PREVENTIVE AND CURATIVE EFFECT OF AFRICAN PEACH (*Nauclea latifolia)* ROOT EXTRACTSON POLOXAMER 407 INDUCED HYPERCHOLESTEROLEMICRATS

BY

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DECLARATION

I hereby declare that this thesis entitled "**Preventive and curative effect of African peach (*Nauclea latifolia)* root extracts on poloxamer 407 induced hypercholesterolemic rats***"*** is a record of my own research work under the supervision of Mr. G. C. Njoku and Prof. E. Onyike. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project has been previously presented elsewhere for the award of a degree in any other university.

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CERTIFICATION

This thesis titled "**Preventive and curative effect of African peach (*Nauclea latifolia)* root extracts on poloxamer 407 induced hypercholesterolemic rats"** by EFFAH, SARAH meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University and is approved for its contribution to scientific knowledge and literacy presentation.

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DEDICATION

This work is dedicated to God Almighty for his provision, guidance and life. Also to my wonderful father, Chief Bassey Asido Effah and my mother, Mrs. Josephine Effah, I say thank you for your love, care, provision and prayers.

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ABSTRACT

The preventive and curative effects of African peach (*Nauclea latifolia)* root extracts (crude, ethyl acetate and n-butanol) on lipid profile, liver, kidney biochemical parameters and antioxidant effect were investigated in Poloxamer 407 induced hypercholesterolemic albino wistar rats. In the preventive study, the total cholesterol and triacylglycerol level in rats was significantly ($p < 0.05$) reduced in all treatment groups when compared with the P407 induced group,with the ethylacetate extract 500 mg/kg having highest activity. The HDL-cholesterol and LDL-cholesterol levels in P407 induced group showed significant ($p < 0.05$) increase compared to the control group. There was also significant ($p < 0.05$) difference between the P407 induced group and the aqueous and ethyl acetate extract treated groups at 100 mg/kg and at 500 mg/kg.

In the curative study, the total cholesterol and triacylglycerol level showed that ethyl acetate extract at 500 mg/kg was found to have the highest activity when compared to P407 induced group. The butanol extractsat 100mg/kg and 500mg/kg showed no significant ($p > 0.05$) difference when compared to P407 induced group. In the case of HDL-cholesterol and LDL cholesterollevel, there was significant increase ($p < 0.05$) in the P407 induced group when compared to the control group. All the treatment groups showed significant ($p < 0.05$) increase when compared to P407 induced group in the HDL-cholesterol study. The atherogenic risk predictor indices (HDL/TC, LDL/HDL) show that none of the treatment groups were at risk of atherosclerosis. During the preventive and curative studies, there was no significant ($p >0.05$) difference in the serum levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total protein and albumin of *Nauclea latifolia* treatment fractions when compared to the P407 induced control group. Based on the findings, the plant demonstrated excellent preventive and curative effects in the treatment of hypercholesterolemia.

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LIST OF ABBREVIATIONS

ALP Alkaline Phosphatase

ALT Alanine Aminotransaminase

ANOAVA Analysis of Variance

AST Aspartate Aminotransaminase

BSA Bovine Serum Albumin

CAD Coronary Artery Disease

CETP Cholesteryl Ester Transfer Protein

CO Cholesterol Oxidase

CVD Cardiovascular Disease

DBP Diastolic Blood Pressure

DPPH 2,2-diphenyl-1-picrylhydrazyl

DSBMTDiscrete Subband Multicarrier Transceiver

FDA Food and Drug Agency

FFA Free Fatty Acids

HBP High Blood Pressure

HDL-cHigh-Density Lipoprotein cholesterol

HL Hepatic Lipase

HMG-CoA 3-hydroxy-3- methylglutaryl-CoA reductase

IDL Intermediate Density Lipoprotein

LCAT Lecithin Cholesterol Acyltransferase

LDL-cLow-Density Lipoprotein cholesterol

LPL Lipoprotein Lipase

MI Myocardial Infarction

NCDs Non-Communicable Diseases

NCEP National Cholesterol Education Programme

P407Poloxamer 407

POD Peroxidase

SGOT Serum Glutamic Oxaloacetic Transaminase

SGPT Serum Glutamic Pyruvic Transaminase

SOD Superoxide Dismutase

SBP Systolic Blood Pressure

TAG Triacylglycerides

TC Total Cholesterol

TLC Therapeutic Lifestyle Changes

VLDL Very Low-Density Lipoprotein Cholesterol

WHOWorld Health Organization

CHAPTER ONE

1.0 Introduction

Cardiovascular diseases (CVD) including coronary heart disease and stroke are the leading cause of death for both men and women among all racial and ethnic groups (Smith, 2004). It accounts for nearly 50% of all deaths in the developed world (Thomas and Rich, 2007). If the current trend continues, the future will be even bleaker considering the fact that globalization and wide spread of Western diet to the developing world have been encountered with explosive increase in the rates of obesity and hypercholesterolemia in developing regions. Therefore, hypercholesterolemia and its associated cardiovascular diseases represent one of the greatest worldwide economic, social and medical challenges (Olshanky *et al.,* 2005).The elevation of serum total cholesterol and more importantly low density lipoprotein (LDL) cholesterol have been implicated as primary risk factor for cardiovascular disease (Edijala *et al*., 2005).

Hypercholesterolemia is the presence of high levels of cholesterol in the blood (Ghatak and Asantha, 1995). It is not a disease but a metabolic derangement that can lead to diseases, notably cardiovascular disease. It is closely related to the terms "hyperlipidemia" (elevated levels of lipoproteins in the blood) (Ghatak and Asthana, 1995).It has been reported by Rerkasem*et al.*,(2008) as one of the most important risk factors in the development and progression of atherosclerosis that lead to cardiovascular diseases (CVDs). Hypercholesterolemia poses a major problem to many societies as well as health professionals because of the close correlation between cardiovascular diseases and lipid abnormalities (Ramachandran *et al.,* 2003; Matos *et al.*,2005). Control of cholesterol levels through therapeutic drugs have significantly reduced the risk of developing atherosclerosis and associated cardiovascular diseases (Stacy and Egger, 2006; Ray *et al*., 2006; Khush and Waters, 2006). Notably, statins, a class of cholesterol-lowering drugs inhibiting cholesterol

synthesis, have been the most widely prescribed for treating hypercholesterolemia and reducing cardiovascular diseases (Ray *et al*., 2006; Khush and Waters, 2006). However, adverse effects associated with therapeutic drugs, such as myopathy, liver damages and potential drug-drug interaction have been reported (Parra and Reddy, 2003;Neuvonen *et al*., 2006; Trifiro, 2006; Kiortsis *et al*., 2007). Therefore, development of additional therapies for controlling cholesterol levels is justified, especially for those with better safety profile.

Medicinal plants have been used in Africa for many centuries and today almost every part of the world use herbal plants for treatment of different diseases (Adewunmi and Ojewole, 2004). Medicinal plants are the main sources of chemical substances with potential therapeutic effects. The use of medicinal plants for the treatment of many diseases is associated with folk medicine from different parts of the world. Naturally occurring compounds from plants, fungi and microbes are still used in pharmaceutical preparations in pure or crude forms. Medicinal plants are increasingly being used in most parts of the world as: analgesic and anti-inflammatory (Adeyemi*et al*., 2002), anticonvulsant (Oyewole and Amabeoku, 2006), hypoglycemic and hypocholesterolemic (Brai*et al*., 2007), wound healing (Nayak*et al.*, 2008), and reducing cancer risk (Lu *et al.*, 2005). Herbal remedies have increasingly become attractive alternatives to prevent or treat hypercholesterolemia, especially for those with cholesterol at the borderline levels. Excellent safety profile, cost effectiveness and multiple beneficial effects on improving wellbeing, have collectively contributed to the emerging trend of increasingly usage of herbal supplements. A number of studies have shown that the reduction of LDL-cholesterol with medicinal plants will reduce the incidence of cardiovascular diseases and overall death rate (Schafer *et al*., 1997;Adebayo *et al.*, 2006).

Nauclea latifolia (Smith) belongs to the family Rubiaceae, Genus *Nauclea* and Species *latifolia.* It is a straggling shrub or small tree, native to tropical Africa and Asia. It bears an

interesting flower, large red ball fruit with long projecting stamens. The red fruit is edible but not appealing. *Nauclea latifolia* is an evergreen multi-stemmed shrub or tree. It is widespread in the humid tropical rain forest zones or in the savanna wood lands of west and central Africa (Burkil, 1985). The common name of the plant is African peach .The commonly used parts of the plant include bark, fruit, roots, stem and sap. In Nigeria, *Nauclea latifolia* is called *Egbesi* [Yoruba], *uburuinu/nbitinu* [Igbo] and *tafashiya or tuwonbiri* [Hausa], *Mbom-mbog* (Efik) in the local languages. The plant fruit contains vitamins A, B_1, B_2, C and E (Nkafamiya *et al.*,2006). The wood of *Nauclea latifolia* is termite resistant and is used as live stakes in farms. All parts of the plant species are rich source of mono-terpene indole alkaloid. The fruit is a major source of food for the baboons, livestock, reptiles, birds and man. It is used for the treatment of stomach ache when the decoction of the bark and leaves are infused. It is also used in the treatment of fever, diarrhea and even as anti-parasitic drug. The sticks are used as chewing stick and a remedy against tuberculosis (Burkil, 1985; Esimore *et al.,* 2003).

Biochemical evaluation of the plant (*Nauclea latifolia*) indicates that the plant has high anti-diabetic, high anti-hypertensive and anti-abortifient properties(Gidado*et al.,* 2005;Nworgu *et al.,* 2008; Nworgu *et al.,* 2010). In African traditional medicine, *Nauclea latifolia* has neuropharmacological effects and it is also being used as herbal recipe for its cardiovascular activities. The crude extract of the roots have been shown to have anti-hypertensive effect (Nworgu *et al.,* 2008).

1.1 Statement of Research Problem

Cardiovascular disease is the leading cause of death accounting for nearly 50% of all deaths in the western world (Thomas and Rich, 2007). Due to wide spread of western diet to the developing world there has been explosive increase in the rates of obesity and hypercholesterolemia in the developing world. Control of cholesterol levels through therapeutic drugs (notably statins), have significantly reduced the risk of developing cardiovascular diseases (Stacy and Egger, 2006; Ray *et al*., 2006; Khush and Waters, 2006). However, adverse effects associated with therapeutic drugs, such as myopathy, liver damages have been reported(Neuvonen *et al*., 2006). Therefore, development of additional therapies for controlling cholesterol levels is important, especially for those with better safety profile and lower cost.

1.2 Justification of the Study

The purpose of this study is to evaluate the effect of *Nauclea latifolia*in changes in lipid profile mediated by cholesterol and fatty acid. The present study was prompted by the claim of some traditional health practitioners in parts of Nigeria that the fruits, leaves and roots of *Nauclea latifolia* are effective remedies for the management and/or control of hypertension and certain cardiac disorders. With the increasing incidence of cardiovascular diseases in the urban and rural population throughout the world, there is clear need for development of indigenous, inexpensive botanical sources of anticholesterolemic crude or purified drugs. This, therefore, necessitates the need for research into alternative medicine or therapeutic agents that retain therapeutic efficacy, and can be taken for long durations, devoid of, or with minimal side effects and with low prescription cost.This work will provide pharmacological justification (or otherwise) for the ethno medical uses of the *latifolia* roots in the management, control and/or treatment of certain cardiovascular disorders.

1.3 AIMS AND OBJECTIVES

1.3.1 General objective

This research aims at investigating thepreventive and therapeuticeffects of *Nauclea latifolia*root aqueous extract and fractions on Poloxamer 407 induced hypercholesterolemic rats.

1.3.2 Specific objectives

- To determine the effects of the aqueous extractand different solvent fractions on lipid profile.
- To determine the effects of the aqueous extract and fractions on hepatic and kidney function in the hypercholesterolemic rats.
- To determine the effects of the aqueous extract and fractions on levels of antioxidants (superoxide dismutase, catalase) and TBARS in the hypercholesterolemic rats.
- To determine the effects of the aqueous extract and fractions on body weight change and percentage organ weight of the hypercholesterolemic rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

The use of herbal medicine for the treatment of diseases and infections is as old as mankind (WHO, 1985). Medicinal plants have served as valuable starting materials for drug development in both developing and developed countries (Wong andMcIntosh, 2005; Odey *et al.,* 2012). Today, more than 80 per cent of the people living in Africa depend on medicinal plants and animal based medicines to satisfy their healthcare requirements (WHO, 2000). In developing countries like Nigeria, many people are poor and cannot afford the high cost of medication and have no alternative for primary health care. In recent times, herbal medicines have received much attention as sources of lead compounds since they are relatively safe for human use, environment friendly, cheap, easily available and affordable (Fazly-Bazzaz *et al.,*2005). The World Health Organization supports the traditional medicine provided they are proven to be efficacious and safe. The growing interest in herbal medicine demands information on the various plant preparations used in the treatment of diseases (Sarwar *et al.,* 2011). Scientific evaluation of medicinal plant is important to the discovery of novel drugs and also helps to assess toxicity risks associated with the use of herbal preparations and other conventional drugs of plant origin.

2.1 ***Nauclea latifolia***

2.1.1 *Nuclea latifolia* (Rubiaceae) is a straggling shrub found in the humid tropical rainforest zone or in savannah woodlands of West and Central Africa. It is an ever green multi-stemmed shrub that grows up to an altitude of 2m, with flowers joined to the calyces. The plant parts

used for medical preparations are leaves, bark, roots, stem and fruits. The fruits are syncarpous (Edet*et al.,* 2005). It has sweet scented flower heads. It produces red fruits that resemble a large, rather hard strawberry, with many seeds which have a pleasant taste but act as emetic if eaten in excess (Oliver, 1960). The plant is found in Nigeria, Guinea, Mali, Sierra Leone, Togo, Uganda, Benin, Cameroon, Democratic Republic of Congo, Gambia and Ghana (Lieberman, 1979). It is called African peach in English, Tafashiya in Hausa, Uburu inu or nbitinu in Igbo and Egbesi in Yoruba, *Mbom-mbog* in Akwa Ibom and Cross River States (Burkill, 1997). It is mainly prepared in the form of powder, concoction and decoction. The active constituents of the plant can be extracted by decoction, aqueous extraction, methanol extraction and can be used in powdered form. The methods of administration are internal, particularly by oral absorption and external: poultice/topical application or bathing. The stem bark of *Nauclea latifolia* is bitter in taste. It yields tannins used in dyeing fabrics (Simplice *et al.,* 2011).

2.1.2 **Taxonomical classification**

Family: *Rubiacae*

Subfamily: *Cinchonoideae*

Tribe: *Naucleeae*

Genus: *Nauclea*

Botanical name: - *Nauclea latifolia*

Common name: African peach

2.1.3 **Uses**

2.1.3.1 Traditional uses

It is used for traditional medicinal practices of the East and West African sub-regions of continental Africa where various extracts of the plant are used for the therapeutic management of malaria, hypertension (Akubue and Mittal, 1982); prolonged menstrual flow (Elujoba, 1995); cough, gonorrhoea, stomach disorders, dysentery, ulcers and liver ailments (Traore *et al.*, 2000).

Traditionally, in West and South Africa infusions and decoctions of the stem bark and leaves of the plant are used for the treatment of malaria, stomach ache, fever, diarrhea and nematodes infections in human and animals. In Kano, Nigeria, it is used as a chewing stick and as a remedy against stomach ache and tuberculosis (Deeniand Hussain, 1999).In Hong Adamawa State, concoctions, infusions and decoctions from stem bark and roots are used against jaundice, fever, stomach ache, and dysentery. The Fulani in Mubi use the leaves to de-worm their cattle. In Ivory Coast and Ghana infusions and decoctions are used to cure malaria (Benoit, 1998). The stem is taken as chewing stick to treat tooth decay. It is applied topically to wounds. The decoction is used to wash sores and is gargled for oral troubles. The bark is chewed as a mouth antiseptic, to relieve bad taste resulting from indigestion and as a vermifuge. When taken in excess, it is emetic, purgative, emmenagogic and even abortifacient (Burkill, 1997). The roots are also used to induce abortion and as a purgative (Vasileva, 1969).

2.1.3.2 Pharmacological activities

The anthelmintic efficacy of aqueous extract of stem bark of the *Nauclea latifolia* was investigated in sheep with natural acute and sub-acute parasitic gastro-enteritis due primarily to mixed nematode species. Graded doses of the extract improved haemoglobin and leucocytosis values in worm-infected sheep and significantly reduced faecal egg counts in infected animals. The percentage reduction by 1600 mg kg^{-1} of the extract was comparable to that of 5 mg kg^{-1} of albendazole (Ademola *et al*., 2007; Onyeyili *et al*.,2001).

Njamen *et al*., (2008) evaluated the *in vitro* estrogenic activity of the methanol extract of the plant using the yeast test-system. The extract yielded interesting activity and was then further investigated on alkaline Phosphatase induction in Ishikawa cells(liver cells). The results showed significant stimulatory effects at 10 and 100 mg mL^{-1}doses. *In vivo* the extract had no effect on the uterine epithelial height on ovariectomised rats, although the administration of 200 mg kg^{-1} increased vaginal epithelial height by 15.64%, confirming the estrogenic activity of the plant.

The evaluation of the neuropharmacological effects of the aqueous extract of *N. latifolia* root bark in rodents were assayed by measuring the effects on the Spontaneous Motor Activity (SMA), exploratory behaviour, pentobarbital sleeping time, apomorphine-induced stereotypic behaviour and motor coordination (rota-rod performance). The extract significantly decreased the SMA and exploratory behaviour in mice and prolonged pentobarbital sleeping time in rats dose-dependently. The intensity of apomorphine-induced stereotypy was also attenuated dose-dependently in mice, but no effect on motor coordination as determined by the performance on rota-rod was recorded. This indicates the presence of psychoactive substances in the aqueous extract of the root bark of *N. latifolia* (Amos *et al*., 2005).

2.1.3.2.1 Antihypertensive activity

Looking for possible hypotensive, cardiotropic and vasodilatory properties; Nworgu *et al.* (2008) found that the aqueous extract of *N. latifolia* was found to reduce systolic, diastolic and mean arterial pressure in normotensive and in one kidney one clip hypertensive rats in a dose dependant manner. The extract also reduced the heart rate of normotensive and hypertensive rats. The reduction in blood pressure and heart rate was not affected by prior treatment with atropine or promethazine.

Odey *et al.*, (2012) evaluated the antihypertensive properties of the root and stem barks of *Nauclea latifolia*, by assessing the serum lipid profile of hypertensive animals. Both the stem and root extracts produced a decrease in the Total Cholesterol levels in the treated groups but the effect on triacylglycerol levels of the treated groups were not significant when compared to the hypertensive control. The high density lipoprotein (HDL) levels in all the treated groups and normal control were increased compared to the hypertensive control. Also, the low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were similarly decreased in the treated groups, but these decreases were not significant. These changes in the lipid profile, especially total cholesterol and high density lipoprotein showed that the ethanolic extracts of the root and stem bark of *Nauclea latifolia* have antihypertensive properties.

2.1.3.2.2 Hypocholesterolemic activity

Omale and Haruna (2011) evaluated the crude fruit sample. Plasma cholesterol was lowered at 40, 60 and 80% and this was dose dependent. The result showed that *Nauclea latifolia* possessed hypocholesterolemic potential (85.20±0.05 mg/dl when compared with control 136.25±0.005 mg/dl).

