EFFECT OF SELECTED VARIETIES OF *HIBISCUS ESCULENTUS* (OKRA)FRUITS EXTRACT ON LIPID PROFILE AND OXIDATIVE MARKERS OF RATS FED HIGH-FAT DIET

BY

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DECLARATION

I hereby declare that this work is the product of my research efforts undertaken under the supervision of Prof A.M Wudil and has not been presented anywhere for the award of M.Sc in Biochemistry. All Sources have been duly acknowledged.

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CERTIFICATION

This is to certify that the research work for this dissertation and the subsequent write up of this report by ASMAU AHMAD NUHU (SPS/16/MBC/00003) were carried out under my supervision.

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APPROVAL

This dissertation has been examined and approved for the award of the degree of MASTER OF SCIENCE in BIOCHEMISTRY.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

SYMBOL	INTERPRETATION
A.esculentus	*Abelmochus esculentus*
CAT	Catalase
GR	Glutathione reductase
GSH	Glutathione
GSHPx	Glutathione peroxidase
VLDL-C	Very low-density lipoprotein – cholesterol
MDA	Malondialdehyde
ROS	Reactive Oxygen specie
SD	Standard deviation
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TCA	Trichloro acetic acid
WHO	World Health Organization
TG	Triglyceride
TC	Total Cholesterol
TAG	Triacylglyceride
PPRE	Peroxisome proliferator responsive elements
HDL-C	High density lipoprotein - cholesterol
CVD	Cardiovascular disease
AI	Atherogenic Index
CHD	Coronary heart disease
PPAR-α	Nuclear regulatory protein (alpha subtype)

LIST OF TABLES

LIST OF FIGURES

Figures **Page**

ABSTRACT

This study aimed at investigating hypolipidemic effects of several varieties of *Abelmochus esculentus* fruit extracts on hyperlipidemic-induced rats' model. Five varieties of the okra plant fruit were each extracted with methanol (80%) using Soxhlet extractor. The extracts were concentrated at 30°C under reduced pressure in a rotary evaporator to a semi solid extract and finally air dried. Phytochemical contents of each of the methanol extracts were evaluated followed by proximate analysis of the selected okra fruit varieties. Out of the five varieties, two varieties with highest yield and high fiber content: *yar kolon* and *NHB-AI-B* varieties were selected in which their hypolipidemic effects were investigated on Hyperlipidemic induced rats. Hyperlipidemia was induced by feeding rats with high fats diet for 35 days period. The rats were grouped into nine groups of five rats each. Groups 1-3(hyperlipidemic rats that received different doses of *NHB-AI-B* okra fruit variety), Group 4-6 (hyperlipidemic rats that received different doses of *Yar kolon* okra fruit variety), Group 7 (Standard control), Group 8 (normal control) and group 9 (Negative control). Extracts were orally administered for 21 days. The experiment was performed according to Principles of laboratory animal care, thereafter rats were sacrificed, blood samples collected and separated, and plasma used for the determination of biochemical parameters. Animal body weight, feed and water intake were measured during the experimental period. The results of the study showed different yields of extract where *NHB-AI-B* and *Yar kolon* okra fruit varieties recorded the highest % yield (22.85 and 17.11%) and fibre content (12.51 and 14.74 %)respectively. Presence of phytochemicals like phenolics and flavonoids were detected in all the varieties. Treating hyperlipidemic rats with the methanol extract of *NHB-AI-B* and *Yar kolon* fruit varieties significantly ($p< 0.05$) reverses the altered lipid profile like triglyceride, cholesterol and high-density lipoprotein as well as antioxidant parameters when compared to the untreated hyperlipidemic rats. In conclusion, the study showed that selected varieties of okra fruit extract varied in their quantities of phytochemicals, fiber content and extract yields and *Yar kolon* variety seem to contain components that likely exert better hypolipidemic effect.

CHAPTER ONE

1.0 INTRODUCTION

1.1 CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is a class of diseases that can result from heart and/or blood vessels abnormalities, such as atherosclerosis, coronary heart disease, cerebrovascular disease, peripheral artery disease, congenital heart disease, rheumatic heart disease, pulmonary embolism and deep vein thrombosis (Mendis *et al.,* 2011). The main cause of CVD is inappropriate lifestyle, such as unhealthy diet, smoking, and lack of physical activity. These factors contribute to the development of oxidative stress, atherosclerosis, chronic inflammation and metabolic syndrome (MS) (Dudzinska *et al*., 2015). Epidemiological studies indicate that a diet rich in polyphenols may reduce the risk of CVD without changes in lifestyle (Nothlings *et al*.,2008., Mink *et al*., 2007). Among patients with established CVD, polyphenols can diminish the effects of risk factors and improve on impaired parameters caused by the development of the disease (Keli *et al*., 1996). Experimental evidence has shown that extracts from plants rich in phenolic compounds exert cardioprotective activity due to their strong free-radical scavenging, antioxidant and/or anti-peroxidative properties towards lipids (Dudzinska *et al*., 2015).

Oxidative stress is one of the important factors associated with various diseases such as cancer, cardiovascular diseases, neurodegenerative brain diseases, and diabetes (Gupta and Chari, 2005). Oxidative stress results when oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them. It not only causes hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction (Yoshikawa, 1998). Oxidative stress plays critical roles in the pathogenesis of various diseases (Brownlee, 2001). Increased oxidative stress also

underlies the pathophysiology of hypertension (Nakazono *et al*., 1991) and atherosclerosis (Ohara *et al*.,1993)
by directly affecting vascular cell wall.

Plants have been used as medical treatment since the beginning of civilization and some derivatives such as aspirin and reserpine have become mainstays of human pharmacotherapy (Mashour *et al*., 1998). For cardiovascular diseases, herbal treatments have been used in patients with related ailment (Mashour *et al*., 1998). Among the herbs used are; *Linum usitatissimum (*Flaxseed), it was reported that Flaxseed consumption lowers both total- and LDL-cholesterol concentrations due to its low–saturated fat content, high polyunsaturated fat and phytosterol content as well as mucilage content (Bruneton, 1995; Cunnane *et al*.,1993).

Okra (*Hibiscus esculentus /Abelmochus esculentus*) is the only vegetable crop of significance in the Malvaceae family and is very popular in the Indo-Pak subcontinent. In India, it ranks number one in its consumption, but its original home is Ethiopia and Sudan, the north-eastern African countries. It is one of the oldest cultivated crops and presently grown in many countries and is widely distributed from Africa to Asia, southern Europe and America. It is a tropical to subtropical crop and is sensitive to frost; low temperature, water logging and drought conditions, and the cultivation from different countries have certain adapted distinguishing characteristics specific to the country to which they belong. (Kochlar,1986).

It is an oligo purpose crop, but it is usually consumed for its green tender fruits as a vegetable in a variety of ways. These fruits are rich in vitamins, calcium, potassium and other mineral matters. The mature okra seed is a good source of oil and protein has been known to have superior nutritional quality. Okra seed oil is rich in unsaturated fatty acids such as linoleic acid,

which is essential for human nutrition. Its mature fruit and stems contain crude fiber, which is used in the paper industry (Kochlar,1986).

Phytochemical studies reported that polysaccharides, polyphenols, flavonoids, tannins, sterols and triterpenes are the major components of *A. esculentus* with various biological activities. (Arapitsas, 2008). It has been reported that the okra powder plays antidiabetic and antihyperlipidemic roles in diabetic rats. Dietary fibers and polyphenols which are abundantly found in *A. esculentus*, may contribute to the hypoglycemic and hypolipidemic effects of *A. esculentus* (Gunness and Gidley,2010).

1.1 Statement of the problem

Cardiovascular diseases are among the diseases in which the treatment is still not satisfactory, and the available drugs have restricted efficacies, very costly and advanced side effects (Dixt and Mittal, 2013). Many Plants used today to treat cardiovascular diseases by traditional medicine practitioners have not undergone careful scientific assessment to authenticate their potentials.

1.2 Justification of the study

Several studies have reported that *Abelmoschus esculentus* exert potential antidiabetic and other related diseases activities. (Subrahmanyam *et al*.,2011; Uraku *et al.,*2011, Amin,2011; Uraku *et al*., 2010; Saha *et al*., 2011; Ramachandran *et al*., 2010; Adelakun *et al*., 2009; Reddy *et al*., 2010). But, data on its cardioprotective potential is insufficient. Hence, the need to investigate its cardioprotective potential for its pharmaceutical development. Literature survey on the antidiabetic and hypolipidemic potential of *A esculentus* has not revealed which variety of the plant is most potent, hence the need of the current study to identify the variety with potent cardioprotective effect.

1.3 Aim of the study

The aim of the study is to evaluate the antihyperlipidemic and antioxidant potential of selected varieties of okra fruit (*Abelmochus esculentus)* on hyperlipidemic rats.

1.4 Objectives of the study

The objectives of the study are to;

i. Evaluate phytochemical components (Qualitative and Quantitative) of *Clemson spinless*, *NHB-AI-B*, *LD-88*, *NHAE-47-4* and *Yar Kolon* okra fruit varieties.

ii. Determine the antihyperlipidemic effect of the methanol extracts of okra fruit varieties with high fiber content and yield on serum lipid profile (HDL-C, LDL-C, VLDL-C, TG, and TC) of hyperlipidemic rats.

iii. Determine antioxidant potential of methanol extracts of okra fruit varieties with high fiber content and yield by measuring the level of antioxidant enzymes viz: Catalase, Superoxide Dismutase, and Glutathione peroxidase on hyperlipidemic rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Cardiovascular diseases

According to the World Health Organization (WHO) reports in 2017 Cardio Vascular Diseases (CVDs) are the main cause of death in most of the countries. It has been estimated that 17.7 million people died from CVDs in 2015, representing 31% of all global deaths (WHO,2017). The underlying primary cause of CVD is believed to be arteriosclerosis, coronary heart disease, cerebrovascular disease, peripheral artery disease, congenital heart disease, rheumatic heart disease, pulmonary embolism and deep vein thrombosis. Atherosclerosis is a progressive multifactorial disease of the arterial wall. Central to the pathogenesis of atherosclerosis as a result of cholesterol deposition in the arterial wall (Kumar and Clark, 2016). It has been reported that CVD may be attributable to inappropriate lifestyle, such as unhealthy diet, smoking, and the lack of physical activity.

2.2 Atherosclerosis

The underlying disease process in the blood vessels that results in coronary heart disease (heart attack) and cerebrovascular disease (stroke) is known as atherosclerosis. It is responsible for a large proportion of CVDs. In 2008, out of the 17.3 million cardiovascular deaths, heart attacks were responsible for 7.3 million deaths and strokes were responsible for 6.2 million deaths. (WHO,2008). Atherosclerosis is a complex pathological process in the walls of blood vessels that develops over many years. In atherosclerosis, fatty material and cholesterol are deposited inside the lumen of medium- and large-sized blood vessels (arteries). These deposits (plaques) cause the inner surface of the blood vessels to become irregular and the lumen to become narrow, making it harder for blood to flow through. Blood vessels also become less pliable as a

result. Eventually, the plaque can rupture, triggering the formation of a blood clot. If the blood clot develops in a coronary artery, it can cause a heart attack; if it develops in the brain, it can cause a stroke.

Risk factors include abnormal cholesterol levels, high blood pressure, diabetes, smoking, obesity, family history, and an unhealthy diet. Plaque is made up of fat, cholesterol, calcium, and other substances found in the blood. The narrowing of arteries limits the flow of oxygen-rich blood to parts of the body. Low-density lipoprotein (LDL) particles in blood plasma invade the endothelium and become oxidized, creating risk of cardiovascular disease. A complex set of biochemical reactions regulates the oxidation of LDL, involving enzymes (such as Lp-LpA2) and free radicals in the endothelium. Treatment of established disease may include medications to lower cholesterol such as statins, blood pressure medication, or medications that decrease clotting, such as aspirin. (Berry *et al*.,2012).

2.3 Hyperlipidemia

Hyperlipidemia is a systemic disorder that impairs the body in a generally unnoticeable, gradual, progressive and systemic way. The direct damage of hyperlipidemia can accelerate systemic arteriosclerosis, and it is an important risk factor for many diseases, such as stroke, coronary artery disease, myocardial infarction and cardiac sudden death (Wang *et al.,* 2012).

The close relationship between hyperlipidemia and cardiovascular diseases (CVD) has been well documented (Wang *et al.,* 2012, Daniels ,2012). The lipoprotein of LDL-cholesterol (LDL-c) has been deemed as the primary risk factor of atherosclerosis (AS) and coronary heart disease (CHD) (Baigent *et al* ., 2010, Berry *et al*., 2012), and the elevated circulating levels of free fatty acids (FFA) and triglycerides (TG) shows an important impact on the AS and CHD (Harchaoui *et al*

.,2009, Pilz *et al*., 2006). Literature have shown that modulating dysregulation of lipid metabolism by decreasing the elevated levels of serum TC, TG and LDL-C are beneficial treatment and prevention of CVD (Derosa *et al*., 2006).

