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Research Article

Blood Pressure-lowering activity of Extract and Fractions of *Persea americana* Leaf

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Abstract

Persea americana leaf is used in ethnomedicine to treat hypertension, but with limited scientific justification. This study evaluated the antihypertensive properties of fractions of *P. americana* leaf in rats. *Persea americana* leaf was extracted in 95% methanol and partitioned into five fractions. Thirty-five noradrenaline-induced hypertensive Wistar rats distributed into seven groups (n=5) were treated intravenously with normal saline; 0.6 mL/kg (control), ME (100 mg/kg), fractions (100 mg/kg); n-hexane, dichloromethane, chloroform, ethyl acetate or n-butanol. The ethyl acetate fraction (EF) which was the most active fraction was fractionated on silica gel using column chromatography. Four fractions (EFCF1, EFCF2, EFCF3 and EFCF4) were obtained and evaluated at 25 mg/kg for antihypertensive activity in noradrenaline-induced hypertension using twenty rats. The systolic, diastolic blood pressure, heart rate and mean arterial pressure (MAP) were used as indices for antihypertensive activity. The constituents of EFCF3 were identified by GC-MS. Data were analysed using descriptive statistics and ANOVA at α 0.05. The ME, EF and EFCF3 produced the most pronounced reduction in systolic and diastolic blood pressure in noradrenaline-induced raised blood pressure. Percentage reductions in noradrenaline-induced raised MAP were 31.6, 32.0 and 32.3% for ME (100 mg/kg), EF (100 mg/kg), and EFCF3 (25 mg/kg) respectively. EFCF3 produced better reduction in MAP than standard drugs (labetalol and phentolamine). In addition, only ME produced significant reduction on heart rate. The major constituents of the most active column fraction of *P. americana* leaf (EFCF3) are 11-Tetradecyn-1-ol acetate, 14-methyl-(Z)-8-Hexadecenal and Cyclopropane carboxaldehyde. *Persea americana* demonstrates *in vitro* and *in vivo* antihypertensive activities.

Key Words: *Persea americana*, Hypertension, Mean Arterial Pressure

INTRODUCTION

Hypertension has been described as a non-communicable disease of disordered auto-regulation of blood pressure in which there is persistent elevated pressure in the arteries. Regarded as the most prevalent cardiovascular disease with occurrence varying with age, race and educational background (Abel *et al*., 2015; Gao *et al*., 2017). Hypertension is said to account for about 16.5% of yearly global mortality, and also the major cause of morbidity and mortality linked with cardiovascular diseases (Kjeldsen, *et al*., 2016; Hu, *et al*., 2017). In addition, it has been projected that by the year 2030, the death toll will be close to 23.5 million people (WHO, 2013). With changing patterns of life style, populations with higher prevalence of high blood pressure nowadays include adults aged 65 and older. (CDC, 2021). The prevalence of hypertension in African adults is 46% (WHO, 2018).

Studies have shown that close to 95% of episodes of hypertension termed primary hypertension has no known cause, while the rest of the cases constituting about 5% are known as secondary hypertension as they result from different disorders of the heart, the arteries, the kidneys or the endocrine system (Poulter *et al*., 2015). While the majority of patients with hypertension is without symptoms, cautious and prompt assessments are necessary in identifying possible risk of damage to vital body organs (James *et al*., 2014). This is because high blood pressure injures the blood vessels of the brain, heart and kidney (Beevers, 2001). It has been noted that effective pharmacologic lowering of blood pressure prevents vascular damage and significantly reduces morbidity and mortality rates.

The determinants of blood pressure are diverse and so is the therapeutic approach to the management of hypertension which includes non-pharmacologic and pharmacologic approaches with various groups of antihypertensive drugs available for use (Whelton *et al*., 2017). However, current antihypertensive drug therapy results in many adverse effects, long treatment periods and high cost (Su *et al*., 2017), hence the need for alternative remedies that could be more effective and affordable with fewer side effects and possibly with different mechanisms of action.

Some plants that have demonstrated antihypertensive properties include *Allium sativum* (Al Disi *et al*., 2015), *Annona muricata*, *Apium graveolens*, *Avena sativa*, *Imperata*

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cylindrical (Mak-Mensah *et al*., 2010) and *Persea americana* Mill. family - Lauraceae (Ojewole *et al*., 2007). The therapeutic usefulness of these natural products as anti-hypertensive agents have been proposed to be due to abundance of bioactive phytoconstituents present in them. *Persea americana* Mill., popularly called avocado, alligator pear or butter pear is a deciduous, erect, terrestrial plant 15 to 20 m high (Imafidon and Okunrobo, 2009). Originally found in the Americas, *P. americana* is now cultivated in many countries for its large, edible fruit. The narrow to broad leaves are spirally arranged. Its fruit is often pear-shaped, usually greenish or earthy when matured, with soft, slick, greenish or yellow mash. The plant is currently attracting widespread attention for its antihypertensive properties.

