ANTI-HYPERTENSIVE PROPERTIES AND MECHANISMS OF ACTION OF THE EXTRACT AND FRACTIONS FROM *Persea americana* MILL. LEAF IN RATS

BY

JOSEPH AYOTUNDE BADEJO

Matriculation number 135299

B. Pharm. (Hons) (Zaria), M. Sc. Pharmacology and Therapeutics (Ibadan)

A Thesis in the Department of

PHARMACOLOGY AND THERAPEUTICS

Submitted to the Faculty of Basic Medical Sciences in partial fulfilment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

Department of Pharmacology and Therapeutics
Faculty of Basic Medical Sciences
College of Medicine
University of Ibadan
Ibadan

November, 2018

ABSTRACT

Hypertension is a chronic cardiovascular disease characterised by persistent, elevated arterial blood pressure. High cost and adverse effect burden of current antihypertensive therapies necessitate the need for alternative remedies. *Persea americana* leaf is used in ethnomedicine to treat hypertension, with limited scientific justification. This study therefore evaluated the antihypertensive properties and mechanisms of action of fractions from *P. americana* leaf in rats.

Fresh leaves of *P. americana* collected from the University of Ibadan was authenticated at the University Herbarium (UIH No. 22381), air-dried and extracted in 95% methanol. *Persea americana* methanol leaf extract (PAM) was partitioned with *n*-hexane, dichloromethane, chloroform, ethyl acetate and *n*-butanol. An initial study was carried out using 25, 50 and 100 mg/kg PAM to determine optimal effective dose. Thirty noradrenaline-induced hypertensive Wistar rats, distributed into six groups (n=5) were treated intravenously as follows: control (normal saline; 0.6 mL/kg), PAM (100 mg/kg), PAM fractions (100 mg/kg); n-hexane, dichloromethane, ethyl acetate and n-butanol. The PAM ethyl acetate fraction (PEtOAc) was fractionated on silica gel using column chromatography. Four pooled fractions (CFEt1, CFEt2, CFEt3 and CFEt4) were evaluated intravenously at 25 mg/kg for antihypertensive activity in noradrenaline- or N^G-nitro-L-arginine methyl-ester (L-NAME)-induced hypertension using twenty-five rats. Mean Arterial Pressure (MAP) was used as index for antihypertensive activity. Antihyperlipidemic effect of PAM and CFEt3 were also evaluated in thirty-five rats distributed into 7 groups (n=5): Triton X100-induced hyperlipidemic rats (100 mg/kg; 72h; intraperitoneally) were treated orally with normal saline (10 mL/kg), atorvastatin (10 mg/kg), PAM (50 and 100 mg/kg), CFEt3 (25 mg/kg); non-hyperlipidemic rats treated with normal saline and PAM (100 mg/kg). The effect of PAM (100 mg/mL) and CFEt3 (25 mg/mL) were also tested on noradrenaline-induced contraction of thoracic aortic rings (n=6). The Angiotensin Converting Enzyme (ACE) inhibition by PAM, CFEt3 and captopril (100 µg/mL) was also investigated. The constituents of CFEt3 were identified by GC-MS. Data were analysed using descriptive statistics and ANOVA at α 0.05

The most active column fraction of PAM was the PEtOAc. The percentage reductions in noradrenaline-induced raised MAP were 31.6%, 32.0% and 32.3% observed for PAM (100 mg/kg), PEtOAc (100 mg/kg), and CFEt3 (25 mg/kg) respectively. Similarly, only PAM and CFEt3 caused 21.2% and 23.3% reductions respectively in L-NAME-induced raised MAP. Also, PAM (100 mg/kg), CFEt3 (25 mg/kg) and Atorvastatin significantly lowered Atherogenic Index of plasma from 1.8 ± 0.5 to 0.03 ± 0.001 when compared with Triton X 100. Additionally, PAM (100 mg/kg) and CFEt3 significantly reduced noradrenaline contraction of endothelium-intact (74.4% and 65.0%) and endothelium-denuded (36.6% and 27.8%) aortic rings respectively.

The percentage *in vitro* inhibition of ACE by PAM, CFEt3 and Captopril were 68.1 ± 1.9, 78.7 ± 2.9 and 59.6 ± 5.0 respectively. The GC-MS analysis revealed 11-Tetradecyn-1-ol acetate (16.6%) and 14-methyl-(Z)-8-Hexadecenal (16.5%) as major components of CFEt3.

Persea americana leaf methanol extract and its ethyl acetate column fraction 3 possess *in vitro* relaxant and *in vivo* antihypertensive activities in rats which may be mediated through inhibition of vascular alpha adrenoceptors, angiotensin converting enzyme and amelioration of dyslipidemia.

Keywords: *Persea americana*, Angiotensin Converting Enzyme, Atherogenic Index, Hypertension

Word count: 498

DEDICATION

This work is dedicated to The Omniscient, Omnipotent God; The Alpha and Omega.

ACKNOWLEDGEMENTS

'I will shout Alleluia. Amen, for the Lord is good to me! Oh! My soul, rise up and praise the Lord!!

I give my sincerest gratitude to God Almighty for granting me this particular opportunity and the many blessings He has bestowed upon my life.

My special appreciation goes to my supervisor, Professor O. S. Fagbemi who gave me all the needed guidance, encouragement and invaluable support all the way through this project.

To Professor Iwalewa, Head, Department of Pharmacology & Therapeutics, I say a Big Thank You. Professor Catherine Falade, you spotted me 'under the fig tree', beckoned on me and helped me to remain focused on the task before me. In deed you are a mother in a million in every way.

I owe a huge debt of gratitude to a lot of people whose intervention saw to the success of this project. I deeply appreciate the contributions of Professors A. Sowunmi, O. G. Ademowo, Grace Gbotosho, F. A. Fehintola, O. A. Adaramoye, A. Adeneye, Drs. S. Umukoro, A. O Aderibigbe, Aduragbenro Adedapo, Oyindamola Abiodun, O. S. Michael, A. G. Bakre, A. M. Ajayi, S. A. Onasanwo, O. Omobowale, A. A. Oyagbemi all the staff and students of the Department of Pharmacology & Therapeutics, Mr. S. Odebiyi and friends in IITA, Ibadan.

I express my deep appreciation to Archbishop G. L. Abegunrin. I sincerely thank my siblings and their families: Mrs. Margaret Aderinola, Peter Badejo *OBE,* Mrs. Clara Williams, Mrs. Sade Adediran, Mr. Matthew Badejo and Bishop E. A. Badejo. I am also grateful to Adelakun family, my in-laws. I pray that God will meet you all at your points of need.

I specially thank my gifts from God – Damilola, Adeyosola and Oluwabunmi. You all shared the pains and anxiety that accompanied the struggle. Thank God it is accomplished! May God make you greater than all your imaginations. Amen!

To my dearly belovèd Mary… the only thing I can say is Thank You, Thank You and Thank you. MAY GOD BLESS YOU ABUNDANTLY!!

CERTIFICATION

I certify that this work was carried out by Mr. **J. A. Badejo** in the Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria.

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Supervisor

O. S. Fagbemi

B.Sc., Ph.D. Pharmacology & Therapeutics (Ibadan)

Professor

Department of Pharmacology and Therapeutics,

University of Ibadan,

Ibadan,

Nigeria

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GLOSSARY OF ABBREVIATIONS

ACE	Angiotensin Converting Enzyme
BP	Blood Pressure
CFEt3	Ethyl acetate Column Fraction 3
CH_3Cl	Chloroform
DBP	Diastolic Blood Pressure
DCM	Dichloromethane
Fig	Figure
G	Gram
Hex	Hexane
HHL	Hippuryl-Histidyl- L-Leucine
HR	Heart Rate
HTN	Hypertension
IC_{50}	50% Inhibitory Concentration
JNC7	7^{th} Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure
Kg	Kilogram
MAP	Mean Arterial Pressure
mL	Milliliter
mmHg	millimetres of mercury
NA	Noradrenaline
NaCl	Sodium Chloride
n-BuOH	*n*- Butanol
SBP	Systolic Blood Pressure
SD	Standard Deviation
µg	microgram

WHO	World	Health	Organization

CHAPTER ONE

INTRODUCTION

1.1 Background

The disease hypertension has been described as a non-communicable illness of disordered auto-regulation of blood pressure in which there is persistent elevated pressure in the arteries. It is regarded as the most prevalent cardiovascular disease of the heart with occurrence varying with age, race and educational background (Abel *et al*., 2015). The disease is said to account for about 16.5% of yearly global mortality (WHO, 2013), and also the chief cause of morbidity and death linked with cardiovascular diseases (Kjeldsen, *et al*., 2016). In addition, it has been projected that by the year 2030, the yearly death toll will be close to 23.5 million people (WHO, 2013). The current prevalence of hypertension in African adults is 46% (WHO, 2018).

Measurement of blood pressure is achieved via dual quantities - the systolic and the diastolic pressures which indicate a maximum pressure during cardiac contraction (systole) or the lowest pressure when the muscle of the heart relaxes (diastole) respectively. The 7th Joint National Committee on prevention, detection and treatment of high blood pressure (JNC 7) defined hypertension in terms of systolic pressure values equal to or higher than 140 mmHg and diastolic pressure values from 90 mmHg. The committee also defined systolic pressure less than 120 mmHg and diastolic pressure less than 80 mmHg as normal. However, precise recording is of utmost importance in order to properly control raised blood pressures.

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 (Beevers *et al*., 2001; Poulter *et al*., 2015).

While a bulk 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 therapeutic approach to the management of hypertension includes non-pharmacologic and pharmacologic therapy. Various groups of antihypertensive drugs are available based on the determinants of blood pressure.

However, current antihypertensive drug therapy results in many adverse effects, lengthy treatment periods and high cost, hence the need for alternative remedies that

could be more effective and affordable with fewer side effects and possibly with different mechanisms of action. At the same time the awareness that vegetation comprises countless plant chemicals found to offer protection against different diseases and illnesses is expanding.

In the recent past, herbal medicine has assumed tremendous importance globally, with the World Health Organization estimating about 80% of the global populace using home grown solution as health remedies. The WHO has also documented nearly 20,000 medicinal plants the world over with thousands of phytochemicals that are broadly effective remedies with promising safety profiles (WHO, 2013). Users have also claimed that these plants possess many beneficial activities such as hepatoprotective, anticancer, antimicrobial, antioxidant, analgesic and antihypertensive properties (Ojewole *et al*., 2007).

Therefore, herbal preparations are recently being integrated with evidence-based medicine and medicinal plants have become very promising sources of extracts and candidates for anti-hypertensive drug development. Indeed, accumulating literature continues to provide the scientific evidence for the application of herbal therapy in the management of hypertension (Owolabi *et al.,* 2005)

Some of the plants proposed to have these antihypertensive properties include *Allium sativum* (Al Disi *et al.,* 2015), *Annona muricata, Apium graveolens*, *Avena sativa, 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, also known as avocado is of Lauraceae family, genus Persea. A perennial tree originally grown in Central America, its bark, fruit and leaf are now employed as herbal cures in many countries of the world to treat many ailments including stomach pain, menstrual pain, hyperlipidaemia, diarrhoea and raised blood pressure (Ojewole, *et al.,* 2007). The avocados are associated with reduction of pain and swelling and the mash is likewise utilized as a part of different skin preparations, for example, combinations as moisturisers and shield from bright radioactivity (Adeyemi, *et al*., 2002). Thus, various parts of the plant have become the object of several studies aimed at confirming their biological activities.

Other studies have also revealed that extracts of the leaf of *P. americana* have additional pharmacological properties including relief of pain and swelling, antilipidemic, antidiarrhoeal, blood sugar lowering and antihypertensive effects (Owolabi *et al*., 2005)

The motivation for this current effort came from assertions by different researchers (Adeyemi *et al.,* 2002; Adeneye, 2014) that *P. americana* leaf extract can effectively control hypertension, hence the need to fully study the antihypertensive properties of the leaf of the plant.

1.2 Study Rationale

There exists a variety of pharmacological strategies in the management of hypertension. These strategies derive from consideration of the various determinants of blood pressure and disease pathophysiology. However, these pharmacologic preparations often employed in combination to control the condition have the potential for drug-drug interactions. In addition, diminished response to current antihypertensive drugs is now becoming a common occurrence. It is, therefore necessary to investigate alternative treatments that can aid in and/or shorten the period of administration of the presently available remedies and possibly with fewer side effects.

However, only a very few accessible herbal remedies have strong avowal of what they contain or scientifically linked data, and also only a few have been authenticated by any recognized body. This is of great importance to users who require precise information concerning herbal preparations given the current advances in the relevance of herbal medicine. In addition a good number of the plants in use currently are potent and not necessarily innocuous.

1.3 Aim

The major aim of this study is to provide needed information on the isolation and determination of the possible mechanisms of antihypertensive activity of the constituents of methanol leaf extract and the most active fraction from *Persea americana* leaf.

The specific objectives of the study are to:

- Evaluate the antihypertensive activities of the crude methanol leaf extract of *P. americana* on systemic arterial blood pressures and heart rates of anaesthetised rats
- Partition the active crude methanol leaf extract with hexane, dichloromethane, ethyl acetate and *n*-butanol and perform bioassay guided evaluation of the activity of the fractions on systemic arterial blood pressures and heart rates of anaesthetised rats
- Identify and further purify the most active fraction using column chromatography.
- Determine the mechanism of action of the most-active pooled column fraction(s) on rat isolated thoracic rings.
- Evaluate the effect of the fraction on inhibition of angiotensin-converting enzyme (ACE) in vitro.
- Evaluate the effect of the fraction on the lipid profile of hyperlipidemic rats

- Determine the phytochemical constituents of the most active column fraction using Gas Chromatography-Mass Spectrometry (GC-MS) technique.

CHAPTER TWO

LITERATURE REVIEW

2.1 The human heart and function

The human heart is both a mechanical and electrical organ that serves the function of pumping oxygen and supplements to body tissues and transporting unwanted items to their organs of elimination. It comprises four chambers namely the right and left atria as well as the right and left ventricles of the heart. The human heart also functions by way of two components: the mechanical and the electrical components. The mechanical component pumps the blood, while the electrical component controls the rhythm (Silver, 2001). The two components must work in synchrony with each other to perfuse the body adequately with blood.

Whenever the heart beats, it pushes blood towards the vessels carrying blood to all body organs, perfusing the tissues with oxygen and supplements. The driving force imparts pressure on the vascular walls, the magnitude of which depends on cardiac output which is the quantity of blood pushed into the vessels by the heart, and the peripheral vascular resistance (PVR) which is the resistance by the vessels against blood flow (Chien, 2000). Therefore, blood pressure (BP) is the force exerted by blood on the lumen of the arteries as it is pushed from the heart (Silver, 2001). If the PVR is high due to extrinsic factors, then the heart will have to pump with greater force to propel blood through the vessels. An extrinsic factor such as sympathetic nervous system activity activates both the electrical and mechanical activities of the heart, resulting in higher cardiac output (CO). Blood pressure is thus elevated due to increased PVR and/or increased CO.

2.1.1 Blood pressure measurement

Arterial pressure is estimated in millimeters of mercury (mmHg) and recorded as two numbers, generally one over the other. The number above is the systolic, which indicates the highest pressure in the arteries when the heart contracts and fills the vessels with blood. The number underneath is the diastolic pressure, which represents the lowest pressure in the arteries between the pulse when the heart muscle is relaxed (Abel, 2015).

2.1.2 Determinants of blood pressure and control

Blood pressure must be controlled to permit continuous perfusion of all vital organs. Thus, even transient disturbance in blood flow to the cerebrum may cause unconsciousness, and longer interruptions may cause death of unperfused tissues.

However, higher pressures which supply blood beyond metabolic need provide no added advantage but a risk to vital organs (Abel *et al*., 2015).

Hypertension is the most widely recognized cardiovascular disorder in advanced and upcoming nations, afflicting around one billion individuals worldwide, as well as contributing significantly to the burden of death. Hypertension also accounts for approximately 7.1 million deaths annually (James *et al.*, 2014). Furthermore, there is a global increase in the prevalence of high blood pressure (Alhawassi, *et al*., 2015). Additionally, hypertension also contributes significantly to the burden of health in sub-Saharan African region. Despite this situation, it is worrisome that only one half of sufferers know their blood pressure status in the sub-region. This is why there are many undiagnosed or uncontrolled cases in the community (Musinguzi and Nwuaha, 2013; Gwatudde *et al.* 2015).

What is more, its prophylaxis, recognition and control measures in the sub region are haphazard. This results from absence of facilities and human services frameworks, non-existent successful preventive techniques at populace levels, absence of feasible medication profile, and hindrances to adherence with recommended prescriptions. Furthermore, structural and financial factors identified with poverty directly affect adherence to recommended antihypertensives (Iwelunmor *et al*., 2014).

Achieving acceptable clinical cardiovascular outcomes have been ascribed with effective management of blood pressure, especially in sufferers with comorbidities like chronic kidney failure and thyroid diseases (Lewington *et al*., 2002; Bromfield and Muntner, 2013). Nevertheless, accomplishing the prescribed objective of blood pressure recording of less than140/90 mmHg in all hypertensives and less than 130/80 mmHg in patients with comorbidity of hypertension and diabetes is rather difficult (O'Brien *et al*., 2007). Various studies including the Framingham Heart Study have showed that a 2 mmHg drop in diastolic pressure will possibly bring about approximately 14% reduction in the danger of stroke as well as transient ischaemic attack plus a 6% diminishment in the danger of coronary artery disease (Wright *et al*., 2014).

2.1.3 Epidemiology of Hypertension

Generally, in terms of non-communicable diseases which include cancer, chronic lung disease, diabetes and heart diseases, there is no discrimination between affluent and poor countries as these illnesses have assumed prominence as world's foremost cause of death (Ogihara *et al*., 2010).

Technical reports have indicated that globally, hypertension affects close to 30% of adults and that approximately 57% of mortality from strokes and 24% of deaths from coronary artery disease are consequences of hypertension (Chobanian, 2003). Reports have also shown that hypertension is the fourth and seventh cause of sudden death in both advanced and upcoming nations respectively (Abel *et al*., 2015). Current projection is that by the year 2030, the annual death toll from hypertension would have reached 23.5 million people (WHO, 2013).

However, hypertension can be prevented and one of the objectives considered by specialists is a sizeable decrease in the population of individuals with hypertension. Unfortunately, despite its high incidence the awareness of this disease among the populace is still poor (Wu *et al*., 2016).

2.1.4 Classification of hypertension

Hypertension has been classified by the High Blood Pressure-Joint National Committee Treatment Guidelines (Chobanian *et al*., 2003) as:

Table 2. 1: Stages of Hypertension

Stage	Systolic Blood Pressure (mmHg)		Diastolic Blood Pressure (mmHg)
Normal	<120	and	<80
Prehypertension	120-139	and/or	80-89
Hypertension, Stage 1	140-159	and/or	90-99
Hypertension, State 2	≥ 160	and/or	≥ 100

[Sourced from Joint National Committee 7 Guidelines (JNC7)]

2.1.4.1 Classification of Hypertension by cause

Essential Hypertension

This denotes elevated blood pressure with no principal underlying cause. It is the most predominant type of hypertension accounting for between 90% and 95% types of hypertension (Roger *et. al*., 2012). In this case, even though no direct cause has been identified, hypertension is said to be sequel to intricate interplay of genetic and environmental influences. Additionally, other factors identified with escalating occurrence of essential hypertension comprise sedentary lifestyle, stress, alcohol intake (Djousse, 2009), potassium deficiency (hypokalemia), obesity (Wofford and Hall, 2004), sodium sensitivity (Lackland and Egan, 2007), vitamin D deficiency, aging, family history of hypertension (Blumenthal, *et al*., 2010), elevated serum renin level, sympathetic nervous system over activity, insulin resistance and consuming foods containing high fructose, e.g., corn syrup (Segura and Ruilope, 2007).

Secondary hypertension

Secondary hypertension is an outcome of a discernible cause such as a disease of the heart, arteries, kidneys or the endocrine system. Thus, treating the primary cause usually leads to a resolution of hypertension.

In these conditions, elevated blood pressure is a consequence of a shift in the physiological mechanism, for example, the endocrine system which regulates the

hormone that controls blood plasma volume and cardiac function. Also, in Cushing syndrome, cortisol is produced in excess by the adrenal gland (Beevers, *et al*., 2001). Apart from these, hypothyroidism, renal disease, hyperthyroidism, toxaemia of pregnancy and some congenital defects have also been known to cause secondary hypertension.

Resistant Hypertension

Resistant hypertension is defined as the blood pressure which requires a minimum of four drugs from different antihypertensive classes. In resistant hypertension, blood pressure stays above objective irrespective of simultaneous utilization of 3 antihypertensive drugs of various groups, including a diuretic, and all being prescribed at their usual correct doses (Bromfield and Munter, 2013).

2.1.5 Signs & symptoms of hypertension

Most cases of high blood pressure usually pass undetected in the initial periods of the disease because the condition is usually asymptomatic during these stages. Identification is usually through screening or often picked up on routine hospital visits. In most cases, treatment also eludes many people diagnosed in resource-poor countries and successful control of the illness takes a long time. However, hypertension is now frequently associated with headache, bleeding from the nose, dizziness, shortness of breath, blurring of vision and pain in the chest. Nevertheless, the aforementioned symptoms are not specific to hypertension and may be identified with related tension instead of the hypertension itself (Go, 2013). It is however dangerous to ignore them because of increased risk of fatal consequences. Studies have shown that increased blood pressure also increases the chances of detrimental consequences to vital organs of the brain, eyes and kidneys (Carretero and Oparil, 2000; Ford, 2011). This is why hypertension has been described as a silent killer and everyone should be encouraged to have routine check of their blood pressure.

2.1.6 Pathophysiology of hypertension

Of all the numerous mechanisms involved in the pathology of essential hypertension, the most common involves disturbance in the haemodynamic system of the body, especially in the renin-angiotensin-aldosterone system (Carretero and Oparil, 2000), and/or disordered sympathetic nervous system function (Lohmeier, *et al*., 2010; Tank and Wong, 2015).

In addition, damage to the endothelium resulting from reduced production of nitric oxide coupled with inflammatory vascular diseases could raise peripheral resistance leading to damage to the vessels. Furthermore the role of interleukin 17 is gaining importance in the synthesis of other immune mediators of relevance in hypertension (Versari *et al*., 2009).

Total peripheral resistance is responsible for increased pressure while cardiac output remains normal in most people with primary hypertension. Some grown-ups with prehypertension or 'marginal hypertension' have high cardiovascular yield, a raised heart rate and common peripheral resistance, named hyperkinetic marginal hypertension (Asekun-Olarinmoye *et al*., 2013). These people ultimately progress to essential hypertension as they advance in age because of the fall in their cardiac output with an accompanying increase in peripheral resistance. The rise in peripheral resistance is majorly due to contraction of the minor blood vessels and decreased vessel thickness. Hypertension is likewise connected with diminished compliance in the veins that may ultimately result in impaired cardiac function (Tank and Wong, 2015)

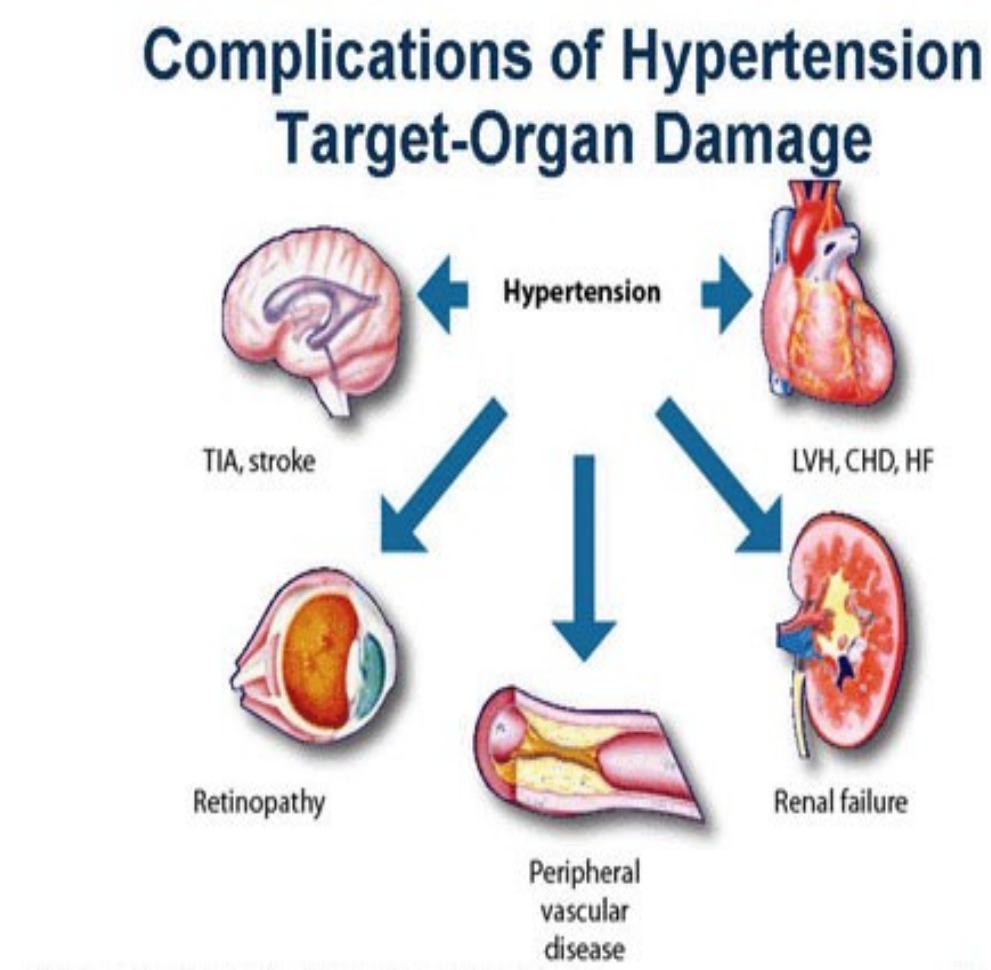
Pulse pressure (the difference between systolic and diastolic pressure) is often raised in the elderly with hypertension. This can imply that systolic pressure is unusually high, yet diastolic might be normal or low - a condition named disconnected systolic hypertension (Chobanian, 2007). The high pulse pressure in older hypertensives or isolated systolic hypertension is sequel to a rise in vascular rigidity which normally goes with old age and might be exacerbated by hypertension (Ford, 2011).

2.1.7 Complications of hypertension

Hypertension can cause severe damage to blood vessels in the brain, heart and kidney and lead to an increased incidence of renal failure, coronary disease, heart failure, stroke, and dementia. Thus hypertension is a risk for coronary heart disease, cardiovascular accidents, cardiac hypertrophy, aortic dissection and renal failure (Mancia, 2013). The prevention of hypertension is therefore important in reducing the risk of these debilitating diseases.

