EFFECTS OF PETROLEUM ETHER AND METHANOLIC FRACTIONS OF AVOCADO PEAR (*PERSEA AMERICANA* MILL*)* SEEDS ON WISTAR RATS FED A HIGH FAT-HIGH CHOLESTEROL DIET

BY

AFAHAKAN, MFONOBONG EFIONG (B.Sc., MAU 2008)
M. SC/SCIEN/07477/2009-2010

BEING A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY ZARIA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN BIOCHEMISTRY

DEPARTMENT OF BIOCHEMISTRY
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY
ZARIA, NIGERIA

DECEMBER, 2012

DECLARATION

I declare that the work in the thesis entitled "Effects of petroleum ether and methanolic fractions of avocado pear *(Persea americana* Mill) seeds on wistar rats fed a high fat-high cholesterol diet" has been performed by me in the Department of Biochemistry under the supervision of Dr I. A. Umar and Dr. H. M. Inuwa. The information derived from literature has been duly acknowledged in the text and list of references provided. No part of this thesis was previously presented for another degree or diploma at any other university.

_____ _____

Afahakan, Mfonobong Efiong Date

CERTIFICATION

This dissertation entitled "EFFECTS OF PETROLEUM ETHER AND METHANOLIC FRACTIONS OF AVOCADO PEAR (*Persea americana* Mill) SEEDS ON WISTAR RATS FED A HIGH FAT-HIGH CHOLESTEROL DIET", by Afahakan Mfonobong Efiong meets the regulations governing the award of the degree of Masters of Science (M.Sc.) in Biochemistry of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

_____ _____

Dr. I. A. Umar Date

Chairman, Supervisory Committee

_____ _____

Dr. H. M. Inuwa Date

Member, Supervisory Commitee

_____ _____

Dr. H. M. Inuwa Date

Head of Department

_____ _____

Prof. A. A. Joshua Date

Dean Postgraduate School

DEDICATION

With gratitude to God, this work is dedicated to my dad, Late Elder E. A. Afahakan.

ACKNOWLEDGEMENTS

My profound gratitude goes to my God for his grace and mercy. My appreciation goes to my able supervisors Dr. I. A. Umar and Dr. H. M. Inuwa for their efforts, patience, support, thoroughness and relentless encouragement throughout the period of this work. I also wish to thank Dr. S. Ibrahim, and all academics Staff of the Department of Biochemistry for their contributions towards the success of this work.

My special thanks goes to Professor G. I. Ndukwe of Department of Chemistry, Professor J. O. Ayo of Faculty of Veterinary Medicine, and Mrs Fatima Dawud of Physiology Department for their countless contributions. I am also grateful to all laboratory staff of Departments of Biochemistry, Organic Chemistry and Animal unit of the Department of Pharmacy.

My appreciations also goes to Dr and Barrister (Mrs) A. Ihuoma, Chief E. Akaiso, Ambassador B. Eyo for their generosity. I will not forget my colleagues Maimuna, Malik, Fatima, Ali, Haruna, Vicky, Atabo and Chidi for always being there for me. Also, to my friends, Chichy, Zioness, Christy, Shade, Mary Esin, Enobong, Ity, Shaineze, Emeka, Taku, Raymond, Mrs Shall, and Mrs Shittu for their love and support.

My heartfelt gratitude goes to my siblings, for their patience, love, support and belief in me. Most of all, my thanks goes to my beloved mother, Mrs A. E. Afahakan for always being there. Finally, to others whose names are not mentioned but have contributed in making this work a success, I am grateful.

ABSTRACT

The effect of petroleum ether and methanolic fractions of Avocado pear (*Persea americana Mill)* in diet-induced hyperlipidemic rats was studied. Eleven groups of five rats each were treated for four weeks and they included normal control, the high fat high cholesterol diet (HFD) control group, HFD + 0.20mg/kgbw Atorvastatin, and groups treated with 125mg/kg, 250mg/kg and 500mg/kg body weight petroleum ether and methanolic fractions. Quantitative phytochemicals analysis revealed significantly ($p<0.05$) higher quantities of saponins (10.30±0.60%), flavonoids (74.30±5.20%), and phenols (27.83±2.47%) in the methanolic fraction. Result of biochemical analysis showed HFD control group had significant ($p<0.05$) increases in the serum total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), triacylglycerols, total lipids, total phospholipids, and the atherogenic index (LDL-c/HDL-c and TC/HDL-c) (268.60±30.40mg/dl, 233.40±30.5mg/dl, 71.70±4.30mg/dl, 13.10±3.00g/dl, 5.70±0.90g/dl, 13.00±1.90, 10.00±3.50 respectively); while it recorded significant ($p<0.05$) decrease in high density lipoprotein cholesterol (HDL-c) (20.70±4.30mg/dl). These effects were reversed in the extracts treated groups. There was a significant ($p<0.05$) increase in thiobarbituric reactive substances (4.73±1.50µmol (NC), and 24.98±5.90µmol (HFD) respectively) and significant ($p<0.05$) decrease in organ catalase (0.04±0.01mol/min/g (NC) and 0.02±0.01mol/min/g (HFD) respectively) and superoxide dismutase activities (152.00±19.10unit/g (NC) and 139.90±8.80unit/g (HFD) respectively) observed in the liver and kidney homogenate in the HFD control group which were reversed in the extracts treated groups. There was no significant ($p>0.05$) difference in the level of reduced gluthathione in both the liver and kidney

homogenate in normal and HFD control groups. Serum alanine and aspartate aminotransferases showed no significant ($p>0.05$) differences between the normal and HFD control groups, while there was significant ($p<0.05$) decrease in alkaline phosphatase level. The serum urea level decrease ($p<0.05$) significantly in the treatment groups when compared with the normal control group. The petroleum ether and methanolic fractions of *Persea americana* seed has been shown to have hypolipidemic and hypocholesterolemic potentials as well as *in vivo* antioxidant activity in diet-induced hyperlipidemic rats.

TABLE OF CONTENTS

LIST OF TABLES

LISTS OF FIGURES

LISTS OF APPENDICES

LIST OF ABBREVIATIONS

%	Percent
µmol	Micromole
ADP	Adenosine diphosphate
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ASVD	Arteriolsclerotic vascular disease
ATP	Adenine triphosphate
BMI	Body Mass Index
CAL	Calibrator
CAT	Catalase
cm	Centimetre
CO_2	Carbon dioxide
conc.	Concentration
DAG	Diacylglycerol
DB	Direct bilirubin
DNTB	Dithio (5' 5' nitro benzoic acid)
EC	Enzymes classification
FDA	Food and Drug Administration
$FeCl_3$	Iron (vi) chloride
g	Gram
g/dl	Gram per decilitre

GK	Glycerol kinase
GPO	Glycerol-3-phosphate
GSH	Reduced gluthathione
H_2O	Water
H_2O_2	Hydrogen peroxide
HCl	Hydrogen chloride
HDL-c	High density lipoprotein cholesterol
HFD	High fat diet
HMG-CoA	3- Hydroxyl 3-methylglutaryl coenzyme A
IP_3	Inositol triphosphate
kg	Kilogram
LD_{50}	Median lethal dose
LDL-c	Low density lipoprotein cholesterol
Ltd	Limited
M	Methanolic extract
m^2	Metre square
MDA	Malondialdehyde
mg/dl	Miligram per decilitre
$MgCl_2$	Magnesium chloride
ml	Millilitre
mmol/L	Milimole per litre
nm	Nanometre
O_2	Oxygen
P	Petroleum ether extract

P. americana	*Persea americana*
PCV	Packed cell volume
pH	Hydrogen ion concentration
PLP	Pyridoxal phosphate
POD	Peroxidase
r.p.m	Revolution per minute
RAAS	Renin Angiotensin Aldosterone System
RI	Reagent 1
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TB	Total bilirubin
TBARS	Thiobarbituric reactive substances
TCA	Trichloroacetic acid
TG	Triacylglycerol
TP	Total protein
UK	United Kingdom
USA	United State of America
VLDL-c	Very low density lipoprotein cholesterol
WHO	World Health Organization
wt	Weight

CHAPTER ONE

INTRODUCTION

1.1 General

Hyperlipidemia is known to be a risk factor for cardiovascular diseases which is one of the leading causes of mortality and morbidity in humans (Krieger, 1998). The modification of lipid concentration has been found to be a useful approach to decreased cardiovascular mortality through prevention of development of atherosclerotic diseases (Frisinghelli and Mafrici, 2007; Laclaustra, *et al*., 2007).

Cardiovascular diseases are a growing health problem all over the world (Crews, 2007). hyperlipideamia is an important risk factor in atherosclerosis (Epstein, 1999). Abnormal high dietary cholesterol which leads to hypercholesterolemia is strongly associated with cardiovascular diseases because it promotes atherosclerosis (Durrington, 2003).

Hypercholesterolemia is the presence of high levels of cholesterol in the blood (Ghanta and Asthana, 1995). It is not a disease but a metabolic derangement that can results in many diseases, notably cardiovascular disease. It is closely related to the terms "hyperlipidemia" (elevated levels of lipids in the blood) and "hyperlipoproteinemia" (elevated levels of lipoproteins in the blood) (Durrington, 2003).

There are several medications that may help to lower plasma lipid. Medications can reduce low density lipoprotein-cholesterol by 20-40% and they can also modestly increase high density lipoprotein-cholesterol (Miller, 2001). Available drugs include niacin, fibrates and 5-hydroxy-3-methylglutaryl-CoA reductase inhibitors (HMGCoA

reductase) among others. However, these synthetic medications are not without their adverse effects. In view of this, the quest for natural products with lipid lowering potential and with minimal or no adverse effects is warranted (Villa and Pratley, 2010)

The use of medicinal plants in medicine, in the treatment of hypertension and related cardiovascular diseases is increasing because of relative cheapness, availability, time-trusted efficacy and absence of adverse effects (Fogari and Zoppi, 2004). Plant medicine or phytomedicine (the sum total of practices, using herbal preparations produced by physical or biological processes, to treat diseases relying on past experience/ observations handed down from generation to generation) has been used in healthcare delivery in many parts of Africa and the rest of the world. At least 80% of Africans depend on plant medicine for their healthcare (Sofowora, 1993; Okwu and Uchegbu, 2009).

Medicinal plants are increasingly being used in most parts of the world as: hypolipidemic (Ugochukwu *et al.*, 2003; Ogbonnia *et al.*, 2008;); contraceptive, abortifacients, or oxytocic (Ritchie, 2001); antihypertensive (Ofem *et al.,* 2007; Ojewole and Adewole, 2007; Nworgu *et al.,* 2008); treatment for skin diseases (Ajose, 2007), wound healers (Biswas and Mukherjee, 2003); antimicrobial (Okigbo and Ajalie, 2005; Osadebe and Akabogu 2006;) and hypoglycaemic (Yadav *et al.*, 2008; Lee *et al.,* 2009) agents.

Persea americana, otherwise known as the avocado pear, Mexican avocado or butter fruit, is a medium-sized, singlestemmed, terrestrial, erect, perennial, deciduous tree 15–20m in height. Although a native of Central America (Mexico), *Persea americana* is now found in most tropical and subtropical countries of the world. The branches are

fissured and grey, but the twigs are green and smooth. The 15–25-cm long and 10–20-cm broad leaves with well-developed petioles are spirally arranged, often clustered near the branch ends, narrowly to broadly elliptical or obolate, and are usually pointed at the tip (Ross, 1999). The greenish-yellow flowers are borne on branched, compact panicles, which are shorter than the leaves. The often pear-shaped, one-seeded fruits are variable in size and shape according to the variety, up to 18 cm long and usually shiny and green, or brownish when ripe. The flesh is soft, oily, greenish or yellow surrounding one large, loose round seed. In many parts of Africa, the fruits of the avocado are much sought after by humans and some animals as valuable foodstuff. Besides the oil, avocado fruit pulp contains carbohydrates and more protein than many other fruits, while its contents of vitamins A and B are high (Watt *and* Breyer-Brandwijk, 1962; Ross, 1999).

The aqueous seed extract of *Persea americana Mill* (Lauraceae) is used by herbalists in Nigeria for the management of hypertension (Ozolua *et al.*, 2009). In addition to the nutritional value of its fruit, the leaves and other morphological parts of *Persea americana* possess medicinal properties and are widely used in traditional medicines of many African countries. For example, the fruit pulp is eaten as an aphrodisiac in South Africa, while a hot-water extract of the leaves is taken orally as a diuretic and for the treatment of hypertension in many West African countries (Ross, 1999). In some other parts of the world, various morphological parts of *Persea americana* have been employed for a wide range of human ailments. Products of the plant have been effectively used for the management, control and treatment of amenorrhoea, anaemia,

insomnia, hyperlipidaemia, hypertension, diabetes mellitus, diarrhoea, dysentery, gastritis, peptic ulcers, bronchitis, cough, hepatitis, and so forth (Ross, 1999).

The fruit in particular has been shown to possess medicinal properties. The edible fruit pulp contains up to 33% oil rich in monounsaturated fatty acids (Ortiz *et al.,* 2004) that are believed to modify the fatty acid contents in cardiac and renal membranes and enhance the absorption of α/β-carotene and lutein (Salazar *et al.,* 2005). The carotenoid content has been reported to play significant role in cancer risk reduction (Lu *et al*., 2005). Other properties of the oil include wound healing (Nayak *et al*., 2008) and hepatoprotection (Kawagishi *et al.,* 2001). Proximate analysis has been conducted on the seeds (Olaeta *et al.,* 2007).

Other parts of the plant have been reported to possess medicinal properties. The aqueous leaf extract for example has analgesic and anti-inflammatory (Adeyemi *et al.,* 2002), anticonvulsant (Ojewole and Amabeoku, 2006), hypoglycaemic and hypocholesterolemic (Brai *et al.,* 2007a), vasorelaxant and blood pressure reducing (Owolabi *et al.,* 2005; Ojewole *et al.,* 2007), activities in animal studies. Although the pulp has been most widely consumed all over the world, in Nigeria, the powdered seed is often mixed with soups, pap and puddings in the belief that it is useful in the management of chronic hypertension.

1.2 Statement of problem

Hypertension has been acknowledged as one of the greatest and established risk factors for cardiovascular diseases. According to the WHO report in 2008, approximately, 1 in 3, or 37.13% or 101 million people in USA live with hypercholesterolemia. And this

trend is increasing in developing countries such as African and Eastern Asia countries. In Nigeria, 6 million people suffer from high cholesterol level while 1.2 million deaths are reported due to hypercholesterolemia related diseases (WHO, 2010)

Search for appropriate hypolipidemic agents had been focused on plants used in traditional medicine because of leads provided by natural products that may be better treatment than currently used drugs which are not affordable, inaccessible and with a lot of side effects including serious muscle and liver problems, erectile dysfunction, loss of memory, insomnia, personality changes and irritability. There is natural cholesterol lowering ingredients, some of which have been shown to outperform the orthodox drugs in lowering cholesterol, without the possible side effects (Fogari and Zoppi, 2004).

1.3 Justification

In Africa, there has been increasing interest in the use of medicinal plants. The use of medicinal plants in modern medicine suffers from the fact that though hundreds of plants are used in the world to prevent or to cure diseases, scientific evidence in terms of modern medicine is lacking in most cases. However, today it is necessary to provide scientific proof as to whether it is justified to use a plant or its active principles (Singh *et al.,* 2007). Throughout the world many traditional treatments for hypercholesterolemia exist. However, few have received scientific or medical scrutiny.

This study was prompted by the claim of some traditional health practitioners in Oron, Akwa Ibom State that decoctions and infusions of avocado seeds are effective remedies for the management and/or control of hypertension and certain cardiac disorders. Hence,

an endeavour will be made to delineate the edifying effect of *Persea americana* in the changes in the lipid profile mediated by cholesterol and fatty acid.

1.4 Aim and objectives

The aim of this study is to investigate the hypolipidemic and antioxidant effects of petroleum ether and methanolic fractions of *Persea americana seed* in experimental animal paradigms fed a high-fat-high-cholesterol diet with a view to providing a pharmacological justification (or otherwise) for the ethnomedical uses of the plant seeds in the management, control and/or treatment of and certain cardiovascular disorders in some rural African communities.

1.5 Specific objectives

1. To carry out the quantitative phytochemical analysis of petroleum ether and methanolic fractions of the seeds of *Persea americana.*

2. To evaluate the effects of the extracts on serum lipid profile of diet-induced hyperlipidemic rats.

3. To examine the effect of the extract on levels of endogenous antioxidants and peroxidation in diet-induced hyperlipidemic rats.

4. To assess the effects of the extracts of *Persea americana* on hepatic and renal function markers in the serum of the diet-induced hyperlipidemic rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Avocado pear

Avocado pear (*Persea americana)* Mill is a tree, native to Central America, cultivated in tropical and subtropical climates around the world, belonging to the family Lauraceae. There are 3 principal races or groups of avocado: Mexican, Guatemalan, and West Indian named for the areas where they were originally cultivated. *P. americana Mill* is commonly known as avocado (Ross, 1999).

2.1.1 Botanical description

Kingdom	*Plantae*
Subkingdom	*Tracheobionta*
Superdivision	*Spermatophyta*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Magnoliidae*
Order	*Laurales*
Family	*Lauraceae*
Genus	*Persea* Mill
Species	*Persea americana* Mill

English: alligator pear, avocado, avocado-pear, butter fruit

Persea americana is a medium to large tree, 15-20 m in height. Avocado tree is distributed geographically. The avocado is classified as an evergreen, although some varieties lose their leaves for a short time before flowering. The tree canopy ranges from low, dense, and symmetrical to upright and asymmetrical. Leaves are 7-41 cm in length and variable in shape (elliptic, oval, and lanceolate). They are often pubescent and reddish when young, becoming smooth, leathery, and dark green when mature (Crane and Walker, 1984). Flowers are yellowish green and 1-1.3 cm in diameter. The many-flowered inflorescences are borne in a pseudoterminal position. The central axis of the inflorescence terminates in a shoot. The fruit is a berry, consisting of a single large seed, surrounded by a buttery pulp. It contains 3-30% oil (Florida varieties range from 3% to 15%). The skin is variable in thickness and texture. Fruit color at maturity is green, black, purple, or reddish, depending on the variety. Fruit shape ranges from spherical to pyriform and weighs up to 2.3 kg (Bekele-Tesemma *et al*., 1993).

Figure 2.1: Avocado pear tree in its fruiting season (Source: Crane and Walker, 1984).

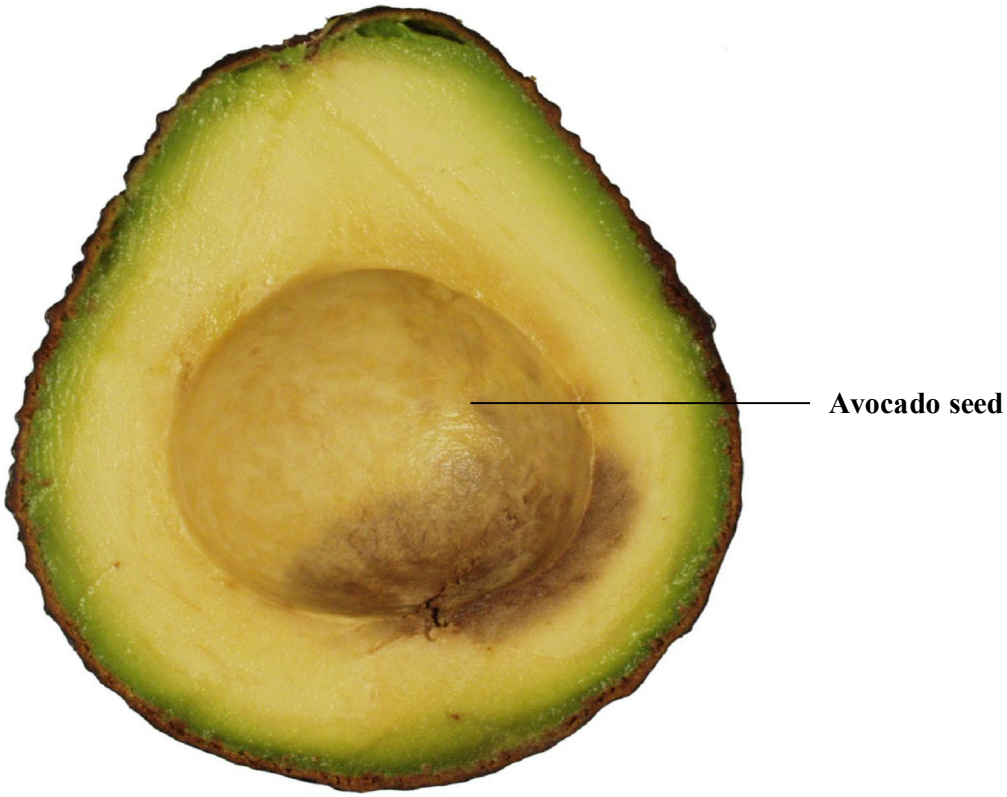


Figure 2.2: Avocado fruit (Source: Crane and Walker, 1984).

2.2 Hypercholesterolemia

Hypercholesterolemia is the presence of high levels of cholesterol in the blood. Elevated cholesterol in the blood is due to abnormalities in the levels of lipoproteins, the particles that carry cholesterol in the bloodstream. This may be related to diet, genetic factors (such as LDL receptor mutations in familial hypercholesterolemia) and the presence of other diseases such as diabetes and an underactive thyroid. The type of hypercholesterolemia depends on which type of particle (such as low-density lipoprotein) is present in excess (Durrington, 2003).

Hypercholesterolemia is treated by reducing dietary cholesterol intake, administration of certain medications, and rarely with other treatments including surgery (for particular severe subtypes). Although hypercholesterolemia itself is asymptomatic, longstanding elevation of serum cholesterol can lead to atherosclerosis. Over a period of decades, chronically elevated serum cholesterol contributes to formation of atheromatous plaques in the arteries. This leads to progressive stenosis (narrowing) or even completes occlusion (blockage) of the involved arteries. Blood supply to the tissues and organs served by these stenotic or occluded arteries gradually diminishes until organ function becomes impaired (Bhatnagar *et al.,* 2008). It is at this point that tissue ischemia (restriction in blood supply) may manifest as specific symptoms.

2.3 Obesity

Obesity is essentially an excessive accumulation of triacylglycerols in fatty tissue that is, the net result of excessive energy intake compared to energy usage. Obesity is a condition of increased adipose tissue mass (Gray, 1998). Obesity can also be defined as an increase in body weight beyond the limits of physical requirement, as the result of an excessive accumulation of fat. Accumulation of fat, or triacylglycerol, is essentially the only way that body weight can become excessive, as other energy storage (e.g. carbohydrate glycogen or protein in liver and muscle) does not have the potential of adipose tissue to exceed the limits of requirement. The definition of obesity cannot be simply made in terms of body weight because we should expect short people to be lighter than tall people. The simplest expression for this is the body mass index (BMI) calculated as weight (kg) divided by height squared (m^2) (Gray, 1998).

One of the consequences of obesity is community stigma where public disapproval may affect education, employment, income, marital status and health care. These are significant detrimental effects on the quality of life and are associated with higher incidence of depression. The risk of diabetes increases by 9% for each kg gained in self reported weight and generally starts to increase at a BMI of 22 (Colditz *et al.,*1996) and is 40 times higher at a BMI over 35 (Hu *et al.,*2001). Insulin resistance is widely recognized as a fundamental defect seen in obesity and Type 2 diabetes. The development of type 2 diabetes is strongly associated with overweight and obesity in both genders and all ethnic groups

Cholesterol production increases as body fat increases and high concentrations of cholesterol relative to bile acids will increase the likelihood of gallstone precipitation. BMI increases the risk of coronary artery disease, and weight gain from any initial BMI further increases the risk (especially weight gain of 20 kg or more). Obesity increases very low density lipoproteins through increased production and decreased clearance of triglyceride rich lipoproteins due to lack of stimulation of lipoprotein lipase. Obesity also lowers HDL in men and women of all ages and ethnicities (Sternfeld *et al.,* 1999).

Hypertension is present in about half of all overweight individuals (Rocchini, 2003) and obesity alone accounts for about 70% of essential hypertension. Cardiac weight increases with increasing body weight, but heart weight as a percentage of total body weight is lower than in normal weight controls (Del, 2000). In obesity, the increase in cardiac output is not explained by the presence of the new adipose tissue, but may be due to increased sympathetic activity. Adrenaline (from the adrenal medulla) tends to be normal to low in obesity and there is a decreased response of adrenaline to

hypoglycaemia and exercise Hypertension in the overweight is associated with increased sympathetic activity and sympathetic blockers have greater effect in obesity (Rocchini, 1999). Sodium reabsorption is increased with high fat diets and the renin/angiotensin/aldosterone system (RAAS) is activated in obesity despite volume expansion and sodium retention (Egan *et al*., 1994). Aldosterone tends to be higher in obese individuals while renin is often relatively normal and there is a positive correlation between BMI and the aldosterone to renin ratio.