Phytochemical analysis has shown that the components of *P. americana* include flavonoids, alkaloids, carotenoids, cellulose, peptone, phenolics, β-galactoside, glycosylated abscisic acid, polygalactourease, protein, fat, ascorbic acid, oxalate, phytate, saponins, tannins, cyanogenic glycoside and mineral elements including zinc (Ejiofor *et al*., 2018; Park *et al*., 2020). The plant seed is also abundantly rich in catechins, procynidins, hydroxycinnamic acids and flavonol glycosides (Park *et al*., 2020). In addition, studies revealed that the seed pulp possesses properties that can reduce body fats (Anaka *et al*., 2009). Also, credited with the plant are various biological activities which include antihyperglycemic and antihyperlipidemic, wound healing, antifungal, antioxidant and antimicrobial, anticancer and anticonvulsant activities (Melgar *et al*., 2017; Rahman *et al*., 2018). Thus, various parts of the plant have become the object of several studies aimed at uncovering their biological activities. This study investigated the blood pressure lowering effect of *P. americana* leaf methanol extract and fractions on noradrenaline-induced hypertension in rats.

MATERIALS AND METHODS

Plant material collection and identification: Fresh leaves of *P. americana* were harvested from University of Ibadan. The leaves were identified by Mr. Donatus Eseimokhuai, curator University Herbarium as *P. americana* Mill. (family: Lauraceae) with specimen identification number UIH22381. A voucher specimen was deposited in the Botany Department Herbarium.

Extraction and partitioning of plant material: Distilled water washed, air-dried leaves of *Persea americana* (1.5 kg) were milled, and extracted with methanol/distilled water (95/5%) at room temperature for 48 hours, with occasional shaking. This methanol extract (ME) was filtered and concentrated to dryness at 40 ±1°C using Buchi Labortechnik® rotary evaporator (Model CH-9230 Switzerland) under reduced pressure to give 158.5 g (10.6 % yield) of a light-brown, powdery extract. The extract was then partitioned into n-hexane (HF), dichloromethane (DF), chloroform (CF), ethyl acetate (EF) and n-butanol (BF) which were also dried as ME above.

Determination of the phytochemical constituents of the ethyl acetate fraction of *P. americana* using Gas Chromatography-Mass Spectrometry (GC-MS): The phytochemical constituents of the ethyl acetate fraction of the methanol leaf extract of *P. americana* was determined using GC-MS Agilent Technologies 7890 system mass spectrometric detector (Agilent technologies 5975 model) according to the method of Al-Nemari and co-workers (Al-Nemari *et al*., 2020). The compounds detected were identified through the comparison of their mass spectra with the reference mass spectra of National Institute of Standard and Technique (NIST) reference library.

Experimental animals: The study protocol was approved by University of Ibadan, Animal Care and Use Research Ethics Committee (UI-ACUREC/17/0071). Healthy male Wistar rats weighing between 250-300 g used for the study were housed in polycarbonate cages with wood shaving as beddings and kept within laboratory specified settings of light, humidity and temperature. The animals were provided with rat pellets (Ace Feeds®) and water ad libitum. The rats were allowed to adapt for two weeks before commencing experiments. The rats were fasted for 18 hours prior to use.

Drugs and Chemicals: (-)-Noradrenaline (Sigma, USA); Labetalol, Phentolamine, NG-nitro-L-arginine methyl-ester (L-NAME) (BDH Chemicals Ltd, Poole, England), Diazepam (Hoffman-La Roche, Switzerland) and Ketamine. All other chemicals used were of analytical grade.

Preparation of animals for experiments Each rat was anaesthetized with intraperitoneal injection of Ketamine/Diazepam 60/5 mg/kg. The right femoral vein was cannulated with a small polythene cannula for administration of plant extract or standard drugs. Heparin (500 Unit/kg) was intravenously administered to the animal and flushed in with 0.2 mL of 0.9 %w/v sodium chloride solution to minimise blood coagulation. The left carotid artery of each rat was also cannulated for systemic arterial blood pressure recording with a pressure transducer (DELTRAN® model DPT-100; Utah Medical Products, Inc. Midvale, USA) connected to a signal manifold (Transbridge, model TBM-4; World Precision Instruments, Sarasota, Florida, USA) and recorded on a data acquisition system (Model LT-4/16S; World Precision Instruments, Sarasota, Florida, USA). The trachea of each rat was cannulated for artificial respiration, but the animal was allowed to breathe spontaneously. Rectal temperature of the rat was monitored with a rectal thermometer. The rat was allowed to stabilize for about 20 min after which baseline systemic arterial pressures were recorded. The animals were thereafter challenged with intravenous administration of noradrenaline 4.0 µg/kg and allowed to recover before commencement of the experiments

Effect of methanol extract and fractions of *P. americana* leaf on blood pressures parameters of anaesthetized rats: Thirty Wistar rats weighing 200-300 g (n=5) were used to evaluate the antihypertensive effect of the methanol extract and fractions (HF, DF, CF, EF and BF) of *P. americana* according to the modified method of Ojewole *et al*., (2007). Each rat was pre-treated with the extract (100 mg/kg) or fractions (100 mg/kg) and then challenged iv, with the noradrenaline 4.0 µg/kg. Thereafter, systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate were measured. This method was followed to measure the antihypertensive activity of the ethyl acetate column fractions of *P. americana*. Labetalol and phentolamine were included as standard controls.