2.3.1 Lipids

Lipids are water insoluble organic compounds, which are essential for many normal functions of living organisms. They are important components of cell membranes, they are used to store energy, and they play a significant role as enzyme co-factors, hormones, and intracellular messengers (Burtis and Ashwood, 1999). Of the many groups of lipids, three are most important from a clinical perspective: fatty acids, sterols (mainly cholesterol), and acylglycerols (mainly triglycerides) (Burtis and Ashwood, 1999, Ginsberg, 1998).

Dietary intake is the major source of cholesterol, but it can also be synthesized endogenously by the liver and other tissues. It plays a fundamental role in central metabolic pathways, such as bile acid metabolism and steroid hormone and vitamin D synthesis (Burtis and Ashwood, 1999, Ginsberg ,1998).

Triglycerides are the most common and efficient form of stored energy in mammal. They can be derived from both dietary sources and endogenous (hepatic) production (Burtis and Ashwood, 1999, Ginsberg,1998). Because lipids are water-insoluble molecules, they cannot be transported in aqueous solutions, such as plasma. For that reason, lipids are transported in plasma as macromolecular complexes known as lipoproteins (Watson and Barrie,1993, Johnson,2005). Lipoproteins are spherical structures that consist of a hydrophobic core containing lipids (i.e. triglycerides and/or cholesterol esters), and an amphophilic (i.e. both hydrophobic and

hydrophilic) outer layer of phospholipids, free cholesterol, and proteins that forms a protective envelope surrounding the lipid core (Burtis and Ashwood,1999, Bauer,2004, Johnson ,2005).

It is worth noting that free fatty acids are transported bound to albumin and do not require incorporation into lipoproteins for transport (Ginsberg, 1998, Whitney,1992, Bauer,2004, Johnson.,2005). Plasma lipoproteins differ in their physical and chemical characteristics such as size, density, and composition.

2.3.2 Obesity

Obesity is a chronic metabolic disorder caused by imbalance in energy intake and energy expenditure. Obesity is associated with an increase in adipogenesis, the process whereby undifferentiated preadipocytes are converted to differentiated adipocytes (Camp *et al*., 2002). Various metabolic syndromes such as non alcoholic fatty liver disease (NAFLD), insulin resistance, and hypertension are known to be associated with obesity (Holland *et al* .,2007, Kubota *et al* ., 1999) .Obesity is most commonly accompanied by liver damage, as dietary obesity promotes liver inflammation and disease (Park *et al* ., 2010). Obesity is the most common metabolic disease in developed nations and has become a global epidemic in recent years (Allan, 2004). It is associated with a variety of chronic diseases, including hyperlipidemia, diabetes mellitus, hypertension coronary artery disease and certain cancers. Furthermore, obesity, especially abdominal obesity, has an association with dyslipidemia characterized by increasing triglyceride (TG) and decreasing high-density lipoprotein cholesterol (HDL-C) concentrations (Paccaud *et al.,*2000). It is known that an oversupply of fat is associated with the development of obesity in mice. (Rebuffe-Scrive *et al.,* 1993).

Long term feeding on a high-fat diet can induce obesity with hyperphagia, hypergluconemia, hyperlipidemia and insulin resistance. (Surwit *et al*.,1988, Widdowson *et al*.,1997). In addition, it has been suggested that high-fat diet induced obesity may contribute to an increase in white and brown adipocytes growth. (Storlien *et al*., 1986).

Visceral obesity leads to insulin resistance in part mediated by adipokines and free fatty acids (FFA). Adipokines such as resistin and retinol-binding protein 4 decrease insulin sensitivity, whereas leptin and adiponectin have the opposite effect. In addition, cytokines like TNF-α and IL-6, which originate from macrophages in adipose tissue, are involved (Flock *et al*., 2011). Obesity, especially central obesity, is probably the main cause of the metabolic syndrome (MetS), which includes insulin resistance, type 2 diabetes mellitus, hypertension, the obstructive sleep apnea syndrome, non-alcoholic fatty liver disease (NAFLD) and dyslipidemia, all risk factors for cardiovascular disease .(Boden,2011, Zalesin *et al*., 2011).The typical dyslipidemia of obesity consists of increased triglycerides (TG) and FFA, decreased HDL-C with HDL dysfunction and normal or slightly increased LDL-C with increased small dense LDL. The concentrations of plasma apolipoprotein (apo) B are also often increased, partly due to the hepatic overproduction of apo B containing lipoproteins (Franssen *et al*.,2011, Wang and Peng,2011).

Hypertriglyceridemia may be the major cause of the other lipid abnormalities since it will lead to delayed clearance of the TG-rich lipoproteins (Ryu *et al.,* 1992, Castro Cabezas *et al*., 1993, Capell *et al*., 1996) and formation of small dense LDL (Hokanson *et al.,* 1995, Capell, *et al*.,1996). Lipolysis of TG-rich lipoproteins is impaired in obesity by reduced mRNA expression levels of LPL in adipose tissue (Clemente-Postigo *et al.,*2011), reductions in LPL activity in skeletal muscle and competition for lipolysis between VLDL and chylomicrons (Klop *et*

al.,2012). Increased postprandial lipemia leads to elevated levels of FFA, resulting in detachment of LPL from its endothelial surface (Peterson *et al*.,1990, Karpe *et al*.,1992). LPL may remain attached to VLDL and IDL contributing to further TG depletion. The exchange of TG from these remnants for cholesterol-esters from HDL by CETP with the concerted action of hepatic lipase, ultimately leads to the formation of small dense LDL (Capell *et al*.,1996, Hokanson *et al*.,1995).

In the presence of hypertriglyceridemia, the cholesterol-ester content of LDL decreases, whereas the TG content of LDL increases by the activity of CETP. However, the increased TG content within the LDL is hydrolyzed by hepatic lipase, which leads to the formation of small, dense LDL particles. The development of small dense LDL in obesity is mainly due to increased TG concentrations and does not depend on total body fat mass (Tchernof *et al*.,1996). Small dense LDL are relatively slowly metabolized with a five-day residence time, which enhances its atherogenicity (Packard, 2003). Chylomicron remnants and LDL may migrate into the sub-endothelium and become trapped in the sub-endothelial space where they can be taken up by monocytes/macrophages (Klop *et al*.,2012, Proctor and Mamo,2003, Proctor *et al*.,2002). Small dense LDL have an increased affinity for arterial proteoglycans resulting in enhanced subendothelial lipoprotein retention (Tabas *et al*.,2007).

It has been described that small dense LDL are more susceptible for oxidation, in part due to less free cholesterol and anti-oxidative content (Subramanian and Chait,2012). Alternatively, LPL-enriched remnants of chylomicrons and VLDL may be transported to the tissues where interaction with proteoglycans and lipoprotein receptors lead to particle removal. This process takes place at the liver and acts as an anti-atherogenic mechanism, but it may also take place in other tissues where cholesterol can not be removed efficiently leading to cholesterol

accumulation and therefore the initiation of the atherosclerotic plaque (Proctor and Mamo,2003, Pacifico *et al*.,2011, Proctor *et al*.,2004).

2.4 Mechanism of lipid regulation

Peroxisome proliferator-activated receptor alpha (PPARa) plays a pivotal role in regulation of lipid metabolism (Haemmerle *et al*.,2011, Deehan *et al.,*2012, Ferre,2004).Activation of PPARa by ligands upregulates the expression of genes involved in fatty acid transport and oxidation, such as acyl-CoA oxidase (ACO)(Pettersen *et al*.,2012, Huang *et al*.,2012), carnitine palmitoyl transferase 1 (CPT1)(Kimura *et al*.,2011, Do *et al*.,2011, Clemenz *et al*.,2008) , fatty acid transport protein (FATP) (Blanquart *et al*.,2002), hormone-sensitive lipase (HSL)(Miranda *et al.,*2011), and lipoprotein lipase (LPL)(Schafer *et al*.,2012) . By altering transcription of these genes, activated PPARa leads to increased breakdown of triglycerides and fatty acids, increased cellular fatty acid uptake, and reduced triglyceride and fatty acid synthesis. Among the PPAR-regulated genes, LPL encodes the rate-limiting enzyme for the hydrolysis of the TG core of circulating TG-rich lipoproteins, such as those found in chylomicrons and very low-density lipoproteins (VLDL) (Davies *et al*.,2012, Erickson *et al*.,2013), playing an important role in TG metabolism.

LPL not only removes TG from VLDL and decreases its size but also serves as a bridge between the cell surface and lipoproteins and promotes the uptake of LDL by cells. Therefore, LPL contributes to the clearance of both VLDL and LDL from the serum (Beisiegel *et al*.,1991). It has been reported that the hypotriglyceridemic action of PPARa agonist fenofibrate results at least in part from induction of the expression and activity of lipoprotein lipase (LPL) (Foger *et al.*,1994).

AMP-activated protein kinase (AMPK) is another key regulator of lipid metabolism, imposing profound influence on lipid oxidation, synthesis, and storage (Slack *et al*.,2012, Niu *et al*.,2012). AMPK activation turns on ATP-generating mechanisms such as lipid oxidation while switches off energy-consuming processes like TG and protein synthesis. The phosphorylation at threonine (Thr-172) on the alpha-subunit of AMPK has been deemed as an index of activation of this kinase which in turn promotes the phosphorylation and inhibition of acetyl-CoA carboxylase (ACC), a critical enzyme for controlling fatty acid biosynthesis and oxidation (Guo *et al.,*2010). AMPK has now been proposed as a major therapeutic target for obesity and obesity-linked metabolic disorders such as hyperlipidemia (Lage *et al*.,2008).

2.4.1 Lipoprotein Metabolism

Numerous metabolic processes are involved in the uptake, transport and storage of lipids. After the ingestion of a meal containing fat, TG are lipolyzed in the intestinal lumen into FFA and 2-monoacylglycerols (MAG) and are taken up by the enterocytes via passive diffusion and specific transporters like CD36 (Pan and Hussain,2011). Cholesterol is taken up by the enterocytes via the specific cholesterol transporter Niemann-Pick C1 Like 1 protein (NPC1L1) (Altmann *et al*., 2004, Davis *et al*.,2004). Once in the enterocyte, cholesterol is transformed into cholesterol-esters, whereas FFA and MAG are assembled into TG again. Finally, cholesterol-esters and TG are packed together with phospholipids and apolipoprotein (apo) B48 to form chylomicrons (Pan and Hussain,2011, Klop *et al*.,2012). After assembly, the chylomicrons are secreted into the lymphatics and finally enter the circulation via the thoracic duct.

The liver synthesizes TG-rich lipoproteins called very low-density lipoproteins (VLDL), which increase postprandially when food derived TG and FFA reach the liver (Klop *et al*.,2012). The assembly of VLDL is almost identical to the synthesis of chylomicrons, but apo B100 is the

structural protein of VLDL (and its remnants, i.e., intermediate density lipoproteins (IDL) and low density lipoproteins (LDL)) (Klop *et al*.,2012).The human liver lacks the editing complex necessary to change the apo B100 molecule into the smaller apoB48, by post-transcriptional modification of one base leading to a premature stop codon (Innerarity *et al*.,1987). Chylomicrons and VLDL deliver FFA to the heart, skeletal muscle and adipose tissue for energy expenditure and storage. Adequate lipolysis of TG-rich lipoproteins is necessary for FFA to be released in the circulation.

Lipoprotein lipase (LPL) is the primary enzyme for TG lipolysis in the circulation and is strongly expressed in tissues that require large amounts of FFA like the heart, skeletal muscle and adipose tissue (Goldberg *et al.,*2009).LPL serves as the docking station for chylomicrons and VLDL for adherence to the endothelium via glycosyl-phosphatidylinositol-anchored high-density-binding protein 1 (GPIHBP1), which is present on the luminal side of the endothelium (Dallinga-Thie *et al.,*2010, Davies *et al*.,2010). The amount of liberated FFA from chylomicrons and VLDL depends on the activity of LPL, which is stimulated by insulin (Karpe *et al*.,2011, McQuaid *et al*.,2011). In contrast, apo C-III is an inhibitor of LPL, but also of hepatic lipase. Plasma apo C-III concentrations correlate positively with plasma TG (Ooi *et al*.,2008). In addition, chylomicrons compete with endogenous VLDL for the action of LPL (Brunzell *et al.,*1973). The liberated FFA are avidly taken up by adipocytes and re-synthesized into TG within the cytoplasm where the acylation-stimulating protein (ASP)/C3adesArg pathway plays an important role (Baldo *et al.,*1993). The scavenger receptor CD36 is the best characterized FFA transporter and is abundant in muscle, adipose tissue and the capillary endothelium (Abumrad, and Davidson, 2012). Insulin and muscle contractions increase the CD36 expression thereby facilitating FFA uptake (Goldberg *et al.,*2009).