2.4 Atherosclerosis

Atherosclerosis (also known as arteriosclerotic vascular disease or ASVD) is a condition in which an artery wall thickens as a result of the accumulation of fatty materials such as cholesterol. It is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries, caused largely by the accumulation of macrophage white blood cells and promoted by low-density lipoproteins (plasma proteins that carry cholesterol and triacylglycerols) without adequate removal of fats and cholesterol from the macrophages by functional high density lipoproteins (HDL). It is commonly referred to as a hardening or furring of the arteries. It is caused by the formation of multiple plaques within the arteries (Maton *et al.,* 1993).

Over the course of years and decades, plaque build up narrows the arteries and makes them stiffer. These changes make it harder for blood to flow through them. Clots may form in these narrowed arteries and block blood flow. Pieces of plaque can also break off and move to smaller blood vessels, blocking them. Either way, the blockage starves tissues of blood and oxygen, which can result in damage or tissue death (necrosis). This

is a common cause of heart attack and stroke. If a clot moves into an artery in the lungs, it can cause a pulmonary embolism.

In some cases, the plaque is part of a process that causes a weakening of the wall of an artery. This can lead to an aneurysm. Aneurysms can break open (rupture), and cause bleeding that can be life threatening (Gennest and Libby, 2011). Hardening of the arteries is a process that often occurs with aging. However, high blood cholesterol levels can make this process happen at a younger age.

For most people, high cholesterol levels are the result of an unhealthy lifestyle -- most commonly, eating a diet that is high in fat. Other lifestyle factors are heavy alcohol use, lack of exercise, and being overweight (Libby, 2011). Other risk factors for hardening of the arteries include; diabetes, family history of hardening of the arteries, high blood pressure and Smoking

2.4.1 Symptoms

Hardening of the arteries does not cause symptoms until blood flow to part of the body becomes slowed or blocked. If the arteries to the heart become narrow, blood flow to the heart can slow down or stop. This can cause chest pain (stable angina), shortness of breath, and other symptoms. Narrowed or blocked arteries may also cause problems and symptoms in the intestines, kidneys, legs, and brain.

2.5 Atorvastatin

Atorvastatin® is a drug marketed by Pfizer as a calcium salt under the trade name Lipitor (McCrindle *et al.,* 2003). It belongs to a class of drugs referred to as statins,

which includes lovastatin (Mevacor), simvastatin, (Zocor), fluvastatin (Lescol), and pravastatin (Pravachol). All statins, including atorvastatin, are used for lowering blood cholesterol. It also stabilizes plaque and prevents strokes through anti-inflammatory and other mechanisms.

2.5.1 Drug class and mechanism of action

Atorvastatin is an oral drug that lowers the level of cholesterol in the blood. Statins reduce total cholesterol as well as LDL cholesterol in blood. LDL cholesterol is believed to be the "bad" cholesterol that is primarily responsible for the development of coronary artery disease. Reducing LDL cholesterol levels retards progression and may even reverse coronary artery disease. Atorvastatin also reduces the concentration of triglycerides in the blood and raises the concentrations of HDL ("good") cholesterol. High blood concentrations of triglycerides also have been associated with coronary artery disease. The FDA approved atorvastatin in December 1996.

Atorvastatin works by inhibiting HMG-CoA reductase, an enzyme found in liver tissue that plays a key role in production of cholesterol in the body. It is a competitive inhibitor of HMG-CoA reductase. Unlike most others, however, it is a completely synthetic compound. HMG-CoA reductase catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate, which is the rate-limiting step in hepatic cholesterol biosynthesis. Inhibition of the enzyme decreases *de novo* cholesterol synthesis, increasing expression of low-density lipoprotein receptors (LDL receptors) on hepatocytes. This increases LDL uptake by the hepatocytes, decreasing the amount of LDL-cholesterol in the blood. Atorvastatin also reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol.

In clinical trials, drugs that block cholesterol uptake like ezetimibe combine with and complement those that block biosynthesis like atorvastatin or simvastatin in lowering cholesterol or targeting levels of LDL (Villa and Pratley, 2010).

Atorvastatin is used for the treatment of elevated total cholesterol, LDL, triglycerides and to elevate HDL cholesterol. The effectiveness of Atorvastatin in lowering cholesterol is dose-related, meaning that higher doses reduce cholesterol more. Atorvastatin prevents angina, stroke, heart attack, hospitalization for congestive heart failure, and revascularization procedures in individuals with coronary heart disease. It also prevents heart attacks and strokes in patients with type 2 diabetes with multiple risk factors for coronary artery disease.

2.5.2 DOSAGE

Atorvastatin comes in 10, 20 to 80mg. Atorvastatin is prescribed once daily. The usual starting dose is 10-20 mg per day, and the maximum dose is 80 mg per day. Individuals who need more than a 45% reduction in LDL cholesterol may be started at 40 mg daily. Atorvastatin may be taken with or without food and at any time of day.

2.5.3 Atorvastatin interactions with other drugs

Decreased elimination of Atorvastatin could increase levels of Atorvastatin in the body and increase the risk of muscle toxicity from Atorvastatin. Therefore, Atorvastatin should not be combined with drugs that decrease its elimination. Examples of such drugs include erythromycin (E-Mycin), ketoconazole (Nizoral), itraconazole), and HIV protease inhibitors such as indinavir (Crixivan) and ritonavir (Norvir).

2.6 Total lipids

Lipids are usually defined as those compounds that are soluble in organic solvents (such as ether, hexane or chloroform), but are insoluble in water. This group of substances includes triacylglycercols, diacylglycercols, monoacylglycercols, free fatty acids, phospholipids, sterols, caretonoids and vitamins A and D. Each type of fat has a different profile of lipids present which determines the precise nature of its nutritional and physiochemical properties.

Lipids are one of the major constituents of foods, and are important in our diet for a number of reasons. They are a major source of energy and provide essential lipid nutrients. Nevertheless, over-consumption of certain lipid components can be detrimental to our health, *e.g.* cholesterol and saturated fats. In many foods the lipid component plays a major role in determining the overall physical characteristics, such as flavor, texture, mouthfeel and appearance. For this reason, it is difficult to develop low-fat alternatives of many foods; because once the fat is removed some of the most important physical characteristics are lost. Also, many fats are prone to lipid oxidation, which leads to the formation of off-flavors and potentially harmful products (Nelson and Cox, 2000).

2.7 Cholesterol

Cholesterol measurements are used in diagnosis and treatments of lipid lipoprotein metabolisms disorders. Lipids play an important role in the body; they serve as hormones or hormone precursors, aid in digestion, provide energy, storage and

metabolic fuels, act as functional and structural components in biomembranes and form insulation to allow nerve conduction and prevent heat loss (Haines, 2001).

Cholesterol is a fat (lipid) which is produced by the liver and is crucial for normal body functioning. Cholesterol exists in the outer layer of every cell in our body and has many functions. It is a waxy steroid and is transported in the blood plasma of all animals. In humans, cholesterol serves three aims. It is used by certain glands to manufacture steroid or cortisone-like hormones, including sex hormones; it helps the liver to produce bile acids, which are essential to the digestion of fats; and, most important, it is a main component of cell membranes and structures, a kind of building block for bodily tissues. Without it, mammalian life would not be possible. The problem with cholesterol arises when the body has too much of it, or has deposits of it in the wrong places. Coronary heart disease results when cholesterol is deposited inside the walls of the heart's coronary arteries, the main suppliers of oxygen to the hearts own muscle tissue. There it contributes to the formation of fatty, toughened blockages called plaque. This build up of plaque is variously called arteriosclerosis, hardening of the arteries, and atherosclerosis (Olson, 1998).

Both dietary cholesterol and that synthesized *de novo* are transported through the circulation in lipoprotein particles. The same is true of cholesteryl esters, the form in which cholesterol is stored in cells. The synthesis and utilization of cholesterol must be tightly regulated in order to prevent over-accumulation and abnormal deposition within the body. Of particular importance clinically is the abnormal deposition of cholesterol and cholesterol-rich lipoproteins in the coronary arteries. Such deposition, eventually

leading to atherosclerosis, is the leading contributory factor in diseases of the coronary arteries (Lecerf and Lorgeril, 2011).

Cholesterol can also be deposited within arteries elsewhere in the body, where it may contribute to the occurrence of stroke (from blocked arteries in the brain) and peripheral vascular disease (from arterial blockage in the legs).

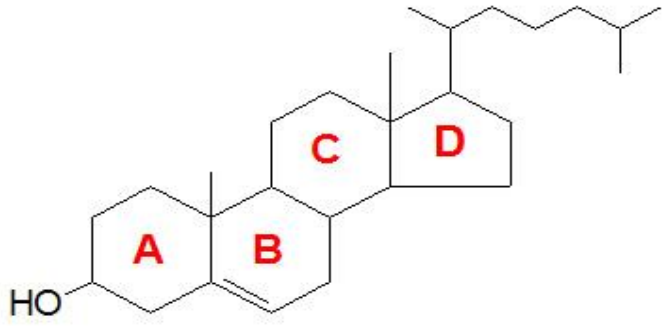


Figure 2.3: Structure of cholesterol (Source: Jonathan, 2003)

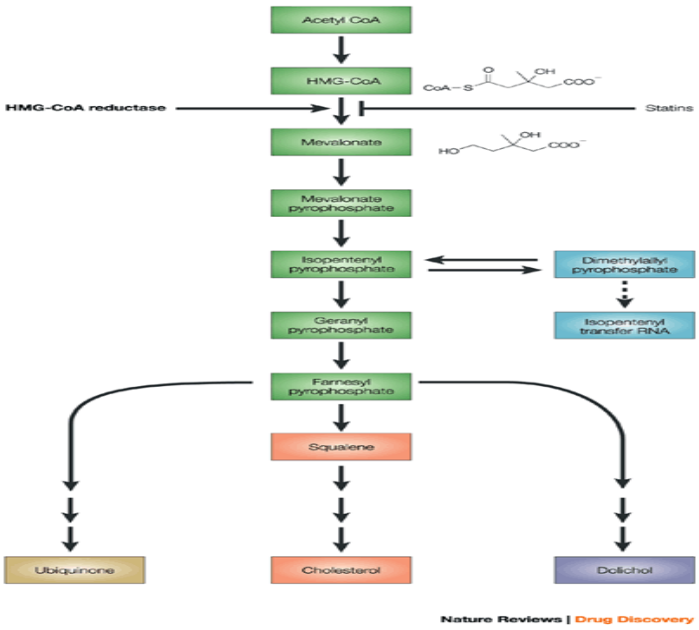


Figure 2.4 Cholesterol synthesis (Source: Jonathan, 2003).

The process of cholesterol synthesis has five major steps:

1. Acetyl-CoAs are converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)

2. HMG-CoA is converted to mevalonate

3. Mevalonate is converted to the isoprene based molecule, isopentenyl pyrophosphate (IPP), with the concomitant loss of CO_2

4. IPP is converted to squalene

5. Squalene is converted to cholesterol.

Regulation of Cholesterol Biosynthesis+

1. Synthesis of LDL receptors decreases with increased cholesterol.
2. Synthesis of HMG CoA reductase decreases with increased cholesterol.

2.8 Phospholipids

Phospholipids are a class of lipids that are a major component of all cell membranes as they can form lipid bilayers. Most phospholipids contain a diglyceride, a phosphate group, and a simple organic molecule such as choline; one exception to this rule is sphingomyelin, which is derived from sphingosine instead of glycerol. The structure of the phospholipid molecule generally consists of hydrophobic tails and a hydrophilic head. It is usually found with cholesterol molecules which are found in-between the spaces of the phospholipid. The 'head' is hydrophilic (attracted to water), while the hydrophobic 'tails' are repelled by water and are forced to aggregate. The hydrophillic

head contains the negatively charged phosphate group, and may contain other polar groups. The hydrophobic tail usually consists of long fatty acid hydrocarbon chains.

When placed in water, phospholipids form a variety of structures depending on the specific properties of the phospholipid. These specific properties allow phospholipids to play an important role in the phospholipid bilayer. In biological systems, the phospholipids often occur with other molecules (e.g., proteins, glycolipids, cholesterol) in a bilayer such as a cell membrane (Campbell *et al.,* 2006). Lipid bilayers occur when hydrophobic tails line up against one another, forming a membrane hydrophilic heads on both sides facing the water. Such movement can be described by the Fluid Mosaic Model that describes the membrane as a mosaic of lipid molecules that act as a solvent for all the substances and proteins within it, so proteins and lipid molecules are then free to diffuse laterally through the lipid matrix and migrate over the membrane. Cholesterol contributes to membrane fluidity by hindering the packing together of phospholipids.

Phospholipid synthesis occurs in the cytosol adjacent to endoplasmic recticulum membrane that is studded with proteins that act in synthesis and allocation (flippase and floppase). Eventually a vesicle will bud off from the endoplasmic recticulum containing phospholipids destined for the cytoplasmic cellular membrane on its exterior leaflet and phospholipids destined for the exoplasmic cellular membrane on its inner leaflet (Lodish *et al.,* 2008).

Some types of phospholipid can be split to produce products that function as second messengers in signal transduction. Examples include phosphatidylinositol (4,5)-bisphosphate (PIP_2), that can be split by the enzyme Phospholipase C into inositol triphosphate (IP_3) and diacylglycerol (DAG), which both carry out the functions of the

G_q type of G protein in response to various stimuli and intervene in various processes from long term depression in neurons (Se-Young, 2005) to leukocyte signal pathways started by chemokine receptors (Darran and Cronshaw, 2006).

2.9 Triacylglycerols

Triacylglycerols are highly concentrated stores of metabolic energy because they are reduced and anhydrous. Triacylglycerols constitute about 11 kg of his total body weight. If this amount of energy were stored in glycogen, his total body weight would be 55 kg greater. The glycogen and glucose stores provide enough energy to sustain biological function for about 24 hours, whereas the triacylglycerol stores allow survival for several weeks (Nelson and Cox, 2000).

2.10 Low density lipoprotein - cholesterol

Low density lipoprotein – cholesterol (LDL) cholesterol is called "bad" cholesterol, because elevated levels of LDL cholesterol are associated with an increased risk of coronary heart disease, stroke, and peripheral artery disease. LDL lipoprotein deposits cholesterol along the inside of artery walls, causing the formation of a hard, thick substance called cholesterol plaque. Over time, cholesterol plaque causes thickening of the artery walls and narrowing of the arteries, a process called atherosclerosis, which decreases blood flow through the narrowed area (John *et al*., 2008).

2.11 High density lipoprotein-cholesterol

High density lipoprotein-cholesterol **(**HDL) cholesterol is called the "good cholesterol" because HDL cholesterol particles prevent atherosclerosis by extracting cholesterol

from the artery walls and disposing of them through the liver. Thus, high levels of LDL cholesterol and low levels of HDL cholesterol (high LDL/HDL ratios) are risk factors for atherosclerosis, while low levels of LDL cholesterol and high levels of HDL cholesterol (low LDL/HDL ratios) are desirable and protect against heart disease and stroke (Rahilly, 2011).

High-density lipoproteins HDL are one of plasma lipoproteins. They are composed of a number of heterogenous particles, including cholesterol and vary with respect to size and content of lipid and apolipoprotein. HDL serve to remove cholesterol from the peripheral cells of the liver, where the the cholesterol is converted to bile acids and excreted into the intestine.

An inverse relationship between HDL-cholesterol (HDL-C) levels in the serum and the incidence of coronary heart disease has been demonstrated in a number of epidemiological studies (Bertel *et al*., 2007)

2.12 Lipid peroxidation

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lies methylene-CH_2 groups that possess especially reactive hydrogens (Muller *et al.,* 2007).

Aerobic organisms possess antioxidant defense systems that deal with reactive oxygen species (ROS) produced as a consequence of aerobic respiration and substrate

oxidation. Low levels of ROS are indispensable in many biochemical processes, including intracellular signaling, defense against microorganisms and cell function. In contrast high dose and/ or inadequate removal of ROS, especially superoxide anion (O), results in 'oxidative stress', which has been implicated in the pathogenesis of many cardiovascular diseases, including hypercholesterolemia, atherosclerosis, hypertension, diabetes, and heart failure (Shewfelt and Purvis, 1995).

Reactive oxygen species, including nitric oxide and related species, commonly exert a series of useful physiological effects. However, imbalance between prooxidant and antioxidant defences in favor of prooxidants results in oxidative stress. This results in damage to lipids, proteins, and nucleic acids. Alone or in combination with primary factors, free radicals are involved in the cause of hundreds of diseases.

Oxidative stress has been implicated in aging and in the pathogenesis of a number of disorders. The extent of injury is generally related to an increase, or decrease of one or more free radical scavenging enzymes (Ambrosone *et al*., 2005).

2.13 Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) are formed as a by product of lipid peroxidation (i.e. as degradation products of fats) which can be detected by the TBARS assay using thiobarbituric acid as a reagent.

Because reactive oxygen species (ROS) have extremely short half-lives, they are difficult to measure directly. Instead, what can be measured are several products of the damage produced by oxidative stress, such as TBARS (Pryor, 1991)

Assay of TBARS measures malondialdehyde (MDA) present in the sample, as well as malondialdehyde generated from lipid hydroperoxides by the hydrolytic conditions of the reaction. MDA is one of several low-molecular-weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products. However, only certain lipid peroxidation products generate MDA, and MDA is neither the sole end product of fatty peroxide formation and decomposition, nor a substance generated exclusively through lipid peroxidation (Janero, 1990).

2.14 Superoxide dismutases

Superoxide dismutases (SOD, EC 1.15.1.1) are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. Superoxide Dismutase is an enzyme that repairs cells and reduces the damage done to them by superoxide, the most common free radical in the body. Superoxide is one of the main reactive oxygen species in the cell. Consequently, SOD serves a key antioxidant role. Superoxide Dismutase is found in our skin and it is essential in order for our body to generate adequate amounts of skin-building cells called fibroblasts (McCord, 1998).

Our bodies naturally generate SOD but only on bare levels, so to increase the effectiveness; it is recommended to consume superoxide dismutase dietary supplements to fulfill the lack.

2.15 Catalase

Catalase is an antioxidant enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani *et al.,* 2004). It is a very important enzyme in reproductive reactions.

Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long (Boon *et al*., 2007). It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. The optimum pH for human catalase is approximately 7, (Maehly and Chance, 1954). The reaction of catalase in the decomposition of hydrogen peroxide is:

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Hydrogen peroxide is a harmful by-product of many normal metabolic processes: to prevent damage to cells and tissues, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules (Gaetani *et al.,* 1996).

2.16 Reduced glutathione

Gluthathione is a polypeptide consisting of glycine, cysteine, and glutamic acid that occurs widely in plant and animal tissues. It is important in cellular respiration in both plants and animals, and serves as a cofactor for many enzymes. It is a major protective mechanism against oxidative stress. For example, it protects red blood cells from hydrogen peroxide, a toxic byproduct of certain metabolic reactions. The majority of glutathione in the body is present in its reduced form because this is the only way it can perform its critical role.

Glutathione protects cells from the free radicals produced through oxidation. It can only do this by remaining in its naturally reduced state so that it is readily available to neutralize free radicals by bonding with them. As GSH bonds it converts to its oxidized

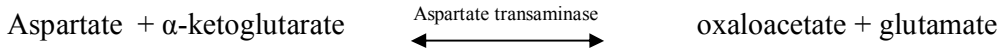
form, called glutathione disulfide. Then an enzyme-glutathione reductase---reverts it back to its reduced state. The ratio of reduced GSH to oxidized GSH within the cells can be used to measure cellular toxicity. In healthy cells, 90 percent of the GSH should be in its reduced form (Pastore *et al.,* 2003).

GSH is produced naturally in the body from cysteine, glutamate and glycine. Since cysteine is the building block that enables its participation in redox reactions, the focus for supplements is on using cysteine to ensure the body has sufficient quantities to produce GSH. Without this powerful antioxidant, the cells in the body would not be protected from oxidative injury. They would be damaged and killed, setting off an inflammation response. GSH also has several other roles. In the liver it helps to transport amino acids across cell membranes so that they can be converted into proteins. It is also essential for the detoxification of acetaminophen, so if the supply of GSH becomes too low, liver toxicity results (Mannervik and Carlberg, 1985).

2.19 Aspartate transaminase

Aspartate transaminase (AST), also called aspartate aminotransferase or serum glutamic oxaloacetic transaminase (SGOT), is a pyridoxal phosphate (PLP)-dependent transaminase enzyme (EC 2.6.1.1). AST catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health (Berg *et al.,* 2006).

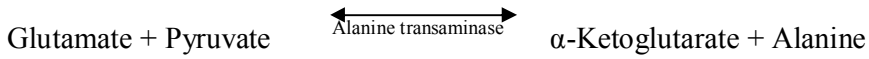
Aspartate transaminase catalyzes the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate (Kirsch *et al.,* 1984). The amino group transfer catalyzed by this enzyme is crucial in both amino acid degradation and biosynthesis.



AST is similar to alanine transaminase (ALT) in that both enzymes are associated with liver parenchymal cells. The difference is that ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart, and skeletal muscle, while AST is found in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells. As a result, ALT is a more specific indicator of liver inflammation than AST, as AST may be elevated also in diseases affecting other organs, such as myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma (Gaze, 2007).

2.20 Alanine aminotransaminase

Alanine aminotransaminase or ALT is a transaminase enzyme (EC 2.6.1.2). ALT is found in serum and in various bodily tissues, but is most commonly associated with the liver. It catalyzes the transfer of an amino group from alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate.



It is commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health. Significantly elevated levels of ALT often suggest the existence of other medical problems such as viral hepatitis, diabetes, congestive heart

failure, liver damage, bile duct problems, infectious mononucleosis, or myopathy. For this reason, ALT is commonly used as a way of screening for liver problems. Fluctuation of ALT levels is normal over the course of the day, and ALT levels can also increase in response to strenuous physical exercise (Wang, 2012).

2.21 Alkaline phosphatase

Alkaline Phosphatases (ALP) are a group of enzymes found primarily in the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2). There are also small amounts produced by cells lining the intestines (isoenzyme ALP-3), the placenta, and the kidney (in the proximal convoluted tubules). As the name implies, this enzyme works best at an alkaline pH (a pH of 10), and thus the enzyme itself is inactive in the blood. Alkaline phosphatases act by splitting off phosphorus (an acidic mineral) creating an alkaline pH.

The primary importance of measuring alkaline phosphatase is to check the possibility of bone disease or liver disease. Since the mucosal cells that line the bile system of the liver are the source of alkaline phosphatase, the free flow of bile through the liver and down into the biliary tract and gall bladder are responsible for maintaining the proper level of this enzyme in the blood. When the liver, bile ducts or gall bladder system are not functioning properly or are blocked, this enzyme is not excreted through the bile and alkaline phosphatase is released into the blood stream. Thus the serum alkaline phosphatase is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine.

Alkaline phosphatase is typically used in the lab to remove phosphate monoester to prevent self ligation (Maxam and Gilbert, 1980). It is also used as a label for enzyme

immunoassays. An increased serum alkaline phosphatase may be due to liver or bone damage.

2.22 Total protein

A total serum protein test measures the total amount of protein in the blood. It also measures the amounts of two major groups of proteins in the blood: albumin and globulin. Albumin is made mainly in the liver. It helps keep the blood from leaking out of blood vessels. Albumin also helps carry some medicines and other substances through the blood and is important for tissue growth and healing.

Globulin is made up of different proteins called alpha, beta, and gamma types. Some globulins are made by the liver, while others are made by the immune system. Certain globulins bind with hemoglobin. Other globulins transport metals, such as iron, in the blood and help fight infection. Serum globulin can be separated into several subgroups by serum protein electrophoresis.

Albumin is tested to check how well the liver and kidneys are working, find out if your diet contains enough protein, help determine the cause of swelling of the ankles (edema) or abdomen (ascites) or of fluid collection in the lungs that may cause shortness of breath (pulmonary edema).