Arise *et al.,* (2012) investigated the effects of administration of aqueous extract of *Nauclea latifolia* stem on lipid profile of rats. The results showed a significant concentration dependent decrease in total cholesterol and triglyceride and increase in high density lipoprotein cholesterol and lipoprotein cholesterol.

2.1.3.2.3 Hypoglycemic activity

Gidado *et al*.,(2005) screened *N. latifolia* for it fasting blood glucose lowering effect in normoglycaemic and streptozotocin induced diabetic wistar albino rats. The aqueous and ethanolic extracts significantly lowered the fasting blood glucose levels of the diabetic rats in a dose-dependent manner; however, the aqueous extract did not significantly lower the glucose levels of normoglycaemic rats. The hypoglycaemic and antihyperglycaemic potentials of the aqueous and ethanolic extracts were comparable to that of glibenclamide supporting the traditional use of the plant in the treatment of diabetes mellitus.

2.1.3.2.4 Wound healing activity

Aqueous extract of the root bark of *N. latifolia* was evaluated for its anti-nociceptive (analgesic), anti-inflammatory and anti-pyretic activities in mice and rats. The results showed that the extract significantly attenuated writhing episodes induced by acetic acid and increased the threshold for pain perception in the hot-plate test in mice, dose-dependently. The extract

also remarkably decreased both the acute and delayed phases of formalin-induced pain in rats and also caused a significant reduction in both yeast-induced pyrexia and egg-albumin-induced oedema in rats. These effects were produced in a dose-dependent manner (Abbah *et al*., 2010).

2.2 **Bioactive Components**

Nauclea latifolia contains diverse phytochemicals such as alkaloids, flavonoids, steroids and glycosides, cardiac glycosides, terpenes, cyanogenic glycosides, carbohydrates, anthraquinone, polyphenols, saponins, tannins, phlobatanins, resins and balsams (Trease and Evans, 2002). Early workers on the plant isolated a series of alkaloids from it. Naucleafoline, nauclechine and naufoline were isolated from the leaves. Other alkaloids isolated from the plant include naucletine, nauclefine, naucledidinal and epinaucleidinal, augustine and card-ambine (Hotellier *et al*., 1979). Naucleidal and epinaucleidal have been isolated from an antiviral preparation produced by roasting *Nauclea latifolia* fruits (Morah, 1994), furthermore, five monoterpene indole alkaloids, naucleamides A to E, were found to be present in the bark and wood of the plant. Naucleamide E was the unique monoterpene indole alkaloid possessing a pentacyclic ring system with an amino acetal bridge (Shigemori *et al*., 2003).

2.3 **Dyslipidemia**

Cholesterol and triglycerides, known as lipids, are fatty substances normally produced by the body. Dyslipidemia is an abnormal amount of lipids (e.g. cholesterol and/or fat) in the blood.

2.3.1**Classification** Dyslipidemia can be the result of a genetic predisposition, secondary causes, or a combination of both. Cholesterol and triglycerides can produce three forms of dyslipidemia: hypercholesterolemia, hypertriglyceridemia, and a combination of both. In each case, the dyslipidemia is the result of an elevation in either the number or composition of specific lipoproteins, which is an important determinant when selecting the appropriate drug therapy (Rodondi*et al.,* 1996).

2.3.2 **Types**

The most common types of dyslipidemia are high cholesterol (called hypercholesterolemia or "hyperlipoproteinemia") and high triglycerides (hypertriglyceridemia). When high cholesterol and triglycerides occur together, the condition is called "combined hyperlipidemia". Low levels of cholesterol may also occur (called "hypocholesterolemia" or "hypolipoproteinemia"), usually for genetic reasons (Fredrickson and lees, 1965).

2.4 **Hypercholesterolemia**

Hypercholesterolemia is a condition characterized by very high levels of cholesterol in the blood. Cholesterol is a soft, waxy, fat-like substance that is a natural component of all the cells of the body. Cholesterol circulates in the blood stream and is an essential molecule for the human body. Cholesterol is a molecule from which hormones and steroids are made. It is also used to maintain nerve cells. About 75 - 80% of the cholesterol that circulates in a person's bloodstream is made in that person's liver. The remainder is acquired from outside sources. Cholesterol is found in animal sources of food. It is not found in plants. High

cholesterol raises the risk of heart disease, heart attack, and stroke. People with hypercholesterolemia have a high risk of developing a form of heart disease called coronary artery disease. This condition occurs when excess cholesterol in the bloodstream is deposited in the walls of blood vessels, particularly in the arteries that supply blood to the heart (coronary arteries). The abnormal buildup of cholesterol forms clumps (plaque) that narrow and harden artery walls. As the clumps get bigger, they can clog the arteries and restrict the flow of blood to the heart. The buildup of plaque in coronary arteries causes a form of chest pain called angina and greatly increases a person's risk of having a heart attack (National Cholesterol Education Program, 2001).

2.4.1 **Classification of hypercholesterolemia**

Classically, hypercholesterolemia was categorized by lipoprotein electrophoresis and the Fredrickson classification. They are Familial hypercholesterolemia, polygenic hypercholesterolemia.

2.4.1.1 Familial Hypercholesterolemia

In 1938, the Norwegian scientist Dr Carl Muller recognized the link between elevated serum cholesterol levels, tendon xanthomas, and the lesions in coronary arteries in patients with FH (Familial Hypercholesterolemia) (Muller, 1938). In the early 1970s and 1980s, the genetic cause for FH was described by Dr Joseph L. Goldstein and Dr Michael S. Brown of Dallas, Texas. Initially, they found increased activity of HMG-CoA reductase, but studies showed that this did not explain the very abnormal cholesterol levels in FH patients (Goldstein and Brown, 1973). The focus shifted to the binding of LDL to its receptor, and effects of impaired binding on metabolism; this proved to be the underlying mechanism for FH (Goldstein and Brown, 1973). Subsequently numerous mutations in the protein were directly identified by

sequencing.

Familial hypercholesterolemia (FH) is an autosomal dominant disorder of clearance of LDL cholesterol and is associated with premature coronary artery disease. Its clinical features include hypercholesterolemia, tendon xanthomata, arcus, and premature atherosclerosis. It is a genetic disorder characterized by high cholesterol levels, specifically very high levels of low-density lipoprotein (LDL, "bad cholesterol"), in the blood and early cardiovascular disease. Many patients have mutations in the LDLR gene that encodes the LDL receptor protein, which normally removes LDL from the circulation, or apolipoprotein B (ApoB), which is the part of LDL that binds with the receptor; mutations in other genes are rare. Patients who have one abnormal copy (heterozygous) of the LDLR gene may have premature cardiovascular disease at the age of 30 to 40. Having two abnormal copies (being homozygous) may cause severe cardiovascular disease in childhood. Heterozygous FH is a common genetic disorder, inherited in an autosomal dominant pattern, occurring in 1:500 people in most countries; homozygous FH is much rarer, occurring in 1 in a million births (Rader*et al.*, 2003). The LDL cholesterol normally circulates in the body for 2.5 days, and subsequently binds to the LDL receptor on the liver cells, undergoes endocytosis, and is digested. LDL is removed, and synthesis of cholesterol by the liver is suppressed in the HMG-CoA reductase pathway(Goldstein and Brown, 1973). In FH, LDL receptor function is reduced or absent, and LDL circulates for an average duration of 4.5 days, resulting in significantly increased level of LDL cholesterol in the blood with normal levels of other lipoproteins (Durrington, 2003). In mutations of ApoB, reduced binding of LDL particles to the receptor causes the increased level of LDL cholesterol. It is not known how the mutation causes LDL receptor dysfunction in mutations of proprotein convertase subtilisin/kexin type 9 and autosomal recessive hypercholesterolemia (PCSK9 and ARH) (Rader*et al.*, 2003).

Although atherosclerosis occurs to a certain degree in all people, FH patients may develop

accelerated atherosclerosis due to the excess level of LDL. The degree of atherosclerosis approximately depends on the number of LDL receptors still expressed and the functionality of these receptors. In many heterozygous forms of FH, the receptor function is only mildly impaired, and LDL levels will remain relatively low. In the more serious homozygous forms, the receptor is not expressed at all (Rader*et al.,* 2003). Some studies of FH cohorts suggest that additional risk factors are generally at play when an FH patient develops atherosclerosis (Scientific Steering Committee on behalf of the Simon Broome Register Group, 1991). In addition to the classic risk factors such as smoking, high blood pressure, and diabetes, genetic studies have shown that a common abnormality in the prothrombingene (G20210A) increases the risk of cardiovascular events in patients with FH (Jansen *et al.,* 2005). Several studies found that a high level of lipoprotein (a) was an additional risk factor for ischemic heart disease (Wiklund *et al.,* 1990). The risk was also found to be higher in patients with a specific genotype of the angiotensin-converting enzyme (ACE) (O'Malley *et al.,* 1998).

Signs and Symptoms of Familial Hypercholesterolemia

Physical signs

High cholesterol levels normally do not cause any symptoms. Cholesterol may be deposited in various places in the body that are visible from the outside, such as in yellowish patches around the eyelids (xanthelasma palpebrarum), the outer margin of the iris (arcus senilis corneae) and in the form of lumps in the tendons of the hands, elbows, knees and feet, particularly the Achilles tendon (tendon xanthoma)(Rader*et al.,* 2003).

Accelerated deposition of cholesterol in the walls of arteries leads to atherosclerosis, the underlying cause of cardiovascular disease. The most common problem in FH is the development ofcoronary artery disease (atherosclerosis of the coronary arteries that supply

the heart) at a much younger age than would be expected in the general population. This may lead to angina pectoris(chest pain or tightness on exertion) or heart attacks. Less commonly, arteries of the brain are affected; this may lead to transient ischemic attacks (brief episodes of weakness on one side of the body or inability to talk) or occasionally stroke. Peripheral artery occlusive disease (obstruction of the arteries of the legs) occurs mainly in people with FH who smoke; this can cause pain in the calf muscles during walking that resolves with rest (intermittent claudication) and problems due to a decreased blood supply to the feet (such as gangrene) (Durrington, 2003). Atherosclerosis risk is increased further with age and in those who smoke, have diabetes, high blood pressure and a family history of cardiovascular disease(Rader*et al.,* 2003).

Diagnosis

Lipid measurements

Cholesterol levels may be determined as part of health screening for health insurance or occupational health, when the external physical signs such as xanthelasma, xanthoma, arcus are noticed, symptoms of cardiovascular disease develop, or a family member has been found to have FH. A pattern compatible with hyperlipoproteinemia type IIa on the Fredrickson classification is typically found: raised level of total cholesterol, markedly raised level of low-density lipoprotein (LDL), normal level of high-density lipoprotein (HDL), and normal level of triglycerides. The LDL is typically above the 75th percentile, that is, 75% of the healthy population would have a lower LDL level(Rader*et al.,* 2003). Cholesterol levels can be drastically higher in FH patients who are also obese (Durrington, 2003).

Mutation analysis

On the basis of the isolated high LDL and clinical criteria (which differ by country), genetic testing for LDL receptor mutations and ApoB mutations can be performed. Mutations are detected in between 50 and 80% of cases; those without a mutation often have higher triglyceride levels and may in fact have other causes for their high cholesterol, such as combined hyperlipidemia due tometabolic syndrome (Durrington, 2003).

Differential diagnosis

Familial hypercholesterolemia needs to be distinguished from familial combined hyperlipidemia and polygenic hypercholesterolemia. Lipid levels and the presence of xanthomata can confirm the diagnosis. Sitosterolemiaand cerebrotendineous xanthomatosis are two rare conditions that can also present premature atherosclerosis and xanthomas. The latter condition can also involve neurological or psychiatric manifestations, cataracts, diarrhea and skeletal abnormalities (Durrington , 2003).

Treatment

Heterozygous FH

Familial hypercholesterolemia is usually treated with statins. Statins act by inhibiting the enzyme hydroxymethylglutaryl CoA reductase (HMG-CoA-reductase) in the liver. In response, the liver produces more LDL receptors, which remove circulating LDL from the blood. Statins effectively lower cholesterol and LDL levels, although sometimes add-on therapy with other drugs is required, such as bile acid sequestrants (cholestyramine or colestipol), nicotinic acid preparations or fibrates(Rader*et al.*, 2003). Control of other risk factors for cardiovascular disease is required, as risk remains somewhat elevated even when cholesterol levels are controlled. Professional guidelines recommend that the decision to treat an FH patient with statins should not be based on the

usual risk prediction tools (such as those derived from the Framingham Heart Study), as they are likely to underestimate the risk of cardiovascular disease; unlike the rest of the population, FH have had high levels of cholesterol since birth, probably increasing their relative risk (National Institute for Health and Clinical Excellence, 2008). Prior to the introduction of the statins, clofibrate (an older fibrate that often caused gallstones), probucol (especially in large xanthomas) andthyroxine were used to reduce LDL cholesterol levels.More controversial is the addition of ezetimibe, which inhibits cholesterol absorption in the gut. While it reduces LDL cholesterol, it does not appear to improve a marker of atherosclerosis called the intima-media thickness. Whether this means that ezetimibe is of no overall benefit in FH is unknown(Kasteleine*t al.,* 2008).

The latest research published in 2008 based on the largest cohort of patients with heterozygous familial hypercholesterolaemia with over 44,000 personof working age, suggested that treatment of FH with statins led to a 48% reduction in death from coronary heart disease, to a point where people with FH are no more likely to die of coronary heart disease than the general population. However if the patient already had coronary heart disease the reduction was 25%. The results emphasize the importance of early identification of FH and treatment with statins (Scientific Steering Committee on behalf of the Simon Broome Register Group, 1991).

Homozygous FH

Homozygous FH is harder to treat. The LDL receptors are minimally functional, if at all. Only high doses of statins, often in combination with other medications, are modestly effective in improving lipid levels(Maraise*t al*., 2002).If medical therapy is not successful at reducing cholesterol levels, LDL apheresis may be used; this filters LDL from the bloodstream in a process reminiscent of dialysis(Rader*et al.,* 2003). Very severe cases may be considered for a liver transplant; this provides a liver with normally functional LDL

receptors, and leads to rapid improvement of the cholesterol levels, but at the risk of complications from any solid organ transplant (such as rejection, infections, or side-effects of the medication required to suppress rejection). Other surgical techniques include partial ileal bypass surgery, in which part of the small bowel is bypassed to decrease the absorption of nutrients and hence cholesterol, and portacaval shunt surgery, in which the portal vein is connected to the vena cava to allow blood with nutrients from the intestine to bypass the liver(López*et al.,* 2000). Lomitapide drug which is an inhibitor of the microsomal triglyceride transfer protein was approved by the United States of America Food and Drug Administration (FDA) in December 2012, as an orphan drug for the treatment of homozygous familial hypercholesterolemia(Cuchel*et al.,* 2007). In January 2013, The US FDA also approved mipomersen, which inhibits the action of the gene apolipoprotein B, for the treatment of homozygous familial hypercholesterolemia (Pollack, 2013). Other treatment options under investigation include infusion of recombinant human apolipoprotein A1(Eriksson*et al.*,1999). Gene therapy is a possible future alternative(Grossman*et al.*,1995).

In children

Given that FH is present from birth and atherosclerotic changes may begin early in life(Mabuchi*et al.*,1989).It is sometimes necessary to treat adolescents or even teenagers with agents that were originally developed for adults. Due to safety concerns, many doctors prefer to use bile acid sequestrants and fenofibrate as these are licensed in children (Greene and Durrington, 2004). Nevertheless, statins seem safe and effective(Rodenburg*et al.*,2004)and in older children may be used as in adults (Greene and Durrington, 2004). A multidisciplinary expert panel in 2006 advised on early combination therapy with LDL apheresis, statins and cholesterol absorption inhibitors in children with homozygous FH at the highest risk(Kavey*et al.*,2006).

2.4.1.2 **Polygenic Hypercholesterolemia**

Polygenic hypercholesterolemia is the most common cause of elevated serum cholesterol concentrations. Low-density lipoprotein cholesterol (LDL-C) elevations are moderate (140-300 mg/dL) with serum triglyceride concentrations within the reference range.This condition is caused by a susceptible genotype aggravated by one or more factors, including atherogenic diet (excessive intake of saturated fat, trans fat, and, to a lesser extent, cholesterol), obesity, and sedentary lifestyle. The involved genes have yet to be discovered. Polygenic hypercholesterolemia is associated with an increased risk for coronary heart disease (CHD) (National Cholesterol Education Program, 1993).

Pathophysiology

Low-density lipoprotein (LDL) particles are the major plasma carriers of cholesterol. Therefore, in patients with normal or minimally elevated triglyceride levels and average high-density cholesterol levels (HDL-C), the total serum cholesterol measurement can be used as a surrogate for the LDL-C level. Hypertriglyceridemia is caused by excessive numbers of very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and/or chylomicron particles; and in this situation the total cholesterol level is not a reflection of the LDL-C level. Elevated LDL-C concentrations may be the consequence of elevated LDL production or decreased LDL hepatic uptake. Diets high in saturated fat, trans fat, and cholesterol cause a reduction in LDL receptors in the liver, thus retarding LDL catabolism (Weghuber and Widhalm, 2008).Some patients with mixed dyslipidemias (elevations of both LDL-C and triglycerides) may have polygenic hypercholesterolemia along with some other condition such as insulin resistance or obesity that causes high triglyceride values.

2.4.2**Causes of Hypercholesterolemia**

Both hereditary and environmental factors affect the cholesterol level.Cholesterol levels can run in families. If the inherited cholesterol levels are very high, this is called familial hypercholesterolaemia (FH) (Bhatnagar *et al*., 2008).

Secondary causes

There are a number of secondary causes for high cholesterol:

- Diabetes mellitus and metabolic syndrome
- Kidney disease (nephrotic syndrome)
- Hypothyroidism
- Cushing's syndrome
- Anorexia nervosa
- Sleep deprivation
- Zieve's syndrome
- Family history
- Antiretroviral drugs, like protease inhibitors and nucleoside reverse transcriptase inhibitors.
- Diet
- Body weight
- Physical activity(Bhatnagar *et al.,* 2008).

2.4.3 **Signs and Symptoms**

Although hypercholesterolemia itself is asymptomatic, longstanding elevation of serum cholesterol can lead to atherosclerosis (Bhatnagar *et al.,* 2008). Over a period of decades, chronically elevated serum cholesterol contributes to formation of atheromatous plaques in the arteries. This can lead to progressive stenosis(narrowing) or

evencomplete occlusion (blockage) of the involved arteries. Alternatively smaller plaques may rupture and cause a clot to form and obstruct blood flow (Finn *et al.*, 2010). A sudden occlusion of a coronary artery results in a myocardial infarction or heart attack. An occlusion of an artery supplying the brain can cause a stroke. If the development of the stenosis or occlusion is gradual blood supply to the tissues and organs slowly diminishes until organ function becomes impaired. At this point that tissue ischemia (restriction in blood supply) may manifest as specific symptoms. For example, temporary ischemia of the brain (commonly referred to as a transient ischemic attack) may manifest as temporary loss of vision, dizziness and impairment of balance, aphasia (difficulty speaking), paresis (weakness) and paresthesia (numbness or tingling), usually on one side of the body. Insufficient blood supply to the heart may manifest as chest pain, and ischemia of the eye may manifest as transient visual loss in one eye. Insufficient blood supply to the legs may manifest as calf pain when walking, while in the intestines it may present as abdominal pain after eating a meal (Grundy *et al.,* 1998). Some types of hypercholesterolemia lead to specific physical findings. For example, familial hypercholesterolemia (Type IIa hyperlipoproteinemia) may be associated with xanthelasma palpebrarum (yellowish patches underneath the skin around the eyelids) (Shields and Shields, 2008) arcus senilis (white or gray discoloration of the peripheralcornea), and xanthomata (deposition of yellowish cholesterol-rich material) of the tendons, especially of the fingers. Type III hyperlipidemia may be associated with xanthomata of the palms, knees and elbows (James and Berger, 2006).