In 2012, the World Health Assembly endorsed an important new health goal- the 25 by 25 goal which is aimed at reducing avoidable mortality from non-communicable diseases (NCDs) by 25% in the year 2025 (Hernandez-Villa, 2015). Unfortunately, due to the obstacles to achieving this goal, chronic non-communicable diseases still remain largely unknown of all the conditions that undermine fate of human wellbeing and prosperity world-wide.

As hypertension is interconnected with cardiovascular ailment, this has prompted hypertension being the main source of harmful cardiovascular results, because of patients living unsuspectingly with uncontrolled hypertension. Therefore in Africa, hypertension is the main source of heart failure; though at worldwide levels, hypertension is accountable for more than half of deaths arising from stroke and coronary artery disease (WHO, 2018).



Transient Ischaemic Attack (TIA); LVH, Left Ventricular Hypertrophy; CHD, Coronary Heart Disease; HF, Heart Failure

Complications of Hypertension (Source; Medscape)

Fig 2.1: Schematic diagram of complications of hypertension

2.1.8 Diagnosis of hypertension

Estimations of blood pressure should be recorded for a few days before a conclusion of hypertension can be made. Hence, determination of hypertension depends on the repeat of constant elevated blood pressures and needs three distinct sphygmomanometer estimations at one month to month intervals (Padwal *et al*., 2007). Ideally, blood pressure is recorded two times a day - early in the day and night. Two back to back estimations are taken, with at least one minute interval and with the individual in sitting position with the arm supported. Blood pressure measurement should be made to the nearest 2 mmHg, and repeated after 5 minutes of rest if the first recording is high (WHO, 2003). The fundamental appraisal of the hypertensive patient ought to incorporate an entire history and physical examination. Self-checking of blood pressure is prescribed for hypertension in patients where recording equipment are available. There are electronic, mercury and aneroid devices that are available to measure blood pressure (WHO, 2003).

As mercury is harmful, it is suggested that mercury apparatuses be replaced with electronic appliances. Aneroid appliances, for example, sphygmomanometers could be utilized provided that they are calibrated twice a year and clients are taught to use them to measure blood pressure. In the event that hypertension is identified early it is likely to limit the danger of heart disease, stroke and renal failure.

Immediately hypertension is diagnosed, attempt should be made at identifying the underlying cause based on the presenting symptoms and other risk factors. It has been documented that renal disease is the foremost reason for paediatric hypertension while, in young adults with primary hypertension multiple risk factors such as a positive family history and obesity are the common causes (Hemmelgarn *et al*., 2006).

2.1.9 Laboratory tests

Laboratory examinations should be considered to distinguish likely bases for auxiliary hypertension, and to decide if hypertension has damaged other vital organs. Besides, further examinations for elevated lipids and diabetes should be done as these are additional hazards for hypertension that may necessitate intervention.

Measurement of serum creatinine is an invaluable indicator of renal disease which may either cause or result from hypertension. However, because serum creatinine results may give misleading results when used alone, it is advisable to use predictive equations to support the estimation of glomerular filtration rate (Chobanian *et al*., 2003). In addition, accurate assessment of the glomerular function offers an

opportunity to obtain primary information on renal activity which can predict the presence of the adverse effects of certain drug used to lower elevated blood pressure. Urine sample tests also give added indication to envisage diseases of the kidney.

Electrocardiogram (EKG/ECG) testing is done to verify that the heart is under stress from hypertension. It might likewise indicate left ventricular hypertrophy or whether the heart has encountered an earlier minor aggravation, for example, a quiet heart attack.

A chest X-ray or an echocardiogram may likewise be done to search for indications of cardiac hypertrophy or injury to the heart (O'Brien *et al.*, 2007).

2.1.10 Prevention

Over the years, the disease has continued to be the foremost avoidable hazard for unexpected death. And the risk of cardiovascular diseases and its attendant morbidities have been on the increase. It is therefore imperative that countries employ effective population strategies to lessen the consequences of hypertension and diminish the requirements for antihypertensive medication. The following routine modifications have been suggested for the primary prevention of hypertension by the British Hypertension Society (Weber *et al.,* 2014).

- Maintenance of normal body weight for adults (e.g. body mass index 20-25 kg/m^2)
- Reduction of dietary sodium intake to <100 mmol/ day
- Engaging in regular aerobic physical activity such as brisk walking daily, at least 3 times a week
- Limiting alcohol consumption to no more than 3 units/day in men and no more than 2 units/day in women
- Consuming a diet rich in fruit and vegetables.

2.2 Management of hypertension

2.2.1 Non-Pharmacologic Approach: Lifestyle modifications

The initial approach to the treatment of hypertension is lifestyle modifications. This method of treatment indicates preventive lifestyle changes comprising weight reduction, dietary changes, physical exercise, moderating alcohol consumption and relaxation therapy (Go *et al.*, 2013). Not only have these measures remarkably controlled mild cases of hypertension, they have also been found useful in conjunction with the use of medication for the treatment of hypertension.

To maintain compliance with a therapeutic regimen, adequate precaution must be taken to ensure that the intervention does not diminish the value of life. Therefore, it is advisable to avoid drug treatment in patients whose hypertension can be satisfactorily maintained with slight modification in daily activity and diet as there is

no drug that is completely free of adverse effects. Additionally, non-drug approaches which involve active participation of patients in their own therapy are well known to achieve better results. Thus, salt restriction, weight reduction, reduced alcohol intake and regular isotonic exercise have given positive results in reducing raised blood pressures and improving drug efficacy in the treatment in hypertensive patients (Lawes *et al*., 2008).

Weight Reduction

Extracellular fluid expansion sequel to reabsorption of Na^+ in the kidneys is positively correlated with the incidence of hypertension in obese patients. However, obese hypertensives have been known to benefit tremendously from weight reduction irrespective of their status of salt consumption (Chobanian *et al*., 2003). Because of the difficulty in maintaining loss of weight, it is advisable to combine physical exercise with counselling on diet in order to ensure compliance (Bacon *et al*., 2004; Blumenthal *et al*., 2010).

Sodium Restriction

Diets low in sodium content is generally good for optimum healthy living (Whelton *et al*., 2012). Compliance with severe salt restriction is very tedious for non-hospitalized patients. However, this approach has been of great benefit in most hospitalized hypertensive patients and patients with mild hypertension. Salt restriction has also been associated with better response to drug therapy in hypertension (Frohlich *et al*., 2005; Strom *et al*., 2013).

Alcohol Restriction

Increased risk of hypertension and cerebrovascular accident correlate with heavy intake of alcohol. Better outcomes have been observed in alcohol restriction (Whelton *et al*., 2012).

Physical Activity

Chobanian *et al*., (2003) have noted that inadequate physical activity is related with increased frequency of hypertension, whereas improved exercise reduced the occurrence of cardiovascular illness in males. Also reported by these researchers is an approximately 10 mmHg reduction in systolic and diastolic pressures.

Relaxation Therapy

Empirically, whereas long-term stress has been known to cause sustained hypertension in animal models, relaxation therapy was equally found to reduce the blood pressure of some hypertensive patients. This approach is useful in patients with

mild hypertension who also require close monitoring and supported with drugs if need be (Ford, 2011).

Potassium Therapy

It has been proposed that if the dietary consumption of potassium is increased this will likely lower blood pressure by augmenting Na^+ elimination, suppressing renin secretion and decreasing sensitivity to endogenous vasoconstrictors (Chobanian *et al*., 2003). Also, in hypertensive rats, supplementation with K^+ decreased the frequency of blood pressure and cardiovascular accidents. Therefore it has been suggested that except in patients on ACE inhibitors, diets rich in K^+ and adequate Na^+ could be of great benefit in effectively controlling hypertension (Blumenthal *et al*., 2010).

2.2.2 Pharmacologic therapy

Drugs decrease blood pressure by actions on critical cardiovascular parameters and classification of antihypertensive preparations are based on sites of action or mechanisms. The principles of treatment are to bring the blood pressure to as near the normotensive range as possible without producing undue side-effects. The objectives of treatment therefore are to prolong life, diminish the occurrence of complications and to lessen the burden of existing complications. Therefore, from the consideration of the various determinants of blood pressure, there are various ways of reducing blood pressure, and achieving an antihypertensive effect (Musini *et al*., 2017). However, even though taking the decision of antihypertensive medications for specific patients can be mind boggling; many motives impact decision-making (Hanselin *et al*., 2011). While rules might be valuable for achieving proper choices, it is usually hard to apply rules on the purpose of care and regularly the rules do not give adequate data on the suggested drugs. Besides, the passionate promotion of particular medications to doctors and patients can befuddle right judgement. Moreover, urging patients to keep taking medications that are often costly for an asymptomatic illness is difficult. Specialists might be hesitant to recommend and patients may likewise be hesitant to accept the quantity of drugs that might be important to correctly control the illness. For these and different reasons, perhaps close to half of the patients on treatment for hypertension may not accomplish the right therapeutic objectives of blood pressure control (Ding *et al*., 2013).

2.2.2.1 Major classes of anthihypertensive drugs

Diuretics:

Diuretics are agents that cause increased flow of urine and loss of Na^+ and Cl^- ions. They are very important in high blood pressure management and are useful as single agents or when combined with other antihypertensives where they influence the usefulness of almost every other drug (Chobanian *et al*., 2003).
Diuretics in low doses practically reduce the danger of mortality, stroke and other heart diseases, with thiazides being the most frequently used. The combined use of loop diuretic agents and potassium-sparing antihypertensives is known to reduce the side effect of hypomagnesemia and potassium depletion. With the clinical use of diuretics, particularly potassium-sparing diuretics, the threat of unexpected death becomes significantly lessened. For example, with long term use of spironolactone, heart failure patients are now known to live longer. Bendroflumethiazide 2.5mg or cyclopenthiazide 0.5mg are typical daily doses. Furosemide 40mg or bumetanide 1 mg daily are also employed (Calhoun *et al*., 2008; Hanselin *et al*., 2011)

Beta Blockers:

This group of drugs is valuable in managing mild to moderate hypertension. Also in cases of severe hypertension, they offer benefit in averting reflex tachycardia which usually follows direct vasodilator use. However, they are no longer used to start antihypertensive management, except in cases where their use is particularly indicated use e.g. angina pectoris. Currently their clinical applications include, low dose β-blockers, frequently in combination with other drugs in the effective management of hypertension. The side effects of β-blockers comprise muscle fatigue, depression, muscle tremor, and decreased libido. Examples of β-blockers are propranolol, metoprolol, atenolol, bisoprolol and labetolol (Beever *et al*., 2001; Lindholm *et al*., 2005; Varon, 2008).

Calcium-Channel Blockers:

The calcium channel blockers comprise two classes of antihypertensives. These are the dihydhropyridines and the non-dihydropyridines. Examples of the dihydropyridines include nifedipine, felodipine and amlodipine, while the examples of the non-dihydropyridines are verapamil and diltiazem. Reduction in peripheral vascular resistance is common to the two groups, even though, verapamil and dilitiazem also possess negative chronotropic and ionotropic effects. Clinically, the choice of drug for each patient depends on the haemodynamic variances that exist between different groups of calcium channel blockers. However, nifedipine has the side effect of initiating tachycardia and spontaneous sensitization of the sympathetic system while analogues of amlodipine are safer in this respect (Kojima, 2004). Inhibitors of calcium channels are of good use in older patients and even as single drugs, in managing Raynaud's illness, peripheral vascular sickness and asthma because

β-blockers are contraindicated in such patients. Oral short-acting nifedipine is powerful in severe hypertension yet patients ought to be observed for the danger of extreme hypotension. Diltiazem and verapamil can be valuable when hypertension exists together with angina, however they may precipitate bradycardia (Zhou *et al*., 2002; Ozawa *et al*., 2006).

Angiotensin Converting Enzyme Inhibitors (ACEIs):

The ability of the ACEIs to lessen the levels of angiotensin II (an intense vasoconstrictor) is a significant improvement for managing hypertension. This group of antihypertensive agents is by and large utilized as first line treatment because of their safety record, moderately few untoward effects and contraindications, aside from cases of bilateral artery stenosis. Angiotensin converting enzyme inhibitors are drugs of first choice in patients who have both diabetes and hypertension as these drugs slow down the development of renal dysfunction that usually accompanies diabetic nephropathy. In hypertensive patients who also have cardiac failure, angiotensin converting enzyme inhibitors are likewise first line drugs. Examples of ACEIs are captopril, ramipril, and Lisinopril (Kobori *et al*., 2003; Navar, 2010).

Angiotensin Receptor Blockers:

This group of drugs blocks angiotensin AT_1-receptors, thus promoting vasodilation, increased sodium and water excretion and a decrease in cardiac output, hence their use as antihypertensive drugs. Examples of drugs in this class include candesartan, losartan and valsartan. They have the added advantage of causing fewer cough episodes. By blocking the effects of angiotensin II, ARBs relax smooth muscle and promote vasodilation, increase renal salt and water excretion, reduce plasma volume and decrease cellular hypertrophy. Adverse effects of ARBs include hypotension, hyperkalemia, and renal artery stenosis (Heran *et al*., 2008; Catanzaro & Frishman, 2010).

Direct Vasodilators:

Vasodilators act by relaxing blood vessels to improve blood flow and decreasing blood pressure. Hydralazine and minoxidil are directly acting vasodilator antihypertensive agents. Hydralazine, the prototype vasodilator causes direct relaxation of the smooth muscles of the arteries. However, hydralazine-induced vasodilation causes a powerful stimulation of the sympathetic nervous system, resulting in side effects which include hypotension, dizziness, increased heart rate and contractility, fluid retention, increased plasma renin activity and lupus syndrome, and hirsutism associated with minoxidil. Thus, their clinical use in the management of hypertension has declined tremendously in the past two decades. Vasodilators are however more effective when administered with β- adrenergic receptor antagonists which prevent unwanted compensatory responses by the heart (Duprez & Cohn, 2006; Cohn *et al*., 2010).

Centrally-Acting Adrenergic Antagonists:

These are agents whose antihypertensive effect is mediated through reduction in sympathetic outflow from vasomotor centres in the brainstem. They include methyldopa, clonidine and dexmedetomidine. Methyldopa is a false neurotransmitter as well as α_2-adrenoceptor agonist while clonidine and dexmedetomidine are agonists at halfway found α_2-adrenoceptors. Both clonidine and dexmedetomidine stabilise circulation, decrease the discharge of catecholamines triggered by pressure, and cause sedation. For this reason, dexmedetomidine is currently utilized for sedation in special care units (James *et al*., 2014).

2.2.2.2 Adjuvant Drug Therapy

Aspirin

This antiplatelet drug is a potent means of reducing cardiovascular risk but its prolonged use may be associated with spontaneous bleeding, particularly, intracerebral haemorrhage, in a small patient population. The benefits, however, outweigh the risk in hypertensive patients aged 50 years and above whose blood pressure are well controlled (Nawarskas *et al*., 1999).

Statins

It has been found out that effective treatment of hyperlipidemia can produce significant and substantial reduction in cardiovascular risk and effectively control hypertension. Statins are cholesterol-lowering agents. They reversibly inhibit HMG-CoA reductase which is the enzyme that catalyses the first committed step in cholesterol biosynthesis. Statins have therefore found use in patients diagnosed with diseases of the vessels and heart. Examples of statins include atorvastatin, simvastatin and lovastatin (Rizzo *et al*., 2012).

2.3 Medicinal plants in therapy

Natural products, for example, unadulterated plant parts or refined products offer infinite chances to develop innovative medicines as a result of unequalled accessibility of different chemicals in plants (Al Disi *et. al.,* 2015). At present a growing number of people across the globe depends on herbal remedies for their immediate need. Plants used as traditional remedies comprise an extensive variety of ingredients which are now being utilized in treating many ailments (Jaarin *et al*., 2015). It was thus discovered that a great many phytochemicals from plants are indeed pharmacologically active. However, it is essential to prove the effectiveness of bioactive compounds to confirm the claim by users. Therefore, clinical trials are on-going focused on studying the pharmacological parameters of new drug candidates.

2.3.1 Herbal remedies with antihypertensive properties

In the recent past, researchers have investigated the claim that some plants possess blood pressure-lowering potentials. The therapeutic benefits of a number of these plants were authenticated while many others are still being studied. Also people

living outside urban cities have extolled the usefulness of a number of these plants as effective home grown cure (Kang *et al.,* 2002). Be that as it may, more logical research still should be done to confirm the adequacy and explain the safety profile of such natural cures in hypertension.

Some plants with antihypertensive activities are as follows:

Allium sativum

Allium sativum, also called garlic belongs to the Liliaceae family. Garlic is locally known as "Lahasun" among the Hindi (India) while the Yoruba (Southwest Nigeria) and Hausa (Northern Nigeria) know it as "Ayu", among whom it is commonly used as a food condiment. Its effects on cardiovascular disorders, particularly hyperlipidemia and blood pressure-lowering potential through increased nitric oxide production, resulting in smooth muscle relaxation and vasodilatation are well known. Previous studies have revealed that the principal active compound responsible for the distinctive aroma and healing benefits is called allicin (Reid *et al.,* 2008; Adeneye, 2014).

Apium graveolens

Popularly known as Celery, *A. graveolens* seed was reported to cause a reduction in the systolic and the diastolic pressures. Fruit juice mixture from celery and vinegar was also used to relieve headaches, dizziness and shoulder pain linked to hypertension (Gharooni and Sarkarati, 2000).

Cassia occidentalis

Commonly known as Coffee weed, *Cassia occidentalis* (family: Caesalpiniaceae) is utilised as a folkloric medication to lower elevated blood pressure. Investigations of the leaf have displayed a relaxant effect on rat aorta. These investigations confirmed the relaxant effect and the ability of *C. occidentalis* to block calcium ions and thereby decrease BP (Ajagbonna *et al*., 2001).

Cuscuta reflexa

Gilani *et al.* (1992) showed that crude extract of *C. reflexa* caused a decrease in systolic and diastolic blood pressures of anaesthetised rats significantly depending on the dose administered. They also demonstrated that the reduction in HR was noticed at higher doses. These cardiovascular responses were not altered by pre-treatment with atropine (1mg/kg).

Fuchsia magellanica

Fuchsia magellanica is a plant native to Southern Argentina and Chile. Reports have it that infusion of the extract has antipyretic, antidiuretic and blood pressure lowering effects. Investigations on the ethanol or aqueous extracts of *F. magnellanica*

also caused pronounced decreases in the mean arterial pressure of normotensive rats (Al Disi *et al*., 2015).

Hibiscus sabdariffa

H. sabdariffa is one of the most widely researched plants for its cardiovascular effects. Researchers have reported the antihypertensive properties of the plant parts including the direct relaxation effect of the petal crude extract on smooth muscles of rat aorta (Mojiminiyi *et al*., 2000). Acetylcholine and histamine dependent mechanisms have been postulated to be responsible for the direct vasorelaxant effects. In addition, prolonged use of *H. sabdariffa* extract was said to have caused a reversal of cardiac hypertrophy in renovascular hypertensive rats (Al Disi *et al*., 2015).

Moringa oleifera

Moringa oleifera (family Moringaceae) leaf extract has been shown to cause a brief decrease in systolic blood pressure, diastolic blood pressure and mean arterial pressure in a dose-dependent fashion. This lowering effect was apparent in heart rate at high doses with slight bradycardia (Anaka *et al.,* 2009).

Phyllanthus amarus

P. amarus has been ascribed with diuretic and blood pressure-lowering effects. In a study, Amaechina and Omogbai (2007) reported a significant reduction on the cardiovascular parameters of normotensive male rabbits treated with intravenous injection of *P. amarus* leaf extract. The lowest blood pressure lowering effect was exhibited at 5 mg/kg dose while 80 mg/kg dose gave the maximum response. The depressant effect in diastolic blood pressure was higher than those of systolic blood pressure (Amaechina and Omogbai, 2007).

Theobroma cacao

It is popularly found in Chocolate or Cocoa butter and is rich in flavonoids. The high flavonoid content of coca powder has been attributed with prevention of cardiovascular diseases. Flavonoids have been known to stimulate nitric oxide synthesis and to cause vasodilation and decrease endothelial dysfunction. Research also has it that a reduction of about 5 mmHg in systolic and 3 mmHg of diastolic blood pressure have been ascribed with consumption of dark or milk chocolate (Blumenthal *et al,* 2010).

Zingiber officinale

Ginger (family Zingiberaceae) acts to improve circulation and relax vascular smooth muscles. In different animal paradigms administration of crude extract of ginger has induced dose-dependent reduction in blood pressure, rate and force of vascular contractions and elicited cardiodepressant activity. These cardiovascular

effects have been attributed to blockage of voltage-dependent calcium channels (Fugh-Berman, 2000).

Fig 2.2: ***Persea americana*** **fruits and leaves**

Description of *Persea americana*

(1) Taxonomy / Classification

Kingdom – Plantae
Subkingdom – Tracheobiophyta
Superdivision – Spermatophyta
Division – Magnoliophyta
Class – Magnoliopsida
Subclass – Magnoliidae
Order – Laurales
Family – Lauraceae
Genus – Persea
Species – *Persea americana*
Authority: Miller

(2) Synonym(s)

Laurus persea L, *Persea drymifolia* Schlecht. and cham, *Persea gratissima* Gaertn.f., *Persea nubigena*

(3) Common Names

English: alligator pear, avocado, avocado-pear, butter fruit
Yoruba: Igba/apoka
Igbo: Ube-beke

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 (Adeyemi *et al*., 2002; Owolabi *et al*., 2010). The plant seed is also abundantly rich in catechins, procynidins, hydroxycinnamic acids and flavonol glycosides (Adeyemi *et al*., 2002). In addition, studies also revealed that its 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 (Ojewole and Amabeoku, 2006).

Furthermore Adeneye (2014), affirmed that in animal studies there were observed pronounced decreases in average cardiovascular parameters of hypertensive rats when *P. americana* aqueous leaf extract was injected intravenously. These effects were said to be mediated via endothelium-dependent vasodilatory mechanism. These results were in agreement with *in vitro* studies which showed that the aqueous leaf extract of the same plant at 25-800 mg/ml caused concentration-dependent relaxation of isolated thoracic aortic rings of rats pre-contracted with noradrenaline as well as concentration-dependent negative ionotropic and chronotropic effects on guinea pig isolated atrial muscle strips (Ojewole *et al*., 2007). Related whole animal studies also showed that oral administration of 200, 500 and 700 mg/kg of the aqueous seed extract of *P. americana* Mill. to 8% salt-induced hypertensive Wistar rats for 5 weeks resulted in profound reductions in the mean systolic blood pressure from 166.00 ± 5.20 mmHg (in the untreated 8% salt-induced hypertensive rats) to 145.00 ± 2.74 mmHg, 125.10 ± 3.54 mmHg and 91.40 ± 5.54 mmHg in 200, 500, and 700 mg/kg *P. americana* Mill. extract-treated rats, respectively (Imafidon and Okunrobo, 2009). Similarly, the plant seed extract significantly reduced the mean diastolic blood pressure from 128.60 ± 4.16 mmHg to 76.68 ± 5.68 mmHg, 65.00 ± 3.75 mmHg and 56.56 ± 4.74 mmHg at 200, 500 and 700 mg/kg of the plant extract, respectively (Imafidon, 2009). Similar effects were observed in normotensive Sprague Dawley rats and hypertensive Wistar rats treated with the plant extract by Anaka and co-workers (2009) and Imafidon and Amaechina, (2010) respectively.

2.4 Some selected methods of induction of hypertension

2.4.1 Noradrenaline induction method

Noradrenaline (NA), also called norepinephrine (NE), is an organic chemical catecholamine that functions in the brain and body principally as a neurotransmitter and to a lesser extent as a hormone. It is an endogenous catecholamine with direct alpha and beta adrenergic receptor agonist activity, whose major site of storage and release are the neurons of the sympathetic nervous system (Louis *et al*,. 1969). The binding of noradrenaline to α- and β-adrenergic receptors (or adrenoceptors) in the blood vessels triggers vasoconstriction of both capacitance and resistance vessels and increases blood pressure. Blood pressure is further raised by noradrenaline as a result of its effects on the heart muscle, which increase cardiac output. In addition, the rat aorta is known to respond biphasically to noradrenaline (NA) and the two components of the contraction (phasic followed by tonic) are mediated by adrenoceptors (Champlain *et al.,* 1967). For these reasons, noradrenaline can be used as a candidate for studying hypertension in experimental animal models.

2.4.2 Nitric oxide inhibition method

Nitric oxide (NO) is a vasodilating factor synthesized in vascular endothelial cells by conversion of L-arginine to NO and L-citrulline by the enzyme Nitric Oxide Synthase (NOS) (Moncada, 1992). There are three identified NOS isoforms; Neuronal

(nNOS or NOS1) and Endothelial (eNOS or NOS3 or cNOS) isoforms which are constitutive (cNOS) and calcium-dependent; whereas, the last one is inducible (iNOS or NOS2) and Ca^{2+} independent (Pacher *et al.,* 2007, Choudhari *et al*., 2013). Endothelial Nitric Oxide Synthase (eNOS) produces nitric oxide in blood vessels and plays a role in regulation of vascular function, cardiac function, angiogenesis, insulin secretion and airway tone. The nitric oxide in blood vessels is produced in physiologic amounts in normotensive animals to enable the endothelium oppose the vasoconstriction produced by sympathetic nervous system activity.

However, administration of L-arginine analogues such as N^G-L- arginine methyl ester hydrochloride (L-NAME) inhibits nitric oxide synthase (NOS) activity and hence nitric oxide biosynthesis, leading to hypertension (Beratova *et al.,* 1999). Therefore, treating rats with L-NAME causes metabolic distruption to the vascular endothelium, and this model is widely used to study hypertension, as well as cardiovascular and kidney diseases (Ribeiro *et al*., 1992). L-NAME causes endothelium-dependent contraction in arteries with intrinsic basal tone in rats and inhibits endothelium-dependent relaxation to a variety of agonists (Toba *et al*., 2005). This is why assessment of nitric oxide is a relevant model in the study of hypertension.

2.4.3 Angiotensin converting enzyme inhibition method

Angiotensin converting enzyme (ACE) is a zinc-containing, nonspecific dipeptidilpeptidase. It cleaves the His-Leu from the C-terminal of the decapeptide angiotensin I to octapeptide angiotensin II. Angiotensin II is a potent vasoconstrictor which plays a major role in the regulation of blood pressure and also increases aldosterone secretion which leads to reabsorption of water and salts in kidneys. In addition, ACE inactivates bradykinin, a vasodilator. In this manner, hindrance of ACE prompts abatement in angiotensin II generation, a rise in bradykinin and thus a reduction in blood pressure (Chen *et al*., 2009). ACE inhibitors, consequently, form a very important group in the control of hypertension.

There are different *in vitro* systems for the assessment of ACE inhibitory activity. James *et al*., (2014) applied a modification of the spectrophotometric technique developed by Cushman and Cheung in 1971. In this strategy, the measure depends on the hydrolysis of hippuryl-L-histidyl-L-leucine (HHL) by ACE. The quantity of hippuric acid formed is found by estimating the absorbance at 228 nm, the absorption maximum of hippuric acid. The distinction between absorbance in the absence and presence of inhibitor is relative to the inhibitory action of tested sample.