2.23 Bilirubin

Bilirubin is the yellow breakdown product of normal heme catabolism. Heme is found in hemoglobin, a principal component of red blood cells. Bilirubin is excreted in bile and urine, and elevated levels may indicate certain diseases. It is responsible for the

yellow color of bruises, the yellow color of urine (via its reduced breakdown product, urobilin), the brown color of faeces (via its conversion to stercobilin), and the yellow discoloration in jaundice. It has also been found in plants (Pirone *et al.,* 2005).

2.23.1 Function

Bilirubin is created by the activity of biliverdin reductase on biliverdin, a green tetrapyrrolic bile pigment that is also a product of heme catabolism. Bilirubin, when oxidized, reverts to become biliverdin once again. This cycle, in addition to the demonstration of the potent antioxidant activity of bilirubin, (Baranano *et al.,* 2002) has led to the hypothesis that bilirubin's main physiologic role is as a cellular antioxidant (Liu *et al.,* 2008).

Total and direct bilirubin levels can be measured from the blood, but indirect bilirubin is calculated from the total and direct bilirubin. Indirect bilirubin is fat-soluble and direct bilirubin is water-soluble. There are no normal levels of bilirubin as it is an excretion product, and levels found in the body reflect the balance between production and excretion.

2.24 Creatinine

Creatinine is a nitrogenous waste product formed from the metabolism of creatine and creatine phosphate in skeletal muscle. Serum or plasma creatinine level is proportional to muscle mass of an individual. Creatinine diffuses freely throughout the body water. It is filtered from the extra cellular fluid by the kidney and excreted in the urine. The excretion of creatinine is mainly renal and in absence of diseases relatively constant. Measurement of serum creatinine is an important test of kidney function. It is a better

indication of overall renal function and progress in renal failure. Serum creatinine level is less affected by age, dehydration and catabolic stress example, fever, sepsis and internal bleeding. Creatinine level is also less influenced by changes in diet as low intake of protein (Chessbrough, 2005). Depressed levels of plasma creatinine are rare and not clinically significant

2.25 Urea

Urea is the major end product of nitrogen metabolism in humans and mammals. Ammonia, the product of oxidative deamination reactions, is toxic in even small amounts and must be removed from the body. The ammonia is detoxified by combining with carbondioxide to form urea which passes into the circulation and excreted by kidney. The overall urea formation reaction is:

2 Ammonia + carbon dioxide + 3ATP ---> urea + water + 3 ADP

Urea is routinely measured in the blood as: Blood Urea Nitrogen (BUN). BUN levels may be elevated (a condition called uremia) in both acute and chronic renal (kidney) failure. Various diseases damage the kidney and cause faulty urine formation and excretion. Congestive heart failure leads to a low blood pressure and consequent reduced filtration rates through the kidneys, therefore, BUN may be elevated. Urinary tract obstructions can also lead to an increased BUN.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

All chemicals were of analytical grade. Assays kits were purchased from Randox laboratories Ltd. Ardmore, Co. Antrm UK. Adrenaline and 5'5'dithiobis (nitro benzoic acid) reagents were purchased from Sigma Chemical Company, St. Louis, U.S.A.

3.1.2 Animals

A total of 81 apparently healthy Wistar rats of both sexes weighing between 150 – 200g were obtained from the Animal house, Department of Pharmacology, Ahmadu Bello University, Zaria. The animals were kept in well ventilated laboratory cages in the Pharmacology Department animal house and were allowed to adjust to the laboratory environment for a period of two weeks before the commencement of the experiment. They were fed with grower mash from Vital Feeds Grand Cereal Plc. and water was provided *ad libitum* during the stabilization period.

3.1.3 Formulation of the feed

The high-fat-high-cholesterol diet was formulated as described by Mukundh *et al.,* (2008) and Peterson *et al*., (1954) with some modification.

Table 3.1 Formulation of high-fat-high-cholesterol diet

Contents of food	Normal diet (%)	HFD (%)
Protein	15.0	12.23
Fat	7.0	22.71
Fibre	10.0	8.15
Calcium	1.0	0.82
Phosphorus	0.35	0.29
Cholesterol	-	1.5
Soluble carbohydrate	66.65	54.32

3.1.4 Plant material

Persea americana seeds were collected from Kafanchan, Kaduna state in the month of July, 2011 during the fruiting season. The plant was identified at the herbarium unit of Biological Sciences Department, Ahmadu Bello University, Zaria where a voucher specimen with number 992 was deposited.

The seeds were chopped into small pieces and shade dried for 5 days. The pieces were ground into powder form with a grinding mill. The powdered sample was Soxhlet defatted with petroleum ether (60-80^{0}c) then extracted with 100% methanol. The filtrates were concentrated in a rotary evaporator and were further dried at room

temperature to constant weight. The fractions were then packed into an amber-coloured bottle and stored at 4^0C until required for experiments.

3.2 Methods

3.2.1 Quantitative determination of the phytochemical constituents

3.2.1.1 Total Phenols

To 5 g of the sample, 100 ml of diethyl ether was added to defat for 2 h. The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. Then 5 ml of the extract was pipetted into a 50 ml flask, and 10 ml of distilled water was added. Then 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505 nm. The concentration of phenol was extrapolated from standard curve (Harborne, 1973).

Calaculation:

$$\text{Concentration of phenols (\%)} = \frac{\text{Weight of phenols}}{\text{Weight of extracts used}} \times 100$$

3.2.1.2 Alkaloids

Into a 250 ml beaker, 5 g of the sample was weighed and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a waterbath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate

was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harborne, 1973).

Calaculation:

Concentration of alkaloids = (Weight of crucible + extract) – (weight of crucible)

$$\text{Concentration of alkaloids (\%)} = \frac{\text{Weight of alkaloids}}{\text{Weight of extracts used}} \times 100$$

3.2.1.3 Tannins

The sample (500 mg) was weighed into a 50 ml plastic bottle; 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.2 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min. The concentration of tannins was estimated from standard curve (Van-Burden and Robinson 1981).

Calaculation:

$$\text{Conc. of tannins (\%)} = \frac{\text{Weight of tannins}}{\text{Weight of extracts used}} \times 100$$

3.2.1.4 Saponin

Into a conical flask, 20 g of each extracts was weighed and 100 cm^3 of 20% aqueous ethanol added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted

with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage (Obadoni and Ochuko, 2001).

Calaculation:

Concentration of saponins = (Weight of crucible + extract) – (weight of crucible)

$$\text{Concentration of saponins (\%)} = \frac{\text{Weight of saponins}}{\text{Weight of extracts used}} \times 100$$

3.2.1.5 Flavonoids

Plant sample weighing 10 g was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight (Boham and Kocipai, 1994).

Calaculation:

Concentration of flavonoid = (Weight of crucible + extract) – (weight of crucible)

$$\text{Concentration of flavonoids (\%)} = \frac{\text{Weight of flavonoids}}{\text{Weight of extracts used}} \times 100$$

3.2.2 RECONSTITUTION OF THE FRACTION AND ATORVASTATIN

The petroleum ether fraction was dissolved in distilled water using 2 drops of tween 80. Methanolic fraction was dissolved in distilled water. A stock solution of 2500mg of the fractions in 10 ml of distilled water was prepared. A fraction of 0.2 ml of the stock solution was drawn for each weight/volume to give doses equivalent to 500mg/kg body weight of the rats. The above was diluted two times and four times to give equivalent doses of 250 and 125mg/kg body weight of the rats.

One tablet of Lipitor® contained 10mg Atorvastatin. This is equivalent to 10,000 µg. This was crushed into powdered form and was dissolved in 100 ml of distilled water. An aliquot of 0.2 ml of the solution was drawn for each weight/volume to give a dose equivalent to 200 µg/kg body weight of the rats.

3.2.3 Acute toxicity study

The median lethal dose (LD_{50}) of the plant fraction was conducted in order to select a suitable dose for the evaluation of antilipidemic activity. This was done using the method described by Lorke, (1983) using 13 rats for each extract. In the initial phase, rats were divided into 3 groups of 3 rats each and were administered with 10mg, 100mg and 1000mg of the fraction per kg body weight orally. They were observed for 24 h for signs of toxicity, including death. In the final phase, 4 rats were divided into 4 groups of one rat each, and were administered with 2000 mg, 3000mg, 4000mg and 5000mg per kg body weight of the fraction based on the observations in the first phase. The LD_{50} was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose, that is, the geometric mean of the consecutive doses with 0 and 100% survival rates were recorded.

3.2.4 Experimental design

The rats were divided into 11 groups of 5 rats each and were treated for four weeks as follows:

Group 1: Rats were fed on normal feed *ad libitum* and served as control.

Group 2: Rats were given high-fat-high-cholesterol diet with no treatment. This group served as HFD control.

Group 3: Rats were fed with high-fat-high-cholesterol diet *ad libitum* and given daily 200µg/kg body weight of standard drug (Atorvastatin®) orally. This served as a positive control.

Group 4: Rats were given high-fat-high-cholesterol diet *ad libitum* and daily 125mg/kg body weight of methanolic extract orally.

Group 5: Rats were given high-fat-high-cholesterol diet *ad libitum* and daily 250mg/kg body weight of methanolic extract orally.

Group 6: Rats were given high-fat-high-cholesterol diet *ad libitum* and daily 500mg/kg body weight of methanolic extract orally.

Group 7: Rats were fed normal rat feed *ad libitum* and given daily 500mg/kg body weight of methanolic extract orally.

Group 8: Rats were given high-fat-high-cholesterol diet *ad libitum* and daily 125mg/kg body weight of petroleum ether extract orally.

Group 9: Rats were given high-fat-high-cholesterol diet *ad libitum* and daily 250mg/kg body weight of petroleum ether extract orally.

Group 10: Rats were given high-fat-high-cholesterol diet *ad libitum* and daily 500mg/kg body weight of petroleum ether extract orally.

Group 11: Rats were fed normal rat feed and daily 500mg/kg body weight of petroleum ether extract orally.

3.2.5 Collection and preparation of sera samples and organs extract

At the end of four weeks of treatment, the animals were anaesthetized under chloroform vapour, sacrificed and blood samples were drawn from the heart of each sacrificed animal from all groups. Blood samples were collected in plain tubes, allowed to clot and the serum separated by centrifugation using Denley BS400 centrifuge (England) at 3000 rpm for 10minutes and the supernatant (serum) collected were subjected to biochemical analysis.

Immediately after the blood was collected, liver and the kidney were quickly dissected out and weighed. The liver and kidney were crushed using mortar and pestle in phosphate buffered saline (pH 7.2), The homogenates were centrifuge at 4000 rpm for ten minutes. The supernatant was collected using Pasteur pipette.

3.3 Analyses

3.3.1 Packed cell volume

Packed cell volume was determined by microhaematocrit methods described by Alexander and Griffins, (1993).

Principle

A volume of uncoagulated blood is centrifuged at high speed in a capillary tube. The level of packed cell is read directly from the graphic reader and the reading is expressed as a percentage.

Procedure:

A heparinized capillary tube was filled with blood to 80% of its length. One end of the capillary tube was sealed with sealing clay. The tube was place in a microheamatocrit centrifuge. The sealed end was kept out. The centrifuge was covered and centrifuged at 1000 rpm for 5 minutes. The packed cell was read directly from the graphic reader.

3.3.2 Serum cholesterol

The serum level of total cholesterol was quantified by spectrophotometric methods as described by Stein, (1987) by the addition of enzyme present in reagent kit.

Principle

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol peroxidase.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{cholesterol -esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{Cholestene-3-one} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O$$

Reagent composition

Contents	Initial concentration of Solutions
R1. Reagent	
4-Aminoantipyrine	0.30 mmol/l
Phenol	6 mmol/l
Peroxidase	≥ 0.5 U/ml
Cholesterol esterase	≥ 0.15 U/ml
Cholesterol oxidase	≥ 0.1 U/ml
Pipes buffer	80 mmol/l; pH 6.8
CAL Standard	195 mg/dl

Procedure

Into a test tube (sample) 10µl of serum was measured and 1000µl of working reagent was added. To another test tube (standard), 10µl of standard and 1000µl of working reagent was measured. The contents were mixed and incubated for 5 minutes at 37^0C. The absorbance of the sample was measured against reagent blank within 60 minutes at 500nm.

Calculation:

Concentration of cholesterol in sample = $\frac{A_{sample}}{A_{standard}} \times$ Concentration of standard

Concentration of standard = 195mg/dl.

3.3.3 Serum high density lipoprotein cholesterol

The serum level of high density lipoprotein cholesterol (HDL-c) was measured by the method of Wacnic and Alber, (1978). The value was expressed in mg/dl.

Principle

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnessiun ions. After centrifugation, the cholesterol concentration in the HDL fraction which remains in the supernatant is determined.

Reagent composition

Contents	Initial concentration of Solutions
R1. Phosphotungstic acid	0.55 mmol/l
Magnessium Chloride	25 mmol/l

Procedure

Into a test tube (sample) 200µl of serum was measured and 1000µl of R1was added**.** The contents were mixed and allowed to stand for ten minutes at room temperature. This was centrifuge for 10 minutes at 4,000 rpm. The clear supernatant was separated within two hours. The absorbance of the sample was measured against the absorbance of the blank within 60 minutes.

Calculation

Absorbance of sample × Factor (Factor = 210 mg/dl)

3.3.4 Serum triacylglycerols

The serum triacylglycerols level was determined by method of McGowan *et al*., (1983). In these methods triglyceride is converted into quinoneimine dye. The value was expressed in the unit of mg/dl.

Principles

The amount of quinoneimine dye formed from hydrogen peroxide, 4 aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase is proportional to triglyceride concentration in the serum. Since it is a coloured compound it can be detected and quantified by a colorimeter at 500nm.

$$\text{Triacylglycerols} + H_2O \xrightarrow{\text{lipases}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{GK} \text{glycerol-3-phosphate} + ADP$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{GPO} \text{dihydroxyacetone} + \text{phosphate} + H_2O_2$$

$$H_2O_2 + 4\text{ aminophenazone} + \text{4-chlorophenol} \xrightarrow{POD} \text{quinoneimine} + HCl + 4H_2O$$

Where GK = Glycerol-kinase, GPO = Glycerol-3-phosphate oxidase, POD = Peroxidase

Reagent composition

Contents	Initial concentration of Solutions
R1a. Buffer	
Pipes buffer	40 mmol/l; pH 7.6
4-chloro-phenol	5.5 mmol/l
Magnessium ions	17.5 mmol/l

R1b. Enzyme Reagent	
4 aminophenazone	0.5 mmol/l
ATP	1.0 mmol/l
Lipases	≥ 150 U/ml
Glycerol-kinase	≥ 0.4 U/ml
Glycerol-3-phosphate oxidase	≥ 0.4 U/ml
Peroxidase	≥ 0.4 U/ml
CAL Standard	1.96mg/dl

Procedure

Into a test tube (sample) 10µl of serum was measured and 1000µl of R1was added. To another test tube (standard), 10µl of standard and 1000µl of R1 was measured. The contents were mixed and incubated for 5 minutes at 37^0C. The absorbance of the sample was measured against reagent blank within 60 minutes.

Calculation:

Concentration of triacylglycerols in sample $= \frac{A_{sample}}{A_{standard}} \times$ concentration of standard

Concentration of standard = 1.96 mg/dl.

3.3.5 Serum low density lipoprotein- cholesterol

The serum level of low density lipoprotein- cholesterol (LDL-c) was measured according to protocol of Friedewald, (1972) using the equation below:

$$\text{LDL-c} = \text{TC} - \frac{\text{TGL}}{5} - \text{HDL-c}$$

The value was expressed in the unit of mg/dl.

3.3.6 Antherogenic ratio

The antherogenic ratio was measured by using the equation:

$$\frac{TC}{HDL\text{-}c} \quad \text{and} \quad \frac{LDL\text{-}c}{HDL\text{-}c}$$

3.3.7 Serum total lipid concentration

The serum total lipid was determined using the method described by Stroev and Makarova, (1989).

Reagents:

Concentrated Sulphuric acid

Phosphovanillin reagents: 80 ml of concentrated orthophosphoric acid were mixed with 20mls of 0.6% aqeous vanillin (0.6g dissolved in distilled water and made up to 100ml). The reagent was kept in a brown bottle at room temperature.

Materials: Blood serum sample

Principle:

This is based on the ability of unsaturated lipid metabolites to produce, by reaction with phosphovanillin reagent, a coloured compound whose intensity of colouration is proportional to the total lipid concentration in blood serum.

Procedure: Into a dry test tube (sample) 0.1 ml of blood serum was measured 2.9 ml of concentrated sulphuric acid was added. To another test tube (control), 0.2ml of distilled water and 5.8 ml of concentrated sulphuric acid was added. The contents of both test

tubes were mixed with a glass rod and placed in boiling water bath for 10 minutes then cooled promptly under a stream of running tap water. Into two new test tubes, 3 ml and 6 ml of phosphovanillin reagent was measured. To the former test tube, 0.2 ml volume of the solution from the cooled sample test tubes was added and to the latter, a 0.4 ml volume of the solution from cooled control test tube was added. The contents were mixed thoroughly with a glass rod and were placed in the dark for 45 minutes at room temperature for colouration to develop. The absorbance for sample solution was measured against control solution at wavelength of 500 nm.

Calculation:

The total lipids concentration in blood serum x (g/litre) was calculated from the formula

$$x = \frac{m1000 \times 3}{0.2 \times 1000}$$

Where m = the mass of lipid as estimated by the standard curve.

3.3.8 Determination of total phospholipids concentration

The serum total phospholipid was determined using the method described by Stroev and Makarova, (1989).

Principle:

This is based on a concentrational measurement of organic phosphate released by acid hydrolysis. The measurement of inorganic phosphate is carried out by ammonium molybdate reaction.

Reagents:

Trichoroacetic acid, concentrated percholic acid

Aqueous solution of ammonium molybdate (0.5 M): 2.5g of ammounium molybodate was dissolved in distilled water and made up to 60 ml and then filtered through a paper filter. The filtrate was transferred to a graduated flask of 100 ml capacity. 25 ml of distilled water was poured into a clean flask and 7.5 ml of concentrated sulphuric acid was added. The latter solution was poured into the former; the mixture was cooled and made up to mark with distilled water.

Reducing agent: 1% solution of ascorbic acid was prepared using 0.016% aqeous copper sulphate solution as a solvent.

Material: Blood serum sample.

Procedure: Into a sample test tube, 0.2 ml of blood serum and 2.8 ml of distilled water was added. To another test tube (control), 3 ml of distilled water was added. To each of the test tubes, 3 ml of trichloroacetic acid was added and the content was mixed by shaking.

The solution was centrifuge at 3000 rpm for 15 minutes. The supernatant was decanted and the test tubes were placed upside down on a piece of filter paper to let the residual liquid flow out. Then 1 ml of concentrated percholic acid was added into both test tubes and the contents were thoroughly mixed. The test tubes were placed in a sand bath at 180^0C for 20-30 minutes until the solution decolorized.

The test tubes were allowed to cool at room temperature and then 3 ml of distilled water was added, after that, 1 ml of ammonium molybdate reagent and 1 ml of freshly prepared reducing agent were added. The contents were mixed thoroughly and let to stand for 10 minutes at room temperature. The absorbance for sample solution was measured against control solution using wavelength of 630 nm.

Calculation: The total phospholipid concentration x (g/litre) in blood serum is calculated by making use of the formula

$$X = \frac{m5000 \times 25}{1000 \times 10}$$

Where m = the mass of phospholipids derived from the standard curve.

3.3.9 Superoxide dismutase

Superoxide Dismutase (SOD) activity was determined by a method described by Fridovich, (1978).

Principle

The ability of superoxide dismutase (SOD) to inhibit auto oxidation of adrenaline at pH 10.2 forms the bases of this assay.

Reagents

Carbonate buffer (0.05M): 14.3g of Na_2CO_3 and 4.2g of $NaHCO_3$ were dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 10.2.

Adrenaline (0.3 mM): 0.01 g of adrenaline was dissolved in 17 ml of distilled water. The solution was prepared fresh.

Procedure

Tissue homogenate of 0.1 ml was diluted in 0.9 ml of distilled water to make 1:10 dilution. An aliquot (0.20 ml) of the diluted homogenate was added to 2.5 ml of 0.05 M Carbonate buffer. The reaction was started with the addition of 0.3ml of 0.3 mM Adrenaline. The reference mixture contained 2.5 ml of 0.05 M Carbonate buffer, 0.3ml of 0.3 mM Adrenaline and 0.20 ml of distilled water. Absorbance measured at 30 s intervals up to 150 s at 480nm.

Calculations:

$$\text{Increase in absorbance per minute} = \frac{(A_5 - A_1)}{2.5}$$

$$\% \text{ inhibition} = 100 - \frac{\text{Increase in absorbance for substrate}}{\text{Increase in absorbance of blank}} \times 100$$

1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adenochrome in 1 minute.

$$\text{SOD activity} = \frac{\% \text{ inhibition}}{50 \times \text{weight of tissue}}$$

3.3.10 Catalase

Catalase activity was determined using the method described by Sinha, (1972).

Principle

The method is based on the reduction of Dichromate in acetic acid to chromic acetate, when heated in the presence of hydrogen peroxide with the formation of perchromic acid as unstable intermediate. Chromic acetate so produced is measured colorimetrically using spectrophotometer at 570 nm. This is because dichromate has no absorbance at 570 nm and does not interfere with the determination of catalase.

Reagents

0.01 M Phosphate buffer (pH 7.0): 1.2g of NaH_2PO_4 and 1.41g of Na_2HPO_4 were dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 7.0.

5 % Potassium heptaoxochromate (VI), $K_2Cr_2O_7$: 5 g of $K_2Cr_2O_7$ was dissolved in little quantity of distilled water and made up to 100 ml.

0.2 M H_2O_2: 0.6 ml of H_2O_2 was dissolved in little quantity of distilled water and made up to 100 ml. It was stored at 4^oC.

Dichromate/Acetic acid solution: 5 % Potassium heptaoxochromate (VI), $K_2Cr_2O_7$, was mixed with gacial acetic acid in the ratio 1: 3, and was stored in brown bottle at room temperature.

Procedure

Into two test tubes T0 and T1, 2ml of homogenate supernatant, 2ml of phosphate buffer and 0.4ml of H_2O_2 was added and mixed. Immediately after, the above, 1 ml of potassium dichromate/ glacial acetic acid was added to T0. After 10 minutes, 1 ml of potassium dichromate /glacial acetic acid was also added to T1. The contents was mixed and incubated at 80^0C for 10 minutes. The absorbance was measured at 570nm against blank.

Calculations:

The quantity of H_2O_2 consumed was calculated using

$C_{T/}C_s = A_{T/}A_S$

C_T = concentration of the test

C_S =Standard concentration = 0.2M

A_T = Absorbance of test (T0 - T1)

A_S = Absorbance of blank (T0)

$$\text{Catalase activity} = \frac{C_T \times \text{total volume of homogenate}}{10 \text{ minutes} \times \text{volume of homogenate used X wt of tissue}}$$

Catalase activity was expressed in moles of H_2O_2/min/g tissue.

3.3.11 Reduced glutathione

Reduced glutathione (GSH) concentration measurement was done according to Ellman, (1959).

Principle

It is based on the reaction of the thiol with 5,5' -dithiobis (nitro benzoic acid) (DNTB) to give the mixed disulphide and 2-nitro-5-thiobenzoic acid (TNB) which is quantified by the absorbance of the anion (TNB^{2-}) at 412nm).

Reagents

Ellman's reagent: (19.8 mg of 5, 5'-dithiobis (nitro benzoic acid) (DNTB) in 100 ml of 0.1 % sodium nitrate)

Trichloroacteic (TCA) 10% (10g of TCA in 100ml of distilled water). Phosphate buffer (0.2 M, pH 8): 27.6g of NaH_2PO_4 and 28.4g of Na_2HPO_4 was dissolved in distilled water and made up to 1000 ml mark in a volumetric flask. The buffer was adjusted to pH 8.

Procedure

To 150 µl of tissue homogenate, 1.5ml of 10 % TCA was added and centrifuged at 1500g for 5min. To a fresh test tube, 1 ml of the supernatant was added then 0.5 ml of

Ellman's reagent was added and mixed then and 3 ml of phosphate buffer was added. The contents were mixed and the absorbance was read at 412 nm against the blank.

Calaculation:

$C_T/C_s = A_{T/}A_S$

C_T = concentration of the test

C_S =Standard concentration

A_T = Absorbance of test

A_S = Absorbance of standard

$$\text{Reduced gluthathione} = \frac{C_T \times \text{total volume of homogenate}}{\text{Volume of homogenate used} \times \text{weight of tissue}}$$

3.3.12 Thiobarbituric acid reactive substances determination

Lipid peroxidation of LDL was assessed by Thiobarbituric acid reactive substances determination (TBARS) formation (Ohkawa *et.al.*, 1979).