2.4.4 **Treatment of Hypercholesterolemia**

Lifestyle

A number of lifestyle changes are recommended in those with high cholesterol including: smoking cessation, limiting alcohol consumption, increasing physical activity, maintaining a

healthy weight, and a diet low in saturated fats, trans fat-free and low cholesterol foods (Tang*et al.,* 1998). In strictly controlled surroundings, a diet can reduce cholesterol levels by 15%. In practice, dietary advice can provide a modest decrease in cholesterol levels and may be sufficient in the treatment of mildly elevated cholesterol (Tang*et al.,* 1998).

Medication

Statins (or HMG-CoA reductase inhibitors) are commonly used to treat hypercholesterolemia if diet is ineffective. Other agents that may be used include: fibrates, nicotinic acid andcholestyramine. These however are only recommended if statins are not tolerated. Statins can reduce total cholesterol by approximately 50% in the majority of people; effects appear similar regardless of the statin used (Tonelli*et al.,* 2011). While statins are effective in decreasing mortality in those who have had previous cardiovascular disease, there is debate over whether or not they are effective in those with high cholesterol but no other health problems.With respect to quality of life there is limited evidence of improvement when statins are used in people without existing cardiovascular disease (i.e. for primary prevention) (Taylor*et al.,* 2011). Statins decrease cholesterol in children with hypercholesterolemia but no studies as of 2010 show improved clinical outcomes and diet is the mainstay of therapy in childhood (Reiner*et al*., 2011).

2.4.5 **Diagnosis**

Cholesterol is measured as milligrams per deciliter (mg/dL) of blood in the United States and some other countries. In the United Kingdom, most European countries,Canada, millimoles per liter of blood (mmol/L) is the measure (High Cholesterol – Tests and Diagnosis; 2013). In Nigeria system internationale (SI) unit for measuring cholesterol is millimoles per liter of blood (mmol/L) (Erasmus *et al.,* 1994).

For healthy adults, the UK National Health Service recommends total cholesterol of 5mmol/L or less, and low-density lipoprotein cholesterol (LDL) of 3mmol/L or less. For people at high risk of cardiovascular disease, the recommendation for total cholesterol is 4mmol/L or less, and 2mmol/L or less for LDL (Diagnosing High Cholesterol, NHS Choices; 2013).In the United States, the National Heart, Lung and Blood Institute within the National Institutes of Health classifies total cholesterol of less than 200 mg/dL as "desirable," 200 to 239 mg/dL as "borderline high," and 240 mg/dL or more as "high (National Cholesterol Education Progam, 2013).There is noabsolute cutoff between normal and abnormal cholesterol levels and interpretation of values needs to be made in relation to other health conditions and risk factors.Higher levels of total cholesterol increase the risk of cardiovascular disease, particularly coronary heart disease. Levels of LDL or non-HDL cholesterol both predict future coronary heart disease(Reiner*et al.*,2011). High levels of small dense LDL may be particularly adverse, although measurement of small dense LDL is not advocated for risk prediction (Reiner*et al.,* 2011). In the past LDL and VLDL levels were rarely measured directly due to cost. Levels of fasting triglycerides were taken as an indicator of VLDL levels (generally about 45% of fasting triglycerides is composed of VLDL), while LDL was usually estimated by the Friedewald formula:

LDL =Total cholesterol - HDL - (0.2 × fasting triglycerides).

However, this equation is not valid on non-fasting blood samples or if fasting triglycerides are elevated >4.5 Mmol/L (>∼400 mg/dL). Recent guidelines have therefore advocated the use of direct methods for measurement of LDL wherever possible. It may be useful to measure all lipoprotein subfractions (VLDL, IDL, LDL, HDL) when assessing

hypercholesterolemia and measurement ofapolipoproteins and lipoprotein (a) can also be of value. Genetic screening is now advised if a form of familial hypercholesterolemia is suspected (Reiner*et al.*, 2011).

2.5 **Hypercholesterolemia and Cardiovascular Diseases**

Link between Cholesterol and Coronary heart disease (CHD)

The processes by which lipids and lipoproteins participate in atherosclerotic plaque formation and CHD events continue to be an area of controversy and research. One of the initiating events of atherosclerotic plaque formation appears to be the entrance of lipoproteins LDL and lipoprotein(a) into the subendothelial space with their oxidatively modified free radicals produced by smooth muscle cells, activated macrophages, and endothelial cells. These oxidatively modified lipoproteins enter macrophages through a scavenger receptor pathway, ultimately yielding lipid-rich foam cells. Circulating monocytes are also attracted to smooth muscle and endothelial cells by chemoattractant (chemical substance that influences the migration of cells) that is augmented by the oxidatively modified lipoproteins (Isreal *et al.*, 1995). As the macrophage scavenger receptor continues to uptake oxidatively modified lipoproteins, foam cells continue to form and progress to the next level of atherogenesis, which is the formation of the fatty streak. At the same time, smooth muscle cells migrate into the subendothelial space and begin proliferating within the intima, contributing to the overall atherogenic process. As the process continues, lesions continue to grow by increased smooth

muscle cell proliferation and collagen synthesis. At this point, necrosis of the foam cell and formation of an extracellular lipid core occurs, as long as plasma LDL levels are elevated. The final phase appears to involve an autoimmune inflammatory response that causes T lymphocyte infiltration of the adventitia (the outermost connective tissue covering of a vessel). This inflammatory response appears to complete the process of plaque formation that is the underlying culprit in CHD (Isreal *et al.,* 1995).

2.6 **Atherosclerosis**

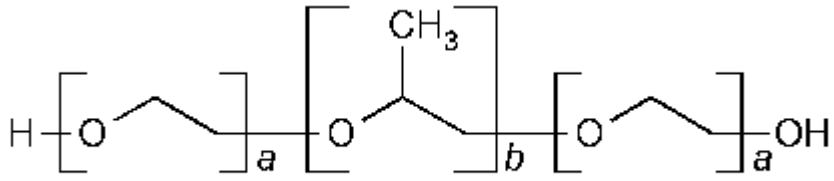
Theories of Atherogenesis

Arteries are blood vessels that carry oxygenated blood from the heart to all tissues of the body. The arterial wall is composed of three layers, namely the intima (inner lining), media and adventitia. A single layer of endothelial cells line the inner surface of the intima, forming a barrier to blood cells and plasma flowing within the blood vessel. Atherosclerosis is characterized by lesions in the intima of arteries, seen as raised fibrous plagues ranging in color from pearly gray to yellowish gray. The cellular components of the plaque include a cell similar to the adjacent endothelial cell, macrophages, fibrinogen from which fibrin is formed and white blood cells interspersed between dense connective tissues which consist majorly of collagen fibers. The cells within and around the plaque are usually lipid ladened.

Atherosclerosis poses a high risk not just because it can close up an artery, slowing down or entirely restricting blood flow, but may also lead to thrombus formation. A thrombus is a complex aggregation of platelets, red and white blood cells in a fibrin network. Several theories have emanated, suggesting the actual pathogenesis of atherosclerosis. Schoenhagen (2006) documented the different theories that have been postulated in the course of history. In 1851, a scientist, Rokitansky suggested the encrustation theory or thrombogenesis in which it

is said that atherosclerosis began in the intima of arteries with the deposition of thrombus. This is followed by the organisation of the thrombus through infiltration of fibroblast, secondarily followed by deposition of lipid. The German pathologist, Rudolf Virchow postulated the insudation or inflammation theory in 1856, a different initiation of atherosclerosis. It was suggested that infiltration of fatty substances from the blood stream into the arterial wall leads to deposition of cholesterol which acts as an irritant, causing inflammation and the proliferation of cells. The cholesterol deposits act as irritant in the arterial intima, initiating inflammatory process as macrophages are incriminated as key role players in the phases of the disease. This theory was further supported by the work of N.N. Anitschkow in 1933 where he discovered that a disease resembling human atherosclerosis could be reproduced in rabbits with high serum cholesterol or LDL levels. He thus stated that this occurrence may be as a result of defects in metabolism of lipids (Schoenhagen, 2006). All the theories above were established based on three methods. Atherosclerotic plaques from autopsy findings from individuals of both sexes, various ages and race with different diseases which included hyperlipidaemia, diabetes and hypertension were considered. Epidemiological studies of factors which promote or prevent development of atherosclerosis, and finally experimental pathology which established the sequence of lesion development or regression were considered. None of these theories entirely explains the pathogenesis of atherosclerosis, but each has explained an aspect of this process.

2.7 **Poloxamer 407**



$HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_aH$

Figure 2.1: Structure of Poloxamer 407

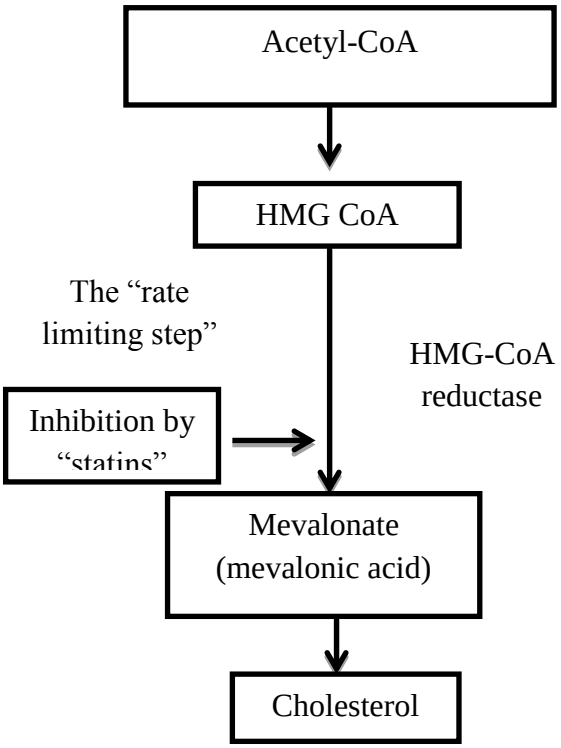
Poloxamer 407 is a hydrophilic non-ionic surfactant of the more general class of copolymers known as poloxamers. Poloxamer 407 is a triblock copolymer consisting of a central hydrophobic block of polypropylene glycol flanked by two hydrophilic blocks of polyethylene glycol. The approximate length of the two PEG blocks is 101 repeated units while the approximate length of the propylene glycol block is 56 repeated units (Tania, 2007). This particular compound is also known bythe BASF (Baden Aniline and Soda Factory) trade name Pluronic F127.

Poloxamer 407 (Pluronic RF-127) has been used to induce hyperlipidemia and hypercholesterolemia in rats, mice and rabbits. P407 is a biocompatible, non-ionic surfactant, considered non-toxic and safe during chronic administration for long term studies (Megalli *et al*., 2005). In rats and mice, the elevations in plasma triacylglycerols appear to be more sensitive to the effects of P-407 when compared to plasma cholesterol. However to date the mechanism by which P-407 modifies plasma lipid concentrations remains unknown (Zjumira *et al.* 1992). The accumulation of plasma triacyglycerols following the administration of P-407 has been suggested to be due to inhibition of capillary-bound lipoprotein lipase (LPL) activity (Johnston and Palmer, 1993). Other studies have indicated that in rats, the increase in plasma cholesterol following P-407 administration,may be due to inhibition of cholesterol

7α-hydroxylase, but not stimulation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (Johnston *et al.* 2001).

2.8 **Statins (Atorvastatin Lopitor)**

The statins, or HMG-CoA reductase inhibitors, have taken a major role in the management of dyslipidemia, especially in the treatment of elevated LDL cholesterol. More specifically, this family of agents is considered first line for the treatment of hypercholesterolemia in patients who have failed to adequately respond to dietary therapy (Stein, 1998). There are currently six FDA-approved HMG-CoA reductase inhibitors marketed in the United Statesatorvastatin (Lipitor), fluvastatin (Lescol), lovastatin (Mevacor),pitavastatin (Livalo), p ravastatin (Pravachol)and simvastatin (Zocor) (Sweetman and Sean, 2009). They all work by inhibiting HMG-CoA reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate, the rate-limiting step in the biosynthesis of cholesterol.



HMG CoA = 3-hydroxy-3-methylglutaryl coenzyme A. Adapted with permission from from the American college of Clinical pharmacy Kansus City, MO(Isreal and Mc Kenny, 1995).

Figure 2.2: Biosynthetic Pathway for Cholesterol, the rate limiting-enzyme is HMG-COA Reductase, which is Inhibited by the Statins.

After administration, the HMG-CoA reductase inhibitors concentrate in the liver, the major site of cholesterol synthesis. All of the available HMG-CoA reductase inhibitors have the ability to decrease LDL and triglyceride levels, while increasing HDL cholesterol. Their main differences lie in their pharmacokinetic profiles, the amount of lipoprotein alterations, and cost. The statins are well tolerated, and there does not appear to be major differences in toxicity or adverse-effect profiles (Sever *et al.*,2003).The HMG-CoA reductase inhibitors are currently used as adjuncts to diet therapy and in combination with other lipid-altering agents. They are also important in the treatment of dyslipidemia in patients with other comorbid conditions, such as diabetes. Experts have defined the ideal HMG-CoA reductase inhibitor as one that is well absorbed, reaches the liver unchanged, undergoes complete hepatic transformation, and is excreted via the hepatobiliary system. In addition, their once-daily dosing intervals, high efficacy, and tolerability with a low potential for drug interactions are other important criteria (Farnier and Davignon, 1998). Ofthe six statins currently available, Atorvastatin (Lipitor), Fluvastatin (Lescol), Lovastatin (Mevacor), Pitavastatin (Livalo), Pravastatin (Pravahol) and Simvastatin (Zocor), slight differences among them help determine the selection of the most appropriate statin for each patient.

2.8.1 **Mechanism of Action**

The HMG-CoA reductase inhibitors are oral agents that competitively inhibit HMG-CoA reductase, the catalytic enzyme in the conversion of HMG-CoA to mevalonic acid in the rate-

limiting step of cholesterol biosynthesis (Israel and McKenney, 1995). This inhibition leads to the up-regulation of LDL receptors in the liver, enhancing LDL clearance from the plasma (Grundy, 1988). In addition, these agents may also reduce LDL production by decreasing the hepatic production of VLDL, and increasing the catabolism of VLDL remnants in the plasma (Slater and MacDonald,1988). In addition to their inhibitory mechanism in the biosynthesis of cholesterol, numerous clinical trials have demonstrated possible mechanisms for their beneficial effects on coronary events that involve endothelial function, plaque stabilization, and thrombogenicity. Under normal circumstances, the endothelium regulates vasomotor tone, thrombosis, fibrinolysis, inhibition of platelet activity, and inflammatory cell responses, all mediated through endothelium-derived relaxing factor. However, this regulatory process is impaired in patients with CHD. Various clinical trials that tested the effects of lovastatin, pravastatin, and simvastatin have indicated that a reduction of cholesterol with the statins improves this impaired endothelial function (O'Driscoll*et al.,* 1997). Furthermore, by improving this endothelial dysfunction, lovastatin was found to reduce myocardial ischemia in patients conducting their activities of daily living (Andrews *et al.,* 1997). Similarly, the effects of statins on plaque stabilization are currently being studied (LaRosa*et al.*, 2005).The majority of coronary events are caused by coronary thrombosis, resulting from the breakage of an atherosclerotic plaque, which ultimately triggers platelet aggregation and thrombus formation. These vulnerable atherosclerotic plaques are characterized by a fibrous cap, made up of a lipid-rich core containing extracellular lipids and inflammatory cells that include macrophage foam cells. These cholesterol-laden foam cells increase density within the plaques and secrete proteinases that can weaken the fibrous cap, causing it to rupture and activate the coagulation cascade, leading to occlusion of the vessels (Williamson and Pharand,1998). It is postulated that statins can prevent this weakening of the fibrous cap by reducing the plaque's rich lipid and inflammatory cell content (Vaughan*et al.*,1996). In

addition, HDL cholesterol, which is increased by the statins, has been implicated in enhancing this process by transporting cholesterol from lipid-rich lesions to the liver for disposal (Gordon and Rifkind, 1989). Finally, patients with CHD have hypersensitive platelets, increasing their risk of coronary thrombosis. Studies with pravastatin suggest that cholesterol reduction can decrease platelet aggregation caused by hypersensitive platelets (Lacoste*et al*.,1995). Additional evidence indicates that pravastatin and simvastatin decrease thrombus generation. Further research is necessary to confirm this antithrombotic property in other statins (Szczeklik*et al*., 1999).

2.8.2 **ATORVASTATIN**

Atorvastatin is marketed by Pfizer as a calcium salt under the trade name Lipitor (PPPP, 2011). It is a member of the drug class known as statins, used for lowering blood cholesterol. It also stabilizes plaque and prevents strokes through anti-inflammatory and other mechanisms. Like all statins, 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.Atorvastatin was first synthesized in 1985 by Bruce Roth of Parke-Davis Warner-Lambert Company (now Pfizer). The best selling drug in pharmaceutical history, sales of Lipitor since it was approved in 1996 exceed US$125 billion, and the drug has topped the list of best-selling branded pharmaceuticals in the world for nearly a decade (CNN Wire Staff, 2011).

2.8.2.1 USES

The primary uses of atorvastatin is for the treatment of dyslipidemia and the prevention of cardiovascular disease. It is recommended to be used only after other measures such as diet, exercise, and weight reduction have not improved cholesterol levels (ACD, 2011).

Dyslipidemia includes:-

- Hypercholesterolemia (heterozygous familial and nonfamilial) and mixed dyslipidemia (Fredrickson types IIa and IIb) to reduce total cholesterol, LDL-C, apo-B, triglycerides levels, as well as increase HDL levels.
- Heterozygous familial hypercholesterolemia in pediatric patients
- Homozygous familial hypercholesterolemia
- Hypertriglyceridemia (Fredrickson Type IV)
- Primary dysbetalipoproteinemia (Fredrickson Type III)
- Combined hyperlipidemia(McCrindle *et al.,* 2003)

Cardiovascular disease

- Secondary prevention in people with coronary heart disease and multiple risk factors for myocardial infarction, stroke, unstable angina, and revascularization
- Myocardial infarction and stroke prophylaxis in patients with type II diabetes

Concomitant therapy considerations may be used in combination with bile acid resins. It is not recommended to combine statin treatment withfibrates because of the increased risk of myopathy-related adverse reactions. Drug dose must be adjusted according to age of patient, and must be lowered in hepatic insufficiency.

2.8.2.2 ADVERSE EFFECTS

As stated earlier, myopathy with elevation of creatinine kinase (CK) and rhabdomyolysis are the most serious side effects, although rare at <1% (LPI, 2009). Headache is the most common side effect, occurring in more than 10% of patients. Side effects that occur in 1–10% of patients taking atorvastatin include:

- Weakness
- Insomnia and dizziness
- Chest pain and peripheral edema
- Rash
- Abdominal pain, constipation, diarrhea, dyspepsia, flatulence, nausea
- Urinary tract infection
- Arthralgia, myalgia, back pain, arthritis
- Sinusitis, pharyngitis, bronchitis, rhinitis
- Infection, flu-like syndrome, allergic reaction (Lipitor, 2009)

Atorvastatin and other statins are associated with anecdotal reports of memory loss by consumers, which have been seen in clinical practice in a tiny percentage of users, particularly women. Evidence is conflicting with anecdotal reports contrasting with a well-established association of high cholesterol with dementia. However, it is known that cholesterol synthesis is necessary for normal neuron functioning. According to Pfizer, the manufacturer of Lipitor, clinical trials "do not establish a causal link between Lipitor and memory loss (Wagstaff *et al.,* 2003). Elevation of alanine transaminase (ALT) and aspartate transaminase (AST) has been described in a few cases (Ghirlanda *et al.,* 1993). High-dose atorvastatin had also been associated with worsening glycemic control.

