EVALUATION OF THE EFFECT OF CAMEL MILK ON PHYSIOLOGICAL PARAMETERS OF POLOXAMER 407 INDUCED HYPERLIPIDEMIC WISTAR RATS

BY

Jibril ZUBERU, BSc.(ABU)

P13MDHP8007

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER DEGREE IN HUMAN PHYSIOLOGY

DEPARTMENT OF HUMAN PHYSIOLOGY,
COLLEGE OF HEALTH SCIENCES,
FACULTY OF BASIC MEDICAL SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

DECEMBER, 2017

DECLARATION

I declare that the work in this dissertation entitled; "EVALUATION OF THE EFFECT OF CAMEL MILK ON PHYSIOLOGICAL PARAMETERS OF POLOXAMER 407 INDUCED HYPERLIPIDEMIC WISTAR RATS" has been carried out by me in the Department of Human Physiology under the supervision of Dr M.I.A Salehand Dr A. Alhassan. The information derived from the literature has been duly acknowledged in text and list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

ZUBERU, Jibril	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled "EVALUATION OF THE EFFECT OF CAMEL MILK ON PHYSIOLOGICAL PARAMETERS OF POLOXAMER 407 INDUCED HYPERLIPIDEMIC WISTAR RATS" meets the regulation governing the award of the degree of Masters of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Chairman, Supervisory Committee: _____ _____

Dr M. I.A Saleh (MBBS, M.Sc., PhD.) — Signature — Date

Member, Supervisory Committee: _____ _____

Dr A. Alhassan (B.Sc., MBBS, M.Sc., PhD.) — Signature — Date

Head of Department _____ _____

Dr M.A. Dewu (B.Sc., M.Sc., PhD.) — Signature — Date

Dean, School of Post Graduate Studies_____ _____

Professor S.Z. Abubakar (B.Eng., MSc., PhD.) — Signature — Date

DEDICATION

This work is dedicated to my Mum and late Dad,I say thank you for the love, care, provision, prayers and up bring.

ACKNOWLEDGMENT

I wish to express my profound gratitude and appreciation to Allah the exalted for his favor, love, mercy, protection and providence all through my life, also for his divine knowledge, wisdom, and guidance through this research work. My profound and sincere gratitude to my supervisors Dr. M.I.A Saleh and Dr A. Alhassan for theirthorough supervision, patience, kindness, high level of understanding and advicewhich led to successful completion of this research work. May the almighty reward you abundantly both in this world and hereafter. I also want to acknowledge the effort of the Head of Department of Human Physiology, Dr M.A. Dewu,the Post Graduate coordinator Dr F. Dawud, Dr Yusuf Tanko, Dr I. Sherrif, senior staffs and all other technical staffs of the department of Human Physiology, Ahmadu Bello University Zaria.

I also appreciate the Head of Department of PharmacologyProfessorN.M. Danjuma, the chief Technologist and other technical staffs ofpharmacology laboratory animal house (especially Mallam Aliyu mallam Salihu and Mallam Yau,).I wish to thankMr Bamidele Adetiba of the department ofAnatomy, Dr Kawu Muhammad of Veterinary Physiology, Mrs Hafsat and Brother Salihu from the department of biochemistry and Dr Gabriel Browne of BM Diagnostics Services.

My success would not be complete without my heartthrob and best friend, Mrs. Maryam Oyiza Abdullah for her love and support throughout my study period and thereafter my son Abdullahi Assim Ogirima and my siblings who prayed and supported me in diverse ways; Brother Nasir, Ummu Habila,Ummu Rauf, Ummu Rashad, Sadiya, Nana, Abdulhamid, Brother Abdulaziz, may God in his infinite mercies guide and reward them abundantly. To my friends and post graduate colleagues, Mal Gidado N., Mal Bello Y., Pastor Emma N., Haj Munira A., Haj Fatima Y.,Bilqis U., Billi I., Nabila S., Deborah E. to mention a few, thank you all.