Principle

Malondialdehyde (MDA) is a product of lipid peroxidation and is used as an indicator of tissue damage. The MDA form a 1:2 adduct with thiobarbituric acid (TBA) and produces a pink coloured product which has absorption maximum at 532nm.

Working reagents

Thiobarbituric acid (100mg of TBA in 30ml distilled water and 30ml of acetic acid)

Trichloroacteic (TCA) 10% (10ml of TCA in 100ml of distilled water).

Normal saline solution (0.9%): 0.9g 0f NaCl was dissolved in distilled water and made up to 100ml

Procedure

Into a sample test tube 0.2ml of normal saline, 0.2ml of serum, 0.5ml of TCA and 0.1ml of TBA was added. In a sample blank test tube, 0.2ml serum was substituted in the above with distilled water. The contents in the two test tubes were mixed and heated at 95^0C for 60 minutes. After then, 3.0ml of n-butanol was added to the different test tubes. They were centrifuged and the absorbance was read at 532nm against the sample blank.

Calculation:

The concentration of TBARS was expressed in terms of Malondialdehyde (MDA) in µmol/ml. molar extinction of MDA 1.56 X 10^5 cm^{-1} m^{-1}.

$$\text{MDA conc.} = \frac{\text{Absorbance}}{1.56 \times 10^5 \text{ cm}^{-1} \text{ m}^{-1}}$$

$$\text{TBARS} = \frac{\text{Volume of homogenate} \times \text{MDA concentration}}{\text{weight of tissue}}$$

The values are expressed as µmol/g tissue.

3.3.13 Serum alanine aminotransferase

Principle

Alanine Aminotransferase (ALT) is measured by monitoring the concentration of pyruvate hydrazone form with 2,4-dinitophenylhydrazine (Reitman and Frankel, 1957).

$$\alpha\text{-oxoglutarate} + \text{L-alanine} \xrightarrow{\text{ALT}} \text{L-glutamate} + \text{pyruvate}$$

Reagent composition

Contents	Initial concentrations of solution
R1. Buffer	
Phosphate buffer	100mmol/l, pH 7.4
L-alanine	200mmol/l
α-oxoglutarate	2.0mmol/l
R2 2,4-dinitophenylhydrazine	2.0mmol/l

Procedure

Sample (0.1ml) was added to a 0.5ml of solution R1. The contents were mixed and incubated for exactly 30 minutes at 37°C. solution R2 (0.5ml) was added into the mixture and allowed to stand for exactly 20 minutes at 25°C, then 0.5ml of sodium hydroxide was added and mixed. The absorbance was read at 546nm after 5 minutes.

Calculation

The activity of ALT in the serum was obtained from the standard table.

3.3.14 Serum aspartate aminotransferase

Principle

Aspartate aminotransaminase (AST) is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine (Reitman and Frankel, 1957).

α-oxoglutarate + L-aspartate $\xrightarrow{\text{AST}}$ L-glutamate + oxaloacetate

Reagent composition

Contents	Initial concentrations of solution
R1. Buffer	
Phosphate buffer	100mmol/l, pH 7.4
L-aspartate	100mmol/l
α-oxoglutarate	2 mmol/l
R2 2,4-dinitophenylhydrazine	2 mmol/l

Procedure

Sample (0.1ml) was added to a 0.5ml of solution R1. The contents were mixed and incubated for exactly 30 minutes at 37°C. solution R2 (0.5ml) was added into the mixture and allowed to stand for exactly 20 minutes at 25°C, then 0.5ml of sodium hydroxide was added and mixed. The absorbance was read at 546nm after 5 minutes.

Calculation

The activity of AST in the serum was obtained from the standard table.

3.3.15 Serum alkaline phosphatase

Principle

Alkaline phosphatase (ALP) reacts with p-nitrophenyl phosphate to form p-nitrophenol, the rate of formation of which is directly proportional to the level of alkaline phosphatase (Reitman and Frankel, 1957).

P- nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ Phosphate + p-nitrophenol.

Reagent composition

Contents	Concentrations in the test
R1a. Buffer	
Diethanolamine buffer	1 mol/l, pH 9.8
$MgCl_2$	0.5 mmol/l
R1b. Substrate	
P- nitrophenylphosphate	10 mmol/l

Preparation of reagents

One vial of Substrate R1b was reconstitute with 10 ml of buffer R1a

Procedure

Sample 0.01ml was added into 0.50ml of reagent. The contents were mixed and the initial absorbance was taken. The timer was started simultaneously and the absorbances were read at 1, 2 and 3 minutes.

Calculation:

U/I = 2790 × change in absorbance (nm/min).

3.3.16 Serum albumin

Principle

Albumin in the presence of bromocresol green at a slightly acid pH produces a colour change of the indicator from yellow-green to green-blue. The intensity of the colour formed is proportional to the albumin concentration in the sample (Rodkey, 1965).

Reagents composition

R.	Bromocresol green pH 4.2	50mmol/L.
Albumin Cal	Albumin aqeous primary calibrator	5g/dL.

Procedure

Into a sample test tube, 5.0µl of sample and 1.0ml R1 was added and mixed. To another test tube, 5.0µl of calibrator and 1.0ml of R1 was added. It was mixed and incubated for 5 minutes at room temperature. The absorbance of the samples (A_{sample}) was measured against blank at wavelength of 630nm.

Calculations

$$\text{Albumin (g/dL)} = \frac{A_{sample}}{A_{standard}} \times \text{Standard concentration}$$

Standard concentration = 5g/dl

3.3.17 Serum bilirubin

This was done using colorimetric method based on that described by Jendrassik and Grof, (1938).

Principle

Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Reagent composition

Contents	Initial Concentration of Solutions
R1. Sulphanilic acid	29 mmol/l
Hydrochloric acid	0.17 N
R2. Sodium nitrate	38.5 mmol/l
R3. Caffeine	0.26 mol/l
Sodium benzoate	0.52 mol/l

R4. Tartate	0.93 mo/l
Sodium Hydroxide	1.9 N

3.17.1 Total bilirubin

Procedure

Into a sample test tube 200µl of R1, 50µl of R2, 1000 µl of R3 and 200µl of sample was added, mixed and incubated for 10 minutes at 25°C. R4 (1000µl) was added, mixed and allowed to stand for 5 -30 minutes at 25°C. The absorbance of the sample was read at wavelength of 578nm.

Calculation:

Total bilirubin (mg/dl) = 10.8 × Absorbance of the sample

3.17.2 Direct bilirubin

Procedure

Into a sample test tube 200µl of R1, 50µl of R2, 2000µl of 0.9% NaCl and 200µl of sample was added, mixed and incubated for 10 minutes at 25°C. The absorbance of the sample was read at wavelength of 578nm.

Calculation:

Direct bilirubin (mg/dl) = 14.4 × Absorbance

3.3.18 Serum total protein

This was done using the method described by Tietz, (1995).

Principle

Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex.

Reagent composition

Contents	Concentration of Solutions
R1. Biuret reagent	
Sodium hydroxide	100 mmol/l
Na-K-tartrate	16 mmol/l
Potassium iodide	15 mmol/l
Cupric sulphide	6 mmol/l
R2. Blank reagent	
Sodium hydroxide	100 mmol/l
Na-K-tartrate	16 mmol/l
CAL. Standard	
Protein	5.98 g/dl

Preparations of reagents

R1. Biuret reagent

The contents of bottle R1 was diluted with 400 ml of distilled water and the bottle was rinsed thoroughly.

The contents of bottle R2 was diluted with 400 ml of distilled water, and the bottle was rinsed thoroughly.

Procedure

Into a sample test tube, 0.02ml of sample and 1.0ml of R1 was added and mixed. To another test tube, 0.02ml of standard and 1.0ml of R1 was added. The contents were mixed and incubated for 30 minutes at 20-25^{0}C. The absorbance of the sample and standard were measured against the reagent blank at 530 nm.

Calculation

$$\text{Total Protein Concentration} = \frac{A_{sample}}{A_{standard}} \times \text{Standard concentration}$$

Standard concentration = 5.98 g/dl.

3.3.19 Serum urea

Serum urea level was determined using the method described by Fawcett and Scout, (1960).

Principle

Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.

$$\text{Urea} + H_20 \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{hypochlorite} + \text{Phenol} \longrightarrow \text{indophenols (blue compound)}$$

Reagent composition

Contents		Initial concentration of Solutions
R1.	EDTA	116 mmol/l
	Sodium nitroprusside	6 mmol/l
	Urease	1 g/l
R2.	Phenol (diluted)	120 mmol/l
R3.	Sodium hypochlorite (diluted)	27 mmol/l
	Sodium hydroxide	0.14N
CAL	Standard	80.5mg/dl

Procedure

Into a sample test tube, 10µl of sample and 2.50ml of R1 was added, to another test tube, 10µl of distilled water and 2.50ml of standard was added. They were mixed and incubated at 37°C for 10 minutes. Then, 2.50ml of R2 and 2.50ml of R3 was added to

the both test tubes. They were mixed incubated at 37°C and the absorbances of the measured at 546nm.

Calculation:

Urea Concentration $= \frac{A_{sample}}{A_{standard}} \times$ Standard conc. (mmol/l).

Standard concentration = 80.5 mg/dl

3.3.20 Serum creatinine

This was determined using method described by Bartels and Bohmer, (1972).

Principle

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Reagent composition

Contents		Initial concentration of Solutions
R1a	Picric acid	35 mmol/l
R1b	Sodium hydroxide	0.32 mol/l
CAL	Standard	1.97 mg/dl

Preparation of working reagent

Equal volumes of Solutions R1a and R1b were mixed together.

Procedure

In a test tube, 100µl sample was mixed with 1000µl of working reagent. Into another test tube, 100µl of standard was mixed with 1000µl of working reagent. The contents were mixed, and absorbance A_1 of the sample and standard were measured against the sample blank after 30 seconds. Exactly 2 minutes later, absorbance A_2 of the standard and sample was measured.

Calculation:

$A_2 - A_1$ = change in A_{sample} or change in $A_{standard}$

$$\text{Concentration of creatinine in serum} = \frac{\text{Change in } A_{sample}}{\text{Change in } A_{standard}} \times \text{standard concentration}$$

Standard concentration =1.97mg/dl.

3.3.21 Daily and weekly parameter estimation

1. Daily feed intake: Initial feed (F_i) was measured and placed on the different cages, on the next day, the remnant was also weighed and the value recorded as (F_r). The daily feed was calculated using:

   Daily feed intake = (F_i) - (F_r)
2. Blood sample was collected from the tail vein for packed cell volume on a weekly basis.
3. Animal weight was taken weekly using weighing balance

3.3.22 Statistical analysis

Data are expressed as mean ± S.D. statistical analyses were performed with one-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test using SPSS 17 statistical package. The values of $p<0.05$ were considered as significant (Duncan *et al.*, 1972).

CHAPTER 4

RESULTS

4.1 Concentrations of some phytochemicals of the petroleum ether and methanolic fractions of *Persea americana* seed.

The mean saponins, tannins, flavonoids, phenols and alkaloids concentrations are presented in Table 4.1. The methanoloic fraction had significantly ($p< 0.05$) higher quantities of saponins, flavonoids and phenols than the petroleum ether fraction.

4.2 Acute toxicity test

The petroleum ether and methanolic fractions were not toxic at dose of 5000mg/kg body weight. The extracts of the seed may be regarded as relatively safe (Table 4.2).

4.3 Effects of petroleum ether or methanolic fractions of *Persea americana* seeds on relative organs weight, body weight, daily feed intake and packed cell volume.

4.3.1 Daily feed intake

The daily feed intake data in Table 4.2 showed there was no significant ($p>0.05$) difference in the feed intake of the HFD control group and the normal control group. On the administration of either of the fractions, the groups with HFD and 500P showed a significant ($p<0.05$) increase in the feed intake. The standard drug and other treatment groups showed no statistical difference with the control groups.

4.3.2 Relative weight of kidney and liver

The mean relative weight of liver and kidney are presented in Table 4.2. There was no significant ($p>0.05$) difference in the relative kidney and liver weights of all the groups.

Table 4.1: Phytochemical Contents (%) of the Fractions of *Persea americana* seeds

Extract	Saponins	Tannins	Flavonoids	Phenols	Alkaloids
Petroleum	4.30±0.58[a]	17.60±0.80[a]	12.80±2.43[a]	10.83±1.04[a]	5.60± 0.40[a]
Methanolic	10.30±0.58[b]	21.07±2.76[a]	74.30 ± 5.20[b]	27.83±2.47[b]	7.60± 0.60[a]

Values are means ± standard deviation of triplicate determination. Values with different superscript along the same column are significantly ($p<0.05$) different.

Table 4.2 Acute Toxicity Studies Effects of Petroleum Ether and Methanolic Fractions of *Persea americana* seeds on wistar rats

Phase 1

Dosage	No of rats	No of toxicity
10 mg	3	0/3
100 mg	3	0/3
1000 mg	3	0/3

Phase 2

Dosage	No of rats	No of toxicity
2000mg	1	0/1
3000mg	1	0/1
4000mg	1	0/1
5000mg	1	0/1

Table 4.3: Effects of Petroleum Ether and Methanolic Fractions of *Persea americana* Seeds on Relative Organs Weight (ROW) and Daily Feed intake of Diet-induced Hyperlipidemic Wistar Rats

	NC	HFD	HFD+A	HFD+125M	HFD+250M	HFD+500M	ND+500M	HFD+125P	HFD+250P	HFD+500P	ND+500P
ROW OF LIVER	4.3 ± 0.5^{ab}	4.4 ± 0.5^{ab}	4.2 ± 0.4^{ab}	4.2 ± 0.5^{ab}	4.6 ± 0.5^{b}	4.1 ± 0.3^{ab}	4.3 ± 0.6^{ab}	3.7 ± 0.3^{a}	4.3 ± 0.5^{ab}	4.9 ± 0.5^{b}	3.8 ± 1.2^{a}
ROW OF KIDNEY	1.2 ± 0.1^{a}	1.4 ± 0.2^{ab}	1.3 ± 0.8^{ab}	1.2 ± 0.1^{ab}	1.2 ± 0.2^{ab}	1.2 ± 0.1^{a}	1.3 ± 0.1^{ab}	1.3 ± 0.1^{ab}	1.4 ± 0.2^{b}	1.7 ± 0.4^{c}	1.7 ± 0.4^{c}
DAILY FEED INTAKE (g/100g/day)	13.4 ± 3.2^{a}	13.6 ± 0.4^{a}	13.1 ± 1.0^{a}	14.1 ± 1.4^{a}	16.0 ± 1.0^{ab}	14.5 ± 1.0^{a}	17.9 ± 2.4^{ab}	13.5 ± 0.9^{a}	16.1 ± 1.5^{ab}	20.8 ± 2.3^{c}	15.8 ± 2.5^{a}

NC= Normal control, HFD= High-fat high-cholesterol diet, A=Atorvastatin, ND= Normal diet, M= mg/kgbody weight of methanolic fractions, P= mg/kgbody weight of petroleum ether extracts.
Values are mean ± S.D. (n=5). Values with different superscript along the same row are significantly ($p<0.05$) different.

4.3.3 Body weight

The mean changes in the body weight of rats are presented in Figure 4.1. There were increases in the final weight compared with the initial weight in all the groups. Higher weight gain was observed in HFD control group compared to other groups. The petroleum ether and methanolic fractions significantly ($p<0.05$) reduced the increase in body weight caused by HFD.

4.3.4 Packed cell volume

The initial and final packed cell volumes are presented in Figure 4.2. There was a decrease in final PCV of the different groups except the group fed a HFD and 500 mg/kg body weight of petroleum ether fraction which showed an increase in the final PCV. The magnitude of decrease in PCV of the fractions-treated groups was lower than that observed in the HFD control group.

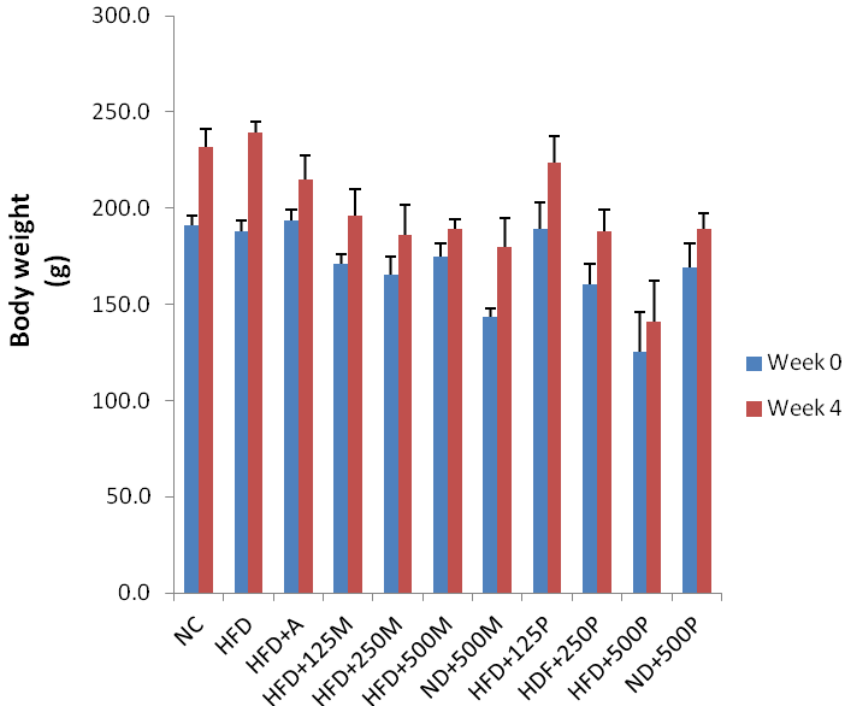


Figure 4.1: Change in body weight of rats administered petroleum ether or methanolic fractions of *Persea americana* seeds

NC= Normal control, HFD= High-fat high-cholesterol diet, A=Atorvastatin, ND= Normal diet, M= mg/kgbody weight of methanolic fractions, P = mg/kgbody weight of petroleum ether fraction Values are mean ± S.D. (n=5).

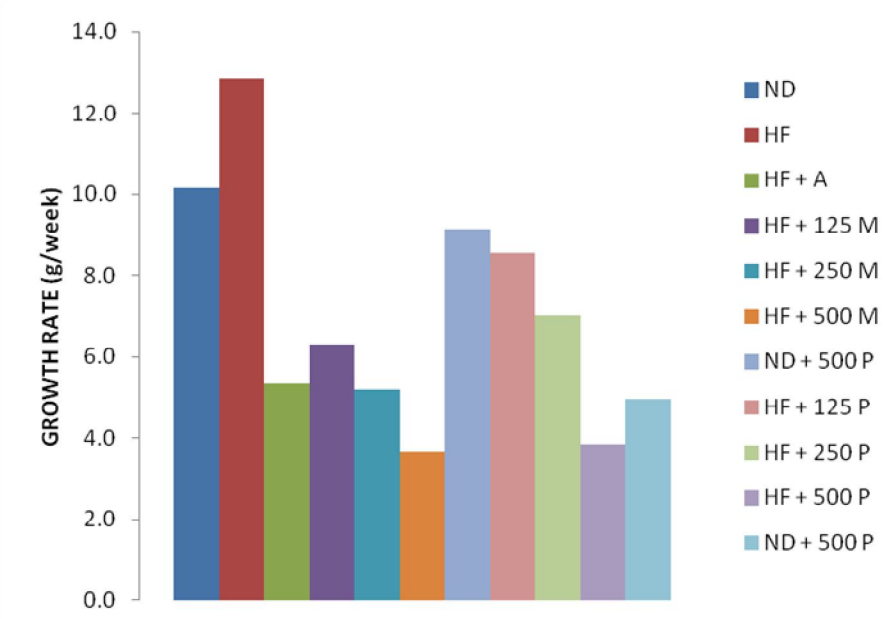


Figure 4.2: Change in Growth rate of rats administered petroleum ether or methanolic fractions of *Persea americana* seeds

NC= Normal control, HFD= High-fat high-cholesterol diet, A=Atorvastatin, ND= Normal diet, M= mg/kgbody weight of methanolic fractions, P = mg/kgbody weight of petroleum ether fraction Values are mean ± S.D. (n=5).

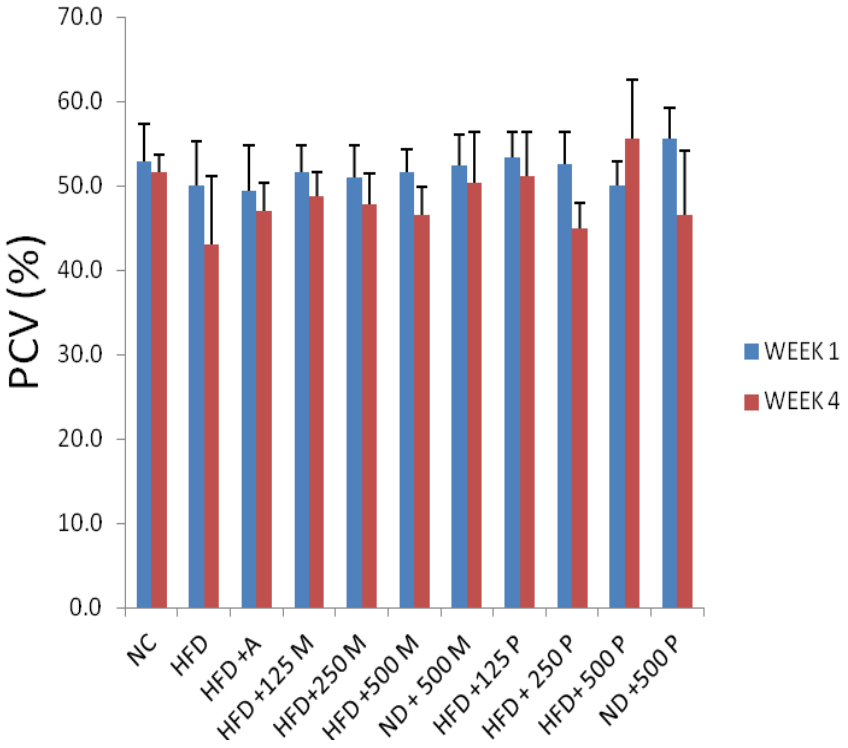


Figure 4.3: Initial and final packed cell volume of rats administered petroleum ether or methanolic extract of *Persea americana*

Values are mean ± S.D. (n=5). NC= Normal control, HFD= High-fat high-cholesterol diet, A=Atorvastatin, ND= Normal diet, M= mg/kgbody weight of methanolic extract, P = mg/kgbody weight of petroleum ether extract

4.4 Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on lipid profile of diet-induced hyperlipidemic wistar rats

4.4.1 Total cholesterol (TC)

Table 4.3 presents data of mean serum TC for rats in all the groups. The serum TC level in the HFD control group was significantly ($p<0.05$) higher than that of the normal control group. This was significantly ($p<0.05$) decreased in a dose dependent manner in the fractions treated groups compared to the HFD control group. The petroleum ether fraction appeared to be more effective in decreasing the serum TC level than the methanolic fraction. The effect of the standard drug was the same with that of the HFD group treated with 125mg/kg body weight of the methanolic fraction.

4.4.2 High density lipoprotein-cholesterol (HDL-c)

The mean serum HDL-c for rats in all the groups is shown in Table 4.3. The serum HDL-c level in the HFD control group showed a significant decrease ($p<0.05$) compared to the normal control group. There was a significant ($p<0.05$) increase in the administration of the 250 and 500mg/kg body weight of methanolic fraction, while the petroleum ether fraction showed a dose dependent significant increase when compared with the HFD control group. Administration of the standard drug or 125mg/kg body weight dose of methanolic fraction to HFD rats had no effect on the serum HDL-c level of rats.

4.4.3 Triacylglycerols (TG)

Triacylglycerol data in Table 4.3 showed that the HFD control group had significantly increased ($p< 0.05$) serum TG levels as compared to the normal control group. The

administration of either of the fractions or the standard drug, caused significant ($p<0.05$) decreases in TG level compared to the HFD control group. The effect caused by the petroleum ether fraction was dose dependent and at the 500mg/kg dose the TG level was virtually same as in the normal controls.

4.4.4 Low density lipoprotein-cholesterol (LDL-c)

Higher level of LDL-c was observed in HFD control group compared to the normal control group. The fractions and standard drug administered groups had significantly ($p<0.05$) lower levels compared to the HFD control group. This decrease was in a dose dependent manner and petroleum ether fraction tended to be more effective in decreasing the level of LDL-c than the methanolic fractions (Table 4.3).

