**USMANU DANFODIYO UNIVERSITY, SOKOTO**

**(POSTGRADUATE SCHOOL)**

# ANTIOXIDANT EFFECT OF FISH OIL AND MANNITOL ON CARDIOVASCULAR DISEASES RISK MARKERS IN EXPERIMENTALLY INDUCED OBESE RATS

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**BY**

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## DEDICATION

This research work is dedicated to God almighty and my beloved parent Mr. and Mrs Joseph A. Bako for their love, kindness, prayers, support and encouragement during the course of my study.

## CERTIFICATION

This dissertation by JOSEPH, Abigail Yok (Adm. No: 15211227013), has met the requirement for the award of the degree of Master of Science in Medical Laboratory Sciences (Chemical Pathology) of the Usmanu Danfodiyo University, Sokoto and is approved for its contribution to knowledge.

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ACRONYMS/DEFINITION OF TERMS AD Alzheimer's Disease

| | |
|---|---|
| AF | Atrial Fibrillation |
| ACS | Acute Coronary Syndrome |
| AKI | Acute Kidney Infarction |
| ALT | Alanine Transaminase |
| AMI | Acute Myocardial Infarction |
| Arf-CopI | ADP-ribosylation factor I-coat protein complex I |
| AST | Aspartate Transaminase |
| ATH | Atherosclerosis |
| AVD | Atherosclerotic Vascular Disease |
| BMI | Body Mass Index |
| Bp | Blood pressure |
| B3-AR | B-3 Adrenergic receptors |
| CAD | Coronary Artery Disease |
| CHD | Coronary Heart Disease |
| CRP | C-Reactive Protein |
| CVD | Cardiovascular disease |
| DHA | Docosahexaneoic acid |
| EC | Endothelial Cell |
| ELISA | Enzyme Linked Immunosorbent Assay |
| EPA | Eicosapentaenoic acid |
| FBS | Fasting Blood Sugar |
| FIT | Fat Storage Inducing Transmembrane |
| Fsp27 | Fat Specific Protein |
| GFR | Glomerular Filteration Rate |
| GGT | y-Glutaryl Transferase Activity |

| | |
|---|---|
| GOD | Glucose Oxidase |
| HbA1C | Glycated Haemoglobin |
| $H_0$: | Null Hypothesis |
| $H_0$FH | Homozygous Familiar Hypocholesterolemia |
| H2O2 | Hydrogen Peroxide |
| $H_A$ | Alternate Hypothesis |
| HDL-C | High Density Lipoprotein- Cholesterol |
| HFD | High-Fat Diet |
| HR | Heart Rate |
| IFN-Y | Interferon –Y |
| IHD | Ischaemic Heart Disease |
| IL-1 | Interlukin-1 |
| IL-6 | Interlukin-6 |
| IL-8 | Interlukin-8 |
| IR | Insulin Resistance |
| IRSI | Insulin Resistance Substrate 1 |
| LDs | Lipid Droplet |
| LDL-C | Low Density Lipoprotien-Cholesterol |
| LMM | Low-Molecular Mass |
| LCPUFA | Omega -3 Long chain Polyunsaturated fatty acid |
| MI | Myocardial Infarction |
| MRF | Modifiable Risk Factor |
| MRNA | Messenger RNA |
| MS | Metabolic Syndrome |
| NMRF | Non-Modifiable Risk Factor |

| | |
|---|---|
| NO | Nitric Oxide |
| NPY | Neuropeptide Y |
| PAPP-A | Pregnancy Associated Plasma Protein Protein-A |
| PAD | Peripheral Arterial Disease |
| PiHDL | Proinflammatory High Density Lipoprotein |
| PUFA | Polyunsaturated Fatty Acid |
| POD | Peroxidase |
| RBF | Renal Blood Flow |
| RH | Rheumatoid Heart Disease |
| SLE | Systemic Lupus Erythromatosus |
| TNF | Tumour necrosing factor |
| SIP | Sphingosine -1-Phosphate |
| T2DM | Type II Diabetes Mellitus |
| TC | Total Cholesterol |
| TLC | Therapeutic Lifestyle Changes |
| TG | Triglyceride |
| TLR4 | Toll-Like Receptor 4 |
| TNF-A | Tumor Necrosis Factor-Alpha, |
| VLDL-C | Very Low Density Lipoprotein- Cholesterol |
| WBC | White Blood Cells |
| WHO | World Health Organization |

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## ABSTRACT

Cardiovascular disease is associated with high blood pressure, lipidemia, obesity, and diabetes mellitus. The effect of fish oil and mannitol on cardiovascular disease risk markers in experimentally induced obese rats is still obscure. Obesity was induced by administration of HFD for 84 days and was given 0.5ml/kg of fish oil and 0.75ml/100g mannitol respectively. Erythrocyte aggregation, fibrinogen, Serum glucose, and lipid profile were measured using standard techniques. The mean fibrinogen level was significantly increased in groups fed with normal diet and treated with fish oil or mannitol ($P < 0.05$), however, it significantly decreased in groups fed with high fat diet and treated with fish oil or mannitol ($P < 0.05$). There was no statistical significance in the erythrocyte aggregation level of all the treated groups when compared to the control group ($P > 0.05$). The lipid profile result shows no statistical significance between the treated groups and the control groups. The histological of the liver of  shows evidence of inflammation due to fatty liver, the group treated with fish oil showed higher healing processes inducted by ballooning degeneration and microvesicular steatosis. Conclusively, the results of this study proves that fish oil and mannitol had cardioprotective effect.

# CHAPTER ONE

## 1.0 INTRODUCTION

## 1.1 BACKGROUND TO THE STUDY

Cardiovascular diseases refers to a variety of ailments that affect the heart as well as all of the blood vessels in the body. Atherosclerosis is one of these ailments and is caused by a buildup of plaque in the arteries; this buildup can accumulate to the point that a clot forms and clogs the artery completely, leading to either a stroke or a heart attack (NHLBI, 2013). These disorders of CVD are caused by consumption of high calories diet, decreased function of nervous system and circulatory system due to imbalance of homeostasis regulators, dyslipidemia, sedentary life style, family history, medication (steroids), technological advances, lack of exercise, genetic polymorphism of insulin receptor substrate (IRSI), (Critchley *et al.*,1999) The cause of the disorder determine the type of CVDs , these include coronary artery diseases (CAD) such as angina and myocardial infarction (commonly known as a heart attack). Other CVDs include stroke, heart failure, hypertensive heart disease, rheumatic heart disease, cardiomyopathy, heart arrhythmia, congenital heart disease, valvular heart disease, carditis, aortic aneurysms, peripheral artery disease, thromboembolic disease, and venous thrombosis (Gupta *et al.,* 2008). Risk factors associated with CVDs are hypertension, metabolic syndrome, cigarette smoking, diabetes mellitus, elevated glucose and cholesterol levels, and obesity or being overweight are the top causes of death globally (Wong *et al.,* 2014). It is a global disease which is predicted to increase and impose a greater economic burden on the health care services all over the world (WHO, 2015). High blood pressure, tobacco smoking, diabetes mellitus, lack of exercise, and obesity are the major risk of factors of cardiovascular disease. (WHO, 2001) cardiovascular

diseases are preventable by improving risk factor through healthy: eating, exercise, avoidance of tobacco and alcohol intake (Wu *et al;* 2012). CVD are the leading cause of death globally, and this so true in all areas of the world except Africa (WHO,

2011). Together they resulted in 17.9 million deaths (32.1%) in 2015, up from 12.3 million (25.8%) in 1990 (GBD, 2013, 2016). Deaths, at a given age, from CVD are more common and have been increasing in much of the developing world, while rates have declined in most of the developed world since the 1970s (Bridget, 2010; Moran *et al.*, 2010). Coronary artery disease and stroke account for 80% of CVD deaths in males and 75% of CVD deaths in females (WHO, 2011). Most cardiovascular disease affects older adults. In the United States 11% of people between 20 and 40 have CVD, while 37% between 40 and 60, 71% of people between 60 and 80, and 85% of people over 80 have CVD (Mozaffarian *et al.*, 2013). The average age of death from coronary artery disease in the developed world is around 80 while it is around 68 in the developing world (Bridget, 2010). Disease onset is typically seven to ten years earlier in men as compared to women (Mendis *et al.*, 2011). In Nigeria, reports on the prevalence of modifiable cardiovascular disease (CVD) risk factors are scarce. In addition, socio-economic status (SES), an important component of the socioeconomic gradient in CVD and its risk factors has not been clearly elucidated. Socio-economic status (SES) is a predictor of CVD and its risk factors. However, the nature of this relationship varies depending on the economic development of the countries (Ogden, 2010; Secrest *et al.*, 2011; Jones-Smith *et al.*, 2011; Wang and Lim, 2012). It has been predicted that CVD will become the ultimate cause of disability in the world between years 2000 – 2025 (Murray and Lopez 1997). Complications of CVD could lead to metabolic syndrome: a condition of at least three of the cardiovascular risk factors: obesity, excessive visceral fat storage, dyslipidemia, hypertension and hyperglycaemia or type II diabetes which may occur due to

insulin resistance, oxidative stress and chronic inflammation. Fish oil have several effects which are thought to result from a reduction in inflammatory and thrombotic prostaglandins and leukotrienes and inflammatory cytokines via its anti-inflammatory, antithrombotic, hypolipidaemic and vasodialatory properties (Bernstein *et al*., 2012). Extensive research has established that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) play a vital role in the prevention of Alzheimer's disease, atherosclerosis, heart attack, angina, stroke, congestive heart failure, depression and cancer (Zheng *et al.*, 2013). Its mechanism of action includes alteration of lipoprotein metabolism: reduced triglycerides; mixed effects on low and high density lipoprotein (LDL and HDL) cholesterol, inhibition of atherosclerosis, prevention of thrombosis, reduction in heart rate, influence on arrhythmias, inhibition of inflammation and immune response (Zheng *et al.*, 2013). EPA and DHA in fish oil may decrease the rate of atherosclerosis and is recommended as a therapeutic strategy to reduce cardiovascular disease (Kris-Etherton *et al*., 2002). Mannitol is an osmotic diuretic widely used in the management of raised intracranial pressure (ICP), for renal protection in cardiac,vascular and renal transplantation surgery. It also acts as free radical scavenger and reduces the harmful effect of free radicals during ischaemia-reperfusion injury. mannitol increases coronary blood flow and reduces the extent of myocardial damage (Willerson *et al*.,1972).

## 1.2 STATEMENT OF THE PROBLEM

Cardiovascular diseases is a major contributor to the world's burden of diseases, ranging currently as the most important cause of death and producing substantial disability and reduced well-being among surviving people (Yusuf *et al.*, 2001,). It is a global disease which is predicted to increase and impose a greater economic burden on the health care

services around the world (WHO, 2015). Studies shows that there is 11% prevalence of CVD in sub-sahara Africa and 24% in North western Nigeria (Mukadas and Mishau 2009). It is postulated that combination of cardiovascular risk factors, obesity, dyslipidemia, hypertension and hyperglycaemia or type 2 diabetes can lead to metabolic syndrome which leads to insulin resistance, oxidative stress and inflammatory conditions.

CVD is increasing due to technological advancement making life easier, sedentary life style, rise in consumption of high fat diet (calories), lack of appropriate physical exercise, medical and genetic factors, alcohol and tobacco intake (Hussain *et al.,* 2007). Complications of risk factors, are likely to vasoconstriction, thrombosis, chronic inflammation, plague accumulation and rapture, vascular lesion and remodeling, angina, atherosclerosis, heart failure and eventually death (Hussain *et al.,* 2007).

## 1.3 JUSTIFICATION

Fish oil can influence many aspects of the pathogenesis of CVD, including arrythmias, lipid concentrations, blood pressure, platelet aggregation, vascular relaxation, inflammation and likely arterial cholesterol delivery (Knapp, 1995; Friedman and Moe, 2006; Bloch and Hannestad, 2012; Cabré *et al.,* 2012; Gerber, 2012). The overall effects of n-3 fatty acids in fish oil are related to multiple interactive mechanisms, including modulation of eicosanoids and other immune pathways, which lead to attraction of inflammatory responses, modulation of molecules or enzymes associated with various signaling pathways involving normal and pathologic cell function, incorporation of n-3 fatty acids into membrane phospholipids and direct effects on gene expression (Guidetti and Cagnazzo, 2012). N-3 fatty acid affect the expression of several key proteins as modulators of many genes involved in lipids metabolism, inflammation, and smooth muscle cell proliferation, genes that can play a

pivotal note in prevention and treatment of CVD and atherosclerosis (Matsuyama *et al.*, 2005; Zulfakar *et al.*, 2007; Miles and Calder, 2012; Zheng *et al.*, 2013).

Mannitol is widely used in the management of raised intracranial pressure (ICP), for renal protection in cardiac, vascular and renal transplantation surgery. It also acts as free radical scavenger and reduces the harmful effect of free radicals during ischaemia-reperfusion injury.

During experimental myocardial ischemia, mannitol increases coronary blood flow and reduces the extent of myocardial damage (Willerson *et al*.,1972). Accordingly, the purpose of this investigation was to assess the extent to which a small, mannitol-induced increase in serum osmolality alters left ventricular function and coronary blood flow in patients with and without cardiovascular disease (Kosh *et al.*,1973)

## 1.4. AIM AND OBJECTIVES

The aim of this study is to investigate the effect of fish oil and mannitol supplementation in rats fed with high fat diet.

1.4.1 The Specific Objectives of the study were:

a. To determine the effect of fish oil and mannitol on body mass index of the experimental rats.
b. To evaluate the fibrinogen level and erythrocyte aggregation in the experimental rats.
c. To evaluate the effect of fish oil and mannitol on the histology of the heart, liver and kidney of experimental rats.
d. To evaluate the effect of fish oil and mannitol on lipid profile and glucose in the experimental rats.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 CARDIOVASCULAR DISEASES

Cardiovascular disease (CVD) is an umbrella term for a number of linked pathologies, commonly defined as coronary heart disease (CHD), cerebrovascular disease, peripheral arterial disease, rheumatic and congenital heart diseases and venous thromboembolism (Ahmadzadeh and Azizi, 2014). The underlying mechanisms vary depending on the disease in question. Coronary artery disease, stroke, and peripheral artery disease involve atherosclerosis. This may be caused by high blood pressure, smoking, diabetes, lack of exercise, obesity, high blood cholesterol, poor diet, and excessive alcohol consumption, among others. It is estimated that 90% of CVDs are preventable (McGill *et al.*, 2008). Prevention of atherosclerosis involves improving risk factors through: healthy eating, exercise, avoidance of tobacco smoke and limiting alcohol intake (WHO, 2011). Treating risk factors, such as high blood pressure, blood lipids and diabetes is also beneficial (WHO, 2011). Studies has shown that high blood pressure results in 13% of CVD deaths, while tobacco results in 9%, diabetes 6%, lack of exercise 6% and obesity 5%. Rheumatic heart disease may follow untreated strep throat (WHO, 2011).

### 2.2 CLASSES OF CARDIOVASCULAR DISEASES

CVD are classified depending on the site of involvement, either the heart directly or blood vessels. They include the following: coronary artery diseases (CAD) such as angina and myocardial infarction (commonly known as a heart attack), stroke, heart failure, hypertensive heart disease, rheumatic heart disease, cardiomyopathy, heart arrhythmia,

congenital heart disease, valvular heart disease, carditis, aortic aneurysms, peripheral artery disease, thromboembolic disease, and venous thrombosis.

### 2.2.1 Coronary heart diseases

Coronary Heart Disease, also known as coronary artery disease, is the narrowing of the blood vessels that supply blood and oxygen to the heart. CHD can lead to unstable angina, myocardial infarction (MI), and heart failure (Imes and Austin, 2012). According to World Health Organization (WHO) estimates, 17.3 million people died from CVDs in 2008, representing 30% of all global deaths. Of these deaths, an estimated 7.3 million were due to CHD and 6.2 million were due to stroke (WHO, 2011). CHD is decreasing in many developed countries (due to improved prevention {in particular reduced cigarette smoking among adults, and lower average levels of blood pressure and blood cholesterol}, diagnosis, and treatment), but is increasing in developing and transitional countries, partly as a result of increasing longevity, urbanization, and lifestyle changes. In developed countries, CHD is predicted to rise from 30–60% between 1990 and 2020. In developing countries, rates are predicted to increase by 120% in women and 137% in men from 1990 to 2020 (Torpy *et al.*, 2009).During the past decades a great deal of knowledge concerning the pathophysiology of CHD has been achieved, and age (older than 40 years for men, 45 years for women), male sex, family history of CHD, smoking, hypertension, diabetes, obesity, high total cholesterol, low high density lipoprotein cholesterol (HDL-C), high low density lipoprotein cholesterol (LDL-C), high triglycerides, low physical activity, and accumulation of abdominal fat are some of the major risk factors (Torpy *et. al.*, 2009). However, despite the identification of important risk factors, CHD remains the leading cause of death worldwide. Up to half of all events associated with CHD are reported to occur in apparently healthy individuals who

have few or none of the traditional risk factors, including dyslipidemia. As a result, attention has increasingly turned to the role of other factors, such as inflammation, in the development of atherosclerosis and CHD (Clearfield, 2005).

Atherosclerosis, the underlying pathology responsible for CHD, is an inflammatory disease. Recent observations suggest that the atherosclerotic process is characterized by a low-grade inflammation altering the endothelium of the coronary arteries and is associated with an increase level in markers of inflammation such as acute phase proteins and cytokines. Cumulative evidence indicates that inflammation, at both focal and systemic levels, plays a key role in destabilization and rupture of atherosclerotic plaques, leading to acute cardiovascular events (Majid and Willerson, 2011). In consideration of the important role that inflammatory processes play in determining plaque stability, recent work has focused on whether biomarkers of inflammation may help to improve risk stratification and identify patient groups who might benefit from particular treatment strategies. Among them, Creactive protein (CRP), a prototype marker of the inflammatory process, is the most studied both as a causal factor and in the prediction of CHD (Calabro *et al.,* 2012).

### 2. 2. 2 Strokes

Strokes are caused by disruption of the blood supply to the brain. This may result from either blockage (ischemic stroke) or rupture of a blood vessel (hemorrhagic stroke). The main risk factor for stroke is high blood pressure (NHIBI, 2015). Other risk factors include tobacco smoking, obesity, high blood cholesterol, diabetes mellitus, previous TIA, atrial fibrillation (a heart rhythm disorder), unhealthy diet, physical inactivity, and advancing age (Donnan *et al.,* 2008).