Bioassay-guided fractionation of the most active fraction using column chromatography: The ethyl acetate fraction (EF), the most active of the five fractions tested, was further fractionated using silica gel (size 60-200 µm) in column chromatography. The EF was mixed with silica gel, dried and packed in a column of 50cmin length and 4mm in diameter. The EF was eluted with isocratic solvent; Ethyl acetate: Chloroform: Methanol: Water (15:8:2:0.5). Two hundred and four fractions were collected which were pooled into four, based on their appearance on thin layer chromatography giving four Ethyl acetate Column Fractions: EFCF1, EFCF2, EFCF3 and EFCF4. The column was then washed with methanol 100% after fractionation.

Effect of the ethyl acetate column fractions on systemic arterial blood pressures and heart rates of anaesthetized rats: The effect of the fractions alone and with noradrenaline (4.0 µg/kg iv) on systemic arterial blood pressures and heart rates of the anaesthetized rats were also evaluated using the method described above. Each rat was injected with 25 mg/kg iv of the ethyl acetate column fraction and the systemic blood pressure and heart rate were recorded. In each case, noradrenaline (4.0 µg/kg) iv was administered after each of the fractions. Labetalol and phentolamine were included as standard controls.

Statistical Analysis: Data analysis was performed using GraphPad® prism version 8.0 software. Data were expressed as mean ± standard error of mean and analysed using Student t-test. Test doses were compared with control values via one-way analysis of variance (ANOVA) and post-hoc Dunnett's Multiple Comparison Test. P-values less than 0.05 ($p < 0.05$) were considered statistically significant.

RESULTS

Identification of chemical components of ethyl acetate fraction of *Persea americana* using GC-MS

The analysis identified 11 compounds as shown in Table 1. 11-Tetradecyn-1-ol acetate (16.6%), 14-methyl-(Z)-8-Hexadecenal (16.5%) and Cyclopropane carboxaldehyde (12.92%) were the most abundant components of the fraction.

Table 1:
Identification of chemical components of ethyl acetate fraction of *Persea americana* using GC-MS

Peak No	Compound	GC-MS-RT (min)	Percentage of total/Abundance (%)	M+1 Value
1	9-Tetradecen-1-ol,acetate	21.864	8.196	252
2	Cyclopropane carboxaldehyde	22.023	12.198	70
3	Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl-	22.041	2.146	170
4	1,14-Tetradecanediol	22.059	1.762	230
5	8-Hexadecenal, 14-methyl-, (Z)-	22.191	16.455	250
6	11-Hexadecen-1-ol, (Z)-	22.213	3.216	240
7	Chloroacetic acid, 10-undeceny ester	22.236	4.870	246
8	7-Methyl-Z-8,10-dodecadienal	22.301	9.048	170
9	11-Tetradecyn-1-ol acetate	22.416	16.593	252
10	2,7-Octadiene, 4-methyl-`	22.447	7.031	124
11	Cyclohexene, 3-(2-methylpropoxy)-	22.487	7.571	154

Effect of Noradrenaline (NA) 4.0 µg/kg on rat systolic blood pressure: The intravenous administration of noradrenaline 4.0 µg/kg on anaesthetised rats elicited a mean increase in systolic blood pressure (SBP) from 117.0 ± 2.6 mmHg to 151.8 ± 2.6 mmHg which lasted for about 3 mins (Fig. 2)

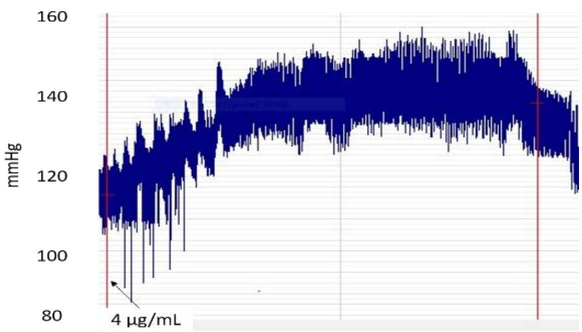

Fig 2:
Tracing of the effect of Noradrenaline (NA) 4.0 µg/kg on rat blood pressure

Effect of methanol extract and fractions of the leaf of *Persea americana* on noradrenaline-induced raised Systolic Blood Pressure (SBP) in rats: Intravenous injection of noradrenaline (4.0 µg/kg) raised the SBP of anaesthetised rats from control value of 117.0 ± 2.6 mmHg to 151.8 ± 2.6 mmHg (Fig. 2 & 3A). This effect lasted for about 3 min before returning to the resting SBP. Furthermore, the SBP values in rats pre-treated with extract and fractions of *P. americana* before exposure to noradrenaline, ranged from 102.2 to 131.8 mmHg (Fig. 3 A). The ethyl acetate column fraction (EF) of *P. americana* produced the most significant reduction in SBP (Fig. 3A).