4.4.5 Total lipids

Total lipids were significantly ($p<0.05$) increased in HFD control group compared to the normal control group. When the fractions or the standard drug was administered, the total lipid level was significantly ($p<0.05$) decreased compared to the HFD control group (Table 4. 3).

4.4.6 Total phospholipids

The serum total phospholipids for rats in all the groups is represented in Table 4.3 and it showed a significant ($p<0.05$) increase in HFD control group compared to the normal control. The fractions treated groups showed dose dependent significant ($p<0.05$) decreases in total phospholipids values as compared with the value seen in HFD

controls. Also the standard drug caused significant decrease ($p< 0.05$) in this parameter as compared to the HFD control group.

4.4.7 Antherogenic ratio

The antherogenic ratios TC/HDL-c and LDL-C/HDL-c for rats in all the groups are presented in Table 4.3. The antherogenic ratio in the HFD control group was significantly ($p<0.05$) higher than that of the normal control group. The standard drug and the fractions caused dose dependent significant ($p< 0.05$) decreases in this ratio as compared with the HFD control group. The lowest values were observed in normal control rats given 500mg/kg body weight of methanolic or petroleum ether fractions.

Table 4.4: Effects of Petroleum Ether and Methanolic Fractions of *Persea americana* Seeds on Serum Levels of Lipids Profile and Atherogenic Ratio of Diet-Induced Hyperlipidemic Wistar Rats

	NC	HFD	HFD +A	HFD +125 M	HFD +250M	HFD +500M	ND+ 500M	HFD +125P	HFD +250P	HFD +500P	ND +500P
TC (mg/dl)	197.7 ±15.8^{a}	268.6 ±30.4^{b}	179.3 ±27.5^{ac}	179.4 ±26.8^{ac}	153.9 ±12.3^{d}	149.2 ±2.2^{d}	129.4 ±11.2^{d}	160.3 ±20.1^{cd}	138.5 ±24.5^{d}	122.6 ±7.0^{d}	93.7 ±8.0^{e}
TG (mg/dl)	14.6 ±0.7^{a}	71.7 ±4.3^{b}	29.4 ±4.3^{c}	32.1 ± 8.8^{c}	14.5 ±3.9^{a}	39.9 ±4.7^{d}	33.6 ±8.7^{d}	23.3 ±3.6^{c}	21.6 ±7.1^{c}	16.1 ±2.0^{a}	10.3 ±3.9^{e}
HDL-C (mg/dl)	45.4 ±3.5^{a}	20.7 ±0.7^{b}	26.5 ±6.5^{b}	19.7 ±4.3^{b}	46.6 ±3.7^{a}	50.4 ±7.9^{a}	75.4 ±9.7^{c}	33.9 ± 2.7^{d}	44.5 ±2.1^{a}	47.7 ±4.0^{a}	72.8 ±4.7^{c}
LDL-C (mg/dl)	146.8 ±16.0^{a}	233.4 ±30.5^{b}	144.3 ±31.4^{a}	153.3 ±26.0^{a}	107.0 ±12.6^{c}	92.1 ±8.8^{cd}	56.2 ±11.2^{d}	115.6 ±22.6^{c}	79.3 ±11.3^{d}	71.7 ±5.8^{d}	12.5 ±4.0^{e}

Total Lipids (g/l)	5.9 ±0.1[a]	13.1 ±3.0[b]	5.6 ±0.7[ac]	6.0 ± 0.3[a]	5.3 ± 0.2[ac]	4.8 ±0.3[ac]	5.1 ±0.3[ac]	6.03 ±1.0[a]	5.1 ±0.2[ac]	4.2 ±0.2[c]	4.5 ±0.6[ac]
Phospholipids (g/l)	4.3 ±0.8[a]	5.7 ± 0.9[b]	4.1 ±1.0[a]	3.3 ±0.5[c]	3.7 ±0.8[ac]	3.6 ±0.7[a]	3.3 ±0.46[c]	4.4 ±0.6[ab]	3.5 ±0.4[c]	2.8 ±0.4[cd]	2.2 ±0.4[d]
TC/HDL-C	4.4 ±0.3[a]	13.0 ±1.9[b]	6.13 ±1.3[c]	8.5 ±0.8[d]	3.7 ±0.9[ae]	3.0 ±0.5[e]	1.8 ±0.5[fg]	4.8 ±0.8[a]	3.1 ±0.7[e]	2.6 ±0.1[f]	1.3 ±0.1[g]
LDL-C/ HDL-C	3.2 ±0.4[a]	10.0 ±3.5[b]	5.9 ±2.4[c]	8.1 ±2.4[b]	2.6 ±0.9[a]	1.9 ±0.5[ad]	0.7 ±0.5[ad]	3.2 ±1.1[a]	2.0 ±0.7[a]	1.5 ±0.2[ad]	0.2 ±0.1[d]

Values are mean ± S.D. (n=5). NC= Normal control, HFD= High-fat high-cholesterol diet, A=Atorvastatin, ND= Normal diet, M= mg/kgbody weight of methanolic extract, P = mg/kgbody weight of petroleum ether extract. Values with different superscript along the same row are significantly ($p<0.05$) different. TC-total cholesterol, HDL-c –High density Lipoprotein cholesterol, LDL-c low density lipoprotein cholesterol, TG-triacylglycerol.

4.5 Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on lipid peroxidation and endogenous antioxidant of the organs of diet-induced hyperlipidemic wistar rats

4.5.1 Thiobarbituric reactive substances (TBARS)

The liver and kidney homogenate TBARS was significantly ($p<0.05$) higher in HFD control group than the normal control group. The administration of either of the fractions or the standard drug significantly ($p<0.05$) lowers the TBARS level to that of the normal control in both the kidney and liver. The methanolic fraction had a greater effect in the liver while the decrease caused by the petroleum ether fraction in the kidney was in a dose dependant manner (Table 4.4).

4.5.2 Catalase

From Table 4.4, significantly ($p< 0.05$) lower catalase activity was observed in HFD control group in the kidney and liver homogenates compared to the normal control. The fractions and standard drug significantly increased ($p< 0.05$) the catalase activity in the diet-induced hyperlipidemic rats. The fractions caused a dose dependent significant ($p<0.05$) increase in this parameter in the kidney.

4.5.3 Superoxide dismutase (SOD)

The superoxide dismutase activity in liver and kidney homogenate (Table 4.4) of HFD control group were significantly ($p<0.05$) lower than that recorded in the control group. On the administration of either of the fractions, there was a significant ($p<0.05$) increase of SOD activity in HFD rats given the in highest dosage of the methanolic fraction. In

the liver, the petroleum ether fraction-treated groups showed significant ($p<0.05$) increase in a dose dependent manner compared to the standard drug and methanolic fraction groups. In the kidney, the fractions caused significant ($p< 0.05$) increase in the SOD activity compared to the HFD control group. The standard drug caused significant ($p<0.05$) decrease in SOD in the kidney, but caused a significant ($p<0.05$) increase in the liver homogenate compared to the HFD control group.

4.5.4 Reduced gluthathione (GSH)

The reduced gluthathione activity (Table 4.4) in the kidney and liver homogenate showed no significant ($p>0.05$) difference between the HFD control group and the normal control group. In the liver, groups administered HFD and 250 or 500mg/kg body weight of the petroleum ether fraction caused an increase in GSH to levels which were significantly ($p<0.05$) higher than those of control groups and the methanolic fraction-treated groups.

The kidney homogenate showed significantly increased GSH level in methanolic fraction administered HFD and 250 or 500mg/kg body weight of the rats. The petroleum ether fraction caused significant ($p<0.05$) increase in GSH activity in all the dosages groups.

Table 4.5: Effects of Petroleum Ether and Methanolic Fractions of *Persea americana* Seeds on Reduced Gluthathione, (GSH), Superoxide Dismutase (SOD), Catalase (CAT), and Thiobarbituric Reactive Acid (TBARS) of the Liver (L) and Kidney (K) Of Diet-Induced Hyperlipidemic Wistar Rats

	NC	HFD	HFD +A	HFD + 125 M	HFD+ 250 M	HFD + 500 M	ND+ 500 M	HFD+ 125 P	HFD+ 250P	HFD+ 500P	ND+ 500P
TBARS-L (µmolMDA/gtissue)	2.14± 0.37^{a}	4.73± 1.55^{b}	1.66± 0.26^{a}	1.34± 0.55^{a}	1.45± 0.22^{a}	1.91± 0.27^{a}	1.69± 0.16^{a}	1.89± 0.47^{a}	2.62± 0.35^{c}	2.11± 0.22^{ac}	2.29± 0.17^{ac}
TBARS K (µmolMDA/gtissue)	13.68± 4.24^{a}	24.98± 5.89^{b}	15.49± 5.23^{b}	12.69± 3.44^{a}	7.55± 1.79^{ac}	8.85± 1.07^{a}	7.29± 2.92^{ac}	13.65± 5.09^{a}	10.29± 6.12^{a}	7.26± 3.60^{ac}	7.63± 0.83^{a}
GSH-L (mg/gtissue)	3.94± 0.09^{a}	4.04± 0.13^{ab}	3.92± 0.04^{a}	3.92± 0.04^{a}	3.86± 0.10^{a}	3.98± 0.04^{ab}	3.84± 0.05^{a}	3.86± 0.05^{a}	4.10± 0.12^{b}	4.14± 0.11^{b}	4.04± 0.05^{ab}
GSH K (mg/gtissue)	5.64± 0.05^{a}	5.88± 0.16^{a}	5.84± 0.05^{a}	5.80± 0.07^{a}	6.26± 0.13^{b}	6.50± 0.87^{b}	6.20± 0.00^{b}	6.28± 0.13^{b}	6.18± 0.04^{b}	6.78± 0.78^{c}	6.02± 0.11^{ab}
CAT. L (moles of H_2O_2/min/gtissue)	0.04± 0.02^{a}	0.02± 0.01^{b}	0.038± 0.001^{a}	0.05± 0.01^{a}	0.04± 0.02^{a}	0.07± 0.01^{c}	0.06± 0.01^{c}	0.03± 0.01^{ab}	0.08± 0.01^{c}	0.06± 0.01^{c}	0.06± 0.01^{c}
CAT. K (moles of H_2O_2/min/gtissue)	0.08± 0.00^{a}	0.04± 0.01^{b}	0.09± 0.03^{a}	0.06± 0.01^{b}	0.07± 0.03^{b}	0.09± 0.03^{a}	0.10± 0.02^{a}	0.05± 0.02^{b}	0.08± 0.01^{ab}	0.11± 0.01^{c}	0.11± 0.01^{c}
SOD L (unit/g)	152.00 ±18.14^{a}	139.9 ±8.83^{b}	154.40 ±15.50^{a}	112.75 ±9.58^{b}	124.50 ±25.40^{b}	155.20 ±12.58^{a}	197.60 ±6.89^{c}	157.10 ±8.45^{a}	166.60 ±24.78^{a}	194.50 ±9.52^{c}	172.90 ±16.73^{ac}
SOD K (unit/g)	242.13± 12.81^{a}	228.15± 37.00^{b}	54.10± 12.28^{c}	237.00± 41.64^{ab}	260.7± 19.77^{a}	289.30± 44.88^{d}	247.11 ±23.48^{a}	270.2± 11.74^{ad}	266.10± 20.78^{a}	297.60 ±48.90^{d}	257.78± 16.36^{a}

NC= Normal control, HFD= High-fat high-cholesterol diet, A=Atorvastatin, ND= Normal diet, M= mg/kgbody weight of methanolic extract, P = mg/kgbody weight of petroleum ether extract. Values are mean ± S.D. (n=5). Values with different superscript along the same row are significantly ($p<0.05$) different.

4.6 Effects of petroleum ether and methanolic fractions of *Persea americana* seed on liver functions markers of diet-induced hyperlipidemic rats

4.6.1 Serum alkaline phosphatase (ALP)

From Table 4.5, significantly ($p<0.05$) higher activity of ALP activity was observed in HFD control group compared to the normal control group. The standard drug and the HFD rat given 250 and 500mg/kg body weight of methanolic fraction showed a significant ($p<0.05$) decrease in the ALP level compared with the HFD control group. There was no significant ($p>0.05$) difference between the petroleum ether fraction treated groups and the HFD control group. HFD and 125mg/kg body weight of methanolic fraction showed an increase in ALP actitvity to a level which was significantly ($p<0.05$) higher compared to the control groups.

4.6.2 Serum alanine aminotransferase (ALT)

There was no significant ($p>0.05$) difference between the HFD control group and the normal control group in serum ALT level. The decrease in the activities of ALT in HFD rats given 500 mg/kg body weight of petroleum ether fraction group were significantly ($p<0.05$) lower compared to the control group. There was no significant difference among groups given 125 and 250 mg/kg body weight of methanolic fraction and also group given 500 mg/kg body weight of petroleum ether fraction groups compared to the control groups as shown in Table 4.5.

4.6.3 Serum aspartate aminotransferase (AST)

The AST level of the HFD control group was significantly ($p<0.05$) increased when compared with the normal control group. The AST activities of the fractions treated and standard drug groups were significantly ($p<0.05$) lower compared to HFD control group. Groups treated with HFD and 250mg/kg body weight of methanolic fraction as

well as HFD and 500mg/kg body weight of petroleum ether fraction showed the lowest activity as shown in Table 4.5.

4.6.4 Serum albumin

The serum albumin showed no significant ($p>0.05$) difference in all the groups (Table 4.5). The lowest level was observed in the group treated with normal diet and 500mg/kg body weight of petroleum ether fraction.

4.6.5 Serum total protein

HFD control group showed no significant ($p>0.05$) difference when compared with the normal control group. The standard drug and the fractions treated groups showed significant ($p<0.05$) decrease when compared to the HFD control group (Table 4.5).

4.6.6 Serum direct bilirubin

Table 4.5 also showed significantly ($p<0.05$) higher levels of direct bilirubin in the HFD control group compared to the normal control group. The fractions and standard drug showed a reduced level except the group given HFD and 250 mg/kg body weight of methanolic fraction which showed a significantly ($p<0.05$) increased level compared to the HFD control group. The lowest value was observed in HFD and 500 mg/kg petroleum ether fraction-treated group.

4.6.7 Serum total bilirubin

Table 4.5 present data for serum total bilirubin level. The total bilirubin level of the HFD control group was significantly higher than that of the normal control group. On treatment with the fractions or standard drug, the level was reduced. The HFD group treated with 250 mg/kg body weight of methanolic fraction showed no significant ($p>0.05$) difference when compared with the HFD control group.

Table 4.6: Effects of Petroleum Ether and Methanolic Fractions of *Persea americana* Seeds on Liver Function Markers of Diet-Induced Hyperlipidemic Rats

	NC	HFD	HFD +A	HFD + 125M	HFD + 250M	HF D+ 500M	ND +500M	HFD+ 125 P	HFD + 250 P	HFD + 5 00P	ND+ 500 P
ALP (nmol /min)	213.6 ±36.0^{a}	127.0± 29.8^{bc}	80.6 ± 13.4^{d}	181.5 ± 34.5^{ae}	58.0± 18.61^{d}	82.1± 11.4^{d}	106.6±7 4.0^{c}	131.4± 18.1^{bc}	115.9±4 5.1^{bc}	353.3± 44.4^{f}	112.5± 32.8^{bc}
ALT (U/L)	37.1± 2.7^{a}	35.2± 4.6^{a}	34.8± 2.2^{a}	26.4± 3.1^{b}	29.4± 3.3^{b}	45.5± 3.5^{c}	50.1± 5.0^{d}	42.3± 4.3^{c}	37.1± 4.2^{a}	26.3± 3.1^{b}	17.8± 2.7^{e}
AST (U/L)	51.0± 4.1^{ab}	55.8± 6.5^{b}	48.4± 10.^{5ab}	33.6± 7.1^{cd}	26.8± 5.4^{c}	34.7± 2.3^{d}	40.8± 4.7^{e}	40.8± 3.7^{e}	50.1± 2.7^{ab}	27.8± 3.9^{cd}	45.7± 3.5^{a}
ALBUMIN (g/dl)	4.6± 0.3^{a}	4.5± 0.9^{a}	4.5± 0.6^{a}	4.3± 0.8^{a}	4.6± 0.4^{a}	4.9± 0.4^{a}	4.2± 0.9^{a}	4.8± 0.5^{a}	4.5± 0.1^{a}	4.2± 0.7^{a}	4.1± 0.8^{a}
T.P (g/dl)	6.9± 0.8^{a}	6.67± 0.2^{a}	5.9± 0.6^{c}	7.0± 0.7^{a}	3.9± 0.5^{d}	3.1± 0.6^{d}	6.2± 0.7^{ac}	7.5± 0.2^{ab}	4.7± 0.3^{e}	6.4± 0.4^{ac}	5.9± 1.2^{c}
D.B (mg/dl)	1.2± 0.2^{a}	2.8± 0.4^{b}	1.0± 0.2^{a}	2.1± 0.9^{c}	3.3± 0.4^{d}	2.4± 0.3^{bc}	1.8± 0.4^{e}	1.3± 0.1^{ae}	2.4± 0.8^{bc}	0.4± 0.1^{f}	1.1± 0.2^{a}
T.B (mg/dl)	1.8± 0.1^{ae}	2.7± 0.1^{b}	2.1± 0.2^{ac}	2.2± 0.7^{d}	2.8± 0.3^{b}	2.2± 0.2^{cd}	2.1± 0.5^{a}	1.6± 0.1^{e}	2.6± 0.3^{d}	2.1± 0.1^{a}	2.1± 0.1^{a}

NC= Normal control, HFD= High-fat high-cholesterol diet, A=Atorvastatin, ND= Normal diet, M= mg/kgbody weight of methanolic extract, P = mg/kgbody weight of petroleum ether extract., ALP- alkaline phosphatase, ALT and AST- alanine and aspartate aminotransferase, T.P- total protein, D.B and T.B- direct and total bilirubin. Values are mean ± S.D. (n=5). Values with different superscript along the same row are significantly ($p<0.05$) different.

4.7 Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on serum levels of urea and creatinine of diet-induced hyperlipidemic wistar rats

4.7.1 Serum urea level

The serum urea level showed a significant ($p<0.05$) decrease in all groups. The petroleum ether fraction and the standard drug groups showed a significant decrease when compared to the HFD control group and the methanolic fractions as shown in Table 4.6.

4.7.2 Serum creatinine level

The serum creatinine level showed no significant ($p>0.05$) difference between the HFD control group and the normal control group. Groups treated with standard drug, 500mg/kg body weight of methanolic fraction and groups treated with 125 and 500mg/kg body weight of petroleum ether fractions had significantly ($p<0.05$) increased levels of creatinine when compared with the HFD control group (Table 4.6).

Table 4.7: Effects of Petroleum Ether and Methanolic fractions ff *Persea americana* Seeds on Serum Levels of Urea and Creatinine of Diet-Induced Hyperlipidemic Wistar Rats

	NC	HFD	HFD +A	HFD + 125 M	HFD + 250 M	HFD + 500M	ND + 500M	HFD+ 125P	HFD+ 250P	HFD+ 500P	ND+ 500P
UREA (mg/dl)	45.56 ±5.19^{a}	30.46 ±4.98^{b}	12.78 ±4.57^{c}	8.40± 1.72^{ce}	31.03± 3.83^{b}	25.42± 2.26^{d}	24.73± 4.00^{d}	8.38 ± 2.67^{ce}	9.75± 1.12^{c}	12.55 ± 2.42^{c}	4.84 ± 0.61^{e}
CREATININE (mg/dl)	0.36± 0.07^{a}	0.65± 0.11^{ab}	0.97± 0.21^{c}	0.42± 0.08^{a}	0.55± 0.16^{ab}	1.42 ± 0.28^{d}	1.53 ± 0.24^{d}	1.66 ± 0.56^{d}	0.50± 0.07^{a}	0.83± 0.23^{b}	0.67± 0.05^{ab}

NC= Normal control, HFD= High-fat high-cholesterol diet, A=Atorvastatin, ND= Normal diet, M= mg/kgbody weight of methanolic extract, P = mg/kgbody weight of petroleum ether extract. Values are mean ± S.D. (n=5). Values with different superscript along the same row are significantly ($p<0.05$) different.

CHAPTER FIVE

DISCUSSION

Medicinal plants are of great importance to the health of individual and the communities. The medicinal values of some plants lie in some chemical substances that produce definite physiological actions in the human body. The most important of these bioactive constituents are alkaloids, tannins, flavonoids and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants (Okwu, 2001).

Flavonoids are known for their diverse biological activities including hypolipidemic activity resulting from their antioxidant activity (Afanas'ev *et al*., 1995). Flavonoids are reported to increase HDL-C concentration and decrease in LDL-C levels in hypercholesterolemic rats (Patel *et al.,* 2009). *Persea americana* seeds extracts demonstrated the presence of flavonoids and other different constituents such as saponins, tannins and alkaloids.

The plant fractions were not toxic, so it is generally safe when used in properly designated therapeutic dosages and less costly than the synthetic prescription drugs available for the treatment of hyperlipidemia.

With respect to the lipid lowering capacity of these plant fractions, it could be suggested that the constituents of these plant fractions may act as inhibitors for enzymes such as hydroxyl-methyl-glutaryl-CoA reductase, which participates in *de novo* cholesterol biosynthesis as has been suggested for some plants (Gebhardt and Beck, 1996; Eidi *et al*., 2006). Flavonoids and phenols found in the methanolic and petroleum ether extracts

may be considered favourable in increasing HDL-C concentration and decreasing in LDL-C levels in extracts treated rats.

There was significant decrease in the packed cell volume of HFD control group. HFD had high level of total bilirubin. This shows that there is degradation of heam as a result of red blood cell destruction, which may be due to diet-induced hyperlipideamia. Due to this effect, low PCV indicate oxidative damage to the group, while the extracts ameliorated the decrease, and even increased final PCV in the group given HFD and 500mg/kg body weight of the petroleum ether extract.

Obesity is strongly associated with other cardiovascular disease (CVD) risk factors. To explain this association, it has been postulated that obesity and higher visceral abdominal fat set off a cascade of reactions, which induces or exacerbates hyperlipidemia, hypertension, and insulin resistance (Blaak *et al.,* 1999). The mean body weight of the HFD control group was significantly increased while there was a reduction in the extracts treated groups. According to Brai *et al*., (2007b), the administration of aqueous and methanolic leaf extracts of *Persea americana* leaf caused a reduction in body weight compared with the hyperlipidemic controls. In this study, the extracts of *Persea americana* seeds also reduced the body weight of the hyperlipidemic rats. Pliego and Litz, (2007) reported *Persea americana* leaf extracts increased the catabolism of lipids accumulated in the adipose tissue, resulting in a decrease in the mean body weight.

Cholesterol is a lipid waxy steroid found in cell membranes and maintains the fluidity of the membrane. Abnormally high dietary cholesterol levels lead to hypercholesterolemia, which is strongly associated with cardiovascular disease because

it promotes atherosclerosis (Durrington, 2003). Hyperlipideamia is a major risk factor in the pathogenesis of atherosclerosis, a physiologic disorder that affects the coronary, cerebral and pheripheral arterial circulation. The association between hyperlipideamia and atherosclerosis has been demonstrated in many studies and trials (Gordon *et al.,* 2007). Hyperlipideamia may be manifested by elevation of total cholesterol, low density lipoprotein and triacylglycerols concentration and a reduction in high density lipoproteins concentration. It has also been shown that reducing plasma level of LDL cholesterol sharply reduced the risk of coronary heart disease (Superko and Krauss, 1994). Also, high plasma levels of HDL cholesterol are associated with lower risk of coronary heart disease and it is widely believed that HDL protects against atherosclerosis by facilitating reverse cholesterol transport (Van *et al.,* 2009).

Petroleum ether and methanolic fractions of *Persea americana* seeds significantly reduced serum concentrations of total cholesterol (TC), low density lipoprotein (LDL) and triacylglycerols (TG) in diet-induced hyperlipidemic rats. It also caused a significant increase in HDL concentration. This is in conformity with the report of Kolawole *et al.,* (2012) in which methanolic extract of *Persea americana* leaf decreased the level of total cholesterol, triacylglycerols, low density lipoprotein while there was an increase in HDL-c in hyperlipidemic rats.

Atherogenic ratio is an important prognostic marker for cardiovascular disease. The risk of cardiovascular diseases increases considerably when the ratio is high (Laboratoires Fournier, 1981). In this study, the TC/HDL-c and LDL-c/HDL-c ratio was high in the hyperlipidemic group. This was significantly reduced in a dose dependent way in the extracts treated groups.

