as a remedy can limit the pathogenesis of hypertension in experimental animals, although it is still contentious in humans. Therefore,

research efforts have been intensified on natural products with antihypertensive potentials to curtail oxidative stress progression in hypertensive patients.

Natural antioxidants from plants have drawn a lot of research interest lately because they have protective effects against ROS [10]. This study is focused on two medicinal plants with these pharmacological potentials. *Persea americana* (Family Lauraceae), commonly called avocado, is a medicinal plant with cardioprotective, hypolipidemic and hypoglycemic effects [11]. Also, *Allium sativum* (Family Liliaceae), a common flavoring and spice herb, is known to be antidiabetic, hypolipidemic, anticancer, antioxidant and antihypertensive [12,13,14,15]. Borek [16] inferred that *A. sativum* deterred low density lipoprotein oxidation, atherosclerosis, thrombocyte aggregation, decreased homocysteine, hypotension and increased microcirculation particularly in diabetics and patients at risk of dementia.

In this study, the antihypertensive properties of several extracts of *P. americana* and *A. sativum* as well as their ability to mitigate against oxidative stress in the brain, heart, kidney and liver of experimentally-induced hypertensive Wistar rat was assessed.

2. MATERIAL AND METHODS

2.1. Preparation of Extracts

Persea americana fruit and *Allium sativum* bulb were purchased in Oshodi, Lagos State in May 2019. Seeds of the plants were harvested, cut up into small sizes and air dried. Solvent-partitioned extraction was done for each plant using hexane, ethyl acetate, and methanol by cold maceration for 72 hours in each solvent. The filtrate was concentrated using a rotary evaporator (BUCHI R-210, Switzerland). The remaining moisture was removed over a 30°C water bath, and the six different extracts obtained was refrigerated till use.

2.2. Experimental Animals

Male Wistar rats (n=83, 6-8 weeks, 120-150g) used during the experiments were accommodated at the animal house of Department of Veterinary Pharmacology and Toxicology, University of Ibadan under a 12/12 hour light/dark natural cycle and fed ad libitum. The animals were adapted to their new environment for 7 days prior to treatment commencement. The rats were distributed randomly to seventeen groups (n=17), consisting five (5) rats per group. Group 1 represented the normotensive control and administered only water. Groups of 2 to 16 were nephrectomized, given 1% NaCl for twelve hours, followed by plain water for twelve hours, and fed daily for 42 days. Groups 2 and 3 were administered lisinopril or hydrochlorothiazide. Groups 4 – 16 were administered the six different extracts of *P. americana* or *A. sativum* at 20mg/kg or 50mg/kg. Abdominal incisions were made on group 17 rats and closed without nephrectomy (sham) and given plain water daily.

2.3. Sample Collection

Tail plethysmography was used to measure blood pressure parameters such as systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MAP) in conscious animals. The average of at least nine readings of blood pressure were taken in quiescent state per animal using an automated blood pressure monitor (CODA S1, Kent Scientific Corporation, Connecticut, USA). Blood samples were taken using heparinized capillary tube and centrifuged at 4000rpm for 10 minutes and immediately stored at -4^{o}C . The rats were afterwards humanely sacrificed. Brain, heart, kidney and liver samples were collected into sample bottles and frozen at -180C until analysed. Tissue samples were centrifuged at 1000rpm and also frozen down. Levels of antioxidants (GPx, GSH, GST, SOD and NO) and products of oxidative stress (MDA and H_2O_2) were determined by standard methods [17].

2.4 Statistical Analysis

Descriptive statistical analysis was used to present the results of this study. The data were reported as mean ± standard deviation. The descriptive data was subjected to one-way analysis of variance (ANOVA) with Turkey's post-hoc test for analysis using Graph Pad Prism version 6.00 and the statistical significance was determined (P = .05).

3. RESULTS

3.1. Blood Pressure

Blood pressure parameters including systolic, diastolic and Mean Arterial Pressure (MAP) were significantly higher in untreated hypertensive rats which were reversed in rats administered 50mg/kg hexane and methanol extracts of *Allium sativum* (As) (103.5/67.5mm/Hg, MAP 90.805mm/Hg; 137.25/115.31mmHg, MAP 110.185mm/Hg) and methanol extract of *Persea americana* (Pa) (50mg/kg - 135/109mm/Hg and MAP of 114.335mm/Hg) when compared to the standard antihypertensive drugs (Lisinopril, Hydrochlorothiazide) and normotensive group (Table 1).

3.2. Anti-oxidant Levels

Glutathione peroxidase (GPx) level was increased in heart, brain, kidney and liver tissues of Wistar extract treated hypertensive rats. GPx levels were highest in As ethyl acetate extracts treated rat brains (295.01±17.02 and 282.35±42.57), Pa methanol 50mg/kg treated rat heart (290.49±39), kidneys of rat treated with Pa ethyl acetate 50mg/kg (225.30±54.84) and liver of rats treated with As methanol extract (67.55±15.77 and 64.13±9.74). GPx levels of untreated hypertensive group was decreased in all the organs compared to the physiologically normal and treated hypertensive rats (Table 2).