ABSTRACT

Hyperlipidemia is viewed as a primary mediator of cardiovascular disease. The aim of the study is to evaluate the effect of camel milk on Poloxamer 407 induced hyperlipidemic Wistar rats. Thirty adult male Wistar rats weighing between 150-200g were randomly assigned into six groups of five animals each; Group I: administered distilled water, Group II: Hyperlipidemic negative control, Group III: Hyperlipidemic positive control treated with Atorvastatin (20mg/kg) and Group IV, V and VI:Hyperlipidemic groups treated with camel milk 0.25ml/kg, 0.50ml/kg and 1ml/kg respectively. After three weeks, blood samples drawn fordetermination of Total cholesterol (TC), Triglyceride(TG),High Density Lipoprotein (LDL), Low Density Lipoprotein(LDL), Malondialdhyde(MDA), Catalase(CAT) Superoxide Dismutase(SOD) and Gluthatione Peroxidase(GPx), Total Protein, Albumin, Globulin, A/G ratio, Alkaline Phospatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and serum electrolytes(Na^+, K^+, Cl^- and HCO_3^-) respectively were carried out. Total cholesterol(174.68 ±46.92 mg/dL)and triglyceride (91.38±5.52mg/dL) level in Wistar rats were significantly ($p< 0.05$) reduced in 1ml/kg treated groups compared to the hyperlipidemic negative control group (727.24 ±126.59 mg/dL and 203.16 ± 27.64mg/dL) respectively. Camel milk at the dose of 1ml/kg hadhighest lipid lowering activity especially LDL (from414.09±25.96mg/dL to 114.75±42.83mg/dL) while at 0.25ml/kgit showed an increase in the level of HDL (from 35.97 ± 2.43mg/dL to 208.72±7.88 mg/dL).Camel milk at doses of 0.25ml/kg and 0.5ml/kg showed significant ($p<0.05$) increase in the level of SOD(9.25±0.51 U/ml and 11.04± 1.14 U/ml) compared to the hyperlipidemic negative control (3.25 ± 1.05U/ml). There was also significant ($p <0.05$) decrease in the serum levels of total protein and globulin at 0.25ml/kg(5.55±0.56g/dL, 2.41±0.51mg/dL) and 1ml/kg (6.42±0.53g/dL, 3.43±0.60g/dL)and significant increase in A/G ratio in all camel milk treated groups (1.33±0.11, 0.87±0.11 and 0.94±0.11) compared to the hyperlipidemic negative control group (11.23±1.25g/dL, 7.75±1.28g/dL

and 0.49±0.08)respectively. Serum ALT, ASTand HCO_3^- were significantly decreased in all camel milk treated groups (6.40±1.03u/L, 10.20± 2.26U/L and 11.20± 1.39U/L), (20.00±3.17U/L, 28.40± 4.51U/L and 33.20± 5.51U/L)and (24.40±1.80 mmol/L, 26.8±1.68 mmol/L and 26.00±0.70 mmol/L) compared to the hyperlipidemic negative control group (42.50± 4.54U/L, 122.75±22.45U/L and 40.50±0.50 mmol/L)respectively. In conclusion, camel milk demonstrated a potenthypolipidemic, anti-oxidantand hepatoprotective effect onPoloxamer 407 induced hyperlipidemicWistar rats.