Effect of methanol extract and fractions of the leaf of *Persea americana* on noradrenaline-induced increase in diastolic blood pressure: Noradrenaline (4.0 µg/kg) elevated the diastolic blood pressure (DBP) from 89.4 ± 4.4 to 120.0 ± 4.6 mmHg (Fig. 3B) in anaesthetised rats. The Methanol Extract (ME) and fractions; Dichloromethane (DF), Chloroform (CF), Ethyl acetate (EF) and n-Butanol (BF) at 100 mg/kg significantly ($p < 0.05$) reduced the DBP of rats in comparison with noradrenaline. The ME and EF produced the most significant reduction in noradrenaline-induced increase in diastolic blood pressure with DBP values of 81.7 ± 1.7 and 80.7± 0.3 mmHg respectively (Figure 3 B).

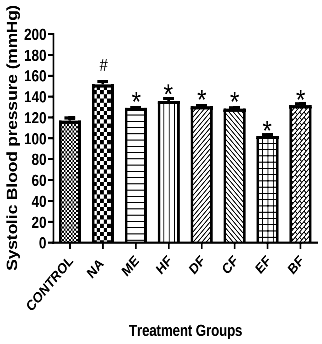

Fig 3A:
Effect of methanol extract and fractions of the leaf of *Persea americana* on noradrenaline-induced raised SBP in rats

NA- Noradrenaline 4.0 µg/kg, ME - *Persea americana* leaf methanol extract 100 mg/kg, Fractions (100 mg/kg): HF - Hexane, DF-Dichloromethane, CF- Chloroform, EF- Ethyl acetate and BF - *n*-butanol. Values represent the mean ± S.E.M of 5 rats per group. #P < 0.05 with NA vs control, *P < 0.05 with NA vs treatment groups, using 1-way ANOVA followed by Dunnett's post hoc test

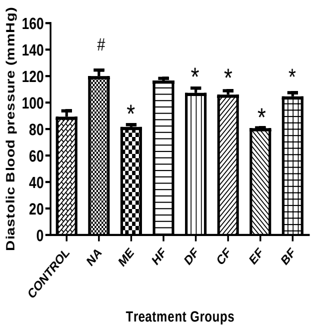

Fig. 3 B*:*
Effect of methanol extract and fractions on NA-induced increase in diastolic blood pressure
NA- Noradrenaline (4.0 µg/kg), ME - *Persea americana* leaf methanol extract (100 mg/kg), HF- Hexane (100 mg/kg), DF-Dichloromethane (100 mg/kg), CF- Chloroform (100 mg/kg), EF-Ethyl acetate (100 mg/kg), BF - *n*-butanol (100 mg/kg). Values represent the mean ± S.E.M of 5 rats per group. . #P < 0.05 with NA vs control, *P < 0.05 with NA vs treatment groups, using 1-way ANOVA followed by Dunnett's post hoc test

Effect of methanol extract and fractions on noradrenaline-induced increase in mean arterial pressure: Noradrenaline (4.0 µg/kg) increased the mean arterial pressure (MAP) from 98.2 to 131.0 ± 2.5 mmHg in anaesthetised rats. The extract and the fractions, except HF, caused significant reductions (p < 0.05) in the mean arterial pressure (MAP) when compared with noradrenaline. EF elicited the highest reduction in MAP (87.3 ± 0.3 mmHg), compared with noradrenaline (131.0 ± 2.5 mmHg) (Fig. 3 C).

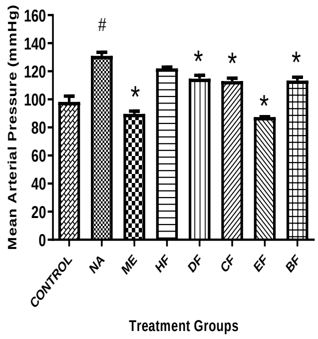

Fig 3 C:
Effect of noradrenaline, methanol extract and fractions of the leaf of *P. americana* on mean arterial pressure of rats
NA- Noradrenaline (4.0 µg/kg), ME- *Persea americana* leaf methanol extract (100 mg/kg), HF- Hexane (100 mg/kg), DF-Dichloromethane (100 mg/kg), CF- Chloroform (100 mg/kg), EF-Ethyl acetate (100 mg/kg), BF - *n*-butanol (100 mg/kg). Values represent the mean ± S.E.M of 5 rats per group. #P < 0.05 with NA vs control, *P < 0.05 with NA vs treatment groups, using 1-way ANOVA followed by Dunnett's post hoc test

Effect of methanol extract and fractions on NA-induced increase in heart rate (HR): Only the methanol extract lowered the mean HR to 333 ± 1.7 beats/min from 410.7 ± 3.5 beats/min in anaesthetised rats. The other fractions increased the HR to between 440 ± 5.0 and 525 ± 2.9 beats/ min (Fig 3 D).