A marginal decrease in GSH levels was observed in all the organs of untreated hypertensive rats but was reversed in rats administered the extract compared to normotensive rats. GSH levels were highest in the brain and heart of rats administered with Pa, while it was highest in kidneys and liver of rats administered As (Table 3). Glutathione-s-transferase level which was significantly decreased in the heart, kidney and liver of untreated hypertensive Wistar rats were reversed by various extracts of *P. americana* and *A. sativum.* Highest GST levels were found in rats treated with Pa methanol 20mg/kg (brain), As ethyl acetate 50mg/kg (heart and liver) and Pa ethyl acetate 50mg/kg (kidney) (Table 4).

Superoxide dismutase (SOD) was significantly higher in the brain, heart, kidney and liver of most extract treated rats compared to untreated hypertensive rats. Normotensive rats had lower SOD levels when matched to the extract-treated rats, with a significant ($p<0.05$) decline compared to brain SOD of rats treated with As ethyl acetate 20mg/kg. However, SOD levels in the kidney of all rats treated with As were significantly declined compared to untreated hypertensive rats (Table 5). Declined nitric oxide (NO) proportions in the brain, heart, kidney and liver tissues of untreated hypertensive rats were restored in extract-treated hypertensive Wistar rats compared to rats with normal BP (Table 6).

3.3. Products of Oxidative Stress

In untreated hypertensive rats, there was increased generation of hydrogen peroxide (H_2O_2) in all tissues compared to rats with normal BP. However, this was reversed in extract-treated hypertensive rats. However, significant increase was recorded in brains of rats treated with As methanol extracts (131.65±53.03 and 177.48±18.56) (Table 7). Malondialdehyde (MDA) levels in all the tissues were consistently lowered by all the extracts doses compared to rats with normal BP. Untreated hypertensive rats had significantly ($P= .05$) higher MDA levels in all the tissues compared to normotensive rats (Table 8).

3.4 Discussion

Pathogenesis and progression of hypertension is now known to involve oxidative stress damage at the micro- and macromolecular levels in the cardiovascular system [18,19]. Treatment should therefore focus on mitigating or reversing oxidative damage as well as lowering of blood pressure (BP), evidenced clinically as a return to normotensive state [20]. This study demonstrated the antihypertensive abilities of *P. americana* and *A. sativum* extracts by significant decline in blood pressure parameters with a return to the normotensive state, particularly at 50mg/kg dose of *P. americana* methanol extract and *A. sativum* hexane extract.

The antihypertensive mechanism of *P. americana* was previously reported to be similar to that of ACE inhibitors with decreased resorption of fluid from the distal convoluted tubule, resulting in natriuresis and decreased plasma volume in high salt-fed Wistar rats [21]. Similarly, an earlier report stated that *A. sativum* lowered BP by stimulating production of vascular gasotransmitter H_2S and enhancing nitric oxide regulation leading to smooth muscle cell relaxation, dilatation and lowered BP [22]. This current study has further fractionated these medicinal plants with evidence of the antihypertensive property traceable primarily to methanol extract for *P. americana* and hexane extract for *A. sativum*.

Furthermore, this study established that these extracts enhanced the antioxidant system (GST, GPx, GSH, SOD) and reduced generation of oxidation products (H_2O_2, MDA) in the brain, heart, kidney and liver of hypertensive rats treated with the extracts. Paravicini *et al*. [6] established the function of oxidative stress in the pathophysiology of high blood pressure, demonstrated as promotion of reactive oxygen species (ROS) production alongside a decline in the bioavailability of NO and antioxidants. ROS are generated by activated NADPH oxidase (Nox), a family of enzymes which catalyze electron transfer to oxygen molecules thereby generating superoxides or H_2O_2 [23]. The endothelial and nephron are unique sources of Nox-derived ROS, playing a crucial role in vascular and renal pathologies as observed in hypertension [24]. Nox functions by donating an electron, therefore, catalyzing oxygen reduction via NADPH, increasing superoxide production and up-regulation of NADPH oxidase in hypertensive states [25]. The primary role of NADPH oxidase-derived superoxide is to inhibit NO in the chemical processes producing peroxynitrite, resulting in dysfunctional endothelial-dependent dilatation [26].

An equilibrium between generated ROS and available antioxidants must be achieved to attain physiological status. Endogenous or exogenous antioxidants are needed to mop up free radicals during oxidative stress [27]. The correlation between oxidative stress and hypertension was expressed in this study with significantly depleted antioxidants and elevated products of oxidation in untreated hypertensive rats, nevertheless was overturned in the extract-treated rats. GPx, SOD and catalase are involved with direct elimination of ROS, while GSH and GST eliminate ROS by decreasing peroxide levels [28]. Gómez-Marcos *et al*. [29] reported a negative correlation between serum SOD and vascular damage in hypertensive patients and diabetic patients which had significant accumulation of superoxide anions leading to vascular impairment caused by ROS. Chrissobolis *et al*. [30] also agreed that SOD, GPx, and catalase protect blood vessels against homocycsteine, which caused oxidative stress in vasculature. These antioxidants were increased in hypertension-

induced Wistar rats treated with extracts of *P. americana* and *A. sativum*, indicating the antioxidant potentials of these plants with the ability to eliminate generated and inhibit further generation of ROS in hypertensive patients.