In 2013 approximately 6.9 million people had an ischemic stroke and 3.4 million people had a hemorrhagic stroke (GBD, 2013). In 2015 there were about 42.4 million people who had previously had a stroke and were still alive (GBD, 2015). Between 1990 and 2010 the number of strokes which occurred each year decreased by approximately 10% in the developed world and increased by 10% in the developing world (Feigin *et al.,* 2010). In 2015, stroke was the second most frequent cause of death after coronary artery disease, accounting for 6.3 million deaths (11% of the total). About 3.0 million deaths resulted from ischemic stroke while 3.3 million deaths resulted from hemorrhagic stroke (GBD, 2015). About half of people who have had a stroke live less than one year (Donnan *et al.,* 2008). Overall, two thirds of strokes occurred in those over 65 years old (Feigin *et al.,* 2014).

### 2.2.3 Congenital heart disease

Malformations of heart structures existing at birth may be caused by genetic factors or by adverse exposures during gestation (Ajdarkosh *et al.,* 2013). Examples are holes in the heart, abnormal valves, and abnormal heart chambers. Risk factors are Maternal alcohol use, medicines (for example thalidomide, warfarin) used by the expectant mother, maternal infections such as rubella, poor maternal nutrition (low intake of folate), close blood relationship between parents (consanguinity) (Shanthi *et al.,* 2011*;* Dean *et al.,* 2014). Having a parent with a congenital heart defect is also a risk factor (Milunsky, 2011). A number of genetic conditions are associated with heart defects including Down syndrome, Turner syndrome, and Marfan syndrome (Shanthi *et al.,* 2011). Congenital heart defects are divided into two main groups: cyanotic heart defects and non-cyanotic heart defects, depending on whether the child has the potential to turn bluish in color (Shanthi *et al.,* 2011). The problems may involve the interior walls of the heart, the heart valves, or the large

blood vessels that lead to and from the heart (NHLBI, 2011). Congenital heart defects are partly preventable through rubella vaccination, the adding of iodine to salt, and the adding of folic acid in certain food products (Shanthi *et al.*, 2011). Some defects do not need treatment (NHLBI, 2011). Other may be effectively treated with catheter based procedures or heart surgery. Occasionally a number of operations may be needed. Occasionally heart transplantation is required. With appropriate treatment, outcomes are generally good, even with complex problems (NHLBI, 2011). Heart defects are the most common birth defect (Shanthi *et al.*, 2011; GBD, 2013). In 2015 they were present in 48.9 million people globally (GBD, 2015). They affect between 4 and 75 per 1,000 live births, depending upon how they are diagnosed (Shanthi *et al.*, 2011; Milunsky, 2011). About 6 to 19 per 1,000 cause a moderate to severe degree of problems (Milunsky, 2011). Congenital heart defects are the leading cause of birth defect-related deaths (Shanthi *et al.*, 2011). In 2015 they resulted in 303,300 deaths down from 366,000 deaths in 1990 (GBD, 2013- 2015).

### 2.2.4 Rheumatic heart disease

Heart muscles and valves damage due to rheumatic fever caused by *Streptococcus pyogenes* a group A streptococcal infection. The damaged valves may result in heart failure, atrial fibrillation and infection of the valves (Marijon *et al.*, 2012). Chronic rheumatic heart disease (RHD) is characterized by repeated inflammation with fibrinous repair. The cardinal anatomic changes of the valve include leaflet thickening, commissural fusion, and shortening and thickening of the tendinous cords (Cotran *et al.*, 2005). It is caused by an autoimmune reaction to Group A β-hemolytic streptococci (GAS) that results in valvular damage (Kaplan *et al.*, 1964). Fibrosis and scarring of valve leaflets, commissures and cusps leads to abnormalities that can result in valve stenosis or regurgitation (Brice *et al.*, 2005).

The inflammation caused by rheumatic fever, usually during childhood, is referred to as rheumatic valvulitis. About half of patients with rheumatic fever develop inflammation involving valvular endothelium (Caldas *et al.,* 2008). The majority of morbidity and mortality associated with rheumatic fever are caused by its destructive effects on cardiac valve tissue (Brice *et al., 2005*). The pathogenesis of RHD is complex and not fully understood, but it is known to involve molecular mimicry and genetic predisposition that lead to autoimmune reactions.

## 2.3 EPIDEMIOLOGY OF CARDIOVASCULAR DISEASES

Cardiovascular diseases are the leading cause of death globally. This is true in all areas of the world except Africa (WHO, 2011). Together they resulted in 17.9 million deaths (32.1%) in 2015, up from 12.3 million (25.8%) in 1990 (GDB, 2013, 2016). Deaths, at a given age, from CVD are more common and have been increasing in most of the developing world, while rates have declined in most of the developed world since the 1970s (Bridget 2010; Moran *et al.,* 2010). Coronary artery disease and stroke account for 80% of CVD deaths in males and 75% of CVD deaths in females (WHO, 2011). Most cardiovascular disease affects older adults. In the United States 11% of people between 20 and 40 have CVD, while 37% between 40 and 60, 71% of people between 60 and 80, and 85% of people over 80 have CVD (Mozaffarian *et al.,* 2013). The average age of death from coronary artery disease in the developed world is around 80 while it is around 68 in the developing world (Bridget 2010). Disease onset is typically seven to ten years earlier in men as compared to women (Mendis *et al.,* 2011). In recent years, the dominance of chronic diseases as major contributors to total global mortality has emerged and has been previously described in detail elsewhere (Adeyi *et al.,* 2007; WHO, 2008). By 2005, the total number of

cardiovascular disease (CVD) deaths (mainly coronary heart disease, stroke, and rheumatic heart disease) had increased globally to 17.5 million from 14.4 million in 1990. Of these, 7.6 million was attributed to coronary heart disease and 5.7 million to stroke. More than 80 percent of the deaths occurred in low and middle income countries (WHO, 2009). The World Health Organization (WHO) estimates there will be about 20 million CVD deaths in 2015, accounting for 30 percent of all deaths worldwide (WHO, 2005). By 2030, researchers project that non-communicable diseases will account for more than three-quarters of deaths worldwide; CVD alone will be responsible for more deaths in low income countries than infectious diseases (including HIV/AIDS, tuberculosis, and malaria), maternal and perinatal conditions, and nutritional disorders combined (Beaglehole and Bonita, 2008). Thus, CVD is today the largest single contributor to global mortality and will continue to dominate mortality trends in the future (WHO, 2009).

Global trends in CVD are based on models that use country-specific data from a diverse range of developed and developing countries including those of the European Union (HEM Project Team, 2008), Saudi Arabia (Alberti and Zimmet, 1998), Pakistan (Albright and Goldstein, 1996), South Africa (Steyn *et al.,* 2006), China (Al-Safi *et al.,* 2006), Indonesia (Appelros *et al.,* 2005), Mexico (Fernald and Neufeld, 2007), India (Reddy, 2007), and the United States (Flegal *et al.,* 2007). Over the past decade, the quality and availability of countryspecific data on CVD risks, incidence, and mortality has increased in accordance with one of the major recommendations of the 1998 IOM report. What emerges are nationally derived data on risks and CVD outcomes. Therefore, in many developing countries, the lack of country-specific data on risks and CVD outcomes that was prominently highlighted in the

1998 IOM report is less an impediment to policy development and action. Nonetheless, before beginning a discussion of CVD trends and risk factor incidence around the world and in specific countries and regions, it is important to note several persistent limitations with the available data. Although many countries have established health surveillance systems with death registration data, the quality of the data collected varies substantially across countries. In many countries, especially in low and middle income countries, health statistics are often based on surveillance that does not cover all areas of the country, is incomplete in the areas it does cover, or is collected by undertrained staff who do not, or cannot, accurately report the pertinent data. These realities limit the reliability of any country health data (Arnold *et al.,* 2014). Despite these limitations, WHO and country health statistics are often the most complete, comparable, or only data available and thus remain a key tool for evaluating the status of a CVD

In high income countries, the evidence points to an inverse relationship between SES and CVD risk factors in the adult population, regardless of indicators of SES used (McLaren, 2007). This trend differs in low-middle-income countries and among those of lower SES in the developed countries where lower SES is a potential marker of poor health outcomes (Cuningham *et al.,* 2008).Few reports in Nigeria showed that the association between SES and cardio-metabolic syndrome varies. High prevalence of cardiometabolic risk factors was found in high SES groups than in low SES groups (Adedoyin *et al,* 2005; Mbada *et al.,* 2009), while Mbada *et al.* (2013) noted a higher prevalence of obesity in the lower SES of a semi-urban Nigerian population. These few reports studied SES as an entity; however, the component of the SES driving the trend of the relationship in prevalence is worth exploring in relation to how it affects risk factors screening.

## 2.4 RISK MARKERS AND SCORES TO CARDIOVASCULAR DISEASES

Cardiovascular diseases are increasing day by day due to over utilization of fats or due to genetic reasons. Though conventional risk prediction algorithms are made available on presence of major cardiovascular risk factors identified in diseased population, authentic and accurate biomarkers of CVDs are lacking. It not only delayed clinical diagnosis, but also increased risk manifold and resulted in the accidental death of patients. Therefore, an early identification and treatment of risk factors are much needed to accelerate disease prevention and morbidity improvement (Bamba, 2014). Numerous risk scores have been developed to predict cardiovascular risk. These scores are based on observations of the relative degree of importance of individual major risk factors. Till date, numerous physiological biomarkers based on serum lipid, glucose and hormone biomarkers serum lipid, glucose and hormone profile have been identified that are associated with increased cardiovascular risks. Some of them are simple traditional biomarkers based on lipid profile and risk factors. More often, levels of plasma, serum, and blood are proved to be best cardiovascular risk biomarkers (Brucker *et al.*, 2014). These markers display cellular lipid interactions and physiological functions of serum lipid bearing proteins and assist in clinical decision making and authenticated risk type (Brown and Bittner, 2008). There are so many established cardiovascular risk markers based on confirmed clinical outcomes related to biomolecules, its structure, and functions. There are new mini and microlevel clinical factors associated with an elevated prospective risk of developing coronary heart diseases. However, various physical factors if known can work as biophysical markers, but all these are not enough to evaluate the disease and the status of emerging risks in patients, hence, other biomarkers to be included in risk analysis. Many of these biomarkers, alone or in combination, can be

incorporated into risk prediction models to determine whether their addition increases the model's predictive ability. Moreover, various cardiovascular risk prediction models have been updated by incorporating traditional risk factors and molecular, immunological genetic, imaging, and biophysical factors for more authentic and reliable estimation of cardiovascular risk.

However, to establish risk status measurement of a standard lipid profile, including total cholesterol, LDL (low-density lipoprotein) cholesterol, HDL (high-density lipoproteins) cholesterol, and triglycerides, is recommended from an integral component of approaches to cardiovascular risk prediction. These old markers and risk factors such as elevated LDL cholesterol, hypertension, diabetes, and low LDL cholesterol, smoking, and family history can predict premature coronary heart diseases in man.

### 2. 4. 1 Framingham 10-year risk score

In addition, numerous risk scores have been developed to predict coronary heart disease risks or cardiovascular risk. These scores are based on observations of the relative degree of importance of individual major risk factors. Most important prediction is made by Framingham 10-year risk score which is commonly used to predict cardiovascular event over the next ten years in the primary prevention of disease. Hence, a need persists for diagnosis of CVDs at two stages: first category of patients stratified as low risk (Framingham 10-year risk score >10%) requires less risk identification, modification, and treatment method, but patients stratified as high risk (Framingham 10-year risk score >20%) need intensive risk factor identification. For more appropriate judgment of CVDs, this score incorporates age, total cholesterol, HDL cholesterol, smoking status, systolic blood pressure,

and gender (Cooper *et al.,* 2005). On the basis of scores obtained in patients, these are classified in three groups as scores of <10% low, intermediate 10–20%, and high >20% risk.

Last category of patients is confirmed as atherosclerotic disease patients and needs early intensive clinical care and factor modification (Cooper *et al.,* 2005). More specifically, patients with a 10-year risk >20% or with diabetes are considered to be coronary heart disease risk equivalents in terms of the approach to Homozygous familial hypercholesterolemia (HoFH) is associated with severe hypercholesterolemia and premature cardiovascular morbidity and mortality.

### 2. 4. 2 Cardiovascular risk factors and complications

More often, increased cardiovascular risk has also been associated with the presence of obesity, hypertriglyceridemia, chronic kidney disease, and elevated levels of LP (a). These patients show abnormal levels of LDL cholesterol, triglycerides, and the HDL cholesterol. Another category of patients is associated with hypertriglyceridemia, low LDL cholesterol, and small dense LDL particles. Furthermore, chronic cholelithiasis and primary biliary cirrhosis are associated with hypercholesterolemia due to elevations in systemic levels of rare lipoprotein X, with xanthomata, and hyperviscosity. Moreover, level of saturated fat is inversely associated with atherosclerosis progression in postmenopausal women, whereas polyunsaturated fat (PUFA) and carbohydrates were positively associated (Kalantarian *et al.*, 2014). Quantification of chlorotyrosine and oxidized methionine in circulating HDL might be useful indicators of the risk of cardiovascular disease that are independent of HDL cholesterol (Shao *et al.,* 2014). A fasting profile that incorporates measurements of the total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides is preferred to simple measurements of total cholesterol alone. However, conventional risk prediction algorithms

are prepared based on the presence of major cardiovascular risk factors identified by population studies, including hypercholesterolemia, hypertension, diabetes, smoking, low levels of HDL cholesterol, and intermediate-risk patients (10-year risk 10–20%) and may require further investigation to categorize their cardiovascular risk. In addition, assessment of non-HDL cholesterol, total HDl cholesterol and triglyceride, and HDL cholesterol ratios are recommended as secondary measures for risk assessment. Despite the use of risk prediction scores, some patients stratified as low risk experience clinical events. More often, hyperglycaemia or type 1 diabetes plays a major role in increased incidence of CVD and mortality in individuals. Patients facing type 1 diabetes showed an increase of premature mortality, primarily from cardiovascular disease (CVD) (Distiller, 2014). It also indicates that severe lipid disorders may occur in patients with type 1 diabetes, but the occurrence of elevated high-density lipoprotein cholesterol is positively associated with longevity of these patients. Similarly, nonrenal hypertension by itself is a significant risk factor for CVD but if adequately treated does not appear to mitigate against longevity (Distiller, 2014). In old ages (55–60) measurement of blood pressure and anthropometric and biochemical parameters such as total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low density lipoprotein (LDL) assist in finding high risk of CVD, dyslipidemia, and metabolic disorders in patients (Distiller, 2014).

### 2. 4. 3 Serum lipid risk markers

Serum lipid biomarkers are also used to access the risk to cardiovascular diseases. They include the following: high cholesterol concentration, hypertriglyceridemia, reduced High density lipoprotein cholesterol, elevated low density lipoprotein cholesterol.

### 2. 4. 4 Interaction between lipid markers in cardiovascular disease

LDL cholesterol plays a pivotal role in the formation and clinical expression of atherosclerotic cardiovascular disease. There is an important connection between HDL and immunity in atherosclerosis (Catapano *et al.,* 2014). LDL is a major transporter of cholesterol in the circulation to peripheral tissues where the cholesterol is used for maintenance of cell membranes. Cholesterol is either reutilized for lipophorin formation or excreted in the bile. LDL cholesterol is directly related to the pathogenesis of atherosclerosis and as a therapeutic target to reduce CVD risks. HDL proteins in the systemic circulation consist of a core of esterified cholesterol and triglyceride surrounded by a surface monolayer of phospholipid and a range of lipoproteins. The HDL also integrates innate and adaptive immunity because during infections or acute conditions high-density lipoprotein cholesterol levels decrease very rapidly and HDL particles increase (Catapano *et al.,* 2014). Thus, low HDL cholesterol levels predict severe CVD risks, more often, ability of HDL to influence the cholesterol availability in lipid rafts in immune cells results in the modulation of Tolllike receptors, MHC-II complex, and B and T cell receptors, while specific molecules shuttled by HDL such as sphingosine-1-phosphate (S1P) contribute to immune cells

trafficking (Catapano *et al.,* 2014). It has also been tried to correlate lipid abnormalities with hypertension, diabetes, and cardiovascular diseases. Moreover, various cardiovascular risk prediction models have been updated by incorporating traditional risk factors and molecular, immunological genetic, imaging, and biophysical factors for more authentic and reliable estimation of cardiovascular risk. Many of these biomarkers, alone or in combination, have been incorporated into risk prediction models to determine whether their addition increases the model's predictive ability. Similarly, myocardial infarction can be assessed by using

circulating microRNAs level in patients (Zampateki *et al.*, 2012) and Omega-3 Index as a risk factor for cardiovascular diseases (Schacky, 2014).

Despite recent treatment advances and clinical methods available, there is an increase in cardiovascular diseases (CVD) mortality cases every year. There are terrible reports of cases of hypercholesterolemia management as the percentage of individuals with LDLc plasma concentration has been alarmingly increased and cardiovascular risk sets are very high. Therefore, both diagnostic and additional therapeutic strategies are highly needed to evaluate CVDs and other lipid abnormalities. Hence, more prompt and continuous efforts are needed to develop new biomarkers for achieving high diagnostic accuracy to predict CVD risks. In addition, good preventive therapies are also needed to stop rising death toll due to CVDs. Thus, risk stratification and assessment of cardiovascular risks in cardiac patients are important areas of research in clinical biology (Heringlake *et al.,* 2014). It warrants further investigations to determine ultramodern emerging risk biomarkers for CVD for more appropriate risk assessment (Grundy, 2014). These biomarkers will not only improve clinical decision making, but also help in exploring CVD risk types in man. Therefore, after seeing the severity and massive increase in CF cases both diagnosis and CVD therapeutics will be highly needful.

### 2. 4. 5 Inflammatory biomakers to Cardiovascular disease

C-Reactive protein is the forerunner in the hunt for inflammatory markers and is subject to intensive research in numerous studies worldwide. Unlike other markers of inflammation, CRP levels are stable over long periods, have no diurnal variation, can be measured inexpensively with available high-sensitivity assays, and have shown specificity in terms of predicting the risk of CHD (Devaki *et al.*, 2011). CRP may have a role in the genesis of

atherosclerotic lesion, since it reduces the expression of nitric oxide (NO) synthase and prostacyclin synthase, and binds LDL-C and promotes its uptake by macrophages, a key step in atherogenesis. CRP also up-regulates the expression of adhesion molecules on endothelial cell (EC). All these phenomena are associated with atherogenesis (Mehta *et al.,* 2007).

Multiple prospective cohort studies have established that increased CRP levels are associated with increased CHD risk in both genders, across a wide age range, and in primary as well as secondary prevention settings. These findings have been consistent in different populations with diverse ethnic backgrounds and in diverse clinical settings, and they have predicted risk of a variety of cardiovascular outcomes, including incident acute myocardial infarction (AMI), stroke, sudden cardiac death, peripheral artery disease and also incident diabetes and new onset hypertension. CRP levels have also been shown to predict the risk of both recurrent ischemia and death among those with stable and unstable angina, those undergoing percutaneous angioplasty, and those presenting to emergency rooms with acute coronary syndrome (ACS) (Greenland *et al.,* 2010; Mahjid and willerson, 2011).