2.4.5 Lipid profile measuring method

Hypertension has been identified to be commonly associated with other cardiovascular risk factors, one of which is dyslipidemia.

Dyslipidemia has been described as high plasma concentrations of lipids - triglycerides (TG) and total cholesterol (TC) and their blood transporting lipoproteins; HDL- Cholesterol, LDL-Cholesterol, VLDL-Cholesterol. This therefore indicates that there is a link between dyslipidemia and hypertension. Since dyslipidemia is important in the progression of hypertension, then treating dyslipidemia should have some effect on BP (Halperin *et al*., 2006); hence the relevance of the study of lipid profile in hyperlipidemia induced in rats.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant material

3.1.1 Plant collection

Fresh leaves of *P. americana* were harvested from University of Ibadan, in April, 2016 and dried at room temperature. The leaves were afterwards identified by Mr. Donatus Eseimokhuai, curator University Herbarium as those of *P. americana* Mill. (family: Lauraceae) with identification number UIH2238. A voucher specimen of the plant has also been deposited in the Botany Departmental Herbarium.

3.1.2 Extraction procedure

Room air-dried leaves of *P. americana* were milled in a Waring commercial blender. One and a half (1.5) kg of the powdered leaf was macerated in methanol/distilled water (95%/5%) and extracted twice, on each occasion with 3.5 L of the solvent at room temperature for 48 hours, with occasional shaking. The combined extracts were concentrated to dryness at 40 ±1°C in Buchi Labortechnik® rotary evaporator Model CH-9230 (Switzerland) under reduced pressure to finally give 158.5 g (10.6 % yield) of a light-brown, powdery leaf extract. The extract was then partitioned into n-hexane, dichloromethane, chloroform, ethyl acetate and n-butanol fractions. The ethyl acetate fraction was further purified by column fractionation using isocratic solution. Aliquot portions from the methanol extract and the different fractions were weighed and dissolved in physiologic saline for use on each day of the experiments in evaluating the antihypertensive effects of extract and fractions from *P. americana* leaf.

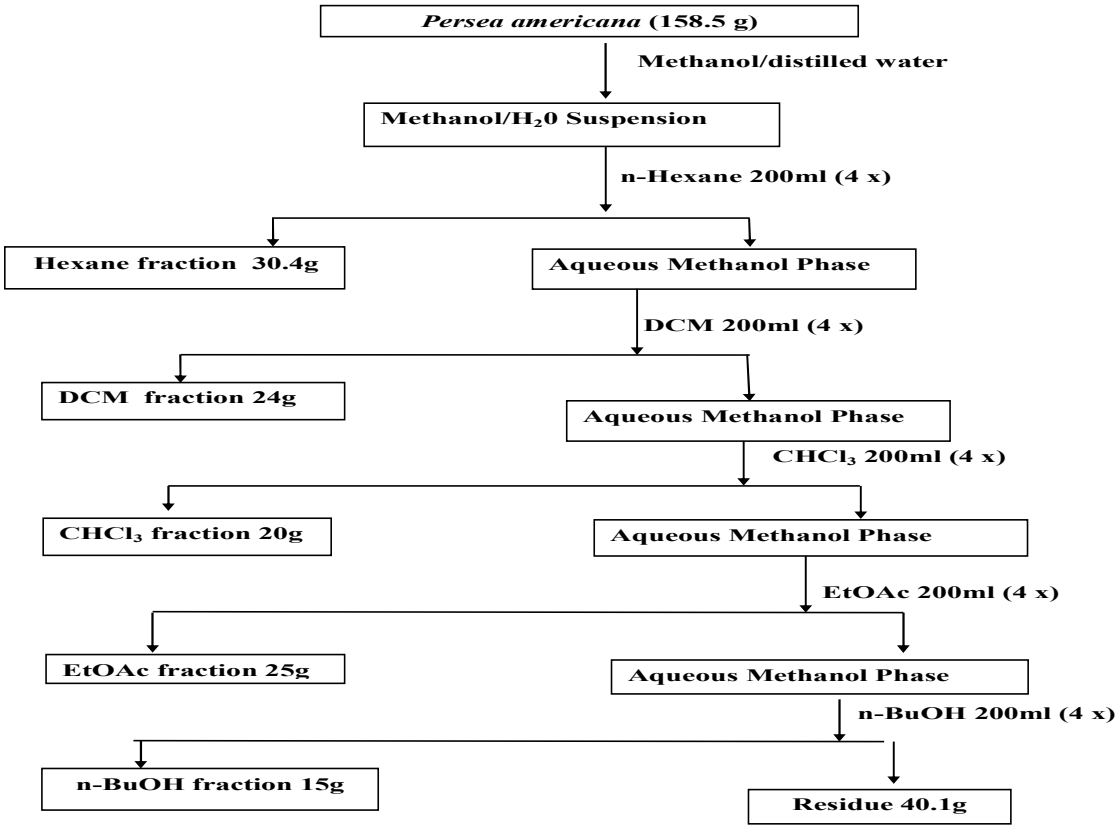


Fig 3.1: Schematic diagram of Partitioning of crude methanol extract of *P. americana*

3.2 Experimental animals

Healthy male Wistar rats (*Rattus norvegicus*) 250–300g were used for the study. The rats were housed within laboratory specified settings of light, humidty and temperature. They were also provided with water and food (standard pellet diet) *ad libitum*. They were permitted to adapt for two weeks before commencement of experiments. Each rat was fasted for 18 hours prior to being used for each experiment. The study protocol was approved by University of Ibadan, Animal Care and Use Research Ethics Committee (UI-ACUREC) No: UI-ACUREC/17/0071.

3.3 Equipment

(a) Rotary Evaporator: Buchi Labortechnik® rotary evaporator Model CH-9230 (Switzerland).
(b) Physiopolygraph: World Precision Instrument 4-channel Physiopolygraph Model Lab Trax-4/16. Sarasota, Florida, USA.
(c) PowerLab®: ADINSTRUMENT PowerLab 26T Model ML4856.
(d) CODA non-invasive blood pressure data acquisition system. Model CODA®: 2096 Kent Scientific, Torrington CT, USA.

3.4 Drugs and Chemicals

Adrenaline, (-)-noradrenaline (Sigma, USA); Labetalol, Phentolamine, Acetylcholine (Sigma, England), heparin (5IU/mL), N^G-nitro-L-arginine methyl-ester (L-NAME) (BDH Chemicals Ltd, Poole, England), Diazepam (Hoffman-La Roche, Switzerland), Captopril (Alpha Laboratories Ltd., India), Ketamine, Atorvastatin, Angiotensin Converting Enzyme (ACE) and Hippuryl-L-Histidyl-L-Leucine (HHL) (Sigma, USA).

3.5 Preparation of working solutions of extract, fractions and standard drugs

Suspensions of the extract and fractions from *P. americana* (100 mg/kg and 25 mg/kg respectively) were prepared fresh on each day of the experiment, using Dimethyl sulphoxide and physiological saline as vehicle. The drug solutions for the other drugs were prepared fresh in physiological saline.

3.6 Evaluation of the antihypertensive effect of crude methanol extract and fractions of *P. americana* leaf on systemic arterial blood pressures and heart rates of anaesthetized rats

The antihypertensive effect of the crude methanol extract and fractions of *P. americana* leaf were measured according to the modified method of Ojewole *et al*., (2007). 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 the administration of the plant extract and reference 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, maintained at 37 ± 0.5°C with an electric lamp placed over the abdomen.

The rat was allowed to stabilize for about 20 min after which were recorded systemic arterial pressures. The effect of *P. americana* methanol leaf extract (PAM) and fractions on noradrenaline (4.0 µg/kg)-induced raised blood pressure was tested on systemic arterial blood pressure and heart rates of the rats. Because PAM and other drugs used were dissolved in normal saline, rats treated with normal saline (2 ml/kg iv) were used as control under the same experimental conditions.

3.7 Evaluation of the activity of the four fractions on systemic arterial blood pressures and heart rates of anaesthetized rats (n=20)

The active methanol extract of *P. americana* leaf was partitioned into hexane, dichloromethane, ethyl acetate and *n*-butanol fractions. The antihypertensive activities of the resulting fractions were evaluated. Twenty rats were used with five rats for each of the four fractions, using the method explained above.

3.8 Further purifying the most active fraction using column chromatography

The ethyl acetate fraction was further fractionated using silica gel (size 60-200µm) column chromatography and extracted with isocratic solvent (EtOAc 15: Chloroform 8: Methanol 2: H_2O 0.5). The column was then washed with methanol 100%. About 200 fractions were collected which were pooled into four, based on their appearance on thin layer chromatography giving CFEt1, CFEt2, CFEt3 and CFEt4 column fractions

3.9 Evaluation of the antihypertensive activity of the pooled ethyl acetate column fractions on systemic arterial blood pressures and heart rates of anaesthetized rats

Five rats were used for each of the pooled column fractions. The effect of the fractions alone and with noradrenaline (4.0 µg/kg iv) on systemic arterial blood pressure and heart rates of the anaesthetized rats were also evaluated using the method described in 3.7 above. Each rat was injected with 25 mg/kg of the fraction and the systemic blood pressure and heart rate were recorded. For the effect of the fraction on raised blood pressure, noradrenaline (4.0 µg/kg) was given after the fraction was injected.

3.10 Evaluation of the effect of the most active ethyl acetate column fraction (CFEt3) on L-NAME-induced increase in blood pressure of rats

Hypertension was induced in rats through a 7-day intraperitoneal administration of L-NAME 80 mg/kg/day (Jaarin, 2015). Rats were then treated with intraperitoneal injection of CFEt3, 25 mg/kg for 7 days. Rat BP and heart rate were measured using a computerised non-invasive system (model CODA®; 2096 Kent Scientific, Torrington, CT, USA) which measures tail BP by means of volume pressure. In this method, the rats were restrained on a preheated platform with the tail exposed. A cuff that occluded the rat tail vein and a cuff for recording volume pressure were fixed on the tail. Afterwards, twenty (20) cycles of recordings were made for each rat to obtain rat blood pressure. Care was taken to ensure that the rats were stable before commencing the recordings which were made by the same person under similar environmental conditions.

3.11 Effect of the extract and most-active ethyl acetate column fraction (CFEt3) on angiotensin-converting enzyme (ACE) in vitro

The ACE inhibitory activity was estimated by a modification of the method of Vermeirssen, *et. al*, 2002. In this experiment, 50 µL of PAM or CFEt3 fraction (the inhibitor solution) and 50 µL of the enzyme were pre-incubated at 37 ^{0}C for 10 min. The substrate-200 µL Hippuryl-L-Histidyl-L-Leucine (HHL) was then added and the reaction was allowed to run for 90 min and later terminated by the addition of 250 µL 1N HCl. The hippuric acid (HA) formed was removed with 0.5 mL ethyl acetate and then centrifuged for 15 minutes at 3000 revolutions /min. From this, 0.2 mL ethyl acetate supernatant layer was put into a bottle. The hippuric acid formed was dissolved again in 1.0 mL distilled water and the absorbance was measured at 228 nm with

Jenway 7315 (UK) ultraviolet/visible spectrophotometer. Percentage inhibition of angiotensin converting enzyme activity was determined using the formula:

% ACE inhibition = [B-A/B-C] x100, where

A = Absorbance of HA generated in the presence of ACE inhibitor

B = Absorbance of HA generated without ACE inhibitor

C = Absorbance of HA generated without ACE

HA = Hippuric Acid

Captopril 100 µg/mL solution served as a positive control for the inhibition of the activity of angiotensin converting enzyme.

3.12 Determination of the mechanism of action of the ethyl acetate column fraction on rat isolated thoracic rings

The rats were euthanized with pentobarbital 120 mg/kg. The thoracic aorta was quickly and carefully extracted, extraneous tissue was removed and then sliced into 3-4mm in width in a petri dish. The rings were held in an organ bath containing Krebs-Henseleit physiological solution, kept at 36 $\pm 1^0$C and consistently bubbled with carbogen (95% O_2 + CO_2). The tissue was allowed to equilibrate before being challenged with graded concentrations of noradrenaline, PAM or CFEt3. The contractile and/or relaxant effect of all the reference drugs, as well as PAM/CFEt3-induced relaxations of the isolated rings were recorded by Powerlab®.

3.13 Evaluation of the effect of PAM and CFEt3 on lipid profile

Thirty-five rats were distributed into 7 groups (n=5): Triton X100- induced hyperlipidemic rats (100 mg/kg/day for 72h; intraperitoneally) treated with normal saline (10 mL/kg), atorvastatin (10 mg/kg), PAM (50 and 100 mg/kg), CFEt3 (25 mg/kg), non-hyperlipidemic rats treated with normal saline and PAM (100 mg/kg).
The blood for serum lipid profile was collected in plain bottles. Serum total cholesterol (TC) and triglyceride (TG) had been decided by way of enzymatic estimation while high density lipoprotein cholesterol (HDL-C) was assessed with the aid of enzymatic estimation after precipitation. Low Density Lipoprotein-Cholesterol (LDL-C) was assessed from the values of the aforementioned by means of Friedewald's formula as follows: VLDL = TG ÷ 5, LDL = Total Cholesterol (TC) – (VLDL + HDL). Atherogenic index was calculated by using base 10 logarithm of the ratio of TG to HDL, components = log (TG/HDL-C) (Dobiasova and Frohlich, 2001)

3.14 Determination of the phytochemical constituents of the most active pooled column fraction using Gas Chromatography-Mass Spectrometry (GC-MS)

The ethyl acetate fraction of the methanol leaf extract of *P. americana* was analysed using GC-MS Agilent Technologies 7890 system mass spectrometric detector (Agilent technologies 5975 model). The GC analysis is a separation technique where there are two phases – the mobile phase and the stationary phase. The mobile phase is

the carrier gas (Helium, 99.99% purity), while the stationary phase is the column. The column is of length 30 m, internal diameter 0.320 mm, while the thickness is 0.25 µm (Thomas *et al*., 2013). The oven was programmed at an initial temperature of 80^0C to hold for 1 minute and to increase by 100 ^{0}C per minute up to the final temperature of 240 ^{0}C to last for 6 minutes. The fraction was injected at a volume of 1µL and detector temperature of 250 ^{0}C. The ionization voltage was 70eV and the mass spectral scan range was 45-500 MHz.

3.15 General procedures for measurement of blood pressure in rat

3.15.1 Non-invasive recording of rat blood pressure

Blood pressure parameters were estimated non-intrusively in rats by the method of Jaarin *et al.,* (2015) utilizing CODA® data acquisition system. An observing cuff was put on the tail close to the rat's body to pick changes in the flow of blood taking place on releasing the cuff. Each rat was placed in a suitable plastic restrainer before commencement of recording so as to enhance ease of measurement. The rat was then placed on a warmer platform for 15 minutes to build up blood stream to the tail. At least 20 estimations were taken for each rat and the mean was used for analysis.

3.15.2 Invasive recording of rat blood pressure

Invasive recording of rat blood pressure denotes direct estimation of pressure in the arteries through surgery whereby a saline-filled cannula is inserted into the vessel. It is the widespread technique used to compare the precision of other non-surgical blood pressure measuring methods. In this study, BP measurements were obtained from anaesthetized Wistar rats.

Materials

The materials comprise the following: adult Wistar rats weighing 250-300g, heparin injection, ketamine injection, diazepam injection, a cannula, needles and syringes (1 mL, 5 mL and 10 mL), 3 inch as well as 5 inch artery forceps, blunt and pointed 5 inch artery forceps, a bulldog clamp, toothed and non-toothed 5 inch Adson analyzing forceps. Others are 18 G needles, scalpel, small-sized insertion tubes, a string, surgical plaster and a source of illumination.

Instruments

Sphygmomanometer and a physiograph with a pressure transducer.

Physiopolygraph

The heart rate of each rat was recorded with a physiopolygraph. The main constituents comprise a console, couplers, pressure transducer (DELTRAN® model DPT-100; Utah Medical Products, Inc. Midvale, USA) with a chart drive.

Data acquisition system

The gadget obtains signals which it then transforms into numerical data. Its primary constituents incorporate an analog input system, pressure transducer with a solitary utility blood pressure arch.

Pressure transducer calibration

Calibrating the pressure transducer is one of the necessary steps for the authenticity and validation of the experiment. Calibration was effected using a known pressure level of a pressure manometer. The pressure transducer of the physiography was coupled with the cuff of the manometer and the cuff was thereafter inflated to the known pressure level. This was used to calculate the conversion factor.

Methods

An overnight fasted rat was anesthetized with ketamine/diazepam (60/5 mg/kg, i.p.). Animal reflexes were tested, and it was placed on an appropriate flat surface which was not electrically conductive. The operation site was then shaved and sterilized with alcohol.

Method for femoral vein cannulation

The right thigh was slit and the femoral vein and nerve were identified. A cannula was introduced through the vein and 0.2ml heparin in saline was used to flush the vein for prevention of clotting.

Method for Tracheostomy and carotid artery cannulation

The neck of the rat was carefully opened and a Y-piece adapter and a rodent tracheal intubation tube were inserted into the trachea and secured in place. Bronchial secretions were discharged slowly using a small polyethylene tube. The carotid artery and the vagus nerve were isolated and cleaned taking care not to excite the nerve and then separated from one another. The end of the artery distal to the heart was ligated while the part proximal to the heart was clamped for cannulation. One end of a cannula was implanted in the carotid artery while a three-way stopcock was connected to the other. The cannula was secured inside the artery with a thread, without impeding blood flow. It is from this end that the parameters were obtained. Haemostasis was maintained, the clamp was slowly removed and a dressing was placed on the operation site.

Baseline recordings were made while system stabilization lasted for 20 minutes. Each time a fraction or reference drug was administered, 0.2 mL of saline was flushed through the femoral vein. The values were allowed to return to baseline before

commencing the next round of tests so as to avoid the response of other drugs interfering with the result.

3.16 Statistical Analysis

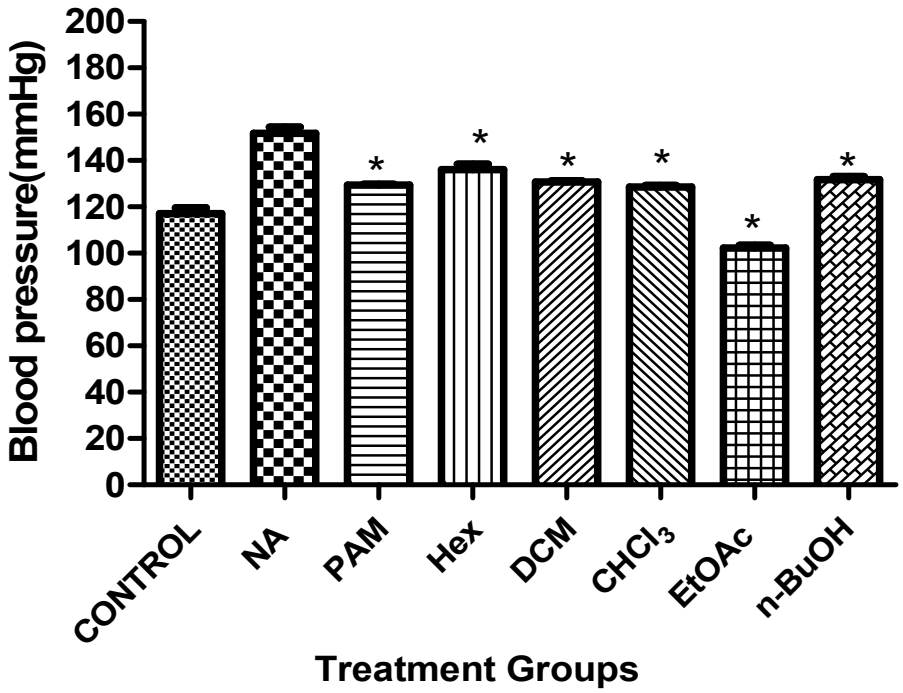
Statistics were stated as Mean ± Standard Error of Mean. Some outcomes were examined utilizing Student t-test. Test doses were compared with control values via one-way analysis of variance (ANOVA) and post-hoc Dunnett's Multiple Comparison Test. Test doses were also compared with the standard drugs (labetalol and phentolamine). P-values less than 0.05 ($p < 0.05$) were considered statistically significant while analysis was performed using GraphPad® prism version 5.0 software

CHAPTER FOUR

RESULTS

4.1: Effect of methanol extract and fractions of the leaf of *Persea americana* on noradrenaline-induced raised systolic blood pressure in rats

The intravenous injection of noradrenaline (4.0 µg/kg) raised the Systolic Blood Pressure (SBP) of rats from 117.0 ± 2.6 mmHg to 151.8 ± 2.6 mmHg. This raised SBP lasted for about 1min before coming down to the resting SBP in about 3min. The extract and fractions of *Persea americana* leaf obtained using various solutions were also tested on systolic blood pressure. The mean systolic blood pressure value for ethyl acetate fraction (100 mg/kg) was 102.2 ± 1.2 mmHg compared to that of noradrenaline (151.8 ± 2.6 mmHg). The values for other fractions ranged between 128 and 136 mmHg (Fig.4.1). The reduction in SBP caused by ethyl acetate fraction was more pronounced than those of other fractions.

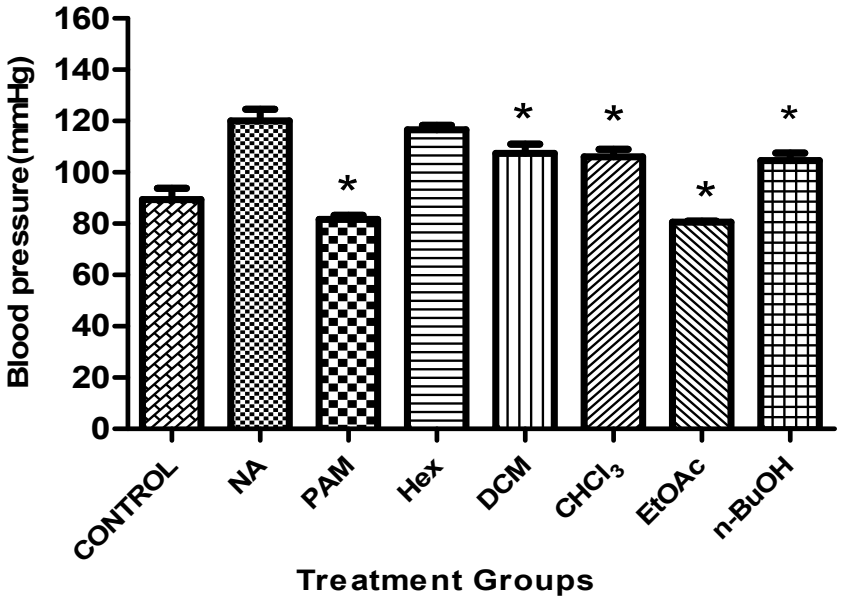


NA- Noradrenaline 4.0 µg/kg, PAM - *Persea americana* leaf methanol extract 100 mg/kg, Hex - Hexane 100 mg/kg, DCM- Dichloromethane 100 mg/kg, $CHCl_3$- Chloroform 100 mg/kg, EtOAc- Ethyl acetate 100 mg/kg, *n*-BuOH - *n*-butanol 100 mg/kg. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test. * $p < 0.05$.

Fig 4.1: Effect of methanol extract and fractions of the leaf of *Persea americana* on noradrenaline-induced raised SBP in rats

4.2: Effect of noradrenaline, methanol extract and fractions of the leaf of *P. americana* on diastolic blood pressure of rats

Noradrenaline (4.0 µg/kg) raised the diastolic blood pressure (DBP) from 89.4 ± 4.4 mmHg to 120.0 ± 4.6 mmHg. Figure 4.2 shows that the extract, Dichloromethane 100 mg/kg (DCM) 107.3 ± 3.7 mmHg; Chloroform 100 mg/kg ($CHCl_3$) 106.0 ± 3.0 mmHg; EtOAc (100 mg/kg) 80.7 ± 0.3 mmHg and *n*-Butanol 100 mg/kg (*n*-BuOH) 104.7 ± 2.9 mmHg fractions significantly reduced the DBP of rats when compared with noradrenaline. However, while the hexane 100 mg/kg (Hex) fraction elicited no observable effect on DBP, the effect of PAM 100 mg/kg (81.7 ± 1.7 mmHg) was similar to that of EtOAc (80.7± 0.3 mmHg).

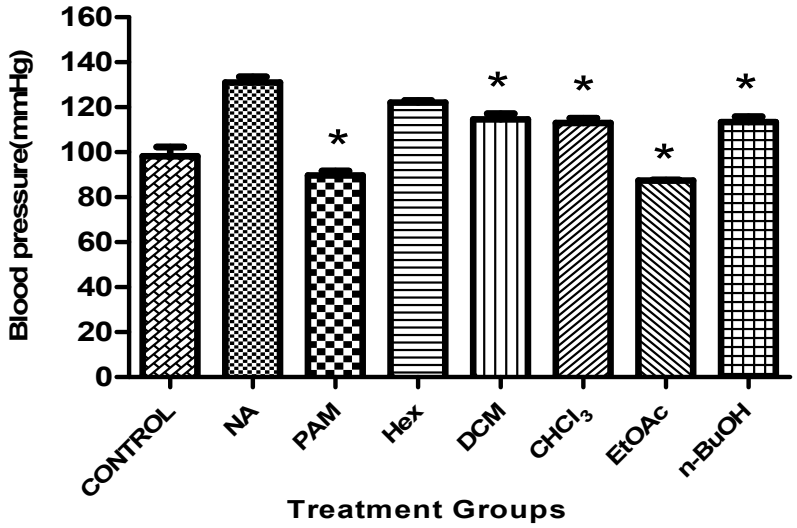


NA- Noradrenaline (4.0 µg/kg), PAM- *Persea americana* leaf methanol extract (100 mg/kg), Hex- Hexane (100 mg/kg), DCM- Dichloromethane (100 mg/kg), $CHCl_3$- Chloroform (100 mg/kg), EtOAc- Ethyl acetate (100 mg/kg), *n*-BuOH - *n*-butanol (100 mg/kg). Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4. 2: Effect of noradrenaline, methanol extract and fractions from the leaf of *P. americana* on diastolic blood pressure of rats

4.3: Effect of noradrenaline, methanol extract and fractions of the leaf of *P. americana* on mean arterial pressure of rats

Noradrenaline (4.0 µg/kg) raised the mean arterial pressure (MAP) from 98.2 to 131.0 ± 2.5 mmHg. The result in figure 4.3 shows that the extract and the fractions caused significant reductions in the mean arterial pressure (MAP) of the test animals when compared with noradrenaline. Of all the groups, EtOAc (100 mg/kg) fraction offered the best reduction in MAP (87.3 ± 0.3 mmHg), compared with noradrenaline (131.0 ± 2.5 mmHg) while hexane (100 mg/kg) fraction caused the least reduction of noradrenaline-induced increase in mean arterial pressure.