TABLE OF CONTENT

Title Page…..…….. i

Declaration……..……..... ii

Certification.....……..…..…...iii

Dedication…..…….……..iv

Acknowledgment…….……..……v

Abstract……..……...…vi

Table of Content…….……..……....……..viii

List of Table...……..…….…….…xv

List ofFigure.…….……..…….….xvi

List of Plates..…….....xvii

List of Abbreviations…….…….…..xviii

CHAPTERONE……..…….....1

1.0 Introduction…….....……1

1.1 Statement of Research Problem…….…….……..2

1.2 Justification……..…….....2

1.3 Aim of the Study……....……..…...3

1.3.1 Specific Objectives of the Study.....……..……..….3

1.5 Research Hypothesis……...…..…5

CHAPTER TWO

2.0 Literature Review……...……..…….....…..6

2.1 Lipids……..……..6

2.1.1 Synthesis and Transportation of Lipids……6

2.1.2 Lipoproteins……..9

2.2 Hyperlipidemia……..……..…11

2.2.1 Classification of Lipid Concentrations …….…..11

2.3 Classification of Hyperlipidemia……...……..……13

2.3.1 Primary Hyperlipidemia…….…….……....…13

2.3.2 Secondary Hyperlipidemia……..……....14

2.4 Pathophysiology of Hyperlipidemia ……..……..……....…..…....15

2.4.1 Pathophysiology of Hypercholesterolemia…….…….....15

2.4.2 Pathophysiology of Hypertriglyceridemia……..…….…...16

2.5 Risk Factors of Hyperlipidemia……...……...……..17

2.5.1 Dietary Cause……..17

2.5.2 Obesity and Hyperlipidemia……...……...……..17

2.5.3 Diabetes Mellitus……... ……..…….18

2.5.4 Genetic Association of Hyperlipidemia……... …….…..19

2.5.5 Alcohol and Hyperlipidemia……...……..…….19

2.5.6 Age and Hyperlipidemia…...…..……..…..…….…..20

2.5.7 Hypothyroidism and Hyperlipidemia…….……..…..……..20

2.5 Signs and Symptoms of Hyperlipidemia…….…….……...…..……20

2.6.1 Complications of Hyperlipidemia…..…….……21

2.7 Treatment of Hyperlipidemia…...…….……..…....…23

2.7.1 Statins…….……..…..……..23

2.7.2 Fibrates……..…….……..…..……..23

2.7.3 Niacin…….……..…..……..24

2.7.4 Bile Acids Resins……..…..……..…..……..25

2.7.5 Ezetimibe……...…….……..…..……..26

2.7.6 Dietary Supplementation……..…..……..26

2.7.7 Exercise……27

2.8 Induction of Hyperlipidemia……..…….……...……...….27

2.8.1 Poloxamer ..……..……..…….…...28

2.8.1 Poloxamer 407 Induced Hyperlipidemic Model....……..…...……..…….28

2.9 Camel Milk……..……30

2.9.1 Composition of Camel Milk…….……...31

2.9.1 Therapeutic Effects of Camel Milk…….……..……..……33

CHAPTER THREE

3.0 Materials and Methods …….……40

3.1 Materials ……...……...……..……40

3.2 Experimental Design……...……..…….41

3.2.1 Experimental Animals……..……..…….41

3.2.2 Camel MilkCollection …….……..41

3.2.3 Proxmate Analysis of camel Milk……..…….…….....41

3.2.4 Acute Toxicity Studies of Camel Milk……..……..……42

3.2.5 Induction of Hyperlipidemia……...…….42

3.2.6 Preparation of Standard Drug……..…….....43

3.2.7 Groupings of Animals……...…….……..44

3.2.8 Collection of Blood samples ……...…44

3.3 Biochemical Assays……...45

3.3.1 Estimation of Serum lipid profile markers……..…..……..……..45

3.3.2 Determination of Oxidative Stress Biomarkers……...…….…….50

3.3.3 Estimation of Serum Proteins……..……53

3.3.4 Determination of Liver Enzymes……..…….…….....55

3.3.4 Detemination of Serum Electrolyte …….……..59

3.4 Statistical Analysis……..……..65

CHAPTER FOUR

4.0 Results…….…….……..……66

4.1 Quantitative Analysis of camel milk ....…….…….66

4.2 Result for Median Lethal Dose (LD_{50}) of Camel Milk in Wistar Rat……..68

4.3 Effect of Camel Milk on lipid profile in P407 Induced Hyperlipidemic WistarRats…….……. …….70

4.3.1 Effect of camel milk on Total Cholesterol level……..……70

4.3.2 Effect of camel milk on Triglyceride level.……...…….…….…70

4.4 Effect of Camel Milk on High Density Lipoprotein and Low Density Lipoprotein on P407 Induced Hyperlipidemic Wistar Rats…...…….……..72

4.4.1 Effect of camel milk on High Density Lipoprotein Cholesterol level……...…..72

4.4.2 Effect of camel milk on Low Density Lipoprotein Cholesterol level..……..72

4.5 Effect of Camel Milk on Serum Oxidative Stress Biomarkers of P407 Induced Hyperlipidemic Wistar Rats…….74

4.5.1 Effect of camel milk on Catalase…….……74

4.5.2 Effect of camel milk on Superoxide Dismutase…….……..……74

4.5.3 Effect of camel milk on Malondialdehyde……..……...…….74

4.5.4 Effect of camel milk on Gluthathione Peroxidase……..……75

4.6 Effect of Camel Milk on Serum Total Protein, Albumin, Globulin and A/GRatio of Poloxamer 407 Induced Hyperlipidemic Wistar Rats…….……...77