### 2. 4. 5. 1 Emerging inflammatory biomarkers of coronary artery disease

There are many known markers available to predict CAD or CVDs including acute coronary syndromes (Mozaffarian *et al.,*2012; Nikus *et al.,* 2014) but seeing severity and rising morbidities in all age groups upgraded biomarkers are to be needed for an earlier assessment of CAD (coronary artery disease) patients. There are many emerging biomarkers for coronary artery diseases which can diagnose the CVD. These important biomarkers are inflammatory mediators (Yayan, 2013), spectral analysis of electrocardiography (Chan *et al.,* 2014), vitamin D status and cardiometabolic (Kew e*t al.,* 2013), aortic wave reflection and pulse pressure amplification, and coronary CT angiography (Mihl *et al.,* 2014). These

are more appropriate risk markers which can evaluate the status of coronary artery diseases in old ages (Cho *et al.,* 2013). Similarly, automated quantification of epicardial adipose tissue (EAT) and manual assessment of coronary CT angiography can establish a correlation with coronary artery disease (Mihl e*t al.,* 2014) and work as clinically important biomarkers in acute coronary syndrome (Chan and Rainer, 2013). Peripheral artery disease (PAD) is a marker disease for generalized atherosclerosis and represents one of the world's major causes of morbidity and mortality (Kloos *et al.,* 2014). Moreover, peripheral venous blood flow rate also functions as important coronary sinus biomarkers (Truong *et al.,* 2014). Similarly, level of proinflammatory markers (hs-CRP, IL-6, and ICAM-1) and pregnancy associated plasma protein-A (PAPP-A) in serum also clearly display myocardial infarction (MI). No doubt, hs-CRP is the potential marker to discriminate cases of the UA from controls while PAPP-A is the reliable marker which can discriminate the cases of MI from UA and controls (Bowring *et al.,* 2008). Similarly, β2-microglobulin, cystatin C, and creatinine are important risk markers of symptomatic peripheral artery diseases (Joosten *et al.,* 2014). P194 Serum uric acid is an independent predictor of the decreased number of circulating proangiogenic progenitor cells in asymptomatic coronary artery disease patients (Berezin and Kremzer, 2014), while P733 regulatory B cells from patients with coronary artery disease display numerical and functional alterations, a novel immune defect in atherosclerosis (Dumitriu *et al.,* 2014). Similarly, phospholipase A2 enzymes can predict ischemic events after acute coronary syndromes (Ryu, *et al.,* 2002), while plasminogenplasmin level is considered as an important biomarker of fibrinolysis (Kwann, 2014). Similarly, atrial fibrosis is a risk stratifier for atrial fibrillation (Velagapudi *et al.,* 2013), while mitogen-activated protein kinases (Bryk *et al.,* 2014) and intercellular adhesion molecule1 gene polymorphism are good markers of coronary heart diseases (Luo *et al.,*

2014) mainly for atherosclerosis (Bryk, 2014). Similarly, aortic vascular functions are also used for assessment of cardiovascular risks (McCall *et al.*, 2014) and provide stratification in asymptomatic severe aortic stenosis (Bhattacharyya *et al.*, 2012). Besides this, traditional cardiac risk factors are also used as markers for disease diagnosis, which are too old to clear out major reasons of cardiovascular problems in different groups of man. More often, several nontraditional biomarkers, including proinflammatory high-density lipoprotein (piHDL) and leptin, have been individually associated with subclinical ATH in SLE. In addition few important biomarkers are combined into a risk profile to predict increased risk of cardiovascular disease in patients with SLE (McMahon and Skaggs, 2014). However, lipid related markers (Chowdhury *et al.*, 2014) or emerging lipoprotein risk factors are used for prediction and assessment of cardiovascular risks in all age groups (Bachorik and Ross, 1995).

### 2.4.6 Fibrinogen as a risk factor

Fibrinogen has been identified as a major independent risk factor for cardiovascular disease **(**Meade *et al.,* 1986, Kannel *et al.,* 1987). The association with CVD has led to evaluation of fibrinogen as a possible causal factor in cardiovascular disease, as a therapeutic target, and as a risk predictor in both healthy persons and those with established cardiovascular diseases. The association of fibrinogen with risks of cardiovascular disease and mortality in healthy individuals has been reliably established through international collaborative individual-person metaanalyses, The strength of fibrinogen's associations with coronary heart disease and stroke is similar to those of classic risk factors, such as blood pressure and serum cholesterol, and to the circulating concentration of C-reactive protein (CRP), which,

like fibrinogen, is a marker of the inflammatory response, including low-grade inflammation in healthy persons (Baena *et al.*, 2005).

Fibrinogen has also been associated with traditional cardiovascular risk factors, (Barasch *et al.,* 1995) suggesting that elevation of fibrinogen may be a pathway by which these risk factors exert their effect. There are several mechanisms by which fibrinogen may increase cardiovascular risk. First, it binds specifically to activated platelets via glycoprotein IIb/IIIa, contributing to platelet aggregation. Second, increased fibrinogen levels promote fibrin formation. Third, it is a major contributor to plasma viscosity. Finally, it is an acute-phase reactant that is increased in inflammatory states.

### 2.4.7 Erythrocyte aggregation as a risk factor

Erythrocyte aggregation is the reversible clumping of red blood cells (RBCs) under low shear forces or at stasis. Erythrocytes aggregate in a special way, forming rouleaux. Rouleaux are stacks of erythrocytes which form because of the unique discoid shape of the cells in vertebrate body. The flat surface of the discoid RBCs give them a large surface area to make contact and stick to each other; thus, forming a rouleaux. Rouleaux formation takes place only in suspensions of RBC containing high-molecular, fibrilar proteins or polymers in the suspending medium (often Dextran-2000 in-vitro). The most important protein causing rouleaux formation in plasma is fibrinogen. RBC suspended in simple salt solutions does not form rouleaux (Chien and Jan, 1973, Chien and Sung, 1987, Mesielman, 1993). Studies show that the type of dietary lipids and the plasma lipid concentrations have an effect on the lipid composition of the erythrocytes (Dougherty *et al.,* 1987). An increased concentration of cholesterol in plasma implies its increased accumulation in the erythrocyte membrane, which leads to altered shape and stiffness (Kanakaraj and Singh. 1989). It has been shown

that increased erythrocyte aggregation is associated with adverse effects in the cardiovascular system (Bamgboye, 2007). A long-standing postulation is that erythrocyte aggregates could form a thrombus in the circulation at low shear stress, which may lead to ischemia and infarction (Stoltz and Donner, 1987). However, this has yet to be considered in clinical applications. It has been reported that the odds of having myocardial infarction in patients with unstable angina was 5.7-times higher among groups with elevated erythrocyte aggregation when compared with groups with normal erythrocyte aggregation (Neumann *et al.,* 1991).

### 2.4.8 Obesity as a risk factor

The rising prevalence of obesity is a worldwide problem affecting not only the developed world, but also developing nation such as Nigeria with the prevalence ranging between 8.122.2% (Chukwuonye *et al., 2013*). Among staff of Usmanu Danfodiyo University, Sokoto is 12%, based on gender male 10% and female 37.5%. Prevalence of obesity in combination with overweight was 47% (Nkwoka.*et al.,* 2014). Ajayi *et al*., (2015) reported the prevalence rate of obesity among Urban and Rural Dwellers in Nigeria. In 2013, more than 1.9 billion adults, 18 years and older, were overweight, of these over 600 million were obese. Overall, about 13% of the world's adult populations (11% of men and 15% of women) were obese in 2014 (WHO, 2015), to be16.5% and 4.0% respectively.

#### 2.4.8.1 Causes of Obesity

The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended. Globally, there has been: an increased intake of energy dense foods that are high in fat, and decrease in physical activity due to the increasingly sedentary nature of many forms of work, changing modes of transportation, and increasing

urbanization. Changes in dietary and physical activity patterns are often the result of environmental and societal changes associated with development and lack of supportive policies in sectors such as health, agriculture, transport, urban planning, environment, food processing, distribution, marketing and education (WHO, 2015).

## CLASSIFICATION OF ADULT UNDERWEIGHT, OVERWEIGHT AND OBESITY

**Table I.** International Classification of Adult Underweight, Overweight and Obesity According to BMI.

| | BMI(kg/m2) | CHRONIC DISEASE RISK |
|---|---|---|
| **Underweight** | <18.5 | Low (but increase mortality and morbidity from other causes |
| • **Severe thinness** | <16.0 | |
| • **Moderate thinness** | 16.0-16.9 | |
| • **Mild thinness** | 17.0-18.5 | |
| **Normal range** | 18.5-24.9 | Average |
| **Overweight** | ≥25.0 | |
| **Pre-obese** | 25.0-29.9 | Increase |
| **Obese** | ≥30.0 | |
| • **Obese class I** | 30.0-34.9 | Moderate |
| • **Obese class II** | 35.0-39.9 | Severe |
| • **Obese class III** | ≥40.0 | Very severe |

**(WHO, 2008)**

## 2. 5 LIPID ABNORMALITIES AND CARDIOVASCULAR RISKS

Cardiovascular diseases are prevalent in human population and most of them are related to diet but genetic lipid abnormalities such as hypercholesterolemia, hypertriglyceridemia, HDL metabolism disorders, and combined hyperlipidemias are more severe. In addition, diseases like dyslipidemia/hyperlipidemia atherosclerosis, familial hypercholesterolemia, hypertriglyceridemia, and diabetes are also prevalent in pediatric groups. Hypertriglyceridemia is commonly found in patients with chronic renal failure, which is largely due to decreased activity of lipoprotein and hepatic lipase and selective enrichment with apolipoprotein C-II. Each of them contributes to reduced hydrolysis of triglyceridecontaining particles. Triglyceride abnormalities are more commonly encountered in patients treated with peritoneal dialysis, which may reflect the presence of glucose in dialysate. Elevated levels of LP (a) and low HDL cholesterol are also encountered and contribute to the accelerated rate of cardiovascular disease. Cardiac implications are also related to hypoglycaemia in patients with diabetes (Hanefield, 2013). Moreover, both type 1 and type 2 diabetes are considered to be high-risk conditions and have stringent cholesterol targets. Similarly, common cholesterol disorders, mainly dyslipidemia, were also found to be specific to the pediatric diabetes population (Bamba, 2014). Altered serum lipid level is the most important risk factor for coronary artery disease (CAD). Nephrotic syndrome is associated with elevated levels of LDL cholesterol, triglycerides, and LP (a). It occurs as a result of increased hepatic apolipoprotein B synthesis, due to reduced oncotic pressure and reduced catabolism of LDL and lipoprotein lipase activity.

Similarly, hypothyroidism is associated with elevated levels of LDL cholesterol and triglyceride, either in isolation or in combination. Reductions in LDL receptor expression and activity, biliary cholesterol excretion, and lipoprotein lipase activity underlie the lipid abnormalities. Hyperthyroidism is associated with excessive activity of each of these factors and is, therefore, typically associated with low levels of LDL cholesterol and triglycerides. Abdominal obesity is also an important visible sign and display of elevated levels of VLDL (very high-density lipoprotein) and of triglycerides and low levels of HDL cholesterol. For fighting obesity, calorie burning by slow and regular exercise results in weight loss. Overweight and obesity are complex health problems that mostly affect adults. There are many health conditions associated with overweight and obesity, including hypertension, coronary heart disease, and type II diabetes. Obesity can be cut down by making dietary modification and therapeutic lifestyle changes (TLC). TLC is an effective lifestyle therapy targeting low-density lipoprotein cholesterol (LDL), a risk factor for coronary heart disease. Along with lowering LDL, TLC also improves risk factors associated with the metabolic syndrome and diabetes, including blood pressure, high-density lipoprotein cholesterol (HDL), serum triglycerides, blood glucose, and weight status. There are so many associating factors which can assist in emerging risks for cardiovascular diseases (Gupta *et al.,* 2013).

Cardiovascular risks such as defects in angiogenesis/vasculogenesis or vessel repair are major complications of coronary artery disease (CAD) which are mostly seen in aged people. Similarly, CVD risks have also increased in women during pregnancy, which is an important issue for management of their cardiovascular health (Rich-Edwards *et al.,* 2014). Cannabis-associated myocardial infarction is observed in young men with normal coronary arteries (Hodcfrot *et al.,* 2011). In developed countries, there is a large population that shows an increased frequency of atherosclerosis (ATH) mainly systemic lupus erythematosus

(SLE). There are paradoxical reports on CAD in South Asian Ethnicity and Cardiovascular Risks (Weiner *et al.,* 2014), but most of atherosclerotic risk factors and atherosclerotic postoperative events are associated with low inflammation with abdominal aortic aneurysms (Hurks *et al.,* 2014). Similarly, the severity of subclinical cardiovascular disease among those with nonalcoholic fatty liver has been alarmingly increased (Oni *et al.,* 2013) with a significant acute myocardial infarction. Hence, there is an urgent need of potential novel cardiac biomarkers for prediction of acute myocardial infarction (Nursalim *et al.,* 2013). More often, highly sensitive cardiac biomarkers are needed to explore cardiovascular risks, morbidity, and mortality in elderly men (Eggers *et al.,* 2013). These are also required to predict acute coronary syndrome (Berenson *et al*., 1998) and for prediction or finding level and type of risk and its assessment in post infarction heart failure (Lippi and Cervellin, 2014) and vasculogenic erectile dysfunction (Miner *et al.,* 2014). Few important body activities such as inflammation, obesity, thrombosis, and autoantibodies also display procardiovascular risks (Samad and Ruf, 2013) and act as emerging biomarkers (LopezMejias *et al.,* 2014). Similarly, sedentary behaviour was also proved as an emerging risk factor for cardiometabolic diseases in children and youth (Saunders *et al.,* 2014).

### 2.5.1 Hypertension and Cardiovascular risks

Hypertension and dyslipidemia are the most prevalent cardiovascular risk factors, with approximately 350 million people having these concomitant conditions throughout the globe. Hypercholesterolemia in midlife relates to an increased risk of Alzheimer's disease (AD) in later life. Another possible mechanism, hypercholesterolemia, may be associated with hypoperfusion through the progression of atherosclerosis (Sukurai and hanyu, 2014). Similarly, dyslipidemias is characterized mainly by elevated levels of total cholesterol and

low-density lipoproteins in cardiovascular patients. High triglyceride levels and lower highdensity lipoproteins are encountered 2 and 1.5 times more frequently, respectively. Agerelated changes and metabolic hepatic disorders associated with alcohol abuse and consequences of prior infectious diseases play an important role in the pathogenesis of dyslipidemias in patients over 40–45 years of age (Paltsev *et al.,* 2014). Vascular dementia is caused by stroke that occurs due to hypertension. More often for evaluation of lipid related disorders, demographic, diagnostic, and medication-related factors are associated with BP (blood pressure) and LDLc goal attainment in patients with concomitant hypertension and dyslipidemia stratified by body mass index BMI. Many more variations were found in therapeutic care in patients with concomitant hypertension and dyslipidemia across different BMI groups. Further, the presence of high body mass index (BMI) has a negative effect on the achievement of blood pressure (BP) and low-density lipoprotein cholesterol (LDLc) targets. Hence, string markers may be needed for improving these disparities (Chopra and Kamal, 2014). In addition, concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, apolipoprotein A-I, apolipoprotein B, and lipoprotein in serum of patients are biomarkers of primary hypertension and with hyperhomocysteinemia (Baszczuk *et al.,* 2014).

### 2.5.2 Diabetes and Cardiovascular risks

Cardiovascular disease is more prevalent in type 1 and type 2 diabetes and continues to be the leading cause of death among adults with diabetes. Diabetes coexists as a more severe risk factor with other associating risk factors, in particular with dyslipidemia. It increases cardiovascular risks due to increased levels of triglycerides, low levels of high-density lipoprotein cholesterol, and postprandial lipidemia. Dyslipidemia is mostly observed in

patients with type 2 diabetes or metabolic syndrome (Jaiswal *et al.,* 2014). In addition, atherosclerotic vascular disease (AVDs) shows obstruction in streaming blood functions due to arterial thickness and high blood pressure. In AVDs, lipid metabolism plays a central role.

However, measurement of arterial stiffness provides assessment of endothelial dysfunction and diagnosis of atherosclerotic burden in patients with MetS (Mitu *et al.,* 2014). Therefore, total serum γ-glutamyl transferase activity (GGT) represents the impact of metabolic disease on vascular injury and atherosclerosis (Mitu *et al.,* 2013). It acts as important biomarkers of arteriosclerosis in the Multiethnic Study of Atherosclerosis (MESA) (Bradley *et al.,* 2013). Similarly, β-trace protein from GFR marker is also used as cardiovascular risk predictor (Orenes-Piñero *et al.,* 2013). There are other markers such as occurrence of a fatty kidney and ectopic lipid in obesity-related renal disease that is also associated with CVDs and are emerging risk predictors (De Vries *et al.,* 2013). Lipid abnormalities are also analysed by MS (Li *et al.,* 2014) and MALDI mass spectrometry imaging and lipidomics for clinical diagnosis (Arafah *et al.,* 2014). Similarly, human serum proteome analysis has been emerging as a new source of markers for knowing metabolic disorders (Girolamo *et al.,* 2012). Similarly, defects in angiogenesis/vasculogenesis or vessel repair are major complications of coronary artery disease (CAD).

## 2.6 SERUM TRIGLYCERIDE TO HDL CHOLESTEROL RATIO

Triglyceride to high-density lipoprotein cholesterol ratio, total cholesterol to high-density lipoprotein cholesterol ratio, and low ankle brachial index in an elderly population can predict risks of cardiovascular diseases (Zhan *et al.,* 2014). The associations of triglyceride (TG) to high-density lipoprotein cholesterol ratio (HDLc) and total cholesterol (TC) to HDLc ratio and low ankle brachial index (ABI) are important biomarkers which predict

CVDs (Zhan *et al.*, 2014). Although triglyceride levels are obtained in more standard lipid panels, but it can be used in CVD risk prediction by using some other lipid profile related markers. Increasing evidence suggests that both fasting and nonfasting triglyceride levels predict prospective cardiovascular risk. Therefore, the triglyceride HDL cholesterol ratio has become increasingly important, providing mixed dyslipidemic patterns in the setting of obesity and the metabolic syndrome (Zhan *et al.*, 2014). A triglyceride: HDL cholesterol ratio >3.5 appears to be associated with increased cardiovascular risk. Similarly, a significant increase in total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol (LDL-C), apolipoprotein B (ApoB), and lipoprotein (a) (LP(a)) and a significant decrease in apolipoprotein A1 (ApoA1), ApoA1/ApoB ratio, and PON1 activity/HDLc ratio are used as important biochemical markers (Fekih *et al.*, 2014). Total cholesterol, LDL-C, ApoB, LP(a), and ApoA1/ApoB ratios are also considered as good biomarkers for prediction of CVDs in man (Shah and Arneja, 2013). More often, a high plasma triglyceride concentration, low HDL cholesterol concentration, and increased concentration of small, dense LDL-cholesterol particles are an indication of diabetic dyslipidemia. The lipid changes associated with diabetes mellitus are attributed to increased free fatty acid flux secondary to insulin resistance. Insulin resistance and type 2 diabetes are associated with a clustering of interrelated plasma lipid and lipoprotein abnormalities, which include reduced HDL cholesterol, a predominance of small dense LDL particles, and elevated triglyceride levels. In fact, patients with higher level of LDL, triglycerides and total cholesterol (hyperlipidemia), affected with type 2 diabetes mellitus (T2DM) remain at higher risk of coronary artery disease (Shah and Arneja, 2013).. Total cholesterol, LDL-C, ApoB, Lp(a), and ApoA1/ApoB ratios are also considered as good biomarkers for prediction of CVDs in man (Shah and Arneja, 2013).