The postprandial rise in insulin is one of the most important regulatory mechanisms for fuel storage. The postprandial increase of insulin results in the effective inhibition of hormone sensitive lipase, which is the key enzyme for hydrolysis of intracellular lipids. Despite the uptake of FFA by adipocytes and myocytes, a proportion of FFA remains in the plasma compartment "spill over" where the FFA are bound by albumin and transported to the liver (Evans *et al*.,2002). When delivery of FFA for energy expenditure is insufficient like in the fasting state, FFA can be mobilized by adipose tissue for oxidation in energy demanding tissues like cardio myocytes.

Insulin is also an important regulator of FFA mobilization from adipose tissue (Karpe *et al*.,2011). Therefore, insulin resistance has a major impact on the metabolism of TG-rich lipoproteins and FFA. Eventually, chylomicrons and VLDL shrink in diameter during the process of lipolysis to form chylomicron remnants and dense LDL, respectively. Chylomicron remnants are taken up by the liver via multiple pathways including apo E, hepatic lipase, the LDL receptor, the LDL receptor-related protein and heparan sulphate proteoglycans (Hussain *et al*.,1991, Beisiegel, *et al*.,1991). In contrast, LDL is primarily taken up by the liver via the LDL receptor (Lambert *et al*.,2012). The LDL receptor is recycled and re-shuttled back to the cell surface.

Besides the above described TG and LDL metabolism, the intestine and liver also play an important role in the reverse cholesterol transport by the synthesis of HDL particles. HDL promotes the uptake of cholesterol from peripheral tissues, including the arterial wall, and returns cholesterol to the liver. Enterocytes and hepatocytes synthesize apo A-I which is the structural protein of HDL. Nascent HDL particles acquire free cholesterol from peripheral tissues. Subsequently, the cholesterol within HDL becomes esterified into cholesterol-esters by

HDL associated lecithin-cholesterol acyltransferase (LCAT) (Abumrad and Davidson,2012). Within the circulation, the HDL particles also become enriched with cholesterol-esters by the action of cholesterylester-transfer-protein (CETP) and phospholipid transfer protein (PLTP).

In this process HDL acquires TG from TG-rich lipoproteins in exchange for cholesterol-esters as a direct consequence of the CETP action (Klop *et al*.,2012). In the liver, hepatic lipase hydrolyses HDL-associated TG and also phospholipids inducing the formation of smaller HDL particles which can contribute again to the reverse cholesterol transport. Therefore, lipid metabolism is highly dynamic and depends on numerous factors including the postprandial state, TG-rich lipoprotein concentrations, HDL levels and function, energy expenditure, insulin levels and sensitivity and adipose tissue function.

2.5 Management of cardiovascular disease

2.5.1 Lifestyle Interventions

Treatment of obesity-associated dyslipidemia should be focused on lifestyle changes including weight loss, physical exercise and a healthy diet. Lifestyle changes synergistically improve insulin resistance and dyslipidemia (Klop and Castro,2012). The amount of ingested fat and total calories are the most important dietary factors to induce obesity and its related postprandial lipemia (Lopez-Miranda *et al*.,2007). Weight loss has been demonstrated to markedly reduce fasting and non-fasting TG concentrations, which can be attributed to an increase in LPL activity with a concomitant reduction in apo C-III levels (Patalay *et al*.,2005), a decrease in CETP activity (Laimer *et al.,*2009, Wang *et al*.,2011) and an increased catabolism of TG-rich lipoproteins (Chan *et al*.,2002).

The type of dietary fat also affects postprandial lipemia (Lopez-Miranda *et al*.,2007). A study in rats showed that a diet high in saturated fats reduced LPL protein levels and LPL activity in skeletal muscle, whereas LPL activity was increased in adipose tissue favoring shunting of lipids from skeletal muscle to adipose tissue (Roberts *et al*.,2002). A plant-based diet that is rich in fruit, vegetables, and legumes and low in saturated fat, along with regular aerobic exercise program, is a typical prescription for anyone with elevated risk of cardiovascular disease.

Physical exercise has been shown to increase LPL and hepatic lipase activity, which stimulates TG lipolysis (Thomas *et al*.,2001, Ferguson *et al*.,1998). The mechanism of exercise-induced LPL activity remains unclear, but it was hypothesized that exercise stimulates especially muscular LPL activity, although this could not be confirmed in a recent study (Harrison *et al*.,2012). A 12-week walking program supplemented with fish oil (1000 mg eicosapentaenoic acid and 700 mg docosahexaenoic acid daily) in subjects with the Mets resulted in lower fasting TG and decreased the postprandial response of TG and apoB48 (Slivkoff-Clark *et al*.,2012). Moreover, intra-hepatic TG content was reduced in overweight men after a low-fat diet for three weeks, whereas a high fat diet increased intra-hepatic TG (van Herpen *et al*.,2012). The plasma TG lowering effect of exercise and weight loss is the most consistent finding in studies concerning blood lipids (Mestek ,2009), whereas increasing HDL-C levels by exercise remains controversial, especially in those subjects with high TG and low HDL-C levels (Thompson and Rader,2001).

Unfortunately, lifestyle modifications are often insufficient to achieve weight loss and improvement of the dyslipidemia.

2.5.2 Pharmacological Treatment

Statins are the first-choice drug of all pharmacological agents to reduce LDL-C, non-HDL-C and/or apo B. However, statins lower TG only marginally and do not fully correct the characteristic dyslipidemia seen in obesity, which may contribute to the residual risk after initiating statin therapy (Watts and Karpe,2011). Statins inhibit the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), which is the rate limiting step in the hepatic cholesterol synthesis. This efficiently increases the fractional catabolic rate of VLDL and LDL together with a slight reduction in hepatic secretion of VLDL. Therefore, statins lower both remnant cholesterol and LDL-C levels (Chan and Watts,2011).

Recently, strategies for combination therapies with statins to achieve even lower cholesterol levels have been reviewed (Watts and Karpe,2011, Chan and Watts,2011, Dujovne *et al*.,2011, Rubenfire *et al*.,2010, Toth, 2010). Combinations can be made with ezetimibe, which inhibits the intestinal cholesterol absorption by interaction with NPC1L1, which results in an additional 20% lowering effect on LDL-C, but without affecting TG or HDL-C concentrations. On the contrary, fibrates are primarily indicated in the case of hypertriglyceridemia and they reduce TG by approximately 30% and LDL-C by 8%, whereas HDL-C is increased by an average of 9% (Rubenfire *et al*.,2010).

Fibrates (fibric acid derivatives) are peroxisome proliferator-activated receptor-α agonists, which transcriptionally regulate lipid metabolism related genes. Fibrates as monotherapy have been shown to reduce cardiovascular mortality, especially in subjects with characteristics of the MetS with TG levels > 2.20 mmol/L (Tenenbaum *et al.,*2005, Tenkanen *et al.,*2006, Rubins *et al*.,2006, Scott *et al*., 2009). However, there is controversy about the effectiveness of fibrate therapy on top of statin therapy since the ACCORD trial was unable to confirm a beneficial

effect on cardiovascular endpoints by fenofibrate combined with statins in diabetic patients (Ginsberg *et al*.,2010). Although subgroup analyses suggested a beneficial effect from combination therapy of fibrates with statins in patients with diabetes and the characteristic dyslipidemia with high TG and low HDL-C (Ginsberg *et al*.,2010). Therefore, fenofibrate may be used to treat residual dyslipidemia in diabetic patients on top of statin therapy (Watts *et al*.,2011).

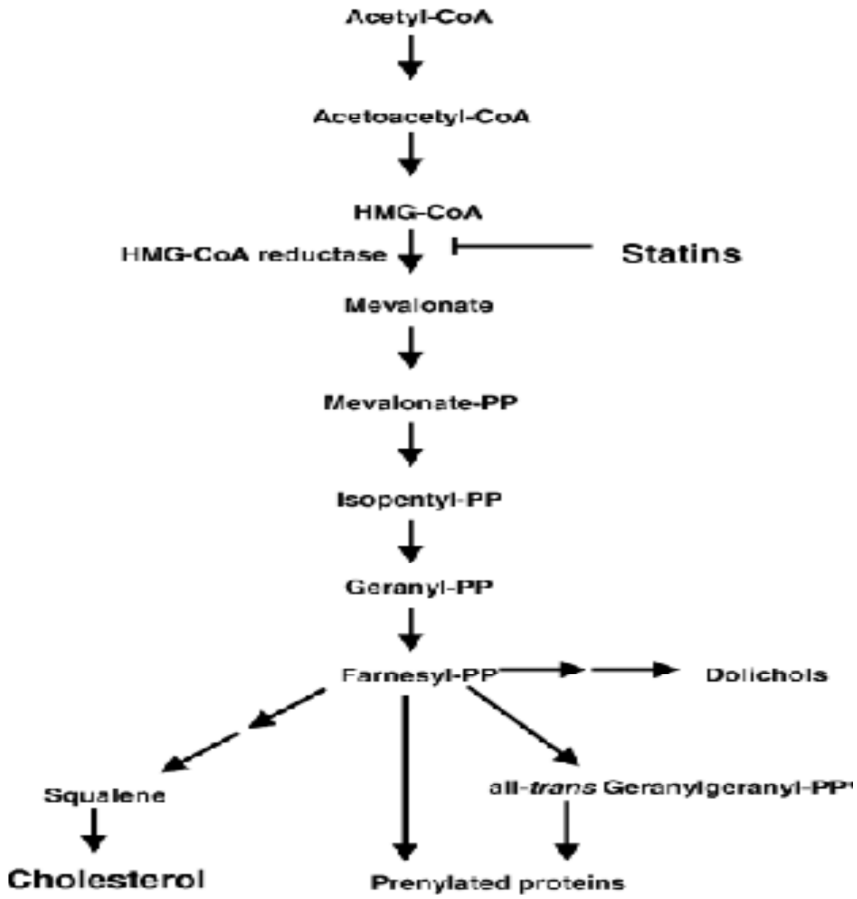
Nicotinic acid inhibits the lipolysis of adipocytes, which results in decreased FFA levels, reduced VLDL synthesis, a slight increase in HDL production rate and decreased catabolism of HDL (. Chan and Watts,2011). These changes by niacin subsequently lead to 15%–35% lower TG levels and 10%–25% higher HDL-C concentrations (Chan and Watts,2011). Recently, it has been shown that the addition of niacin to patients with a known history of cardiovascular disease, typical dyslipidemia and intensively controlled LDL-C levels with statin therapy did not lead to clinical benefit despite a reduction in fasting TG and increase in HDL-C (Boden *et al*.,2011). However, specific data concerning combination therapy of niacin with statins in obesity remains scarce.

Omega-3 fatty acids, which decrease the hepatic synthesis and accumulation of TG (Watts *et al*.,2006), have been shown to reduce plasma TG by 25%–30% by effectively reducing the hepatic secretion of VLDL in insulin resistant subjects (Chan and Watts,2011, Chan *et al*.,2002,). Omega-3 fatty acids have also been shown to increase the conversion of VLDL into IDL, which suggests an additional benefit for combining omega-3 fatty acids with statins by increased catabolism of VLDL, IDL and LDL (Chan *et al*.,2002).

Drugs that increase insulin sensitivity like metformin or thiazolidinedione derivatives, have no (Castro Cabezas *et al*.,2012) or minimal effects on the lipoprotein profile in obesity (Van Wijk *et al*.,2003). In the case of thiazolidinedione derivatives, their mode of action causes an increase of body weight, due to expansion of the subcutaneous fat compartment, which makes these drugs less appropriate in the case of obesity (Van Wijk *et al*.,2003).

2.5.3 Mechanism of Action

3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) act by blocking the HMG-CoA reductase enzyme, which catalyzes the rate-limiting step in *de novo* cholesterol synthesis. All statins are competitive inhibitors of HMG-CoA reductase with respect to the binding of the substrate, HMG-CoA, but not for that of the co-enzyme NADPH, suggesting that their HMG-CoA-like moieties bind to the HMG-CoA-binding portion of the enzyme active site. Comparison of the six statin–enzyme complexes revealed subtle differences in their modes of binding. An additional hydrogen bond was demonstrated in the Atorvastatin and Rosuvastatin enzyme complexes along with a polar interaction unique to Rosuvastatin, such that Rosuvastatin has the most binding interactions with HMG-CoA reductase of all the statins (Istvan, 2001).



2.6 High Fat Diet

A high fat diet is a diet in which the 30% of the energy comes from the fat present in the food. It is usually added to the feed of the animals.