Oxidative damage has a cyclical effect of cellular damage with increasing ROS generation. Superoxide production is enhanced via Nox activation and/or H_20_2-induced endothelial NO synthase (NOS) uncoupling. This triggers endothelial dysfunction following rapid oxidative inactivation of NO by excess superoxide production and the cycle is repeated [31]. In this study, *P. americana* and *A. sativum* inhibited NO depletion which is essentially required for normal endothelial function. There are three isoforms of NOS and they are; inducible NOS (iNOS), endothelial NOS (eNOS) and neuronal NOS (nNOS) with the latter being atheroprotective while (iNOS) is proatherogenic [32]. Hypertensive patients have been reported to have reduced NO levels coupled with impairment of endothelium-dependent vasodilation [19]. Deficiencies in the production of nitric oxide pathway may lead to development of essential hypertension [33].

MDA is one of the important biomarkers for assessing oxidative stress leading to lipid peroxidation. It is a byproduct of polyunsaturated fatty acid peroxidation, as it increases during cellular injury, as well as oxidative damage to other lipid containing molecules [34]. Ayala *et al*. [35] described lipid peroxidation as a chain reaction occurring during oxidative stress with the formation of various reactive compounds which cause cellular damage. MDA levels is reportedly higher in serum of hypertensive patients [36], a feature observed in this study and was reversed by the extracts. Elevated MDA and H_2O_2 levels in the untreated group might be attributed to enhanced lipid peroxidation activity and superoxide generation [37,38].

Table 1: Blood Pressure in experimentally-induced hypertensive Wistar rats treated with solvent-partitioned *Persea americana* or *Allium sativum* extract

Group	SYSTOLIC	DIASTOLIC	MAP
Control	112.55±18.45[a]	100.44±20.99	104.42±9.22
UT	158.40±21.28	151.00±20.36	153.20±19.02
LIS	148.20±27.94	123.20±27.12	125.20±29.80
Hydr	132.00±17.38	94.25±15.61	107.43±24.10
Pa Hex 20	143.73±16.01	110.00±15.61	118.47±15.29
Pa Hex 50	206.87±37.21[a]	159.48±35.02	177.10±30.94
Pa EA 20	216.00±26.91[a]	176.67±50.82	185.13±57.86
Pa EA 50	148.00±27.16	123.00±23.99	135.40±21.71
Pa Meth 20	150.83±20.93	115.19±21.41	123.62±25.74
Pa Meth 50	135.00±22.63	109.00±22.63	114.33±16.65
As Hex 20	152.75±19.61	105.81±36.82	133.41±27.19
As Hex 50	103.50±38.49	67.5±19.07	90.80±33.77
As EA 20	153.20±40.39	105.23±38.08	113.08±35.39
As EA 50	148.18±37.18	103.14±30.06	111.67±40.86
As Meth 20	148.75±12.12	113.40±31.19	137.00±26.26
As Meth 50	137.25±29.27	115.31±35.49	110.18±30.09
Sham	115.00±15.60	102.41±43.37	104.00±31.10

UT- Untreated Hypertensive; LIS – Lisinopril; Hydro- Hydrochlorothiazide; Pa Hexane- *P. americana* Hexane extract; Pa EA- *P. americana* Ethyl acetate extract; Pa Meth- *P. americana* Methanol extract; As Hex- *Allium sativum* Hexane extract; As EA- *Allium sativum* Ethyl acetate extract; As Meth- *Allium sativum* Methanol extract.

Values with the same superscript alphabets as control or untreated hypertensives are statistically significant at *P*= .05

Table 2: Glutathione peroxidase in experimentally-induced hypertensive Wistar rats treated with solvent-partitioned *Persea americana* or *Allium sativum* extracts

Group	Brain	Heart	Kidney	Liver
Control	200.01±39.15	219.89±24.99	172.44±55.82	50.72±4.50
UT	177.21±19.75	198.37±5.13	155.34±7.97	46.34±2.46
LIS	244.51±8.46	292.41±11.56	168.18±5.72	61.90±1.77
Hydr	216.95±17.73	275.54±72.18	186.35±83.36	49.38±1.80
Pa Hex 20	235.47±83.47	231.29±39.49	183.30±16.49	59.71±7.10
Pa Hex 50	234.07±79.25	269.67±37.95	190.57±63.08	56.15±11.85
Pa EA 20	214.29±56.20	252.55±48.00	211.30±59.10	52.21±1.17
Pa EA 50	218.31±63.11	264.55±40.30	225.30±54.84	53.00±1.83
Pa Meth 20	278.11±36.50	233.92±49.05	110.36±6.65	47.63±1.05
Pa Meth 50	202.36±23.88	290.49±39.57	112.80±3.35	56.25±12.56
As Hex 20	254.29±38.00	262.76±40.81	104.64±6.72	56.63±10.27
As Hex 50	238.46±56.26	240.49±50.24	97.38±2.63	53.85±4.89
As EA 20	295.01±17.02	248.59±37.07	101.19±8.85	60.66±10.99
As EA 50	282.35±42.57	245.56±32.73	98.10±11.49	68.12±21.83
As Meth 20	224.35±42.57	248.74±36.87	102.04±17.78	67.55±15.77
As Meth 50	236.26±34.15	227.53±30.83	62.31±13.33	64.13±9.74
Sham	201.27±0.13	216.27±20.23	172.15±23.56	49.86±4.57