### 2.6.1 Lipid-Lipoprotein Ratio

Lipid-lipoprotein ratio can predict severe cardiovascular risks in both early age and old age patients. Moreover, it was also established in population studies that increasing levels of oxLDL are associated with greater cardiovascular risk. Therefore, measurement of antibodies against oxidized forms of LDL (oxLDL) may play a role in risk stratification. But it remains unclear whether oxLDL provides an incremental or greater ability to predict risk compared with measuring LDL cholesterol. More often, LDL cholesterol does not account for the entire cohort of atherogenic lipid particles within the systemic circulation. Therefore, calculation of non-HDL cholesterol has become increasingly popular because it represents the full complement of atherogenic lipids. Similarly, lipoprotein particle size and number, as determined by nuclear magnetic resonance, are used as an alternative approach to evaluation of lipid abnormalities related to cardiovascular risk. However, both low levels of LDL cholesterol and the number of small LDL particles increased or decreased are an indicator of cardiovascular risks. These small, dense LDL particles can be used to establish a relationship with the metabolic syndrome and abdominal obesity in adults. In a similar fashion, raising the concentration of small HDL particles appeared to predict the clinical benefit of drugs on clinical events in patients with established coronary heart disease. Hence, there is a need to increase standardization, validation, and authentication of newly emerging biomarkers in cardiovascular risk prediction and monitoring of therapeutic response. Besides measurement of LDL and HDL cholesterol, quantification of lipid particles, apolipoprotein B (ApoB), and apolipoprotein (AI) provides a direct measure of atherogenic and therapeutic role in human

population. More often, number of lipid particles may directly influence the accumulation of lipid within the artery wall. However, levels of ApoB and ApoAI or ratio of

ApoB : ApoAI is the strongest predictor of myocardial infarction and statin is a predictor of slowing progression of coronary atherosclerosis. In addition, assessment of ApoB and ApoAI may provide incremental risk prediction when used in place of LDL and HDL cholesterol, respectively. More specifically, using an apolipoprotein-based approach to risk prediction will become a cost-effective method. Apolipoproteins are very heterogeneous protein family, implicated in plasma lipoprotein structural stabilization, lipid metabolism, inflammation, or immunity. Hence, apolipoprotein composition and structure may contribute to elucidating lipoprotein roles in atherogenesis. It may also assist in developing new therapeutic strategies for the treatment of lipoprotein-associated disorders. Apolipoprotein E effects free high-density lipoproteins and cholesterol metabolism. Therefore, characterizing the apolipoprotein component of plasma VLDL, LDL, and HDL fractions from patients can predict the vulnerability of disease in atherosclerotic patients. The highest levels of AP SAA found in patients elucidate the role of LDL as AP SAA carrier into the subendothelial space of artery wall, where AP SAA accumulates and may exert noxious effects (Kato *et al.*, 1989). Similarly, lipoprotein-associated phospholipase A2 (Lp-PLA2) circulates on LDL particles and is thought to be involved in the promotion of inflammation, as a result of the release of arachidonic acid metabolites. Moreover, elevated levels of Lp-PLA2 are found to be associated with an increased risk of cardiovascular events in patients which later on evoke cardiovascular disease. Lp-PLA2 levels have also been demonstrated to be reduced by statins, suggesting that they may potentially be a target for therapies in addition to being used as a marker of cardiovascular risk (Lepedda *et al.*, 2014)

## 2.7 HIGH FAT DIET AND CARDIOVASCULAR DISEASES

The association between dietary fat and risk of coronary heart disease is a subject of debate, especially whether coronary heart disease risk relates to both the quantity and the quality of dietary fats. Prospective cohort studies have investigated the associations between the intake of total dietary fat and the major types of dietary fat and the risk of coronary heart disease with inconsistent results. Total fat intake was positively associated with risk of coronary heart disease in four studies (McGee *et al.*, 1984; Posner *et al*., 1991; Esrey *et al*., 1996; Boniface *et al*., 2002), whereas six studies found no association (Morris *et al*., 1977, Fehily *et al*, 1993; Ascherio *et al*., 1996). A positive association between saturated fat intake and risk of coronary heart disease was found in some studies (McGee *et al*., 1984; Posner *et al*., 1991; Esrey *et al.*, 1996; Boniface *et al*., 2002; Kushi *et al*., 1985; Goldbourt *et al*., 2004), whereas others found no association (Garcia-Palmieri *et al*., 1980; Ascherio *et al*.,1997). An inverse association between monounsaturated fat intake and risk of coronary heart disease was found in one study (Hu *et al*., 1997), whereas two other studies found a positive association (Posner *et al*., 1991, Esrey *et al*., 1996). Three studies found that monounsaturated fat intake was not associated with coronary heart disease risk (GarciaPalmieri *et al*., 1980; Pietinen *et al*., 1997, Shekelle *et al*., 1981). Polyunsaturated fat intake was inversely associated with risk of coronary heart disease in three studies (Pietinen *et al.*, 1997; Goldbourt *et al*., 2004; Shekelle *et al*., 1981), whereas eight studies found no association (McGee *et al.*, 1984; Posner *et al*., 1991; Esrey *et al*., 1996; Morris *et al*., 1977; Ascherio *et al*., 1996; Pietinen *et al*., 1997; Kushi *et al*., 1987; Buttner, 1993). One factor potentially contributing to the discrepancies observed across the studies may be the differences in the way of expressing fat intake and incomplete adjustment for intake of total energy and other types of fat .Obesity is when excess fat is accumulated in the body, mainly

in adipose tissues, to an unhealthy extent. An adult with a body mass index (BMI) between 25 and 30 kg/$m^2$ is considered overweight, while an adult with a BMI over 30 kg/m2 is regarded as obesity (WHO, 2005) nonetheless, this classification is debatable when applied to different races (Wang, 1996). Utilizing the historic record provided by American obesity data (CDC) we are able to track the increasing incidence of overweight and obesity in the new world. Not trailing far behind, developing countries are catching up with this trend, which is a direct consequence of overconsumption of energy-dense food and engaging in a sedentary lifestyle (Hussain *et al*., 2007). Obesity is one of the biggest threats to public health in the new century, as it increases the risk of developing type 2 diabetes (Colditz,1995), cardiovascular disease (Willett, 1995) and certain types of cancer (Cabré *et al.*, 2012).

Consequently, considerable financial resources as well as social and scientific efforts have been mobilized towards the war against obesity (Berenson *et al.,* 1998). One focus point has been to promote a reduction in dietary fat intake, This is mainly because fat contains more energy per unit weight than carbohydrate or protein, 9 kcal/g, 4.5 kcal/g and 4 kcal/g respectively. By reducing the energy density of food, the average energy intake of the general public should therefore be reduced. Besides the increase in average energy intake over time (Chitra *et al*., 2000), another major alteration over the thousands of years of dietary history is the dramatic elevation of carbohydrate content in the food at the expense of protein (Cordain, 2005). One recent publication suggested that over a period of two years a low-carbohydrate diet had a similar weight loss effect as a low-fat diet (Foster, 2010). Moreover, a body of evidence pointed to the beneficial effects of high protein diet on whole body metabolism (Paddon-Jones, 2008), implying that macronutrients are not simply

different with regard to their caloric value, but have other impacts on weight gain and maintenance that are not fully elucidated.

Percentage Compositions of Normal and Experimental Diet (High Fat Diet).

| Nutrient Components | Ingredients Used | Normal Diet (ND) (% per 100g of feed) | High Fat Diet (HFD) (%per 100g of feed) |
|---|---|---|---|
| Protein | Fish meal | 50% | 25% |
| Fats and oils | Groundnut cake | 35% | 60% |
| Carbohydrates | Maize | 10% | 10% |
| Fibers | Rice thusk | 0.1% | 0.1% |
| Premix (Vitamins) | Vitamins B, C, D | 0.4% | 0.4% |
| Amino acids | Lysine, Methionine | 0.2% | 0.2% |
| Water | Water | 4.3% | 4.3% |

(Michael *et al.*, 2015)

## 2.8. FISH OIL

### 2.8.1 Fish oil source and constituent

Fish oil (FO) is derived from two sources; Fish oil liver derived from (the liver of the cod, halibut or shark) and fish body oil derived from the flesh of the herring and sardine (Calder, 2014). Fish oil and fish liver oil are sources of omega -3 long chain polyunsaturated fatty acid (LCPUFA); eicosapentaenoic acid (EPA) and docosahexanenoic acid (DHA).They also contain vitamin E. Fish liver oil contains vitamin A (750-1200mcg/daily dose) and vitamin D (2.5- 10mcg/daily dose) (Lenihan- Geel *et al*., 2013).

### 2.8.2 Health benefits of fish oil

Fish oil has been widely recognized due to their beneficial effects on health and are considered as essential supplements in human food (Choi *et al*., 2009). Fish oil has several effects which are thought to result from a reduction in inflammatory and thrombotic prostaglandins and leukotrienes and inflammatory cytokines via its anti-inflammatory, antithrombotic, hypolipidaemic and vasodialatory properties (Bernstein *et al*., 2012). Extensive research has established that EPA and DHA play a vital role in the prevention of Alzheimer's disease, atherosclerosis, heart attack, angina, stroke, congestive heart failure, depression and cancer (Virtanen *et al.,* 2009). Its mechanism of action includes alteration of lipoprotein metabolism: reduced triglycerides; mixed effects on low and high density lipoprotein (LDL and HDL) cholesterol, inhibition of atherosclerosis, prevention of thrombosis, reduction in heart rate, influence on arrhythmias, inhibition of inflammation and immune response (Virtanen *et al*., 2009). EPA and DHA in fish oil may decrease the rate of

atherosclerosis and is recommended as a therapeutic strategy to reduce cardiovascular disease (Kris-Etherton *et al*., 2002).

### 2.8.3 Recommendations for intake of fish oil

Recommendation of fish oil intake by six organisations:

I. UK Food Standard Agency (FSA): recommends two portions of fish per Week (including one oily): equivalent to 450mg omega LCPUFA/day.

II. British Diabetic Association: recommends a variety of fish (preferably oily) at least twice a week for those not documented with coronary heart disease, while those documented with coronary heart disease should consume 1g EPA/DHA daily preferably from fatty fish or consider supplement 1g EPA and DHA (medical advice) is recommended. While those with raised triglycerides 2-4g daily of EPA and DHA (with medical advice) is recommended.

III. International Society for the Study of Fatty Acids and Lipids (ISSFAL): recommends minimum of 500mg daily of EPA and DHA for cardiovascular health.

IV. National Institute for health and Clinical Excellence (NCE): recommends 1g daily LCPUFAs (preferably from oily fish, but from supplements if oily fish not consumed for myocardial infarction.

V. World Health Organisation (WHO): Two portions of fish per week; equivalent to 250-500mg daily of EPA and DHA (Jacobson, 2007).

### 2.8.4 Fish oil and susceptibility to ventricular tachycardia

The lipid composition of the cell membrane can be modified by diet. Fish oil

Supplementation alters myocardial responsiveness to beta-adrenoceptors stimulation and therefore vulnerability ventricular tachycardia/fibrillation (McLennan and Abywarden, 2005). Recently studies on fish oil and ventricular arrhymia (Brouwer *et al.,* 2005, Brouwer *et al.,* 2006) also did not show clear benefit of supplementation with fish oil on prevention of sudden death in patient with an implantable cardioverter defibrillator (ICD). After one year, 30% of patients in the fish oil group had a life threatening arrhythmias compared with 33% in the placebo group (Yokoyama *et al.,* 2007).

### 2.8.5 Fish oil and atherosclerosis

Fish oil intake may influence plaque growth because supplemental fish oil is incorporated into the phospholipids and cholesterol esters in atherosclerotic lesion (Davis *et al.,* 1987). In a recent study of patients awaiting carotid endarterectomy, specimens obtained at surgery showed that fish oil had been incorporated into plaques. This was associated with a significant decrease in macrophage infiltration, which is consistent with greater plaque stability (Thies *et al.,* 2003). As with human trial in animal models fish oil; supplementation inhibits development of experimental atherosclerosis independent of plasma lipid effects (David *et al.,* 1987, Zampoli *et al.,* 2006; Angerer *et al*., 2002; Wang *et al.,* 2004)

### 2.8.6 Fish oil and atrial fibrillation

Fish oil may prevent patients developing episodes of atrial fibrillation. Consumption of fish oil 1-4 times a week was associated with a 28% lower risk of incident atrial fibrillation ($p=0.05$) than consumption of fish less than once a month. The consumption of fish five times a week or more was associated with 31% lower risk ($p=0.08$) (Mozaffarian *et al.,* 2004). Rats study shown that fish oil decreases the synchronus contractile activity in electrically stimulated atrial myocytes (Jahangiri *et al.,* 2000). The decrease in synchronus

contractile activity may be explained by changes in membrane fluidity and could be the reason for the beneficial effects of fish oil in preventing atrial fibrillation. A recent study of rabbits has shown that DHA rich oils can prevent fibrillation induced by atrial stretch (Ninio *et al.*, 2005)

### 2.8.7 Fish oil infarction size and angina

Fish oil may also reduce infarct size and the incidence of large infarct (Landmark *et al.*, 1998), lower the prevalence of myocardial lesion (Burchifiel *et al.*, 1996), reduce cardiac oxygen consumption during experimental ischaemia in a rat ischaemia –reperfusion model (Pepe and Mc lennan, 2002), decrease episodes of angina (Salachas *et al.*,1994; Yamamoto *et al.*, 1995; Saynor *et al.*, 1996). In models of experimental myocardial infarction, fish oil decreases mortality rate, creatinine kinase end myocardial lipid peroxidises (Burchfiel *et al.*, 1996)

### 2.8.8 Effects of fish oil on membrane

Fatty acids (FA) are incorporated into cell membranes increasing the amount of PUFA in the membrane increases the fluidity and deformability (Gawrisch *et al.*, 2003). DHA is the most unsaturated FA and it is particularly effective in transitional change associated with transmembrane protein activation (Rajamoorthi *et al.*, 2005; Salem *et al.*, 2001). Fish oil is a ligand for nuclear receptors such as perixisome proliferator activated receptors and retinoid X receptors. Therefore, they may influence gene regulation (Dekelbaum *et al.*, 2006, Li *et al.*, 2005, Radominska-pandiya *et al.*, 2002).

### 2.8.9 Effects fish oil on lipid profile

Fish oil is effective in lowering the concentration of post prandial TG-rich lipoprotein particles, chylomicrons, chylomicron remnants and very low lipoprotein (VLDL) cholesterol (Weintrabiet *et al.,* 1988). It appears that chylomicron clearance is accelerated with fish oil therapy, probably because of reduced competition with VLDL cholesterol for hepatic receptor uptake after remnant particles are partly metabolised an aquire apolipoprotein E (Weintrabiet *et al.,* 1988). Fish oil supplementation has no significant effect on total serum cholesterol or LDL cholesterol concentrations if the TG level is not elevated (Harris *et al.,* 1997). Fish oil significantly lowers chylomicron level probably by decreasing secretion of VLDL cholesterol from the liver, also decreases chylomicron size, which increases clearance and possibly increases lipoprotein lipase activity. DHA and EPA appear equally effective in improving chylomicron clearance (Raitt *et al*., 2005). Animal and in vitro studies shows that consumption of fish oil increases HDL cholesterol receptors and the turnover of HDL cholesterol (Nestel *et al.,* 1987, Roach *et al.,* 1987).

### 2.8.10 Effects of fish oil on inflammation

Fish oil supplementation inhibits nuclear transcription factor κβ (NF-κβ) a key transcription factor in cytokine gene expression, cellular adhesion, inflammation and carcinogenesis (Schwartz *et al.,* 1999). In a murine model, fish oil decreases NF-κβ activation and TNF-α expression by 46% in lipopolyaccharide –stimulated macrophages (Novak *et al.,* 2003). It is thought that fish oil may decrease production of nitric oxide (NO) and its reactive products, especially in macrophages (Ohata *et al.,* 1997, Khair El- Din *et al.,* 1996). This is a favourable action when there is chronic inflammation or a tumour (Lala and Chatraborty

2001) in endothelial cells, NO is important for normal function. Fish oil is associated with improved endothelial function and presumably does not adversely influence No production in the endothelium.

### 2.8.11 Effects of fish oil on leptin

Leptin is one of the cytokines secreted by adipose tissues. Plasma concentrations of leptin reflect adipose tissue mass and a high leptin level is associated with obesity in human (Considine *et al.,* 1996). Elevated plasma concentration of leptin is independently associated with adverse cardiovasular risk (Soderberg *et al*., 1999; Soderberg *et al.,* 2001; Wallace *et al*., 2001). The mechanism for the increased risk associated with the elevated leptin concentration is not clear. Fish oil supplementation inhibit gene expression in an animal model (Raclot *et al.*, 1997). In a tribal population in Tanzania a diet rich in fish and fish oil is associated with a low plasma leptin concentration independent of body fat level (Winnicki *et al*., 2002).

### 2.8.12 Effects on platelets and coagulation

Fish oil has a mild antiplatelet effect but no significant effect on bleeding time. preoperative supplementation does not significantly decrease postoperative bleeding following coronary artery byepass surgery (Calo *et al*., 2005). In combination with aspirin, high dose of fish oil (>3000mg/day DHA and EPA) May lower fibrinolytic response to venous occlusion (La couviello *et al.,*1992) fish oil protects rather than worsens the gastric mucosa against ulcer induced by non-steroidal anti-inflammatory drugs (Al-Harbi *et al*., 1995). Fish oil supplementation does not change INR levels in patients on warfarin. There have been a few case reports of an idiosyncratic increase in INR after fish oil supplementation commenced.