2.8.2.3 MECHANISM OF ACTION

As with other statins, atorvastatin 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 novocholesterol 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. Like other statins, 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).

2.8.2.4 PHARMACOKINETICS

Atorvastatin undergoes rapid absorption when taken orally, with an approximate time to maximum plasma concentration (T_{max}) of 1–2 hr. The absolute bioavailability of the drug is about 14%, but the systemic availability for HMG-CoA reductase activity is approximately 30%. Atorvastatin undergoes high intestinal clearance and first-pass metabolism, which is the main cause for the low systemic availability. Administration of atorvastatin with food produces a 25% reduction in C_{max} (rate of absorption) and a 9% reduction in Area under curve (AUC) (extent of absorption), although food does not affect the plasma LDL-C-lowering efficacy of atorvastatin. Evening dose administration is known to reduce the C_{max} and AUC by 30% each. However, time of administration does not affect the plasma LDL-c lowering efficacy of atorvastatin.Atorvastatin is highly protein bound (≥98%)(Lipitor, 2009).

The primary proposed mechanism of atorvastatin metabolism isthrough cytochromeP450 3A4 hydroxylation to form active ortho- and parahydroxylated metabolites, as well as various beta-oxidation metabolites. The ortho- and parahydroxylated metabolites are responsible for 70% of systemic HMG-CoA reductase activity. The ortho-hydroxy metabolite undergoes further metabolism via glucuronidation. As a substrate for the CYP3A4 isozyme, it has shown susceptibility to inhibitors and inducers of CYP3A4 to produce increased or decreased plasma concentrations, respectively. This interaction was tested *in vitro* with concurrent administration of erythromycin, a known CYP3A4 isozyme inhibitor, which resulted in increased plasma concentrations of atorvastatin. It is also an inhibitor of cytochrome 3A4(Hermann*et al.*, 2006).Atorvastatin is primarily eliminated via hepatic biliary excretion, with less than 2% recovered in the urine. Bile elimination follows hepatic and/or extrahepatic metabolism. There does not appear to be any entero-hepatic recirculation. Atorvastatin has an approximate elimination half-life of 14 hr. Noteworthy, the HMG-CoA reductase inhibitory activity appears to have a half-life of 20–30 hr, which is thought to be due to the active metabolites. Atorvastatin is also a substrate of the intestinal P-glycoprotein efflux transporter, which pumps the drug back into the intestinal lumen during drug absorption (Williams and Feely,2002). Geriatric patients (>65 years old) exhibit altered pharmacokinetics of atorvastatin compared to young adults, with mean area under curve (AUC) and C_{max} values that are 40% and 30% higher, respectively. Additionally, healthy elderly patients show a greater pharmacodynamic response to atorvastatin at any dose; therefore, this population may have lower effective doses (Lipitor, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Sample Collection

Roots of *Nauclea latifolia* were collected in the month of April from Zaria, Kaduna State. The plant was identified at the herbarium unit of Biological Sciences Department A.B.U Zaria where a voucher specimen number005 was given for future reference.

3.1.2 Chemicals and Reagents

All chemicals and reagents used were purchased from Sigma chemical company St. Louis U.S.A and were of analytical grade. They include: Ethyl acetate, n- butanol, chloroform, tween- 80, sodium hydroxide and randox kits.

3.1.3 Experimental Animals

A total of 86apparently healthy wistar albino rats of both sexes weighing between 150-200g were obtained from the animal house, Department of Pharmacology, ABU, Zaria. The animals were kept in well aerated laboratory cages in the Department of Pharmacology animal house and were allowed to acclimatize for two weeks before the commencement of the experiment. They were fed with growermash (Vital Feeds Company) and water was provided *ad libitum* during the stabilization period.

3.2 Methodology

3.2.1 Plant Extraction and Fractionation

*Nauclea latifolia*root was chopped into small pieces, washed and dried at room temperature. The pieces were ground into powder with a grinding mill. Distilled water (5litres) was heated and at 90^0C, it was poured into a conical flask. Two kilograms (2kg) of dried powdered root was soaked in hot distilled water (decoction) in the conical flask, stoppered with a rubber cork and left for 24hours with occasional shaking. It was filtered using sterile whatman filter paper into a clean conical flask. The filtrate was concentrated by evaporating to complete dryness in a water bath at 50^0C for 48 hours. The dry extract was partitioned using liquid-liquid partitioning. One hundred and sixty grams (160g) of dry*N.latifolia* root extract was suspended in 1.5 litres of distilled water and then transferred to a separating funnel. Seven hundred milliliters (700 ml) of ethyl acetate (EtOAc) was added to the concentrated extract and left standing for 4 hours. The two–liquid phase layers were then separated into the ethyl

acetate-soluble (EAS) and the water soluble (WAS) fractions respectively. The ethyl acetate fractionation was repeated five times. Ethyl acetate fraction was collected and concentrated by evaporating to complete dryness in a water bath at 50^0C for 48 hours. The dried fraction was stored in an air tight container.

One hundred and sixty grams (160g) of dry *N.latifolia* root extract was suspended in 1.5 litres of distilled water and then transferred to a separating funnel.Seven hundred milliliters (700 ml) of n-butanol (n-BuOH) was added to the concentrated extract and left standing for 4 hours. The two–liquid phase layers were then separated into the butanol-soluble (BUS) and the water-soluble (WAS) fractions respectively. The butanol fractionation was repeated five times. The fractions were later concentrated by evaporating to complete dryness in a water bath at 50^0C for 48 hours and stored in an air tight container.

3.2.2 Acute toxicity study (Lorke 1983)

The median lethal dose (LD_{50}) of the plant extract and fractions was conducted using the method described by Lorke (1983). In the initial phase, the rats were divided into 3 groups of 3 rats each and were treated with 10mg, 100mg and 1000mg of *Nauclea latifolia* extract per kg body weight and administered orally. They were observed for 24hrs for signs of toxicity, including death. In the final phase, 4 rats were divided into 4 groups of one rat each and were treated with the extract at doses of 1000mg, 1600mg, and 2900mg, 5000mg per kg body weight. They were also observed for signs of toxicity and death.The LD_{50} was calculated from the results of the final phase as square roots of the product of the lowest lethal dose and the highest non-lethal dose i.e. the geometric mean of the consecutive doses in which 0 and 100% survival rates was recorded.

3.2.3 Induction of Hypercholesterolemia(Johnston and Palmer, 1993)

Poloxamer 407(Lutrol F127; BASF, Ludwigshafen, Germany), a non-ionic surfactant was used as hypercholesterolemic inducing reagent (Johnston and Palmer, 1993).This was administered intraperitoneally (1g/kg body wt). Levels of total cholesterol and triacylglycerol concentrations above 200mg/dl and 160mg/dl respectively were considered hypercholesterolemic (Delvin, 2006).Total cholesterol and triacylglycerol concentrations of all induced rats were determined 2 hours after induction using standard diagnostic kits.

3.2.4 Animal Grouping and Treatment

The rats were randomly divided into 2 major groups and 18 sub-groups of 6 rats each.

Group 1 (control): Rats were fed normal feed*ad libitum.*

Group 2 (hypercholesterolemic control): Intraperitoneal administration of Poloxamer 407 and hypercholesterolemia observed 2 hours later.

Group A (Preventive group)

Animals in sub-groups 3,4,5,6,7,8 and 9 were administered the drug and plant fractions orally on a daily basisfor 11 days and on the 12^{th} day, induced with Poloxamer 407 (1g/kg b. wt) and sacrificed 48 hours after induction.

Group 3(standard): administered atorvastatin (40mg/kg b.wt) + Poloxamer 407 (1g/kg b.wt)

Group 4: administered aqueous extract (100mg/kg b.wt) + Poloxamer 407 (1g/kg b.wt).

Group 5: administered aqueous extract (500mg/kg b.wt) + Poloxamer 407 (1g/kg b.wt).

Group 6: administered ethyl acetate fraction (100mg/kg b.wt) + Poloxamer 407 (1g/kg b.wt).

Group 7: administered ethyl acetate fraction (500mg/kg b.wt) + Poloxamer 407 (1g/kg b. wt).

Group 8: administered n-butanol fraction (100mg/kg b.wt) + Poloxamer 407 (1g/kg b. wt).

Group 9: administered n-butanol fraction (500mg/kg b.wt) + Poloxamer 407 (1g/kg b. wt).

Group B (Curative group)

Animals in sub-groups iii, iv,v,vi,vii,viiiand ix were fed normally with rat chow diet and on the 12th day, they were induced with Poloxamer 407 (1g/kg b. wt). After two hours, oral administration of the drug and extract commenced for two days, once a day.

Group iii(standard): induced with Poloxamer 407 (1g/kg b.wt). + Atorvastatin (40mg/kg b.wt)

Group iv: induced with Poloxamer 407 (1g/kg b.wt). + aqueous extract(100mg/kg b.wt).

Group v: induced with Poloxamer 407 (1g/kg b.wt). + aqueous extract(500mg/kg b.wt).

Group vi:induced with Poloxamer 407 (1g/kg b.wt). + Ethyl acetate fraction (100mg/kg b.wt)

Group vii: induced with Poloxamer 407 (1g/kg b.wt). + Ethyl acetate fraction (500mg/kg b.wt).

Group viii: induced with Poloxamer 407 (1g/kg b.wt). + n-butanol fraction (100mg/kg b.wt).

Group ix: induced with Poloxamer 407 (1g/kg b.wt). + n-butanol fraction (500mg/kg b.wt).

3.2.5 COLLECTION OF SERUM SAMPLES AND ORGANS

On the 14th day of the experiment, the chloroform-inhalation anesthesia was performed on all experimental animals. The anesthetized animals were bled by cardiac puncture and organs (liver and kidney) harvested, weighed and stored in formalin containing plain sample bottles. The blood samples were centrifuged at a speed of 3000×g for 15 minutes and supernatant collected in plain sample bottles for further analysis.Immediately after the blood wascollected, liver and the kidney was quickly dissected out, weighed and kept in the refrigerator. The liver and kidney was crushed separately using blender. 5ml normal saline was added to the homogenate and centrifuged at 4000rpm for ten minutes. The supernatant was collected using Pasteur pipette and filtered to have a pure homogenate.

3.3Determination of Biochemical Parameters

3.3.1 Determination of serum total cholesterol (TC) concentration

Theserum level of TCwas quantified by spectrophotometric methods as described by Stein (1987).

Principle: A large portion of the cholesterol in blood is in the form of cholesteryl esters, which is hydrolysed by cholesterol esterase into cholesterol. Cholesterol is then oxidized by cholesterol oxidase to yield H_2O_2. The coloured complex quinoneimine is formed from

hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase which can be detected spectrophotometrically.

Cholesterol esterase
Cholesteryl ester + H_2O ⟶ Cholesterol + Fatty acids

Cholesterol oxidase
Cholesterol + O_2 ⟶ Cholestene-3-one + H_2O_2

peroxidase
2 H_2O_2 + Phenol + 4-Aminoantipyrine ⟶ Quinoneimine + 4 H_2O

Procedure: Ten microlitre of distilled water, 10µl of cholesterol standard and 10µl of test serum were dispensed into the test tubes labelled reagent blank, standard and sample respectively. One millilitre of the cholesterol reagent (4-Aminoantipyrine) provided was then added to each of the tubes and mixed.The mixture was incubated at 37°C for 5minutes in a water bath. The absorbance of the sample (A_{sample}) and that of the standard ($A_{standard}$) were then measured against the reagent blank at 500nm wavelength using a colorimeter. The values obtained were used to calculate the total cholesterol concentration using the formula provided by the manufacturer (Randox laboratory) viz:

$$\text{Total cholesterol (mg/dl)} = \frac{A_{sample} \times \text{conc. of standard}}{A_{standard}}$$

3.3.2 Determination of serum triacylglycerol (TG) concentration

The serum level of TG was determined by enzymatic method described by Stein (1987).

Principle:The triacylglycerol is determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acids}$$

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

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerophosphate oxidase}} \text{Dihydroxyacetone} + \text{Phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-Aminophanazone} + \text{4-Chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + HCl + 4H_2O$$

Procedure: One millilitre of the randox TG enzyme reagent (containing a mixture of lipases, glycerol-kinase, ATP, 4-aminophenazone, 4-chlorophenol, peroxidise, glycerol-3-phosphate oxidase and magnesium ions) was dispensed into three separately labelled test tubes; standard, sample and reagent blank. The standard, contained 10 µl standard triacylglycerol, the tube labelled sample contained 10 µl test serum while that labelled reagent blank was distilled water. The mixture was then incubated for 5 minutes at 37°C in a water bath. After the period of incubation the absorbance of the sample (A_{sample}) and that of the standard ($A_{standard}$) was then read against the reagent blank within 60 minutes using a colorimeter at 500 nm wavelength. The triglyceride concentration was then calculated using the formula provided by the manufacturer viz:

$$\text{Triglyceride concentration (mg/dl)} = \frac{A_{sample}}{A_{standard}} \times \text{conc. of standard}$$

3.3.3Determination of Serum high density lipoprotein cholesterol (HDL-c) concentration

HDL-c level was determined based on the method described by Naito (2003).

Principle:When a sample is mixed with reagent R1 containing a selective accelerator, cholesterol of non HDL is subject to enzymatic reactions to be eliminated.

$$\text{LDL, VLDL, chylomicrons} \xrightarrow{\text{Accelerator + CO + DSBmT + POD}} \text{Non-reactive LDL, VLDL, Chylomicrons}$$

When R2 HDL is solubilized by a specific detergent, then HDL-c is measured by enzymatic reactions.

$$\text{HDL} \xrightarrow{\text{HDL specific detergent}} \text{Solubilized HDL}$$

$$\text{HDL cholesterol} + O_2 \xrightarrow{\text{Cholesterol esterase + Cholesterol oxidase}} \text{Cholest-4-on-3-one} + H_2O_2$$

$$H_2O_2 + 4\text{ AA} + \text{DSBmT} \xrightarrow{\text{Peroxidase}} \text{Coloured compound}$$

Procedure: Two hundred and forty microliter (240 µl) of reagent 1 (cholesterol oxidase, peroxidise, ascorbate oxidase and 4-N-bis(4-sulphobutyl)-*m*-toludine-disodium) was dispensed into three separate test tubes labelled "blank"(containing 2.4 µl distilled water), "standard" (containing 2.4 µl of HDL-c calibrator) and "sample" (containing 2.4 µl of test serum). The mixture was incubated at 37°C for 4 minutes 40 seconds in a water bath and the absorbance (A_1) measured using a colorimeter at 578 nm wavelength against the blank. Exactly, 80 µl of Reagent R_2 (4-amino-Antipyrine, cholesterol esterase) was then added to each of the tubes and the resultant mixture incubated at 37°C for 4 minutes in a water bath

and the absorbance (A_2) measured using a colorimeter at 578 nm wavelength against the blank. The concentration of HDL-c was then calculated using the formula provided by the manufacturers viz:

$$\text{HDL-c (mg/dl)} = \frac{A_2 - A_{1\,(\text{sample})}}{A_2 - A_{1\,(\text{standard})}} \times \text{conc. of standard}$$

3.3.4 Determination of serum low density lipoprotein- cholesterol (LDL-c) concentration

The serum level of LDL-cwascalculated according to the protocol of Friedewald(1972) using the equation:LDL-c (mg/dl) =TGL/5 – HDL-c

3.3.5Determination of serum alkalinephosphatase (ALP) activity

The ALP activity was determined by a standard method described byHaussament (1977).

Principle:Alkaline phosphatase is measured by monitoring the concentration of phosphate hydrazone formed with 2, 4-dinitrophenylhydrazine at 405 nm and 37^0C.

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

$$\text{Phosphate} + \text{2, 4 - dinitrophenol} \xrightarrow{\text{ALP}} \text{2, 4 - dinitrophenylhydrazone}$$

Procedure: Exactly, 0.01ml of test serum was dispensed into a 1 ml cuvette followed by the addition of 0.05 ml of the reagent (Prethanolamine buffer, $MgCl_2$, p-nitrophenylphosphate). The absorbance of the mixture was immediately taken using a colorimeter at a wavelength of 405 nm, and the timer started simultaneously as the initial absorbance was been recorded. The absorbance was read again at 1, 2, and 3 minutes at 405 nm. The initial absorbance

reading was subtracted from the absorbance readings at 1, 2 and 3 minutes (i.e. $A_{1/2/3} - A_{initial}$). The average of the three values obtained was then computed and used to calculate the ALP activity using the formula provided by the manufacturer as shown below:

ALP (U/I) = 2760 × ΔA at 405nm/min

Where ΔA = Changes in absorbance

3.3.6Determination of serum alanineaminotransferase (ALT) activity

The ALT activity was determined based on the method described by Reitman and Frankel (1957) and expressed in U/L.

Principle: Alanine aminotrasferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine at 540 nm and 37^{o}C.

ALT
α - ketoglutarate + L - AlaninePyruvate+ ~~Glutamate~~

Procedure: Exactly, 0.5 ml of reagent 1 (phosphate buffer, α-oxoglutarate and L-alanine) was dispensed into labelled test tubes: one containing 0.1 ml distilled water (Reagent blank) and the other 0.1 ml of the test serum (sample). The mixture was incubated at 37°C for 30 minutes in a water bath. This was followed by the addition of 0.5ml of reagent 2 (2,4-dintrophenylhydrazine) to each of the test tubes and the mixture allowed to stand in a water bath at 25°C for 20 minutes. At the end of the 20 minutes 5 ml of sodium hydroxide (0.4 Mol/l) was then dispensed into each of the tubes and the absorbance of the sample (A_{sample}) was read against the reagent blank using a colorimeter at 546 nm. The activity of ALT in the serum was obtained by extrapolating the corresponding absorbance from the plot of the standard calibration curve for the enzymes provided in the manual of the kit.

3.3.7 Determination of serum aspartateaminotransferase (AST) activity

The AST activity was determined based on the method described by Reitman and Frankel (1957) and expressed in U/L

Principle:AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine at 540 nm and 37^{o}C.

AST
α - Ketoglutarate + L - Aspartate ⟶ Oxaloacetate + Glutamate
.

Procedure: Exactly, 0.5 ml of reagent 1 (phosphate buffer, α-oxoglutarate and L-aspartate) was dispensed into labelled test tubes: one containing 0.1 ml distilled water (Reagent blank) and the other 0.1 ml of the test serum (sample). The mixture was incubated at 37°C for 30 minutes in a water bath. This was followed by the addition of 0.5 ml of reagent 2 (2,4-dintrophenylhydrazine) to each of the test tubes and the mixture allowed to stand in a water bath at 25°C for 20 minutes. At the end of the 20 minutes, 5 ml of sodium hydroxide (0.4Mol/l) was then dispensed into each of the tubes and the absorbance of the sample (A_{sample}) was read against the reagent blank using a colorimeter at 546 nm. The activity of AST in the serum was obtained by extrapolating the corresponding absorbance from the plot of the standard calibration curve for the enzymes provided in the manual of the kit.