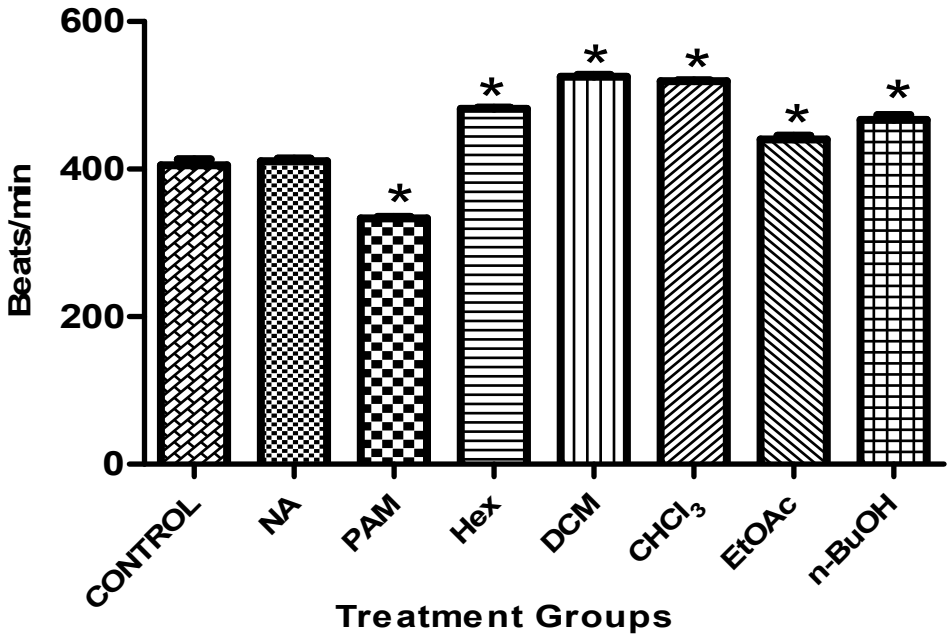


NA- Noradrenaline (4.0 µg/kg), PAM- *Persea americana* leaf methanol extract (100 mg/kg), Hex- Hexane (100 mg/kg), DCM - Dichloromethane, $CHCl_3$- Chloroform, EtOAc- Ethyl acetate, *n*-BuOH-*n*-butanol (100 mg/kg). Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.3: Effect of noradrenaline, methanol extract and fractions of the leaf of *P. americana* on mean arterial pressure of rats

4.4: Effects of noradrenaline, methanol extract and fractions of the leaf of *P. americana* on heart rate of rats

Figure 4.4 compared the effect of the methanol extract, hexane, dichloromethane, chloroform, ethyl acetate and *n*-butanol fractions on the heart rate (HR) of anaesthetized rats. The mean heart rate of the anaesthetized rats was 405 ± 8.2 beats/min but noradrenaline (4.0 μg/kg) raised the heart rate to 410 ± 3.5 beats/min. Only the methanol extract lowered the mean HR of the rats to 333 ± 1.7 beats/min from that of noradrenaline (410.7 ± 3.5 beats/min). In the other treatment groups HR was elevated above that caused by noradrenaline to between 440 ± 5.0 and 525 ± 2.9 beats/min, indicating an induction of tachycardia. This may be due to feedback mechanism induced by lowering of blood pressure or the effect of the fractions.

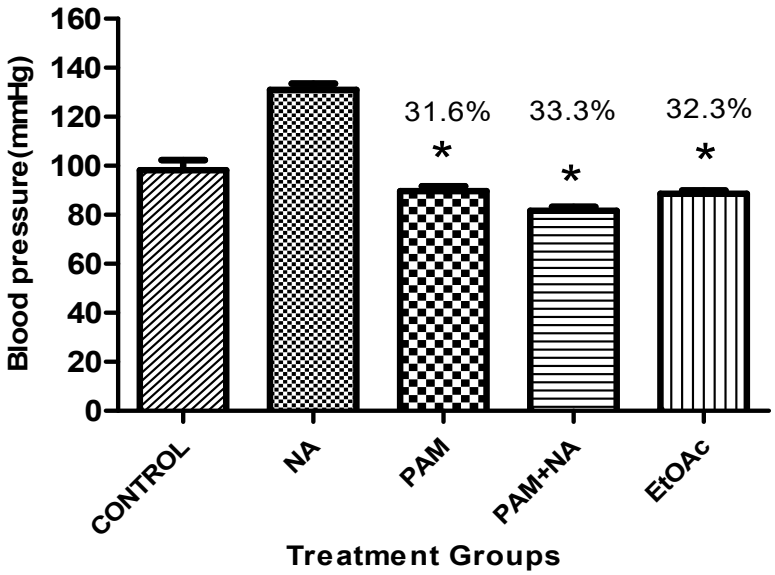


NA- Noradrenaline (4.0 µg/kg), PAM- *Persea americana* leaf methanol extract (100 mg/kg), Hex- Hexane (100 mg/kg), DCM- Dichloromethane (100 mg/kg), $CHCl_3$-Chloroform (100 mg/kg), EtOAc- Ethyl acetate (100 mg/kg), *n*-BuOH -*n*-butanol (100 mg/kg). Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.4: Effects of noradrenaline, methanol extract and fractions of the leaf of *P. americana* on heart rate of rats

4.5: Percentage reduction of mean arterial pressure of rats treated with methanol extract alone and noradrenaline

Noradrenaline (4.0 μg/kg) raised the mean arterial pressure (MAP) from 98.2 ± 4.1 mmHg to 131.0 ± 2.5 mmHg as shown in figure 4.5. However, PAM (100 mg/kg) and EtOAc (100 mg/kg) decreased the rise in MAP significantly to 89.7 ± 2.0 mmHg and 88.6 ± 1.2 mmHg respectively, representing percentage reduction of 31.6% and 32.3% respectively. In addition when administered on anaesthetized rats pretreated with PAM (100 mg/kg), noradrenaline only caused a rise of 81.7 ± 1.7 mmHg in MAP. This represents a 33.3% reduction in the activity of noradrenaline.

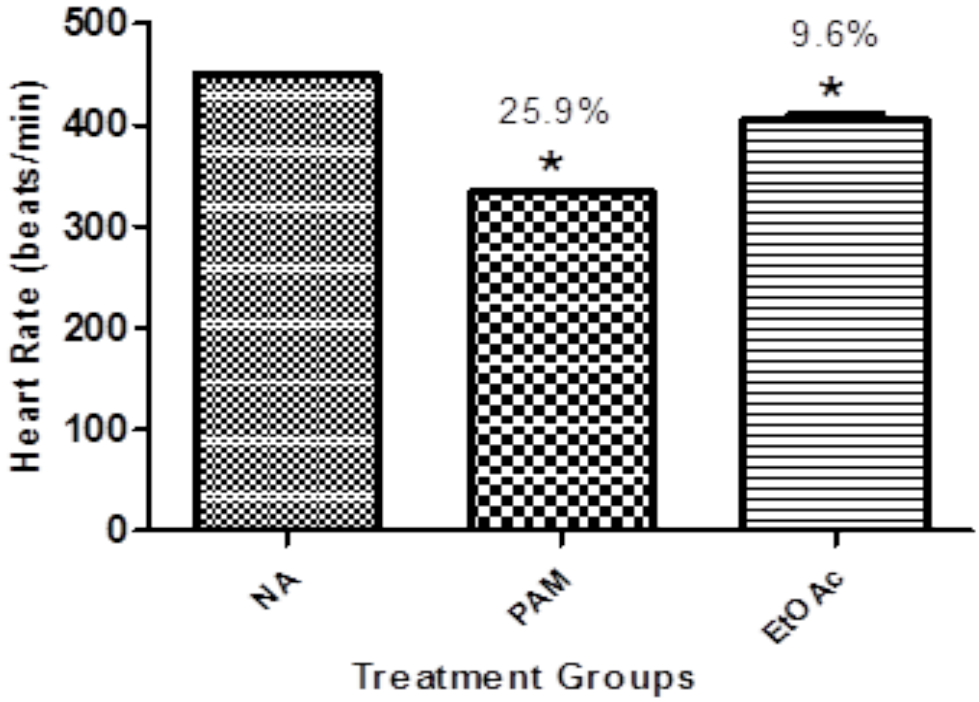


NA- Noradrenaline 4.0 µg/kg, PAM- *P. americana* leaf methanol extract (100 mg/kg), PAM+NA- PAM+ Noradrenaline 4.0µg/kg, EtOAc- Ethyl acetate fraction (100 mg/kg). Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.5: Percentage reduction of mean arterial pressure of rats treated with methanol extract alone and noradrenaline

4.6: Percentage reduction of heart rate by methanol extract and ethyl acetate fraction of the leaf of *P. americana*

Noradrenaline (4.0 µg/kg) raised the heart rate of anaesthetised rats from a mean of 405± 0.1 beats /min to 450 ± 0.1 beats/min. However, PAM (100 mg/kg) and EtOAc (100 mg/kg) reduced this increase to 333.3 ± 1.7 beats/min and 406.7 ± 4.4 beats/min respectively. These represent 25.9 % and 9.6% reduction for PAM and EtOAc respectively. Comparing the effect of PAM and EtOAc on heart rate it was observed that PAM (100 mg/kg) appeared to be more effective in causing a greater reduction in heart rate than the EtOAc fraction (100 mg/kg).



NA- Noradrenaline 4.0 µg/kg, PAM- *P. americana* leaf methanol extract (100 mg/kg), EtOAc- Ethyl acetate fraction (100 mg/kg). Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.6: Percentage reduction of heart rate by methanol extract and ethyl acetate fraction of the leaf of *P. americana*

4.7: Effect of Noradrenaline (NA) 4.0 µg/kg on rat systolic blood pressure

Figure 4.7 shows a representative tracing of the effect of intravenous administration of noradrenaline 4.0 µg/kg on rat systolic blood pressure. The treatment elicited a rise in SBP up to 151.8 ± 2.6 mmHg which lasted for about 3mins.

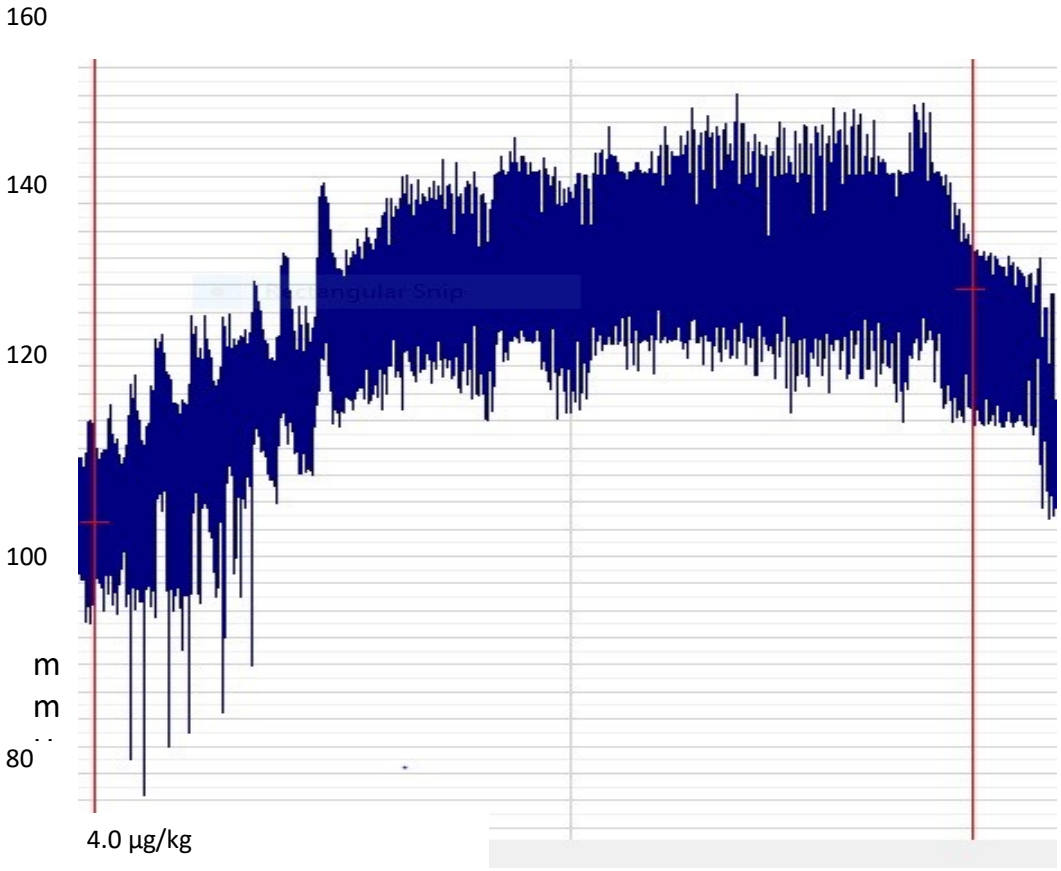


Fig 4.7: Effect of Noradrenaline (NA) 4.0 µg/kg on rat blood pressure

4.8: Effect of column fraction 3 of ethyl acetate - CFEt3 (25 mg/kg) on rat systolic blood pressure

Figure 4.8 shows that the intravenous administration of CFEt3(25 mg/kg) to anaesthetised rat effected a 20 mmHg reduction of the systolic blood pressure from about 120 mmHg to about 100 mmHg.

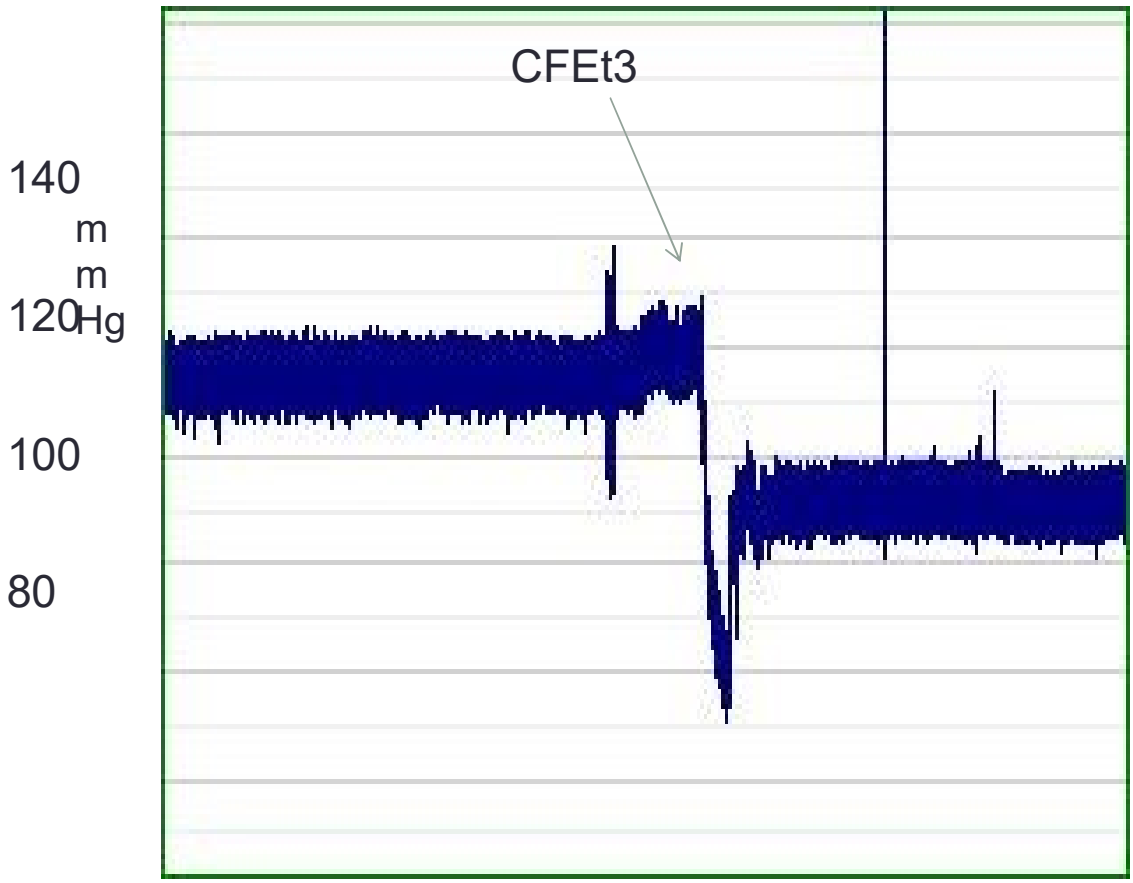
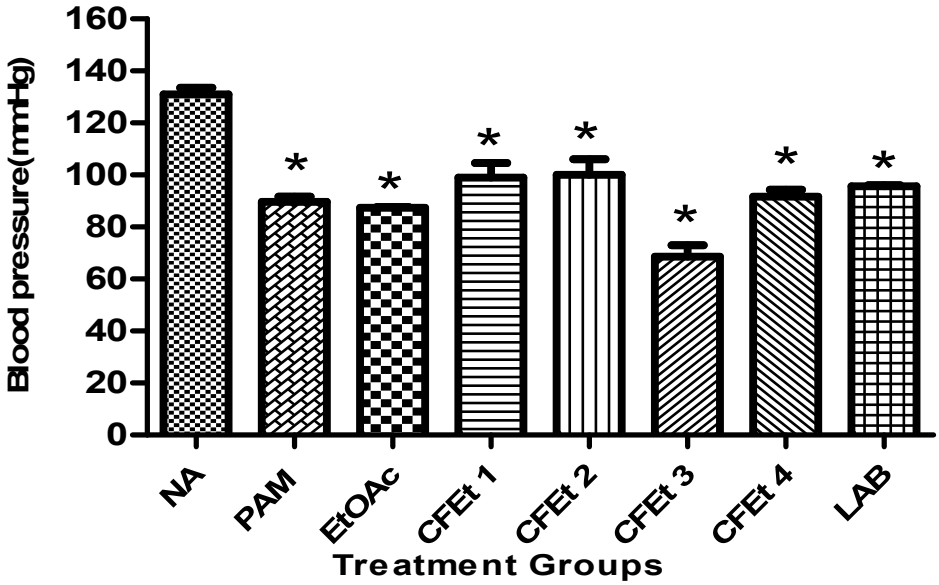


Fig 4.8: Effect of column fraction of ethyl acetate - CFEt3 25 mg/kg on rat blood pressure

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

Figure 4.9 showed the effect of ethyl acetate fraction and column fractions 1-4 compared with the standard drug labetalol on MAP. Each of the fractions reduced the Mean Arterial Pressure of rats comparable to labetalol. However, the lowering effect of EtOAc (100 mg/kg) and $CFEt_3$ (25 mg/kg)were greater than that of the standard drug labetalol. From a MAP of 131.0 ± 2.5 mmHg induced by noradrenaline, labetalol, EtOAc, and $CFEt_3$ reduced MAP to the following respectively: 95.7 ± 0.3 mmHg, 87.3 ± 0.3 mmHg and 68.7± 4.4 mmHg.

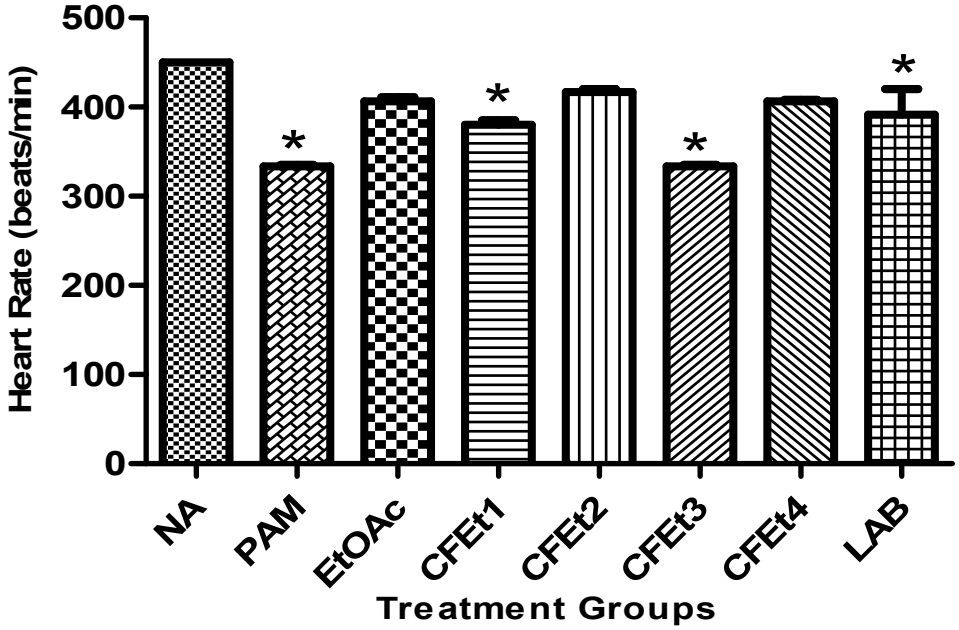


NA- Noradrenaline 4.0 µg/kg, PAM- *P. americana* leaf methanol extract (100 mg/kg), EtOAc- Ethyl acetate fraction (100 mg/kg), Ethyl acetate column fractions 1, 2, 3, 4 (25 mg/kg) LAB- Labetalol 25 mg/kg. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.9: Effect of ethyl acetate fraction, column fractions and labetalol on the mean arterial pressure of rats

4.10: Effect of PAM, ethyl acetate fraction, column fractions and labetalol on the heart rate of rats

Noradrenaline (4.0 µg/kg) raised the heart rate of anaesthetised Wistar rats from 405.0 ± 0.1beats/min to 450.0 ± 0.1 beats/min. Ethyl acetate column fraction 1 25 mg/kg (CFEt1) and column fraction 3 25 mg/kg (CFEt3) significantly reduced the heart rate to 380. 0 ± 5.0 beats/min and 333.0 ± 1.7 beats/min respectively. The results from the other groups were not statistically significant. Labetalol (25 mg/kg) that blocks both α and β adrenoceptors in the heart reduced the heart rate to 391.0 ± 28.3 beats/min. When compared with labetalol CFEt3 and CFEt1 appeared to have better, pronounced lowering effects on heart rate than labetalol – 333.0 ± 1.7 beats/min and 380.0 ± 5.0 beats/min respectively.

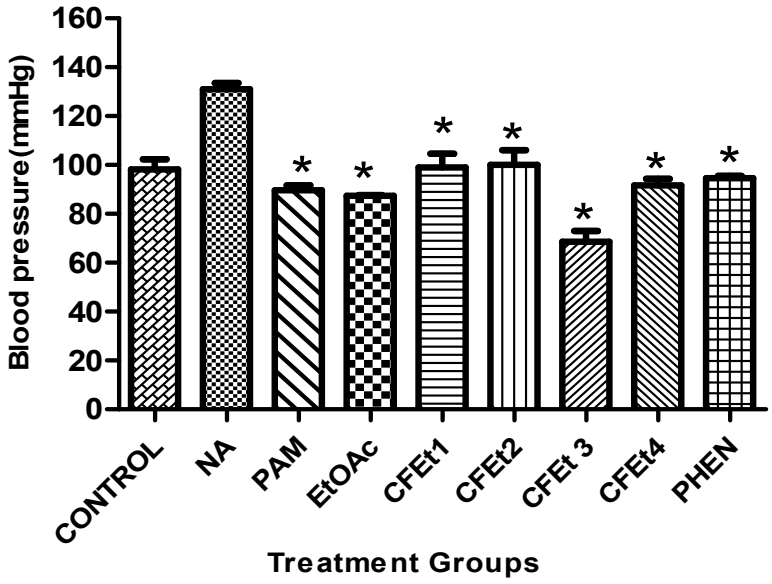


Noradrenaline 4.0 µg/kg, PAM- *P. americana* leaf methanol extract (100 mg/kg), EtOAc- Ethyl acetate fraction (100 mg/kg), Ethyl acetate column fractions 1, 2, 3, 4 (25 mg/kg), LAB- Labetalol (25 mg/kg). Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.10: Effects of ethyl acetate fraction, column fractions and labetalol on the heart rate of rats

4.11: Effect of ethyl acetate fraction, column fractions and phentolamine on the mean arterial pressure of rats

Noradrenaline (4.0 µg/kg) raised the mean arterial pressure in anaesthetised Wistar rats from 98.2 ± 4.1to 131.0 ± 2.5 mmHg. Figure 4.11 compared the effect of ethyl acetate fraction (100 mg/kg) and column fractions 1-4 (25 mg/kg) with the standard drug phentolamine (83.3 µg/kg) on MAP. Ethyl acetate fraction and the column fractions 1-4 significantly reduced MAP raised by noradrenaline. The values for the treatment groups are as follows: EtOAc (87.3 ± 0.3 mmHg), CFEt1 (99.0 ± 5.6 mmHg), CFEt2 (100.0 ± 6.1 mmHg), CFEt3 (68.7 ± 4.4 mmHg), CFEt4 ((91.7 ± 2.7 mmHg). Phentolamine also reduced the value of MAP to 94.7 ± 0.9 mmHg. Except for CFEt1 and CFEt2, the extract and other column fractions reduced MAP better than phentolamine 83.3µg/kg, an α adrenoceptor blocker. However CFEt3 exhibited the most pronounced lowering effect on MAP.

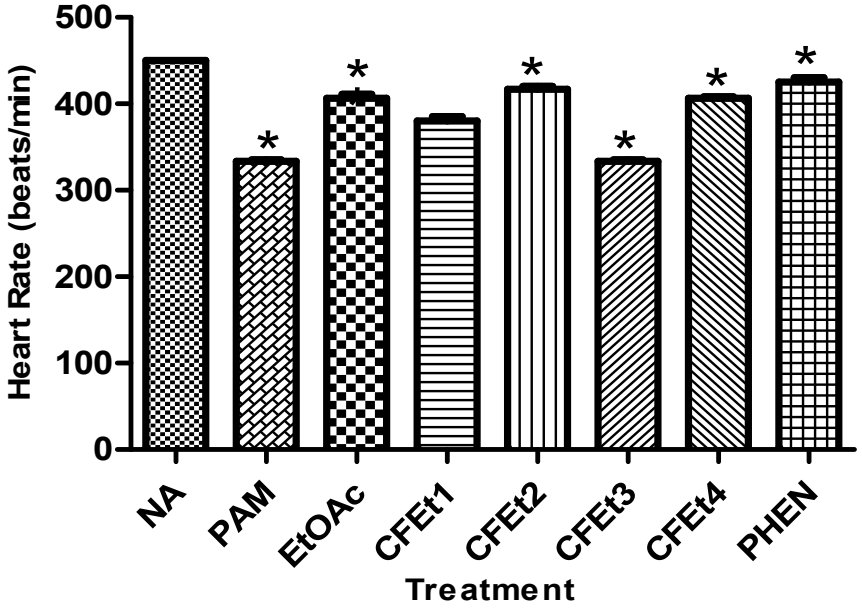


NA- Noradrenaline 4.0 µg/kg, PAM- *P. americana* leaf methanol extract, EtOAc- Ethyl acetate fraction (100 mg/kg), Ethyl acetate column fractions 1, 2, 3, 4 (25 mg/kg), PHEN- Phentolamine 83.3µg/kg. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.11: Effect of ethyl acetate fraction, column fractions 1-4 and Phentolamine on the mean arterial pressure of rats

4.12: Effect of ethyl acetate fraction, column fractions 1-4 and Phentolamine on the heart rate of rats

Noradrenaline (4.0 µg/kg) raised the heart rate of anaesthetised Wistar rats from 405.0 ± 0.1beats/min to 450.0 ± 0.1 beats/min. PAM (100 mg/kg), Ethyl acetate fraction (100mg/kg) and the column fractions CFEt1 CFEt2, CFEt3, CFET4 (25 mg/kg) and phentolamine (83.3 µg/kg) also significantly reduced the heart rate to 406.7 ± 4.4, 380. 0 ± 5.0, 417.0 ± 3.0, 333.3 ± 1.7, 406.7±1.7 and 425.0 ± 5.0 beats/min respectively. Phentolamine blocks α adrenoceptors in the heart. When compared with phentolamine and other treatment groups, PAM and CFEt3 appeared to have offered the best protection against noradrenaline induced increase on heart rate.