## 2.9 EFFECT OF MANNITOL ON THE KIDNEY

Mannitol, an osmotic diuretic, has been used in the belief that it exerts renoprotective properties in patients undergoing surgery. However, results from studies in which mannitol has been evaluated in the perioperative setting, for prevention or treatment of acute kidney infection, are divergent. Although mannitol has failed to show a prophylactic effect in patients undergoing abdominal aortic or cardiac surgery (Smith *et al.,* 2008) , mannitol has been shown to reduce the incidence of postoperative (Molitoris *et al.,* 2002) Acute Kidney Infection in the setting of renal transplantation, along with volume expansion (Tiggeler *et al*., 1985: van Valenberg *et al.,*1987) Furthermore, mannitol treatment has been shown to increase the glomerular filtration rate (GFR) in patients after severe trauma or surgery (Valdes *et al.,* 1979) . In addition, it showed that mannitol increases GFR in postoperative cardiac surgery patients (Redfors *et al.*, 2009), possibly by a deswelling effect on tubular cells. Kurnik *et al* studied the effect of mannitol (15%) on RBF in patients with moderate chronic renal failure and found that mannitol did not affect RBF (Kurnik *et al.,* 1990)

### 2.9.1 Effect of Mannitol on the Heart

Recent study shows that plasma volume expansion with mannitol was not large enough to cause increased cardiac filling pressures at the time of renal blood flow measurements. However, there is a possibility that mannitol bolus plus infusion induced a transient increase in cardiac filling pressures and distention, causing a release of natriuretic peptides. Previous study shows that in postoperative uncomplicated cardiac patients with normal renal function, by using an identical protocol, it was found that mannitol did not affect renal blood flow (Redfors *et al.,* 2009) suggesting that mannitol-induced plasma volume expansion and the

consequent cardiac release of renal vasodilatory cardiac peptides is not the main mechanism behind the renal vasodilation, as demonstrated in the present study. Experimental studies have shown that renal ischemia causes endothelial cell injury and dysfunction followed by endothelial cell edema (Molitoris *et al.,* 2002**)**. Flores et al showed in an animal study that ischemia-induced endothelial cell swelling can be reversed and prevented by mannitol (Flores *et al.,* 1972). Hypertonic mannitol increases myocardial contractility in isolated cat papillar muscle (Koch *et al.*, 1963; Widenthal *et al.,* 1969; Willerson *et al*., 1974) and in the intact anesthetized and the conscious dog (Atkins *et al*., 1973; Hutton *et al*., 2017)

During experimental myocardial ischemia, mannitol increases coronary blood flow and reduces the extent of myocardial damage (Willerson *et al*., 1972). However, there has been no reported extensive study of the cardiovascular effects of mannitol in human subjects.

Accordingly, the purpose of this investigation was to assess the extent to which. a small, mannitol-induced increase in serum osmolality alters left ventricular function and coronary blood flow in patients with and without cardiovascular disease (Collier *et al.,* 2011). Flores et al suggested that mannitol maintains the GFR in renal ischemia primarily by an osmotic effect that reduces vascular endothelial cell swelling, which would reduce renal vascular resistance and increase renal blood flow (Flores *et al*., 1972). A study on healthy human volunteers showed no effect of mannitol on GFR (Goldberg *et al.,* 1965). Whereas mannitol increased creatinine clearance in patients with severe trauma/surgery (Valdes *et al*., 1965). In a recent study on uncomplicated postcardiac-surgery patients with normal renal function, by using a protocol identical to that in the present study, we showed that mannitol induced a 20% increase in GFR and filtration fraction with no change in RBF (Redfors *et al.*, 2009).

Those findings were interpreted as deswelling effect on tubular cells, subjected to intraoperative hypotensive episodes, and recruitment of functional nephrons that are opened up by mannitol, which will increase tubular flow and restore GFR (Lindstrom *et al*., 1999). In the present study, mannitol tended to increase GFR (16%), but the increase in GFR did not reach statistical significance (P = 0.16).

### 2.9.2 Effect of Mannitol on severe head trauma

Mannitol is frequently used for severe head trauma (SHT) treatment. Favorable results have been recently reported for the prophylactic use of aminoglycosides in patients with SHT. Medical treatment of SHT aims to prevent or minimize secondary brain damage following injury (Proccacio *et al.,* 2000). Mannitol has substituted other osmotic diuretics in the last 20 years for the treatment of patients with SHT (Kharitonova *et al*., 1984), especially when increased intracranial pressure (ICP) is suspected, or is in fact present (Suarez, 2001). In many studies, mannitol has proved to benefit cases of high ICP, deficient blood flow and brain metabolism, and short-term benefit for the patient's neurological prognosis (Mendelow, 1985). The immediate plasma expansive factor of mannitol decreases hematocrit and blood viscosity, increasing brain blood flow (BBF) as well as oxygen distribution. This decreases ICP a few minutes after mannitol administration, especially in patients with low perfusion pressure in cerebral (PPC) (< 70 mmHg) (Muizelaar *et al*. 1984). Mannitol increases the plasma osmolar pressure and is excreted in the urine, which implies significant risk of acute renal failure by acute tubular necrosis when plasmatic osmolarity reaches values above 320 mOsm/l (Czupryniak *et al.,* 2008).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

## 3.1 EXPERIMENTAL ANIMALS

Sixty (60) male Wistar rats (100-120g) were purchased from the Animal House, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. All animals were kept at constant room temperature (25-30 $^{o}$C) and 12:12 hr light: dark cycle with free access to food and water ad libitum. The rats were acclimatized on normal diet for one week before introducing the experimental diets for 12weeks (84 days) for the induction of obesity. The rats were divided into the six (6) groups, they are Group I: Normal diet, Group II: Normal diet with fish oil fed rats, Group III: Normal diet with mannitol, Group IV: High fat diet, Group V: High fat diet with fish oil, Group VI: High fat diet with mannitol.

## 3.2 INDUCTION OF OBESITY

Normal diet was sourced from Grand Cereals and Oil Mills Ltd Jos, and high fat diet (HFD) was prepared by mixing 40% of the normal diet (Grow Maxx) from Grand Cereals and Oil Mills Ltd, Jos and 60% of groundnut cake (Daubail *et al.*, 2014). Obesity was induced by feeding the rats with HFD for twelve (12) weeks (84days). BMI was calculated before, during and after the research.

### 3.3 RESEARCH DESIGN

Table 1.1 Research design

| Group | Treatment | Dose of treatment |
|---|---|---|
| I | Normal diet | - |
| II | High fat diet | 0.5 ml/kg body weight |
| III | Normal diet and fish oil | 0.75 ml per 100 g body weight |
| IV | High fat diet and fish oil | - |
| V | Normal diet and mannitol | 0.5 ml/kg body weight |
| VI | High fat diet and mannitol | 0.75 ml per 100 g body weight |

The experiment runs for the period of twelve weeks

### 3.4 SOURCE OF EXPERIMENTAL CHEMICALS AND ASSAY KITS

Fibrinogen and Erythrocyte Aggregation Kits (ELISA) was obtained from Sunlong Biotechnology Company LTD, Hangzhou, China. While glucose, total cholesterol, high density lipoprotein, triglyceride and 20% mannitol was source from royal Chemical Company, Kaduna, Nigeria

### 3.4.1 Source of Fish oil

Fish oil was sourced from Carlson laboratory fish oil in Norway, the constituent includes eicosapentaenoic acid, docosahexaenoic acid, omega 3 fatty acid and vitamin E extracted from sardine and mackerel. Each ml contains 160mg EPA,100mg DHA and 60mg of omega 3 fatty acid. The required dose to be administered to laboratory rats was calculated according to the body weight.

### 3.4.2 Source of Mannitol

Mannitol was sourced from Royal diagnostics in Kaduna, Nigeria.

## 3.5 CALCULATION OF BODY MASS INDEX IN RATS

The BMI in the rats was determined at the beginning and the end of the study was taken by measuring the body weight (g) and the length (cm) of the rats using a weighing balance and a measuring tape from the nose to the anus respectively. The weight of the rats is divided by the length per square (De Vries *et al.*, 2014).

$$Body\ mass\ index\ (g/cm^2) = \frac{wight\ of\ the\ rat\ in\ (g)}{the\ length\ of\ the\ rats\ (cm^2)}$$

## 3.6 SAMPLE COLLECTION

At the last day of the treatment, the rats were fasted overnight and anaesthetized by dropping each in transparent plastic jar saturated with chloroform vapour. Seven millitres (7mls) of Blood sample was obtained through cardiac puncture. Three 3mls were dispensed into sodium citrate and 4mls into lithium heparin containers and spun with a centrifuge at 3000 rpm for 5 minutes, the supernatant was removed and used for the biochemical parameters ..

For histology sample (Tissue); the heart, kidney and liver from all experimental rats were harvested and preserved in a close container with 10% neutral buffered formalin for histological examination.

## 3.7 LABORATORY INVESTIGATION

### 3.7.1 Fibrinogen level (ELISA)

#### 3.7.1.1 Principle

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate has been precoated with an antibody specific to EMP. Standards or samples was added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)- conjugated antibody specific for EMP was added to each Microelisa stripplate well and incubated. Free components were washed away. The TMB substrate solution was added to each well. Only those wells that contain EMP and HRP conjugated EMP antibody appeared blue in colour and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of EMP. concentration of EMP were calculated in the samples by comparing the OD of the samples to the standard curve.

#### 3.7.1.2. Sample preparation (plasma)

Whole blood was collected into tubes with anticoagulant (sodium citrate). After incubated at room temperature for 10-20 minutes, it was centrifugated for 20 min at 2,000-3,000 rpm. the supernatant was collected carefully as plasma samples. precipitates were observed if it appears during reservation, the sample was then centrifugated again.

### 3.7.1.3 Procedure

- Dilution of Standards Ten wells was set for standards in a Microelisa stripplate. In Well

1 and Well 2, 100 µl Standard solution and 50 µl Standard Dilution buffer was added and mixed properly. In Well 3 and Well 4, 100 µl solution from Well 1 and Well 2 was added respectively. Then 50 µl Standard Dilution buffer were added and mixed properly. 50 µl solution will be discarded from Well 3 and Well 4. In Well 5 and Well 6, 50 µl solution from Well 3 and Well 4 was added respectively. Then 50 µl Standard Dilution buffer was added and mixed properly. In Well 7 and Well 8, 50 µl solution from Well 5 and Well 6 was added respectively. Then 50 µl Standard Dilution buffer was added and mixed appropriately. In Well 9 and Well 10, 50 µl solution from Well 7 and Well 8 was added respectively. Then 50 µl Standard Dilution buffer was added and mixed properly. 50 µl solution was added discarded from Well 9 and Well 10. After dilution, the total volume in all the wells was 50 µl and the concentrations are 1800 pg/ml, 1200 pg/ml 600 pg/ml 300 pg/ml 150 pg/ml, respectively.

- An empty well was left in the Microelisa stripplate, as blank control. In sample wells, 40 µl Sample dilution buffer and 10 µl sample was added (dilution factor is 5). Samples was loaded on to the bottom without touching the well wall. The sample was Mixed well with gentle shaking.
- Incubation: incubate 30 min at 37 ℃ after it was sealed with Closure plate membrane.

  Dilution: The concentrated washing buffer was diluted with distilled water (30 times for 96 T and 20 times for 48 T).

- Washing: Closure plate membrane was carefully peel off, aspirated and refilled with the wash solution. Wash solution was discarded after resting for 30 seconds and the washing procedure was repeated for 5 times.
- 50 µl HRP-Conjugate reagent was added to each well except the blank control well.
- Incubation as described in Step3.
- Washing as described in Step5.
- Colouring: 50 µl Chromogen Solution A and 50 µl Chromogen Solution B was added to each well, it was mixed and was gently shaked and incubated at 37℃ for 15 minutes.
- Termination: 50 µl stop solution was added to each well to terminate the reaction. The colour in the well changed from blue to yellow.
- Absorbance O.D. was read at 450 nm using a Microtiter Plate Reader. The OD value of the 1800pg/ml 1200pg/ml 600pg/ml 300pg/ml 150pg/ml2700p g/ml blank control well was set as zero. Assay was carried out within 15 minutes after adding stop solute (Fibrinogen ELISA Kit. Catalogue number: SL0676Hu. *SunLong Biotech Co., LTD*. Retrieve from www.sunlongbiotech.com)

### 3.7. 2 Erythrocytes aggregation (ELISA)

#### 3.7.2.1 Principle

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate was precoated with an antibody specific to EMP. Standards or samples were added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)- conjugated antibody specific for EMP was added to each

Microelisa stripplate well and incubated. Free components were washed away. The TMB substrate solution was added to each well. Only those wells that contain EMP and HRP conjugated EMP antibody appeared blue in colour and then turn yellow after the stop solution added. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of EMP. the concentration of EMP in the samples was calculated by comparing the OD of the samples to the standard curve.

**3.7.2.2. Sample preparation (plasma)** the whole blood was collected into tubes with anticoagulant (sodium citrate). After incubated at room temperature for 10-20 minutes, centrifuged for 20 min at 2,000-3,000 rpm. the supernatant was collected carefully as plasma samples. precipitates was observed if it appeared during reservation, the sample was centrifuged again.

**3.7.2.3 Procedure**

• Dilution of Standards Ten wells was set for standards in a Microelisa stripplate. In Well 1 and Well 2, 100 µl Standard solution and 50 µl Standard Dilution buffer were added and mixed properly. In Well 3 and Well 4, 100 µl solution from Well 1 and Well 2 were added respectively. Then 50 µl Standard Dilution buffer was added and mixed properly. 50 µl solution was discarded from Well 3 and Well 4. In Well 5 and Well 6, 50 µl solution from Well 3 and Well 4 was added respectively. Then 50 µl Standard Dilution buffer was added and mixed properly. In Well 7 and Well 8, 50 µl solution from Well 5 and Well 6 was added respectively. Then 50 µl Standard Dilution buffer was added and mixed appropriately. In Well 9 and Well 10, 50 µl solution from Well 7 and Well 8 was added respectively. Then 50 µl Standard

Dilution buffer was added and mixed properly. 50 µl solution was added discarded from Well 9 and Well 10. After dilution, the total volume in all the wells will be 50 µl and the concentrations are 1800 pg/ml, 1200 pg/ml, 600 pg/ml, 300 pg/ml, 150 pg/ml, respectively.

- An empty well in the Microelisa stripplate, was labelled as blank control. In sample wells, 40 µl Sample dilution buffer and 10 µl sample was added (dilution factor is 5). Samples should be loaded on to the bottom without touching the well wall. Mix well with gentle shaking.
- Incubation: incubate 30 min at 37 ℃ after sealed with Closure plate membrane.
- Dilution: The concentrated washing buffer was diluted with distilled water (30 times for 96 T and 20 times for 48 T).
- Washing: Closure plate membrane was carefully peel off, aspirated and refilled with the wash solution. Wash solution was discarded after resting for 30 seconds and the washing procedure will be repeated for 5 times.
- 50 µl HRP-Conjugate reagent was added to each well except the blank control well.
- Incubation as described in Step3.
- Washing as described in Step5.
- Colouring: 50 µl Chromogen Solution A and 50 µl Chromogen Solution B was added to each well, it was mixed and was gently shaked and incubated at 37℃ for 15 minutes.
- Termination: 50 µl stop solution was added to each well to terminate the reaction. The colour in the well would change from blue to yellow.

- Absorbance O.D. was read at 450 nm using a Microtiter Plate Reader. The OD value of the 1800pg/ml 1200pg/ml 600pg/ml 300pg/ml 150pg/ml 2700p g/ml blank control well is set as zero. Assay was carried out within 15 minutes after adding stop solution.

(Human Erythrocyte Membrane Protein (EMP) ELISA Kit. Catalogue number: SL0676Hu. *SunLong Biotech Co., LTD*. Retrieve from www.sunlongbiotech.com)

**3.7.3 Plasma Glucose—Oxidase- Peroxidase Method** (Burtis, 1999).

**3.7. 3 .1 Principle**

Glucose oxidase (GOD) catalyzes the oxidation of glucose to give hydrogen peroxide ($H_2O_2$) and gluconic acid. In the presence of the enzyme Peroxidase (POD), the hydrogen peroxide is broken down and the oxygen released reacts with 4-aminophenazone (4aminoantipyrine) and phenol to give a pink colour. The absorbance of pink colour is directly proportional to the concentration of glucose in the sample.

**3. 7. 3. 2 Procedure**

Ten microlitres (10µl) of each samples were added into respective label test tube for the samples ten microlitre of the glucose standard was added to test tube labelled standard. Five milliliters (5mls) of glucose reagent was added into all the tube including that of the blank test tube. The mixtures were incubated for 15 minutes at 37 oC. (After the incubation absorbance was read at 490nm spectrophotometrically

**3. 7. 3. 3 Calculation**

$$\frac{\text{absorbance of Test}}{\text{absorbance of standard}} \text{ x con c of standard (5.5mmol/L)}$$

**3.7. 4. Total cholesterol (TC) Enzymatic Colorimetric Method (**Trinder, 1969**).**

**3.7. 4.**1**Principle**

Cholesterol ester + $H_20$ —cholesterol esterase→ cholesterol + fatty acid

Cholesterol + $O_2$ —cholesterol oxidase→ 4-cholesten-3-one + $H_2O_2$

$2H_2O_2$ + phenol + aminoantipyrin —Peroxidase→ Red quinine + $4H_2O$

The absorbance of red colour is proportional to the concentration of cholesterol in the sample.

**3.7. 4.2 Procedure**

One mililitre (1ml) of the cholesterol reagents was added to test, standard and blank tubes. Ten micrometres (10 µl) of serum sample was added to the test tube and ten microlitres (10 µl) of standard into standard tube. The tubes were mixed and incubated at 37oc for 5 minutes. Absorbance was read within 60 minutes at 505nm.

**3.7. 4.3 Calculation**

$$\frac{\text{absorbance of Test}}{\text{absorbance of standard}} \times \text{conc. of standard (200mg/dl)}$$

**3.7.5 High Density Lipoprotein-Cholesterol (HDL-C) (**Tinder , 1969**).**

**3.7. 5.1 Principle**

The chylomicrons, very low density lipoprotein (VLDL) and low density lipoprotein (LDL) are precipitated by Phosphotungstic acid and magnesium ions. After centrifugation HDL remain in the supernatant and was measured by enzymatic method as in cholesterol above.

### 3.7. 5.2 Procedure

### 3.7. 5.2 .1 Precipitation

Three hundred microlitres (300µl) of sample was pipette into a test tube. Three hundred microlitres (300µl) of HDL reagent was added and mix well. It was incubated at room temperature for 10 minutes and mix and centrifuge at 4000rpm for 10 minutes. The mixture was Separate to collect the clear supernatant for Determination of the HDL-C concentration using cholesterol reagent.

### 3.7. 5.2. 2 *HDL-C Determination*

One thousand microlitres (1000µl) of cholesterol reagent was pipette in to a labeled test, standard and blank test tubes. Fifty microlitres (50µl) of HDL-C supernatant was added to test tube and fifty microlitres (50µl) of standard into standard tube; they were w mixed and incubated at $37^0$ C for 5 minutes. The absorbance was read at 505nm.