4.6.1 Effect of camel milk on Total Protein……...77

4.6.2 Effect of camel milk on Albumin…….…….77

4.6.3 Effect of camel milk on Globulin…….….77

4.6.4 Effect of camel milk on Albumin/Globulin Ratio…….78

4.7 Effect of camel milk on liver enzymes of poloxamer 407 induced Hyperlipidemic Wistar Rats……..……..81

4.7.1 Effect of camel milk on Alkaline Phospatase (ALP)…...……..81

4.7.2 Effect of camel milk on Alanine Aminotransferase (ALT)……..…….81

4.7.2 Effect of camel milk on Aspartate Aminotransferase (AST)……..……..…….81

4.8 Effect of camel milk on Serum Electrolytes of P407 Induced Hyperlipidemic Wistar Rats……....……83

4.8.1 Effect of camel milk on Sodium and Potassium…….....…….83

4.8.2 Effect of camel milk on Chloride…….....…….83

4.8.3 Effect of camel milk on Bicarbonate……...…..…….83

CHAPTER FIVE

5.0 Discussion……..……..…..85

CHAPTER SIX

6.0 Conclusion, Recommendations, Contributions to knowledge and Limitations…….…..……..92

6.1 Conclusion……...……,……..…..……92

6.2 Recommendations…….....……...…..……...……92

6.3 Contributions to Knowledge…….…….……....…….93

6.4 Limitations……..94

References……..……..……..95

Appendix…….116

LIST OF TABLES

Table 4.1:Quantitative analysis of milk from one humped camel of Kaura Namoda farm……..…..67

Table 4.2(a): Result for LD_{50} of oral administration of camel milk on Wistar rats during Phase 1…….69

Table 4.2(b):Result for LD_{50} of oral administration of camel milk on Wistar rats during Phase 2……...……..69

Table 4.5: Effect of camel milk on Oxidative Stress Biomarkers of Poloxamer 407 Induced Hyperlipidemic Wistar rats……76

Table 4.8.1: Effect of Camel Milk on Serum Electrolytes of Poloxamer 407 Induced Hyperlipidemic Wistar Rats……..84

LIST OF FIGURES

Figure 4.3.1: Effect of Camel Milk on Total Cholesterol and Triglyceride in P407 Induced Hyperlipidemic Wistar Rats…….71

Figure 4.4.1: Effect of Camel Milk on High Density Lipoprotein and Low Density Lipoprotein in P407 Induced Hyperlipidemic WistarRats…….……73

Figure 4.6.1: Effect of Camel Milk on Serum Total Protein, Albumin and Globulin of oloxamer 407 Induced Hyperlipidemic Wistar Rats…..…..……..……..……..79

Figure 4.6.2: Effect of Camel Milk on Serum Albumin/Globulin Ratio of Poloxamer 407 Induced Hyperlipidemic Wistar Rats……..80

Figure4.7.1: Effect of camel milk on serum liver enzymes of poloxamer 407 induced hyperlipidemic WistarRats……82

LIST OF PLATES

Plate I: A brown one humped camel from Kaura Namoda, Zamfara state……...38

Plate II: A bottle of raw camel milk collected from one humped camel at Kaura Namoda, Zamfara State……..……..……..…….39

LIST OF ABBREVIATIONS

A/G	Albumin/Globulin Ratio
B	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
AT	Atorvastatin 20mg/kg
CAD	Coronary Artery Disease
CAT	Catalase
CM	Camel Milk
C(0.25)	Camel Milk (0.25ml/kg)
C(0.50)	Camel Milk (0.50ml/kg)
C (1)	Camel Milk (1ml/kg)
CVD	Cardiovascular Disease
CETP	Cholesteryl Ester Transfer Protein
GLB	Globulin

GPx	Gluthathion Peroxidase
HDL	High Density Lipoprotein
HDL-C	High Density Lipoprotein-cholesterol
HL	Hepatic Lipase
HMG-COA	3-Hydroxy-3- Methylglutaryl-CoA Reductase
IDL	Intermediate Density Lipoprotein
LCAT	Lecithin Cholesterol Acyltransferase
LDL:	Low Density Lipoprotein
LDL-C	Low-Density Lipoprotein Cholesterol
LPL	Lipoprotein Lipase
MDA	Malondialdehyde
NCEP	National Cholesterol Education Programme
P407	Poloxamer 407
SOD	Super Oxide Dismutase
TC	Total Cholesterol
TCA	Trichloroacetic Acid
TG	Triglyceride