UT- Untreated Hypertensive; LIS – Lisinopril; Hydro- Hydrochlorothiazide; Pa Hexane- *P. americana* Hexane extract; Pa EA- *P. americana* Ethyl acetate extract; Pa Meth- *P. americana* Methanol extract; As Hex- *Allium sativum* Hexane extract; As EA- *Allium sativum* Ethyl acetate extract; As Meth- *Allium sativum* Methanol extract.

Values with the same superscript alphabets as control or untreated hypertensives are statistically significant at *P*= .05

Table 3: Reduced glutathione levels in experimentally-induced hypertensive Wistar rats treated with solvent-partitioned *Persea americana* or *Allium sativum* extract

Group	Brain	Heart	Kidney	Liver
Control	96.13±7.82	107.85±8.07	105.58±5.96	71.84±0.66
UT	85.99±1.63	100.70±0.75	101.87±0.30	69.37±1.32
LIS	88.68±3.31	109.82±6.96	106.84±4.46	75.06±2.55
Hydr	99.13±29.18	110.05±12.85	110.22±4.69	74.92±3.26
Pa Hex 20	101.62±25.36	113.05±14.46	108.82±6.14	79.20±10.94
Pa Hex 50	101.23±15.04	115.94±13.74	106.94±2.52	83.70±11.26
Pa EA 20	99.59±12.60	115.86±15.33	107.19±7.89	77.56±7.95
Pa EA 50	96.98±15.67	116.84±7.99	106.87±4.51	77.92±3.02
Pa Meth 20	102.51±15.77	109.96±14.74	115.54±16.71	85.06±17.49
Pa Meth 50	87.72±3.76	98.04±1.81	112.20±17.38	76.02±4.47

As Hex 20	88.89±2.11	94.78±0.54	111.38±14.15	82.27±10.67
As Hex 50	84.85±1.51	98.58±1.05	106.72±9.61	81.61±6.07
As EA 20	85.28±4.51	101.98±5.57	118.89±18.03	88.10±8.67
As EA 50	80.88±1.28	102.19±4.97	110.90±15.17	85.23±7.51
As Meth 20	93.26±2.86	99.00±1.35	120.27±11.95	86.23±21.68
As Meth 50	82.72±4.84	108.26±5.42	111.02±9.39	83.24±6.61
Sham	96.30±1.81	107.19±2.41	105.28±0.60	72.66±1.23

UT- Untreated Hypertensive; LIS – Lisinopril; Hydro- Hydrochlorothiazide; Pa Hexane- *P. americana* Hexane extract; Pa EA- *P. americana* Ethyl acetate extract; Pa Meth- *P. americana* Methanol extract; As Hex- *Allium sativum* Hexane extract; As EA- *Allium sativum* Ethyl acetate extract; As Meth- *Allium sativum* Methanol extract.

Values with the same superscript alphabets as control or untreated hypertensives are statistically significant at *P*= .05

Table 4: Gluthathione-s-transferase levels in experimentally-induced hypertensive Wistar rats treated with solvent-partitioned *Persea americana* or *Allium sativum* extracts

Group	Brain	Heart	Kidney	Liver
Control	7.50±0.86	2.59±0.22[a]	3.63±1.29[a]	3.92±0.79[a]
UT	6.93±0.32	1.75±0.01[b]	2.80±0.05[b]	3.30±0.25[b]
LIS	8.93±1.50	3.08±0.53	4.01±0.59	5.08±0.34
Hydr	8.05±2.80	3.09±0.07	3.96±0.08	4.02±0.09
Pa Hex 20	8.85±1.20	2.66±0.18	3.91±0.45	5.80±1.21[b]
Pa Hex 50	7.48±1.12	2.79±0.34	6.72±2.41[ab]	4.97±0.41
Pa EA 20	8.61±0.64	2.83±0.09	7.03±0.93[ab]	4.47±0.36
Pa EA 50	8.01±0.40	2.46±0.06	7.06±1.22[ab]	4.88±0.50
Pa Meth 20	9.72±1.42	2.30±0.28	2.04±0.17	4.84±0.43
Pa Meth 50	8.17±4.67	1.17±0.41	2.05±0.33	5.43±0.79
As Hex 20	3.64±1.58	3.21±0.34	1.55±0.33	7.20±0.58[ab]
As Hex 50	2.89±0.35	1.02±0.44	1.32±0.28	5.66±0.53[b]
As EA 20	3.73±0.97	1.14±1.21	3.20±0.36	4.99±0.70
As EA 50	4.19±0.72	6.46±2.18[ab]	0.52±0.09[a]	9.53±1.89[ab]
As Meth 20	3.61±0.41	4.34±1.98	0.39±0.16[a]	5.53±0.82
As Meth 50	4.23±1.38	5.20±3.51	0.43±0.13[a]	4.83±0.52
Sham	7.31±0.34	3.40±0.31	3.53±0.01	4.28±0.38

UT- Untreated Hypertensive; LIS – Lisinopril; Hydro- Hydrochlorothiazide; Pa Hexane- *P. americana* Hexane extract; Pa EA- *P. americana* Ethyl acetate extract; Pa Meth- *P. americana* Methanol extract; As Hex- *Allium sativum* Hexane extract; As EA- *Allium sativum* Ethyl acetate extract; As Meth- *Allium sativum* Methanol extract.