**Calculation**

$$\frac{\text{absorbance of Test}}{\text{absorbance of standard}} \times N \times 2$$

N= Standard concentration (50mg/dl)

2= Dilution factor

**3.7.6 Low Density Lipoprotein-Cholesterol (LDL-C)** (Friedewald *et al.*, 1972)

$$LDL - C = TC - HDL - C + \frac{TG}{5}$$

**3.7.7 Very Low Density Lipoprotein Cholesterol (VLDL-C)** (Friedewald, *et al.*, 1972).

VLDL-C = TG/5

**3.7.8 Atherogenic Index**

Atherogenic index = TC/ HDL-C

**3.7.9 Triglycerides (TG) (**Trinder, 1969**).**

**3.7.9.1 Principle**

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

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

Glycerol-3-phosphate + $O_2$ $\xrightarrow{\text{glycerol-3-phosphate}}$ Dihydroxy acetone phosphate + $H_2O_2$

$H_2O_2$ + 4-aminoantipyrine + P-chlorophenol $\xrightarrow{\text{Peroxidase}}$ Red quinoimine.

The absorbance is proportional to the concentration of TG in the sample.

**3.7.9.2 Procedure**

0ne mililitres (1ml) each of TG reagents was pippetted into test, standard and blank tube. Ten micolitres (10µl) of serum, standard and distilled water was pippetted in to test, standard and blank respectively. It was mixed and incubated at $37^0$ C for 5 minutes. The absorbance was read against blank within 60 minutes at 505nm. **3.7.8.3 Calculation**

$\frac{\text{absorbance of Test}}{\text{absorbance of standard}}$ x conc of standard (200mmol/l)

**Determination of TC/HDL-C ratio** (Atherogenic index) (Tiez, 1994)

Atherogenic Index = TC/HDL-C

## 3.8 BODY WEIGHT AND LENGTH

Body weight of the animals was taken by a weighing balance, while the length was measured using a measuring tape before and after the study.

### 3.8.1 Histology and Histochemistry

The experimental rats were anesthetized with chloroform in a glass jar post supplementation with antioxidants. Freshly excised heart, kidney and liver of the rats from each group were preserved in 10% formal saline solution for histopathological studies. They were processed for 12 hr. Using Leica Automatic tissue Processor and embedded in paraffin for light microscopic study. The embedded tissues were sectioned at 5µ thickness, stained to demonstrate the general tissue structure using Haematoxylin and Eosin (H&E) staining method technique for morphological assessment of injury and detection of inflammatory cells, including polymorphonuclear cells (neutrophils, monocytes and macrophages-like cells). Photomicrographs were captured at a magnification appropriate to the features to be described.

## 3.9 DATA ANALYSIS

The results were analyzed using analysis of variance (ANOVA). Post Hoc Tests was used for Multiple Comparisons using LSD was utilized to identify differences in means. Difference was considered statistically significant at 5% ($p < 0.05$) only. Statistical Package for Social Science (SPSS) windows version 21 was used for the analysis

# CHAPTER FOUR

## 4.0 RESULTS

The results obtained in the current study are presented in tables 4.1 to 4.7

**Table 4.1** The Mean weight was significantly lower ($p<0.05$) in groups fed with normal diet and fish oil, normal diet and mannitol when compared with the normal diet control group, likewise with high fat diet with fish oil, high fat diet with mannitol when compared with the high fat diet control group.

The Mean post body mass index (BMI) was significantly lower ($p<0.05$) in groups fed with normal diet with fish oil, normal diet with mannitol, high fat diet with fish oil, high fat diet with mannitol when compared with the control groups

**Table 4.1 Anthropometric measurements at baseline and twelve weeks in experimental rats and control.**

| GROUPS | PRE-BMI ($g/cm^2$) | POST BMI ($g/cm^2$) |
|---|---|---|
| GROUP 1 | 0.262 ± 0.009 | |
| GROUP 2 | 0.242 ± 0.006 | 0.370 ± 0.005 0.360 |
| GROUP 3 | 0.257 ± 0.008 0.258 | ± 0.007 0.358 ± |
| GROUP 4 | ± 0.005 0.260 ± | 0.007 0.467 ± 0.010 |
| GROUP 5 | 0.006 | 0.452 ± 0.007 |
| GROUP 6 | 0.257 ± 0.006 | 0.422 ± 0.006 |
| F Values | 1.049 | 43.897 |
| P values | 0.408 | 0.000* |

Table 4.2 shows one-way analysis of variance using Post Hoc test, showing comparison between groups. $P < 0.05$, shows statistical significance in comparison between the two groups involved while $P < 0.05$, shows no statistical significant

**Table 4.2 Differences in mean of Length, Weight and BMI within the groups using Post Hoc Test Multiple comparison using LSD**

| GROUPS | Pre- BMI ($g/cm^2$) | Post- BMI ($g/cm^2$) |
|---|---|---|
| Post HOC Using LSD | 0.62 | 0.27 |
| Grp1 vs Grp 4 | 0.74 | 0.00* |
| Grp 1 vs Grp 5 | 0.87 | 0.00* |
| Grp 1 vs Grp 6 | 0.62 | 0.00* |
| Grp 2 vs Grp 3 | 0.14 | 0.87 |
| Grp 2 vs Grp 4 | 0.10 | 0.00* |
| Grp 2 vs Grp 5 | 0.08 | 0.00* |
| Grp 2 vs Grp 6 | 0.14 | 0.00* |
| Grp 3 vs Grp 4 | 0.87 | 0.00* |
| Grp 3 vs Grp 5 | 0.74 | 0.00* |
| Grp 3 vs Grp 6 | 1.00 | 0.00* |
| Grp 4 vs Grp 5 | 0.87 | 0.16 |
| Grp 4 vs Grp 6 | 0.87 | 0.00* |
| Grp 5 vs Grp 6 | 0.74 | 0.01* |

Values are mean ± SEM, n = number of subjects, group 1 = normal diet, group 2 =normal diet + fish oil, group 3 = normal diet + mannitol, group 4 =high fat diet, group 5= high fat diet+ fish oil, group 6= high fat diet + mannitol * = value differ significantly when compared with controls.

Table 4.3: Shows mean serum cholesterol concentration in the studied animals. Mean Serum cholesterol was (59.83± 7.57) mmol/l in control, 53.83±4.68 in Group 2, (55.0±3.62) mmol/l in group 3, (64.67±8.89) mmol/l in group 4, (64.67±8.89) mmol/l in group 5 and 49.33±1.71 in group 6.

The mean triglycerides concentration was (49.17 ±9.23) mmol/l in control, (43.83 ± 8.54) mmol/l in group 2, (31.00 ± 4.96) mmol/l in group 3, (33.33 ± 5.99) mmol/l in group 4, (37.00 ± 9.31) mmol/l in group 5, (32.50 ± 6.17) mmol/l in group 6. The mean total serum Triglyceride concentration results show no significant difference (P>0.05) in all the experimental groups when compared with those of the control.

The mean serum High density lipoprotein – cholesterol (HDL-C) concentration was (34.20 ± 4.69) mmol/l in control group, (38.50 ± 4.20) mmol/l in group 2, (40.83 ± 1.28) mmol/l group 3, (35.83 ± 3.75) mmol/l group 4, (30.83 ± 2.98) mmol/l group 5, (41.00 ± 2.28) mmol/l in group 6. Results shows no significant difference (P>0.05) in all the experimental groups when compared with those of the control

The mean serum low density lipoprotein – cholesterol (LDL-C) concentration was (10.67 ± 3.01) mmol/l in control group, (11.50 ± 3.95) mmol/l group 2, 9.17 ± 3.32 in group 3, (22.00 ± 5.98) mmol/l in group 4, (7.67 ± 1.48) mmol/l in group 5, (11.50 ± 3.98) mmol/l in group 6. Results shows no significant difference (P>0.05) in all the experimental groups when compared with those of the control

The mean serum very low density lipoprotein – cholesterol (VLDL-C) concentration was 9.83 ± 1.85 in control group, 8.77 ± 1.71 in group 2, 6.20 ± 0.99 in group 3, 6.67 ± 1.19 in

group 4, 7.40 ± 1.86 in group 5, 6.67 ± 1.19 in group 6. Results shows no significant difference (P>0.05) in all the experimental groups when compared with those of the control.

**Table 4.3 Serum lipid profile in experimental rats and controls**

| GROUPS | TC (mmol/l) | TGs (mmol/l) | HDL-C (mmol/l) | LDL-C (mmol/l) | Atherogenic index | VLDL-C (mmol/l) |
|---|---|---|---|---|---|---|
| GROUP 1 | 59.83 ± 7.57 | 49.17 ± 9.23 | 34.20 ± 4.69 | 10.67 ± 3.01 | 1.31 ± 0.1 | 9.83 ± 1.85 |
| GROUP 2 | 53.83 ± 4.68 | 43.83 ± 8.54 | 38.50 ± 4.20 | 11.50 ± 3.95 | 1.23 ± 0.31 | 8.77 ± 1.71 |
| GROUP 3 | 55.00 ± 3.62 | 31.00 ± 4.96 | 40.83 ± 1.28 | 9.17 ± 3.32 | 1.29 ± 0.21 | 6.20 ± 0.99 |
| GROUP 4 | 64.67 ± 8.89 | 33.33 ± 5.99 | 35.83 ± 3.75 | 22.00 ± 5.98 | 1.57 ± 0.81 | 6.67 ± 1.19 |
| GROUP 5 | 64.67 ± 8.89 | 37.00 ± 9.31 | 30.83 ± 2.98 | 7.67 ± 1.48 | 1.48 ± 0.61 | 7.40 ± 1.86 |
| GROUP 6 | 49.33 ± 1.71 | 32.50 ± 6.17 | 41.00 ± 2.28 | 11.50 ± 3.98 | 1.55 ± 0.51 | 6.67 ± 1.19 |
| F Values | 0.874 | 0.911 | 1.281 | 1.732 | 0.527 | 0.887 |
| P values | 0.510 | 0.487 | 0.298 | 0.158 | 0.120 | 0.502 |

Values are mean ± SEM, n = number of subjects, group 1 = normal diet, group 2 =normal diet +fish oil, group 3 = normal diet + mannitol, group 4 =high fat diet, group 5= high fat diet+ fish oil, group 6= high fat diet + mannitol * = value differ significantly when compared with controls.
TC- Total Cholesterol, TG- Triglycerides, HDL-C, High Density Lipoprotein-Cholesterol, LDL-C, Low Density Lipoprotein- Cholesterol, VLDL-C, Very Low Density LipoproteinCholesterol

**Table 4.4** shows one-way analysis of variance using Post Hoc test, showing comparison between groups. P< 0.05, shows statistical significance in comparison between the two groups involved while P>0.05, shows no statistical significance**.**

**Table 4.4 Differences in mean of Total Cholesterol, Triglycerides, HDL-C, LDL-C and VLDL-C within the groups using Post Hoc Test Multiple comparison using LSD**

| Groups | TC mmol/l | TGs mmol/l | HDL-C mmol/l | LDL-C mmol/l | VLDL-C mmol/l |
|---|---|---|---|---|---|
| Post HOC Using LSD | | | | | |
| Grp 1 vs Grp 2 | 0.466 | 0.622 | 0.891 | 0.880 | 0.621 |
| Grp 1 vs Grp 3 | 0.557 | 0.100 | 0.731 | 0.785 | 0.099 |
| Grp1 vs Grp 4 | 0.557 | 0.149 | 0.494 | 0.047 | 0.148 |
| Grp 1 vs Grp 5 | 0.206 | 0.264 | 0.093 | 0.587 | 0.263 |
| Grp 1 vs Grp 6 | 0.935 | 0.130 | 0.706 | 0.880 | 0.148 |
| Grp 2 vs Grp 3 | 0.887 | 0.240 | 0.631 | 0.672 | 0.238 |
| Grp 2 vs Grp 4 | 0.193 | 0.334 | 0.583 | 0.064 | 0.333 |
| Grp 2 vs Grp 5 | 0.584 | 0.528 | 0.121 | 0.488 | 0.527 |
| Grp 2 vs Grp 6 | 0.517 | 0.298 | 0.607 | 1.000 | 0.333 |
| Grp 3 vs Grp 4 | 0.244 | 0.829 | 0.307 | 0.026 | 0.828 |
| Grp 3 vs Grp 5 | 0.491 | 0.579 | 0.046* | 0.785 | 0.578 |
| Grp 3 vs Grp 6 | 0.612 | 0.889 | 0.973 | 0.672 | 0.828 |
| Grp 4 vs Grp 5 | 0.069 | 0.734 | 0.307 | 0.013 | 0.733 |
| Grp 4 vs Grp 6 | 0.504 | 0.938 | 0.291 | 0.064 | 1.000 |
| Grp 5 vs Grp 6 | 0.236 | 0.677 | 0.043 | 0.488 | 0.733 |

Values are mean ± SEM, n = number of subjects, group 1 = normal diet, group 2 =normal diet + fish oil, group 3 = normal diet + mannitol, group 4 =high fat diet, group 5= high fat diet+ fish oil, group 6= high fat diet + mannitol * = value differ significantly when compared with controls.

TC- Total Cholesterol, TG- Triglycerides, HDL-C, High Density Lipoprotein-Cholesterol, LDL-C, Low Density Lipoprotein- Cholesterol, VLDL-C, Very Low Density LipoproteinCholesterol

Table 4.5: The Mean Serum Fasting Blood Glucose level was significantly higher ($p<0.05$) in groups fed with normal diet with fish oil (2.18 ± 0.25), normal diet with mannitol (2.42 ± 0.16), high fat diet with fish oil (2.47 ± 0.42), high fat diet with mannitol (3.43 ± 0.47) when compared with the control groups

The mean plasma Erythrocytes aggregation activities results shows no significant difference ($P>0.05$) in all the experimental groups when compared with those of the control. The mean plasma Erythrocytes aggregation concentration was (35.00 ± 2.92) in control group, (50.33 ± 4.02) in group fed with normal diet with fish oil, (39.83 ± 5.50) in group fed with normal diet with fish oil, (34.33 ± 5.68) in group fed with high fat diet, (48.83 ± 7.34) in group fed with high fat diet with fish oil, (49.17 ± 6.39) in group fed with high fat diet with mannitol.

The Mean serum fibrinogen level was significantly higher ($p<0.05$) in groups fed with normal diet with fish oil, normal diet with mannitol, high fat diet with fish oil, high fat diet with mannitol when compared with the control groups

The Mean serum fibrinogen level was (23.00 ± 1.29) in control group, (32.00 ± 3.02) in group fed with normal diet with fish oil, (24.17 ± 1.22) in group fed with normal diet with mannitol, (21.50 ± 1.63) in group fed with high fat diet, (19.33 ± 1.28) in group fed with high fat diet with fish oil, (16.83 ± 0.87) in groups fed with high fat diet with mannitol

**Table 4.5 Serum concentration of Fasting Blood Glucose, Erythrocytes Aggregation and fibrinogen in experimental rats and control**

| Groups | FBG (mmol/L) | Erythrocytes Aggregatio | (pg/ml) Fibrinogen(pg/ml) |
|---|---|---|---|
| GROUP 1 | 4.08 ± 0.32 | 35.00 ± 2.92 | 23.00 ± 1.29 |
| GROUP 2 | 2.18 ± 0.25 | 50.33 ± 4.02 | 32.00 ± 3.02 |
| GROUP 3 | 2.42 ± 0.16 | 39.83 ± 5.50 | 24.17 ± 1.22 |
| GROUP 4 | 3.50 ± 0.43 | 34.33 ± 5.68 | 21.50 ± 1.63 |
| GROUP 5 | 2.47 ± 0.42 | 48.83 ± 7.34 | 19.33 ± 1.28 |
| GROUP 6 | 3.43 ± 0.47 | 49.17 ± 6.39 | 16.83 ± 0.87 |
| F Values | 4.54 | 1.812 | 9.396 |
| P values | 0.003* | 0.141 | 0.000* |

Values are mean ± SEM, n = number of subjects, group 1 = normal diet, group 2 =normal diet + fish oil, group 3 = normal diet + mannitol, group 4 =high fat diet, group 5= high fat diet+ fish oil, group 6= high fat diet + mannitol * = value differ significantly when compared with controls.

**Table 4.6** shows one-way Analysis of variance using Post Hoc test, showing comparison between groups. P< 0.05, shows statistical significance in comparison between the two groups involved while P>0.05, shows no statistical significanc**e**

**Table 4.6 Differences in mean of Fasting Blood Glucose, Erythrocytes Aggregation and Fibrinogen within the groups using Post Hoc Test Multiple comparison using Least Significant Difference.**

| Gro (pg/ml) (pg/ml) | FBG (mmol/L) | Erythrocytes Aggregation | Fibrinogen |
|---|---|---|---|
| Grp 1 vs Grp 2 | 0.001* | 0.058 | 0.001* |
| Grp 1 vs Grp 3 | 0.003* | 0.540 | 0.631 |
| Grp1 vs Grp 4 | 0.258 | 0.932 | 0.538 |
| Grp 1 vs Grp 5 | 0.003* | 0.086 | 0.138 |
| Grp 1 vs Grp 6 | 0.208 | 0.079 | 0.016* |
| Grp 2 vs Grp 3 | 0.648 | 0.188 | 0.003* |
| Grp 2 vs Grp 4 | 0.014* | 0.049* | 0.000* |
| Grp 2 vs Grp 5 | 0.579 | 0.849 | 0.000* |
| Grp 2 vs Grp 6 | 0.019* | 0.882 | 0.000* |
| Grp 3 vs Grp 4 | 0.040* | 0.486 | 0.276 |
| Grp 3 vs Grp 5 | 0.922 | 0.257 | 0.054 |
| Grp 3 vs Grp 6 | 0.053 | 0.240 | 0.005* |
| Grp 4 vs Grp 5 | 0.050 | 0.073 | 0.375 |
| Grp 4 vs Grp 6 | 0.896 | 0.067 | 0.062 |
| Grp 5 vs Grp 6 | 0.065 | 0.966 | 0.307 |

Values are mean ± SEM, n = number of subjects, group 1 = normal diet, group 2 =normal diet + fish oil, group 3 = normal diet + mannitol, group 4 =high fat diet, group 5= high fat diet+ fish oil, group 6= high fat diet + mannitol * = value differ significantly when compared with controls.