Other possible mechanisms by which the extracts lowers serum lipid could be by reducing the biosynthesis of cholesterol by inhibiting the activity of 3-hydroxy-3-methylglutaryl Coenzyme- A reductase (HMG-CoA reductase), the key enzyme in cholesterol synthesis. They could also act by increasing the activity of lecithin-cholesterol acyl transferase (LCAT). This enzyme plays an important role in incorporating free cholesterol into HDL (Geetha *et al*., 2011). This will promote reverse cholesterol transport and competitively inhibits the uptake of LDL by endothelia cells.

Hyperlipideamia has been reported to increase production of reactive oxygen species (ROS) by polymorphonuclear leukocytes and monocytes (Wilson and Gelb, 2002). These ROS cause damage to subcellular structures including membrane components, DNA and certain proteins (Flora, 2007). Oxidative damage caused by free radicals is related to various diseases (Halliwell and Gutteridge, 1984). Almost all organisms are well protected against free radical damage by enzymes, such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione (Niki *et al*., 1994; Mau *et al*., 2002). Medicinal plants are good sources of antioxidants, such as polyphenols which scavenge free radicals and limit the harmful effect of these radicals (Burits and Bucar, 2000). Oxidative stress plays a major role in the pathogenesis of atherosclerosis (Adaramoye *et al.,* 2005).

There was a significant increase in lipid peroxidation in the HFD control group. Increased lipid peroxidation would lead to the generation of harmful free radicals which impair membrane function and ultimately results in microvascular and macrovascular complications (Virella-Lopes and Virella, 2003). The increased lipid peroxidation

observed in HFD group was reversed by the methanolic and petroleum ether of *Persea americana* seeds extracts.

Oxidative stress occurs when the rate of reactive oxygen species generation exceeds the capacity of the cell for their removal. Conversion of superoxide anion to H_2O_2 and O_2 by SOD is often called the primary defense against oxidative stress because superoxide is such a strong initiator of chain reactions. H_2O_2, once formed, must be reduced to water and molecular oxygen to prevent it from forming the hydroxyl radical. Catalase converts H_2O_2 to water and oxygen (Collen *et al.,* 2003).

In this study, the catalase and superoxide activities were reduced in HFD control group. But when the extracts were administered, the catalase activity was increased, and the SOD activity was also increased in the groups given the highest dosages of the extracts. The reduced gluthathione also showed an increase in the extracts administered groups. This suggests that the extracts may possess *in vivo* antioxidant effects. This is most likely the case because isolation of bioactive phytoconstituents from the leaves of *Persea americana* has produced compounds with antioxidant properties such as lutein, rutin, quercetin and apigenin (Owolabi *et al*., 2010).

The activities of some enzymes were studied to determine the functional indices of liver, heart and skeletal muscles; the enzymatic activity of alanine aminotransferase (ALT) aspartate aminotransferases (AST) and alkaline phosphatase (ALP) were studied to evaluate liver functions. AST is found virtually in every tissue of the body, serum AST level concentrations increases shortly after myocardial infarction and hepatic parenchymal injury ALT is an enzyme found mostly in the liver, smaller amounts are found in the kidney, heart and muscle. Under normal conditions, an ALT level in the

blood is low, however, when the liver is damaged, ALT is released into the bloodstream before more obvious damage occur (Kaplan and Szabo, 1979). AST of the HFD was significantly increased when compared to the normal control. But the extracts caused a decrease in the activity of this enzyme. The standard drug used also, caused similar effect. Although there was no statistical difference in the ALT activity of the HFD control group and the normal control group, the group given HFD and 500mg/kg body weight of petroleum ether extract showed a significant decrease in the activity. This shows that extracts may possess hepatoprotective properties.

Alkaline phosphatase test is usually required if a patient has symptoms of liver or bone disorder. It is often associated with growth and developmental stages. There was decrease in ALP concentrations in all the groups showing there was no liver or bone damage.

It is not unusual that the kidneys bear a significant burden from the assault of xenobiotic exposure, since they are involved with maintenance of homeostasis. One of these roles is elimination of metabolic waste. Blood urea is derived from normal metabolism of protein and is excreted in the urine. Elevated blood urea usually indicate glomerular damage, however, its level can also be affected by poor nutrition and hepatotoxicity which are common effects of many toxicants.

Creatinine is a metabolite of creatine and also excreted completely in the urine via glomerular filtration. An elevation of its level in the blood is an indication of kidney damage. Also, high level of the serum creatinine concentration indicates low clearance rate of creatinine which signifies kidney dysfunction (Ukoha, 1998; Murray *et al.,* 2000). Impairment in renal function is detected by the measurement of creatinine and

urea clearance, which gives an accurate estimate of the clearance and glomerular filtration rate. The serum urea was significantly decreased in the HFD. The standard drug and petroleum ether extract caused a further decreased in the serum urea concentration.

Atorvastain which was used as positive control in this study is a HMG-CoA reductase inhibitor, HMG-CoA reductase reduces serum triglyceride levels through the modulation of apolipoprotein lipase. Rats treated with atorvastatin showed marked reduction in TC, LDL-C and triacylglycerols and increase in HDL-C level as compared with HFD control group. The standard drug was seen to lower the level of TBARS while increasing the catalase activity in liver and kidney homogenates of the hyperlipidemic rats.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

- Petroleum ether and methanolic fractions of seeds *of Persea americana* contain phytochemicals like flavonoids, phenols, saponins, alkaloids and tannins with methanolic fraction having higher values of saponins (10.3±0.6%), flavonoids (74.3±5.2%) and phenols (27.83±2.4%) than the petroleum ether fraction.
- The seeds extracts of *P. americana* may be regarded as relatively safe at dose of 5000mg/kg body weight.
- High-fat-high-cholesterol diet (HFD) caused an increase in body weight of experimental rats. The HFD caused decrease in PCV of the experimental rats. The plant fractions ameliorate the decrease in PCV of rats.
- High-fat-high-cholesterol diet caused an increase in TC, TG, LDL-c, total lipids, total phospholipids and antherogenic ratio (268.6±30.4mg/dl, 233.4±30.5mg/dl, 71.7±4.3mg/dl, 13.1±3.0g/dl, 5.7±0.9g/dl, 13.0±1.9, 10.0±3.5 respectively), while decreasing HDL-c (20.7±4.3mg/dl) in hyperlipidemic rats. The petroleum ether and methanolic fractions of *Persea americana* reversed the effects of HFD.
- The petroleum ether and methanolic fractions of *Persea americana* seeds significantly ($p<0.05$) decreased the level of TBARS, while significantly ($p<0.05$) increasing the level of catalase, superoxide dismutase and reduced gluthathione in hyperlipidemic rats.

- The seeds fractions decreased levels of serum AST, total bilirubin and serum urea in hyperlipidemic rats.
- Both the petroleum ether and methanolic fractions possess the hypolipidemic and antioxidant effects.

6.2 Conclusion

- High-fat-high-cholesterol diet had been shown to induce hyperlipidemia in wistar rats. The administration of petroleum ether and methanolic fractions of *Persea americana* seeds was shown to reduce the level of hyperlipidemia in wistar rats.
- The seed fractions had led to improvement of the antherogenic ratio of hyperlipidemic wistar rats.
- The high-fat-high-cholesterol diet had also induced oxidative stress. The extracts of *Persea americana* seeds appeared to have reduced oxidative stress caused by high-fat-high-cholesterol diet in the rats.

6.3 Recommendation

- The period of the study was four weeks; it is recommended that in further study, the period should be extended.
- In this work, the crude extract was used, further study should employ the purified extract and also steps should be taken to isolate the active components that possess this hypolipidemic and antioxidant properties.

REFERENCES

Adaramoye, O. A., Nwaneri, V. O., Anyanwu, K. C., Farombi, E. O. and Emerole, G. O, (2005). Possible anti-antherogenic effect of kolaviron (a Garicana kola seed extract) in hypercholesterolemic rats. *Clinical Experimental Pharmacology and Physiology*, **32**: 40-46.

Adeyemi, O. O., Okpo, S. O. and Ogunti, O. O. (2002). Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). *Fitoterapia*, **73**: 375–380.

Afanas'ev, I. B., Ostrachovitch, E. A., Abramova, N. E. and Korkina, L. G. (1995). Different antioxidant activities of biflavonoid rutin in normal and iron overloading rats. *Biochem. Pharmacology*, **80**: 627-635.

Ajose, F. O. A. (2007). Some Nigeria plants of dermatologic importance. *International Journal of Dermatology,* **46**: 48-55.

Alexander, R. R. and Griffins, J. M. (1993). Haematocrit. In R. R. Alexander R. R and J. M. Griffins (Eds.). *Basic biochemical method.* New York: John Wiley and Sons pp. 186-187.

Ambrosone, C. B., Ahn, J., Singh, K. K., Rezaishiraz, H., Furberg, H., Sweeney, C., Coles, B. and Trovato, A. (2005). Polymorphisms in genes related to oxidative stress (MPO, MnSOD, CAT) and survival after treatment for breast cancer. *Cancer Research*, **65**:1105–1111.

Baranano D. E., Rao, M., Ferris, C. D. and Snyder S. H. (2002). Biliverdin reductase: a major physiologic cytoprotectant. *Proceedings of National Academy of Sciences in U.S.A.,* **99**: 16093–16098.

Bartels, H. and Bohmer, M. (1972). Serum creatinine dertimination without protein precipitation. *Clinica Chimica Acta,* **37**:193.

Bekele-Tesemma, A., Birnie, A. and Tengnas, B.(1993). Useful trees and shrubs for Ethiopia.Regional Soil Conservation Unit (RSCU), Swedish International Development Authority (SIDA).

Berg, J. M., Tymoczko, J. L. and Stryer, L. (2006). *Biochemistry*. W. H. Freeman. New York. pp. 656–660.

Bertel, P., Gotto, A. M., LaRosa, J. C., Maroni, J., Szarek, M., Grundy, S. M., Kastelein, J. J. and Bittner, V. (2007). HDL Cholesterol, Very Low Levels of LDL Cholesterol, and Cardiovascular Events. *New England Journal of Medicine,* **357** (13): 1301–10.

Bhatnagar, D., Soran, H. and Durrington, P. N. (2008). Hypercholesterolaemia and its management. *British Medical Journal*, **337:** 45-47.

Biswas, T. K. and Mukherjee, B. (2003). Plant Medicines of Indian Origin for Wound Healing Activity: A Review. *The International Journal of Lower Extremity Wounds,* **2**(1): 25-39.

Blaak, E. E., Saris, W. H. M. and Wolffenbuttel, B. H. R. (1999). Substrate utilization and thermogenic responses to -andrenergic stimulation in obese subjects with NIDDM. *International Journal of Obesse Related Metabolism Disorder*; **23**:411–8.

Boham, A. B. and Kocipai, A. C. (1994). Flavonoid and condensed tannins from Leaves of *Hawaiian vaccininum vaticulum* and *vicalycinium. Pacific Sci*ence, **48**: 458-463.

Boon E. M., Downs, A. and Marcey, D. (2007). Catalase: H_2O_2: H_2O_2 Oxidoreductase. *Catalase Structural Tutorial Text*. *Journal of Research in Bioinformatics*, **1**:3-4

Brai, B. I., Odetola, A. A. and Agomo, P. U. (2007a). Hypoglycemic and hypocholesterolemic potential of *Persea americana* leaf extracts. *Journal of Medicinal Food*, **10**:356–360.

Brai, B. I., Odetola, A. A. and Agomo, P. U. (2007b). Effects of *Persea americana* leaf extracts on body weight and liver lipids in rats fed hyperlipidaemic diet. *African Journal of Biotechnology*, **6:**1007–11.

Burits, M. and Bucar, F. (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy Research,* **14**: 323-328.

Campbell, N. A., Brad, W. and Robin, J. H. (2006). *Biology: Exploring Life***.** Boston, Massachusetts: Pearson Prentice Hall. pp. 45-89.

Chelikani, P., Fita, I. and Loewen, P. C. (2004). Diversity of structures and properties among catalases. *Cellular Molecular Life Science,* **61** (2): 192–208.

Chessbrough, M. (2005). *District laboratory Practice in Tropical Countries Part 1* 2nd ed. Cambridge University Press, London, pp. 333-338.

Collen, S., Allan, M. D. and Micheal, L. (2003). *Marks Basic Medical Biochemistry*: A Clinical Approach. 2nd ed. New York, PP. 453.

Colditz, I. G., Watson, D. L., Gray, G. D. and Eady, S. J. (1996). Some relationships between age, immune responsiveness and resistance to parasites in ruminants. *International Journal of Parasitology*, **26**:869–877.

Crane E and Walker P. (1984). Pollination directory for world crops. London, UK: International Bee Research Association; **5:**1-3**.**

Crews, D. E. (2007). Senescence, aging and disease. *Journal of Physiological Anthropology,* **26**:365-372.

Darran, G. and Cronshaw, E. (2006). Evidence that phospholipase C-dependent, calcium-independent mechanisms are required for directional migration of T lymphocytes in response to the CCR4 ligands CCL17 and CCL22. *Journal of Leukocyte Biology,* **79**: 34-35.

Del, R. G. (2000). Adrenomedullary function and its regulation in obesity. *International Journal of Obesity Related Metabolism Disorder*, **24:** S89–91.

Duncan, O. D., David, I. Featherman D. and Beverly, D. (1972). *Socioeconomic Background and Achievement.* New York Academic Press, pp. 35-42.

Durrington, P. (2003). Dyslipidaemia. *The Lancet***, 362:** (9385): 717–31.

Egan, B. M., Stepniakowski, K. and Goodfriend, T. L. (1994). Renin and aldosterone are higher and the hyperinsulinemic effect of salt restriction greater in subjects with risk factors clustering. *American Journals of Hypertension*, **7**:886–93.

Eidi, A., Eidi, M. and Esmaeili, E. (2006). Antidiabetic effect of garlic in normal and STZ-induced diabetic rats. *Phytomedicine*, **13**(9-10): 624-629.

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics,* **82** (1): 70–7.

Epstein, F. H. (1999). Atherosclerosis- An inflammatory disease. *The New England Medicine*; **340**: 115-123.

Fawcett, J. K. and Scoutt, J. E. (1960). A rapid and precise method for the determination of urea. *Journal of Clinical Pathology*, **13**:156.

Flora, S. J. (2007). Role of free radicals and antioxidants in health and disease. *Cell Biology*, **53**: 1-2.

Fogari, R. and Zoppi, A. (2004). Effect of antihypertensive agents on quality of life in the elderly. *Drugs Aging,* **21**:377-393.

Fridovich, I. (1978). Biological oxygen radicals. *Science*, **201**: 875-880.

Friedwald, W. T. (1972). Methods for the determination of LDL Cholesterol. *Clinical Chemistry,* **18**:499-502.

Frisinghelli, A. and Mafrici, A. (2007). Regression or reduction in progression of atherosclerosis, and avoidance of coronary events, with lovastatin in patients with or at high risk of cardiovascular disease: A review. *Clinical Drug Investigation,* **27**:591-604.

Gaetani, G., Ferraris, A., Rolfo, M., Mangerini, R., Arena, S., Kirkman H. (1996). Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes. *Blood,* **87** (4): 1595–9.

Gaze, D. C (2007). The role of existing and novel cardiac biomarkers for cardioprotection. *Current Opinion on Investigational Drugs,* **8** (9): 711–7.

Gebhardt, R. and Beck, H. (1996). Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. *Lipids,* **31**(12):1269-1276.

Geetha, G., Kalavalarasariel, G. P. and Sankar, V. (2011). Antidiabetic effect of *Achyranthes rubrofusca* leaf extracts on alloxan induced diabetic rats. *Pakistan Journal of Pharmaceutical Sciences, **24***(2):193-199.

Gennest, J. and Libby, P. (2011). Lipoprotein disorders and cardiovascular disease. In: Bonow R. O., Mann, D. L., Zipes, D. P and Libby, P. (Ed). *Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine*. 9th ed. Philadelphia, Pa: Saunders Elsevier, 237-289.

Ghanta, A. and Asthana, O. P. (1995). Recent trends in hyperlipoprotenemias and its Pharmacotherapy, *Indian Journals of Pharmac*ology, **27**:14-29.

Gordon, T., Castelli, W. P., Hjortland, M. C. , Kannel, W. B., Dawber, T. R. (2007). High density lipoprotein as a protective factor against coronary heart diseases: The Framingham study. *American Journal of Medicine,* **62**(5): 705-714.

Gray, D. S. (1998). Diagnosis and prevalence of obesity. *Medical Clinics of North America,* **73**:1–13.

Haines, T. H. (2001). Do sterols reduce proton and sodium leaks through lipid bilayers? *Progress-in-lipid-researh,* **40** (4): 299–324

Halliwell, B. and Gutteridge, J. M. C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Journal of Biochem*istry, **219:** 1-14.

Harborne, J. B. (1973). *Phytochemical Methods*. Chapman and Hall, London, p. 113.

Hu, F. B., Manson, J. E. and Stampfer, M. J. (2001). Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *New England Journal of Medicine*, **345**:790–7.

Janero, I. (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radical Biology and Medicine*, **515**:40.

Jendrassik. L. and Grof. L. (1938). *In-vitro* determination of total and direct bilirubin in serum. *Journal of Biochem*istry, **299**:81-88.

John, D. B., Michael, D., Curt D. F., Ronald, B. G., Barbara, V., James H. S., and Joseph L. W. (2008). Lipoprotein Management in Patients With Cardiometabolic Risk. *Journal of the American College of Cardiology*, **51**:1512-1524.

Jonathan, A. T. (2003). Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. *Nature Reviews Drug Discovery*, **2**:517-526.

Kaplan, B. and Szabo, L. L. (1979). Enzymes and the Liver Tests of Hepatic Function. In: *Clinical Chemistry. Interpretation and techniques*. Lea and Febiger. Philadelphia, pp: 203.

Kawagishi, H., Fukumoto, Y., Hatakeyama, M., He, P., Arimoto, H.and Matsuzawa, T. (2001).Liver injury suppressing compounds from avocado (*Persea americana*). *Journal of Agricultural and Food Chemistry* , **49**:2215 -21.

Kawagishi, J., Kumabe, T., Yoshimoto, T. and Yamamoto, T. (1995). Structure, organization, and transcription units of the human alpha-platelet-derived growth factor receptor gene, PDGFRA. *Genomics,***30**(2):224-32

Kirsch, J. F., Eichele, G., Ford, G. C., Vincent, M. G., Jansonius, J. N. and Gehring, H. (1984). Mechanism of action of aspartate aminotransferase proposed on the basis of its spatial structure. *Journal of Molecular Biology,* **174** (3): 497–525.

Kolawole, O. T., Kolawole, S. O., Ayankunle, A. A. and Olaniran, I. O. (2012). Metahnol Leaf extract of *Persea americana* Protects Rats against Cholesterol-Induced Hyperlipideamia. *British Journal of Medical Research*, **2**(2): 235-242.

Krieger, M. (1998). The "best" of cholesterols, the "worst" of cholesterol: a tale of two receptors. *Proceedings of national academy of science*, **95**:4077-4080.

Laboratories Fournier (1981). Why, When and How to Treat Hyperlipopoteinemia.. Fournier-Dijon 9, Rue Petitot 211OO Dijon.p.111.

Laclaustra, M., Frangi, A. F., Casasnovas, J. A., Cia, P. (2007). Association of endothelial function and vascular data with LDL-C and HDL-C in a homogenous population of middle-aged, healthy military men: Evidence for a critical role of optimal lipid levels. *International Journal of. Cardiology,* **2**:23-26.

Lecerf, J. M., and de Lorgeril, M. (2011). Dietary cholesterol: from physiology to cardiovascular risk. *British* Journal of *Nutrition,* **106** (1): 6–14.

Lee, S. I, Oh, S. H., Park, K. Y., Park, B. H., Kim, J. S. and Kim, S. D. (2009). Antihyperglycemic effects of fruits of privet (*Ligustrum obtusifolium*) in streptozotocin –induced diabetic rats fed a high fat diet. *Journalof Medicinal Food,* **12** (1): 109-117.

Libby, P. (2011).The vascular biology of atherosclerosis. In: *Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine* (Bonow, R. O, Mann, D. L., Zipes, D. P. and Libby, P., Eds). 9th ed. Philadelphia, Pa: Saunders Elsevier. p. 43.

Liu, Y., Li, P., Lu, J., Xiong, W., Oger, J., Tetzlaff, W. and Cynader, M. (2008). Bilirubin possesses powerful immunomodulatory activity and suppresses experimental autoimmune encephalomyelitis. *Journal of Immunology,* **181** (3): 1887–97.

Lodish, H., Berk, K., Kaiser, S. and Ploegh, M. (2008). *Molecular Cell Biology***.** W.H. Freeman and Company. pp 43-49.

Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. *Archives of Toxicology*, 275-287.

Lu, Q. Y., Arteaga, J. R., Zhang, Q., Huerta, S., Go, V. L, Heber, D. (2005). Inhibition of prostate cancer cell growth by an avocado extract: role of lipid-soluble bioactive substances. *Journal of Nutritional Biochemistry,***16**:23–30.

Maehly, A. and Chance, B. (1954). The assay of catalases and peroxidases. *Methods of Biochemical Analysis,* **1**: 357–424.

Mannervik, B. and Carlberg, I. (1985). Glutathione Reductase. *Methods in Enzymology*, **13**:113.

Maton, A., Roshan, L., Jean, H., Charles, W. M., Susan, J., Maryanna, Q. W., David, L., and Jill, D. W. (1993). *Human Biology and Health*. Englewood Cliffs, NJ: Prentice Hall, pp: 48-67.

Mau, J. L., Lin, H. C. and Song, S. F. (2002). Antioxidant properties of several specialty mushrooms. *Food Research International,* **35**: 519-526.

Maxam, A. M. and Gilbert, W. (1980). Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods in Enzymology,* **65** (1): 499–560.

McCord, J. M. (1998). Human disease, free radicals, and the oxidant/antioxidant balance. *Clinical Biochem*istry, **26:**351–357.

McCrindle, B. W., Ose, L. and Marais, A. D. (2003). Efficacy and safety of atorvastatin in children and adolescents with familial hypercholesterolemia or severe hyperlipidemia: a multicenter, randomized, placebo-controlled trial. *Journal of Pediatric,* **143** (1): 74–80.

McGowan, M. W., Artiss, J. D., Strandbergh, D. R. and Zak, B. A. (1983). Peroxidase-Coupled Method for the Colorimetric Determination of Serum Triglycerides. *Clinical Chem*istry, **29** (3): 538.

Miller, C. A. (2001). Update on statins and other lipid-lowering drugs. *Geriatric Nursing*, **22**:276-277.

Mukundh, N. B., Muralidharan, P. and Balamurugan, G. (2008). Antihyperlipidemic activity of *Pedalium murex (Linn)* fruits on high fat diet fed rats. *International Journal of Pharmacology*;**4**:310–3.

Muller, F. L., Lustgarten, M. S., Jang, Y., Richardson, A. and Van Remmen, H. (2007) Trends in oxidative aging theories. *Free Radical Biology and Medicine*, **43**: 477-503.

Murray, R. K., Grammer, D. K., Mayes, P. A. and Rodwell, V. W. (2000). *Harpers Biochemistry* (25th ed.). McGraw-Hill, New York. pp. 264-269.

Nayak, B. S., Raju, S. S. and Chalapathi, R. A. V. (2008). Wound healing activity of *Persea americana* (avocado) fruit: a preclinical study on rats. *Journal of Wound Care*, **17**:123-126.

Nelson, D. L. and Cox, M. M. (2000). Lehninger, *Principles of Biochemistry,* 3rd ed.. Worth Publishing: New York, pp. 341-349.

Niki, E., Shimaski, H. and Mino, M. (1994). Antioxidantism-free radical and biological defence. Gakkai Syuppan Center, Tokyo.

Nworgu, Z. A. M., Onwukaeme, D. N., Afolayan, A. J., Amaechina, F. C. and Ayinde, B. A. (2008). Preliminary Studies of blood pressure lowering effect of *Nauclea latifolia* in rats. *African Journal of Pharmacy and Pharmacology*. **2**(2): 37-41.

Obadoni, B. O. and Ochuko, P.O. (2001). Phytochemical studies and Comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Science*, **8**: 203-208.

Ofem, O. E., Eno, A. E., Imoru, J., Kanu, E., Unoh, F. and Ibu, J. O. (2007). Effect of crude aqueous leaf extract of *Viscum album* (mistletoe) in hypertensive rats. *Indian Journal of Pharmacology*, **39**(1):15-19.