Values with the same superscript alphabets as control or untreated hypertensives are statistically significant at *P*= .05

Table 5: Superoxide dismutase levels in experimentally-induced hypertensive Wistar rats treated with solvent-partitioned *Persea americana* or *Allium sativum* extract

Group	Brain	Heart	Kidney	Liver
Control	6.82±1.30[a]	7.63±3.07	7.16±2.44	1.92±0.12
UT	5.98±0.65[b]	6.84±0.29	5.77±0.13	1.69±0.05
LIS	8.26±0.26[ab]	10.04±0.49	5.84±0.07	2.33±0.13
Hydr	7.28±0.60	9.51±3.69	8.55±2.29	1.80±0.08
Pa Hex 20	8.54±2.71	9.52±2.44	6.89±0.59	2.25±0.16
Pa Hex 50	7.92±2.70	9.60±1.48	9.47±3.41	2.09±0.36
Pa EA 20	7.26±1.91	9.55±2.06	8.97±1.25	1.86±0.02
Pa EA 50	7.41±2.08	8.60±2.49	9.08±1.31	2.02±0.09
Pa Meth 20	9.59±1.28	8.92±2.60	3.68±0.26	1.58±0.03
Pa Meth 50	7.31±0.68	10.21±1.34	4.27±0.24	2.27±0.24
As Hex 20	8.57±1.39	10.39±3.04	3.35±0.33	2.11±0.12
As Hex 50	8.14±2.28	12.33±2.43	3.32±0.14	1.98±0.07
As EA 20	10.13±0.81	9.47±2.72	3.49±0.16	2.22±0.28
As EA 50	9.75±0.65	9.87±1.79	3.28±0.39	2.56±0.68
As Meth 20	7.65±1.61	8.62±2.23	3.51±0.72	2.38±0.48
As Meth 50	8.16±1.03	7.90±2.09	2.17±0.50	2.32±0.26
Sham	6.87±0.17	7.66±0.77	7.19±5.48	1.91±0.56

UT- Untreated Hypertensive; LIS – Lisinopril; Hydro- Hydrochlorothiazide; Pa Hexane- *P. americana* Hexane extract; Pa EA- *P. americana* Ethyl acetate extract; Pa Meth- *P. americana* Methanol extract; As Hex- *Allium sativum* Hexane extract; As EA- *Allium sativum* Ethyl acetate extract; As Meth- *Allium sativum* Methanol extract.

Values with the same superscript alphabets as control or untreated hypertensives are statistically significant at *P*= .05

Table 6: Nitric oxide levels in experimentally-induced hypertensive Wistar rats treated with solvent-partitioned *Persea americana* or *Allium sativum* extract

Group	Brain	Heart	Kidney	Liver
Control	0.75±0.37[a]	0.51±0.14	0.98±0.28	3.01±0.57
UT	0.59±0.06[b]	0.37±0.10	0.76±0.04	2.50±0.42
LIS	0.68±0.17	0.54±0.09	0.93±0.12	4.46±1.00
Hydr	2.28±0.79[ab]	0.80±0.42	1.70±0.61	3.19±1.19
Pa Hex 20	1.45±0.66	0.39±0.07	1.21±0.15	3.61±0.97
Pa Hex 50	0.99±0.42	0.67±0.29	1.36±0.61	3.29±1.04
Pa EA 20	1.16±0.44	0.81±0.33	1.41±0.59	2.70±0.23
Pa EA 50	0.69±0.30	0.56±0.15	1.00±0.42	2.95±1.05
Pa Meth 20	0.53±0.07	0.52±0.06	1.55±0.69	4.08±1.20
Pa Meth 50	0.76±0.12	0.49±0.09	1.20±0.27	3.68±1.21
As Hex 20	0.83±0.24	0.45±0.08	1.02±0.12	3.95±1.26
As Hex 50	0.80±0.32	0.45±0.04	1.81±0.81	5.22±1.22

As EA 20	0.41±0.04	0.52±0.12	1.25±0.36	3.20±0.93
As EA 50	0.74±0.32	0.56±0.08	0.98±0.04	3.60±1.54
As Meth 20	0.74±0.32	0.53±0.10	1.55±0.66	3.98±1.19
As Meth 50	0.64±0.06	0.53±0.11	1.21±0.16	3.22±0.71
Sham	0.74±0.17	0.52±0.37	1.05±0.01	3.05±0.96

UT- Untreated Hypertensive; LIS – Lisinopril; Hydro- Hydrochlorothiazide; Pa Hexane- *P. americana* Hexane extract; Pa EA- *P. americana* Ethyl acetate extract; Pa Meth- *P. americana* Methanol extract; As Hex- *Allium sativum* Hexane extract; As EA- *Allium sativum* Ethyl acetate extract; As Meth- *Allium sativum* Methanol extract.