3.3.8 Determination of serumtotal protein (TP) concentration

The serum TP concentration was determined based on the method described by Fine *et al*. (1935).

Principle: The protein assay is based on a two-step reaction of protein with an alkaline copper tartarate solution and Folin reagent leading to colour development. First is the reaction

between protein and copper at alkaline pH, and subsequently, the reduction of Folin reagent by the copper-treated protein. Colour formation is due to amino acids that reduce the Folin reagent, yielding reduced species that impart a characteristic blue colour. Bovine serum albumin is used as standard.

Procedure: Exactly, 0.02 ml of distilled water, 0.02 ml of standard and 0.02 ml of test serum were dispensed into the test tubes labelled reagent blank, standard and sample respectively and 1ml of the Biuret reagent was then added to each of the test tubes and the mixture incubated at 25°C for 30 minutes in a water bath. After the period of incubation, the absorbance of the sample (A_{sample}) and that of the standard ($A_{standard}$) were then read against the reagent blank using a colorimeter at a wavelength of 546 nm and the values recorded. The total protein concentration was then calculated using the formula provided by the kit manufacturer viz:

$$\text{Total protein concentration (g/dl)} = \frac{A_{sample}}{A_{standard}} \times \text{conc. of standard}$$

3.3.9 Determination of serum albumin (ALB) concentration

The serum ALB concentration was determined based on the method described by Doumas *et al*., (1971).

Principle:The measurement of serum albumin is based on its quantitative binding to the indicator 3, 3′,5, 5′– tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The absorbance of the blue green colour of albumin-BCG-complex at 630nm is directly proportional to the concentration of albumin in the sample.

Procedure: Three ml of the Bromocresol green (BCG) was dispensed into each of the tubes labelled reagent blank, standard and sample containing 0.01 ml distilled water, 0.01 ml standard albumin and 0.01 ml test serum respectively. The mixture was then incubated for 5 minutes at 25°C in a water bath. After the incubation, the absorbance of the sample (A_{sample}) and that of the standard ($A_{standard}$) against the reagent blank were read using a colorimeter at a wavelength of 578 nm. The values obtained were used to calculate the albumin concentration using the formula provided by the manufacturer viz:

$$\text{Albumin concentration (g/dl)} = \frac{A_{sample}}{A_{standard}} \times \text{conc. of standard}$$

3.3.10 Determination of serum creatinineconcentration

The serum creatinine concentration was determined based on the method described by Bartels and Bohmer (1973).

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

Procedure: Exactly, 0.1 ml of creatinine standard solution and 0.1 ml of test serum were dispensed into separate cuvettes labelled standard and sample respectively. This was followed by addition of 1.0 ml of the working reagent (picuric acid + NaOH). The absorbances of the mixtures were then taken after 30 seconds (A_1) and at exactly 2 minutes later (A_2) using a colorimeter at 492 nm wavelength. The ΔA of the sample or standard was then calculated by subtracting A_2 from A_1 i.e. $A_2 - A_1 = \Delta A$ of sample or standard. The values obtained were then used for calculating the serum creatinine concentration with the aid of the formula provided by the manufacturer viz:

$$\text{Creatinine concentration (mg/dl)} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{conc. of standard}$$

3.3.11Determination of serum ureaconcentration

The serum urea concentration was determined based on the method of Berthelot's reaction (Fawcett and Scout, 1960).

Principle: Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia formed is then measured using a colorimeter at wavelength of 546 nm.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

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

Procedure: Exactly, 100 µl of reagent 1 (sodium nitroprusside [6 mMol/l] + urease [1 g/l]) was dispensed into three separately labelled test tubes: reagent blank, standard and sample containing 10 µl of distilled water, 10 µl urea standard and 10 µl test serum respectively. The mixture was then incubated at 37°C for 10 minutes in a water bath. After the period of incubation 2.5 ml of reagent 2 (phenol [120 mMol/l]) was added to the contents of each test tube followed by 2.5 ml of reagent 3 (Sodium hypochlorite [27 mMol/l]). The mixture was then incubated for 15 minutes at 37°C in a water bath. The absorbance of the sample (A_{sample}) and that of the standard ($A_{standard}$) were then read against the reagent blank using a colorimeter at wavelength of 546 nm and the readings obtained were used for the calculation of the serum urea concentration with the aid of the formula provided by the manufacturer viz:

$$\text{Urea concentration(mg/dl)} = \frac{A_{sample}}{A_{standard}} \times \text{conc. of standard}$$

3.3.12 *Determination of Catalase*

Catalase Activity Assay was based on the method of Beers and Sizer (1952).

Principle: Catalase enzyme activity can be measured by monitoring the consumptionof H_2O_2substrate at 240 nm.

$2H_2O_2$Catalase $\longrightarrow$ $2H_2O + O_2$

(Absorbance at 240nm) (No absorbance at 240nm)

Unfortunately, H_2O_2 levels above 0.1 M cause rapid inactivation of catalase even though enzyme saturation requires up to 5M H_2O_2substrate. For this reason, accurate measurement of catalase activity requires that substrate be present at fairly low concentration. Accordingly, one unit of catalaseactivity is classically defined as the amount of enzyme that will decompose 1.0 KMole H_2O_2 substrate (starting concentration = 10.3 mM) per minute at pH 7.0 and 25°C. The precise starting substrate requirement requires that a tedious calibration of H_2O_2 be performed prior to assay.

Procedure: Assay Protocol:

Cuvette Assay:

To a clean cuvette, one thousand micro litre (1000 µL) of Sample Dilution Buffer was added, and placed in the reference cuvette holder. Wavelength was set to 240 nm and the instrument was set at zero.To a clean semi-micro UV cuvette, 950 µL of Working Assay Buffer was added. 50 µL of diluted standard or sample was pipettedinto the cuvette, mixed as quickly as possible by repeated pipetting (~10 times) with the same pipette tip, or by capping/inverting the cuvette. This was then followed by immediate recording of the absorbance at 240 nm, every 2 second or at the smallest time interval allowed for 0.25 minutes (15 seconds).

Note:Because data must be recorded within 30 seconds, do not allowpipetting/mixing time to exceed 15 seconds. Keep the same pace of pipetting/mixing throughout the whole experiment. A practice run with a timer using Sample Dilution Buffer is recommended.

3.3.13 **Superoxide dismutase (SOD)**

Superoxide Dismutase activity was measured using the method described by Martin *et al*., (1978).***Principle*:** Auto-oxidation of heamatoxylin (with increase in absorbance at 560 nm) is inhibited by SOD activity at the assay pH 7.8; the percentage of inhibition is proportional to the amount of SOD present within a specific range. SOD activity in the sample was determined by measuring the amount of heamatin at 560 nm.

Heamatoxylin $\xrightarrow[\text{Auto-oxidation}]{}$ H_2O + Heamatin (active form of heamatoxylin)

***Procedure*:** Assay Protocol:

Microplate Assay

Sample/standard layout was recorded. To each well used for testing, 230 µL of Assay Buffer was added.Next 10 µL of Assay Buffer (for blank) or 10 µL Sample was added. Shake to mix and incubated for 2 minutes.10 µL of Hematoxylin Reagent was added to begin reaction. A multi-channel pipette was preferably used. The mixture was mixed quickly using the instrument's shaker function and immediately began recording the absorbance at 560 nm every 10 seconds or smaller time interval for at least 5 minutes.

Note: The reaction rate should be linear for approximately 10 minutes. Undialyzedsamples may need longer recording time.

3.3.14: Estimation of Lipid Peroxidation by Measuring Malondialdehyde (MDA) level

Thiobarbituric acid reactive substances (TBARS) determination

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

Procedure: Assay Protocol for MDA

Ten microlitres (10 µL)of Butylated hydroxytoluene (BHT) Reagent was added to microcentrifuge vial. 250 µL or sample was added to the vial. 250 µL Acid Reagent was also added to the vial. 250 µL Thiobarbituric acid (TBA) Reagent was added to the vial. It was then vortex vigorously (5-count) and incubated for 60 minutes at 60°C. The mixture was centrifuged at 10,000 xGfor 2-3 minutes. The reaction mixture was then transfered to the cuvette.

3.4 STATISTICAL ANALYSIS

The results were expressed as mean ± standard deviation (SD) and data was analyzed by one way analysis of variance (ANOVA). The difference between the various *Nauclea latifolia* fractions and animal groups were compared using the Duncan Multiple Range Test. *p* value less than 0.05 was considered significant ($p < 0.05$).

CHAPTER FOUR

RESULTS

4.1 Lethal Dose (LD_{50}) Determination of *Nauclea latifolia* RootBark Fractions

The Lethal dose (LD_{50}) determination for root bark fractions of *Nauclea latifolia*is shown in Table 4.2. No sign of toxicity or death was recorded up to 5000mg per kg body weight.

Table 4.1:Lethal Dose (LD_{50}) Determination for *Nauclea latifolia* Bark RootExtract and Fractions

Dose of *N. latifolia* Fractions (mg/kg)	*AE	*EAF	*BF
10	No death	No death	No death
100	No death	No death	No death
1000	No death	No death	No death
1600	No death	No death	No death

2900	No death	No death	No death
5000	No death	No death	No death

*AE: Aqueous extract, *EAF: Ethyl acetate fraction, *BF: n-Butanol fraction
LD_{50} = √ (highest non-lethal dose) × (lowest lethal dose)

4.2 Preventive Effect of *Nauclea latifolia* Root Bark Fractions on lipid profile in P407 Induced Hypercholesterolemic Albino Rats

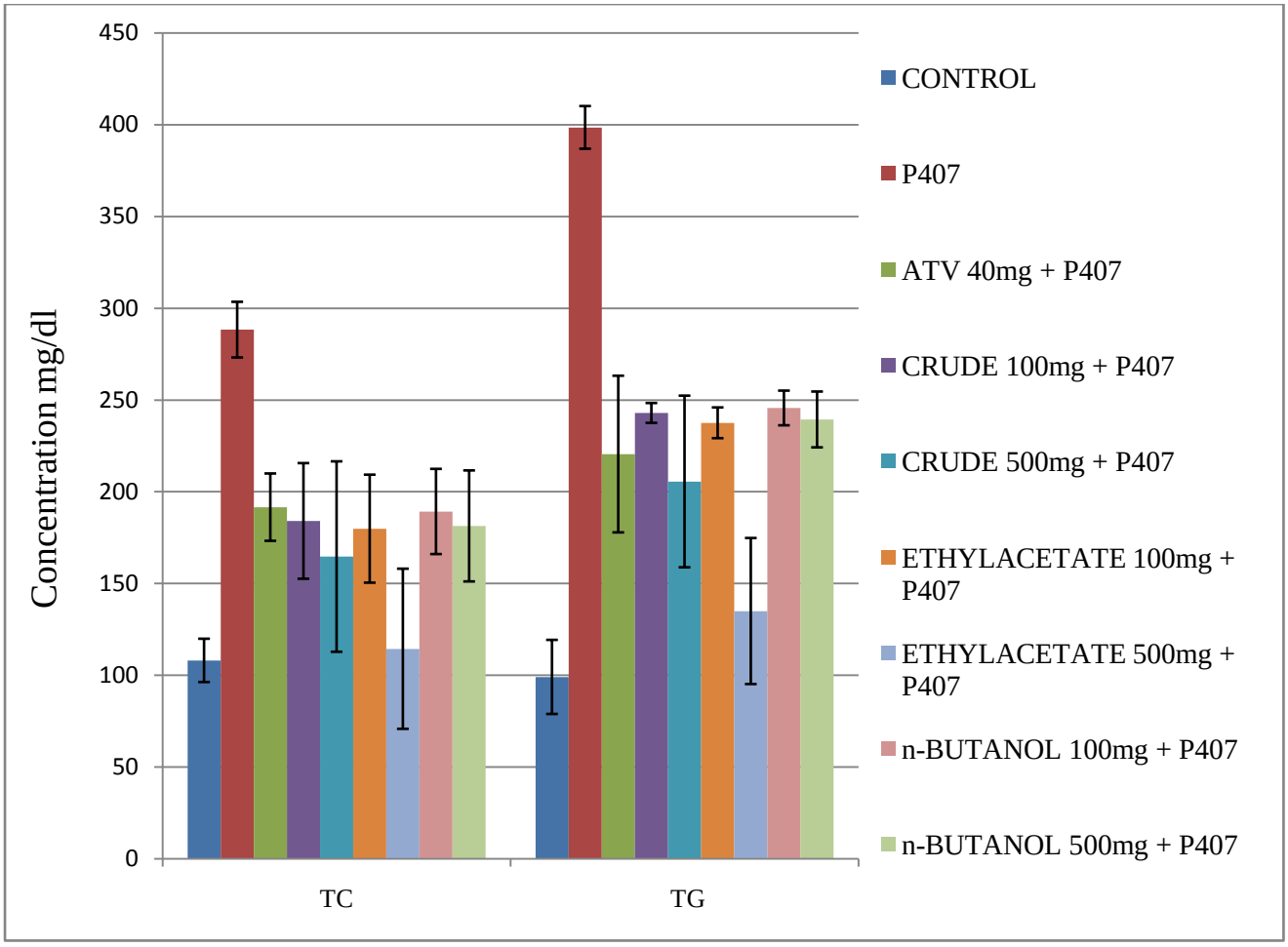
4.2.1 Effect on Total Cholesterol level

The total cholesterol level in rats within the control group was (108.08 ±11.79) as shown in Fig 4.1. After induction with P407, cholesterol level in P407 induced group was significantly ($p < 0.05$) higher (288.29 ±15.17) than all the other groups. Treatment with ethyl acetate fraction at 500 mg/kg gave the highest reduction in cholesterol level (114.41 ±43.63) followed by the aqueous extract 500 mg/kg (164.65 ±51.88). There was no significant ($p < 0.05$) difference between the aqueous extract treatment groups and the atorvastatin treatment group. The n-butanol fractions 100mg (189.21±23.21) and 500mg (181.34±30.25) showed significant ($p < 0.05$) difference when compared with P407 induced group.

4.2.2 Effect on Triacylglycerol level

The triacylglycerol level in rats within the control group was (99.07±20.18) as shown in Fig 4.1. After induction with P407, triacylglycerol level in P407 induced group increased (398.49

±11.62). Treatment with ethyl acetate fraction at 500 mg/kg had the highest reduction (134.98 ±39.78) followed by the aqueous extract 500 mg/kg (205.57 ±46.76). There was no significant ($p > 0.05$) difference in the reduction by the aqueous extract treatment groups, n-butanol fractions and the atorvastatin extract treatment groups. The n-butanol fractions (100mg and 500mg) treated groups showed significant ($p < 0.05$) difference when compared with P407 induced group without treatment.



P407: Poloxamer 407 Induced Rats, ATV+P407: 40mg of Atorvastatin+P407 Induced Hypercholesterolemic Rats, TC: Total cholesterol, TG: Triglycerides

Fig 4.1: Preventive Effect of *Nauclea latifolia* Root Bark Fractions on Total Cholesterol and Triacylglycerol of P407 induced hypercholesterolemic Albino Rats

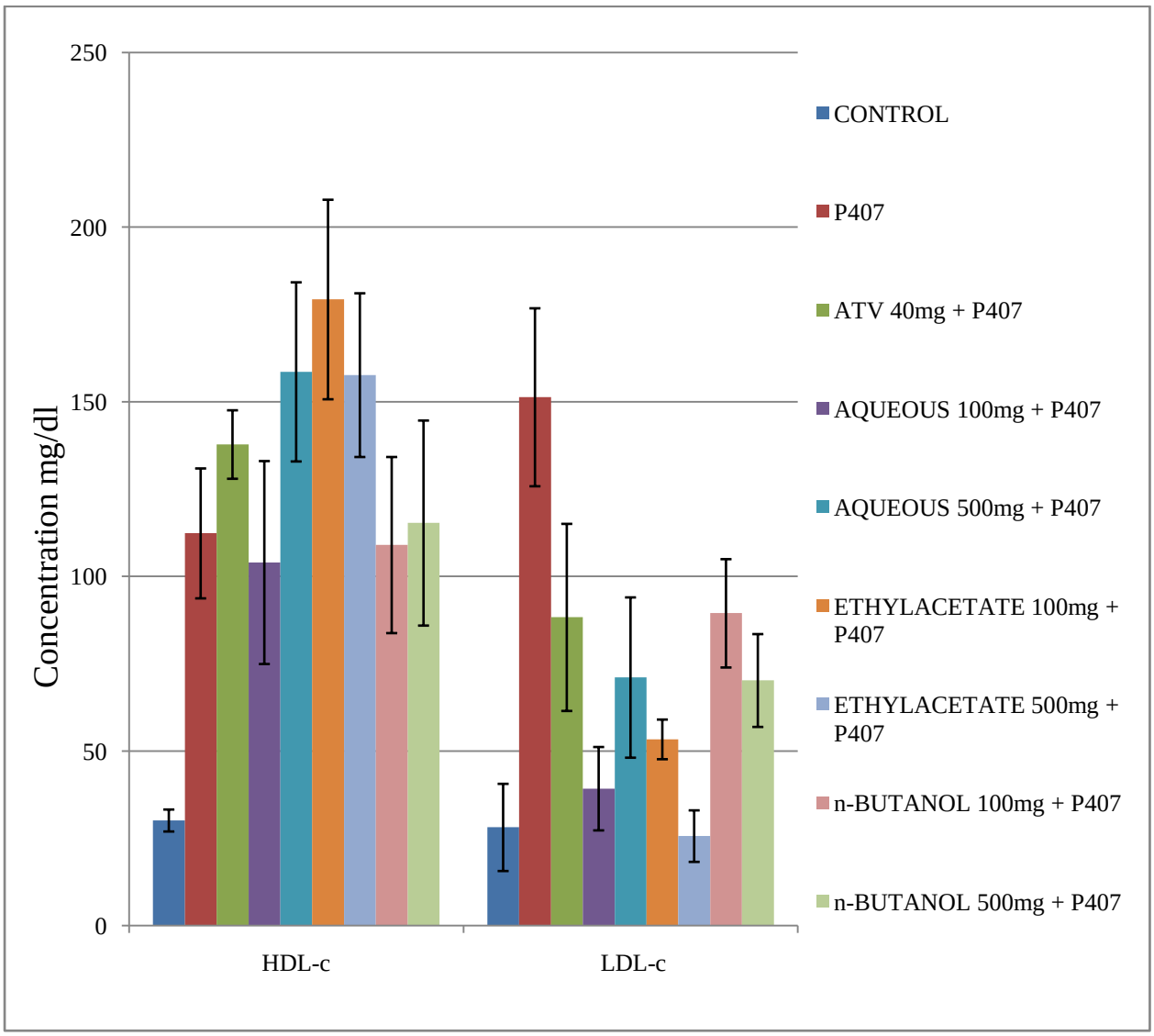
4.2.3 Effect on High Density Lipoprotein Cholesterol level

Figure 4.2 show that the HDL-cholesterol level in the control group was 30.13 ± 3.15mg/dl. The P407 induced group showed significant ($p < 0.05$) increase (112.35 ± 10.04mg/dl)

compared to the control group. There was also no significant ($p < 0.05$) difference between the P407 induced group and the treatment fraction groups except for aqueous 500 mg/kg, ethyl acetate at 100 mg/kg and at 500 mg/kg.