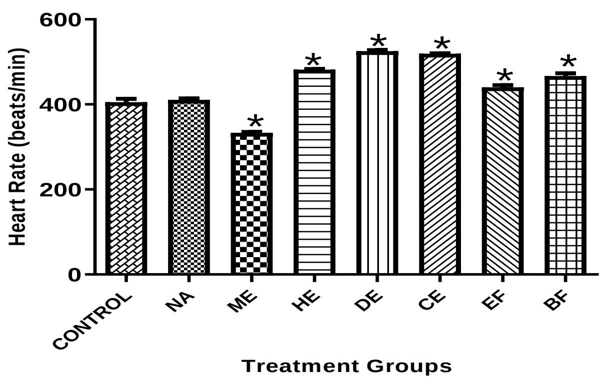

Fig 3 D:
Effect of noradrenaline, methanol extract and fractions of the leaf of *P. americana* on heart rate of rats
NA- Noradrenaline (4.0 µg/kg), ME- *Persea americana* leaf methanol extract (100 mg/kg), HF- Hexane (100 mg/kg), DF-Dichloromethane (100 mg/kg), CF- Chloroform (100 mg/kg), EF-Ethyl acetate (100 mg/kg), BF - *n*-butanol (100 mg/kg). Values represent the mean ± S.E.M of 5 rats per group. * P < 0.05 with NA vs treatment groups, using 1-way ANOVA followed by Dunnett's post hoc test.

Effect of ethyl acetate fraction, column fractions 1-4 on the mean arterial pressure of rats: Treatment with EF and its column fractions reduced the Mean Arterial Pressure (MAP) of anaesthetised rats compared to the control. Interestingly, the lowering effect of EF (100 mg/kg) and EFCF3 (25 mg/kg) were greater than that of the standard drug labetalol (25 mg/kg). From a MAP of 131.0 ± 2.5 mmHg induced by noradrenaline, labetalol, EF, and EFCF3 reduced MAP significantly (Fig. 4A).

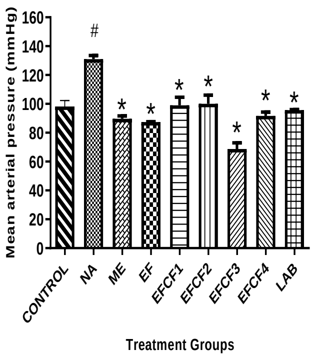

Fig 4 A:

Effect of ME, EF, column fractions and labetalol on the mean arterial pressure of rats

Noradrenaline 4.0 µg/kg, ME- *P. americana* leaf methanol extract (100 mg/kg), EF- Ethyl acetate fraction (100 mg/kg), EFCF -Ethyl acetate column fractions 1, 2, 3, 4 (25 mg/kg), LAB- Labetalol (25 mg/kg). Values represent the mean ± S.E.M of 5 rats per group. * P < 0.05 when NA is compared with the treatment groups, using 1-way ANOVA followed by Dunnett's post hoc test.

Effect of ME, EF, EFCF 1-4 and labetalol on NA-induced increase in the heart rate of rats: In anaesthetised rats, ME, EFCF 1, 3 and labetalol, significantly reduced the heart rate with values ranging from to 333.0 ± 1.7 to 391.0 ± 28.3 beats/min (Fig.4 B). Labetalol (25 mg/kg) reduced the heart rate to 391.0 ± 28.3 beats/min (Fig.4 B).

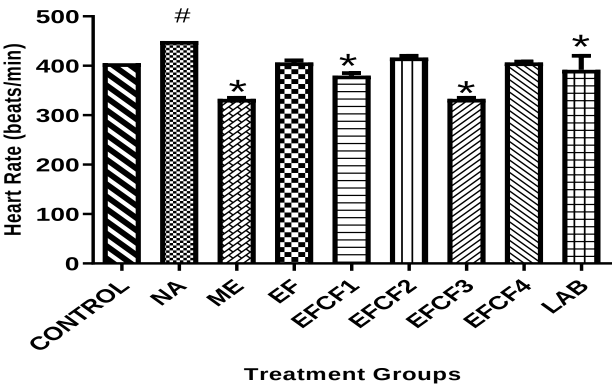

Fig 4 B.:

Effect of ME, EF, column fractions and labetalol on the heart rate of rats

Noradrenaline 4.0 µg/kg, ME- *P. americana* leaf methanol extract (100 mg/kg), EF- Ethyl acetate fraction (100 mg/kg), EFCF -Ethyl acetate column fractions 1, 2, 3, 4 (25 mg/kg), LAB- Labetalol (25 mg/kg). Values represent the mean ± S.E.M of 5 rats per group. . #P < 0.05 with NA vs control, *P < 0.05 with NA vs treatment groups, using 1-way ANOVA followed by Dunnett's post hoc test.