Values with the same superscript alphabets as control or untreated hypertensives are statistically significant at *P*= .05

Table 7: Hydrogen peroxide levels in experimentally-induced hypertensive Wistar rats treated with solvent-partitioned *Persea americana* or *Allium sativum* extract

Group	Brain	Heart	Kidney	Liver
Control	75.26±18.13	115.77±49.40[a]	40.75±5.96[a]	97.39±23.26
UT	107.52±44.53[b]	157.38±28.78[b]	46.93±5.04[b]	114.93±16.70[b]
LIS	29.36±3.05[b]	36.09±4.91[ab]	31.23±2.65[b]	56.93±5.57[b]
Hydr	56.07±19.59	88.94±19.08[b]	44.25±1.03	69.15±17.38
Pa Hex 20	60.55±23.30	82.41±18.02[b]	33.63±1.03[b]	66.69±12.76
Pa Hex 50	56.28±7.41	87.79±12.82[b]	34.88±4.27	83.32±25.64
Pa EA 20	55.40±4.87	77.38±17.53[b]	38.84±3.39	88.84±1.92
Pa EA 50	34.57±7.96[b]	92.90±9.72[b]	34.54±5.25	63.06±12.90
Pa Meth 20	55.82±2.95	30.40±5.68[ab]	35.35±4.15	74.98±23.54
Pa Meth 50	87.20±30.49	28.39±5.27[ab]	26.28±3.81[ab]	59.88±3.09
As Hex 20	69.98±27.70	36.23±7.31[ab]	32.55±3.95[b]	87.40±25.90
As Hex 50	69.88±19.30	32.04±4.60[ab]	31.02±1.98[b]	97.69±1.46
As EA 20	61.44±18.27	37.61±9.35[ab]	37.69±2.67	77.83±8.31
As EA 50	90.19±10.90	36.83±7.30[ab]	33.02±4.54[b]	88.66±7.63
As Meth 20	131.65±53.03	34.46±7.51[ab]	28.65±4.77[b]	75.05±21.69
As Meth 50	177.48±18.56	35.14±8.18[ab]	33.88±2.36[b]	67.22±13.71
Sham	76.88±1.92	144.77±28.87	38.63±6.92	96.30±43.34

UT- Untreated Hypertensive; LIS – Lisinopril; Hydro- Hydrochlorothiazide; Pa Hexane- *P. americana* Hexane extract; Pa EA- *P. americana* Ethyl acetate extract; Pa Meth- *P. americana* Methanol extract; As Hex- *Allium sativum* Hexane extract; As EA- *Allium sativum* Ethyl acetate extract; As Meth- *Allium sativum* Methanol extract.

Values with the same superscript alphabets as control or untreated hypertensives are statistically significant at *P*= .05

Table 8: Malondialdehyde levels in experimentally-induced hypertensive Wistar rats treated with solvent-partitioned *Persea americana* or *Allium sativum* extract

Group	Brain	Heart	Kidney	Liver
Control	3.15±1.15	1.32±0.18	2.02±0.75[a]	2.18±0.40[a]
UT	5.46±1.72[b]	1.69±0.17[b]	2.96±0.66[b]	3.00±0.43[b]
LIS	0.86±0.30[b]	0.76±0.16[b]	0.31±0.13[ab]	1.60±0.35[b]
Hydr	1.55±0.50[b]	0.67±0.03[b]	1.33±0.65[b]	1.81±0.22[b]
Pa Hex 20	2.31±0.92[b]	0.84±0.37[b]	0.99±0.22[b]	1.83±0.15[b]
Pa Hex 50	2.03±0.79[b]	1.64±0.08	1.38±0.30[b]	1.02±0.37[ab]
Pa EA 20	2.76±0.40[b]	1.12±0.04	1.21±0.19[b]	0.21±0.07[ab]
Pa EA 50	1.69±0.61[b]	1.03±0.28	1.74±0.10[b]	1.85±0.12[b]
Pa Meth 20	1.82±0.26[b]	1.00±0.70	0.91±0.12[ab]	1.20±0.51[b]
Pa Meth 50	2.28±0.65[b]	0.87±0.16[b]	0.87±0.27[ab]	1.76±0.34[b]
As Hex 20	2.47±0.66[b]	0.80±0.12[b]	0.41±0.06[ab]	1.67±0.16[b]
As Hex 50	2.03±0.76[b]	1.10±0.23	0.42±0.11[ab]	0.96±0.45[ab]
As EA 20	2.30±0.63[b]	0.65±0.01[b]	0.66±0.13[ab]	0.72±0.19[ab]
As EA 50	1.72±0.61[b]	1.73±0.27	0.48±0.19[ab]	1.52±0.11[b]
As Meth 20	2.75±1.11[b]	1.30±0.04	0.46±0.13[ab]	1.61±0.74[b]
As Meth 50	2.54±0.79[b]	1.26±0.04	0.47±0.17[ab]	1.63±0.26[b]
Sham	3.17±1.37	1.37±0.44	2.31±0.35	2.16±0.27