4.2.4 Effect on Low Density Lipoprotein Cholesterol level

The effect on LDL- cholesterol is also shown in Fig 4.2. From the figure; it is shown that the LDL- cholesterol level in rats within the P407 induced group increased with about 5-folds (151.33±25.48mg/dl) when compared with the control group (28.15±12.48mg/dl). There was significant ($p < 0.05$) difference in reduction between the P407 induced group and the control group and there was also significant ($p < 0.05$) difference in reduction between the atorvastatin group (88.29 ±26.79mg/dl) and the P407 group (151.33 ±25.48mg/dl). Ethyl acetate fraction at 500 mg/kg showed the lowest reduction at 25.66±7.38mg/dl. The ethyl acetate, aqueous 100 and 500mg/kg had significant reduction ($p < 0.05$) when compared to P407 induced group.



P407: Poloxamer 407 Induced Rats, ATV+P407: 40mg of Atorvastatin+P407 Induced Hypercholesterolemic Rats, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol

Fig4.2: Preventive Effect of *Nauclea latifolia* Root Bark Fractions on High Density Lipoprotein Cholesterol and Low Density Lipoprotein cholesterol of P407 induced Hypercholesterolemic Albino Rats

4.3 Preventive Effects of *Nauclea latifolia*RootBark Fractions on Serum Atherogenic Risk Predictor Indices of P407 Induced hypercholesterolemic Albino Rats

The values of atherogenic risk indices of wistar rats are shown in Table 4.2. The HDL-cholesterol/total cholesterol of rats in the control group was 1.76± 0.74; however there was significant ($p < 0.05$) decrease in the atherogenic risk factor in P407 induced group. Rats administered atorvastatin showed no significant ($p > 0.05$) difference (1.34± 0.03) when compared to all treatment groups at 100 mg/kg and 500 mg/kg. All treatment groups had significant increase ($p < 0.05$) when compared to P407 induced group.

The ratio of the LDL-cholesterol to HDL-cholesterol in the control group was 0.46± 0.09 as shown in Table 4.2. However, in the P407 induced group, there was significant ($p < 0.05$) increase (2.93 ± 0.20) when compared to other treated groups. There was significant ($p < 0.05$)difference in atorvastatin group when compared to the aqueous and ethyl acetate groups at 100 mg/kg and at 500 mg/kg but no significant difference ($p > 0.05$) when compared to butanol groups at 100 mg/kg and at 500 mg/kg.

Table 4.2: Preventive Effects of *Nauclea latifolia*RootBark Fractions on Serum Atherogenic Risk Predictor Indices of P407 Induced hypercholesterolemic Albino Rats

GRP (n=6)	HDL-c/TC*	LDL-c/HDL-c*
Control	1.76 ± 0.74[b]	0.46 ± 0.09[a]
P407	0.19 ± 0.02[a]	2.93 ± 0.20[c]
ATV 40mg + P407	1.34 ± 0.03[b]	1.08 ± 0.27[b]
Aqueous 100mg + P407	1.79 ± 0.46[b]	0.50 ± 0.29[a]
Aqueous 500mg + P407	1.72 ± 1.44[b]	0.48 ± 0.26[a]
Ethyl 100mg + P407	1.75 ± 1.27[b]	0.31 ± 0.86[a]
Ethyl 500mg + P407	1.62 ± 0.47[b]	0.16 ± 0.05[a]
Butanol 100mg + P407	1.60 ± 0.52[b]	0.77 ± 0.33[b]
Butanol 500mg + P407	1.72 ± 1.41[b]	0.65 ± 0.21[b]

*Values are means ± Standard deviation Values with different superscripts down the column differ significantly ($P<0.05$)P407: Poloxamer 407 Induced Rats, ATV+P407: 40mg of Atorvastatin+P407 Induced Hypercholesterolemic Rats, HDL-c/TC: High density lipoprotein cholesterol/Total cholesterol, LDL-c TG/ HDL-c: Low density lipoprotein cholesterol /High density lipoprotein cholesterol

4.4 Curative effect of *Nauclea latifolia* root bark fractions on Lipid Profile of P407 Induced hypercholesterolemic Albino Rats

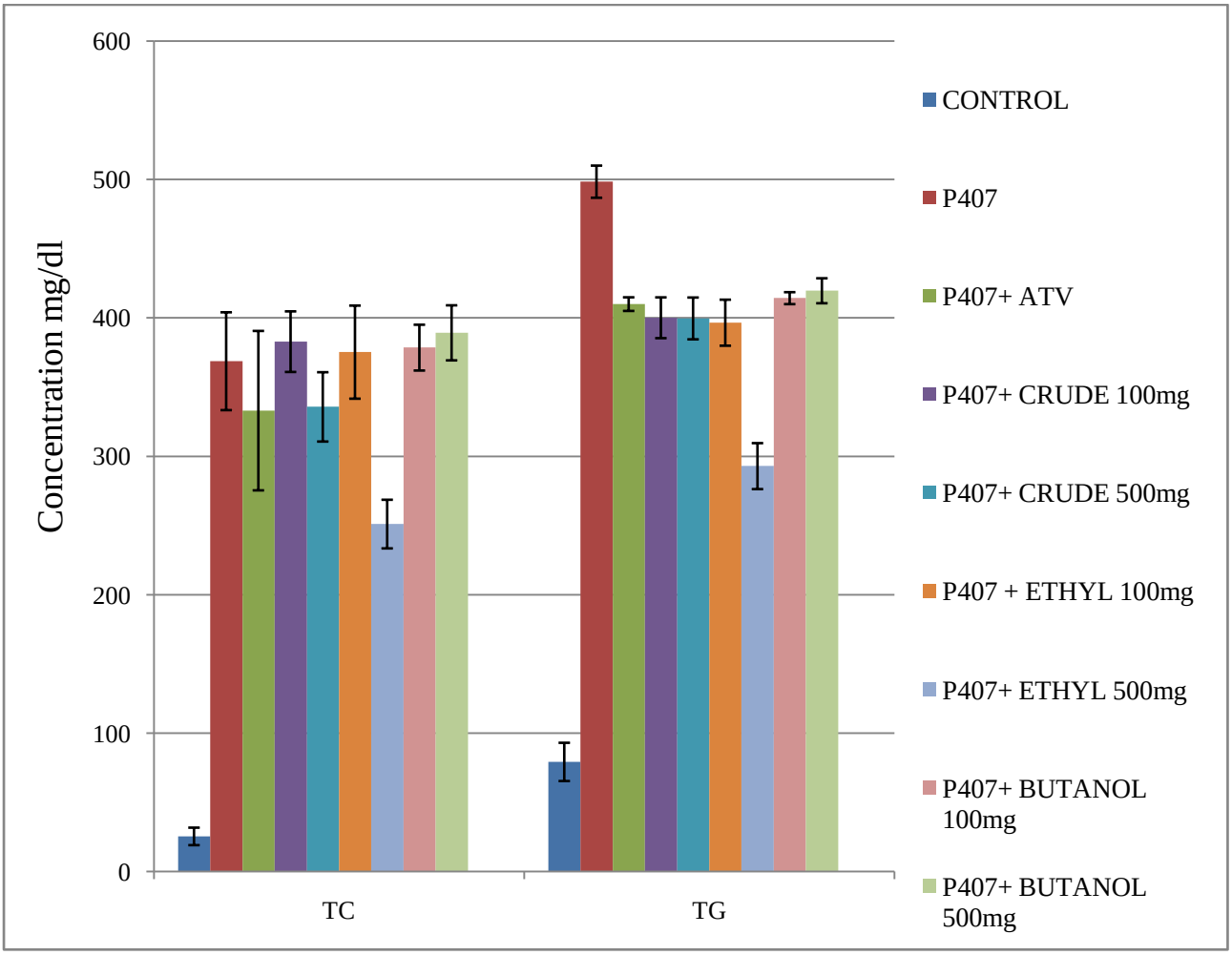
The effects of a fourteen day administration of *Nauclea latifolia* root extract and its fractions at different concentrations on the lipid profile of P407 induced hypercholesterolemic albino rats were presented in Fig.4.3 and 4.4.

4.4.1 Effect on Total Cholesterol level

The total cholesterol level in rats within the control group was 24.45 ± 6.32 mg/dl as shown in Fig 4.3. After induction with P407, cholesterol level in P407 induced group was 368.78 ± 35.35 mg/dl and this showed no significant ($p > 0.05$) difference when compared to the treatment groups except ethyl acetate fraction at 500 mg/kg. Treatment with ethyl acetate fraction at 500 mg/kg gave the highest reduction in cholesterol level (251.10 ± 17.57 mg/dl) followed by the aqueous extract 500 mg/kg (335.78 ±25.05 mg/dl). There was no significant ($p > 0.05$) difference between the aqueous extract 500 mg/kg fraction group and the atorvastatin treated group. The butanol fractions 100mg and 500mg showed no significant ($p > 0.05$) difference (378.59±16.55 mg/dl) (389.28±19.88 mg/dl) when compared to P407 induced group.

4.4.2 Effect on Triacylglycerol level

The triacylglycerol level in rats within the control group was 79.27 ± 13.82 mg/dl as shown in Fig 4.3. After induction with P407, triacylglycerol level in P407 induced group increased to 498.49± 11.62 mg/dl. Treatment with ethyl acetate fraction at 500 mg/kg is found to have the highest reducing activity (292.98 ±16.62 mg/dl) followed by the aqueous extract (500 mg/kg) 399.69 ±15.11 mg/dl. There was no significant ($p > 0.05$) difference between the aqueous extract and the atorvastatin group. The butanol fractions 100mg and 500mg showed significant ($p < 0.05$) difference when compared to P407 induced group.



P407: Poloxamer 407 Induced Rats, ATV+P407: 40mg of Atorvastatin+P407 Induced Hypercholesterolemic Rats, TC: Total cholesterol, TG: Triglycerides

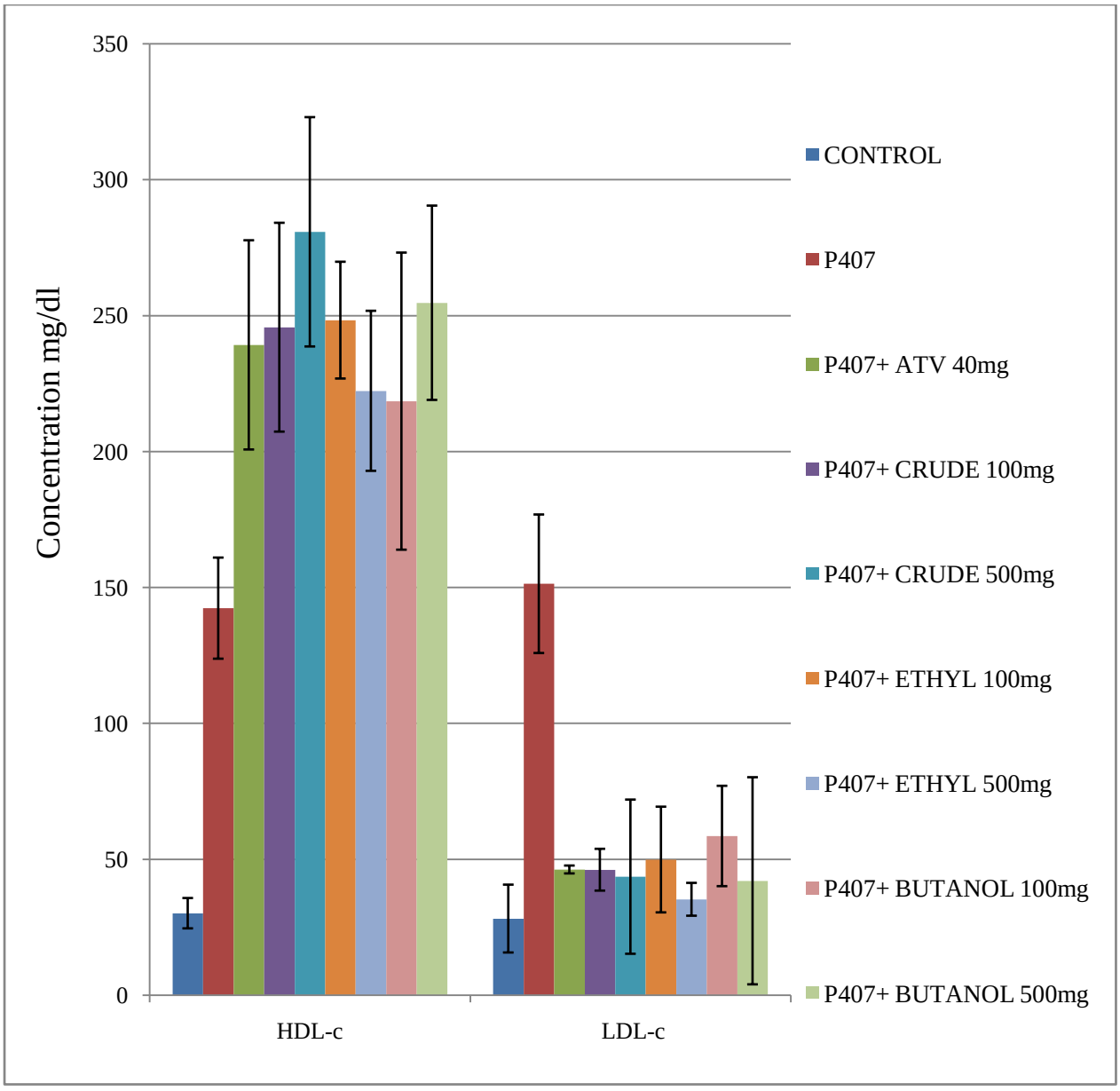
Fig 4.3: Curative Effect of *Nauclea latifolia* Root Bark Fractions on Total Cholesterol and Triacylglycerol of P407 induced hypercholesterolemic Albino Rats

4.4.3 Effect on High Density Lipoprotein Cholesterol level

The HDL-cholesterol level of rats within the control group was 30.13 ± 5.58 mg/dl as shown in Fig 4.4. There was significant increase ($p < 0.05$) in the P407 induced group (142.35 ± 18.60 mg/dl) when compared to the control group. All the treatment groups showed significant ($p < 0.05$) increase when compared to P407 induced group. There was no significant ($p > 0.05$) difference between the atorvastatin group and all the treatment groups.

4.4.4 Effect on Low Density Lipoprotein-cholesterol level

On the other hand, level of LDL-cholesterol of rats within the control group was 28.15 ± 12.48 mg/dl as shown in Fig 4.4. There was significant increase ($p < 0.05$) in the P407 induced group (151.33 ± 25.48mg/dl) when compared to the control group but there was no significant difference ($p > 0.05$) between the control and treatment groups. There was a significant ($p < 0.05$) reduction in the LDL-cholesterol for all the treatment groups when compared to the P407 induced group.



P407: Poloxamer 407 Induced Rats, ATV+P407: 10mg of Atorvastatin+P407 Induced Hyperlipidemic Rats, TC: Total cholesterol, TG: Triglycerides, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol

Fig 4.4: Curative Effect of *Nauclea latifolia* Root Bark Fractions on High Density Lipoprotein-cholesterol and Low Density Lipoprotein-cholesterol of P407 induced Hypercholesterolemic Albino Rats.

4.5 Curative Effects of *Nauclea latifolia*RootBark Fractions on Serum Atherogenic Risk Predictor Indices of P407 Induced hypercholesterolemic Albino Rats

The values of atherogenic risk indices of wistar rats are shown in Table 4.3. The HDL-cholesterol/total cholesterol of rats in the control group was 1.76± 0.74, however there was significant ($p < 0.05$) decrease in the P407 control group. Rats administered atorvastatin showed no significant ($p > 0.05$) difference (0.62± 0.71) when compared to all treatment groups except aqueous 500mg which was significantly ($p < 0.05$) higher (0.72± 0.18). The result shows there was a general increase in all treatment groups when compared to P407 induced group.

The ratio of the LDL-cholesterol to HDL-cholesterol in the control group was 0.12± 0.69 as shown in Table 4.3. There was no significant difference ($p > 0.05$) in atorvastatin group when compared to all treatment groups, however the treated groups had significant reduction ($p < 0.05$) when compared to the P407 induced group with ethyl acetate fraction having the highest.

Table 4.3: Curative Effects of *Nauclea latifolia*RootBark Fractions on Serum Atherogenic Risk Predictor Indices of P407 Induced hypercholesterolemic Albino Rats

GRP (n=6)	HDL-c/TC*	LDL-c/HDL-c*
Control	1.76 ± 0.74^{d}	0.12 ± 0.19^{a}
P407	0.19 ± 0.02^{a}	1.07 ± 0.18^{b}
P407 + ATV 40mg	0.62 ± 0.71^{b}	0.19 ± 0.03^{a}
P407 + Aqueous 100mg	0.62 ± 0.10^{b}	0.19 ± 0.04^{a}
P407 + Aqueous 500mg	0.72 ± 0.18^{c}	0.24 ± 0.22^{a}
P407 + Ethyl 100mg	0.63 ± 0.09^{b}	0.21 ± 0.10^{a}
P407 + Ethyl 500mg	0.63 ± 0.62^{b}	0.16 ± 0.04^{a}
P407 + Butanol 100mg	0.58 ± 0.17^{b}	0.39 ± 0.04^{a}
P407 + Butanol 500mg	0.60 ± 0.13^{b}	0.56 ± 0.19^{a}

*Values are means ± Standard deviation Values with different superscripts down the column differ significantly (P<0.05)P407: Poloxamer 407 Induced Rats, ATV+P407: 40mg of Atorvastatin+P407 Induced Hypercholesterolemic Rats, HDL-c/TC: High density lipoprotein cholesterol/Total cholesterol, LDL-c TG/ HDL-c: Low density lipoprotein cholesterol /High density lipoprotein cholesterol

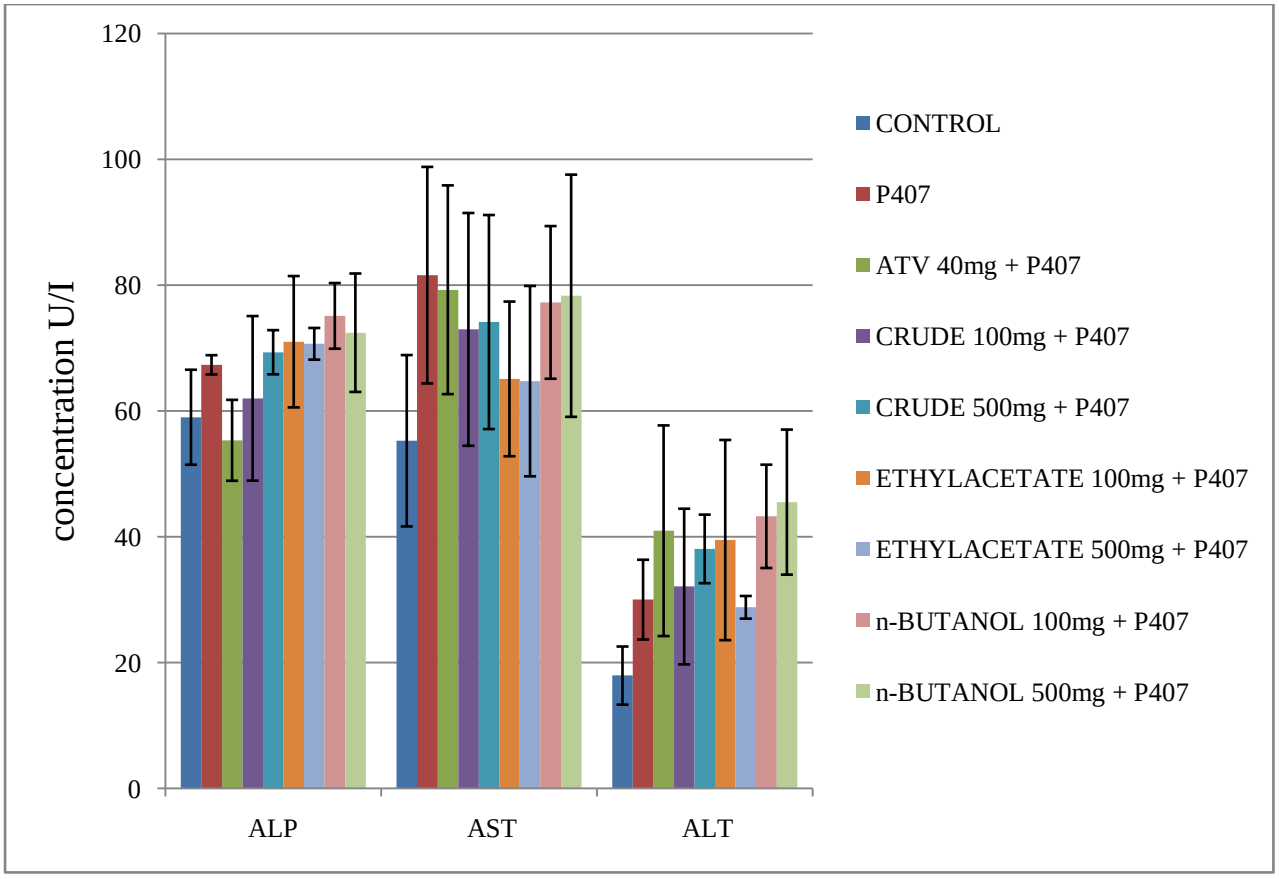
4.6 Effects of *Nauclea latifolia*RootBark Fractions on Serum Liver Damage Indicators and Liver Function Parameters of P407 Induced Hypercholesterolemic Albino Rats

4.6.1Preventive effect of *Nauclea latifolia* on serum liver damage indicators

The preventive effect of *Nauclea latifolia* fractions on serum liver damage indicators of P407 induced hypercholesterolemic rats is presented in Fig 4.5. AST showed no significant difference ($p > 0.05$) in the serum liver activity between control and all hypercholesterolemic groups treated with root bark fractions.