Effect of ethyl acetate fraction, column fractions and phentolamine on NA-induced increase in the mean arterial pressure of rats: Ethyl acetate fraction and the column fractions 1-4 significantly reduced NA-induced, increase in MAP in anaesthetised rats. The values of MAP in the extract and fraction treated groups ranged from 68.7 ± 4.4 to 100.0 ± 6.1 mmHg with EFCF3 exhibiting the most significant reduction in MAP (Fig.5). In addition, MAP in Phentolamine treated group was 94.7 ± 0.9 mmHg.

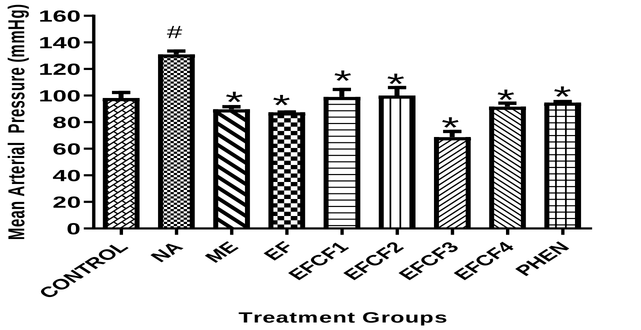

Fig 4 C:

Effect of ethyl acetate fraction, column fractions 1-4 and Phentolamine on the mean arterial pressure of rats

NA- Noradrenaline 4.0 µg/kg, ME- *P. americana* leaf methanol extract, EF- Ethyl acetate fraction (100 mg/kg), EFCF - Ethyl acetate column fractions 1, 2, 3, 4 (25 mg/kg), PHEN- Phentolamine 83.3µg/kg. Values represent the mean ± S.E.M of 5 rats per group. #P < 0.05 with NA vs control, *P < 0.05 with NA vs treatment groups, using 1-way ANOVA followed by Dunnett's post hoc test

Effect of ethyl acetate fraction and column fractions 1-4 on the heart rate of rats: Noradrenaline (4.0 µg/kg) raised the heart rate of anaesthetised rats from 405.0 ± 0.1 beats/min to 450.0 ± 0.1 beats/min (Fig. 4 D). Only, pre-treatment with the plant extract and fraction, ME (100 mg/kg) and EFCF3 (25 mg/kg) significantly reduced the heart rate to 406.7 ± 4.4 and 333.3 ± 1.7 beats/min respectively (Fig. 4 D).

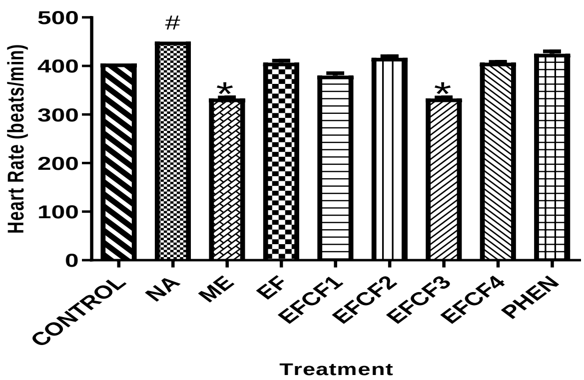

Fig 4 D:

Effects of ethyl acetate fraction, column fractions 1-4 on the heart rate of rats

NA- Noradrenaline 4.0µg/kg, ME- *P. americana* leaf methanol extract (100 mg/kg), EF- Ethyl acetate column fractions 1, 2, 3, 4 (25 mg/kg), PHEN- Phentolamine 83.3µg/kg. Values represent the mean ± S.E.M of 5 rats per group. #P < 0.05 with NA vs control, *P < 0.05 with NA vs treatment groups, using 1-way ANOVA followed by Dunnett's post hoc test.

Effect of column fraction of ethyl acetate 3 on the heart rate of rats: From an original heart rate value of 405.0 ± 0.1 beats/min, noradrenaline (4.0 µg/kg) increased HR to 450.0 ± 0.1 beats/min. Thereafter, the administration of ME (100 mg/kg) and EFCF3 (25 mg/kg) reduced the heart rate to 333.0 ± 2.0 beats/min. In addition, the heart rate of the rats after administration of EFCF3, 25 mg/kg followed by NA was

332.0 ± 2.0 beats/min. Thus EFCF3 inhibited the increase in heart rate caused by NA. (Figure 5 A)