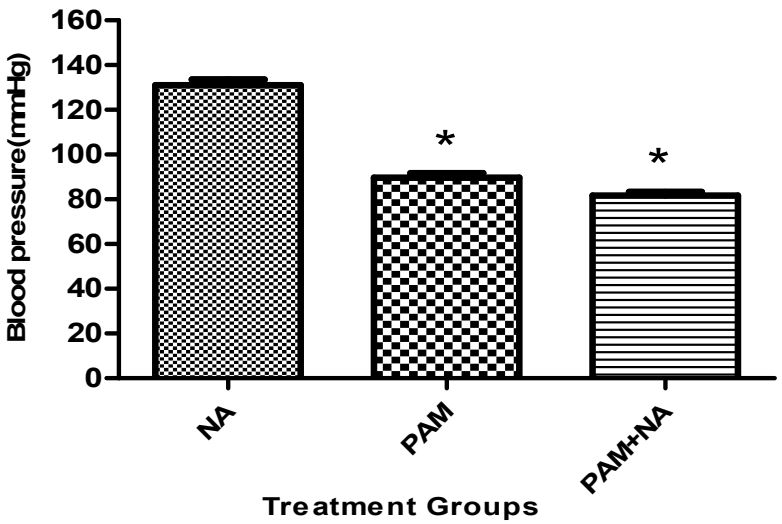


NA- Noradrenaline 4.0µg/kg, PAM- *P. americana* leaf methanol extract (100 mg/kg), EtOAc- Ethyl acetate fraction (100 mg/kg), Ethyl acetate column fractions 1, 2, 3, 4 (25 mg/kg), PHEN- Phentolamine 83.3µg/kg. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.12: Effects of ethyl acetate fraction, column fractions 1-4 and phentolamine on the heart rate of rats

4.13: Reduction in mean arterial pressure of rats treated with methanol extract alone or in the presence of noradrenaline

Noradrenaline (4.0 µg/kg) raised the Mean Arterial Pressure (MAP) to 131.0 ± 2.5 mmHg as shown in figure 4.14. However, PAM (100 mg/kg) alone and pre-treatment of the rats with PAM (100 mg/kg) decreased the noradrenaline-induced rise in MAP significantly to 89.7 ± 2.0 mmHg and 81.7 ± 1.7 mmHg respectively. This indicated that the methanol extract of the leaf of *Persea americana* either alone or as pre-treatment antagonised the elevation in MAP caused by noradrenaline.

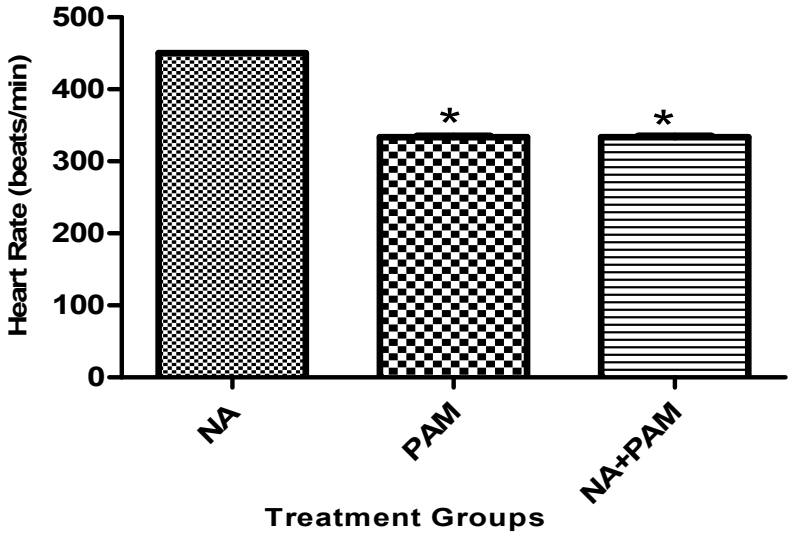


NA- Noradrenaline 4.0 µg/kg, PAM- *P. americana* leaf methanol extract (100 mg/kg), PAM+NA- PAM+ Noradrenaline. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.*$p < 0.05$.

Fig 4.13: Reduction in mean arterial pressure of rats treated with methanol extract alone or in the presence of noradrenaline

4.14: Reduction in heart rate of rats treated with methanol extract alone or rats pretreated with the extract before administering noradrenaline

Noradrenaline (4.0 µg/kg) raised the heart rate of anaesthetised rats from a mean of 405± 0.1 beats/min to 450 ± 0.1 beats/min. However, both PAM (100 mg/kg) and NA + PAM reduced this increase to 333.0 ± 2.0 beat/min. Even though the methanol extract of *Persea americana* and ethyl acetate fraction significantly decreased the heart rate of the anaethetised rats, there was no difference in the effects of the treatments when compared with one another.

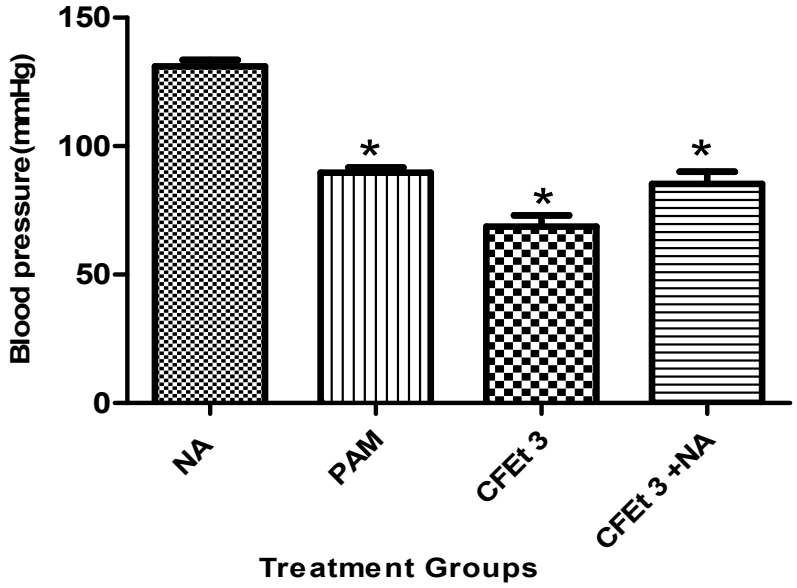


NA- Noradrenaline 4.0 µg/kg, PAM- *P. americana* leaf methanol extract 100 mg/kg, PAM+NA- PAM+ Noradrenaline. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig. 4.14: Reduction in heart rate of rats treated with methanol extract alone or rats pretreated with the extract before administering noradrenaline

4.15: Effect of noradrenaline alone or in the presence of ethyl acetate column fraction 3 (CFEt3) on mean arterial pressure of rats

Figure 15 shows the effect of noradrenaline alone or in the presence of ethyl acetate column fraction 3 (CFEt3) on mean arterial pressure of rats or when the animals were first pretreated with ethyl acetate column fraction 3 before being challenged with noradrenaline. After an initial rise of MAP from 98.2 ± 4.1 mmHg to 131.0 ± 2.5 mmHg caused by noradrenaline (4.0 µg/kg), mean arterial pressure value fell to 68.7 ± 4.4 mmHg after CFEt3 was administered alone. On the intravenous administration of CFEt3 followed by the administration of noradrenaline the mean arterial pressure value was 85.3 ± 4.7 mmHg. The result obtained showed that CFEt3 alone demonstrated a more pronounced reduction on MAP than when used as pretreatment.

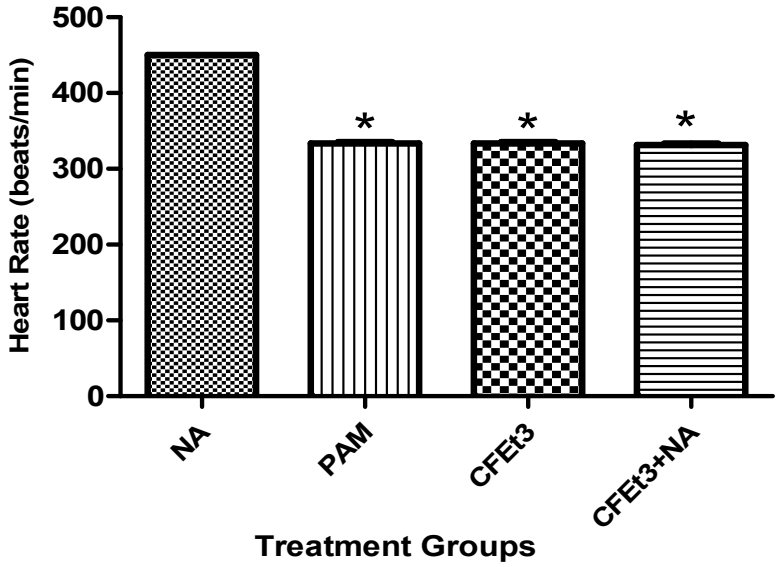


NA- Noradrenaline 4.0 µg/kg, PAM- *P. americana* leaf methanol extract (100 mg/kg), CFEt3- Column Fraction of Ethyl acetate fraction 3 (25 mg/kg), CFEt3+ NA- CFEt3+ Noradrenaline 4.0 µg/kg. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.15: Effect of noradrenaline alone on mean arterial pressure or in the presence of ethyl acetate column fraction 3 (CFEt3)

4.16: Effect of noradrenaline alone or in the presence of ethyl acetate column fraction 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. However, the administration of PAM (100 mg/kg) and ethyl acetate column fraction 3 (25 mg/kg) reduced the heart rate to 333.0 ± 2.0 beats/min. The heart rate on the administration of ethyl acetate column 3 (25 mg/kg) followed by noradrenaline was 332.0 ± 2.0 beats/min. Thus CFEt3 inhibited the increase in heart rate caused by noradrenaline.

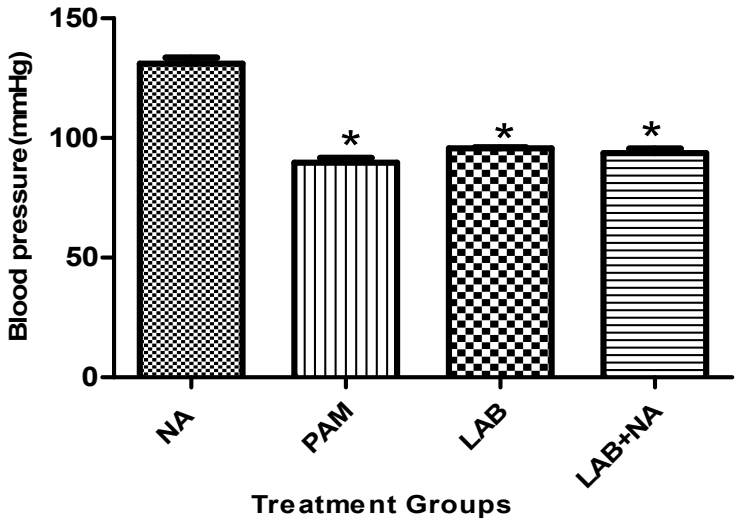


NA- Noradrenaline 4.0 µg/kg, PAM- *P. americana* leaf methanol extract (100 mg/kg), CFEt3- Column Fraction of Ethyl acetate fraction 3 (25 mg/kg), CFEt3+ NA- CFEt3+ Noradrenaline. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.16: Effect of noradrenaline alone or in the presence of ethyl acetate column fraction 3 on the heart rate of rats

4.17: Effect of noradrenaline alone or in the presence of labetalol 25 mg/kg on mean arterial pressure of rats

Noradrenaline (4.0 μg/kg) raised the Mean Arterial Pressure (MAP) to 131.0 ± 2.5 mmHg as shown in figure 4.18. The effect of the administration of labetalol (25 mg/kg) alone on the mean arterial pressure of the anaesthetised Wistar rats is (95.7 ± 0.8 mmHg). The administration of labetalol followed by noradrenaline produced a mean arterial pressure of 93.7 ± 3.2 mmHg. The blood pressure raising effect of NA was significantly attenuated by labetalol.

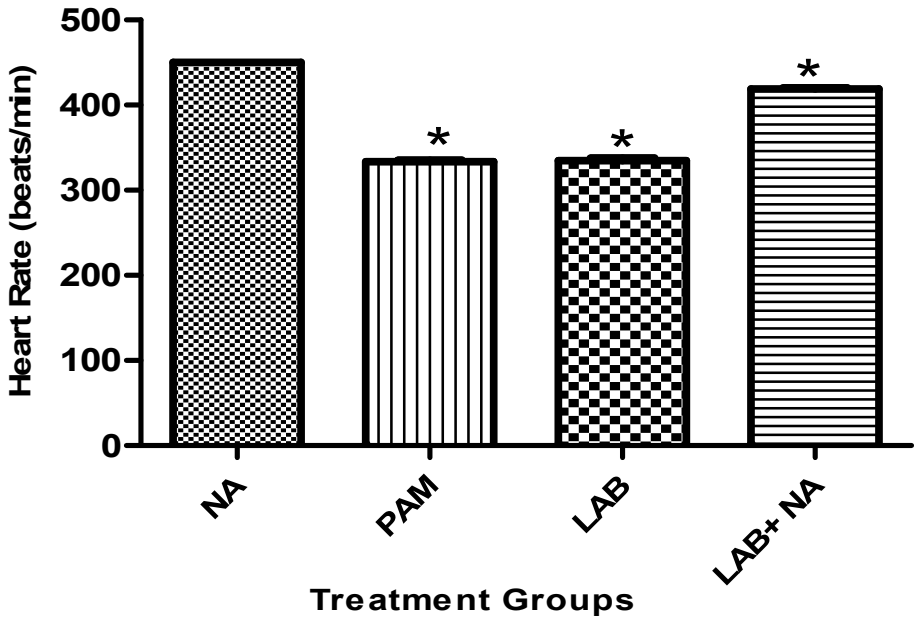


NA- Noradrenaline 4.0 µg/kg, PAM (100 mg/kg), LAB-Labetalol 25 mg/kg, LAB+NA – Labetalol + Noradrenaline. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.*$p < 0.05$.

Fig 4.17: Effect of noradrenaline alone or in the presence of labetalol 25 mg/kg on mean arterial pressure of rats

4.18: Effect of noradrenaline alone or in the presence of labetalol 25 mg/kg on heart rate of rats

Figure 4.18 showed the effect of labetalol 25 mg/kg on the heart rate of anaesthetized Wistar rats. Labetalol reduced heart rate by 25.6% (from 450.0 ± 0.1beats/min to 335.0 ± 3.0 beats/min). The administration of labetalol 25 mg/kg followed by the intravenous administration of noradrenaline resulted in a heart rate of 419.0 ± 1.0 beats/min.

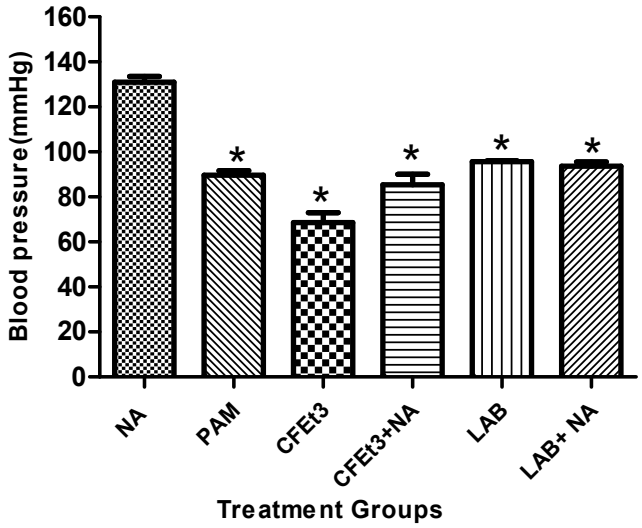


NA- Noradrenaline 4.0 µg/kg, PAM 100 mg/kg, LAB-Labetalol 25 mg/kg, LAB+NA – Labetalol + Noradrenaline. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.18: Effect of noradrenaline alone or in the presence of labetalol 25 mg/kg on heart rate of rats

4.19: Comparison of the effect of ethyl acetate column fraction 3 or labetalol on noradrenaline-induced rise in mean arterial pressure of rats

Figure 19 showed the effect of ethyl acetate column fraction 3 (25 mg/kg) or labetalol (25 mg/kg) on noradrenaline-induced rise in mean arterial pressure. Noradrenaline (4.0 μg/kg) increased the mean arterial pressure to 131.0 ± 2.5 mmHg. Labetalol or ethyl acetate column fraction 3 significantly ($p > 0.05$) reduced the elevation of mean arterial pressure. The effect of ethyl acetate column fraction 3 was however greater than that of labetalol 25 mg/kg alone.

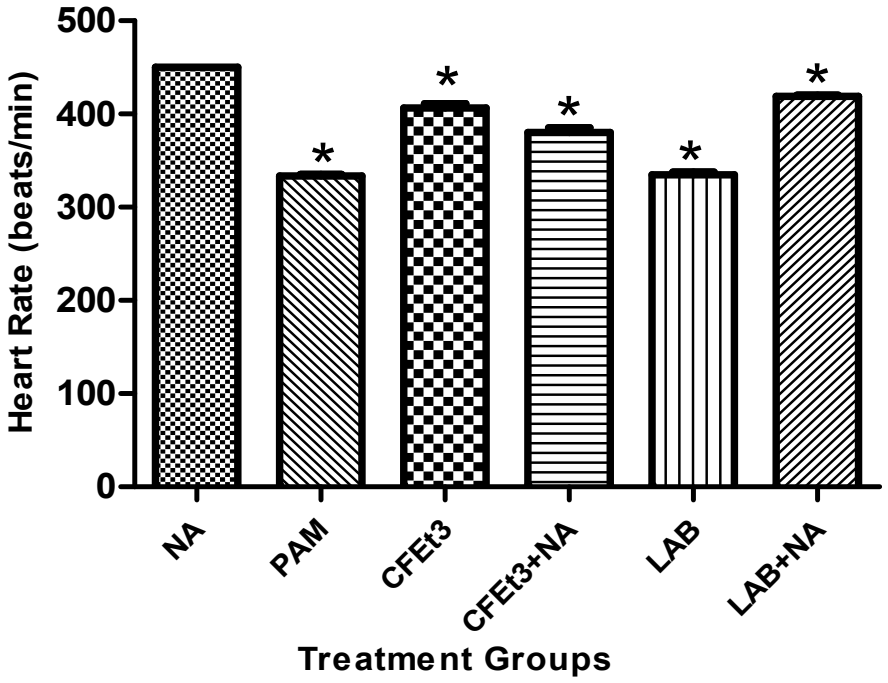


NA- Noradrenaline 4.0 µg/kg, PAM 100 mg/kg,CFEt3- ethyl acetate column fraction 3 25 mg/kg, LAB-labetalol 25 mg/kg. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.*$p < 0.05$.

Fig 4.19: Comparison of the effect of ethyl acetate column fraction 3 or labetalol on mean arterial pressure of rats

4.20: Comparison of the effect of ethyl acetate column fraction 3 or labetalol on heart rate of rats

Noradrenaline (4.0 µg/kg) increased heart rate from 405.0 ± 8.2 beats/min to 450.0 ± 0.1 beats/min. Figure 20 shows the effects of ethyl acetate column fraction 3 or labetalol on heart rate. Ethyl acetate column fraction 3 reduced the heart rate from 450.0 ± 0.1 beats/min to 406.7 ± 4.4 beats/min and labetalol reduced the heart rate to 335.0 ± 5.0 beats/min. The reduction in noradrenaline-induced increase in heart rate caused by labetalol (25 mg/kg) was more than that of ethyl acetate column fraction 3 (25 mg/kg). Labetalol blocks both alpha and beta adrenoceptors in the heart and this may be responsible for its efficacy.

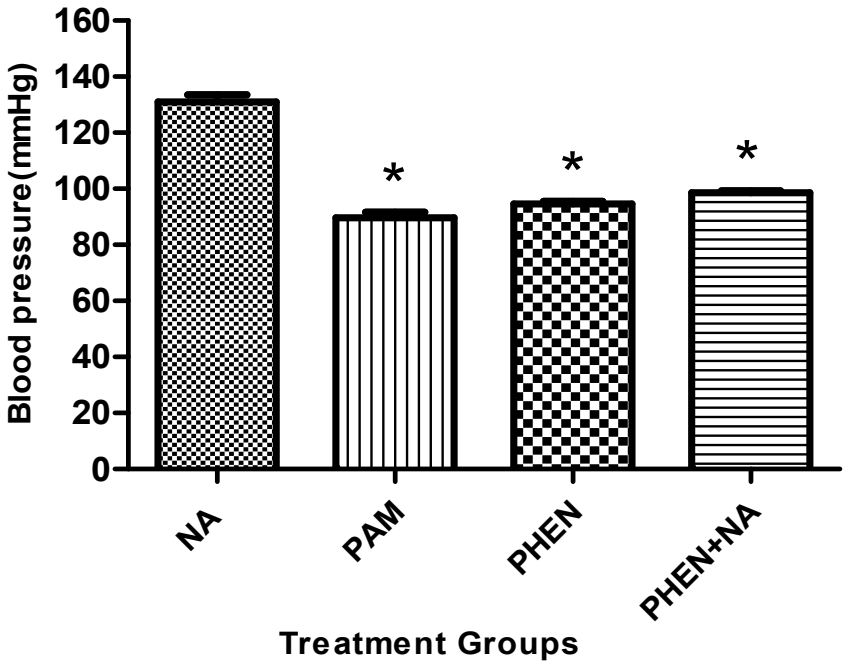


NA- Noradrenaline 4.0 µg/kg, PAM 100 mg/kg, CFEt3- ethyl acetate column fraction (25 mg/kg), LAB-labetalol 25 mg/kg. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.*$p < 0.05$.

Fig 4.20: Comparison of the effect of ethyl acetate column fraction 3 or labetalol on heart rate of rats

4. 21: Effect of noradrenaline alone or in the presence of phentolamine 83.3 µg/kg on mean arterial pressure of rats

Phentolamine (83.3 µg/kg) significantly reduced the increase in mean arterial pressure caused by noradrenaline. The mean arterial pressure increased from a baseline value of 98.4 ± 4.1 mmHg to 131.0 ± 3.0 mmHg when noradrenaline (4.0 µg/kg) was injected. Phentolamine reduced the mean arterial pressure by 27.7 % (from 131.0 ± 3.0 mmHg to 94.7 ± 0.9 mmHg). On the injection of phentolamine followed 5 min later by administration of noradrenaline, the mean arterial pressure became 98.7 ± 0.7 mmHg.

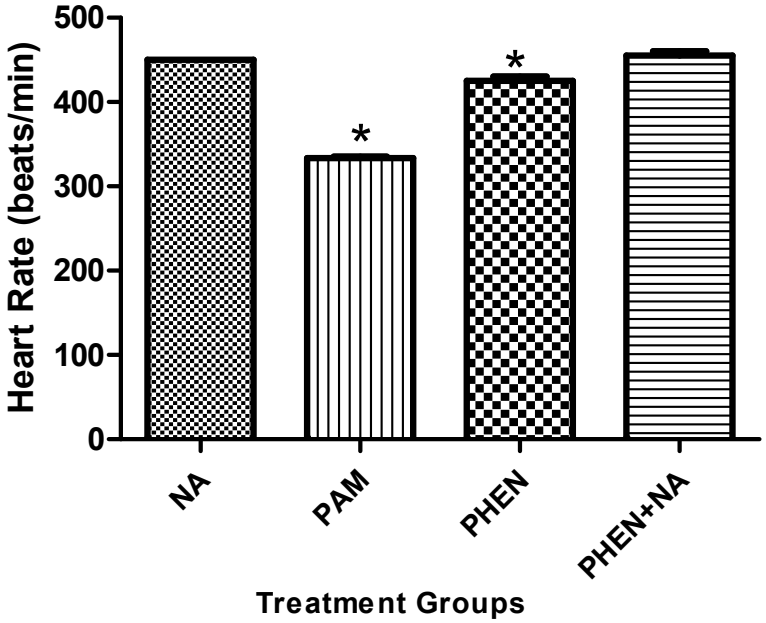


NA- Noradrenaline 4.0 µg/kg, PAM 100 mg/kg, PHEN – Phentolamine 83.3µg/kg, PHEN+N A – Phentolamine + Noradrenaline. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.21: Effect of noradrenaline alone or in the presence of phentolamine 83.3µg/kg on the mean arterial pressure of rats

4.22: Effect of noradrenaline alone or in the presence of phentolamine 83.3 µg/kg on the heart rate of rats

Phentolamine alone demonstrated only a slight reduction to 425.0 ± 5.0 beats/min in HR that was earlier increased by noradrenaline to 450.0 ± 0.1beats/min from a baseline value of 405.0 ± 0.1beats/min, while phentolamine in combination with NA caused a higher value of HR (455.0 ± 5.0 beats/min). This shows that phentolamine has no effect on the β adrenoceptors on the heart.

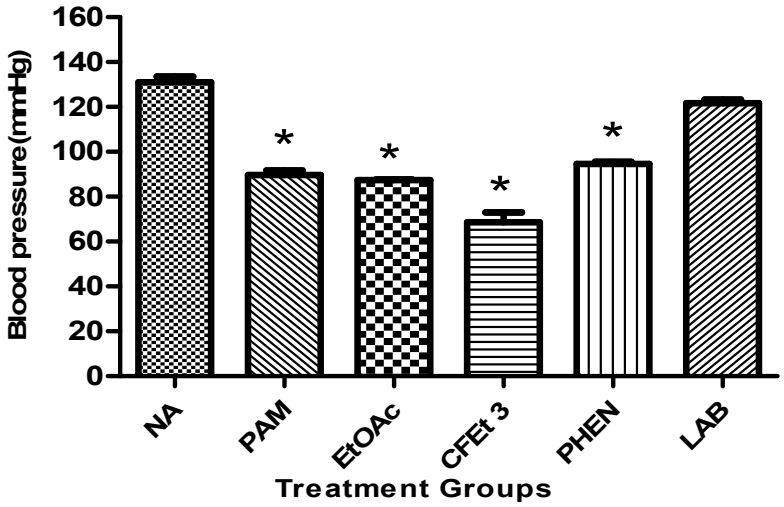


NA- Noradrenaline 4.0 µg/kg, PAM 100 mg/kg, PHEN – Phentolamine 83.3 µg/kg, PHEN+NA – phentolamine + Noradrenaline. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.22: Effect of noradrenaline alone or in the presence of phentolamine 83.3 µg/kg on the heart rate of rats.