**Table 4.7a:** Histology of the liver showing changes in different groups observed

| Liver | Microscopy |
|---|---|
| Group 1 | Normal, portal triad, central vein and hepatocytes (Fig 1a) |
| Group 2 | Normal, portal triad, central vein and hepatocytes (Fig 2a) |
| Group 3 | Normal, portal triad, central vein and hepatocytes (Fig 3a) |
| Group 4 | Normal, portal triad, central vein and hepatocytes showing balooning degeneration with areas of microvesicular steatosis (Fig 4a) |
| Group 5 | Normal, portal triad, central vein and hepatocytes showing balooning degeneration (Fig 5a) |
| Group 6 | Normal, portal triad, central vein and hepatocytes showing balooning degeneration with areas of microvesicular steatosis (Fig 6a) |

**Table 4.7b**: Histology of the kidney showing changes in the different groups observed

| Kidney | Microscopy |
|---|---|
| Group 1 | Normal glomeruli, tubules and interstitium (Fig 1b) |
| Group 2 | Normal glomeruli, tubules and interstitium (Fig 2b) |
| Group 3 | Normal glomeruli, tubules and interstitium (Fig 3b) |
| Group 4 | Normal glomeruli, tubules and interstitium (Fig 4b) |
| Group 5 | Normal glomeruli, tubules and interstitium (Fig 5b) |
| Group 6 | Normal glomeruli, tubules and interstitium (Fig 6b) |

**Table 4.7c**: Histology of the heart showing changes in the different groups observed

| Heart | Microscopy |
|---|---|
| Group 1 | Normal myocardiac fibers and coronary artery (Fig 1c) |
| Group 2 | Normal myocardiac fibers and coronary artery (Fig 2c) |
| Group 3 | Normal myocardiac fibers and coronary artery (Fig 3c) |
| Group 4 | Normal myocardiac fibers and coronary artery (Fig 4c) |
| Group 5 | Normal myocardiac fibers and coronary artery (Fig 5c) |
| Group 6 | Normal myocardiac fibers and coronary artery (Fig 6c) |

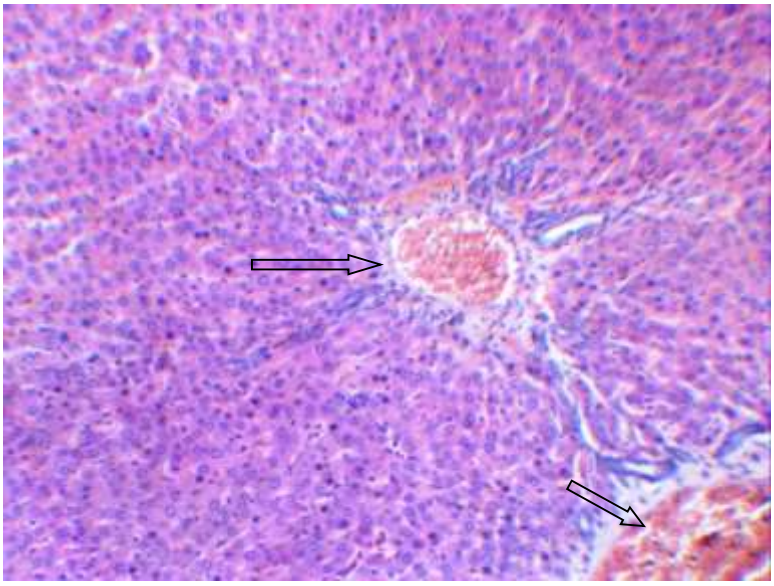

Figure 1a Liver: Section show Normal, portal triad (white arrow), central vein (Short arrow) and hepatocytes. H & E X 100

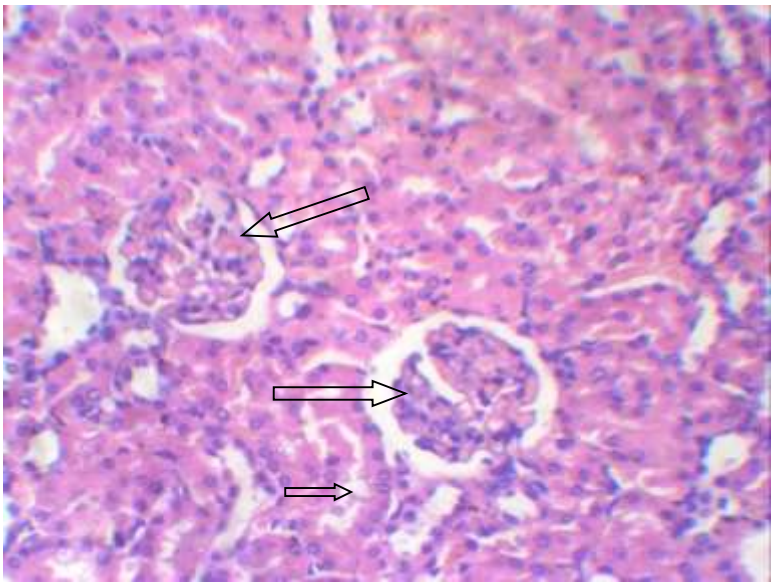

Figure 1b Kidney: Section show Normal glomeruli (white arrow), tubules (short arrow). H & E X100

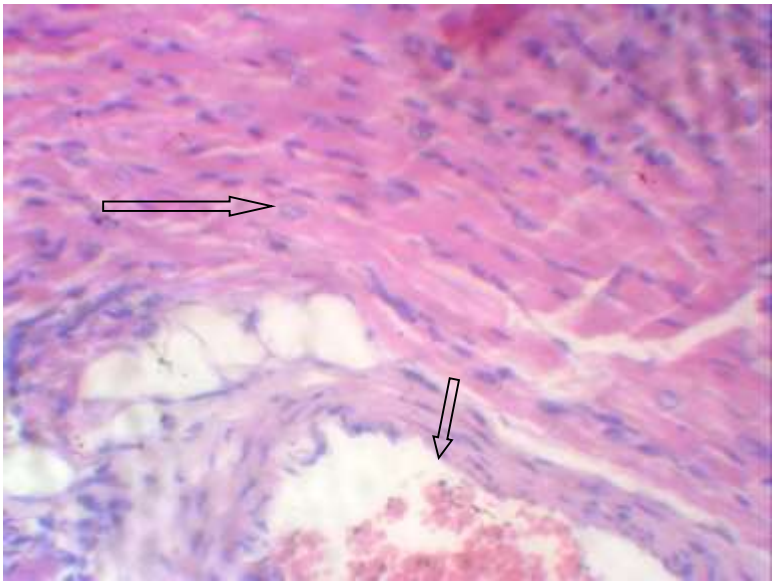

Figure 1c, Heart: Section show Normal myocardiac fibers (arrow) and coronary artery (Short arrow). H & E X 100

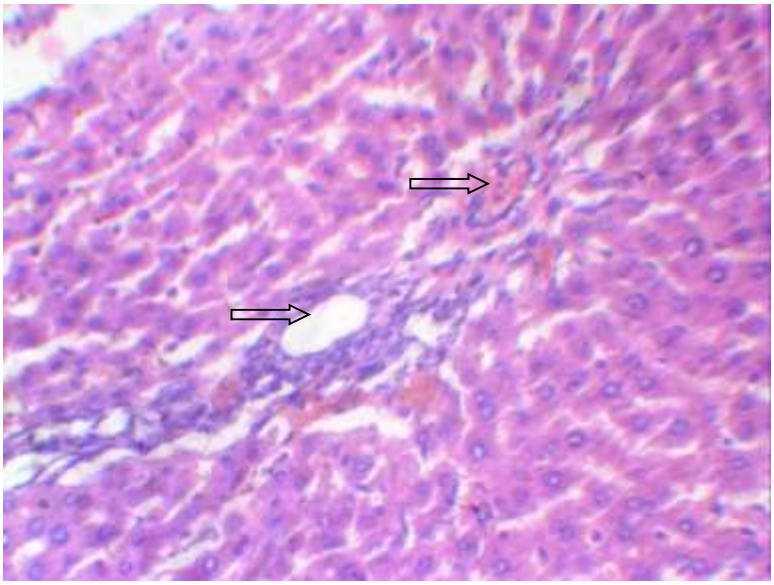

Figure 2a Liver: Section show Normal, portal triad (white arrow) and hepatocytes. H & E X 100

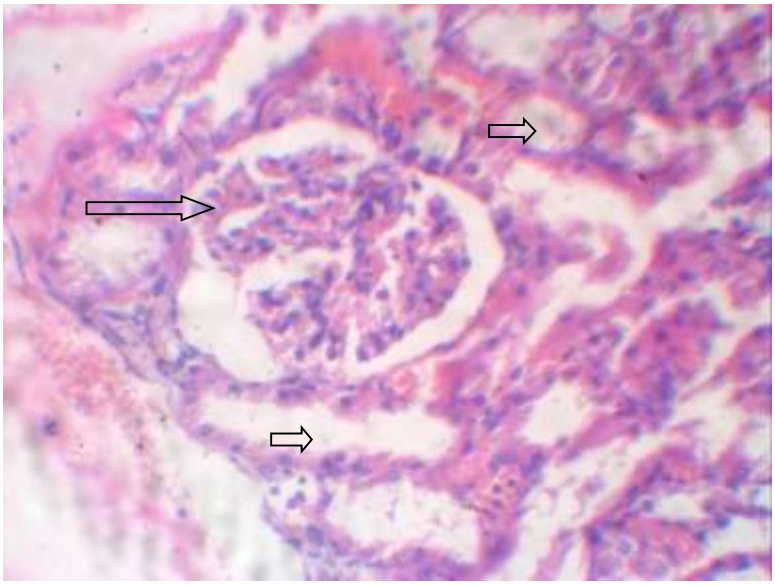

Figure 2b Kidney: Section show Normal glomerulus (long arrow), tubules (short arrow). H & E X100

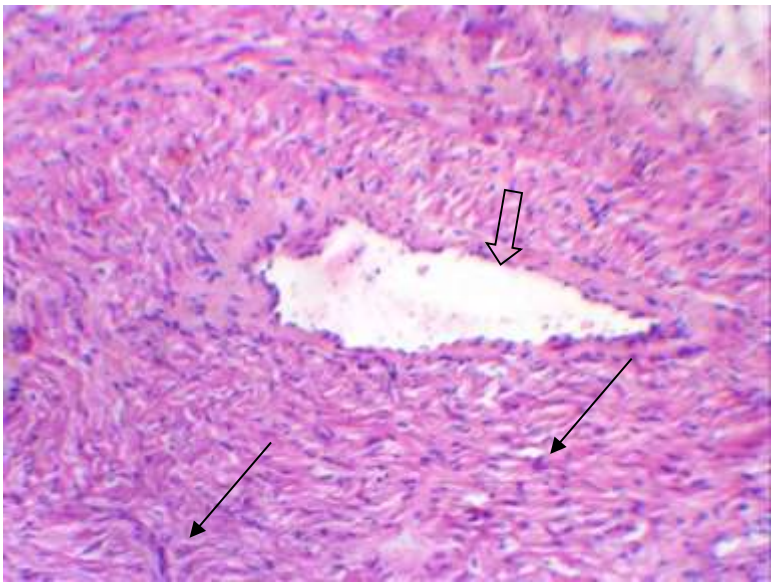

Figure 2c. Heart: Section show Normal myocardiac fibers (arrow) and coronary artery (Short arrow). H & E X 100

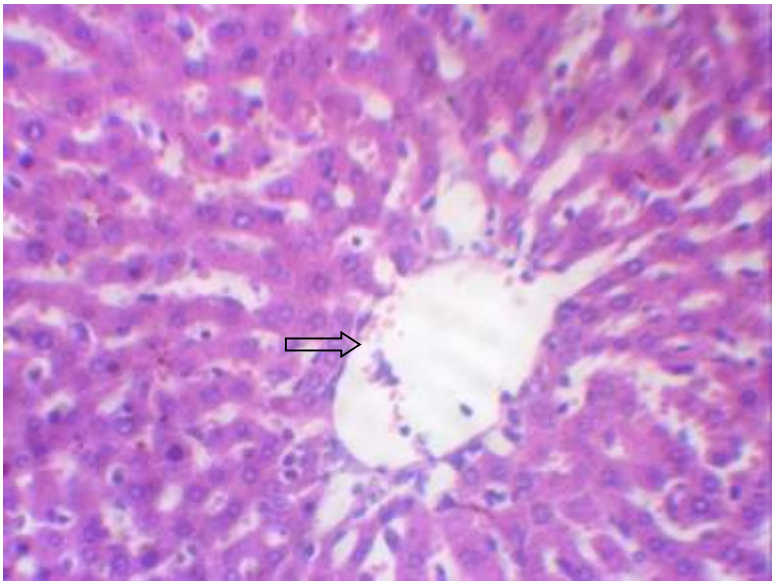

Figure 3a Liver: Section show Normal hepatocyte and central vein (white arrow) H & E X 100

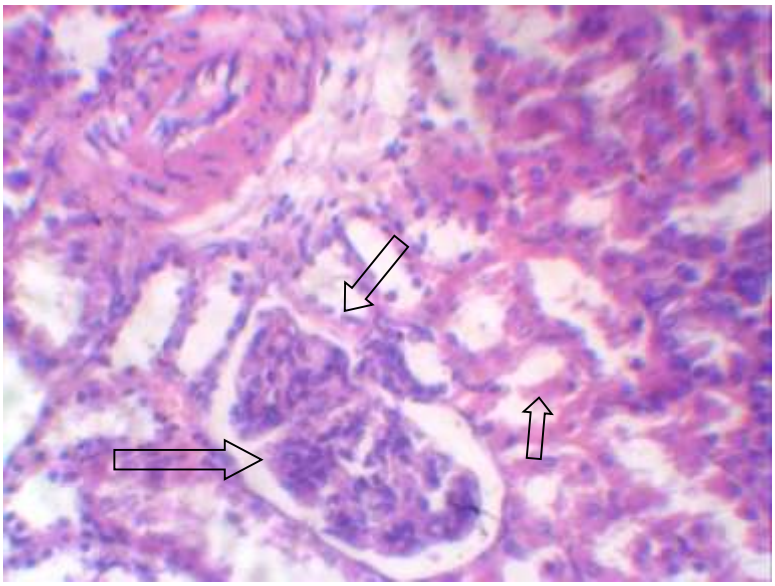

Figure 3b Kidney: Section show Normal glomerulus (long arrow), tubules (short arrow). H & E X100

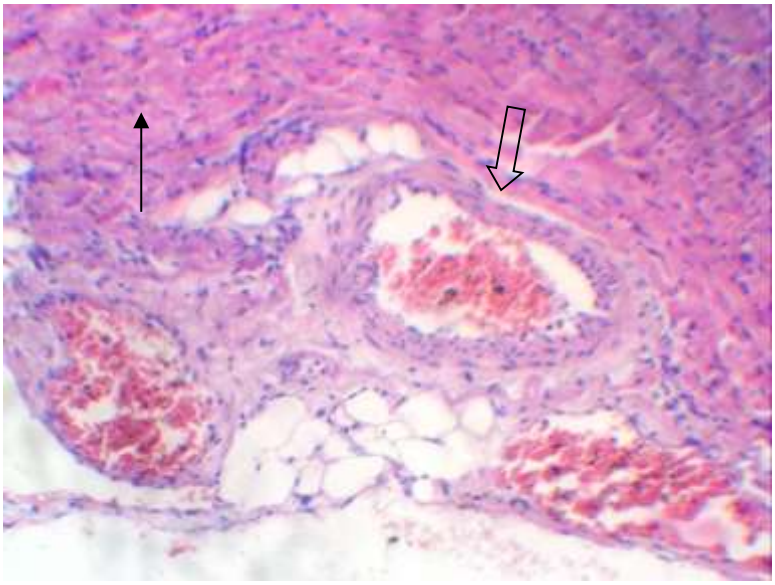

Figure 3c Heart: Section show Normal myocardiac fibers (arrow) and coronary artery (Short arrow). H & E X 100

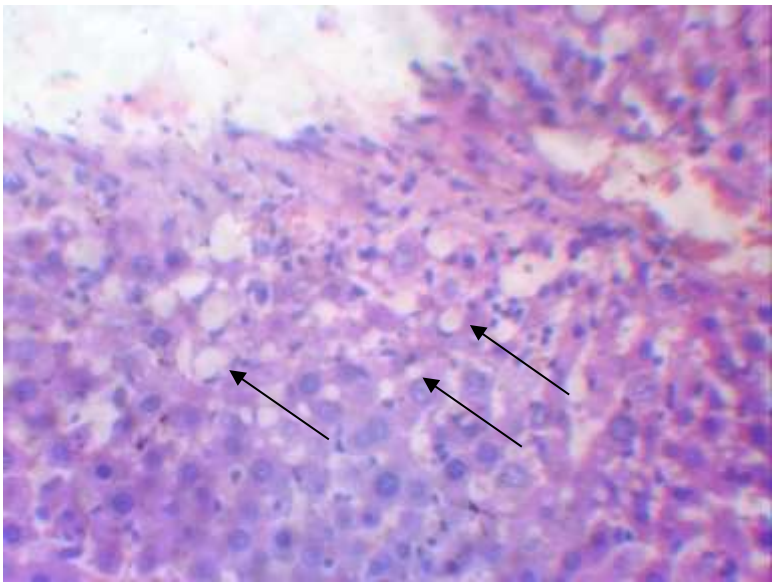

Figure 4a Section show hepatocytes showing balooning degeneration with areas of microvesicular steatosis. (arrow). H & E X 100

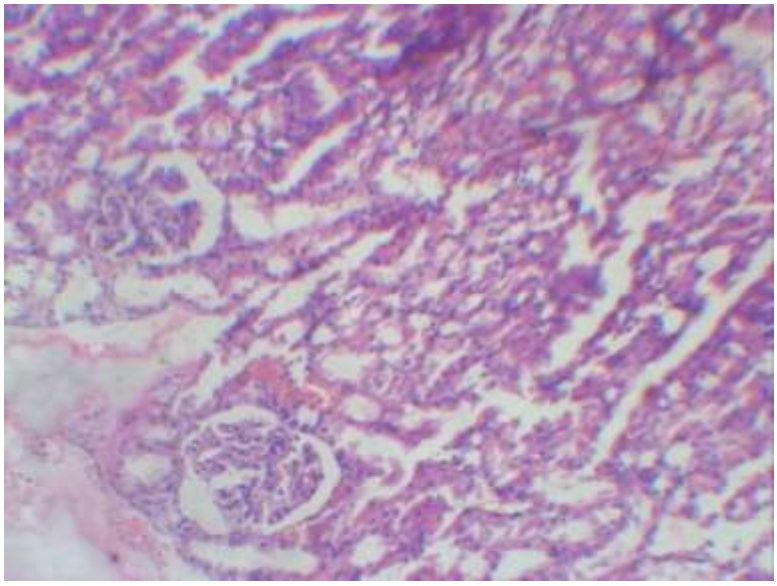

Figure 4b Kidney: Section show Normal glomeruli (long arrow), tubules (short arrow). H & E X100