UT- Untreated Hypertensive; LIS – Lisinopril; Hydro- Hydrochlorothiazide; Pa Hexane- *P. americana* Hexane extract; Pa EA- *P. americana* Ethyl acetate extract; Pa Meth- *P. americana* Methanol extract; As Hex- *Allium sativum* Hexane extract; As EA- *Allium sativum* Ethyl acetate extract; As Meth- *Allium sativum* Methanol extract.

Values with the same superscript alphabets as control or untreated hypertensives are statistically significant at *P*= .05

4. CONCLUSION

In conclusion, the antihypertensive effect of *P. americana* and *A. sativum* was not limited to the blood pressure lowering effects, remarkably at 50mg/kg doses of *P. americana* methanol and *A. sativum* hexane extract. These extracts also enhanced the antioxidant systems and inhibited oxidative stress in hypertensive subjects. Further study of these extracts as drug candidates for management of hypertension is thus warranted.

CONSENT (WHERE EVER APPLICABLE)

Not applicable

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of the University of Ibadan.

REFERENCES

1. Anonymous. Hypertension. World Health Organization (WHO), 2021. Accessed 25 August, 2021. Available: https//www.who.int/news-room/fact-sheets/detail/hypertension.
2. Saba AB, Oridupa OA. Lipoproteins and cardiovascular diseases. In: Lipoproteins - Role in Health and Diseases, S. Frank and G. Kostner (editors). Published by In-Tech Open Access, Rijeka, Croatia. 2012; Chapter 8;197–222. Available:http://www.intechopen.com/articles/show/title/lipoproteins-and-cardiovascular-diseases ISBN 978-953-51-0773-6
3. Verloop WL, Voskuil M, Doevendans PA. Renal denervation: a new treatment option in resistant arterial hypertension. Neth Heart J. 2013;21(2):95-98. doi: 10.1007/s124471-012-0357-8
4. Rodrigo R, Passalacqua W, Araya J, Orellana M. and Rivera G. Homocysteine and essential hypertension. J Clin Pharmacol. 2003;43:1299–1306.
5. Lassègue B, Griendling K. Reactive oxygen species in hypertension, an update. Am J Hypertens. 2004;17:852–860.
6. Paravicini TM, Touyz RM. Redox signalling in hypertension. Cardiovasc Res. 2006; 71:247–258
7. Rodrigo R, González J, Paoletto F. The role of oxidative stress in the pathophysiology of hypertension. Hypertens Res. 2011;34:431–440. https://doi.org/10.1038/hr.2010.264
8. Lee SK, Arunkumar S, Sirajudeen KN, Singh HJ. Glutathione system in young spontaneously hypertensive rats. J Physiol Biochem. 2010;66:321–327.
9. Zhan CD, Sindhu RK, Pang J, Ehdaie A, Vaziri ND. Superoxide dismutase, catalase and glutathione peroxidase in the spontaneously hypertensive rat kidney: effect of antioxidant-rich diet. J Hypertens. 2004;22:2025–2033.
10. Gulcin İ. Antioxidants and antioxidant methods: an updated overview. Arch Toxicol. 2020;94**:**651–715. https://doi.org/10.1007/s00204-020-02689-3
11. Ojewole J, Kamadjaapa GMM, Moodley K, Musabayane CT. Cardiovascular effects of *Persea americana* Mill (Lauracéae) aqueous leaf extract in experimental animals. Cardiovasc J S Afr. 2007;18:69–76.
12. Nwokocha CR, Ozolua RI, Owu DU, Nwokocha MI, Ugwu AC. Antihypertensive properties of *Allium sativum* (garlic) on normotensive and two kidney one clip hypertensive rats. Niger J Physiol Sci. 2011; 26:213–8
13. Ranjani R, Ayya Raju M. Anticancer properties of *Allium sativum*—Review. Asian J Biochem Pharm Res. 2012;3(2):191–6.
14. Goswami S, Verma RK, Tripathi P, Singh AP, Ojha G, Rai M. A review on Hypoglycemic, Hypolipidemic and Anti-obesity effect of Allium sativum. J Chem Pharmaceut Sci. 2015;7(4):321
15. Vafaee K, Dehghani S, Tahmasvand R, Abadi FS, Irian S. Potent antitumor property of Allium bakhtiaricum extracts. BMC Complement Altern Med. 2019;19:116 . https://doi.org/10.1186/s12906-019-2522-8
16. Borek C. Garlic reduces dementia and heart disease risk. J Nutr. 2006;136:810–812.
17. Patro G, Bhattamisra SK, Mohanty BK, Sahoo HB. In vitro and in vivo antioxidant evaluation and estimation of total phenolic, flavonoidal content of *Mimosa pudica* L. Pharmacog Res. 2016;8(1):22–28. Doi: 10.4103/0974-8490.171099
18. Sinha N, Kumar DP. Oxidative stress and antioxidants in hypertension–a current review. Curr Hypert Rev 2015;11(2):132-142.
19. Ahmad A, Dempsey SK, Daneva Z, Azam M, Li N, Li PL, Ritter JK. Role of Nitric Oxide in the Cardiovascular and Renal Systems. Int J Mol Sci. 2018;19(9):2605. doi: 10.3390/ijms19092605
20. Johnson J, Jaggers RM, Gopalkrishna S, Dahdah A, Murphy AJ, Hanssen NM, Nagareddy PR. Oxidative stress in neutrophils: Implications for diabetic cardiovascular complications. Antioxid Red Signal. 2022;36(10):652-666.