2.6.1 High fat diet and hyperlipidemia

Dietary fat intake often has been claimed as responsible for the increase in adiposity. Human studies have shown that high-fat diets (30% of energy from fat) can easily induce obesity (Jequier ,2002, Hill *et al*.,2000). Epidemiological studies conducted in countries such as China, Canada and the USA have shown that, when the average amount of fat in the diet increases, the incidence of obesity also increases (Popkin *et al*.,1993, Saris *et al.,*2000). This has led to a worldwide effort to decrease the amount of fat in the human diet. Diets rich in fat not only

induce obesity in humans but also make animals obese (Buettner *et al*.,2007). In both rats (Ghibaudi *et al*.,2002) and mice (Takahashi *et al*.,1999). A positive relationship has been found between the level of fat in the diet and body weight or fat gain. In the scientific literature it was first shown that rats consuming diets containing high proportions of fat gained weight faster than those on diets containing minimal amounts of fat (Deuel *et al*.,1947). Usually high-fat diets within the range of 30–78% of total energy intake are used(Buettner *et al.,*2007),either by adding a particular fat to the animal's diet or using an assortment of fat- and sugar-rich supermarket foods (cafeteria diet) – for studying obesity in rats(Ghibaudi *et al.,*2002),and mice(Huang *et al*.,2004). The use of high-carbohydrate–low fat diets has not been found as efficient as high-fat– low-carbohydrate diets in inducing obesity (Ellis *et al*.,2002).

Other factors that may contribute to obesity induced by a diet rich in fat include failure to adjust oxidation of fat to the extra fat in the diet (Schrauwen and Westerterp,2000), increase in adipose tissue lipoprotein lipase activity (Preiss *et al*.,2002), increased meal size and decreased meal frequency (Westerterp-Plantenga .,2004), as well as overconsumption of energy attributed to high energy density of the diet (Rolls,2000), orosensory characteristics of fats and poorly satiating properties of the high-fat diets (Warwick and Schiffman,1992).

Obesity occurs when energy uptake surpasses energy expenditure in the individual animal and so the stores of energy in body fat are enlarged, particularly in adipose tissues. Obesity involves both or either an increase in the number of adipocytes (hyperplasia) and their size (hypertrophy)(de Ferranti and Mozaffarian,2008).In humans, a significant positive relationship has been found between the amount of dietary energy from fat and the proportion of the population who are overweight (in epidemiological studies), and in clinical studies between the level of dietary fat and body-weight gain as well as between the reduction in the dietary fat and weight loss (Popkin

et al.,1993).These associations have also been shown in animal studies (Ghibaudi *et al*.,2002).This relationship in humans or in animal models of more dietary fat leading to greater obesity shows that the fat content of the diet is an important factor in energy balance. In general, diets containing more than 30% of total energy as fat lead to the development of obesity.

Some reports have attributed obesity induced by high-fat diets to their high food efficiency (g bodyweight gain per kJ food consumed). Energy from fat has a larger effect on body-weight gain than has energy from non fat sources (Bray and Popkin,1998, Prpic *et al*.,2002).Diet-induced thermogenesis is the energy for digesting, absorbing and storing nutrients and produces a loss of energy for the body which is 2–3% for fats, 25–30% for proteins and 6–8% for carbohydrates. Therefore, the efficiency of nutrient utilisation differs among macronutrients and fats have an efficiency of 97–98%, whereas efficiency is 70–75% for proteins and 92–94% for carbohydrates (Jequier,2002).In addition, it costs energy to build long-chain fatty acids from glucose or amino acids, whereas dietary fat contains long-chain fatty acid pre-formed.

2.6.2 High fat diet and oxidative stress

A link between high-fat diet (HFD) and oxidative stress (OS) has been recognized for long (, Turpeinen *et al*.,1998, Oliveros *et al.,*2004, Vijayakumar *et al*.,2004). It has been suggested that long-term feeding of a high-saturated fat diet acts as an inducer of OS, since it significantly attenuates the hepatic enzyme antioxidant system, and increases the levels of lipid peroxidation (LPO) products in the liver and plasma (Oliveros *et al.,*2004, Vijayakumar *et al*.,2004). Other reports suggest a role for the OS in pathogenesis of metabolic derangements leading to IR and obesity (Matsuzawa-Nagata *et al*.,2008) and diabetes mellitus (Simmons, 2006). It has been argued that an increase in OS precedes the development of obesity and metabolic derangements that are induced by an HFD (Matsuzawa-Nagata *et al*.,2008) and as such it would be conceivable

that amelioration of the elevated OS at this stage could possibly prevent or limit the extent of the subsequent metabolic perturbations and its sequelae. OS as well has been shown to play a major role in organ pathophysiology and is associated to organ dysfunctions (Murdolo *et al*.,2013, Rashid *et al*.,2013).

Plant-derived polyphenols and polyphenol metabolites have long been recognized for its prominent antioxidative benefits, with relatively minimal adverse actions. Antioxidant therapies with phytochemicals have been shown to be effective in suppressing multiple OS pathways, particularly in relation to obesity and its pathophysiological sequels (Rodrigo *et al*.,2014).

2.7 Medicinal plants used for the management of cardiovascular disease

Herbs have been used as food and for medicinal purposes for centuries. Research interest has focused on various herbs that possess hypolipidemic, antiplatelet, antitumor, or immune-stimulating properties that may be useful adjuncts in helping reduce the risk of cardiovascular disease and cancer. In different herbs, a wide variety of active phytochemicals, including the flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, and phthalides have been identified. Several of these phytochemicals either inhibit nitrosation or the formation of DNA adducts or stimulate the activity of protective enzymes such as the Phase II enzyme glutathione transferase. (Cook *et al*., 2000).

Several researches have centered around the biochemical activity of the Allium sp. and the Labiatae, Umbelliferae, and Zingiberaceae families, as well as flaxseed, licorice root, and green tea. Many of these herbs contain potent antioxidant compounds that provide significant protection against chronic diseases. These compounds may protect LDL cholesterol from oxidation, inhibit cyclooxygenase and lipoxygenase enzymes, inhibit lipid peroxidation, or have

antiviral or antitumor activity. The volatile essential oils of commonly used culinary herbs, spices, and herbal teas inhibit mevalonate synthesis and thereby suppress cholesterol synthesis and tumor growth.

The World Health Organization estimated that <80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts or their active components. Furthermore, many Western drugs had their origin in a plant extract. Reserpine, which is widely used for the treatment of high blood pressure, was originally extracted from the plant Rauwolfia serpentina, whereas digitalis, used as a heart stimulant, was derived from the foxglove plant (Digitalis purpurea) (WHO, 2017).

Studies of fenugreek (Trigonella foenum-graecum) have shown that it may also have hypocholesterolemic activity. Subjects with elevated blood cholesterol concentrations who consumed powdered fenugreek seeds experienced a significant reduction in LDL cholesterol and triacylglycerol concentrations without any change in HDL-cholesterol concentrations (Sharma and Raghuram, 1990, Sharma *et al*., 1991). Asian ginseng (Panax ginseng) is another medicinal plant with a long history of use. Researchers have discovered a non saponin fraction in ginseng root that inhibits platelet aggregation by potently inhibiting thromboxane A2 production (Park *et al*.,1995).

Phytochemicals like flavonoids have extensive biological properties that promote human health and help reduce the risk of disease. Flavonoids extend the activity of vitamin C, act as antioxidants, protect LDL cholesterol from oxidation, inhibit platelet aggregation, and act as anti-inflammatory and antitumor agents (Manach *et al*.,1996, Smith and Yang,1994, Cook and Samman,1996). The Zutphen study of elderly men in the Netherlands found that flavonoid intake

from fruit, vegetables, and tea was inversely associated with heart disease mortality and incidence of heart attack and stroke over a 5-years period. Subjects who had the highest consumption of flavonoids had 60% lower mortality from heart disease and a 70% lower risk of stroke than those who consumed low amounts of flavonoids (Hertog *et al*.,1993, Keli *et al*.,1996).

Studies shows that licorice extract and the isoflavan glabridin, a major polyphenolic compound found in licorice, were shown to markedly inhibit LDL oxidation via a mechanism involving scavenging of free radicals (Fuhrman *et al*.,1997). Several epidemiologic studies have suggested that drinking either green or black tea may lower blood cholesterol concentrations and blood pressure, thereby providing some protection against cardiovascular disease (Imai and Nakachi,1995, Kono *et al*.,1996). When rats were fed green tea polyphenols, blood cholesterol concentrations declined in hypercholesterolemic animals and blood pressure decreased in spontaneously hypertensive animals (Dreosti,1996). Some of these effects may be explained by the capacity of green tea catechins and gallate esters to reduce intestinal cholesterol absorption, lower blood coagulability, and inhibit proliferation of human aortic smooth muscle cells (Dreosti,1996).

A variety of phenolic compounds, in addition to the flavonoids, are found in fruit, vegetables, and many herbs. These phenolics influence the quality and stability of foods by acting as flavorants, colorants, and antioxidants. The phenolic compounds (such as caffeic, ellagic, and ferulic acids, sesamol, and vanillin) also exhibit anticarcinogenic activity and inhibit atherosclerosis (Decker, 1995). Many of the phenolic phytochemicals that have been shown to provide protection against heart disease and cancer are metabolites of the shikimic acid pathway.

Anthocyanins are the water-soluble pigments responsible for the red, pink, mauve, purple, blue, and violet hues of many types of flowers and fruit.

2.8 The plant "*Abelmoschus esculentus/ Hibiscus esculentus*"

Abelmoschus esculentus L. (Moench) also referred to as ladies finger is commonly known as "*Kubewa"* (Hausa), Ookro (Igbo) and l"laa (Yoruba). It is an important vegetable crop in tropical, subtropical and warm temperate regions around the world with total trade estimated to over $5 billion (NRC,2006, Kumar *et al*., 2010, Benchasri, 2012, Lim, 2012). Okra is an annual or perennial tall (around 2 meters) dicotyledonous plant related to species such as cotton, cocoa and Hibiscus.

Abelmochus esculentus is the only vegetable crop of significance in the Malvaceae family and is very popular in the Indo-Pak subcontinent. In India, it ranks number one in its consumption, but its original home is Ethiopia and Sudan, the north-eastern African countries (Kumar *et al*.,2013). The plant grows preferably in well-drained humus rich fertile soil in full sun with pH ranging from 6 to 6.7, but it can tolerate a wide range of soil types and pH from 5.5 to 8.0 (Jain *et al*.,2012). The leaves are long-petiole, orbicular or orbicular-ovate around 10–20 cm long, broad and rough, palmately lobed with 5–7 lobes. Flowers of this plant are axillary and solitary, 4–8 cm in diameter having five white to yellow petals, often with a red or purple spot at the base of each petal. Fruit is elongated, 10 to 25 cm long, 1.5 to 3 cm in diameter, tapering to a blunt point and containing rows of rounded, and kidney shaped seeds. Depending on the cultivar, fruits of Okra mature after 60-180 days of sowing (alternatively can also be counted 5-10 days after flowering of plant). Fruits are detached from the stacks by applying slight twist (Tindall,1986). Irritating hairs are sometimes present on leaves, stems and on the fruit surface. Immature fresh and green seed pods are consumed as vegetable. It offers mucilaginous consistency after

cooking. Often the extract obtained from the fruit is added to different recipes like soups, stews and sauces to increase the consistency.

The immature pods are also used in making pickle. The entire plant is edible and is used to have several foods (Babu and Srinivasan,1995, Madison,2008, Lim,2012, Maramag,2013) and non food applications (Camciuc *et al*.,1998). Okra leaves are to some extent edible and are used as salad when fresh or cooked for edible purposes as the greens of beets or dandelions.

2.8.1 Nutritional composition of Okra

K, Na, Mg and Ca are the principal elements in pods, which contain about 17 % seeds; the presence of Fe, Zn, Mn and Ni also has been reported (Moyin-Jesu,2007). Fresh pods are low in calories (20 per 100 g), practically no fat, high in fiber, and have several valuable nutrients, including about 30% of the recommended levels of vitamin C (16 to 29 mg), 10 to 20% of folate (46 to 88 g) and about 5% of vitamin A (14 to 20 RAE) (NAP,2016). Both pod skin (mesocarp) and seeds are excellent source of zinc (80 g/g) (Glew,1997, Cook *et al*., 2000).

Okra seed is mainly composed of oligomeric catechins (2.5 mg/g of seeds) and flavonol derivatives (3.4 mg/g of seeds), while the mesocarp is mainly composed of hydroxylcinnamic and quercetin derivatives (0.2 and 0.3 mg/g of skins). Pods and seeds are rich in phenolic compounds with important biological properties like quarcetin derivatives, catechin oligomers and hydroxycinnamic derivatives. (Arapitsas,2008). These properties, along with the high content of carbohydrates, proteins, glycoprotein, and other dietary elements enhance the importance of this foodstuff in the human diet. (Manach *et al*., 2005, Arapitsas,2008).

Dried okra sauce (pods mixed with other ingredients and regularly consumed in West Africa) does not provide any beta carotene (vitamin A) or retinol. (Avallone *et al*., 2008). However, fresh okra pods are the most important vegetable source of viscous fiber, an important dietary

component to lower cholesterol. Seven-days-old fresh okra pods have the highest concentration of nutrients. (Habtamu F *et al.,*2015).