TOPS	N-ethyl-N-sulfopropyl-m-toluidine
WHO	World Health Organization

CHAPTER ONE

1.0 Introduction

Cardiovascular diseases (CVD) are the leading cause of death for both men and women among all racial and ethnic groups (Smith, 2004). It accounts for nearly 50% of all deaths in the developed world (Thomas and Rich, 2007) and has also been predicted by the World Health Organization to remain the leading causes of deaththat will affect approximately 23.6 million people globallyby 2030(Ooi and Liong,2010).Hyperlipidemia is viewed as the primary mediator of a cascade of heart diseases such as atherosclerosis, stroke, myocardial infarction and pancreatitis (Olorunnisola *et al.,* 2012; Harikumar *et al.,* 2013). It may be due to heredity or acquired secondarily from underlying disease such as; type 2 diabetes mellitus, liver cholestasis, alcohol, nephrotic syndrome, chronic renal failure, hypothyroidism, cigarette smoking, drugs, hormone and obesity (Olorunnisola *et al.,* 2012; Harikumar *et al.,* 2013; Onwe *et al.,* 2015). Irrespective of the etiology of hyperlipidemia, common features include elevated serum total cholesterol, triglyceride, low density lipoprotein, very low density lipoprotein, and reduced high density lipoprotein (Olorunnisola *et al.,* 2012). The current hypothesis suggests oxidative stress as an underlying mechanism through which hyperlipidemia induce tissue damage with several studies paying attention to the oxidative modification of LDL, protein glycation, glucose auto-oxidation and lipid peroxidation (Bansal and Jaswal 2009; Olorunnisola *et al.,* 2012; Adekunle *et al.,* 2013; Ngoc 2015).

Camel milk has been utilized since ancient times due to its therapeutic benefit. It is used in countries such India and Egypt in the treatment of wide range of diseases, some of which include; edema, jaundice, asthma, splenic disorders, anemia, hemorrhoids, diabetes and fatigue (Redwan *et al*., 2003; Chakrapany and Chandan, 2014). In ancient Russia camel milk was used in sanitoria for the treatment of tuberculosis (Guakhar and Bernand, 2004). Camel milk does not form coagulum in acidic environment (Hassan and Bayoumi, 2010) and can be tolerated by individuals with lactose intolerance and those with weakened immune system (Yateem *et al.,* 2008; Abbas *et al.,* 2013; Gizachew *et al.,* 2014).

1.1 Statement of Research Problem

Hyperlipidemia is a major risk factor for cardiovascular diseases (Oguejiofor *et al.,* 2012; Olorunnisola *et al.,* 2012). The World health Organization reported that it accounts for more than four million deaths annually (Oguejiofor *et al.,* 2012). Influx of western diet to the developing world has increased the rates of hyperlipidemia in Africa (Lim *et al.,* 2012). According to Oguejiofor *et al.,* (2012), hyperlipidemia is highly prevalent in all geopolitical zones of Nigeria, with the overall the prevalence ranging from 60% among apparently healthy Nigerians to 89 % among diabetic Nigerians.

1.2 Justification

Several allopathic hypolipidemic drugs are currently available in the market, but there is a lag behind in terms of efficacy, cost and simplicity of administration and safety on long term use. Some of these factors do not fulfill conditions for patient's compliance (Davidson and Tooth, 2004). Consumption of some of these drugs have been reported to cause hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function (Arun Kumar *et al.,* 2013). The search for

potential cardiovascular protective agent from natural sources had been gaining universal acceptance with many studies paying attention to natural products as hypolipidemic candidates as well as antioxidant effects but have no side effects(Bansa and Jaswal 2009; Sefi *et al.,* 2010; Olorunnisola *et al.,* 2012).

1.3 Aim of the Study

To determine the effect of camel milk on physiological parameters of Poloxamer 407 induced hyperlipidemia in Wistar rats.

1.3.1Specific Objectives of the Study

To evaluate the effect of camel milk on;

a) Serum lipid profile of Poloxamer 407 induced hyperlipidemic male Wistar rats;

    Total cholesterol (TC),
    Triglycerides (TG),
    Low Density Lipoprotein (LDL),
    High Density Lipoprotein (HDL).

b) Oxidative stress biomarkers of Poloxamer 407 induced hyperlipidemic male Wistar rats;

    Malondialdehyde (MDA)
    Glutathione Peroxidase (GPx),
    Superoxide Dismutase (SOD),
    Catalase (CAT).

c) Total protein and albumin/globulin ratio of Poloxamer 407 induced hyperlipidemic male Wistar rats.

d) Liver Enzymes of Poloxamer 407 induced hyperlipidemic male Wistar rats

    Alkaline Phosphatase (ALP)
    Alanine Trasaminase (ALT)
    Aspartate Aminotransferase (AST)

e) Serum electrolytes of Poloxamer 407 induced hyperlipidemic male wistar rats;

    Sodium (Na^+)
    Potassium (K^+)
    Chloride (Cl^-)
    Bicarbonate (HCO_3^-)

1.5 Research Hypothesis

H_0: Camel milk does not have protective effect on Poloxamer 407 induced hyperlipidemic Wistar rats.