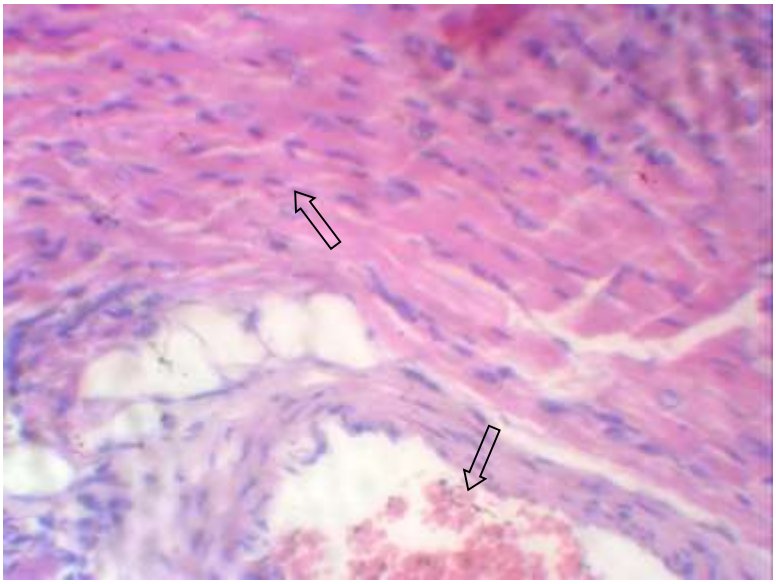

Figure 4c Heart: Section show Normal myocardiac fibers (arrow) and coronary artery (Short arrow). H & E X 100

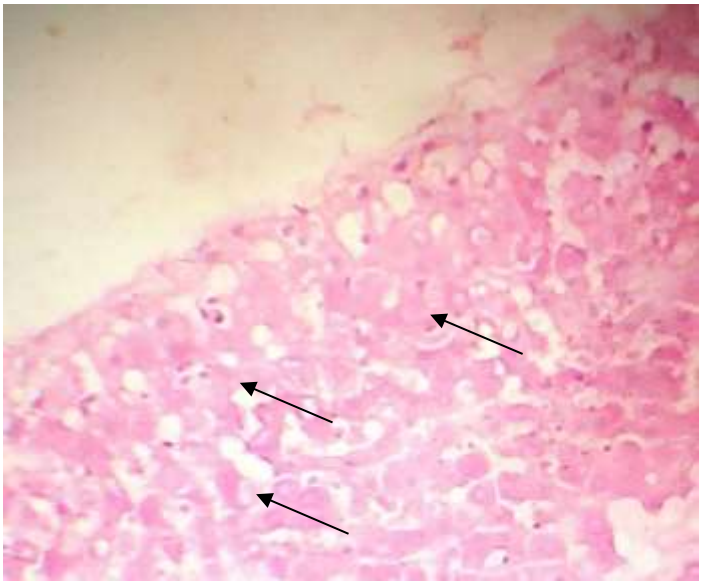

Figure 5a Section show hepatocyte showing ballooning degeneration (arrow). H & E X 100

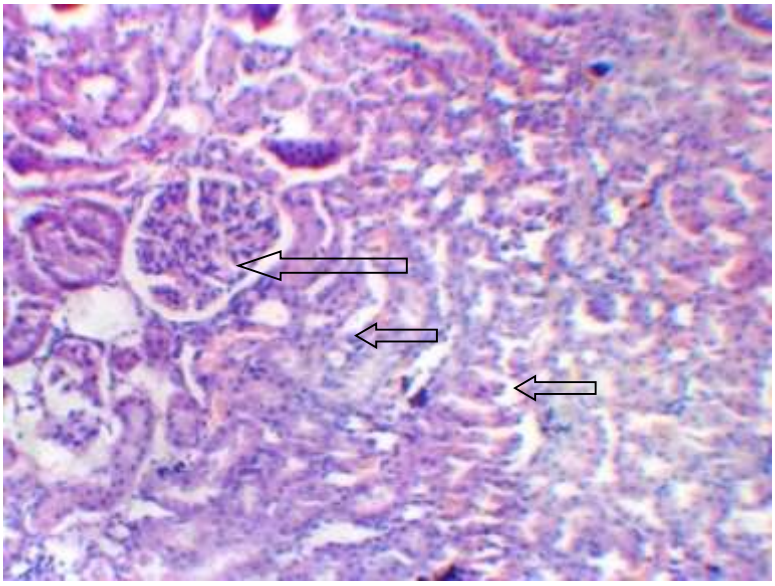

Figure 5b Kidney: Section show Normal glomerulu (long arrow), tubules (short arrow). H & E X100

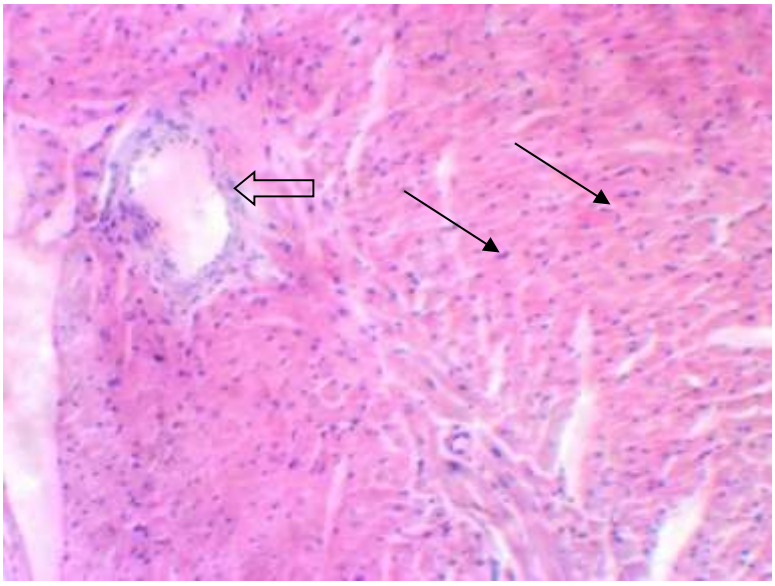

Figure 5c Heart: Section show Normal myocardiac fibers (arrow) and coronary artery (Short arrow). H & E X 100

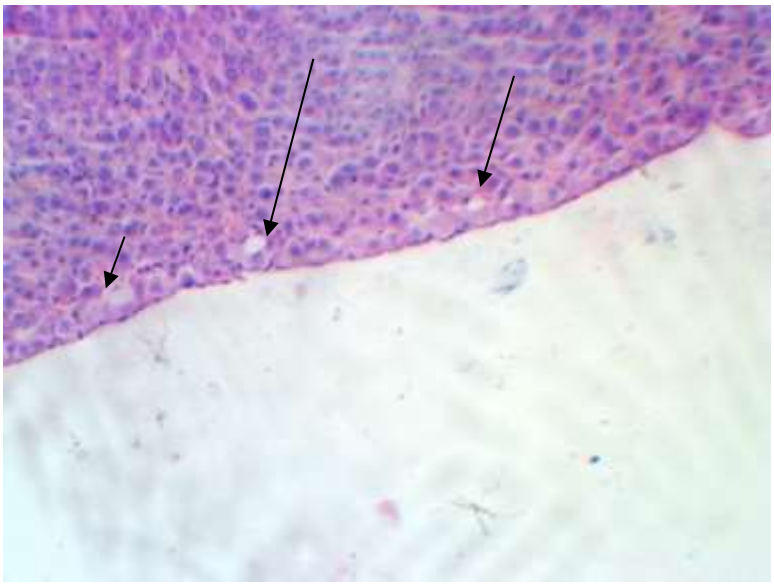

Figure 6a. Section show hepatocytes showing balooning degeneration (arrow) with areas of microvesicular steatosis. (long arrow). H & E X 100

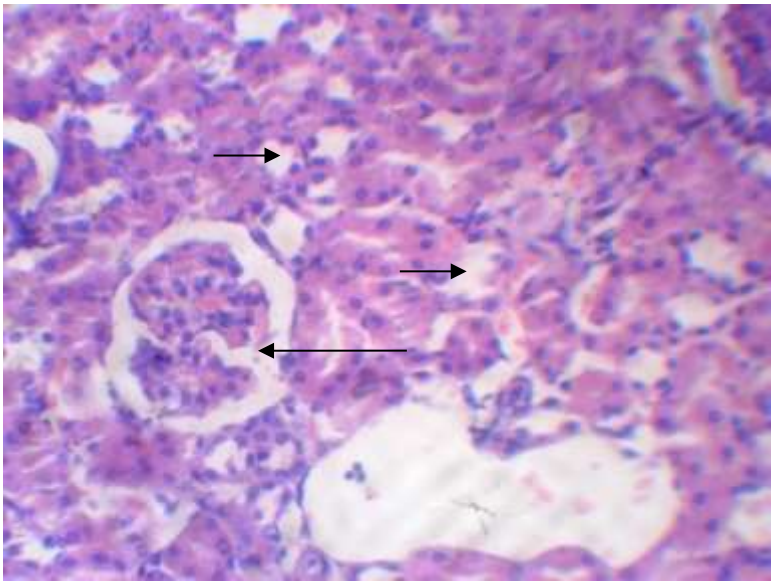

Figure 6b Kidney: Section show Normal glomeruli (long arrow), tubules (short arrow). H & E X100

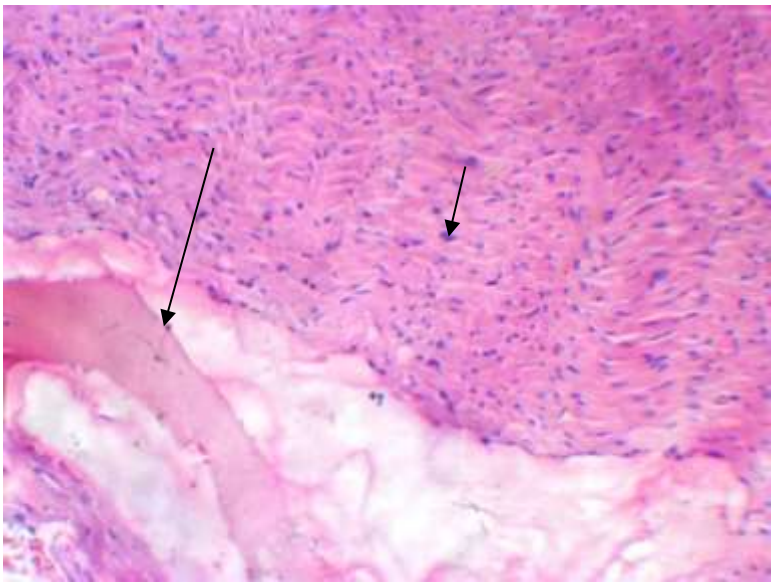

Figure 6c Heart: Section show Normal myocardiac fibers (arrow) and coronary artery (Short arrow). H & E X 100

# CHAPTER FIVE

## 5.0 DISCUSSION

In this study, we are able to show a significant variation in body weight and BMI of control and the experimental rats. Increase in the body weight invariably increases the BMI which directly influence the adipose tissue to secret adipocytokines (Christine *et al.,* 2005). High fat fed rats developed significant increase in weight which led to increase in Body Mass index, this studies is in agreement with other studies who shows that increase in body weight and BMI in fat fed animals (Yongbin *et al.,* 2014). This increase is as a result of the energy dense containing high fat diet. High BMI is associated with the risk of developing diabetes mellitus and cardiovascular disorders. Obesity and diabetes mellitus are associated with significant increase in serum Triglycerides (TG), Very Low density lipoprotein (LDL), Total cholesterol, Low Density Lipoprotein (LDL), with decrease in High Density Lipoprotein (HDL-C) (Marie *et al,* 2015; Shoaib *et al.,* 2015 and Gholizalleh *et al.,* 2015).

In this study there was a significantly low fasting blood glucose concentration in the treated group when compared with the high fat diet control group. Rats fed with normal diet with fish oil had significantly lower fasting glucose level as well as those fed with normal diet and mannitol when compared with normal diet control group. This shows the effect of fish oil and mannitol on glycaemic control. This is in consistency with a study carried out by *Paola et al.,* (2017) indicated Fish oil supplementation did not attenuate the elevation in triacylglycerol caused by fructose intake, but the interruption of sugar consumption normalized this parameter. Also rats fed with high fat diet with fish oil had significantly lower fasting glucose level as well as those fed with high fat diet and mannitol. A study by

Ayae *et al.,* 2009 suggests that mannitol consumption dose-dependently lowered the digestibility of crude fat and crude protein, the ratio of body fat accumulation to energy absorbed and the hepatic and serum triglyceride levels in normal rats. Since mannitol lowers body fat accumulation, it furthermore suggests it has an effect on high fat diet consumption, which in turn could lead to lower glucose level amongst high fat fed rats. Mannitol although is partially absorbed, has a very low glycemic blood glucose and insulin levels.

This study is contrary to a study by Lawrence *et al.,* (1995) which indicated that Fish oil treatment showed no change in glycaemic control as assessed by glycosylated haemoglobin and LDL although a rise in fasting blood glucose showed no statistical significance ($P = 0.06$). They also indicated that treatment with olive oil did not change levels of Thiobarbituric acid reactive substances (TBARS), vitamin E or indices of glycaemic control compared with baseline.

From this study there was no significant change in lipid profile: Total cholesterol, Triglycerides, high density lipoprotein, low density lipoprotein-cholesterol, and very low density lipoprotein. Rats fed with high fat diet with fish oil showed no change in serum total cholesterol, while those fed with high fat diet with mannitol had lower serum total cholesterol level but failed to reach significance. This study is in agreement with a study carried out by Lawrence *et al.,* 1995, which indicated that total cholesterol and triglyceride (TG) content of plasma and lipoprotein fractions were not significantly altered in treatment with fish oil, although it resulted in elevation of TBARS ($P < 0.001$) and reduction of vitamin E ($P < 0.01$) compared with baseline and olive oil treatment. Plasma cholesterol was unchanged. A reduction in plasma TG compared with baseline occurred but failed to reach significance ($P = 0.07$). With respect to the level of total cholesterol in mannitol fed rats it shows mannitol it is in agreement with Ayae *et al.,* 2009 which indicates that mannitol

lowers fat digestibility and body fat accumulation in normal rats and at such could lower serum total cholesterol. Ricardo *et al.,* (2011) indicated that low dose of fish oil supplementation (1 g/kg/day) was able to reduce TC and TG levels, in addition to improved systemic and muscle insulin sensitivity. These results lend credence to the benefits of n-3 fatty acids upon the deleterious effects of insulin resistance mechanisms

Rats fed with high fat diet with fish oil had increased serum TG while those fed with high fat diet and mannitol had lower serum TGs when compared with control. Its shows mannitol has an effect on TGs. However, it is contrary to the study carried out by cylla *et al.,* (1998) which connotes that the use of fish oil lowers triglyceride levels effectively by almost 30%. However, this may be accompanied by a slight increase in LDL cholesterol concentration which makes fish oil useful in treating dyslipidemia in diabetes.

Rat group fed with high fat diet with fish oil had lower HDL-C, while those fed with high fat diet with mannitol had an increased HDL-C when compared with the control. No significant changes were observed in the concentration or composition of high density lipoprotein (HDL).

Rats fed with high fat diet with fish oil had decreased LDL-C as well as those who were fed with high fat diet with mannitol when compared with control but failed to reach statistical significance. This study is contrary with a study carried out by farmer *et al.,* (2001) which indicated that fish oil supplementation in type 2 diabetes lowers triglycerides, may raise LDL cholesterol (especially in hypertriglyceridemia patients on higher doses of fish oil) and has no statistically significant effect on glycemic control. It is also contrary to a study by Cai *et al.,* (2015) which indicated that the ratio of EPA/DHA and early intervention with omega 3 fatty acids may affect their effects on glucose control and lipid levels, which may serve as a dietary reference for clinicians or nutritionists who manage diabetic patients. This disparity

in outcome could be as a result of location in which the studies were carried out, animal or human studies and different method of investigation employed in lipid analysis

In this study, there is no significant in erythrocytes aggregation between the test and control groups relative to the risk of cardiovascular diseases. This is contrary to a study Gyawali *et al.,* 2014, which indicated that erythrocyte aggregates increase blood viscosity at low shear rates and increase the risk of atherothrombosis which predisposes to cardiovascular diseases most likely by a mechanism called decreased erythrocyte zeta potential. In this manner, hyperuricemia and decreased zeta potential may be risk factors for atherosclerotic cardiovascular disease. Another study by Prajwal Gyawali indicated that the strong association of triglyceride and HDL-cholesterol with erythrocyte aggregation emphasizes the role of these lipids in the cardiovascular system. Studies has it that High fibrinogen levels have been identified as a relevant cardiovascular risk factor, but the biological mechanisms remain unclear. Increased aggregation of erythrocytes (red blood cells) has been linked to high plasma fibrinogen concentration. Fonville *et al.,* 2014 reported that Erythrocyte aggregation was highly significantly increased in the CRVO group when compared with the control group ($P < 0.0001$), as was the hematocrit level ($P < 0.05$). In this study, there is significance difference in fibrinogen level between the treated groups, the normal control group and high fat diet control group. This is in consistency with a study by Kannel *et al.,* 1984 which indicated that elevated fibrinogen level is a predictor of cardiovascular disease that should be added to the cardiovascular risk factor profile. It is also in consistency with a study carried out by James *et al.,* (2000) which indicated that Fibrinogen was associated with traditional cardiovascular risk factors. With the immunoprecipitation method, there were significant linear trends across fibrinogen tertiles

($P$<0.001) for age, body mass index, smoking, diabetes mellitus, total cholesterol, HDL cholesterol, and triglycerides in men and women. Fibrinogen levels were higher for those with cardiovascular disease when compared with those without cardiovascular disease.

# CHAPTER SIX

## 6.0 CONCLUSIONS AND RECOMMENDATIONS

## 61. CONCLUSIONS

At the end of this study the following conclusions were made:

1. groups fed on high fat diet has a significantly higher body weight and body mass index when compared with those of the control group which is indicative of obesity as a result of prolonged intake of high calories diet.
2. Fasting blood glucose was significantly higher in obese rats (high fat diet) compare to Non obese group (Normal diet). groups treated with fish oil and mannitol were significantly lower when compared to the high fat diet control group.
3. Fibrinogen level was significantly lower in groups treated with fish oil compared to the normal diet and high fat diet control groups.
4. Erythrocyte aggregation results show no significant difference in all experimental groups when compared with those of the control group.
5. The histology of the kidney and heart of the control group were similar to those of the experimental group while histology of the liver shows inflammation in the hepatocytes of experimental group which is due to fatty liver which occurred due to obesity in rats.

## 6.2 RECOMMENDATIONS

Considering the findings of this study it is recommended that;

1. Fish oil is recommended for management of overweight (obese) and cardiovascular disease patient.
2. Further studies should be carried out to have a clear understanding of a more prolonged feeding with high fat diet.
3. Fish oil has antiglycemic effect and hence it can use to treat diabetes mellitus which is also a risk factor of cardiovascular disease.
4. Further studies should be done on the activities of liver enzyme (AST, ALT) due to the inflammation caused by the prolonged high fat diet on the rats.
5. Further studies should be carried out on female rats and on other laboratory animals to observe the hormonal and species variation.
6. Extraction of fish oil from various species of fish and its phytochemical analysis should be considered.

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