4.23: Effect of noradrenaline, ethyl acetate, ethyl acetate column fraction 3, phentolamine and labetalol on the mean arterial pressure of rats

Figure 4. 23 summarized the effect of ethyl acetate fraction (100 mg/kg), column fraction of ethyl acetate 3 (25 mg/kg), phentolamine (83.3 µg/kg) and labetalol (25 mg/kg) on noradrenaline-induced raised mean arterial pressure. From a raised mean arterial pressure of 131.0 ± 2.5 caused by noradrenaline 4.0 µg/kg, the treatment groups lowered the elevated pressure as follows: ethyl acetate fraction 100 mg/kg (87.3 ± 0.3 mmHg), ethyl acetate column fraction 3 25 mg/kg (68.7 ± 4.4 mmHg) and phentolamine 83.3 µg/kg (94.7 ± 0.9 mmHg). Labetalol 25 mg/kg resulted in a mean arterial pressure value of 121.7 ± 1.7 mmHg and thus demonstrated no significant effect at all, while ethyl acetate column fraction 3 offered the best significant lowering effect (68.7 ± 4.4 mmHg).



NA- Noradrenaline 4.0 µg/kg, PAM 100 mg/kg, EtOAc- Ethly acetate 100 mg/kg, CFEt3- Column fraction of Ethyl acetate 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. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

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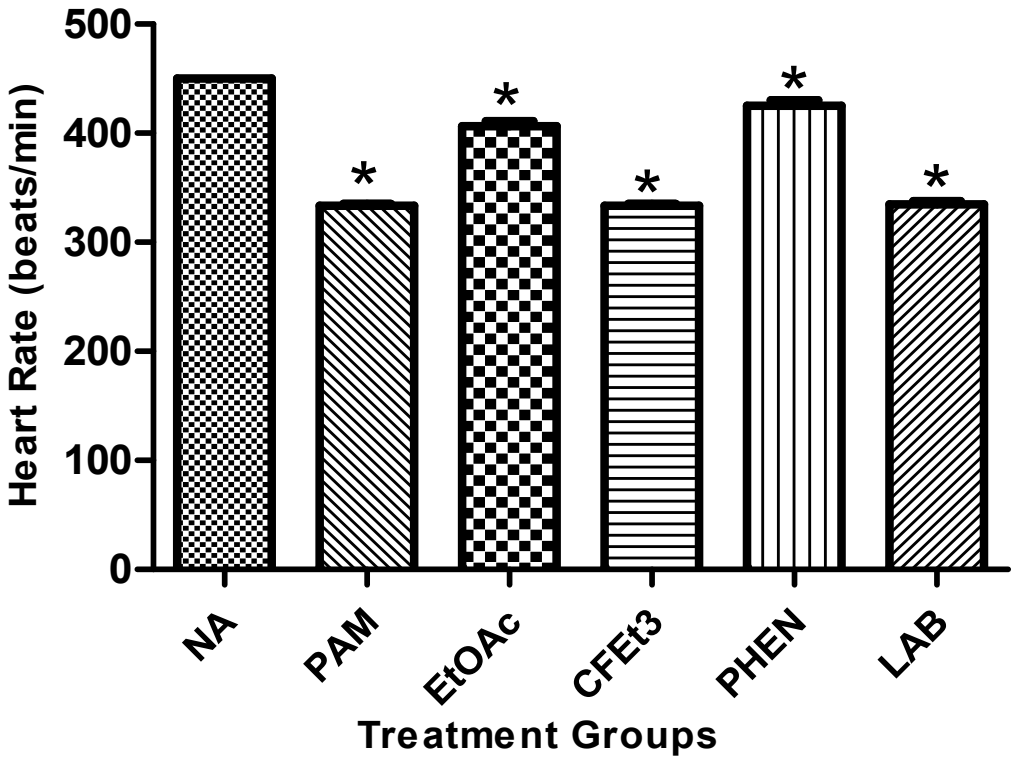
Fig 4. 23: Effect of noradrenaline, ethyl acetate, column fraction of ethyl acetate, phentolamine and labetalol on the mean arterial pressure of rats

4.24: Effect of noradrenaline, ethyl acetate fraction, column fraction of ethyl acetate 3, phentolamine and labetalol on the heart rate of rats

The effect of ethyl acetate fraction (100 mg/kg), column fraction of ethyl acetate 3 (25 mg/kg), phentolamine (83.3 µg/kg) and labetalol (25 mg/kg) on heart rate are shown in figure 4.24. The result obtained showed that the decrease in heart rate by ethyl acetate column fraction 3 (333.0 ± 2.0 beats/min) is similar to that of labetalol (335.0 ± 3.0 beats/min). The values for the other groups were also significant EtOAc 100 mg/kg (406.7 ± 4.4 beats/min) and phentolamine (425.0 ± 5.0 beats/min) compared to that of noradrenaline (450.0 ± 0.1 beats/min).

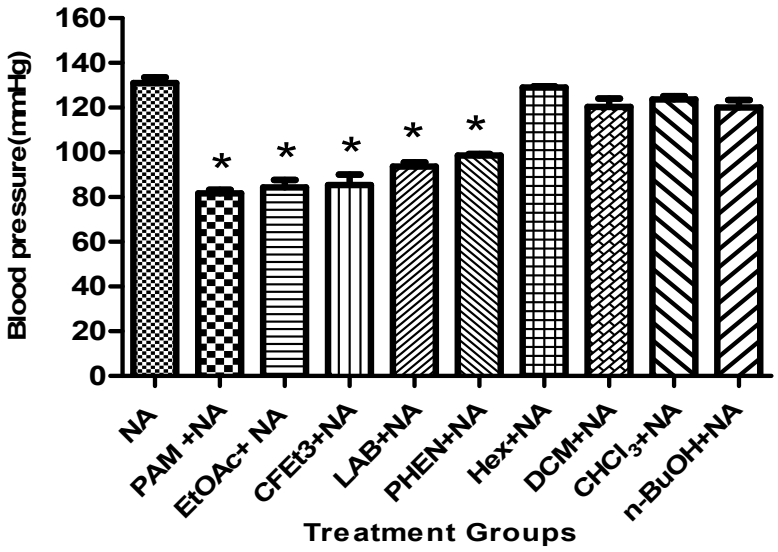


NA- Noradrenaline 4.0 µg/kg, PAM 100 mg/kg, EtOAc- Ethly acetate 100 mg/kg, CFEt3- Column fraction of Ethyl acetate 25 mg/kg, PHEN – phentolamine 83.3 µg/kg, LAB-labetalol 25 mg/kg. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.24: Effect of noradrenaline, ethyl acetate, column fraction of ethyl acetate, phentolamine and labetalol on the heart rate of rats

4.25: Comparison of the effects of noradrenaline on mean arterial pressure after pretreatment with the extract and fractions

Figure 4.25 shows the mean arterial pressure in response to noradrenaline (4.0 µg/kg) and noradrenaline administered after the intravenous injection of *Persea americana* methanol leaf extract (PAM 100 mg/kg) and various fractions of PAM. From the results it could be seen that the response to noradrenaline in the presence of *Persea americana* methanol leaf extract, ethyl acetate fraction (100 mg/kg), ethyl acetate column fraction 3 (25 mg/kg), labetalol (25 mg/kg) and phentolamine (83.3 µg/kg) were attenuated (81.7 ± 1.7, 84.3 ± 3.3, 85.3 ± 4.7, 93.7 ± 1.7 and 98.7 ± 0.7 mmHg respectively) and all had significant lowering effects on raised mean arterial pressure. However, the hexane fraction, chloroform fraction, dichloromethane fraction and n-butanol fractions had no such effect.



NA- Noradrenaline (4.0 µg/kg), PAM- *P. americana* leaf methanol extract (100 mg/kg), EtOAc- Ethyl acetate (100 mg/kg), Hex- Hexane (100 mg/kg), DCM- Dichloromethane (100 mg/kg), $CHCl_3$- Chloroform (100 mg/kg), *n*-BuOH- *n*-butanol (100 mg/kg), LAB- labetalol 25mg/kg, PHEN- phentolamine 83.3µg/kg, CFEt3- Column fraction of Ethyl acetate 25 mg/kg. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test.* $p < 0.05$.

Fig 4.25: Comparison of the effects of all the treatments in combination with noradrenaline on mean arterial pressure

4.26: Effect of N^G-nitro-L-arginine methyl ester alone or with ethyl acetate column fraction 3 on systolic blood pressure of rats

Noradrenaline 4.0 μg/kg caused a rise in systolic blood pressure (SBP) of anaesthetised rats from 117.0 ± 2.6 mmHg to 151.8 ± 2.6 mmHg and N^G-nitro-L-arginine methyl ester (L-NAME) 80 mg/kg/day for 7days also raised SBP to 150.2 ± 1.0 mmHg. When ethyl acetate column fraction 3 25 mg/kg was administered to L-NAME-pretreated rats, the systolic blood pressure dropped to 133.6 ± 0.9 mmHg compared with the effect of L-NAME alone (150.2 ± 1.0 mmHg). This showed the ability of ethyl acetate column fraction 3 to reverse the L-NAME-induced rise in systolic blood pressure.

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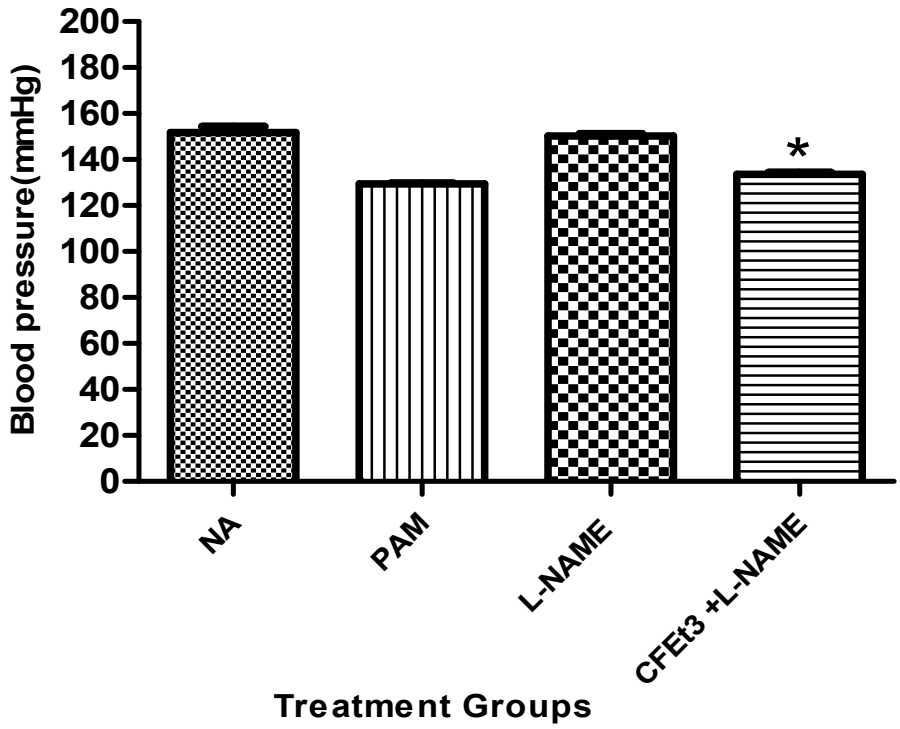
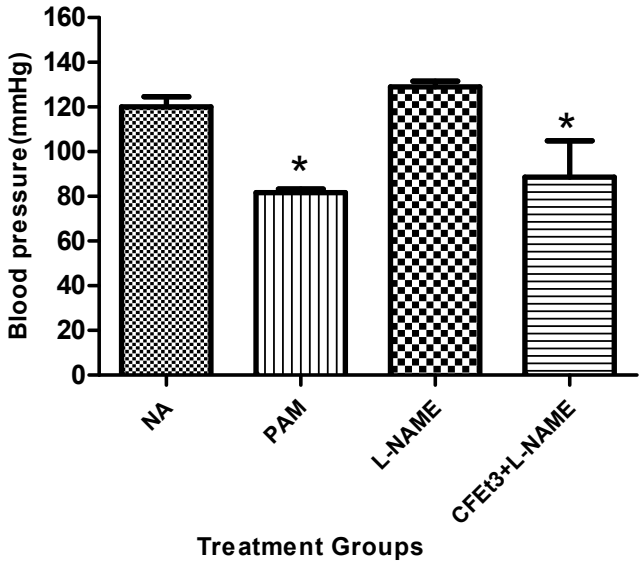


NA- Noradrenaline 4.0 µg/kg, PAM 100 mg/kg, L-NAME-N^G-nitro-L-arginine methyl ester 80 mg/kg/ day for 7 days, CFEt3- ethyl acetate column fraction 3 25 mg/kg. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test. * $p < 0.05$

Fig 4.26: Effect of N^G-nitro-L-arginine methyl ester or with ethyl acetate column fraction 3 on systolic blood pressure of rats

4.27: Effect of N^G-nitro-L-arginine methyl ester alone or with ethyl acetate column fraction 3 on diastolic blood pressure of rats

Figure 4.27 showed the blood pressure-raising effect of noradrenaline and N^G-nitro-L-arginine methyl ester (L-NAME) 80 mg/kg/day for 7 days on diastolic blood pressure. Noradrenaline raised diastolic blood pressure from 89.4 ± 16.2 mmHg to 120.0 ± 4.6 mmHg and L-NAME raised the diastolic blood pressure to 129.0 ± 2.5 mmHg. However, when L-NAME was injected after administration of ethyl acetate column fraction 3 (25 mg/kg), the diastolic blood pressure fell from 129.0 ± 2.5 mmHg to 88.7 ± 16.2 mmHg representing 31.2% reduction in the activity of L-NAME on diastolic blood pressure. This is an indication of the ability of ethyl acetate column fraction 3 to block the action of L-NAME in raising diastolic blood pressure.

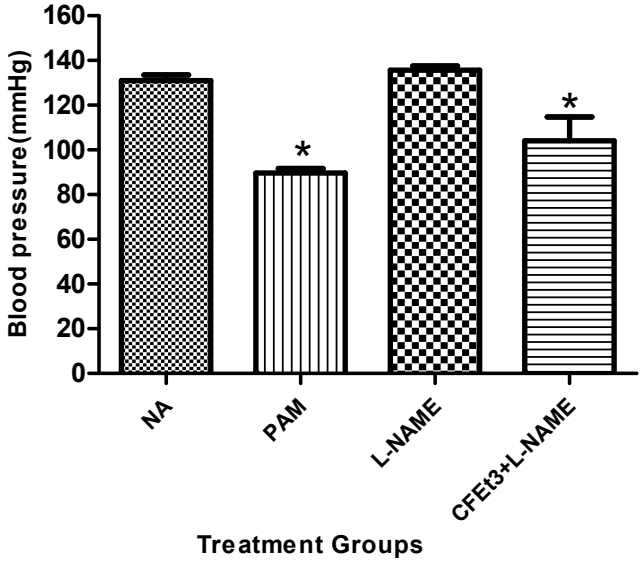


NA- Noradrenaline 4.0 µg/kg, PAM 100 mg/kg, L-NAME- N^G-nitro-L-arginine methyl ester 80 mg/kg/day for 7 days, CFEt3- ethyl acetate column fraction 3 25 mg/kg. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test. * $p < 0.05$

Fig 4.27: Effect of N^G-nitro-L-arginine methyl ester alone or with ethyl acetate column fraction 3 on diastolic blood pressure of rats

4.28: Effect of N^G-nitro-L-arginine methyl ester alone or with ethyl acetate column fraction 3 on mean arterial pressure of rats

In figure 4.28, noradrenaline (4.0 µg/kg) increased mean arterial pressure of tested rats from 98.4 ± 4.1 mmHg to a mean of 131.0 ± 2.5 mmHg, while N^G-nitro-L-arginine methyl ester **(**L-NAME) 80 mg/kg/day for 7 days raised mean arterial pressure to 135.7 ± 1.9 mmHg. However, when ethyl acetate column fraction 3 25 mg/kg (CFEt3) was administered before L-NAME was injected, there was a significant 23.3% reduction in mean arterial pressure previously raised by L-NAME (104.0 ± 10.7 mmHg). This indicates the inhibitory effect of ethyl acetate column fraction 3 on the efficacy of L-NAME.

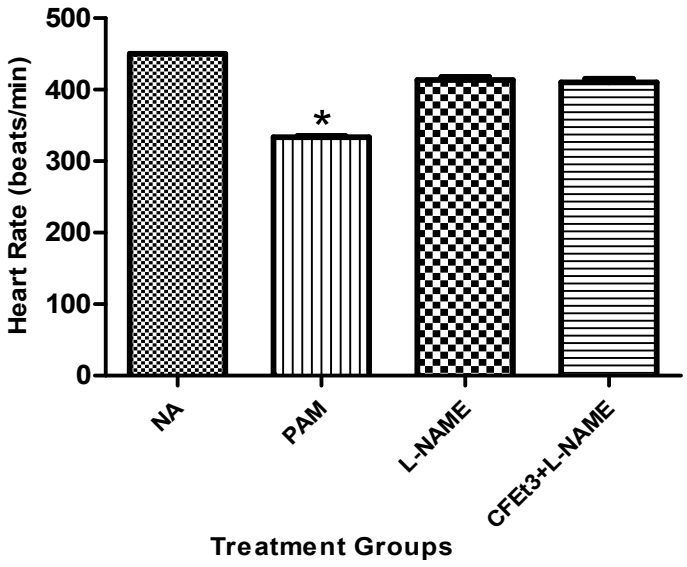


NA- Noradrenaline 4.0 µg/kg, PAM 100 mg/kg, L-NAME-N^G-nitro-L-arginine methyl ester 80 mg/kg/day for 7 days, CFEt3- ethyl acetate column fraction 3 25 mg/kg. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test. * $p < 0.05$

Fig 4.28: Effect of N^G-nitro-L-arginine methyl ester or with ethyl acetate column fraction 3 on mean arterial pressure of rats

4.29: Effect of N^G-nitro-L-arginine methyl ester alone or with ethyl acetate column fraction 3 on heart rate of rats

Noradrenaline 4.0 µg/kg and N^G-nitro-L-arginine methyl ester (L-NAME) 80 mg/kg/day for 7 days raised the heart rate of anaesthetised Wistar rats from a baseline value of 405.0 ± 8.2 beats/min to 450.0 ± 0.1 and 413.0 ± 4.4 beats/min respectively. The rats were then pretreated with ethyl acetate column fraction 3 25 mg/kg, followed with intravenous injection of N^G-nitro-L-arginine methyl ester L-NAME. There was observed a slight drop of heart rate to 410.0 ± 5.0 beats/min. However, this drop was not statistically significant. Only PAM (100 mg/kg) caused a significant drop of the heart rate to 333.3 beats/min.

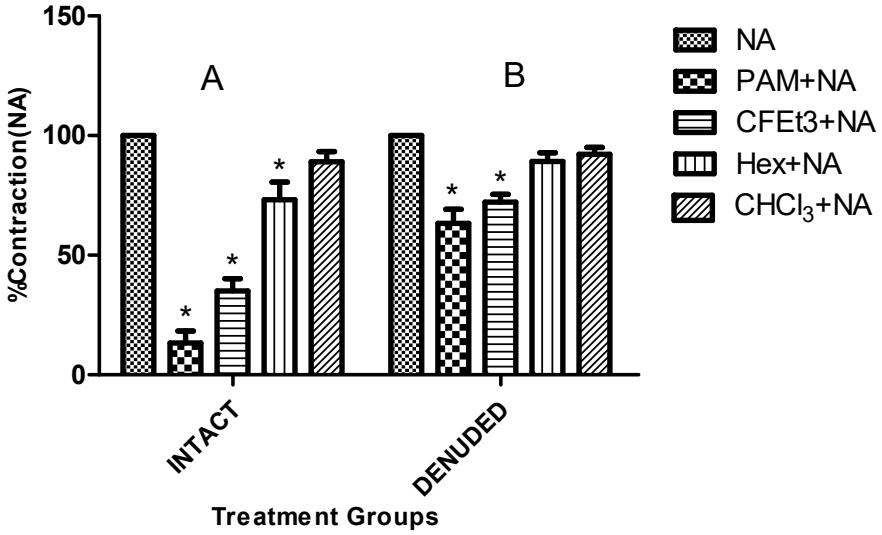


NA- Noradrenaline 4.0 µg/kg, PAM 100mg/kg, L-NAME-N^{G}-nitro-L-arginine methyl ester 80 mg/kg/day for 7 days, CFEt3- ethyl acetate column fraction 3 25 mg/kg. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test. * $p < 0.05$.

Fig 4.29: Effect of N^{G}-nitro-L-arginine methyl ester alone or with ethyl acetate column fraction 3 on heart rate of rats

4.30: Percentage contraction of endothelium intact and endothelium denuded aorta to noradrenaline in the presence of the extract and fractions

Noradrenaline (10^{-5} M) was used to contract the endothelium intact and endothelium denuded aorta. Noradrenaline contracted the endothelium intact aorta by 100%. The contraction produced by noradrenaline in the presence of *Persea americana* leaf methanol extract 2 mg/ml (PAM), ethyl acetate column fraction 3 2 mg/ml (CFEt3), hexane fraction 2 mg/ml and chloroform fraction 2 mg/ml were 13%, 35%, 73% and 89% respectively. Thus *Persea americana* leaf methanol extract and ethyl acetate column fraction 3 reduced the contractile effect of noradrenaline significantly in the endothelium intact aorta. Conversely, in the endothelium denuded aortic ring, the contraction caused by noradrenaline was higher than that of endothelium intact. Noradrenaline caused greater contractions when the aortic rings were pretreated with *P. americana* leaf methanol extract (63.4%), ethyl acetate column fraction 3 (72.2%), hexane fraction (89.2%) and chloroform fraction (91.5%). This underscores the fact that the endothelium is necessary for the activity of the extract and fraction.

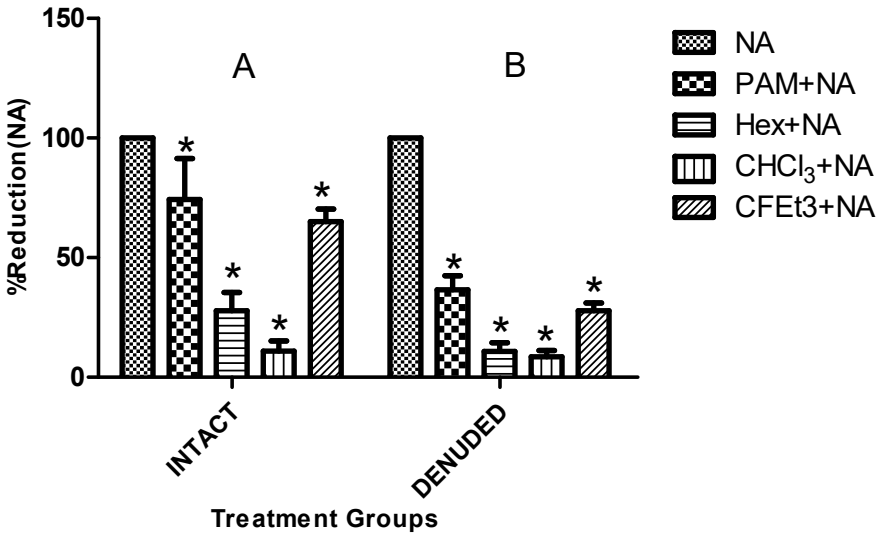


NA- Noradrenaline (10^{-5} M), PAM- *P. americana* leaf methanol extract (2 mg/ml), CFEt3- Column fraction of ethyl acetate fraction 3 (2 mg/ml), Hex- Hexane (2 mg/ml), $CHCl_3$- Chloroform fractions (2 mg/ml). Values represent the mean ± S.E.M of 5 aortic rings per group. ANOVA followed by Dunnett's post hoc test. * $p < 0.05$

Fig 4.30: Percentage contraction of endothelium intact and endothelium denuded aorta to noradrenaline in the presence of the extract and fractions

4.31: Percentage reduction in the contraction of endothelium intact and endothelium denuded aorta to noradrenaline in the presence of *Persea americana* methanol leaf extract and fractions

The result of percentage reduction in contraction of endothelium intact and endothelium denuded aorta to noradrenaline in the presence of *Persea americana* methanol leaf extract and fractions (2 mg/ml) are presented in figure 4.31. The results obtained for endothelium intact aortic rings are as follows: noradrenaline 10^{-5}M (0%), *Persea americana* methanol leaf extract (74.4%), column fraction of ethyl acetate fraction 3 2 mg/ml (65%), hexane fraction 2 mg/ml (27.8%) and chloroform fraction 2 mg/ml (11%). The values for percentage reduction in contraction for endothelium denuded aorta are also as follows: noradrenaline (0%), *Persea americana* methanol leaf extract (36.6%), column fraction of ethyl acetate fraction 3 (27.8%), hexane fraction (10.8%) and chloroform (8.5%). The results further emphasised the role of the endothelium on the activity of the extract and fractions.

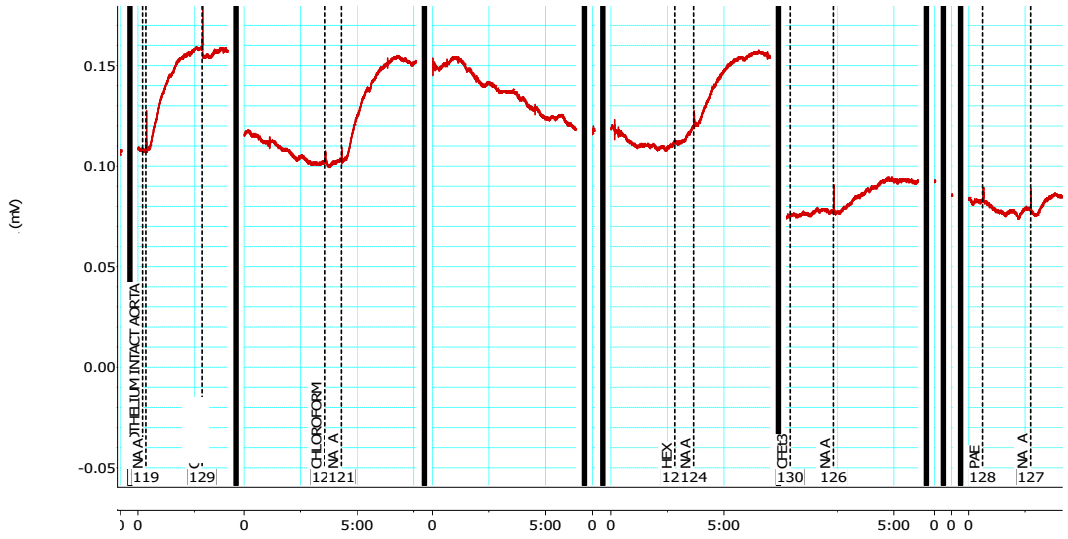


NA- Noradrenaline (10^{-5} M), PAM- *P. americana* leaf methanol extract 2 mg/ml, Hex- Hexane 2 mg/ml, $CHCl_3$- Chloroform fraction 2 mg/ml, CFEt3- Column fraction of ethyl acetate fraction 3 (2 mg/ml). Values represent the mean ± S.E.M of 5 aortic rings per group. ANOVA followed by Dunnett's post hoc test. * $p < 0.05$.