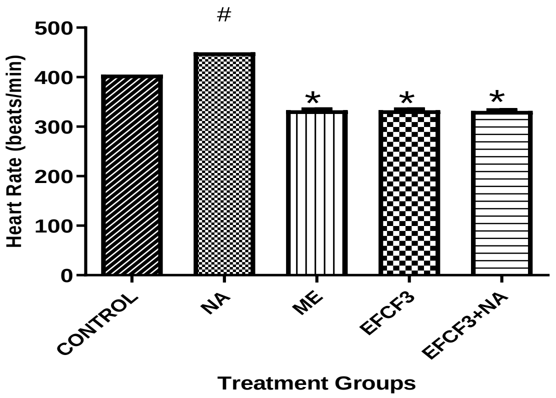

Fig 5 A:
Effect of noradrenaline alone or in the presence of ethyl acetate column fraction 3 on the heart rate of rats
NA- Noradrenaline 4.0 µg/kg, ME- *P. americana* leaf methanol extract (100 mg/kg), EFCF3- Ethyl acetate fraction Column Fraction 3 (25 mg/kg), EFCF3+ NA - EFCF3 + Noradrenaline. Values represent the mean ± S.E.M of 5 rats per group. #P < 0.05 with NA vs control, *P < 0.05 with NA vs treatment groups, using 1-way ANOVA followed by *Dunnett's post hoc test*

Blocking effect of ethyl acetate column fraction 3 on noradrenaline-induced MAP in rats: This is a summary of the effect of EF (100 mg/kg), EFCF3 3 (25 mg/kg), phentolamine (83.3 µg/kg) and labetalol (25 mg/kg) on noradrenaline-induced raised mean arterial pressure in figure 6. From a raised mean arterial pressure of 131.0 ± 2.5 caused by 4.0 µg/kg noradrenaline, the treatment groups lowered the elevated pressure (figure 6). EFCF3 produced the best significant lowering effect (68.7 ± 4.4 mmHg).

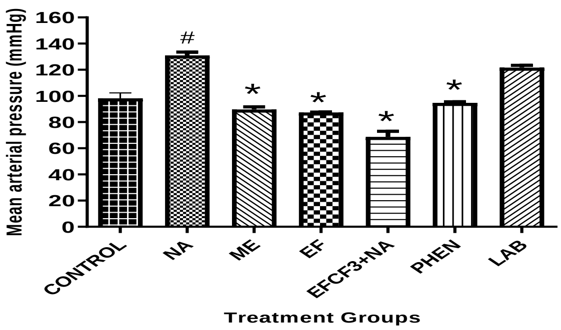

Fig 6:
Effect of noradrenaline, ethyl acetate and ethyl acetate column fraction 3 on the mean arterial pressure of rats
NA- Noradrenaline 4.0 µg/kg, ME100 mg/kg, EF- Ethyl acetate 100 mg/kg, EFCF3- Ethyl acetate column fraction 3 25 mg/kg, PHEN – phentolamine 83.3 µg/kg, LAB-labetalol 25 mg/kg. Values represent the mean ± S.E.M of 5 rats per group. * P < 0.05 when NA is compared with the treatment groups, using 1-way ANOVA followed by Dunnett's post hoc test.

Effect of ethyl acetate column fraction 3 (EFCF3) 25 mg/kg on rat blood pressure: Intravenous administration of 25 mg/kg of EFCF3 to anaesthetised rat effected a 20 mmHg reduction of the systolic blood pressure from about 120 mmHg to about 100 mmHg (Fig. 7).

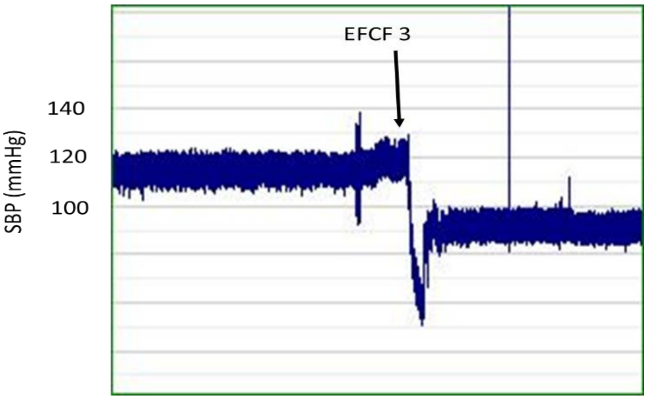

Fig. 7:
Effect of ethyl acetate column fraction 3- EFCF3 (25 mg/kg) on rat systolic blood pressure (SBP)

DISCUSSION

Hypertension is a non-communicable disease of the heart and blood vessels, which used to be uncommon but has now assumed prominence globally with about 1.28 billion people with hypertension (Hypertension, 2021). The encouraging achievements made in understanding the pathology, diagnosis and management of hypertension (Leontsinis *et al*. 2020), notwithstanding, there is still the need to overcome the challenges of cost and adverse effects of the drugs in use which render many of the current pharmacotherapies ineffective for most patients. To do this effectively, there is the need to re-examine the inherent variability in the factors that determine blood pressure and understand their role and mechanisms in the pathophysiology of hypertension, with the aim of finding new therapies for the disease. Thus this study investigated the antihypertensive property of methanol leaf extract and fractions of *P. americana* in noradrenaline-induced raised blood pressure in rats.