The serum ALT activity showed no significant difference ($p > 0.05$) between the control (17.94± 4.62) and P407 induced group (30.01± 6.34), there was also no significant difference ($p > 0.05$) between the P407 control group and all treatment groups.

In ALP there was no significant difference ($p > 0.05$) between control (59.00± 7.55) and P407 induced group (67.33± 1.53). There was also no significant difference ($p > 0.05$) between the P407 induced group and all the treatment groups.



P407: Poloxamer 407 Induced Rats, P407+ATV: P407 Induced Hypercholesterolemic Rats+40mg of Atorvastatin, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

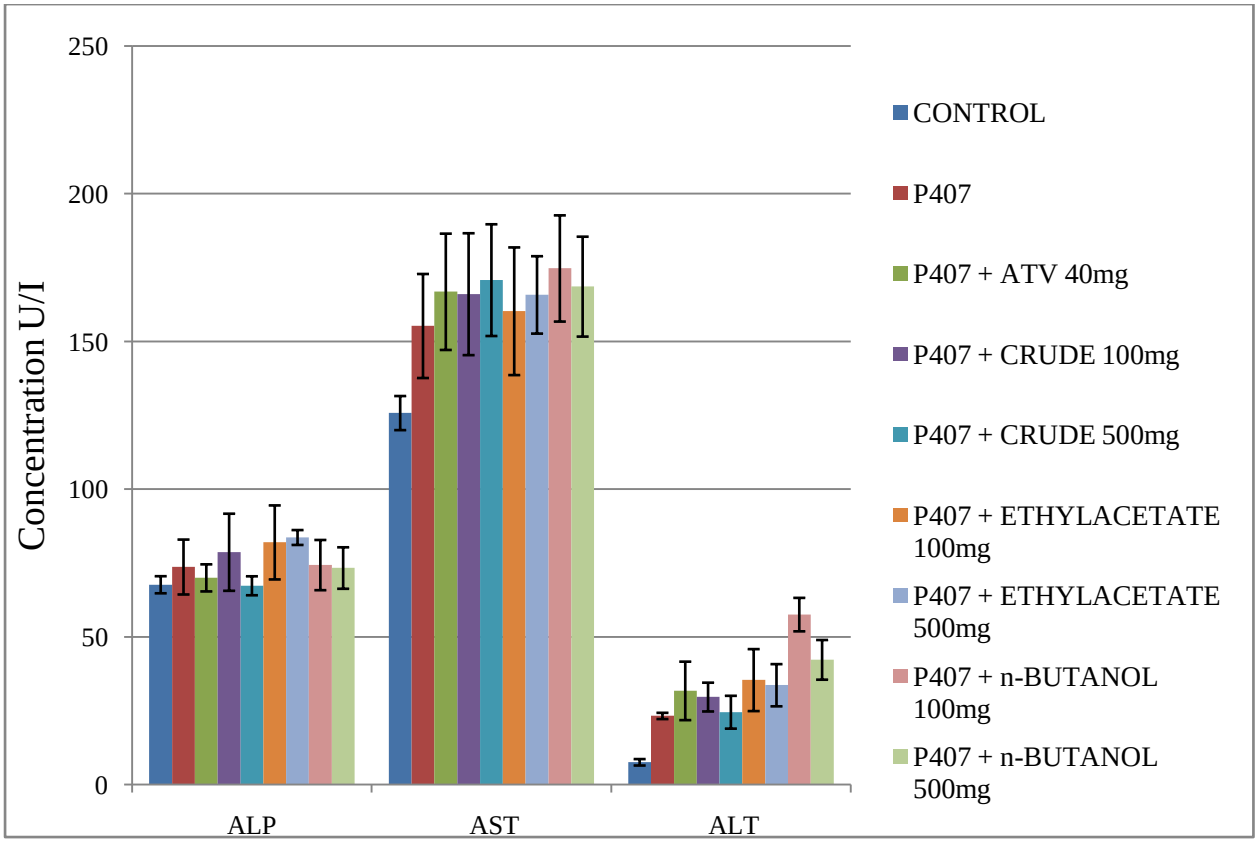
Fig 4.5: Preventive Effects of *Nauclea latifolia* Root Bark Fractions on Serum Liver Damage Indicators of P407 Induced Hypercholesterolemic Albino Rats

4.6.2 Curative Effect of *Nauclea latifolia* Root Fraction on Serum Liver Damage.

The curative effect of *Nauclea latifolia* fractions on serum liver damage indicators of P407 induced hypercholesterolemic rats is presented in Fig 4.6. It represents the effect of *Nauclea latifolia* root fractions on alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

Serum ALP showed no significant difference ($p > 0.05$) in the serum liver level between control (67.67± 2.89) and P407 induced group (73.67± 9.29), there was also no significant difference ($p > 0.05$) between the P407 induced group (73.67± 9.29) and all treatment groups. Serum ALT level for control group showed a value of (7.57± 1.09) while P407 induced group was (23.26± 1.06). This value indicates a significant ($p < 0.05$) increase in the P407 induced group compared to the control group. There was also significant ($p < 0.05$) increase between the P407 induced group (23.26± 1.06) and treatment groups ethyl acetate 100mg(35.39± 10.49), and n-butanol 100mg (57.57± 5.66), and 500mg (42.25± 6.72).

In AST there was significant ($p < 0.05$) increase between control (125.79± 5.77) and P407 induced group (125.79± 5.77). There was no significant ($p > 0.05$) difference between the P407 induced group and all the treatment groups.



P407: Poloxamer 407 Induced Rats, P407+ATV: P407 Induced Hypercholesterolemic Rats+40mg of Atorvastatin, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Fig 4.6: Curative Effects of *Nauclea latifolia* Root Bark Fractions on Serum Liver Damage Indicators of P407 Induced Hypercholesterolemic Albino Rats

4.7 Serum Total Protein and Albumin Concentration

Serum concentration of total protein (TP) and albumin (ALB) of the preventive study are shown in Table 4.4. There was significant ($P<0.05$) increase in the concentrations of serum

TP between normal control and P407 induced group. There was however no significant (P>0.05) difference between P407 control group and all treatment groups. The level of total protein in the ethyl acetate and butanol fractions were reduced to values not significantly (*P*>0.05) different from the control group.

There was no significant (P>0.05) difference in the serum ALB concentration of P407 induced group when compared to the normal control though all treatment groups had no significant (*P*>0.05) effect when compared to P407 induced group. There was a dose dependent increase for aqueous fraction 100mg/kg (2.64± 0.32) to aqueous fraction 500mg/kg (3.48± 0.53).

Table 4.4: Preventive Effect of *Nauclea latifolia*RootBark Fractions on Serum Total Protein and Albumin of P407 Induced Hypercholesterolemic Albino Rats

GRP (n=6)	TP (g/dl) *	ALB (g/dl) *
Control	7.51 ± 0.34[a]	3.17 ±0.09[ab]
P407	9.39 ± 0.38[b]	2.97 ± 0.18[ab]
ATV 40mg + P407	9.39 ± 0.69[b]	2.97 ± 0.59[ab]
Aqueous 100mg + P407	8.82 ± 0.82[b]	2.64 ± 0.32[a]
Aqueous 500mg + P407	9.09 ± 0.68[b]	3.48 ± 0.53[b]
Ethyl 100mg + P407	8.72 ± 1.31[ab]	2.79 ± 0.35[a]
Ethyl 500mg + P407	8.08 ± 0.82[ab]	2.89 ± 0.04[ab]
Butanol 100mg + P407	8.72 ± 0.55[ab]	3.52 ± 0.34[b]
Butanol 500mg + P407	8.64 ± 0.75[ab]	2.78 ± 0.50[a]

*Values are means ± Standard deviation
Values with different superscripts down the column differ significantly ($P<0.05$)
P407: Poloxamer 407 Induced Rats, P407+ATV: P407 Induced Hypercholesterolemic Rats+40mg of Atorvastatin, TP: Total protein, ALB: Albumin

Table 4.5 Shows serum TP and ALB concentrations of the curative study. There was significant (P<0.05) difference in the concentrations of serum TP between the normal control and P407 induced group. The value of normal control (7.39± 0.13 g/dl) when compared to

P407 induced group (9.26± 0.55 g/dl) indicates a significant increase. Treatments however had no significant (*P*>0.05) effect on serum TP concentration when compared to P407 induced group and all treatment groups.

There was no significant (P>0.05) difference in the serum ALB concentration of P407 induced group when compared to the normal control group. All treatment groups had no significant (*P*>0.05) effect when compared to P407 induced group but treatment with ethyl acetate 500mg/kg showed a significant (P<0.05) decrease when compared with standard drug treatment (3.24± 0.36 g/dl) and aqueous 100mg/kg (3.22± 0.23 g/dl).

Table 4.5: Curative Effect of *Nauclea latifolia*Root Bark Fractions on Serum Total Protein and Albumin of P407 Induced Hypercholesterolemic Albino Rats

GRP (n=6)	**TP (g/dl) ***	**ALB (g/dl) ***

Control	7.39 ± 0.13[a]	3.06 ± 0.28[ab]
P407	9.26 ± 0.55[b]	2.83 ± 0.24[ab]
P407 + ATV 40mg	9.72 ± 0.53[b]	3.24 ± 0.36[b]
P407 + Aqueous 100mg	9.62 ± 0.55[b]	3.22 ± 0.23[b]
P407 + Aqueous 500mg	9.39 ± 0.21[b]	2.85 ± 0.13[ab]
P407 + Ethyl 100mg	9.67 ± 0.87[b]	2.91 ± 0.32[ab]
P407 + Ethyl 500mg	9.17 ± 0.66[b]	2.63 ± 0.17[a]
P407 + Butanol 100mg	9.72 ± 0.55[b]	3.02 ± 0.42[ab]
P407 + Butanol 500mg	9.64 ± 0.73[b]	2.95 ± 0.30[ab]

*Values are means ± Standard deviation
Values with different superscripts down the column differ significantly ($P<0.05$)
P407: Poloxamer 407 Induced Rats, P407+ATV: P407 Induced Hypercholesterolemic Rats+40mg of Atorvastatin, TP: Total protein, ALB: Albumin

4.8 Effects of *Nauclea latifolia* Fractions on Kidney Function Parameters of P407 Induced Hypercholesterolemic Albino Rats

Preventive Effect

Serum creatinine and urea concentrations after a 14 day oral administration of root barkfractions of *Nauclea latifolia*before induction are shown in Table 4.7. The result showed no significant (P>0.05) difference in creatinine and urea concentrations of all the groups.

Table 4.6:Preventive Effect of *Nauclea latifolia* Root Bark Fractions on Kidney Function Parameters of P407 Induced Hypercholesterolemic Albino Rats

GRP (n=6)	**CREATININE (mg/dl) ***	**UREA (mg/dl) ***
Control	0.56 ± 0.21^{a}	23.62 ± 1.98^{a}
P407	0.59 ± 0.18^{a}	25.42 ± 10.54^{a}
ATV 40mg + P407	0.40 ± 0.18^{a}	18.63 ± 6.16^{a}
Aqueous 100mg + P407	0.41 ± 0.15^{a}	19.14 ± 4.81^{a}
Aqueous 500mg + P407	0.47 ± 0.11^{a}	15.69 ± 1.07^{a}
Ethyl 100mg + P407	0.40 ± 0.14^{a}	21.09 ± 3.56^{a}
Ethyl 500mg + P407	0.42 ± 0.20^{a}	24.61 ± 10.47^{a}
Butanol 100mg + P407	0.49 ± 0.17^{a}	23.26 ± 5.41^{a}
Butanol 500mg + P407	0.48 ± 0.16^{a}	20.31 ± 8.29^{a}

*Values are means ± Standard deviation
Values with different superscripts down the column differ significantly (P<0.05)
P407: Poloxamer 407 Induced Rats, P407+ATV: P407 Induced Hypercholesterolemic Rats+40mg of Atorvastatin

Curative Effect

The kidney function parameters of therapeutic study are presented in Table 4.8. The result also showed no significant (P>0.05) change in serum creatinine and urea concentrations for all the groups.

Table 4.7:Curative Effect of *Nauclea latifolia* RootBark Fractions on Kidney Function Parameters of P407 Induced Hypercholesterolemic Albino Rats

GRP (n=6)	CREATININE (mg/dl) *	UREA (mg/dl) *
Control	0.56 ± 0.15[a]	23.62±1.98[a]
P407	0.59 ± 0.18[a]	25.42±0.88[a]
P407 + ATV 40mg	0.48 ± 0.32[a]	22.72±1.98[a]
P407 + Aqueous 100mg	0.49 ± 0.17[a]	18.87±1.25[a]
P407 + Aqueous 500mg	0.51 ± 0.15[a]	20.27±5.65[a]
P407 + Ethyl 100mg	0.48 ± 0.21[a]	17.09±3.29[a]
P407 + Ethyl 500mg	0.50 ± 0.14[a]	21.32±1.75[a]
P407 + Butanol 100mg	0.49 ± 0.19[a]	18.77±1.79[a]
P407 + Butanol 500mg	0.47 ± 0.30[a]	19.86±5.61[a]

*Values are means ± Standard deviation
Values with different superscripts down the column differ significantly (P<0.05)
P407: Poloxamer 407 Induced Rats, P407+ATV: P407 Induced Hypercholesterolemic Rats+40mg of Atorvastatin

4.9 Effects of *Nauclea latifolia*RootBark Fractions on Serum Antioxidant Enzymes and Lipid Peroxidation of P407 Induced Hypercholesterolemic Albino Rats

Preventive Effect

The preventive effect of *Nauclea latifolia* root fraction administration on catalase activity shows a significant (P<0.05) decrease in P407 induced group when compared to the normal

control group as shown in Table 4.8. The value of normal control (60.00± 3.61) when compared to P407 induced group (43.67± 6.81) indicates a significant decrease. There was however no significant (P>0.05) difference between P407 induced group and all treatment groups.

There was a significant (P<0.05) decrease in superoxide dismutase in P407 induced group compared to normal control group. The value of normal control (2.37± 0.15) when compared to P407 induced group (1.67± 0.21) indicates a significant decrease. Atorvastatin, aqueous 500mg and ethyl acetate 500mg treatment groups showed no significant difference but significantly differ (P<0.05) when compared to P407 induced group. Aqueous 100mg/kg, ethyl acetate 100mg/kg and butanol 100mg/kg showed no significant difference (P>0.05) but differ from P407 induced group.

Serum malondialdehyde level showed significant (P<0.05) increase in P407 induced group when compared to normal control group. The value of normal control (1.90± 0.10) when compared to P407 induced group (2.50± 0.30) indicates a significant increase. There was also significant decrease in all treatment groups when compared to P407 induced group.

Table 4.8:Preventive Effect of *Nauclea latifolia* RootBark Fractions on Serum Antioxidant Enzymes and Lipid Peroxidation of P407 Induced Hypercholesterolemic Albino Rats

GRP (n=6)	**CAT (U/I) ***	**SOD (U/I) ***	**MDA (umol/l) ***

Control	60.00 ± 3.61[b]	2.37 ± 0.15[c]	1.90 ± 0.10[a]
P407	43.67 ± 6.81[a]	1.67 ± 0.21[b]	2.50 ± 0.30[c]
ATV 40mg + P407	53.67 ± 3.79[ab]	2.17 ± 0.21[c]	1.77 ± 0.15[a]
Aqueous 100mg + P407	43.33 ± 8.96[a]	1.37 ± 0.31[a]	1.80 ± 0.10[a]
Aqueous 500mg + P407	46.33 ± 2.52[a]	2.05 ± 0.12[c]	2.13 ± 0.21[b]
Ethyl 100mg + P407	45.33 ± 3.06[a]	1.53 ± 0.21[a]	2.20 ± 0.20[b]
Ethyl 500mg + P407	45.67 ± 5.51[a]	2.23 ± 0.15[c]	1.73 ± 0.15[a]
Butanol 100mg + P407	40.23 ± 6.43[a]	1.40 ± 0.35[a]	2.25 ± 0.24[b]
Butanol 500mg + P407	43.52 ± 5.26[a]	1.98 ± 0.26[b]	2.00 ± 0.32[b]

*Values are means ± Standard deviation
Values with different superscripts down the column differ significantly ($P<0.05$)
P407: Poloxamer 407 Induced Rats, P407+ATV: P407 Induced Hypercholesterolemic Rats+40mg of Atorvastatin
CAT: catalase, SOD:superoxide dismutase, MDA: malodialdehyde

Curative Effect

The curative effect also indicates the same trend. Effect of *Nauclea latifolia* root fraction administration on catalase activity shows a significant ($P<0.05$) decrease in P407 induced group when compared to the normal control group (table 4.9). The value of normal control (64.00± 3.61) when compared to P407 induced group (43.00± 1.00) indicates a significant

decrease. There was however no significant (P>0.05) difference between P407 induced group and all treatment groups.

There was a significant (P<0.05) decrease in superoxide dismutase in P407 induced group compared to normal control group. The value of normal control (2.17± 0.12) when compared to P407 induced group (1.17± 0.45) indicates a significant decrease. There was however no significant (P>0.05) difference between P407 induced group and all treatment groups.

Serum malondialdehyde showed significant (P<0.05) increase in P407 induced group when compared to normal control group. The value of normal control (1.87± 0.15) when compared to P407 induced group (2.53± 0.06) indicates a significant increase. There was also significant decrease in all treatment groups when compared to P407 induced group.