Fig 4.31: Percentage reduction in the contraction of endothelium intact and endothelium denuded aorta to noradrenaline in the presence of *Persea americana* methanol leaf extract and fractions

4.32: Figure showing the effect of some fractions on the contractions of rat aorta

Representative tracing of rat thoracic aortic contraction of some fractions are presented in figure 4.32.

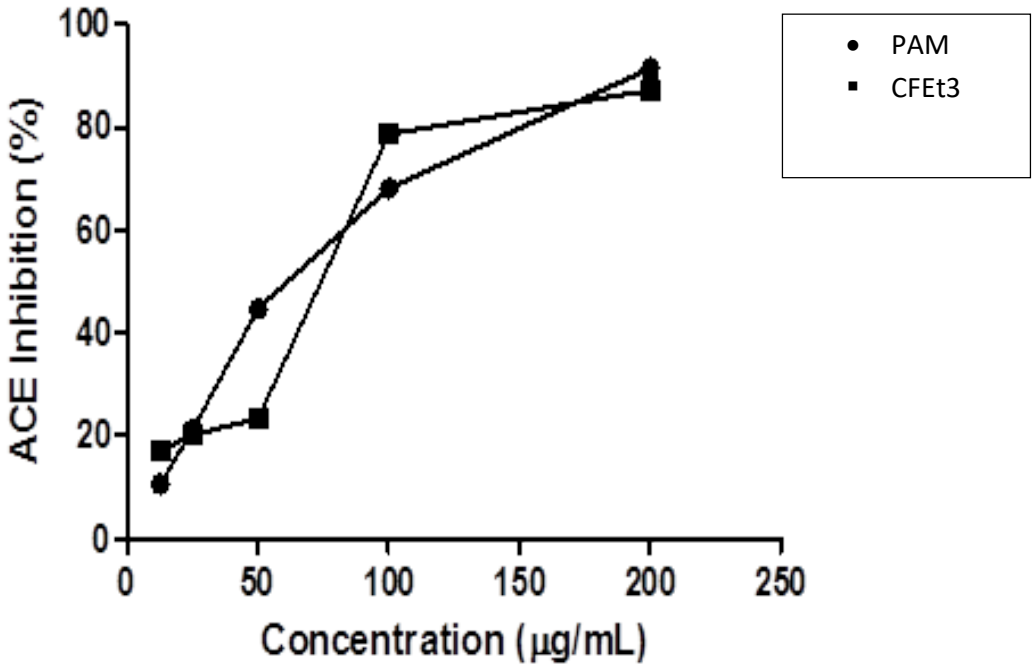


NA -Noradrenaline 10^{-5}M, PAM- *Persea americana* methanol leaf extract 2 mg/ml, Chloroform extract 2 mg/ml, Hex- Hexane 2 mg/ml CFEt3- ethyl acetate column fraction 3 (2mg/ml).

Fig 4.32: Figure showing the effect of some fractions on the contractions of rat aorta

4.33: Percentage angiotensin converting enzyme inhibition by *P. americana* crude leaf extract and ethyl acetate column fraction 3

The *in vitro* angiotensin converting enzyme inhibitory activities of *Persea americana* methanol leaf extract and column fraction of ethyl acetate fraction 3 were matched with captopril. From available results, activity of both extract and fraction are similar. The column fraction has the advantage of being devoid of the components that may possibly cause adverse effects that may be present in the crude extract.

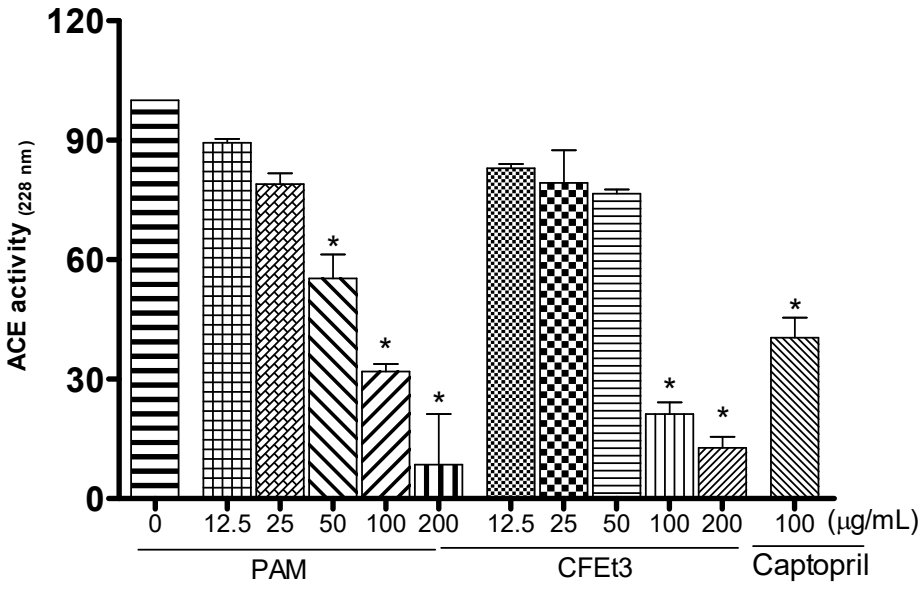


PAM- *P. americana* methanol leaf extract, CFEt3- ethyl acetate column fraction 3.

Fig 4.33: Percentage angiotensin converting enzyme inhibition by *P. americana* methanol leaf extract and ethyl acetate column fraction 3

4.34: Angiotensin converting enzyme activity in the presence of *P. americana* methanol leaf extract, ethyl acetate column fraction 3 and captopril

The activity of angiotensin converting enzyme in the presence of *P. americana* methanol leaf extract, ethyl acetate column fraction 3 and captopril at different concentrations are presented in Figure 34. The extract and column fraction 3 significantly suppressed the activity of angiotensin converting enzyme in a concentration-dependent manner. The extract caused concentration-related decreases in ACE activity that were significant at the 50 µg/mL,100 µg/mL and 200 µg/mL doses, while CFEt3 reduced the activity at 100 µg/mL and 200 µg/mL doses, compared to that of Captopril at 100 µg/mL.

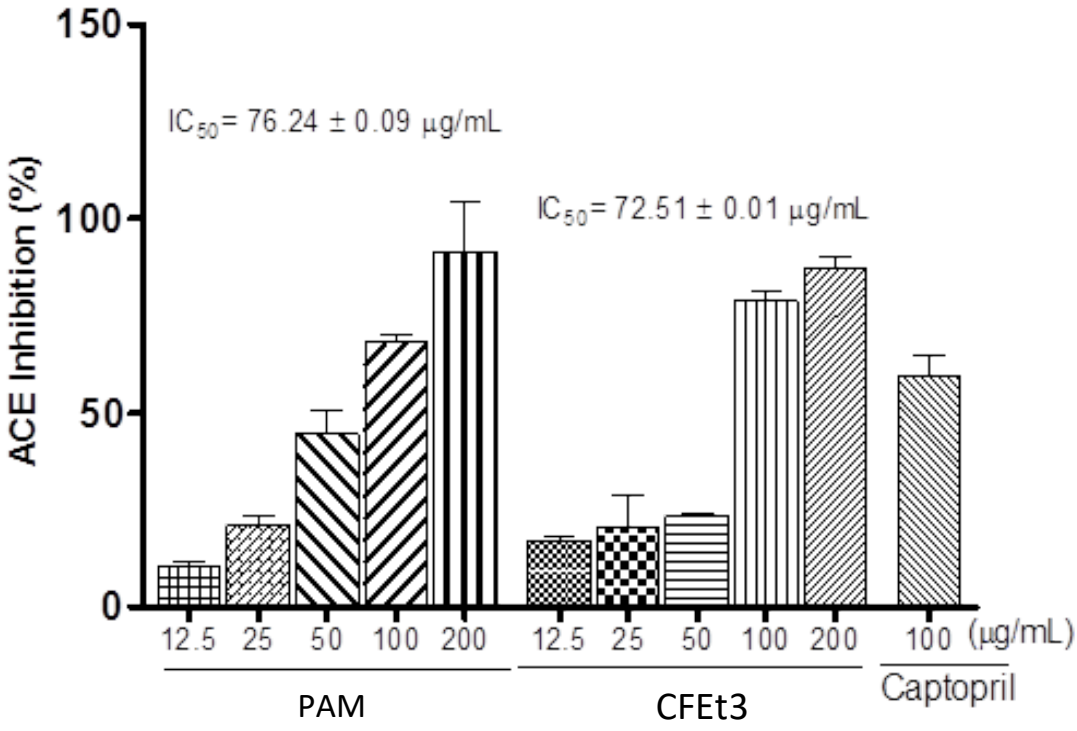


PAM- *P. americana* methanol leaf extract, CFEt3- ethyl acetate column fraction 3.

Fig 4.34: Angiotensin converting enzyme activity in the presence of *P. americana* methanol leaf extract, ethyl acetate column fraction 3 and captopril

4.35: Percentage angiotensin converting enzyme inhibition by *P. americana* methanol leaf extract, ethyl acetate column fraction and captopril

Figure 4.35 gives the result of the percentage inhibition of ACE by *P. americana* methanol leaf extract, ethyl acetate column fraction 3 and captopril. The extract and CFEt3 inhibited angiotensin converting enzyme by 70% and 73. 6% respectively compared with that of 100 µg/ml of captopril (68.1%). Also IC_{50} of PAM was 76.24 ± 0.09 µg/mL, CFEt3 was 72.51 ± 0.01 µg/ml, while that of captopril was 61.92 ± 0.02 µg/mL



PAM- *Persea americana* crude leaf extract, CFEt3-*Persea americana* ethyl acetate column fraction 3.

Fig 4.35: Percentage angiotensin converting enzyme inhibition by *P. americana* methanol leaf extract, ethyl acetate column fraction 3 and captopril

The effect of *P. americana* methanol leaf extract and ethyl acetate column fraction 3 on angiotensin converting enzyme inhibition is presented in Table 4.1 The result of the IC_{50} is as follows: PAM (76.24 ± 0.09 µg/mL), CFEt3 (72.51 ± 0.01ug/mL) compared with that of captopril (61.92 ± 0.02 µg/mL).

Table 4.1: Angiotensin converting enzyme Inhibition assay for *P. americana* methanol leaf extract and ethyl acetate column fraction 3

	Concentration	%Inhibition(Mean ± S.E.M)	IC50(ug/ml)
PAM	12.5	10.64 ± 1.000	76.24 ± 0.09
	25	20.98 ± 2.702	
	50	44.71 ± 6.061	
	100	68.09 ± 1.915	
	200	91.73 ± 12.73	
CFET3	12.5	17.01 ± 1.011	72.51 ± 0.01
	25	20.72 ± 8.213	
	50	23.40± 1.000	
	100	78.72 ± 2.889	
	200	87.23 ± 2.766	
CAPTOPRIL	100	59.57 ± 5.041	61.92 ± 0.02

IC_{50} of Captopril, Vermeissen *et al*., 2002.

PAM- *P. americana* leaf methanol extract, CFEt3- Ethyl acetate column fraction 3

Table 4. 2: Fifty Percent Angiotensin Converting Enzyme Inhibitory Concentration of PAM and CFEt3

ASSAYS	IC_{50} ± SEM (µg/mL)
IC_{50} Captopril	61.92 ± 0.02
IC_{50} PAM	76.24 ± 0.09
IC_{50} CFEt3	72.51 ± 0.01

IC_{50} of Captopril, Vermeissen *et al*., 2002.

Table 4.3: Effect of oral administration of *Persea americana* methanol leaf extract and CFEt3 on lipid profile when hyperlipidemia was induced in rats using Triton X-100

Treatment	T CHOL (mg/dL)	TRIG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	AI
Normal control	89.58 ± 7.69	115.46 ± 2.84	62.30 ± 4.01	4.19 ± 4.37	23.09 ± 1.01	0.43 ± 0.03
TX- 100	150.94 ± 5.92#	144.60 ± 2.66	56.12 ± 7.28	65.89 ± 20.96#	28.92 ± 0.62	1.81 ± 0.51
PAM 100	87.11 ± 4.49	63.10 ± 1.40*	127. 67 ± 6.83*	-22.53 ± 15.86*	16.25 ± 1.02*	0.03 ± 0.12*
TX +AT 10	126.89 ± 9.82	142.70 ± 12.82	94.72 ± 3.28*	3.63 ± 27.84*	28.54 ± 2.56	0.03 ± 0.001*
TX+PAM 50	149.467 ± 5.61	141.68 ± 3.07	97.58 ± 8.16*	23.55 ± 21.90*	28.34 ± 0.61	0.57 ± 0.28
TX+ PAM 100	115.30 ± 8.64*	81.23 ± 0.66*	121.59 ± 5.38*	-22.53 ± 15.86*	16.25 ± 1.02	0.03 ± 0.001*
TX + CFEt3	87.13 ± 4.49*	63.11 ± 1.40*	125. 67 ± 6.83*	-22.50 ± 15.86*	15.25 ± 1.02*	0.03 ± 0.01*

TCHOL- Total Cholesterol, TRIG - Triglycerides, HDL- High Density Lipoprotein, LDL-Low Density Lipoprotein, VLDL- Very Low Density Lipoprotein, AI- Atherogenic Index, PAM 50,100- *P. americana* leaf methanol extract, 50 mg/kg, 100 mg/kg respectively; AT 10- Atorvastatin 10 mg/kg., CFEt3- ethyl acetate column fraction 3 25 mg/kg. # $p < 0.05$ vs NC, * $p < 0.05$ vs TX-100

4.36: GC-MS Chromatogram of ethyl acetate column fraction 3 of the leaf of *P. americana* showing a plot of Retention time (min) vs. Intensity

GC-MS mass spectrum was conducted using the database of the National Institute of Standard and Technique (NIST) reference library which contains about 62,000 patterns. The mass spectra of the unknown components in the ethyl acetate column fraction 3 were compared with those of the components stored in the library to ascertain their individual names, molecular weights, molecular formula and structures.

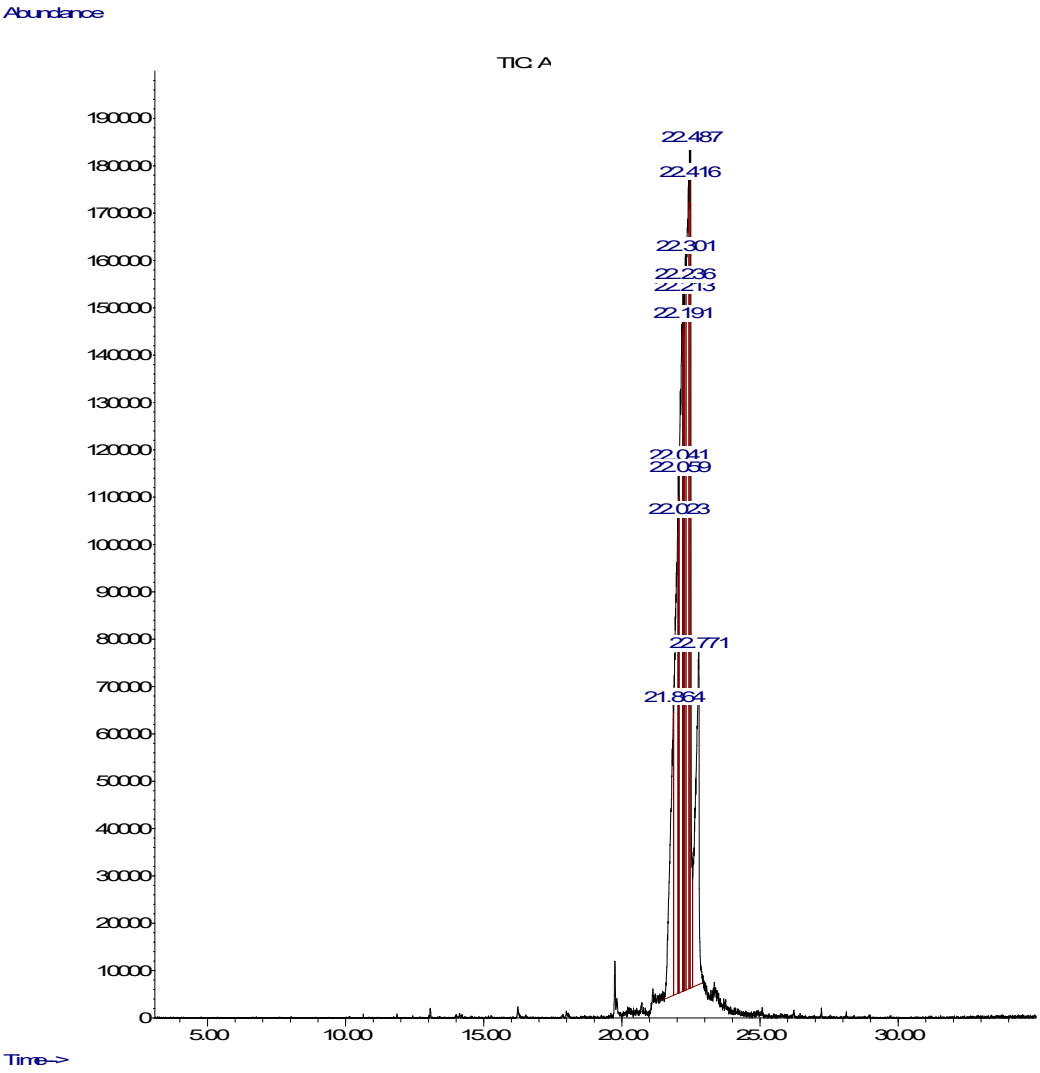


Fig 4.36: GC-MS Chromatogram of ethyl acetate column fraction 3 of the leaf of *P. americana* showing a plot of Retention time (min) vs. Intensity

Table 4.4: 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	Cyclopropanecarboxldehyde	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