Noradrenaline (NA) is an endogenous catecholamine with direct alpha and beta adrenergic receptor agonist activities. When noradrenaline binds to adrenoceptors in the blood vessels, it activates vasoconstriction of both capacitance and resistance vessels thereby causing an increase in blood pressure (Foulon, 2018). Noradrenaline-induced contractions of blood vessels have been shown to be partly due to calcium release from intracellular storage sites and partly due to extracellular calcium influx into the cell by receptor-gated channels following alpha1 (α1)-adrenoceptor activation (Fischer, *et al*, 2021).

The mean arterial pressure was used, in this study, as the major index for antihypertensive activity as it is the pressure at which organs are supplied with blood. It expresses a relationship between the systolic and diastolic pressures. Its significance lies in the fact that it reflects the perfusion pressure, i.e, the force with which the blood in circulation supplies the vital organs of the body with oxygen and important nutrients (DeMars and Wachs, 2021). The mean arterial pressure is influenced by differences in cardiac output and systemic vascular resistance, which alludes to the force against blood flow by all blood vessels, incorporating arteries, veins and capillaries. The most important mechanism for changing systemic vascular resistance consists of alteration in vessel lumen diameter.

The *Persea americana* methanol leaf extract and the purified ethyl acetate Column Fraction 3 (EFCF3) used in this study exhibited significant reduction in noradrenaline-induced raised systolic, diastolic and mean arterial pressures. This is in agreement with previous studies by Ozolua *et al*., 2009; Sokpe, *et al*. 2020). When rats were pre-treated with the plant fractions and then challenged with noradrenaline, the plant fractions significantly lowered the mean arterial pressure thereby blocking noradrenaline-induced alpha 1 adrenoceptor activation. Also, when the plant extract was used to pre-treat the rats before the administration of noradrenaline it prevented a rise in mean arterial pressure when compared with control. However, none of the fractions except the methanol leaf crude extract demonstrated a significant reduction on the heart rate.

Intravenous injection of *P. americana* methanol leaf extract prompted a distinct lowering in mean arterial pressure lasting about 3 minutes. Rapid metabolism and reflex action from the animal may be responsible for the transient duration of action. Attenuation of the hypertension-inducing effect of noradrenaline suggests the ability of the extract and fraction 3 to block the effect of noradrenaline on adrenoceptors and protect against raised blood pressure. This effect of *P. americana* methanol leaf extract and ethyl acetate column fraction 3 on mean arterial pressure was more pronounced than those of phentolamine and labetalol - the standard adrenoceptor blockers (Odubanjo, *et al*, 2016; Al-Akwaa, *et al*, 2020). The observed significant reduction in the systolic blood pressure, diastolic blood pressure and mean arterial pressure, by the extract and its fractions indicating their vasoconstricting effect on the blood vessels is important in their blood pressure-lowering effect (Brai, *et al*. 2020).

From this study therefore, it is suggested that the ethyl acetate column fraction 3 reduced the blood pressure better than phentolamine and labetalol, hence it shows that ethyl acetate column fraction 3 contains compounds which possess beneficial blood pressure lowering effect, as mean arterial pressure has been said to be a better measure of tissue perfusion (Wehrwein, *et al*., 2013; Sokpe *et al*., 2020). This therefore offers a great advantage in protecting target organs against end organ damage that is common in hypertension (Lohmeier, *et al*., 2010; Kyada et. al, 2017).

The impact of the plant extract and fractions were for the most part low on heart rate, although ethyl acetate column fraction 3 still exhibited a slight reducing effect on heart rate. In addition, the fraction alone or when used to pre-treat the rats before administration of noradrenaline offered a better lowering effect of mean arterial pressure compared with phentolamine alone or when phentolamine was used as pre-treatment before noradrenaline administration. This shows that ethyl acetate column fraction 3 can still lower the blood pressure despite noradrenaline administration. It was noted also that in some of the cases, the effect of ethyl acetate column fraction 3 was similar to that of labetalol (a mixed α- and β- adrenoceptor blocker) on heart rate (Chobanian *et al*., 2003).

From the phytochemical study it was found out that 8-Hexadecenal, 14-methyl-, (Z)-, 11-Tetradecyn-1ol acetate and Cyclopropane carboxaldehyde are the three most abundant constituents obtained from the GC-MS profile of *Persea americana* leaf. It can thus be suggested that the presence of these major phytocompounds in *P. americana* leaf might contribute immensely to its antihypertensive property exhibited in the study (Dzeufiet *et al*, 2014; Al-Nemari, *et al*, 2020; Sokpe *et al*, 2020)

In conclusion, the administration of *P. americana* methanol leaf extract and its ethyl acetate column fraction 3 significantly reduced the systolic blood pressure, diastolic pressure and the mean arterial pressure previously raised by noradrenaline This may be mediated by inhibition of alpha and beta adrenergic receptors, an effect similar to the activity of labetalol, a standard, antihypertensive with non-selective alpha and beta antagonist activity.

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