Okra is more a diet food than staple (National Research Council, 2006). Okra seeds have been used on a small scale for oil production. Lipid components greatly contribute to the nutritional and sensory value of almost all types of foods. Okra seed oil is a rich source of linoleic acid, a polyunsaturated fatty acid essential for human nutrition (Savello *et al*., 1980). Proteins play a particularly important role in human nutrition. Okra has been called "a perfect villager's vegetable" because of its robust nature, dietary fiber, and distinct seed protein balance of both lysine and tryptophan amino acids (unlike the proteins of cereals and pulses) (Holser and Bost, 2004, Sanjeet *et al*., 2010).

2.8.2 Medicinal importance of Okra

In recent years, increasing attention has been paid to the role of diet in human health (Ohr,2004). The high intake of plant products is associated with a reduced risk of a number of chronic diseases, such as atherosclerosis and cancer (Gosslau and Chen, 2004). These beneficial effects have been partly attributed to the compounds which possess antioxidant activity. Atawodi *et al*., (2009) has reported *in vitro* antioxidant assay of methanolic extract of okra fruits. In addition, Arapitsas, (2008) reported that Okra seed is rich in Phenolic compounds, mainly composed of flavonol derivatives and oligomeric catechins. Other antioxidants are vitamins C and E, carotenoids etc. These antioxidants scavenge radicals and inhibit the chain initiation or break the chain propagation (the second defense line). Vitamin E and carotenoids also contribute to the first defense line against oxidative stress, because they quench singlet oxygen (Krinsky, 2001). Flavonoids as well as vitamin C showed a protective activity to α-tocopherol in human LDL, and

they can also regenerate vitamin E, from the α chromanoxy radical (Davey *et al*.,2000). Okra is used in folk medicine as antiulcerogenic, gastroprotective, diuretic agents (Gurbuz,2003)

Okra is used to treat digestive disorders. The polysaccharides present in immature okra pods possessed considerable antiadhesive properties (i.e. they help remove the adhesion between bacteria and stomach tissue, preventing the cultures from spreading). Okra's polysaccharides were particularly effective at inhibiting the adhesion of Helicobacter pylori, a bacterium that dwells in the stomach and can cause gastritis and gastric ulcers if left unchecked. Therefore, eating more okra can keep our stomach clean and create an environment that prevents destructive cultures from flourishing (Messing *et al*., 2014).

The soluble fiber of okra helps to reduce serum cholesterol and therefore decreases the chance of cardiovascular disease. Consuming okra is an efficient method to manage the body's cholesterol level. Okra is additionally loaded with pectin that can help in reducing high blood cholesterol simply by modifying the creation of bile within the intestines (Ngoc *et al*., 2008). Okra is also used to improves good eyesight. The okra pods are fantastic options for Vitamin A and also beta carotene that are both important nourishment for sustaining an excellent eyesight along with healthy skin. (Messing *et al*.,2014).

Literature shows that administration of different doses of peel and seed powder of okra significantly increased liver, kidney and pancreas superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) levels and decreased thiobarbituric acid reactive substances (TBARS) (Liao *et al*.,2012). A study has reported the antioxidant potentials of different organs; flower, fruit, leaf, and seed of fractions of water extracts of *A. esculentus* plant where it was linked with their phenolics and flavonoids contents. The study

found that, the flower of okra has the highest amount of total phenolics and total flavonoids (Liao *et al*.,2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment

UV spectrophotometer, centrifuge, Rotary evaporator, Soxhlet extractor, Micropipette, Weighing balance, water bath, Muffle Furnace, Desiccator, Buchner funnel, Oven.

3.1.2 Chemicals

All chemicals used for the study were of analytical grade and were obtained from Sigma Aldrich, England and British Drug House (BDH), London. Lipid profile and Antioxidant assay kits were obtained from Randox Lab., UK, Atorvastatin drug, Methanol, Folin–Ciocalteu reagent, sodium carbonates, Gallic acid, ethanol aluminum nitrate, potassium acetate, quercetin, tannin acid Folin-Denis reagent, sodium carbonate, vanillin reagent, Sulphuric acid, Diosgenin, Sodium hydroxide, Hydrochloric acid.

3.1.3 Experimental Animals

Forty-five Wistar albino rats weighing 65-85g used in this study were obtained from the Animal House, Department of Plant Biology, Bayero University, Kano. The rats were kept in cages and fed with animal feed (Vital feed, Jos) and water for two weeks to acclimatize before the commencement of the experiment. Principles of laboratory animal care (NIH, 1996) and ethical guidelines for investigation of experimental pain in conscious animals were observed during experimentation (Zimmermann,1983).

3.1.4 Plant Collection/Identification

Abelmochus esculentus fruit Varieties were obtained from a farm at Faculty of Agricultural Sciences, Abubakar Tafawa Balewa University, Bauchi. The plant material was identified and authenticated at the Department of Biological Science, Abubakar Tafawa Balewa University, Bauchi. (Voucher number:1914)

3.2 Methods

3.2.1 Plant Extraction

Okra fruit varieties were each extracted following the method described by Doreddula *et al* (2014). with modification in extraction time (12 h). The okra was grounded using pestle and mortar. The powdered material was then sonicated in 80 % methanol for 1 h and then extracted using Soxhlet for 12 h at room temperature. The extract was filtered, and the filtrate was concentrated at 30^0C under reduced pressure in a rotary evaporator until a crude solid extract was obtained which was then air dried. The dried extract was put in an air-tight container and kept in a refrigerator at 4^oC until used.

3.2.2 Phytochemical Screening

Preliminary qualitative phytochemical tests for the detection and quantification of phenols, flavonoids, alkaloids, tannins, saponins, and glycosides was conducted using methods descried by AOAC (1984).

3.2.3 Quantitative phytochemicals Screening

Determination of total phenolic content

The total phenolic content of extract was measured using Folin–Ciocalteu reagent. The extract was solubilized in distilled water. After that ,1 ml of sample was mixed with Folin–Ciocalteu reagent (5 ml), sodium carbonates (4 ml) and distilled water (5 ml). This solution was kept at

room temperature for 30 min, and the absorbance of solution was measured at 760 nm in a spectrophotometer. A set of reference standard solutions of gallic acid (1, 2, 3, 4 and 5 ppm) were prepared in the same manner as described earlier. Total phenolic content was calculated using gallic acid as standard (Madaan *et al*.,2011).

Determination of total flavonoid content

Aqueous extract (500 µl) was mixed with ethanol (1.5 ml), aluminum nitrate (100 ml, 10 %), potassium acetate (100 ml, 1 M) and water (2.8 ml). The solution was kept at ambient temperature for 40 min and the absorbance of solution was measured at 425nm using a spectrophotometer. Total flavonoid content was recorded according to a standard established curve with quercetin (Mohsen and Ammar, 2008).

Determination of tannin content

Stock solution of 1 mg/ml of tannin acid was prepared by dissolving 10 mg of accurately weighed tannic acid in water. Aliquots(10ml) were taken in clear test tube and 1 ml of Folin-Denis reagent, 1 ml of sodium carbonate solution were added to each test tube. Each tube was made up to 10ml with distilled water. All the reagents in each tube were mixed well and kept undisturbed for about 30 min and read at 760 nm against blank reagent in a spectrophotometer. A set of reference standard solutions of Tannic acid (1, 2, 3, 4 and 5 ppm) were prepared in the same manner as described earlier (Polshettiwar *et al*.,2007).

Quantitative determination of total saponin

Extracts (10ml) were dissolved in 5 ml of 50% aqueous methanol. 2.5 ml of aliquot was transferred to test tubes into which an equal volume of vanillin reagent (8 %) was added followed by 72% (v/v) sulphuric acid. The mixture was mixed and placed in a water bath adjusted at 60 °C for 10 min. The tubes were cooled on an ice-cold water bath for 3 to 4 min and

absorbance of yellow color reaction mixture was measured at 544 nm using a UV–Vis spectrophotometer

(UV–1800 Shimadzu) against a blank containing 50% aqueous methanol instead of sample extract. A set of reference standard solutions of Diosgenin (1, 2, 3, 4 and 5 ppm) were prepared in the same manner as described earlier. The saponin concentrations were calculated from standard curve and expressed as mg Diosgenin equivalents (DE) per g crude extract. (Hiai *et al*;1976, Baccou *et al*.,1977).

3.2.4 Proximate Analysis of Okra Varieties

Determination of Moisture content (AOAC 2000)

Okra(2g) were placed in the crucible and heated at 105^{o}C, until a constant weight was attained. The moisture content of each of the sample was calculated as loss in weight of the original sample and expressed as percentage moisture content.

$$(\%)\ \text{Moisture} = \frac{\text{Initial weight of sample} - \text{Final weight of samples}}{\text{Initial weight of sample}} \times 100$$

Determination of Ash content (AOAC 2000)

The total ash content of a substance is the percentage of inorganic residue remaining after the organic matter has been ignited. Okra samples (2g) were placed in a crucible and ignited in a muffle furnace at 550^{o}C for 6 hours. It was then cooled in a desiccator and weighed at room temperature to get the weight of the ash using the formula.

$$(\%)\ \text{Ash content} = \frac{\text{Weight of ash}}{\text{Initial weight of sample}} \times 100$$

Determination of Crude fiber content

The estimation was done using the method of AOAC (1990). Okra varieties (5 g) were boiled in 200ml of 1.25% H_2SO_4 for 30 minutes, and afterwards filtered with a Buchner funnel. The residue was washed with distilled water until it was acid free. It was then boiled in 200ml of 1.25% NaOH for another 30 minutes, filtered and washed until it was also alkaline free. It was then rinsed with 10% HCl and twice with ethanol. The residue was dried at 105^{o}C in an oven. After cooling in a desiccator, it was ignited in a muffle furnace at 550^{o}C for 90 minutes to obtain the weight of the ash.

$$(\%)\ \text{Crude fiber content} = \frac{\text{Weight loss on ignition}}{\text{Initial weight of sample}} \times 100$$

3.2.5 Feed formulation

A modified method of Vesselinvitch *et al.* (1980) was used to induce hyperlipidemia. Fully grown rats were exposed to high fat diet formulation for six weeks. The diet was formulated by adding 5% egg yolk, 20% palm oil to 75% pelletized super starter feed. Body weight gain was recorded weekly.

3.2.6 Pre-treatment

Prior to treatment, animals were made hyperlipidemic by feeding them with high fat diet for 35 days period. Thereafter, hyperlipidemic rats were selected and grouped into eight as follows; group I, II, III, IV, V, VI, VII, and IX. The animals were confirmed hyperlipidemic by ascertaining their serum lipid profile levels in comparison to the normal rats.

3.2.7 Animal grouping/treatment

Following the grouping from the pre-treatment, animals were divided into nine groups of five rats each. The grouping details are:

Groups I, II and III: rats fed high fat diet and treated with 250mg/kg, 500mg/kg and 750mg/kg of *NHB-AI-B* okra fruit extract variety respectively.

Groups IV, V, and VI: rats fed high fat diet and treated with 250mg/kg, 500mg/kg and 750mg/kg of *Yar kolon* okra fruit extract variety respectively.

Group VII served as standard control: rats fed high fat diet and treated with standard drug (10mg/kg Atorvastatin)

Group VIII served as normal control: rats fed normal diet

Group IX served as negative control: rats fed high fat diet and no treatment.

3.2.8 Determination of water and feed intake

Water and feed intake of the experimental animals were determined daily, water was measured using measuring cylinder before giving to the animals and after 24hrs. Feed was also weighed using weighing balance before giving to the animals and after 24hrs.

3.2.9 Determination of body weight

The weight of the animals was determined weekly throughout the experimental period.

3.2.10 Collection of Blood Samples and Preparation of Serum

Twenty-four hours after the last treatment, the animals were subjected to 12 hours fasting after which the animals were anaesthetized by dropping each individual animal in a plastic jar saturated with chloroform vapor. The animals were then sacrificed and blood samples collected into labelled plastic specimen bottles containing EDTA (disodium ethylinediamine tetraacetate)

The sera obtained were pipetted into labelled specimen test tubes for estimation of serum lipid profiles and antioxidant enzymes.

3.2.11 Estimation of Total Cholesterol

Serum total cholesterol (TC) was estimated by enzymatic method using Randox kit (Allain *et al.,* 1974).

Principle

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4 – aminoantipyrine in the presence of phenol and peroxidase. The absorbance of the dye is measured spectrophotometrically at 500nm. (Cholesterol ester in serum is hydrolyzed by cholesterol esterase. The total cholesterol is then oxidized by cholesterol oxidase to the corresponding ketone. The H_2O_2 formed is decomposed by peroxidase in the presence of 4 – aminoantipyrine and phenol to yield a quinoneimine dye. The absorbance of the dye was measured spectrophotometrically at 500nm which is proportional to the concentration of cholesterol).

The equations for the reaction are:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\textit{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acids}$$

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

$$2H_2O_2 + \text{Phenol} + 4 - \text{aminoantipyrine} \xrightarrow{\textit{proxidase}} \text{Quinoneimine} + 4H_2$$

Procedure

Three test tubes were set up and labelled blank, test and standard as follow:

	Blank	Test	Standard
Serum (µl)	-	10	-
Standard cholesterol (µl)	-	-	10
Distilled water (µl)	10	-	-
Reagent (µl)	1000	1000	1000

Into test tubes labelled test, standard and blank, 10 µl of serum, standard (200 mg/dl) and distilled water were respectively pipetted in to the test tubes. Each test tube was then followed by 1000 µl of the reagent as shown above.