CHAPTER TWO

2.0 Literature Review

2.1 Lipids

Lipids are hydrophobic small molecules which are naturally occurring compounds that are insoluble in water but soluble in non-polar solvents. In all living cells, lipids are required to maintain cellular structure, provide energy and cell signaling. Lipid metabolism generates a variety of intermediate compounds, many of which are signaling molecules or second messengers (Mattes, 2005). Several chemical compounds in foods and in the body are classified as lipids. They include; cholesterol, triglycerides, phospholipids and few others. The basic lipid moiety of both triglycerides and phospholipids is fatty acids with the following: CH3(CH2)14COOH(Guyton 2006). Cholesterol has no fatty acid but sterol nucleus synthesized from portions of fatty acid molecules. This gives it many of the physical and chemical properties of other lipid substances (Guyton 2006).

2.1.1Synthesis and Transportation of Lipids

Lipids are found exogenously from diets or synthesized endogenously from the liver and intestines (Amuamuta, 2014).Numerous metabolic processes are involved in the absortion, transport and storage of lipids.

2.1.1.1 Synthesis and Transportation of Lipids in the Small Intestine

After the ingestion of a fatty meal, bile acids cause emulsification of lipids rendering them accessible to lipases (Dashty, 2014). The digestion of lipids begins from the stomach by gastric lipase but major parts of lipid degradation occur in the small intestine, via pancreatic lipase and pancreatic phospholipase A2 (Dashty, 2014). Triglycerides (TG) are hydrolyzed by pancreatic lipase in the intestinal lumen into free fatty acids (FFA) and2-monoacylglycerols (MAG). They are taken up by the enterocytes via passive diffusion and specifictransporters like clusters of differentiation 36 (CD36) (Klop *et al.*, 2013). Following absorption, FFA and MAG are assembled again to form TG (Guyton, 2006; Klop *et al.*, 2013). Most dietary cholesterol is esterified by combination of cholesterol and one molecule of fatty acids (Guyton 2006). Cholesterol esters converted to the free form by cholesterol esterase (Dashty 2014). Cholesterol is then taken up by the enterocytes via the specific cholesteroltransporters such as Niemann-Pick C1 like 1 protein (NPC1L1) scavenger receptor B1 (SRB1) and CD36 (Klop *et al.*, 2013). Once in the enterocyte, cholesterol is transformed into cholesterol-esters. Finally, both cholesterol-esters and TG are packed together with phospholipids and apolipoprotein (apo) B48 toform nascent chylomicrons (CMs). This process is mediated by microsomal triglyceride transfer protein (Nirosha *et al.,* 2014). The CMs are secreted into the lymphatics which finally enter the circulation via the thoracic duct(Klop *et al.*, 2013).

In the blood stream,lipoprotein lipase hydrolyses TG component of CMs into free FFA and MAG with concomitant release of cholesterol esters into the liver via portal circulation (Nirosha *et al.,* 2014). This enzyme is expressed on endothelial cells of the heart, muscle, adipose tissue, macrophages and lactating

mammary gland (Shattat, 2014). A multifunctional protein known as hepatic lipase is also synthesized by hepatocytes to hydrolyze phospholipids and triglycerides of lipoproteins (Shattat, 2014). In addition, hepatic lipase facilitates lipid delivery by stimulating lipoprotein absorption by cell surface receptors and proteoglycans (Shattat, 2014). Free fatty acids are transported to the heart, skeletal muscle and adipose tissue for energy expenditure and storage (Klop, 2013). Cholesterol binds to low density lipoprotein receptor-like-protein (LRP) receptors or Apo-E receptor in the liver (Nirosha *et al.,* 2014).