Ogbonnia, S. O., Odimegwu, J. I. and Enwuru, V. N. (2008). Evaluation of hypoglycemic and hypolipidemic effects of ethanolic extracts of *Treculia africana* Decne and *Bryophyllum pinnatum* Lam. and their mixture on streptozotocin (STZ) - induced diabetic rats. *African Journal of Biotechnology*, **7**(15): 2535-2539.

Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, **95**:351-358.

Ojewole, J.A.O. and Adewole, S.O. (2007). Hypoglycemic and hypotensive effects of *Globimetula cupulata* leaf extract in rats. *Cardiovascular Journals of South Africa,* **18**(1):9-15.

Ojewole J. A. and Amabeoku, G. J. (2006). Anticovulsant effect of *Persea americana* Mill (Lauraceae) (Avocado) leaf aqueous extract in mice. *Phytothererapy Research,* **20**:696-700.

Ojewole J. A., Kamadyaapa, D. R., Gondwe, M. M., Moodley K, Musabayane, C. T. (2007). Cardiovascular effects of *Persea americana* Mill (Lauraceae) (avocado)

aqueous leaf extract in experimental animals. *Cardiovascular Journal of African,* **18**:69–76.

Okigbo, R. N. and Ajalie, A. N. (2005). Inhibition of some human pathogens with tropical plant extracts *Chromolaena odorata* and *Citrus aurantifolia* and some antibiotics. *International Journal of Molecular Medicine and Advanced Sciences*, **1** (1):34-41.

Okwu, D. E. (2001). Evaluation of the chemical composition of indigenous spices and flavouring Agents. *Global Journal of Pure and Applied Sci*ence, **7**(3): 455- 459.

Okwu, D. E. and Uchegbu, R. (2009). Isolation, characterization and antibacterial activity screening of methoxyamine tetrahydroxyanthocyanidines from *Detarium senegalense* gmelin stem bark. *African Journal of Pure and Applied Chemistry*, **3** (1): 1-5.

Olaeta, J. A., Schwartz, M., Undurraga, P. and Contreas, S. (2007). Use of Hass avocado (*Persea americana* Mill) seed as a processed product. *Proceedings of the VI World Avocado Congress, pp.* 1–8.

Olson, R. E. (1998). Discovery of the lipoproteins, their role in fat transport and their significance as risk factors. *Journal of Nutrition*. **128** (2): 439S–443S.

Ortiz, M. A., Dorantes, A. L., Gallndez, M. J. and Cardenas, S. E. (2004).Effect of a novel oil extraction method on avocado (*Persea americana* Mill) pulp microstructure. *Plant Foods for Human Nutrition*, **59**:11–14.

Osadebe, P. O. and Akabogu, I. C. (2006). Antimicrobial activity of *Loranthus micranthus* harvested from kola nut tree. *Fitoterapia,* **77**(1):54-56.

Owolabi, M. A., Jaja, S. I., Coker, H. A. (2005). Vasorelaxant action of aqueous extract of the leaves of *Persea americana* on isolated thoracic rat aorta. *Fitoterapia,* **76**:567–573.

Owolabi, M. A., Coker, H. A. B., Jaja, S. I. (2010). Bioactivity of the phytoconstituents of the leaves of *Persea americana*. *Journal of Medicinal Plants Research*, **4**(12): 1130-1135.

Ozolua, R. I., Anaka, O. N., Okpo, S. O. and Idogun, S. E. (2009). Acute and subacute toxicological assessment of the aqueous seed extract of *Persea americana Mill* (Lauraceae) in rats. *African Journal of Traditional Complement to Alternernative Medicine,* **6**(4): 573-578.

Pastore, A., Piemonte, F., Locatelli, M., Russo, A. L., Gaeta, L. M., Tozzi, G. and Federici, G. (2003). Determination of blood total, reduced, and oxidized glutathione in pediatric subjects. *Clinical Chemistry,* **47** (8): 1467–9.

Patel, D. K., Patel, K. A., Patel., U. K., Thounaoja, M. C., Jadeja, R. N., Padate, G. S., Salunke, S. P., Devkar, R. V. and Ramachandra, A. V. (2009). Assessment of lipid lowering effect of *Sida rhomboidea*. Roxb methanolic extract in experimentally induced hyperlipidemia. *Journal of Young Pharma*cists, **1**(3): 233-238.

Peterson, D. W., Shneour, E. and Peck, N. F. (1954). Effects of dietary sterols and sterol esters on plasma and liver cholesterol in the chick. *Journal of Nutrition,* **53**:451-459.

Pirone, C., Quirke, J. M. E., Priestap, H. A. and Lee, D. W. (2005). The Animal Pigment Bilirubin Discovered in Plants**.** *Journal of the American Chemical Society***, 131** (8): 2830.

Pliego, A. F. and Litz, R. E. (2007). California Avocado Society Yearbook, 90:149–67.

Pryor, W. (1991). The antioxidant nutrients and disease prevention— what, do we know and what, do we need to find out? *American Journal of Clinical Nutrition*, **6**:34-37.

Rahilly, C. (2011). Relation between high-density lipoprotein cholesterol and survival to age 85 years in men (from the VA normative aging study). *American Journal of Cardiology,* **107** (8): 1173–7.

Ritchie, H. E. (2001). The safety of herbal medicine use during pregnancy. Frontiers in Fetal Health. **3**: 259-266.

Reitman, S. and Frankel, A. S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *American Journal of Clinical Pathology*, **28**:53-82.

Rocchini, A. P. (2003). Obesity and blood pressure regulation. In: Bray G. A. and Bouchard, C., (Ed). *Handbook of Obesity*. New York: Marcel Dekker, pp 54-78.

Rocchini, A. P. (1999). Clonidine prevents insulin resistance and hypertension in obese dogs. *Hypertension*, **33**:548–53.

Rodkey, F. L. (1965). Direct spectrophotometric determination of albumin in human serum. *Clinical Chememistry*, **11**: 478-487.

Ross, I. A. (1999). *Medicinal Plants of the World – Chemical Constituents, Traditional and Modern Uses.* Totowa, New Jersey: Humana.

Salazar, M. J., El Hafidi, M., Pastelin, G., Ramírez-Ortega, M. C., Sánchez-Mendoza, M. A. (2005). Effect of an avocado oil-rich diet over an angiotensin II-induced blood pressure response. *Journal of Ethnopharmacology,* **98**:335– 338.

Se-Young, C. (2005). Multiple Receptors Coupled to Phospholipase C Gate Long-Term Depression in Visual Cortex. *The Journal of Neuroscience*, **25**(49): 11433-45.

Shewfelt, R. L. and Purvis, A. C. (1995). Toward a comprehensive model for lipid peroxidation in plant tissue disorders. *HortScience*, **20**:213–218.

Singh, I. M., Shishehbor, M. H. and Ansell, B. J. (2007). High-density lipoprotein as a therapeutic target: a systematic review. JAMA 298:786-798. The aqueous extract of leaves of *Persea americana* mill (Lauraceae). *Fitoterapia*. **73**:375-380.

Sinha, A. K. (1972). Colorimetric assay of catalase. *Analytical Biochemistry*, **47**: 389-394.

Sofowora, A. (1993). *Medicinal Plants and Traditional Medicine in Africa*, 2nd ed., Spectrum Books, Ibadan, Nigeria, pp. 26 -100.

Stein, E. A. (1987). Lipids, lipoproteins and Apolipoproteins. In:Treitz,N.W. (Ed). *Fundamentals of Clinical Chemistry*. (3rd Ed.) W . B Sauders Philadelphia, pp. 470-479.

Sternfeld B., Sidney, S., Jacobs, D. R., Sadler, M. C., Haskell, W. L. and Schreiner, P. J. (1999). Seven-year changes in physical fitness, physical activity, and lipid profile in the CARDIA study. Coronary Artery Risk Development in Young Adults. *Annals of Epidemiology,* **9**:25–33.

Stroev , E. A. and Makarova, V. G. (1989). *Laboratory Manual in Biochemistry*. MIR Publishers Moscow, pp 148-156.

Superko, H. R. and Krauss, R. M. (1994). Coronary artery disease regression. Convincing evidence for the benefit of aggressive lipoprotein management. *Circulation*, **90**: 1056-69.

Tietz, N. W. (1995). *Clinical Guide to Laboratory Tests*. 3rd Ed. WB Saunders Company. Philadelphia, pp. 518-519.

Ugochukwu, N. H., Babady, N. E., Cobourne, M. and Gasset, S. R. (2003). The effect of *Gangronema latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. *Journal of Biosciences* **28** (1): 1-5.

Ukoha, A. J. (1998). *Modern Enzymology*. FUTO Press Association with Brumine Publishers. Owerri, pp: 162-163.

Van, E. C., Lievens, J. Feng, Y., Snoeys, J. and Geest, B. A. (2009). Apoprotein A-I and lecithin cholesterol acyltransferase transfer induce cholesterol unloading. *Gene therapy*, ***16***, 757-765.

Van-Burden, T.P. and Robinson, W. C. (1981). Formation of complexes between protein and tannin acid. *The Journal of Agricultural and Food Chemistry,* 1: 77-82.

Villa, J. and Pratley, R. E. (2010). Ezetimibe/simvastatin or atorvastatin for the treatment of hypercholesterolemia in patients with the metabolic syndrome: the VYMET study. *Current Diabetes Report,* **10** (3): 173–5.

Virella-Lopes, M. F. and Virella, G. (2003). The Role of immune and Inflammatory processes in the development of macrovascular disease in diabetes. *Frontiers in Bioscience*, **8**: 750-768.

Wang, C. S. (2012). Impact of increasing alanine aminotransferase levels within normal range on incident diabetes. *Journal of the Formosan Medical Association,* **111** (4): 201-8.

Wacnic, R. G. and Alber, J. J. (1978). A comprehensive evaluation of the heparin manganese precipitation procedure for estimating high density lipoprotein cholestsrol. *The Journal of Lipid Research* **19**:65-76.

Watt, J. M. and Breyer-Brandwijk, M. G.(1962). *The Medicinal and Poisonous Plants of Southern and Eastern Africa*. E. and S. Livingstone, Ltd. London, p. 830.

Wilson, J. and Gelb, A. (2002). Free radicals, antioxidants and neurologic injury: possible relationship to cerebral protection by anesthetics. Journal of Neurosurgery and Anesthesiology, 14(1):66-79.

World Health Organization, (2010). Annual report, New York, 45-46.

Yadav, J. P., Saini, S., Kalia, A. N. and Dangi, A. S. (2008). Hypoglycemic activity of ethanolic extract of *Salvadora oleoides* in normal and alloxan – induced diabetes rats *Indian Journal of Pharmacology*, **40** (1): 23.

APPENDIX 1: Phytochemical contents (%) of the extract of the seeds of *Persea americana*

FLAVONOIDS

	I	II	III	TOTAL	MEAN	S.D
Methanolic	72.80	80.10	70.00	222.90	74.30	5.21
Petroleum ether	14.30		10.00	38.40	12.80	2.43

ALKALOIDS

	I	II	III	TOTAL	MEAN	S.D
Methanolic	5.20	6.00	5.60	16.80	5.60	0.40
Petroleum ether	8.20	7.60	7.00	22.80	7.60	0.60

SAPONINS

	I	II	III	TOTAL	MEAN	S.D
Methanolic	11.00	10.00	10.00	31.0	10.33	0.58
Petroleum ether	5.00	4.00	4.00	13.0	4.33	0.58

PHENOLS

	1	11	111	TOTAL	MEAN	S.D
Methanolic	29.00	25.00	29.50	83.50	27.83	2.466
Petroleum ether	10.00	10.50	12.00	32.50	10.83	1.041

TANNINS

	1	11	111	TOTAL	MEAN	S.D
Methanolic	19.0	20	24.2	63.2	21.07	2.76
Petroleum ether	17.6	16.8	18.4	52.8	17.60	0.80

APPENDIX 2: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on body weight of diet-induced hyperlipidemic wistar rats

	Week 0	Week 1	Week2	Week 3	Week 4
ND	191.0	211.2	218.0	219.4	231.6
HFD	188.0	195.2	219.0	230.2	239.4
HFD + A	193.4	201.0	201.6	202.2	214.8
HFD + 125M	170.8	176.4	176.6	178.8	196.0
HFD +250 M	165.6	166.6	165.6	166.8	186.4
HFD + 500M	174.8	178.0	178.0	180.2	189.5
ND + 500 P	143.2	157.6	157.8	163.8	179.8
HFD + 125 P	189.2	207.2	206.6	211.8	223.4
HFD + 250 P	160.2	170.6	171.4	177.6	188.2
HFD + 500 P	125.6	139.6	130.8	127.6	141.0
ND + 500 P	169.4	177.8	183.0	188.0	189.3

APPENDIX 3: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on total feed intake of diet-induced hyperlipidemic wistar rats

	WEEK 1	WEEK 2	WEEK 3	WEEK 4	TOTAL	MEAN	S.D
ND	18.2	12.4	11.3	11.8	53.7	13.4	3.21
HFD	14.2	13.4	13.2	13.7	54.5	13.6	0.43
HFD + A	14.3	12.0	12.6	13.3	52.2	13.1	0.99
HFD +125 M	12.1	14.5	14.4	15.3	56.3	14.1	1.38
HFD + 250M	14.7	16.6	15.9	16.6	63.8	16.0	0.90
HFD + 500M	13.8	15.9	14.0	14.2	57.9	14.5	0.96
ND + 500 M	21.4	16.2	16.7	17.2	71.5	17.9	2.39
HFD + 125 P	14.8	12.6	13.5	13.1	54.0	13.5	0.94
HFD + 250P	18.0	16.3	14.5	15.4	64.2	16.1	1.49
HFD + 500 P	24.1	20.5	18.9	19.5	83.0	20.8	2.33
ND + 500 P	19.3	15.4	15.2	13.3	63.2	15.8	2.52

APPENDIX 4: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on PCV of diet-induced hyperlipidemic wistar rats

	WEEK 1	WEEK 2	WEEK 3	WEEK 4	TOTAL	MEAN	S.D
ND	52.8	47.6	52.8	51.6	204.8	51.2	2.5
HFD	50.0	52.0	47.0	43.0	192.0	48.0	3.9
HFD + A	49.4	48.4	51.2	47.0	196.0	49.0	1.8
HFD +125 M	51.6	45.8	50.4	48.8	196.6	49.2	2.5
HFD +250 M	51.0	45.8	47.4	47.8	192.0	48.0	2.2
HFD +500 M	51.6	48.2	45.5	46.5	191.8	48.0	2.7
ND + 500 M	52.4	45.6	47.8	50.4	196.2	49.1	3.0
HFD + 125 P	53.4	45.2	50.4	51.2	200.2	50.1	3.5
HFD + 250 P	52.6	46.0	45.2	45.0	188.8	47.2	3.6
HF D+ 500 P	50.0	47.2	53.2	55.5	205.9	51.5	3.6
ND + 500 P	55.6	44.6	44.3	46.5	191.0	47.7	5.3

APPENDIX 5: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on relative weight of liver of diet-induced hyperlipidemic wistar

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	4.67	4.16	4.06	4.93	3.64	21.46	4.29	0.51
HFD	4.64	4.15	5.03	4.44	3.68	21.94	4.39	0.51
HFD + A	4.33	4.62	3.88	4.56	3.81	21.21	4.24	0.38
HFD + 125 M	4.75	3.92	4.41	3.43	4.41	20.92	4.18	0.52
HFD + 250 M	3.90	4.70	5.08	4.45	4.82	22.95	4.59	0.45
HF D+ 500 M	4.54	3.92	3.97	3.91	4.08	20.42	4.08	0.26
ND + 500 M	3.82	4.97	4.59	4.51	3.44	21.33	4.27	0.62
HFD + 125 P	3.90	3.75	3.98	3.38	3.40	18.41	3.68	0.28
HFD + 250 P	4.29	4.16	5.14	3.97	4.05	21.60	4.32	0.48
HFD + 500 P	4.75	4.87	4.42	5.44	4.87	24.35	4.87	0.37
ND + 500 P	2.36	4.87	4.93	2.83	3.75	18.74	3.75	1.16

APPENDIX 6: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on relative weight of kidney of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	1.15	1.08	1.20	1.27	1.14	5.84	1.17	0.07
HFD	1.49	1.26	1.40	1.67	1.18	6.99	1.40	0.19
HFD + A	1.28	1.33	1.21	1.39	1.41	6.62	1.32	0.08
HF D+ 125M	1.08	1.19	1.25	1.26	1.46	6.23	1.25	0.14
HFD +250 M	1.19	1.31	1.46	1.06	1.14	6.16	1.23	0.16
HFD + 500M	1.31	1.24	1.06	1.06	1.17	5.85	1.17	0.11
ND + 500 M	1.17	1.49	1.21	1.28	1.14	6.30	1.26	0.14
HFD + 125 P	1.44	1.34	1.26	1.16	1.29	6.49	1.30	0.10
HFD + 250 P	1.63	1.17	1.53	1.36	1.42	7.12	1.42	0.17
HFD+ 500 P	1.16	1.65	1.60	2.20	1.65	8.26	1.65	0.37
ND + 500 P	1.28	1.18	1.22	1.25	1.23	6.15	1.23	0.04

APPENDIX 7: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on total cholesterol of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	201.30	221.25	198.15	178.35	189.90	988.95	197.79	15.83
HFD	242.70	235.95	286.95	308.85	268.61	1343.06	268.61	30.39
HFD + A	166.80	199.05	148.80	165.75	216.15	896.55	179.31	27.47
HFD +125M	136.50	185.55	174.75	192.75	207.60	897.15	179.43	26.80
HFD +250M	150.60	152.85	171.75	137.55	156.60	769.35	153.87	12.30
HFD +500M	149.25	146.70	148.05	152.70	149.18	745.88	149.18	2.23
ND + 500 M	120.45	139.80	142.95	124.20	119.40	646.80	129.36	11.17
HFD + 125 P	162.60	161.55	181.05	169.20	127.05	801.45	160.29	20.14
HFD + 250 P	128.55	124.95	178.05	145.20	115.95	692.70	138.54	24.50
HFD + 500 P	116.55	133.35	124.05	116.40	122.59	612.94	122.59	6.94
ND + 500 P	94.95	83.70	105.75	90.45	93.71	468.56	93.71	8.02

APPENDIX 8: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on serum triacylglycerols of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	14.05	13.94	14.59	14.59	15.79	72.95	14.59	0.73
HFD	71.76	64.90	81.23	70.34	70.23	358.46	71.69	5.94
HFD + A	24.39	31.03	35.50	29.40	26.68	147.00	29.40	4.25
HF D+ 125M	30.16	38.33	39.42	17.75	34.95	160.61	32.12	8.81
HFD + 250M	12.63	12.82	10.34	20.04	16.77	72.60	14.52	3.85
HFD +500 M	41.70	32.56	39.93	45.52	39.93	199.64	39.93	4.71
ND + 500 M	39.20	44.10	32.01	31.58	21.12	168.02	33.60	8.72
HF D+ 125 P	21.67	29.51	20.80	23.25	21.02	116.24	23.25	3.63
HFD+ 250 P	32.78	12.85	21.64	20.91	20.04	108.21	21.64	7.15
HFD + 500 P	14.81	16.77	13.72	18.95	16.06	80.30	16.06	1.99
ND + 500 P	10.27	16.12	5.01	9.69	10.27	51.36	10.27	3.94

APPENDIX 9: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on HDL-Cholesterol of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	47.88	46.83	49.14	42.21	41.16	227.22	45.44	3.55
HFD	21.21	21.42	20.58	19.74	20.74	103.69	20.74	0.65
HFD + A	23.73	30.45	35.91	22.89	19.74	132.72	26.54	6.53
HFD + 125M	16.38	25.41	20.58	14.49	21.45	98.31	19.66	4.32
HFD + 250M	46.62	42.42	49.14	43.68	51.24	233.10	46.62	3.67
HFD + 500M	44.31	58.59	40.95	57.75	50.40	252.00	50.40	7.87
ND + 500 M	88.35	79.17	61.95	72.24	75.43	377.14	75.43	9.65
HFD + 125 P	38.01	33.86	32.97	30.45	34.02	169.31	33.86	2.72
HFD + 250 P	45.36	43.89	41.58	44.31	47.25	222.39	44.48	2.08
HFD + 500 P	45.57	48.51	53.76	43.05	47.72	238.61	47.72	3.99
ND + 500 P	67.41	70.14	73.71	80.01	72.82	364.09	72.82	4.72

APPENDIX 10: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on LDL-Cholesterol of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	150.33	171.86	132.95	133.02	145.87	734.03	146.81	15.99
HFD	206.98	201.53	250.26	274.94	233.43	1167.14	233.43	30.51
HFD +A	138.10	154.02	105.65	132.91	190.99	721.67	144.33	31.37
HFD + 125 M	114.21	152.29	146.07	174.55	179.23	766.36	153.27	26.01
HFD + 250 M	118.75	103.94	120.32	90.14	101.89	535.03	107.01	12.60
HFD+ 500 M	96.61	81.75	104.03	86.02	92.10	460.51	92.10	8.76
ND + 500 M	52.68	51.64	74.89	45.43	56.16	280.79	56.16	11.16
HFD + 125 P	120.16	97.01	144.03	128.11	88.77	578.07	115.61	22.64
HFD + 250 P	76.88	78.73	79.27	96.55	64.90	396.33	79.27	11.31
HFD + 500 P	68.23	81.75	67.48	69.32	71.69	358.46	71.69	5.84
ND + 500 P	18.99	10.29	12.54	8.33	12.54	62.70	12.54	4.01

APPENDIX 11: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on TC/HDL-C ratio of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	4.20	4.72	4.03	4.23	4.61	21.80	4.36	0.29
HFD	11.44	11.02	13.94	15.65	13.01	65.06	13.01	1.89
HFD + A	7.03	4.43	4.14	7.24	5.71	28.55	5.71	1.43
HFD + 125M	8.33	7.30	8.49	8.45	9.69	42.27	8.45	0.85
HFD + 250 M	5.16	3.60	3.50	3.15	3.06	18.46	3.69	0.85
HFD + 500 M	3.37	2.50	3.62	2.64	3.03	15.16	3.03	0.47
ND + 500 M	2.01	1.77	2.31	1.72	0.97	8.77	1.75	0.50
HFD + 125 P	4.28	4.77	5.49	5.56	3.73	23.83	4.77	0.78
HFD + 250 P	2.83	2.85	4.28	3.28	2.45	15.69	3.14	0.70
HFD + 500 P	2.56	2.75	2.31	2.70	2.58	12.90	2.58	0.17
ND + 500 P	1.41	1.19	1.43	1.13	1.29	6.46	1.29	0.13

APPENDIX 12: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on LDL-c/HDL-C ratio of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	3.14	3.67	2.71	3.15	3.54	16.21	3.24	0.38
HFD	9.76	9.41	12.16	13.93	5.21	50.47	10.09	3.29
HFD + A	5.82	3.43	2.94	5.81	9.68	27.67	5.53	2.67
HFD + 125 M	6.97	5.99	7.10	12.05	8.37	40.48	8.10	2.36
HFD + 250 M	4.07	2.45	2.45	2.06	1.99	13.02	2.60	0.85
HFD + 500 M	2.18	1.40	2.54	1.49	1.90	9.51	1.90	0.48
ND + 500 M	0.88	0.65	1.21	0.63	0.09	3.46	0.69	0.41
HFD + 125 P	3.16	1.66	4.37	4.21	2.61	16.01	3.20	1.13
HFD + 250 P	1.69	1.79	3.07	2.18	1.37	10.11	2.02	0.65
HFD + 500 P	1.50	1.69	1.26	1.61	1.51	7.56	1.51	0.16
ND + 500 P	0.28	0.15	0.42	0.10	0.24	1.20	0.24	0.13

APPENDIX 13: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on total phospholipids of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	3.20	5.15	4.26	3.91	4.79	21.31	4.26	0.76
HFD	5.68	5.50	5.28	5.15	6.57	28.18	5.64	0.56
HFD + A	4.26	4.44	4.79	3.37	3.73	20.60	4.12	0.57
HFD + 125 M	3.91	3.55	3.02	3.37	2.66	16.51	3.30	0.48
HFD + 250 M	3.20	3.73	3.02	4.97	3.73	18.64	3.73	0.76
HF D+ 500 M	4.79	3.20	2.84	3.73	3.64	18.20	3.64	0.74
ND + 500 M	3.20	3.73	2.84	3.02	3.91	16.69	3.34	0.46
HF D+ 125 P	3.91	4.44	5.33	3.73	4.35	21.75	4.35	0.62
HFD + 250 P	3.46	3.20	3.73	3.91	3.02	17.31	3.46	0.37
HFD + 500 P	2.49	3.20	3.02	2.31	2.75	13.76	2.75	0.37
ND + 500 P	1.60	2.49	1.95	2.66	2.18	10.88	2.18	0.42