The test tubes were mixed, incubated at 37^{o}C for 5 minutes and the absorbance of the standard and test were read against the blank at 500 nm against the reagent blank in a spectrophotometer.

Calculation

Cholesterol concentration was obtained using the relation:

$$\text{Serum total cholesterol (mg/dl)} = \frac{Absorbance\ of\ Test}{Absorbance\ of\ \text{Standard}} \times \text{Conc of Standard}$$

3.2.12 Estimation of Serum Triglyceride

This was assayed by the method of Tietz (1990), using Randox Kit.

Principle

The triacylgycerols were estimated after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from H_2O_2, 4- aminophenazone and 4 – chlorophenol under the catalytic influence of peroxidase (POD).

The equations for the reactions are:

$$\text{Triglycerides} + H_2O \xrightarrow{Lipase} \text{Glycerol} + \text{fatty acid}$$

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

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{Glycerol\ Phosphate\ Oxidase} \text{Dihdroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4 - aminophenazone} + \text{4 - chlorophenol} \xrightarrow{Peroxidase} \text{Quinoneimine} + HCl + 4H_2O$$

Procedure

Three test tubes were set up as follows:

	Blank	**Test**	**Standard**
Serum (µl)	-	10	-
Standard triglyceride (µl)	-	-	10
Distilled water (µl)	10	-	-
Reagent (µl)	1000	1000	1000

The tubes were mixed and incubated at 37^{o}C for 5 minutes and the absorbance of the standard and tests were read at 500nm against the blank in a spectrophotometer.

Calculation: The TG levels were calculated using the relation:

$$\text{Serum TG (mg/dl)} = \frac{Absorbance\ of\ Test}{Absorbance\ of\ \text{Standard}} \times \text{Conc. of Standard}$$

3.2.13 Estimation of Serum HDL – C

This was done by enzymatic method of Burstein *et al.,* (1970) using Randox Kit

Principle

Low density lipoproteins, very low-density lipoproteins and chylomicron fractions are precipitated quantitatively by the addition of phosphotungastic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the high-density lipoprotein fraction, which remains in the supernatant, is determined spectrophotometrically at 500 nm.

Procedure

Into centrifuge tubes, 200 µl of serum and 500 µl of precipitant (0.55 mmol/L phosphotungastic acid and 25 mmol/l Magnesium Chloride) were added, mixed and allowed to stand for 10 minutes at room temperature. The tubes were centrifuged for 10 minutes at 4000 rpm. The supernatant was collected and used for the analysis.

Three test tubes were then set up and labelled blank, standard and test, as follows:

	Blank	**Standard**	**Test**
Distilled water (µl)	100	-	-
Supernatant (µl)	-	-	100
Standard supernatant(µl)	-	100	-
Reagent (µl)	1000	1000	1000

The tubes were mixed and incubated for 5 minutes at 37^0 C and the absorbance of the samples and standard were measured against the reagent blank at 500nm in a spectrophotometer.

Calculation

The HDL-C concentration was obtained from the relation:

$$\text{Serum HDL-C (mg/dl)} = \frac{\textit{Absorbance of Test}}{\textit{Absorbance of Standard}} \times \text{Conc. of Standard}$$

3.2.14 Estimation of Serum LDL – C

Low Density Lipoprotein Cholesterol (LDL-C), was calculated using Friedewald *et al* ;(1972) formula

$$\text{LDL} - \text{C(mg/dl)} = [\text{TC} - (\text{HDL-C} + \text{TAG}/5)]$$

3.2.15 Estimation of Serum VLDL – C

Very Low-Density Lipoprotein Cholesterol (VLDL-C) was calculated by Friedewald *et al;* (1972) formula

$$\text{VLDL-C(mg/dl)} = [\text{TAG}/5].$$

3.2.16 Determination of Super-oxide dismutase activity

This was carried out by the method described by, (Misra and Fridovich,1972).

Principle

The ability of superoxide dismutase to inhibit the autoxidation of epinephrine at pH 10.2 makes this reaction a basis for a simple assay for this dismutase. Superoxide ($O_2\bullet^-$) radical generated by the xanthine oxidase reaction caused the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per $O_2\bullet^-$ introduced increased with increasing pH (Valerino and McCormack, 1971) and also increased with increasing concentration of epinephrine. These results led to the proposal that autoxidation of epinephrine proceeds by at least two distinct

pathways, only one of which is a free radical chain reaction involving superoxide ($O_2^{\bullet -}$) radical and hence inhabitable by superoxide dismutase.

Procedure

Sample (0.2ml) was diluted in 0.8 ml of distilled water to make a 1 in 5 dilution. An aliquot of 0.2 ml of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

Calculation

Increase in absorbance per minute = $\frac{A_3 - A_0}{2.5}$

Where A_0 = absorbance after 30 seconds

A_3=absorbance after 150 seconds

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

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline.

3.2.17 Determination of Catalase activity (Claiborne, 1985)

Principle

This method is based on the loss of absorbance observed at 240 nm as catalase splits hydrogen peroxide. Despite the fact that hydrogen peroxide has no absorbance maximum at this wavelength, its absorbance correlates well enough with concentration to allow its use for a quantitative assay. An extinction coefficient of 0.0041 mM^{-1} cm^{-1} (Noble and Gibson,1970) was used.

Procedure

Hydrogen peroxide (2.95 ml of 19 mM solution) was pipetted into a 1 cm quartz cuvette and 50 µl of sample was added. This was done to reduce the dilution of the samples (done according to the other protocols whereby H_2O_2 was prepared separately in distilled water (100ml) and the buffer was also prepared separately. The mixture was rapidly inverted to mix and then placed in a spectrophotometer. Change in absorbance was read at 240 nm every minute for 5 minutes.

Calculation

Catalase activity (µmole H_2O_2/min/mg protein) = $\frac{(\Delta OD/min \times \text{volume of assay system})}{(0.0041 \times \text{Vol. of Sample} \times \text{mg protein})}$.

3.2.18 Determination of Reduced glutathione level (Beutler *et al*., 1963)

The principle

The reduced form of glutathione comprises in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable yellow color when 5', 5'- dithios – (2-nitrobenzoic acid) (Ellman's reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellman reagent with the reduced GSH, 2-nitro 5-thiobenzoic acid possess a molar absorption at 412nm.

Procedure

The reduced glutathione content of serum as non-protein sulfhydryls was estimated by adding 10% tricarboxylic acid to the sample and 1.0ml of the mixture was treated with 0.5ml of Ellmans reagent (19.8mg of 5,5-dithiobisnitro benzoic acid in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0).

3.2.19 Determination of lipid peroxidation

Lipid peroxidation was determined according to the method described by Varshney and Kale (1990), by measuring the levels of Malondialdehyde (product of lipid peroxide during peroxidation).

Principle

This method is based on the reaction between 2-thiobarbituric acid (TBA) and MDA: an end product of lipid peroxide during peroxidation. On heating in acidic pH, the product is a pink complex which absorbs maximally at 532nm and which is extractable into organic solvents such

as butanol. Malondialdehyde (MDA) is often used to calibrate this test and thus the results are expressed as the amount of the free MDA produced

Procedure

An aliquot of 400µl of the sample was mixed with 1.6ml of tris-KCl buffer to which 500 µl of 30% TCA was added. Then, 500 µl of 0.75% TBA was added and placed in a water bath for 45 minutes at 80^0C. This was cooled in ice and centrifuged at 3000 g for 5 minutes. The clear supernatant was collected and absorbance measured against a reference blank at 532 nm in a spectrophotometer. Lipid peroxidation expressed as MDA formed/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ Cm^{-1}$

$$\text{LPO (MDA formed/mg protein)} = \frac{\text{Absorbance} \times \text{volume of mixture}}{E_{532nm} \times \text{volume of sample} \times \text{mg protein}}$$

MDA =mmol/mg protein

STATISTICAL ANALYSIS

The data of the result were expressed as mean ±SD. Means were determined using Anova and significant difference was considered at $p < 0.05$. (Duncan,1975)

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Percentage Yield of Methanol Extracts of *Abelmochus esculentus* Fruit Varieties

Table 4.1 shows percentage yield of varieties of *Abelmochus esculentus* fruit after extraction with methanol. From the result of the study, *NHB-AI-B* had the highest yield (22.85) while *LD-88* had the lowest yield (11.37).

Table 4.1: Percentage Yield of Methanol Extracts of *Abelmochus esculentus* Fruit Varieties

Sample	Weight (g)	Yield	% Yield
Clemson Spinless	240.00	38.00	15.83
LD-88	255.00	29.00	11.37
NHB-AI-B	254.00	58.03	22.85
NHAE-47-4	285.00	40.36	14.04
Yar Kolon	282.00	48.24	17.11

4.2 Phytochemical Content of Methanol Extracts of *Abelmochus esculentus* Fruit Varieties

Table 4.2 shows the qualitative phytochemical analysis of varieties of *Abelmochus esculentus* fruit extracts. The result identified the presence of phytochemicals like saponins, tannins, flavonoids, and phenols in all the varieties. Quantitative phytochemical analysis showed that the okra varieties varied in their quantities of phytochemicals. Highest quantity of phenolic compounds and flavonoids was found in *Yar Kolon* variety (4.066 mg/g and 0.0067mg/g) respectively. *LD-88* had the highest quantity of saponins (164.0mg/g). Higher quantities of Tannins were found in *Clemson spinless* (3.3mg/g) as shown in Table 4.3.

Table 4.2 Phytochemical Profile of Methanol Extracts of *Abelmochus esculentus* Fruit Varieties

Samples	Saponins	Tannins	Flavonoids	Alkaloids	Steriods	Glycosides	Phenolics
CLEMSON SPINLESS	++	++	++	-	+	++	+
LD-88	++	+++	+	-	-	+++	++
NHB-AI-B	++	++	++	-	-	++	++
NHAE-47-4	+	++	-	-	-	+	+
YAR KOLON	+	+	++	+	-	+	+++

+: Present -: Absent

Table 4.3: Phytochemical Content of Methanol Extracts of *Abelmochus esculentus* Fruit Varieties.

	Phytochemicals			
Samples	Saponins (mg/g of Diosgenin)	Tannins (mg/g of tannic acid)	Flavonoids (mg/g of Quercetin)	Phenolics (mg/g of Gallic acid)
CLEMSON SPINLESS	132.8	3.3	0.0041	1.685
LD-88	164.0	2.1	0.0017	3.432
NHB-AI-B	151.1	2.3	0.0017	3.475
NHAE-47-4	156.6	2.4	0.0052	2.142
YAR KOLON	115.1	2.4	0.0067	4.066

4.3 Crude fiber, Ash and Moisture content of *Abelmochus esculentus* Fruit Varieties

Table 4.4 shows the result of crude fiber, ash and moisture content of selected varieties of *Abelmochus esculentus* fruit. The highest quantity of crude fiber was found in *Yar kolon* variety (14.74 ±0.17).