2.1.1.2 Synthesis of Lipids in the Liver

The liver preserves cholesterol homeostasis by synthesizing this molecule and also converting excess cholesterol to bile acids. The pool of cholesterol is tightly regulated;a rise in the concentration of cholesterol 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoAR); the rate-limiting enzyme de novo in cholesterol synthesis (Dashty, 2014). This provides an intrinsic feedbackcontrol system to prevent an excessive increase in plasma cholesterol concentration (Guyton 2006).Cholesterol also stimulates HMG-CoAR polyubiquitination and degradation which is modulated by its transmembrane sterol-sensing domain (SSD) and its role in proteasome degradation. HMG-CoAR is also regulatedby phosphorylation and dephosphorylation(Dashty, 2014).Cholesterol is synthesized from molecules of acetyl Co A; two molecules of acetyl Co A condenses to form Acetoacetyl Co A. This reaction is facilitated by Acetyl Co A synthase enzyme. Acetoacetyl Co A is condensed in the presence of 3-Hydroxy-3-methylglutaryl coenzyme A synthase to form 3-Hydroxy-3-methylglutaryl coenzyme A which is subsequently reduced to mevalonate by HMG-CoAR. Mevalonate in the presence of ATP undergo phosphorylation and decarboxylation leading to the formation of Isopentenyl pyrophosphate. Cholesterol is derived from isopentenyl pyrophosphate after series of reactions have occurred (Rhodes *et al.,* 1995). Eighty percent (80%) of cholesterol in the liver is stored in the gallbladder in the form of bile salts while the remaining 20% is used for generation of other cholesterol-based products like sex hormones, phospholipids, CMs, cell membrane lipids, and vitamin D3(Dashty, 2014). Cholesterol is released from the gallbladder to the intestine and is again absorbed via enterocytes (Dashty, 2014).

2.1.2 Lipoproteins

Lipids due to their hydrophobic nature are transported in the form of biochemical assemblies called lipoproteins (Amuamuta, 2014). This transport facilitates the delivery to other tissues that require them as source of energy or structural materials in their membrane. . The liver synthesizes most of the lipoproteins required by the body. Lipoproteins are smaller than CMs but qualitatively similar in composition. The total concentration of lipoproteins in the plasma averages about 700 mg/dl (Guyton, 2006).The structure of lipoproteins consists of an amphipathic monolayer of lipids (hydrophilic head groups of phospholipids, cholesterol and apolipoproteins) and hydrophobic layer (TG and cholesterol esters) (Dashty, 2014). Lipoproteins are classified based on their densities measured in the ultra-centrifuge;

i. Very low density lipoproteins (VLDL): contain high concentrations of triglycerides but moderate concentrations of both cholesterol and phospholipids (Guyton, 2006).

ii. Intermediate density lipoproteins (IDL): They are very low density lipoproteins from which a share of the triglycerides has been removed making so that the concentrations of cholesterol and phospholipids higher (Guyton, 2006).

iii. Low-density lipoproteins (LDL): Derived from IDL by the removal of almost all the triglycerides, they have high concentration of cholesterol and a moderately high concentration of phospholipids (Guyton 2006).

iv. High-density lipoproteins (HDL) have high concentration of protein (Guyton 2006). They promote the uptake of cholesterol from peripheral tissues, including the arterial wall and returns cholesterol to the liver (Klop *et al.*,2013).

The liver synthesizes and secretes nascent very low density lipoprotein (VLDL) by complexing TG and apo-B100 under the mediation of MTP. Triglyceride-rich nascent VLDL serves as an efficient acceptor of Cholesterol esters from HDL. This transfer takes place under the influence of cholesteryl ester transfer protein (CETP) in the plasma leading to the formation of mature VLDL. Following hydrolyses of TG, VLDL are converted to smaller lipoproteins called intermediate density lipoproteins (IDL) and smaller LDL rich in esterified cholesterol. LDL supplies tissues with cholesterol making it the major site for both cholesterol synthesis and LDL catabolism (Nirosha *et al.,* 2014).