21. Olusola AI, Aderibigbe KO, Saka OS, Odukoya SA. Biochemical effects of aqueous extract of *Persea americana* (Mill) on the myocardium of left ventricle of high salt-fed adult Wistar rats. J Evid Based Complementary Altern Med. 2017;22(4):765-769
22. Reid K, Fakler P. Potential of garlic (*Allium sativum*) in lowering high blood pressure: mechanisms of action and clinical relevance. Integr Blood Press Control. 2014:7;71-82. Doi: 10.2147/IBPC.S51434
23. Taylor JP, Tse HM. The role of NADPH oxidases in infectious and inflammatory diseases. Redox Biol. 2021;48:102159. Doi:10.1016/j.redox.2021.102159
24. Gonzalez J, Valls N, Brito R, Rodrigo R. Essential hypertension and oxidative stress: new insight. World J Cardiol. 2014;6(6):353-366. Doi: 10.4330/wjc.v6.i6.353
25. Feairheller DL, Brown MD, Park JY, Brinkley TE, Basu S, Hagberg JM, et al. Exercise training, NADPH oxidase p22phox gene polymorphisms, and hypertension. Med Sci Sports Exerc. 2009;41(7):1421–8.
26. Lassegue B, Clempus RE. Vascular NAD(P)H oxidases: Specific features, expression, and regulation. Am J Physiol Regul Integr Comp Physiol. 2003;285:277–97.
27. Bhattacharya S. Reactive oxygen species and cellular defense system. In *Free radicals in human health and disease*. 2015; pp. 17-29. Springer, New Delhi.
28. Barati E, Nikzad H, Karimian M. Oxidative stress and male infertility: Current knowledge of pathophysiology and role of antioxidant therapy in disease management. Cell Mol Life Sci. 2020;77(1):93-113.
29. Gómez-Marcos MA, Blázquez-Medela AM, Gamella-Pozuelo L, Recio-Rodriguez JI, García-Ortiz L, Martínez-Salgado C. Serum superoxide dismutase is associated with vascular structure and function in hypertensive and diabetic patients. Oxid Med Cell Longev. 2016; 2016: 9124676. doi: 10.1155/2016/9124676
30. Chrissobolis S, Didion SP, Kinzenbaw DA, Schrader LI, Dayal S., Lentz SR *et al*. Glutathione peroxidase-1 plays a major role in protecting against angiotensin ii-induced vascular dysfunction. Hypertension. 2008;51:872-877. Ahajournals.org/doi/10.1161/HYPERTENSIONAHA.107.103572
31. Incalza MA, D'Oria R, Natalicchio A, Perrini S, Laviola L, Giorgino F. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases, Vasc Pharmacol. 2018;100:1-19. https://doi.org/10.1016/j.vph.2017.05.005.
32. Liu VW, Huang PL. Cardiovascular roles of nitric oxide: a review of insights from nitric oxide synthase gene disrupted mice. Cardiovasc Res. 2008;77:19–29. doi: 10.1016/j.cardiores.2007.06.024.
33. Thoonen R, Sips PY, Bloch KD, Buys ES. Pathophysiology of Hypertension in the Absence of Nitric Oxide/Cyclic GMP Signaling. Curr Hypertens Rep. 2013;15(1):47–58. doi: 10.1007/s11906-012-0320-5
34. Alka C, Amit KM, Manukonda R, Debabrata T, Anupam C, Uttam CB *et al*. Two new stereoisomeric antioxidant triterpenes from *Potentilla fulgens*. Fitoterapia. 2013;91:290–297
35. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014:360438. https://doi.org/10.1155/2014/360438
36. Reis GS, Augusto VS, Silveira APC, Jordão AA, Baddini-Martinez J, Neto OP *et al.* Oxidative-stress biomarkers in patients with pulmonary hypertension. Pulm Circ. 2013;3(4):856–861. https://doi.org/10.1086/674764
37. Achiliya GS, Wadodkar SO, Dorle AK. Evaluation of hepatoprotective effect of *Amakadi ghrita* against carbon tetrachloride induced hepatic damage in rats. J Ethnopharmacol. 2004;90:229- 232
38. Das SK, Varadhan S, Dhanya L, Mukherjee S, Vasudevan DM. Effects of chronic ethanol exposure on renal function tests and oxidative stress in kidney. Indian J Clin Biochem. 2008;23:341–344.