Table 4.4 Crude fiber, Ash and Moisture content of *Abelmochus esculentus* Fruit Varieties

	LD – 88	Clemson Spinless	Yar Kolon	NHB – AI – B	NHAE-47-4
Moisture content (%)	63.47 ±0.14	48.72 ±0.38	61.17±0.00	58.34 ±0.00	45.80 ±0.21
Ash content (%)	8.76 ±0.47	8.48 ±0.27	10.33 ±0.30	9.68 ±0.00	8.10 ±0.06
Crude fiber (%)	8.62 ±0.23	11.83 ±0.13	14.74 ±0.17	12.51 ±0.00	10.27 ±0.54

4.4 Effect of High Fat Diet on Wistar Albino Rats Pre-Treatment with *Abelmochus esculentus* Fruit Extracts Varieties.

A significant increase($p<0.05$) in the level of TC, TG, LDL and VLDL were shown by the group that were fed high fat diet compared to the normal control rats. Further, they had a significantly lower HDL level in comparison with the group that received normal diets. (fig 4.1)

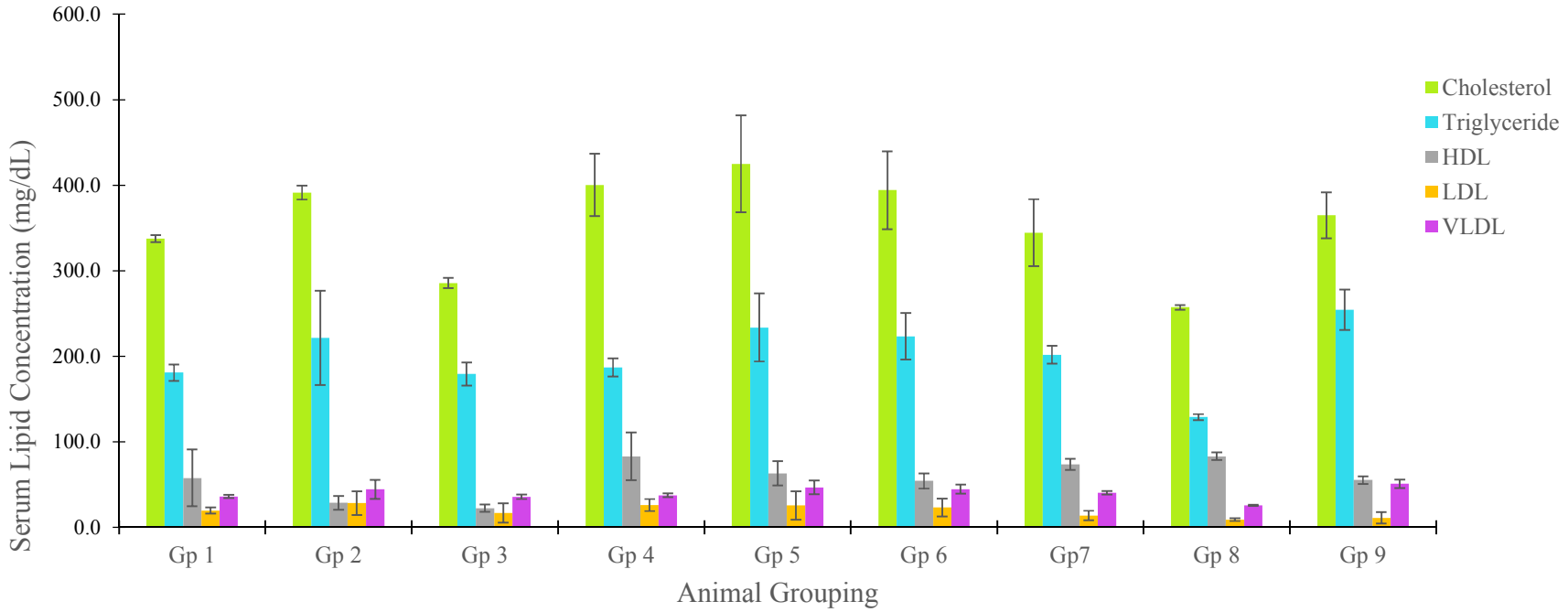


Figure 4.1: Effect of High Fat Diet on Wistar Albino Rats Pre-Treatment with *Abelmochus esculentus* Fruit Extracts Varieties.

Change in Serum Lipid Profile Levels of Wistar Albino Rats Fed Normal and High-Fat Diets for 35 Days Period

Groups 1,2 and 3 = Hyperlipidemic Rats + NHB-AI-B (250 mg,500mg and 750mg respectively).

Groups 4,5, and 6 = Hyperlipidemic Rats + YAR KOLON (250 mg,500mg and 750mg respectively)

Group 7 = Positive Control Group 8 = Normal Control Group 9 = Negative Control

4.5 Effect of High Fats Diet on Water and Feed Intake of Experimental Rats

There was a significant increase ($p<0.05$) in levels of water intake after treatment with Methanol Extracts of *Abelmochus esculentus* Fruit Varieties compared to their water intake before treatment and with the control. However, little or no change was observed in their levels of feed intake before and after treatment with Methanol Extracts of *Abelmochus esculentus* Fruit Varieties.

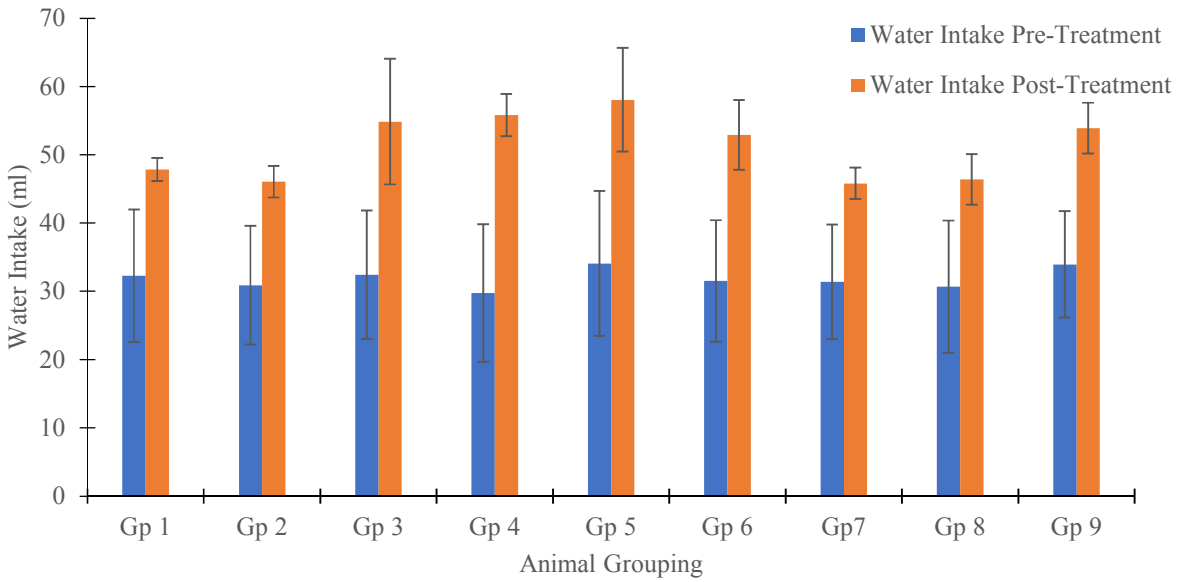


Figure 4.2. Effect of Water Intake of Rats Fed High Fats Diet Before and After Treatment with Methanol Extracts of *Abelmochus esculentus* Fruit Varieties

Groups 1,2 and 3 = Hyperlipidemic Rats + NHB-AI-B (250 mg,500mg and 750mg respectively).

Groups 4,5, and 6 = Hyperlipidemic Rats + YAR KOLON (250 mg,500mg and 750mg respectively)

Group 7 = Positive Control

Group 8 = Normal Control

Group 9 = Negative Control

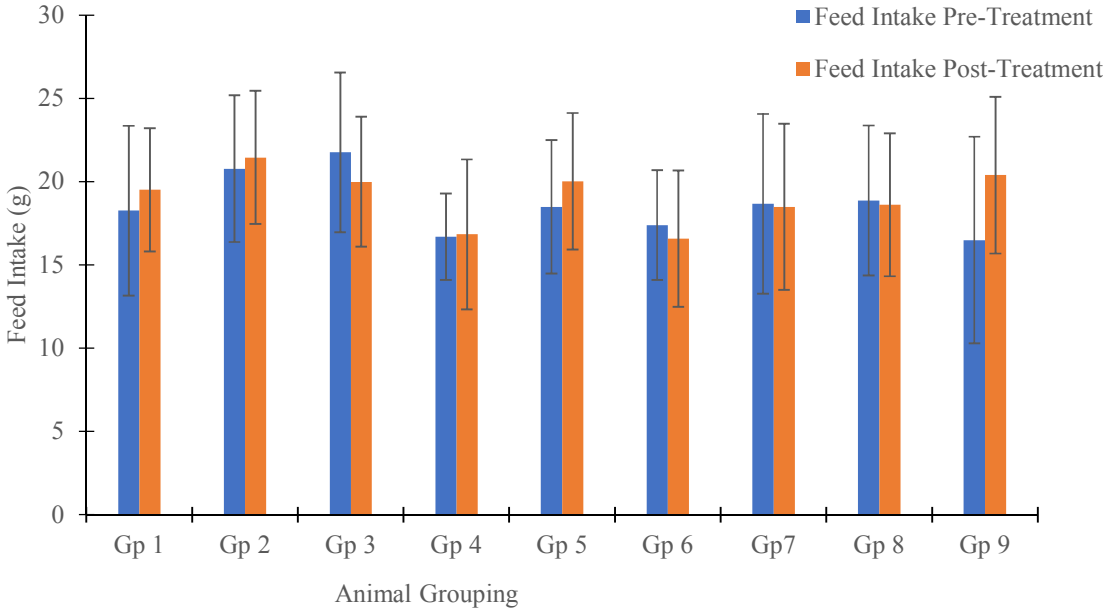


Figure 4.3. Effect of Feed Intake of Rats Fed High Fats Diet Before and After Treatment with Methanol Extracts of *Abelmochus esculentus* Fruit Varieties

Groups 1,2 and 3 = Hyperlipidemic Rats + NHB-AI-B (250 mg,500mg and 750mg respectively).

Groups 4,5, and 6 = Hyperlipidemic Rats + YAR KOLON (250 mg,500mg and 750mg respectively)

Group 7 = Positive Control

Group 8 = Normal Control

Group 9 = Negative Control

4.6 Effect of Oral Administration of Methanol Extracts of *Abelmochus esculentus* Fruit Varieties on Lipid Profile of Rats Fed High Fats Diet

The result showed a reduction in lipid profile (TC, TG, VLDL and LDL) and an increase in HDL in group one that were administered 250mg/kg of *NHB-AI-B* variety and Group 5 that were administered 500mg/kg of *yar kolon* okra fruit extract variety though other tested doses are also effective in modulating blood lipid levels favorably. (Fig 4.4)

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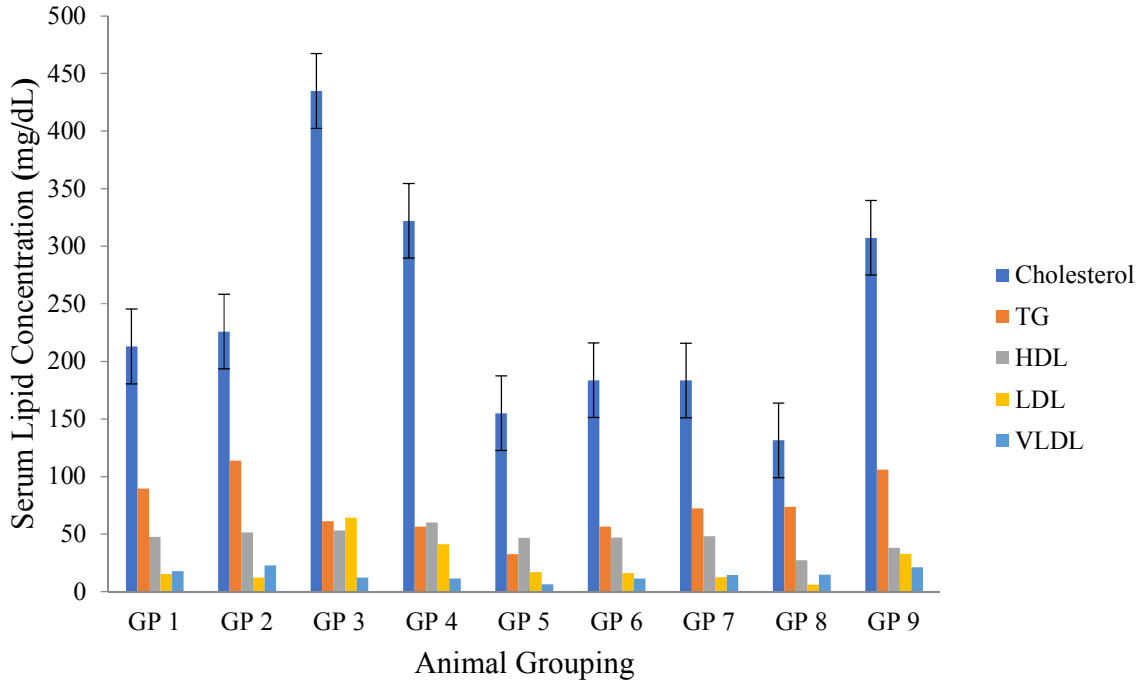

Fig 4.4: Effect of Oral Administration of Methanol Extracts of *Abelmochus esculentus* Fruit Varieties on Lipid Profile of Rats Fed High Fats Diet.

Groups 1,2 and 3 = Hyperlipidemic Rats + NHB-AI-B (250 mg,500mg and 750mg respectively).

Groups 4,5, and 6 = Hyperlipidemic Rats + YAR KOLON (250 mg,500mg and 750mg respectively)

Group 7 = Positive Control

Group 8 = Normal Control

Group 9 = Negative Control

4.7 Effect of Oral Administration of Methanol Extracts of *Abelmochus esculentus* Fruit Varieties on Antioxidant Indices on Rats Fed High Fats Diet

The results showed that both *NHB-AI-B* and *Yar Kolon* fruit extract variety poses antioxidant activity. The MDA levels were elevated in the negative control compared to the treated groups. Administration of *NHB-AI-B* and *Yar Kolon* extracts varieties decreases the MDA levels but elevated SOD, Catalase and glutathione peroxidase activities as shown in fig 4.5.