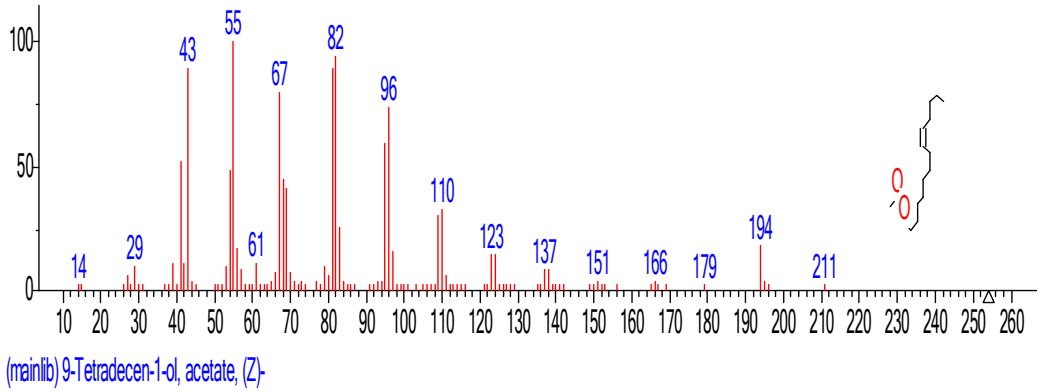


Fig 4.37: Mass spectrum of 9-Tetradecen-1-ol, acetate,(Z)-

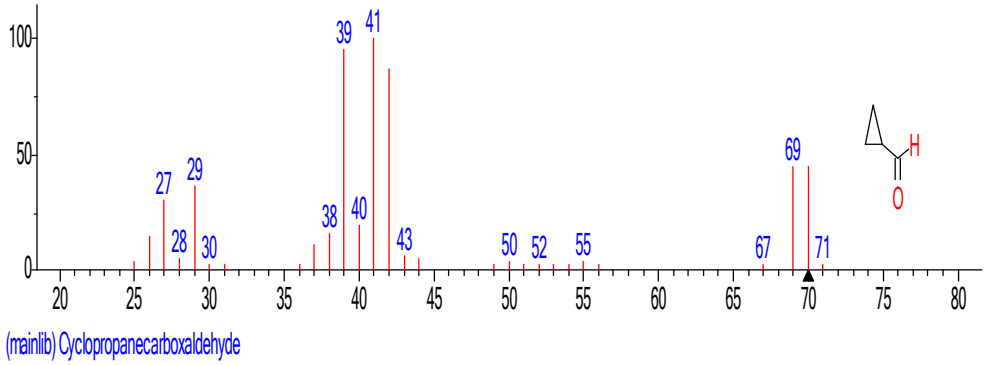


Fig 4.38: Mass spectrum of Cyclopropanecarboxyldehyde

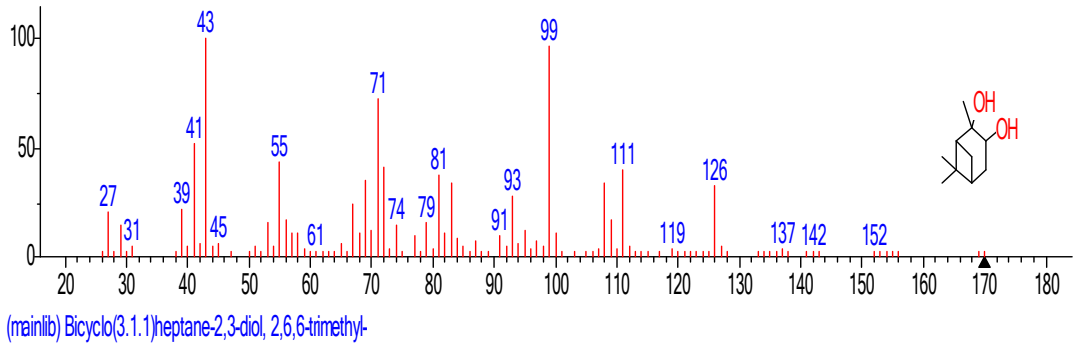


Fig 4.39: Mass spectrum Bicyclo (3.1.1) heptane-2,3-diol,26,6-trimethyl-

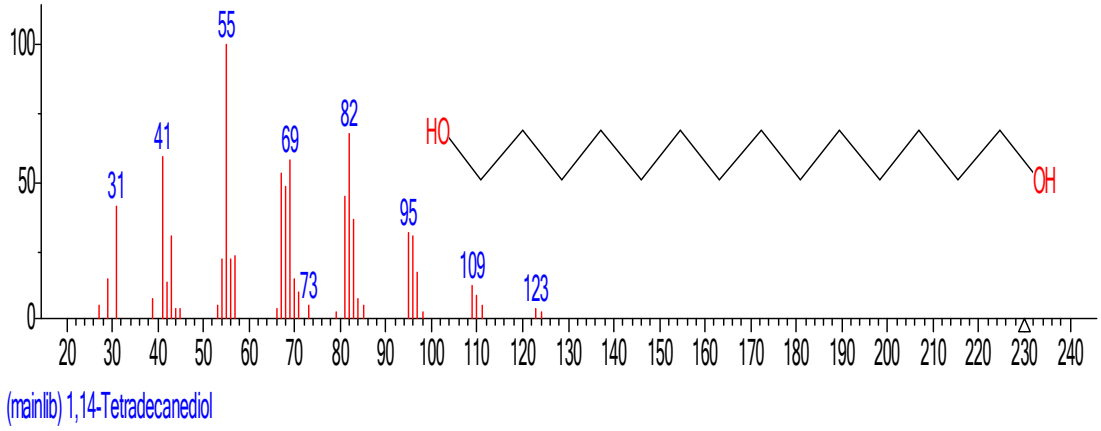


Fig 4.40: Mass spectrum of 1,14-Tetradecanedol

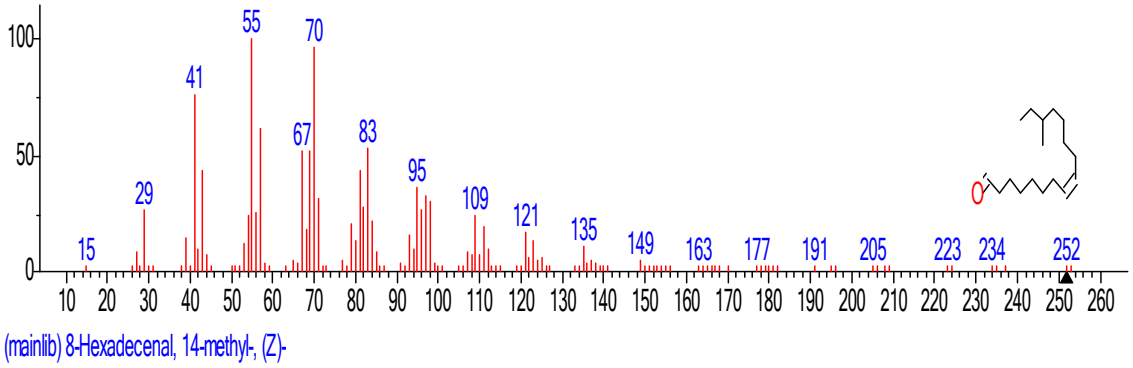


Fig 4.41: Mass spectrum of 8-Hexadecenal, 14-methyl-,(Z)-

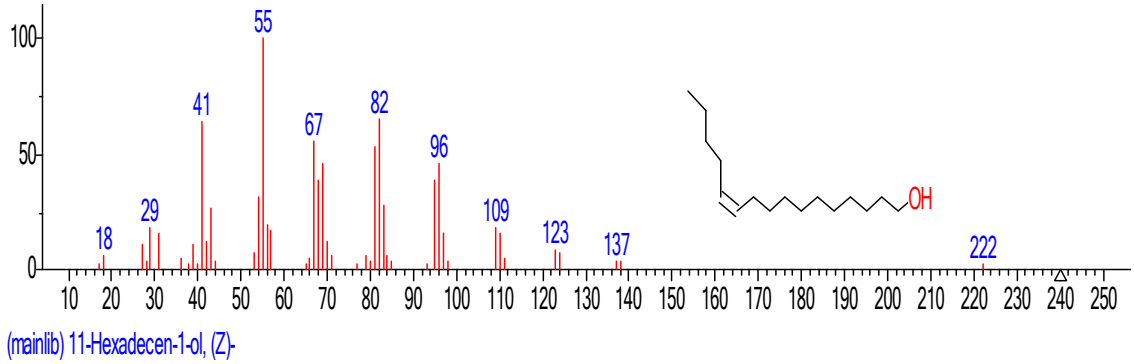


Fig 4.42: Mass spectrum of 11-Hexadecen-1-ol, (Z)-

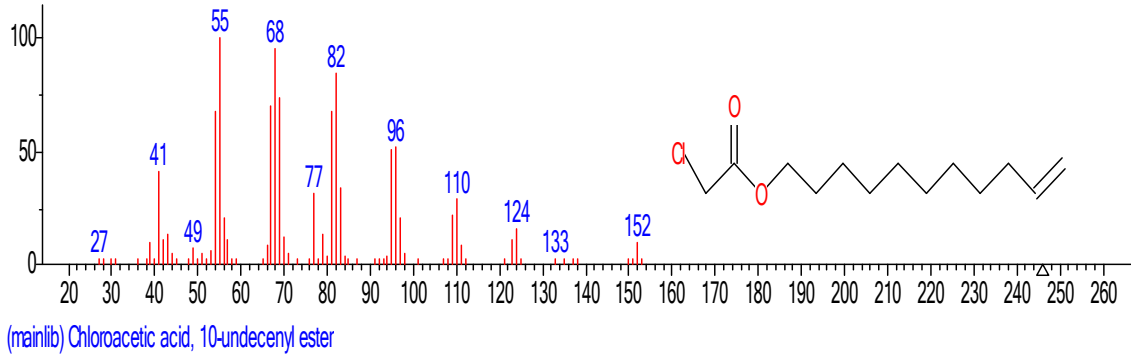


Fig 4. 43: Mass spectrum of Chloroacetic acid, 10-undecenyl ester

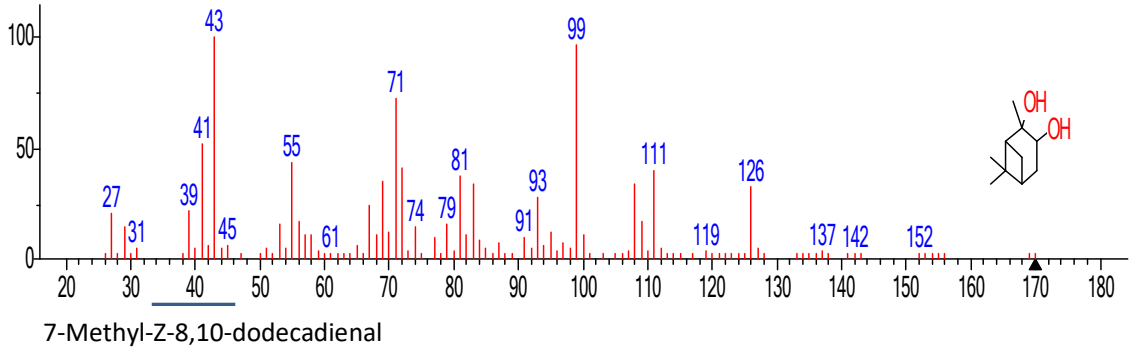


Fig 4.44: Mass spectrum of 7-Methyl-Z-8,10-dodecadienal

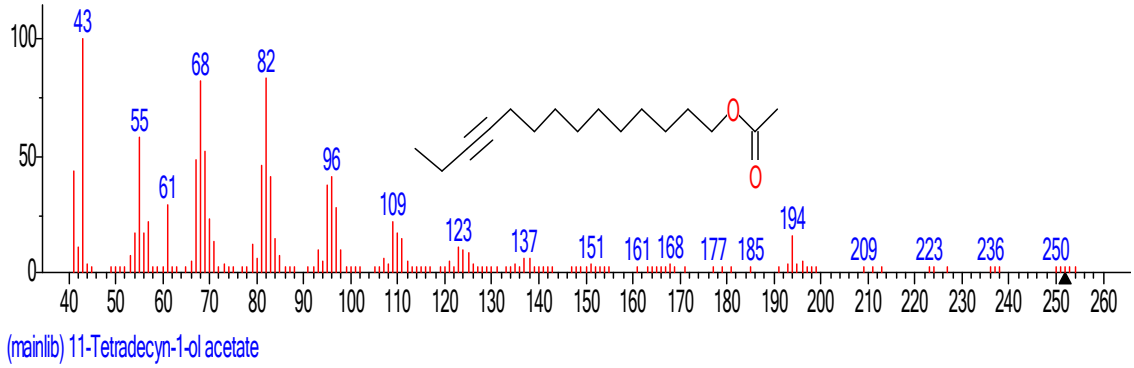


Fig 4.45: Mass spectrum 11-Tetradecyn-1-ol acetate

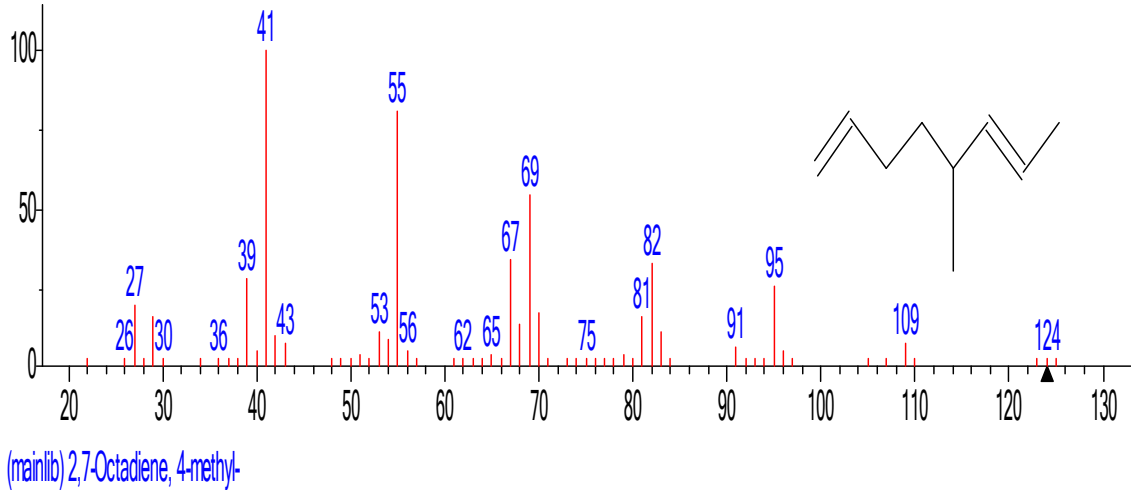


Fig 4.46: Mass spectrum 2,7-Octadiene, 4-methyl-

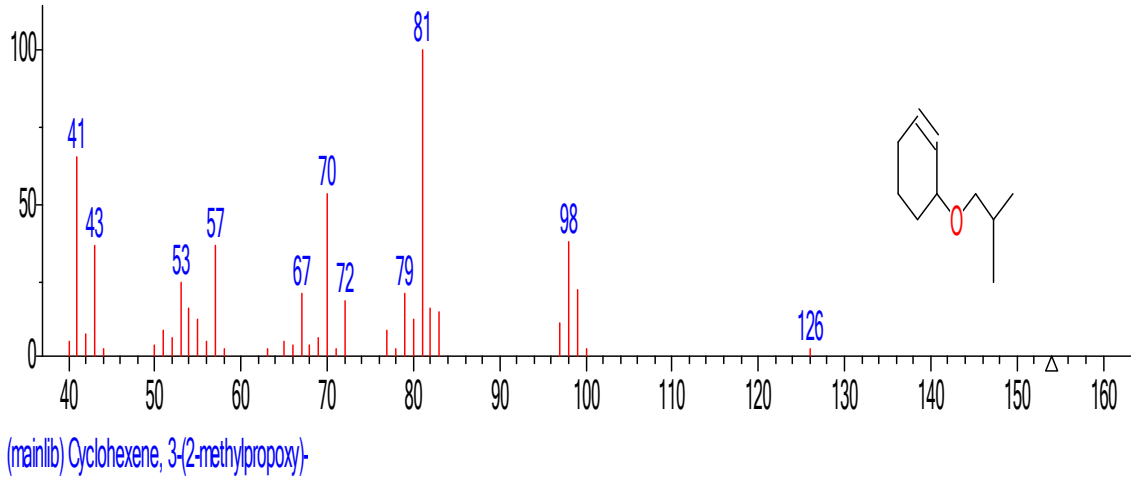


Fig 4.47: Mass spectrum of Cyclohexene, 3-(2-methylpropoxy)-

CHAPTER FIVE

DISCUSSION

Accumulating evidence has indicated that the prevalence of hypertension is on the increase globally. James *et al*., (2014) have noted that hypertension which used to be uncommon has now assumed prominence globally. Despite encouraging achievements made in understanding the pathology, diagnosis and management of hypertension, 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. Hence, there is the need for a continuous search for newer remedies for the management of hypertension. This study investigated the antihypertensive properties of methanol leaf extract as well as fractions from *P. americana* in noradrenaline- and L-NAME-induced raised blood pressure in rats. The antihyperlipidemic effect and inhibitory activity of angiotensin converting enzyme for both extract and fractions were also investigated.

Persea americana Mill. (Lauraceae) avocado pear, is a perennial shrub about 20m in height. It was originally grown in Central America but currently grown in many parts of the world where the leaf extract has been recognised to produce brief, dose-related, lowering of rat systemic arterial blood pressures and heart rates. Nowadays in different countries of the world many parts of the plant parts are now used as herbal remedies for managing many disorders, for example, menorrhagia, abdominal ache, bronchitis, diarrhoea, diabetes and hypertension (Ojewole *et al*., 2007).

Noradrenaline (NA) is an endogenous catecholamine with direct alpha and beta adrenergic receptor agonist activities. Sympathetic nerves are the main location of storing and discharge of noradrenaline. When noradrenaline binds to adrenoceptors in the blood vessels, it activates vasoconstriction of both capacitance and resistance vessels thereby causing increases blood pressure. Noradrenaline further increases blood pressure by its direct effect on the myocardium. It increases the force and rate of contraction which subsequently increase cardiac output. In addition, the rat aorta is known to respond biphasically to noradrenaline (NA) and the two components of the contraction (phasic followed by tonic) are mediated by adrenoceptors (Tank and Wong, 2015). For these reasons, noradrenaline can be used as a candidate for elevating the blood pressure in experimental animal models.

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 (α_1)-adrenoceptor activation. Ojewole *et al*., (2007) in an earlier study had stated that endothelium-containing aortic rings pre-contracted with NA in Krebs-Henseleit solution with and without normal calcium concentrations were relaxed by exogenous additions of *P. americana* aqueous leaf extract or acetylcholine. They also proposed a mechanism of non-competitive α_1- adrenoceptor blockade.

In this study the administration of *P. americana* methanol leaf extract and its ethyl acetate column fraction 3 significantly ($p< 0.05$) reduced the systolic blood pressure, diastolic pressure and the mean arterial pressure previously raised by noradrenaline and N^G-nitro-L-arginine methyl ester (L-NAME) (Ozolua *et al.*, 2009).

The mean arterial pressure is the average arterial pressure for the duration of one cardiac cycle. It is viewed a better indicator of perfusion to crucial organs than systolic blood pressure (SBP). It is determined via direct or oblique measurements of arterial pressure. Its parameters are Systolic Blood Pressure, Diastolic Blood Pressure and Pulse Pressure:

MAP = DBP + 1/3 PP

PP = SBP - DBP

PP = Pulse Pressure

SBP = Systolic Blood Pressure

DBP = Diastolic Blood Pressure

Like blood pressure, the unit of measure of mean arteria blood pressure is millimetre of mercury (mmHg).

In this study, the mean arterial pressure was used as the major index for antihypertensive activities as it is the pressure at which organs are supplied.

The mean arterial pressure (MAP), expresses a relationship between the systolic and diastolic pressures. Its significance lies in the fact that it reflects the perfusion pressure, ie, the force with which the blood in circulation supplies the vital organs of the body with oxygen and important nutrients (Beckett *et al*., 2008).

Normal mean arterial ranges are in the vicinity of 70 and 110 mm Hg. At least 60 mmHg is expected to give enough blood to support the brain, coronary arteries and kidneys. At the point when mean arterial pressure falls under 60 mmHg for a long time, organs may be denied of the sufficient oxygen required for normal functioning (Beckett *et al*., 2008).

The mean arterial pressure is influenced by variances 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.

According to Poiseuille relationship, resistance is conversely correlated with the fourth power of the vessel radius. In long-standing hypertension, the vessel radius is as often lessened because of a thickening of the wall of the vessel prompting a reduction in the size of the lumen. In addition, neurohumoral mechanisms are also significant in modifying systemic vascular resistance and pressure in the arteries, for the most part in

specific categories of secondary hypertension (Beevers *et al*., 2001). These mechanisms are controlled largely by arterial baroreceptors and chemoreceptors. Therefore, inhibiting the action of neurohumoral mechanism is said to be the main action for the therapies used for lowering elevated arterial pressures. However, nitric oxide, endothelin, prostacyclin and other vascular factors have important effects on the diameter of the vessel (Harraz *et al*., 2014).

The protective effect of the extract and its ethyl acetate fractions on blood pressure is evidenced by the significant decrease in the mean arterial pressure of the rat ($p < 0.05$). As said before, a standard range for the mean arterial pressure is 70 to 110 mm Hg in people. An elevated mean arterial pressure could indicate that the heart needs to work substantially harder than normal. This may cause an enormous stress on the heart. Elevated mean arterial pressure can bring about cutting edge coronary illness, blood clots, cardiac arrest, and stroke. With persistent raised mean arterial pressure, heart muscles will expand and become thicker, and endanger life. If mean arterial pressure shoots up abruptly, organs can fail. In addition, when the condition achieves a life-threatening level (around 180 mmHg), the hitherto constricted vessels cannot withstand the pressure, counter-control fails, and a widespread vasodilatation follows (Chien, 2000). Such a leap forward of cerebral blood stream prompts hyperperfusing the cerebrum under intense pressure, and results in cerebral edema and the clinical disorder of hypertensive encephalopathy.

However, it could be fatal if low levels of mean arterial pressure are ignored without treatment. Thus prompt intervention is necessary when pressures fall below 60 mmHg as shock and organ failure can follow when organs are not adequately perfused.

P. americana methanol leaf extract and CFEt3 exhibited significant reduction in noradrenaline-induced raised systolic, diastolic and mean arterial pressure (Ozolua *et al*., 2009). When administered on rats pre-treated with noradrenaline 4.0 ug/kg, all the fractions significantly lowered the mean arterial pressure. However, none of the fractions except the methanol leaf crude extract demonstrated a significant influence on the rate at which the heart was beating. Also, when the crude drug was used to pre-treat the rats before the administration of noradrenaline it prevented a rise in mean arterial pressure when in comparison with control.

Intravenous injection of PAM prompted a distinct lowering in mean arterial pressure lasting about 3 minutes. Rapid metabolism 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 *Persea 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 (Chobanian *et al.*, 2003).

That the result for ethyl acetate column fraction 3 (25 mg/kg) was more marked than those of the standard drugs (phentolamine – 83.3 µg/kg and labetalol - 25 mg/kg) shows that ethyl acetate column fraction 3 contains compounds which may possess

marked blood pressure lowering effect as mean arterial pressure has been said to be a better measure of tissue perfusion (Wehrwein, *et al*., 2013. This therefore offers a great advantage in protecting target organs against end organ damage that is common in hypertension (Lohmeier*, et al*., 2010).

Even though the impact of the extract and fraction were for the most part low on heart rate, ethyl acetate column fraction 3 still exhibited a slight reducing effect on heart rate. In addition, ethyl acetate column fraction 3 alone or when used to pretreat 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 on heart rate. Labetalol is a mixed α- and β- adrenoceptor blocker (Chobanian *et al*., 2003).

In this study, the intraperitoneal administration of N^G-Nitro-l-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor at 80 mg/kg daily for 7 days increased rodent blood pressure. This is akin to the discoveries of prior investigations by Beratova *et al*., (1999) who had earlier reported similar observations. In the L-NAME-pre-treated rats, the injection of ethyl acetate column fraction, 25 mg/kg for 7 days brought down systemic blood pressure parameters in contrast with control. This was in concurrence with different investigations (El Tahir *et al*., 2003, Khattab *et al*., 2007, Sayed *et al*., 2009). Be that as it may, ethyl acetate fraction 3 had no impact on L-NAME-induced increment in heart rate.

It has been documented that marked damage to target organs for the study of hypertension could be achieved by the use of prolonged administration of inhibitors of nitric oxide (Köng *et a*l., 1999).

Nitric oxide is a vasodilating factor synthesized in vascular endothelial cells by conversion of L-arginine to L-citrulline and nitric oxide by the enzyme nitric oxide synthase (Moncada, 1992). Nitric oxide is normally produced copiously to balance the contractile influence of the sympathetic system on vascular tone.

Intravenous administration of *P. americana* methanol leaf extract and its ethyl acetate column fraction 3 significantly reduced L-NAME-induced raised systolic blood pressure, diastolic blood pressure and mean arterial pressure (Ozolua *et al*., 2009). This effectively prevented the dysfunction in the synthesis of nitric oxide caused by L-NAME and therefore the imbalance between the vasoconstricting and vasorelaxing factors which is a common feature in several models of hypertension.

In the study on rat thoracic aorta precontracted with noradrenaline 10^{-5}M, ethyl acetate column fraction 3 offered significant ($p< 0.05$) reduction in the contraction of endothelium-intact aortic rings. The reduction in the contraction in endothelium denuded aortic rings was however not significant. These findings indicate that the action of the ethyl acetate column fraction requires intact endothelium.

In the endothelium-stripped aortic rings, there is decrease in the bioavailability of nitric oxide (Jaarin *et al*., 2015), an endothelium-dependent relaxation factor that

eases blood pressure homeostasis (Albrecht, 2003). Under standard physiological conditions, endothelial nitric oxide synthase produces basal levels of nitric oxide vascular stability. Nitric oxide promotes vascular smooth muscle relaxation by stimulating soluble guanylate cyclase and by augmenting cyclic guanosine - 3',5'-monophosphate (cGMP), which inactivates the release of intracellular calcium. Increases in intracellular calcium are associated with vascular smooth muscle narrowing. Prolonged vascular smooth muscle contraction is thought to start fundamental changes inside the vessel, for instance, thickening, which can incite an irreversible scaling in peripheral resistance. Studies have shown that opened up peripheral resistance is connected with primary hypertension (Munzel *et al*., 2003; Artinia *et al*., 2012; Ko *et al*., 2013; Harraz *et al.,* 2014).

Because of its effect on the relaxation of smooth muscle, nitric oxide plays a vital role in the physiological control of pulse and the reinforcing of the vasculature.

The arterial muscle relaxant effect of the extract disappeared on removal of the functional endothelium. This vasorelaxant property would appear to have contributed, at least in part, to the antihypertensive effect of the plant extract.
These observations therefore indicate that the vasorelaxant effects of *P. americana* and ethyl acetate column fraction 3 were dependent, in part, on the formation, synthesis and/or release of endothelium-derived nitric oxide, since removal of the functional endothelial cells led to the absence of relaxant response.

The present study also suggests that the endothelium-dependent vasorelaxant effect of *Persea americana* methanol leaf extract and ethyl acetate column fraction 3 could be mediated via endothelial nitric oxide signalling in the aortic tissue preparations. Thus, maintaining intact endothelial integrity is necessary for normal blood pressure as distorted endothelium can facilitate vascular damage.

Angiotensin-converting enzyme (ACE) plays a major role in the regulation of blood pressure. In this study, the inhibitory effect of PAM and CFEt3 was determined by a modification of the method described by Vermiessen *et al*., (2002) and expressed as IC_{50} (the concentration of sample needed to inhibit 50% of ACE activity).

ACE inhibition was measured using Hippuryl-L-Histidyl-L-Luecine as substrate, rabbit lung acetone extract as ACE source and Captopril as standard drug. *Persea americana* methanol leaf extract and ethyl acetate column fraction 3 showed significant angiotensin converting enzyme inhibitory activity. The ability of the treatments to block this enzyme indicated that on administration to the anaesthetized rats, there will be less Angiotensin II formed and hence less vasoconstricting effect on blood vessels and thus a decrease in blood pressure. An earlier report by Vermeirssen *et al*., (2002) had noted a comparable effect with Captopril. The IC_{50} value of ACE inhibition assay of methanol leaf extract and ethyl acetate column fraction 3 from *P. americana* leaf and Captopril revealed that Captopril exhibited a superior inhibition over both extract and fraction ($p > 0.05$). The presence of enzyme inhibitory activity in *P. americana* methanol leaf extract and ethyl acetate column fraction 3 is a noteworthy addition to understanding the probable antihypertensive mechanism of *P.americana.* In addition, more potent angiotensin converting inhibitors might well be isolated by

further chemical purification and investigation from this fraction, or from other parts of the plant.

Hypertension has been identified to be frequently connected with other cardiovascular hazard factors, for example, obesity, diabetes, and dyslipidemia (Segura and Ruilope, 2007). Dyslipidemia has been described as elevated plasma concentrations of lipid - triglycerides (TG) and total cholesterol (TC) and their blood transporting lipoproteins; HDL- Cholesterol, LDL-Cholesterol, VLDL-Cholesterol (Nwagha *et al*., 2010).

Additionally, several other researches have also noted the joint occurrence of hypertension and dyslipidemia in the same patients (Thomas and Bean, 2002; Wong *et al*, 2006).

Moreover, higher incidents of hypertension usually follow elevated values of cholesterol whereas increased High Density Lipoprotein-Cholesterol (HDL-C) values have decreased episodes of hypertension Because elevated lipid levels have been found to predate the onset of hypertension, it has thus been suggested that lipids may offer a potentially important screening tool with which to identify high risk individuals for hypertension over time and therefore, proper handling of dyslipidemia will have a positive outcome on blood pressure (Dobiasova and Frohlich, 2001; Halperin, 2006; Ford, 2011).

The atherogenic index of plasma characterized as logarithm [log] of the proportion of plasma convergence of triglycerides to high-density lipoprotein (HDL) cholesterol, has lately been proposed as an insightful marker for plasma atherogenicity and is unequivocally related with cardiovascular disease risk (Dobiasova, *et al*., 2001; George, 2006).

The atherogenic index of plasma reflects the genuine connection amongst defensive and atherogenic lipoprotein and is related with the extent of pro- and anti-atherogenic lipoprotein molecule. It has been proposed that an AIP estimation of under 0.11 is related with generally low incidence of cardiovascular diseases; the values between 0.11 to 0.21 and beyond predict higher incidence (Dobiasova, *et al.,*2011).

The antilipidemic activity of the extract and fraction were tested on Triton X 100- induced hyperlipidemic rats and the results showed that *P. americana* methanol leaf extract and ethyl acetate column fraction 3 demonstrated a positive atherogenic index of the plasma by reducing the levels of total cholesterol and triglycerides with a corresponding elevation in the values of high density lipoprotein. The Artherogenic Index of Plasma for both *Persea americana* methanol leaf extract and ethyl acetate column fraction 3 were 0.3 ± 0.001. This is similar to the Artherogenic Index of Plasma for Atorvastatin, a standard statin lipid lowering drug (Hannan *et al*., 2016). This lipid lowering effect may offer a protection against the damage of vascular endothelium that is common in hypertension resulting from hyperlipidemia.

It is noted that some previous studies have identified flavonoids and tannins; terpenoids and saponins (Adeyemi, *et al*., 2002), as constituents of the leaf of *P. americana*. That the additionally identified compounds from ethyl acetate column

fraction 3 by GC-MS still relaxed aortic rings pre-contracted with noradrenaline, blocked angiotensin converting enzyme *in vitro* and reduced hyperlipidemia in experimental animal models is a strong indication of the antihypertensive effects of leaf of *P. americana*. It also indicates that the leaf of the plant contains compounds that may be of use in the development of antihypertensive drugs.

Therefore, this study supports the fact that *P americana* possesses vasorelaxant and antihypertensive effects in the experimental animal paradigms used. This is in consonance with the reports of some of the earlier investigators (Kang *et al*., 2002; Owolabi *et al.,* 2005; Yin *et al.,* 2005; Ojewole *et al*, 2007) who have reported similar observations.

CHAPTER SIX

SUMMARY AND CONCLUSIONS

Hypertension is an illness of the cardiovascular system characterised by persistent elevation in arterial blood pressure higher than 140/90 mmHg. Thus, blood pressure must be closely controlled to safeguard continuous blood supply to all organs in the body. However, current antihypertensive therapy results in many adverse effects, prolonged treatment and high cost, hence the need for alternative remedies. *Persea americana* leaf has over time found use in herbal medicine for treating hypertension with little scientific justification. This study therefore investigated the antihypertensive properties and mechanism(s) of action of the extract and fractions of *P. americana* leaf in rats.

This study evaluated in rats, the effect of the methanol extract and fractions of *P. americana* on raised blood pressure induced by noradrenaline and N^G -nitro- L -arginine methyl-ester (L-NAME) *in vivo*, the angiotensin converting enzyme inhibitory action of the extract and fractions *in vitro* and the hyperlipidemic effect of the treatments *in vivo*. The findings from the study showed that the treatments afforded significant antihypertensive effects against noradrenaline- and L-NAME-induced hypertension in rats, inhibited ACE activity *in vitro* and lowered the atherogenic index of plasma *in vivo*.

Noradrenaline was used to raise the blood pressure in anaesthetized rats. This rise in blood pressure was effectively reversed by the extract and fractions of the leaf of *P. americana*. The raised blood pressure induced through L-NAME was countered through the extract and fractions. The contraction induced in the thoracic aortic rings by noradrenaline and L-NAME was also effectively relaxed by the extract and column fraction. This indicates that the extract and column fraction possess antihypertensive activity.

In this study, the ability of *Persea americana* leaf methanol extract and column fraction to inhibit angiotensin converting enzyme was investigated. Angiotensin converting enzyme converts angiotensin I to angiotensin II in the kidney. The produced angiotensin II constricts blood vessels and elevates blood pressure. *Persea americana* extracts and fractions inhibited ACE activity *in vitro* with IC_{50} values comparable to that of captopril.

In addition, *P. americana* leaf methanol extract and its ethyl acetate column fraction 3 considerably decreased Atherogenic Index of Plasma. This demonstrated amelioration of hyperlipidemic profile indicates that the extract and fraction offer a protection against the occurrence of factors that may lead to deposition of atherogenic

agents on the blood vessels. When these agents are deposited, there is reduced nitric oxide production and increased incidence of cardiovascular diseases.

Persea americana leaf methanol extract and its ethyl acetate column fraction 3 possess *in vivo* antihypertensive action in rats and *in vitro* vasorelaxant effect which may be mediated by inhibition of vascular alpha adrenoceptors, angiotensin converting enzyme and amelioration of dyslipidemia.

The findings from this study have confirmed the potential of *Persea americana* methanol leaf extract and ethyl acetate column fraction 3 as agent(s) from which useful pharmaceuticals could be developed for the management of hypertension.

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