Table 4.9:Curative Effect of *Nauclea latifolia* RootBark Fractions on Serum Antioxidant Enzymes and Lipid Peroxidation of P407 Induced Hypercholesterolemic Albino Rats

GRP (n=6)	**CAT (U/I) ***	**SOD (U/I) ***	**MDA (umol/l) ***
Control	64.00 ± 3.61[c]	2.17±0.45[b]	1.87±0.06[b]

P407	43.00 ± 1.00[ab]	1.17±0.12[a]	2.53±0.06[c]
P407 + ATV 40mg	46.67 ± 4.04[b]	1.70±0.26[ab]	1.63±0.06[ab]
P407 + Aqueous 100mg	47.67 ± 7.02[b]	1.60±0.20[a]	1.53±0.35[ab]
P407 + Aqueous 500mg	41.67 ± 4.04[ab]	1.43±0.31[a]	1.70±0.10[ab]
P407 + Ethyl 100mg	39.00 ± 3.61[a]	1.37±0.31[a]	1.53±0.15[ab]
P407 + Ethyl 500mg	41.67 ± 2.52[ab]	1.50±0.26[a]	1.57±0.15[ab]
P407 + Butanol 100mg	46.67 ± 3.06[b]	1.50±0.20[a]	1.70±0.20[ab]
P407 + Butanol 500mg	45.67 ± 3.21[ab]	1.33±0.21[a]	1.23±0.67[ab]

*Values are means ± Standard deviation
Values with different superscripts down the column differ significantly ($P<0.05$)
P407: Poloxamer 407 Induced Rats, P407+ATV: P407 Induced Hypercholesterolemic Rats+40mg of Atorvastatin
CAT: catalase, SOD:superoxide dismutase, MDA: malodialdehyde

4.10 Effects of *Nauclea latifolia* Root Bark Fractions on Body Weight of P407 Induced Hypercholesterolemic Albino Rats

Preventive Effect

The change in body weights of the preventive study is shown in Table 4.10. The result showed there was no significant ($P>0.05$) change in the body weight of all the groups.

Table 4.10: Preventive Effect of *Nauclea latifolia* RootBark Fractions on Body Weight of P407 Induced Hypercholesterolemic Albino Rats

GRP (n=6)	MEAN INITIAL BODY WGHT (g)	MEAN FINAL BODY WGHT (g)	CHANGE IN BODY WGHT (g) *
Control	255.00 ± 43.59	278.00 ± 57.45	23.00 ± 14.53[a]
P407	206.33 ± 49.90	223.33 ± 54.17	17.00 ± 7.94[a]
ATV 40mg + P407	150.20 ± 21.43	168.20 ± 22.67	17.80 ± 7.69[a]
Aqueous 100mg + P407	136.33 ± 39.26	147.33 ± 47.65	11.00± 10.82[a]
Aqueous 500mg + P407	138.00 ± 12.53	160.67 ± 13.05	22.67 ± 21.08[a]
Ethyl 100mg + P407	140.40 ±16.82	157.40 ± 24.62	17.00 ± 8.15[a]
Ethyl 500mg + P407	130.00 ± 14.71	147.20 ± 14.62	17.20 ± 8.58[a]
Butanol 100mg + P407	142.31 ± 29.22	147.20 ± 19.32	19.36 ± 5.23[a]
Butanol 500mg + P407	150.20 ± 12.31	170.00 ± 15.58	20.54 ± 9.54[a]

*Values are means ± Standard deviation
Values with different superscripts down the column differ significantly (P<0.05)
P407: Poloxamer 407 Induced Rats, P407+ATV: P407 Induced Hypercholesterolemic Rats+40mg of Atorvastatin

Curative Effect

Table 4.11 presents the change in body weights of rats in the therapeutic study. The result also showed there was no significant (P>0.05) change in the body weight of all the groups, but ethyl acetate 500mg/kg showed an increased body weight when compared with atorvastatin drug.

Table 4.11: Curative Effect of *N.latifolia* Fractions on Body Weight of P407 Induced Hypercholesterolemic Albino Rats

GRP (n=6)	MEAN INITIAL BODY WGHT (g)	MEAN FINAL BODY WGHT(g)	CHANGE BODY IN WGHT(g) *
Control	255.00 ± 14.53	278.00 ± 57.45	23.00 ± 14.53^{ab}
P407	175.83 ± 34.96	188.50 ± 32.96	12.17 ± 10.65^{ab}
P407 + ATV 40mg	201.64 ± 35.26	211.67 ± 40.67	10.00 ± 9.08^{a}
P407 + Aqueous 100mg	198.60 ± 37.71	217.20 ± 46.89	18.60 ± 11.28^{ab}
P407 + Aqueous 500mg	211.33 ± 38.48	222.83 ± 42.17	11.50 ± 5.09^{ab}
P407 + Ethyl 100mg	181.00 ± 68.79	216.67 ± 54.08	35.67 ± 47.96^{b}
P407 + Ethyl 500mg	181.00 ± 51.57	213.60 ± 68.23	30.00 ± 16.61^{ab}
P407 + Butanol 100mg	188.33 ± 39.88	202.67 ± 39.55	13.33 ± 7.77^{ab}
P407 + Butanol 500mg	184.50 ± 30.77	200.33 ± 37.54	15.83 ± 10.59^{ab}

*Values are means ± Standard deviation
Values with different superscripts down the column differ significantly ($P<0.05$)
P407: Poloxamer 407 Induced Rats, P407+ATV: P407 Induced Hypercholesterolemic Rats+40mg of Atorvastatin

4.11 Effects of *Nauclea latifolia* Root Bark Fractions on Percentage Organ Weight of P407 Induced Hypercholesterolemic Albino Rats

Preventive Effect

Table 4.13 shows the percentage of liver and kidney weights of the preventive study of administered*Nauclea latifolia* Root fractions. The weight of the liver significantly (P<0.05) increased when comparing hypercholesterolemic induced group to control group. There was no significant (P>0.05) difference in liver weight between the hypercholesterolemic induced

group and all the treatment groups. Also, there was no significant (P>0.05) change in kidney weight of all the treated groups.

Table 4.12: Preventive Effect of *Nauclea latifolia* RootBark Fractions on Percentage Organ Weight of P407 Induced Hypercholesterolemic Albino Rats

GRP (n=6)	**KIDNEY (%)***	**LIVER (%)***
Control	0.65 ± 0.06^{a}	2.66 ± 0.07^{a}
P407	0.72 ± 0.06^{a}	3.68 ± 0.22^{a}
ATV 40mg + P407	0.79 ± 0.09^{a}	3.28 ± 0.24^{a}
Aqueous 100mg + P407	0.77 ± 0.09^{a}	3.71 ± 0.37^{a}

Aqueous 500mg + P407	0.67 ± 0.01^{a}	2.97 ± 0.05^{a}
Ethyl 100mg + P407	0.68 ± 0.08^{a}	3.20 ± 0.11^{a}
Ethyl 500mg + P407	0.68 ± 0.16^{a}	3.46 ± 0.81^{a}
Butanol 100mg + P407	0.71 ± 0.07^{a}	3.16 ± 0.25^{a}
Butanol 500mg + P407	0.75 ± 0.02^{a}	3.54 ± 0.24^{a}

*Values are means ± Standard deviation
Values with different superscripts down the column differ significantly (P<0.05)
P407: Poloxamer 407 Induced Rats, P407+ATV: P407 Induced Hypercholesterolemic Rats+40mg of Atorvastatin

Curative Effect

The curative study of percentage kidney and liver weights of P407 induced Hypercholesterolemic rats treated with *Nauclea latifolia* root fractions is presented in Table 4.14. The result showed no significant (P>0.05) change in liver and kidney weights of all the groups.

Table 4.13: Curative Effect of *Nauclea latifolia* RootBark Fractions on Percentage Organ Weight of P407 Induced Hypercholesterolemic Albino Rats

GRP (n=6)	KIDNEY (%)*	LIVER (%)*
Control	0.65 ± 0.06^{a}	2.66 ± 0.07^{a}
P407	0.64 ± 0.01^{a}	2.66 ± 0.03^{a}
P407 + ATV 40mg	0.67 ± 0.02^{a}	2.93 ± 0.21^{a}
P407 + Aqueous 100mg	0.61 ± 0.04^{a}	2.75 ± 0.33^{a}
P407 + Aqueous 500mg	0.65 ± 0.01^{a}	2.89 ± 0.21^{a}
P407 + Ethyl 100mg	0.65 ± 0.01^{a}	3.15 ± 0.34^{a}
P407 + Ethyl 500mg	0.75 ± 0.12^{a}	3.66 ± 1.18^{a}
P407 + Butanol 100mg	0.68 ± 0.03^{a}	3.14 ± 0.25^{a}
P407 + Butanol 500mg	0.97 ± 0.23^{a}	4.07 ± 1.11^{a}

*Values are means ± Standard deviation
Values with different superscripts down the column differ significantly ($P<0.05$) P407: Poloxamer 407 Induced Rats, P407+ATV: P407 Induced Hypercholesterolemic Rats+40mg of Atorvastatin

CHAPTER FIVE

5.0 Discussion

The Poloxamer 407 treated rodent is a well-established animal modelof dose dependent hyperlipidemia, hypercholesterolemia and atherosclerosis (Johnston, 2004). P-407 has been utilized in the hypercholesterolemia model due to its convenience, reproducibility and lack of undesirable underlying pathological conditions (Wout *et al.,* 1992).This study agrees with the work of Megalli *et al.,*(2005)where P407 was shown to increase triglycerides, total

cholesterol, LDL-cholesterol and HDL-cholesterol levels in plasma when compared to the control group.

The lipid analysis carried out in this study and results are shown in chapter four. The result of total cholesterol, triacylglycerol and low density lipoprotein (LDL) in the preventive study showed a reduction in all treatment groups when compared to the induced group. This is in agreement with the report of Malinon, (1997) who reported that the action of *Nauclea latifolia* plant in reducing plasmacholesterol concentration could be due to the ability of one or more of the phytochemicals present in the plant to activate the functioning enzymes of the rat responsible for cholesterol absorption. Of all the treatment groups (aqueous extract, ethyl acetate and n-butanol fraction), ethyl acetate at 500mg/kg body weight possessed the highest reducing activity in TC, TG and LDL-C analysis. HDL-cholesterol carries cholesterol and cholesteryl esters from the peripheral tissues and cells to the liver, where cholesterol is metabolized into bile acids. This pathway plays a very important role in reducing cholesterol levels in the blood and peripheral tissues and in inhibiting atherosclerotic plaque formation in the aorta (Kim *et al.*, 2008; Karmarkar, 2008). The result of the preventive study shows increased levels of plasma HDL-cholesterol concentrations with treatment crude extract 500mg, ethyl acetate 100 and 500mg/ kg body weight having the highest activity, this suggests that *Nauclea latifolia* may protect against cardiovascular diseases that result from hypercholesterolemia.

The result of total cholesterol, triacylglycerol and low density lipoprotein (LDL) in the curative study showed reduction in concentration with ethyl acetate 500mg/kg body weight having highest reducing activity, this could also be due to the ability of one or more of the phytochemicals in the plant fraction which activates the functioning enzymes responsible for cholesterol absorption in rat. Since the increase in triacylglycerol (TG) seen following P-407 intraperitoneal (ip) injection results primarily from an inhibition of TG degradation, where P-

407 directly inhibits capillary lipoprotein lipase (LPL) responsible for plasma TG hydrolysis (Johnston and Plamer, 1993; Johnston, 2004), the serum TG lowering effects of the root extract and the fractions of the plant can be attributed to the activation of lipoprotein lipase. The result of the curative study also shows increased levels of plasma HDL-cholesterol concentrations with crude 500mg/kg body weight having highest reducing activity, this suggests that *Nauclea latifolia* may protect against cardiovascular diseases that result from hypercholesterolemia. This is in agreement with the report of Prema *et al.*, (2011) which showed significant increase of HDL-cholesterol concentrations in *Allium sativum*treated hyperlipidemic rats.

The Atherogenic indices predictor (AIP) reflects thedelicate metabolic interactions within the whole lipoprotein complex. When using the results of well standardized assays, AIP provides information about the atherogenicity of plasma and quantifies the response to curative intervention (Ojiako and Nwanjo, 2005). Although values of HDL-cholesterol /total cholesterol <0.3 are believed to be at risk of atherosclerosis, it can be seen that the value in the animals given *Nauclea latifolia* extract and its fractions in the preventive and curative studies were all >0.3. The LDL-cholesterol to HDL-cholesterol ratio also showed that the extract and the fractions reduced the risk to atherosclerosis because they were <2.3. These are in agreement with the report of Ojiako and Nwanjo (2005).

The liver damage indicators carried out in this study are alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Decrease of ALT, AST and ALP may indicate damage or death of the liver cells (Jimoh and Odutuga, 2011) or loss of the enzyme to extracellular fluids or the inactivation of the enzyme in situ by the components of the extract and their metabolites.In the preventive study, there was no

significant difference between the treatment groups and the P407 induced group. This indicates that the extract and its fractions do not possess hepatotoxic potentials.

According toPincus and Schaffner (1996), AST and ALT are released into serum when there is severe hepatocellular injury. In the curative study, there was significant increase in the P407 induced group when compared to the control group but no significant difference between the treatment groups and the P407 induced group. This indicates that the extract and its fractions were unable to reduce the AST, ALT and ALP concentrations, This indicates that the extract and its fractions do not possess hepatotoxic potentials.

This could be attributed to theduration of administration. It is important to note that the choice of duration was made to coincide with all groupsand chosen because P407-induced hypercholesterolemia is atmaximum after 48 h of induction.

Some herbal medicines have been reported to possess hepatotoxic effect due to elevation in clinical chemistry parameters (Ranjna, 1999). Some of these indices are AST, ALT, total proteins and albumin.Any change in the concentration of serum protein and albumin indicates a change in the normal liver function (Ahmad *et al.,* 1992). Serum albumin is a good criterion to assess the function and secretory capacity of the liver (Yakubu *et al.*, 2005).

The non-significant effect of *Nauclea latifolia* extract and its fractions on the level of albumin and total protein in the preventive and curative study gives useful information on the lack of ability of the extract to inhibit protein biosynthesis. The lack of changes in the level of albumin showed that the administration of the plant extract did not cause any impairment of liver function.

The kidney plays a very important role in removal of metabolic wastes from the blood stream. Its functionality therefore can be assessed among many others by determining the

serum concentration of excretory constituents (Spencer *et al*., 2011). The preventive and curative study of *Nauclea latifolia* extract and its fractions on the level of creatinine and urea concentrations in the serum showed no significant difference in all treatment groups after induction with P407. The lack of changes in the level of creatinine and urea showed that the administration of the plant extract and its fractions did not cause any impairment of kidney function.

The increase in superoxide dismutase (SOD) activity after the period of treatment with aqueous extract and ethyl acetate 500mg/kg body weight was suggestive of the ability of *Nauclea latifolia*extract and its fractions to boost the production of the antioxidant S.O.D. within the system of the experimental animals in the preventive study. This is in agreement with the earlier findings of Adaramoye *et al.*, (2005), which showed that the seeds of *Garcinia kola* possess antilipoperoxidative effect. Malondialdehyde (MDA) is a product of lipid peroxidation (Devaki *et al.*, 2004). An increase in the serum MDA levels is an indication of elevated level of lipid peroxidation (Trible *et al*., 1987).*Nauclea latifolia*extract and its fractions treatment which caused a decrease in the levels of MDA is in accordance with the findings of Mathew *et al.*, (2007), which suggests that the seed of *Garcinia kola* may possess the natural antioxidants necessary for protectionagainst free radical damage induced by carbon tetrachloride in rat liver, since marked decrease in the levels of lipid peroxides was recorded in rats pretreated with *Garcinia kola* seeds. *Nauclea latifolia*extract and its fractions treatment however, caused no significant ($p>0.05$) effect in the activity of catalase. This may not be conclusive, as the plant has been shown to have antioxidative properties as stated above. However, more specific research may still be done to ascertain its actual effect on the activity of catalase. Udoh and Lot (1998) reported the presence of flavonoids in the root of *Nauclea latifolia*.Flavonoids have been reported to exhibit antioxidant activity (Ramanathan *et al.,* 1989).This antioxidative effects can be attributed to the presence of natural

antioxidants e.g. flavonoids, present in the root of this plant. In the curative study, there was no significant effect in the activity of catalase and SOD. This could be as a result of the short duration (2 days) of treatment. However there was decrease in the levels of MDA in all treatment groups, this is in accordance with the findings of Mathew *et al.,* (2007).

Organ body weight ratio is an index of swelling, atrophy or hypertrophy (Swanston-Flatt *et al.,*1990). An increase in this parameter is an indication of inflammation while decrease may be due to cell constriction. The preventive and curative study of *Nauclea latifolia* shows non-significant changes in the organ body weight ratios of livers and kidneys. This demonstrates that the extract and its fractions may not cause any inflammation or cellular constriction of these organs following the findings of Moore and Dalley(1999).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1. Summary

Thesummary of this study is itemized into the following

(i) The lipid profile analysis of the preventive and curative study shows that the aqueous extract of *Nauclea latifolia* root bark and its fractions are effective in reducing the level of total cholesterol (TC), triacylglycerol (TG) and low density lipoprotein (LDL-c). Ethyl acetate 500mg had the highest reducing activity amongst all the treatment groups in total cholesterol (TC) and triacylglycerol (TG) study. In

the high density lipoprotein (HDL-c) analysis of preventive and therapeutic study, aqueous extract 500mg/kg body weight, ethyl acetate 100 and 500mg/kg body weight had the highest increase.

(ii) The atherogenic risk predictor indices of the preventive and curative study show that all treatment groups are not at risk of developing atherosclerosis.

(iii) The effect of*Nauclea latifolia* root bark treatment on liver damage indicators (AST, ALT and ALP) in the preventive and curative studies shows that the extract and its fractions had no significant effect ($p>0.05$) on the hypercholesterolemic induced animals. There was also no significant effect ($p>0.05$) of *N. latifolia* extract and its fractions on the level of albumin and total protein in the preventive and therapeutic studies.

(iv) The effect of*Nauclea latifolia* root bark treatment on kidney function parameters (urea and creatinine) in the preventive and curative studies shows that the extract and its fractions had no significant effect ($p>0.05$) on the hypercholesterolemic induced animals.

(v) *Nauclea latifolia* root bark treatments in the preventive and curative study were found to have significant effect ($p>0.05$) in reducing the level of lipid peroxidation (malondialdehyde) and increase superoxide dismutase in the hypercholesterolemic induced animals after treatment.

6.2 Conclusion

The aqueous extract of the *Nauclea latifolia* root bark and its fractions (ethyl acetate and n-butanol) at different concentrations caused a significant reduction in the concentrations of total cholesterol, triacylglycerol and low density lipoprotein cholesterol and an increase in high density lipoprotein cholesterol concentration in the hypercholesterolemic induced

animals. The plant treatment had no effect on AST, ALT, ALP, total protein and albumin concentrations in the hypercholesterolemic induced animals. The plant extract and its fractions (ethyl acetate and n-butanol) showed an increase in the activity ofsuperoxide dismutase, and a decrease in the levels of malondialdehyde of the hypercholesterolemic induced animals. These suggest that the ethyl acetate and n-butanol fractions may contain some phytochemicals that possess hypocholesterolemic effect and a remedy for oxidative stress or damage caused by free radicals in the body.

6.3 Recommendations

- Further chemical and pharmacological analysis are required for isolation, characterization and structure elucidation of the bioactive constituents of the active plant fraction so that the particular isolate with the hypocholesterolemic activity can be identified.
- There is also need for further studies on the effect of *Nauclea latifolia* root fractions on specific diseases that involve free radical-induced oxidative damage

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