Table 4.5: Effect of Oral Administration of Methanol Extracts of *Abelmochus esculentus* Fruits Varieties on Lipid Peroxidation and Antioxidant Indices of Rats Fed High Fats Diet

	Animal Grouping								
	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9
MDA µmol/L/mg protein	69.63 ±9.94^{bc}	68.27 ±14.14^{bc}	64.44 ±13.43^{b}	74.31 ±8.66^{bc}	54.47 ±12.79^{ab}	61.78 ±1.21^{b}	99.99 ±5.06^{bcd}	45.84 ±1.19^{a}	115.67 ±4.68^{bcde}
GSH (µg/ml)	5.32 ±0.64^{abc}	7.36 ±2.46^{bcde}	5.60 ±1.21^{bcd}	6.30 ±0.50^{bcde}	5.16 ±0.79^{abc}	5.00 ±0.15^{abc}	7.20 ±0.52^{bcde}	4.48 ±0.82^{ab}	3.84 ±0.57^{a}
SOD (U/Min/mg Protein)	541.02 ±131.55^{bcd}	497.03 ±19.84^{bc}	520.92 ±27.68^{bcd}	458.12 ±25.58^{bc}	465.99 ±42.32^{bc}	506.27 ±71.39^{bc}	459.46 ±36.91^{bc}	315.92 ±101.12^{a}	411.74 ±30.27^{b}
Catalase (µmolH_2O_2/ Min/mg Protein)	343.67 ±80.29^{ab}	428.47 ±98.99^{bc}	355.98 ±31.58^{ab}	621.30 ±11.51^{bcdef}	571.03 ±23.53^{bcde}	520.11 ±63.41^{bcde}	406.61 ±43.70^{abc}	422.97 ±62.26^{bc}	260.53 ±30.30^{a}

Values are Mean ± SD of 5 determinations. Values with different superscript across the rows are significantly different ($P<0.05$)

Groups 1, 2 and 3 = Hyperlipidemic Rats + NHB-AI-B (250 mg, 500mg and 750mg respectively).

Groups 4, 5, and 6 = Hyperlipidemic Rats + YAR KOLON (250 mg, 500mg and 750mg respectively)

Group 7 = Positive Control Group 8 = Normal Control Group 9 = Negative Control

4.8 Effect of Oral Administration of Methanol Extracts of *Abelmochus esculentus* Fruit Varieties on Body Weight of Rats Fed High Fats Diet

The results showed an increase in body weight of the rats fed high fat diet pre-treated when compared with the values post-treated from each group (Fig: 4.6).

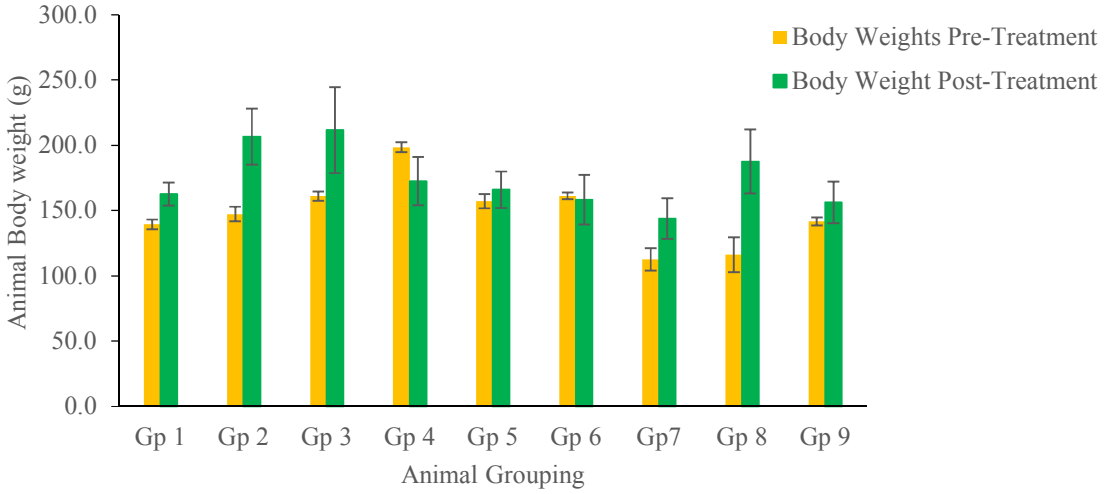

Figure 4.6: Effect of Body Weight of Rats Fed High Fats Diet Before and After Treatment with Methanol Extracts of *Abelmochus esculentus* Fruit Varieties

Groups 1,2 and 3 = Hyperlipidemic Rats + NHB-AI-B (250 mg,500mg and 750mg respectively).

Groups 4,5, and 6 = Hyperlipidemic Rats + YAR KOLON (250 mg,500mg and 750mg respectively)

Group 7 = Positive Control Group 8 = Normal Control Group 9 = Negative Control

4. 9 DISCUSSION

Extraction solvents and techniques used mainly determine the yield outcome of plants. High yield from the *NHB-AI-B* fruit variety in this study indicates that the Okra fruit contain different components with different solubilities. This may suggest that phytocomponents of the plant varies in their solubilities, techniques and extraction time. Based on the result of the present study, one may suggest that *NHB-AI-B* variety has components that are readily extractable with a polar solvent like methanol and hence be of economical importance in terms of phytochemicals extraction.

Literature review shows that there were no reports on Okra fruit toxicity since ancient past (Anupam *et al*., 2014). This finding is in-line with the report of Alqasoumi, (2012) who also performed acute toxicity study of Okra aqueous extract on rats and reported that there was no toxicity symptoms or lethality recorded.

Plants have different phytochemicals as reported by several studies. (Cook and Samman,1996, Manach *et al.,*1996, Liao *et al*.,2012). Okra is one of the plants which contain varieties of phytochemicals as reported in this study by identifying chemical compounds like phenolic and flavonoids. These phytochemicals were reported to be the most abundant and exerted the most pharmacological activities. Presence of these phytochemicals in okra fruit varieties suggest their usefulness in disease intervention. (Cook and Samman,1996).

Literature have reported the potentials of several medicinal plants in disease intervention due to the presence of some bioactive phytochemical constituents that produce definite physiological actions of the human body. Some of the bioactive phytochemical constituents in medicinal plants are alkaloids, flavonoids, saponins, phenolic compounds, tannins, anthracine derivatives and

essential oils (Krishnaiah and Basu,2009). This study also confirmed the presence of phytochemical constituents like saponins, tannins, flavonoids, and phenols in all the varieties of *A. esculentus* fruit extract. Arapitsas, (2008) have also reported the presence of phenolics, flavonol derivatives and oligomeric catechins in Okra plant.

Proximate analysis on okra fruit varieties confirmed the presence of fiber with *Yar kolon* variety having the highest quantity. However, similar research confirmed that okra pods are important vegetable source of viscous fiber, an important dietary component to lower cholesterol (Kendall and Jenkins, 2004, kumar *et al*., 2010). The soluble fiber of okra helps to reduce serum cholesterol and therefore decreases the chance of cardiovascular disease.

Hyperlipidemia is considered as an important risk factor for the development of atherosclerosis and subsequent cardiovascular diseases and stroke (Kumar *et al*.,2016), and has been the major health problem among populations of both affluent and less affluent societies (Neil *et al.,*1990, Smith *et al*.,1993). Several treatments like dietary restriction and exercise, pharmacological intervention with drugs like fibrates, statins and bile acid sequestrants have been used and are reported to have a spectrum of adverse effects in patients and they are also costly (BNF,2017). Research has been directed toward finding safer, inexpensive, and effective agents to combat the disorder (Gosslau and Chen,2004).

The results of this study showed an alternation in all the lipid components assayed like triglyceride, cholesterol, high density lipoprotein in all rats' groups fed high fat diet. Similar scenario has been reported on these parameters; triglycerides and cholesterol as a result of the consumption of diet rich in fats (Murray *et al*.,2009, Ngoc *et al*.,2008). However, following administration of the *Abelmochus esculentus* fruit extracts on rats fed high fats diet, a reversal

effect of the serum lipid profile and atherogenic indices were recorded. Study on okra peel and seed powder was reported to have exerted antihyperlipidemic effect in streptozotocin induced diabetic rats (Sabitha *et al*.,2011). The results of this study suggest that *Abelmochus esculentus* fruit extract possess antihyperlipidemic activity in rats fed high fat diet.

Based on the varieties used in this study, both the *Yar kolo*n and *NHB-AI-B* seem to be of potential in exhibiting antihyperlipidemic effect particularly in lowering cholesterol. Similar finding has reported that both methanol and dichloromethane fractions of the okra fruit lowered cholesterol level (Ngoc *et al*.,2008). In the same vein, level of HDL from the treated rats fed high fats diet were elevated but LDL level were lowered.

Literature survey have shown that accumulation of blood lipids, particularly LDL, enhances the production of reactive oxygen species (ROS). It has also been reported that ROS-induced oxidative stress plays an important role, at least in part, in the etiology of atherosclerosis and coronary heart disease (Paudel *et al*.,2016). In this study, levels of antioxidant markers; SOD, catalase, and glutathione were diminished whereas MDA levels elevated in the rats fed high fats diet.

Treating rats fed high fats diet with extracts from okra fruit varieties reversed the changes in the antioxidant markers. This may suggest that methanol extracts of *Abelmochus esculentus* fruit varieties possesses components that exert antioxidant activities. Also, levels of MDA were found to be reduced in the same rats' groups treated with the methanol extracts of the okra fruit varieties. Study on the peel and seed powder of Okra was reported to have significantly increased superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) levels but decreased thiobarbituric acid reactive substances (TBARS) (Liao *et al*.,2012).

Also, study on different organs; flower, fruit, leaf, and seed of aqueous extracts of *A. esculentus* were shown to exhibit antioxidant activity and were suggested to be due to the presence of phytochemicals likes phenolics and flavonoids. Atawodi *et al*., (2009) has reported *in vitro* antioxidant effect of methanol extract of okra fruits. At this point, one may suggest that presence of the phytochemicals identified in the varieties of the Okra fruit extracts might have contributed to the antioxidant activity observed in this study.

The study also recorded a change in body weights of the rats fed high fats diet after feeding for 35 days possibly due to high fats contents from the diet. A study has reported that increase in body weight of rats after feeding on diets rich in fats was suggested to have induced obesity (Lai *et al.,*2016, Kim *et al*.,2016).

The study also observed that lower dose is more effective than higher dose. This could be due to the fact that the crude plant extract contains complex mixtures with multiple pharmacological activities. The different constituents could have antagonistic, allosteric and synergistic effects. At higher doses as observed in this study, an active substance could have blunted the activity of a second bioactive constituent. It is not unusual to notice dual effect.

In other case, the putative component might be targeting different molecules or pathway evolving cellular proliferation at different concentration. In some cases, the activity of a component manifested at a certain concentration could have been modified at a different concentration owing to overriding of the original effect elicited. some of the presumptive constituent might be redox active, and could display differential effects as well.

CHAPTER FIVE

5.0 SUMMARY, CONCLUSION, AND RECOMMENDATIONS

5.1 SUMMARY

This study was carried out to evaluate the hypolipidemic effects of selected varieties of *Abelmochus esculentus* fruits extracts on hyperlipidemic-induced rats' model.

The findings of the study are summarized as follows:

I. Proximate analysis of okra fruit varieties confirmed the presence of fiber where *NHB-AI-B* and *Yar Kolon* varieties recorded high Fiber content (12 and 14 %) respectively. Presence of phytochemicals like phenolics and flavonoids were detected in all the okra fruit varieties. Quantitative phytochemical analysis showed that the selected okra varieties varied in their quantities of phytochemicals.

II. Treating hyperlipidemic rats with the methanol extract of *NHB-AI-B* and *Yar kolon* fruit varieties significantly ($p< 0.05$) reversed the altered lipid profile like triglyceride, cholesterol and high-density lipoprotein when compared with the untreated hyperlipidemic rats, but *Yar Kolon* variety exert better hypolipidemia effect with about 50% reduction in lipid profile.

III. The methanol extract of *NHB-AI-B* and *Yar kolon* fruit varieties significantly ($p< 0.05$) increases the levels of antioxidants enzymes like SOD, catalase, and glutathione and decreases MDA levels in the treated hyperlipidemic rats in comparable to untreated hyperlipidemic rats, where *NHB-AI-B* variety shows about 40% increase in the level of antioxidant enzymes.

5.2 CONCLUSION

Based on the findings of this study, it is concluded that the methanol extracts of selected varieties of *Abelmoschus esculentus* fruit have components that have the capacity to exert some biological activity hence, their usefulness in disease intervention. The methanol extracts of the two varieties of *Abelmoschus esculentus* fruit both have components that have the ability to lower hyperlipidemia but *Yar Kolon* variety exert better hypolipidemia effect. The methanol extracts of the two varieties of *Abelmoschus esculentus* fruit both exhibit antioxidant activity where *NHB-AI-B* variety seem to contain components that likely have better antioxidant potential.

5.3 RECOMMENDATIONS

Isolate bioactive components of the two potent Okra varieties and compare their effects with available hypolipidemic drugs.

To study the hypolipidemic effect of the active components of the two potent varieties at molecular level.

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