APPENDIX 14: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on total lipids of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	6.75	6.00	5.85	5.40	5.70	29.70	5.94	0.50
HFD	13.35	10.20	18.00	11.10	12.75	65.40	13.08	3.03
HFD + A	6.00	5.40	4.80	6.60	5.40	28.20	5.64	0.68
HF D+ 125 M	5.70	6.60	5.85	6.00	6.00	30.15	6.03	0.34
HF D+ 250 M	5.55	5.10	5.33	5.40	5.25	26.63	5.33	0.17
HFD + 500 M	4.65	4.50	4.80	5.25	4.80	24.00	4.80	0.28
ND + 500 M	5.55	4.80	5.10	4.95	5.10	25.50	5.10	0.28
HFD + 125 P	6.45	5.55	7.20	4.65	6.30	30.15	6.03	0.97
HFD+ 250 P	5.10	4.95	5.14	5.10	5.40	25.69	5.14	0.16
HFD + 500 P	4.35	4.50	3.90	4.20	4.24	21.19	4.24	0.22
ND + 500 P	3.75	5.25	4.20	4.95	4.54	22.69	4.54	0.59

APPENDIX 15: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on TBARS of the liver homogenate of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	Total	Mean	S.D
ND	2.64	1.61	2.05	2.27	2.13	10.71	2.14	0.37
HFD	7.11	4.73	3.59	5.06	3.15	23.65	4.73	1.55
HFD + A	1.69	1.39	1.83	1.39	1.98	8.29	1.66	0.26
HFD + 125 M	1.91	1.34	1.03	0.59	1.83	6.69	1.34	0.55
HFD + 250 M	1.76	1.17	1.32	1.54	1.47	7.26	1.45	0.22
HFD + 500 M	1.91	2.20	2.05	1.47	1.91	9.54	1.91	0.27
ND + 500 M	1.61	1.69	1.76	1.47	1.91	8.44	1.69	0.16
HFD + 125 P	1.32	1.54	2.42	2.27	1.89	9.44	1.89	0.47
HFD + 250 P	2.79	2.13	2.93	2.62	1.98	10.47	2.62	0.35
HFD + 500 P	2.35	1.76	2.20	2.13	2.11	10.54	2.11	0.22
ND + 500 P	2.13	2.57	2.20	2.27	2.29	11.46	2.29	0.17

APPENDIX 16: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on TBARS of kidney homogenate of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	Total	Mean	S.D
ND	16.00	10.99	19.31	13.68	8.43	68.40	13.68	4.24
HFD	31.79	19.20	30.51	19.84	23.57	124.91	24.98	5.89
HFD + A	9.92	10.77	15.49	21.87	19.41	77.46	15.49	5.23
HFD + 125 M	14.40	12.69	17.49	9.71	9.17	63.46	12.69	3.44
HFD + 250 M	8.53	7.55	4.59	9.28	7.79	37.74	7.55	1.79
HFD + 500 M	7.15	8.85	10.43	9.39	8.85	44.26	8.85	1.07
ND + 500 M	7.47	7.29	3.63	10.77	6.40	29.16	7.29	2.92
HFD + 125 P	11.41	20.69	8.85	13.65	12.05	54.61	13.65	5.09
HFD + 250 P	5.12	6.61	18.77	10.67	22.19	41.17	10.29	6.12
HFD + 500 P	3.13	4.27	10.45	11.20	7.26	36.31	7.26	3.60
ND + 500 P	6.61	8.21	8.64	7.04	7.63	38.14	7.63	0.83

APPENDIX 17: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on reduced gluthathione of the liver of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	4.0	4.0	4.0	3.8	3.9	19.5	3.9	0.1
HFD	4.2	3.9	4.1	3.9	4.1	20.2	4.0	0.1
HFD + A	3.9	4.0	3.9	3.9	3.9	19.7	3.9	0.0
HFD + 125 M	3.9	3.9	3.9	3.9	4.0	19.6	3.9	0.0
HFD + 250 M	3.9	3.9	3.9	3.9	3.7	19.4	3.9	0.1
HFD + 500 M	4.0	4.0	4.0	3.9	4.0	19.9	4.0	0.0
ND + 500 M	3.8	3.8	3.8	3.9	3.9	19.3	3.9	0.0
HFD + 125 P	3.8	3.9	3.9	3.8	3.9	19.3	3.9	0.0
HFD + 250 P	3.9	4.2	4.1	4.2	4.1	20.4	4.1	0.1
HFD + 500 P	4.3	4.2	4.0	4.1	4.1	20.6	4.1	0.1
ND + 500 P	4.1	4.0	4.0	4.0	4.1	20.1	4.0	0.1

APPENDIX 18: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on reduced gluthathione of the liver of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	5.7	5.6	5.6	5.6	5.7	28.2	5.6	0.0
HFD	5.8	6.1	6.0	5.8	5.7	29.3	5.9	0.2
HFD + A	5.8	5.9	5.9	5.8	5.8	29.1	5.8	0.0
HFD + 125 M	5.9	5.8	5.7	5.8	5.8	29.1	5.8	0.1
HFD + 250 M	6.2	6.5	6.2	6.2	6.2	31.2	6.2	0.1
HFD + 500 M	6.1	5.8	6.1	8.0	6.5	32.5	6.5	0.9
ND + 500 M	6.2	6.2	6.2	6.2	6.2	31.1	6.2	0.0
HFD + 125 P	6.2	6.1	6.4	6.4	6.3	31.4	6.3	0.1
HFD+ 250P	6.2	6.2	6.2	6.1	6.2	30.9	6.2	0.0
HFD + 500 P	7.7	7.4	6.0	6.0	6.8	33.9	6.8	0.8
ND + 500 P	5.9	6.0	6.0	6.2	6.0	30.1	6.0	0.1

APPENDIX 19: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on SOD activity of the liver of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	139.46	137.50	172.86	139.46	170.89	760.18	152.04	18.14
HFD	139.46	125.71	147.32	139.46	147.32	699.29	139.86	8.83
HFD + A	141.43	166.96	141.43	147.32	174.82	771.96	154.39	15.50
HFD + 125M	110.00	123.75	106.07	102.14	121.79	563.75	112.75	9.58
HFD + 250M	119.82	106.07	104.11	125.71	166.96	622.68	124.54	25.41
HFD + 500M	145.36	172.86	161.07	141.43	155.18	775.89	155.18	12.58
ND + 500 M	190.54	200.36	206.25	200.36	190.54	988.04	197.61	6.89
HFD + 125 P	166.96	153.21	163.04	157.14	145.36	785.71	157.14	8.45
HFD + 250 P	194.46	161.07	174.82	127.68	174.82	832.86	166.57	24.78
HFD + 500 P	184.64	208.21	186.61	198.39	194.46	972.32	194.46	9.52
ND + 500 P	151.25	178.75	165.00	196.43	172.86	864.29	172.86	16.73

APPENDIX 20: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on SOD activity of the kidney of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	226.96	248.89	231.11	245.93	257.78	1210.67	242.13	12.81
HFD	162.96	242.96	254.81	237.04	242.96	1140.74	228.15	37.00
HFD+ A	54.07	53.33	47.41	41.48	74.07	270.37	54.07	12.28
HFD+ 125M	278.52	225.19	228.15	177.78	275.56	1185.19	237.04	41.64
HFD + 250M	234.07	263.70	272.59	284.44	248.89	1303.70	260.74	19.77
HFD + 500 M	287.41	248.89	257.78	362.96	289.26	1446.29	289.26	44.88
ND + 500 M	260.74	242.96	240.00	245.93	245.93	1235.56	247.11	8.01
HFD + 125P	254.81	287.41	266.67	269.63	272.59	1351.11	270.22	11.74
HFD+ 250P	266.67	284.44	278.52	269.63	231.11	1330.37	266.07	20.78
HFD + 500 P	344.44	348.15	254.81	242.96	297.59	1487.96	297.59	48.90
ND + 500 P	231.11	269.63	257.78	272.59	257.78	1288.89	257.78	16.36

APPENDIX 21: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on catalase activity of the liver of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	0.034	0.031	0.080	0.034	0.042	0.220	0.044	0.020
HFD	0.015	0.014	0.022	0.027	0.032	0.110	0.022	0.008
HFD + A	0.030	0.038	0.051	0.038	0.032	0.189	0.038	0.008
HFD +125 M	0.050	0.055	0.060	0.037	0.040	0.243	0.049	0.010
HFD + 250 M	0.056	0.070	0.032	0.026	0.027	0.212	0.042	0.020
HFD + 500 M	0.068	0.068	0.079	0.056	0.068	0.339	0.068	0.008
ND + 500 M	0.061	0.050	0.065	0.062	0.057	0.294	0.059	0.001
HFD + 125 P	0.038	0.040	0.031	0.016	0.031	0.156	0.031	0.009
HFD + 250 P	0.078	0.071	0.084	0.071	0.076	0.381	0.076	0.005
HFD + 500 P	0.055	0.057	0.072	0.049	0.058	0.291	0.058	0.008
ND + 500 P	0.071	0.064	0.052	0.056	0.056	0.279	0.056	0.015

APPENDIX 22: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on catalase activity of the kidney of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	Total	Mean	S.D
ND	0.095	0.078	0.086	0.090	0.057	0.404	0.081	0.015
HFD	0.050	0.043	0.039	0.044	0.041	0.217	0.043	0.004
HFD+ A	0.080	0.089	0.050	0.116	0.109	0.445	0.089	0.026
HFD + 125 M	0.065	0.060	0.064	0.060	0.050	0.300	0.060	0.006
HFD + 250 M	0.095	0.085	0.050	0.031	0.069	0.330	0.066	0.026
HFD + 500 M	0.081	0.047	0.119	0.098	0.086	0.430	0.086	0.026
ND + 500 M	0.099	0.103	0.107	0.117	0.070	0.496	0.099	0.018
HFD + 125 P	0.082	0.064	0.054	0.036	0.033	0.269	0.054	0.020
HFD + 250 P	0.083	0.080	0.081	0.075	0.054	0.373	0.075	0.012
HFD + 500 P	0.132	0.108	0.100	0.114	0.113	0.567	0.113	0.012
ND + 500 P	0.114	0.105	0.108	0.113	0.098	0.538	0.108	0.006

APPENDIX 23: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on AST activity of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	56.0	54.0	48.0	51.0	46.0	255.0	51.0	4.12
HFD	59.0	45.0	56.0	62.0	57.0	279.0	55.8	6.46
HFD + A	62.0	42.0	38.0	57.0	43.0	242.0	48.4	10.45
HFD + 125 M	41.0	33.0	35.0	22.0	37.0	168.0	33.6	7.13
HFD + 250 M	20.0	30.0	26.0	34.0	24.0	134.0	26.8	5.40
HFD + 500 M	37.0	36.0	34.7	31.0	34.7	173.4	34.7	2.27
ND + 500 M	36.0	44.0	47.0	40.0	37.0	204.0	40.8	4.66
HFD + 125 P	46.0	38.0	43.0	40.0	37.0	204.0	40.8	3.70
HFD +250 P	43.0	40.0	37.0	35.5	34.0	189.5	37.9	3.61
HFD + 500 P	28.5	30.5	22.0	30.0	27.8	138.8	27.8	3.40
ND + 500 P	34.0	38.0	41.0	35.0	37.0	185.0	37.0	2.74

APPENDIX 24: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds onALT activity of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	35.0	34.0	37.1	39.0	40.5	185.6	37.1	2.70
HFD	43.5	45.0	36.0	43.0	35.0	202.5	40.5	4.64
HFD + A	35.5	31.0	36.0	35.0	36.5	174.0	34.8	2.20
HFD + 125 M	22.5	27.0	30.0	26.0	26.4	131.9	26.4	2.68
HFD + 250 M	34.0	28.5	30.5	25.0	29.0	147.0	29.4	3.27
HFD + 500 M	35.0	39.0	37.0	40.0	45.5	196.5	39.3	3.96
ND + 500 M	28.5	30.0	34.0	43.0	36.0	171.5	34.3	5.72
HFD + 125 P	37.0	39.0	47.0	46.0	42.5	211.5	42.3	4.32
HFD + 250 P	38.0	37.0	43.5	32.0	35.0	185.5	37.1	4.25
HFD + 500 P	28.0	30.5	23.5	23.0	26.3	131.3	26.3	3.13
ND + 500 P	14.0	17.0	18.5	21.5	17.8	88.8	17.8	2.70

APPENDIX 25: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on ALP activity diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	256.68	223.56	157.32	209.76	220.80	1068.12	213.62	36.02
HFD	154.56	124.20	82.80	154.56	118.68	634.80	126.96	29.79
HFD + A	77.28	80.04	88.32	96.60	60.72	402.96	80.59	13.44
HFD + 125 M	181.47	138.00	201.48	160.08	226.32	907.35	181.47	34.49
HFD +2 50 M	55.20	33.12	57.96	85.56	57.96	289.80	57.96	18.62
HFD + 500 M	88.32	63.48	82.80	93.84	82.11	410.55	82.11	11.44
ND + 500 M	88.32	165.60	88.32	201.48	259.44	803.16	160.63	74.02
HFD + 125 P	115.92	121.44	138.00	160.08	121.44	656.88	131.38	18.06
HFD + 250 P	160.08	135.24	71.76	63.48	149.04	579.60	115.92	45.06
HFD + 500 P	132.48	04.88	113.16	115.92	116.61	583.05	116.61	10.02
ND + 500 P	138.00	107.64	60.72	143.52	112.47	562.35	112.47	32.85

APPENDIX 26: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on direct bilirubin of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	Total	Mean	S.D
ND	1.08	1.15	1.17	1.53	1.28	6.21	1.24	0.18
HFD	2.07	2.29	2.20	2.38	2.51	11.45	2.29	0.17
HFD +A	0.95	0.78	1.34	0.75	0.92	4.74	0.95	0.24
HFD + 125M	1.64	1.73	2.25	2.09	1.90	9.61	1.92	0.25
HFD + 250M	2.38	2.28	2.43	2.22	2.30	11.61	2.32	0.08
HFD + 500M	2.25	2.52	2.04	2.81	2.40	12.02	2.40	0.29
ND + 500 M	1.80	2.29	1.75	1.75	1.17	8.76	1.75	0.40
HFD + 125 P	1.32	1.37	1.28	1.32	1.44	6.74	1.35	0.06
HFD + 250 P	3.48	2.87	1.79	2.19	1.51	11.84	2.37	0.81
HF D+ 500 P	0.30	0.56	0.35	0.45	0.41	2.07	0.41	0.10
ND + 500 P	0.98	1.14	0.91	1.41	1.11	5.55	1.11	0.19

APPENDIX 27: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on total bilirubin of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	Total	Mean	S.D
ND	1.76	1.92	1.71	1.80	1.85	9.04	1.81	0.08
HFD	2.53		2.62	2.76	2.69	10.61	2.65	0.10
HFD +A	2.18	2.29	2.05	2.04	1.85	10.41	2.08	0.17
HFD + 125M	2.10	2.30	3.30	2.20	1.44	11.34	2.27	0.67
HFD + 250M	2.60	2.49	3.16	2.82	2.88	13.96	2.79	0.26
HFD + 500M	2.34	2.14	2.02	2.49	2.25	11.25	2.25	0.18
ND + 500 M	2.37	2.45	2.07	2.21	2.28	11.38	2.28	0.14
HFD + 125 P	1.43	1.68	1.53	1.66	1.49	7.80	1.56	0.11
HFD + 250 P	2.83	2.67	2.46	2.08	2.71	12.75	2.55	0.29
HFD + 500 P	2.13	1.95	2.10	2.35	2.13	10.66	2.13	0.14
ND + 500 P	2.17	2.10	2.04	1.89	2.05	10.25	2.05	0.10

APPENDIX 28: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on albumin of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	5.013	4.268	4.288	4.628	4.589	22.786	4.557	0.304
HFD	4.981	4.018	4.917	3.158	5.237	22.311	4.462	0.862
HFD + A	3.549	5.321	4.666	4.634	4.543	22.714	4.543	0.636
HFD + 125 M	5.295	3.286	4.230	4.724	4.069	21.605	4.321	0.751
HFD + 250 M	4.769	3.986	5.064	4.737	4.217	22.773	4.555	0.441
HFD + 500 M	5.071	5.353	4.929	4.354	4.925	24.632	4.926	0.364
ND + 500 M	3.492	3.569	5.148	5.083	3.479	20.770	4.154	0.879
HFD + 125 P	4.056	4.807	4.807	5.090	5.315	24.076	4.815	0.474
HFD + 250 P	4.422	4.397	4.454	4.454	4.596	22.323	4.465	0.077
HFD + 500 P	5.359	4.121	3.941	3.556	4.244	21.221	4.244	0.675
ND + 500 P	4.743	2.882	4.769	4.131	4.131	20.656	4.131	0.765

APPENDIX 29: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on total protein of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	6.395	6.118	8.105	6.423	7.357	34.40	6.88	0.83
HFD	7.133	6.299	6.390	6.847	6.704	33.37	6.67	0.34
HFD + A	6.647	5.873	5.818	5.308	5.723	29.37	5.87	0.49
HFD + 125 M	7.567	7.762	6.609	6.118	6.871	34.93	6.99	0.68
HFD + 250 M	3.212	4.489	3.912	4.141	3.497	19.25	3.85	0.51
HFD + 500 M	3.455	3.869	2.726	2.197	3.061	15.31	3.06	0.65
ND + 500 M	5.962	6.220	6.938	6.819	5.160	31.10	6.22	0.72
HFD + 125 P	7.520	7.290	7.676	7.743	7.371	37.60	7.52	0.19
HFD + 250 P	4.508	4.755	5.222	4.441	4.479	23.41	4.68	0.33
HF D+ 500 P	6.528	5.785	6.766	6.360	6.360	31.80	6.36	0.36
ND + 500 P	4.732	4.755	6.309	7.743	5.885	29.42	5.88	1.25

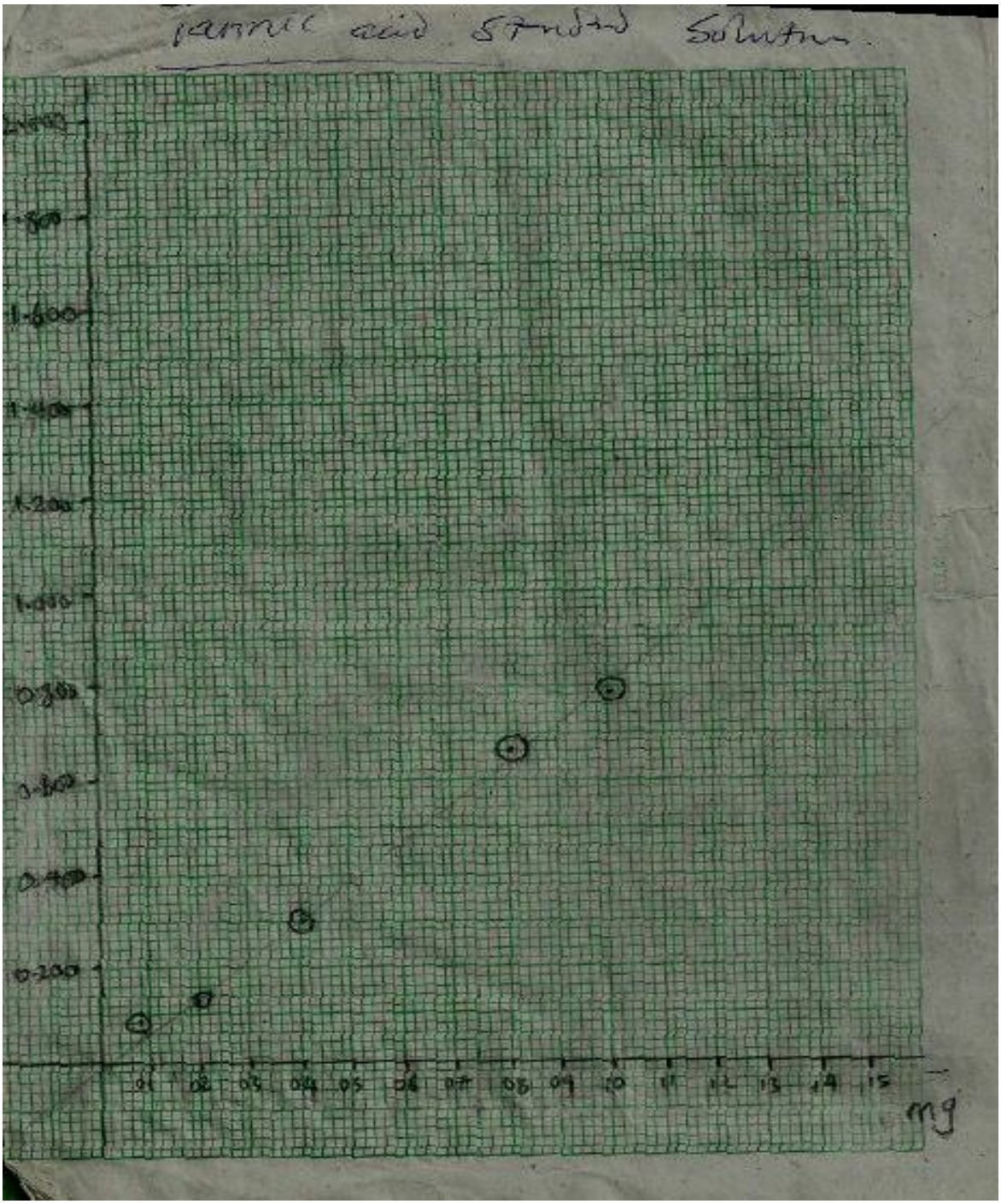
APPENDIX 30: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on urea concentration of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOAL	MEAN	S.D
ND	44.81	45.64	49.79	37.35	50.21	227.81	45.56	5.19
HFD	37.35	28.22	24.07	32.78	29.88	152.29	30.46	4.98
HFD + A	8.30	16.18	18.67	12.03	8.71	63.90	12.78	4.57
HFD + 125 M	6.22	8.40	10.79	7.47	9.13	42.01	8.40	1.72
HFD + 250 M	31.54	26.14	36.10	32.78	28.63	155.19	31.04	3.83
HFD + 500 M	27.80	24.07	27.39	22.41	25.42	127.08	25.42	2.26
ND + 500 M	24.90	21.58	22.41	31.54	23.24	123.65	24.73	4.00
HFD + 125 P	10.79	11.62	6.22	5.81	7.47	41.91	8.38	2.66
HFD + 250 P	11.20	8.30	10.37	9.13	9.75	48.76	9.75	1.12
HFD + 500 P	9.96	13.28	10.79	16.18	12.55	62.76	12.55	2.43
ND + 500 P	4.84	4.15	4.56	5.81	4.84	24.20	4.84	0.61

APPENDIX 31: Effects of petroleum ether and methanolic fractions of *Persea americana* seeds on creatinine concentration of diet-induced hyperlipidemic wistar rats

	1	2	3	4	5	TOTAL	MEAN	S.D
ND	0.261	0.319	0.406	0.406	0.406	1.796	0.359	0.067
HFD	0.579	0.695	0.550	0.608	0.811	3.245	0.649	0.106
HFD + A	1.159	1.043	0.869	0.637	1.130	4.838	0.968	0.216
HFD + 125M	0.550	0.435	0.406	0.348	0.348	2.086	0.417	0.083
HFD + 250M	0.521	0.435	0.377	0.753	0.666	2.752	0.550	0.157
HFD + 500M	0.637	0.724	0.811	0.608	0.695	3.476	0.695	0.079
ND + 500 M	0.637	0.550	0.724	0.695	0.724	2.694	0.674	0.083
HFD + 125 P	0.956	0.550	0.753	0.608	0.666	2.578	0.645	0.082
HFD + 250 P	0.435	0.492	0.608	0.521	0.435	2.491	0.498	0.072
HFD + 500 P	0.782	1.188	0.811	0.521	0.826	4.129	0.826	0.238
ND + 500 P	0.753	0.608	0.637	0.666	0.666	3.331	0.666	0.054

Appendix 32: Standard curve for tannins



Appendix 33: Standard curve for phenols