2.2 Hyperlipidemia

Hyperlipidemia is a condition characterized byexcess lipids, largely cholesterol and triglycerides in the blood (Harikumar *et al.,* 2013; Nirosha *et al.,* 2014). Hyperlipidemia can also be termed hyperlipoproteinemia, due to the transportation of cholesterols and triglycerides in the form of a biochemical assembly known as lipoproteins. Hyperlipdemia is divided into two broad categories; hypercholesterolemia and hypertriglyceridemia (Harikumar *et al.,* 2013). Hyperlipidemia progresses with alteration in the serum lipids profile. Hence, lipid profiles are important risk factors in many degenerative diseases (Kolawole *et al.,* 2012; Sodipo *et al.,* 2012).Lipid abnormality can be qualitative or quantitative. Quantitatively, it is due to elevated Total Cholesterol (TC), elevated Low Density Lipoprotein Cholesterol (LDL-C) elevated Triglyceride (TG) and reduced High Density Lipoprotein Cholesterol (HDL-C) levels occurring singly or in combinations. Qualitatively it implies changes in composition of LDL-C which includes small dense LDL-C increased TG content or increased electronegativity of LDL-C (Oguejiofor *et al.,* 2012).

2.2.1 Classification of Lipid Concentrations

Lipids are classified based on the guidelines of National Cholesterol Education Program/Adult Treatment Panel. Lipids can be classified as TC, triglycerides, LDL, HDL and very low density lipoprotein (VLDL) (Arun Kumar*et al.,* 2013).

2.2.1.1 Total cholesterol

Desirable level of Total Cholesterolconcentrations is below 200mg/dL while concentrations greater than 240mg/dL is termed hyperlipidemic (Rohilla *et al.,* 2012, Arun Kumar *et al.,* 2013; Nirosha *et al.,* 2014).

2.2.1.2 Triglyceride

For triglyceride concentration less than 150 mg/dL, it is regarded as normal, however, concentrations of 200-499 mg/dL are considered as high(Rohilla *et al.,* 2012, Arun Kumar *et al.,* 2013; Nirosha *et al.,* 2014), concentrations of 500 mg/dL and above are prone to the development and progression of various cardiovascular diseases(Rohilla *et al.,* 2012;Arun Kumar *et al.,* 2013).

2.2.1.3 Low Density Lipoprotein Cholesterol

High levels of Low Density Lipoprotein which is also known as “bad cholesterol” indicate more cholesterol in the blood stream (Arun Kumar *et al.,* 2013), it is known to transport 70% of cholesterol but very little triglyceride (Amuamuta, 2014).LDL cholesterol concentrations below 100mg/dL are considered optimal and high when concentrations exceed 160 mg/dL (Arun Kumar *et al.,* 2013; Nirosha *et al.,* 2014). It becomes very high when concentrations exceed 190mg/dL(Nirosha *et al.,* 2014).

2.2.1.4 High Density Lipoprotein Cholesterol

High Density Lipoprotein cholesterol also known as “good cholesterol” transports cholesterol and other lipids from tissues back to the liver for degradation (Arun Kumar *et al.,* 2013).High Density Lipoprotein makes up 30% of total cholesterol(Amuamuta, 2014). Elevated level of HDL cholesterol is cardio protective; it acts by preventing oxidation of LDL-C, protects against cytotoxicity induced by LDL-C and also possessanti-inflammatory properties such as suppression of cytokines-induced endothelial adhesion molecules function (Thalia *et al.,*2015). The concentrations of 60 mg/dL or above have been considered high but when the concentration falls below 40 mg /dL, it becomes a major risk factor for cardiovascular disease(Arun Kumar *et al.,* 2013).

2.3 Classification of Hyperlipidemia

Hyperlipidemia may be classified as either Primary or Familial if it results from specific genetic abnormalities, secondary if there is an underlying disorder that leads to elevation of plasma lipid and lipoprotein metabolism. Hyperlipidemia may be idiopathic if the underlying cause is not known(Harikumar *et al.,* 2013; Nirosha *et al.,* 2014).

2.3.1 Primary Hyperlipidemia (Familial Hyperlipidemia)

Primary hyperlipidemia is also called familial hyperlipidemia due to genetic defect which could be either monogenic or polygenic (Harikumar *et al.,* 2013). Primary hyperlipidemia is an autosomal dominant disorder of LDL cholesterol clearance which is associated with marked hypercholesterolemia, tendon/cutaneous xanthoma, premature coronary artery disease and arcus cornea in some cases (Teramoto *et al.,* 2013). Many patients with mutations in the LDLR gene that encodes the LDL receptor protein, result in delay in the removal of LDL from circulation. Also implicated are proprotein para convertase family known as proprotein convertase subtilisin/kexin type 9 gene (PCSK9) (Austin *et al.,* 2004; Soutar and Naoumova, 2007) and mutation of apolipoprotein B (ApoB), which is the part of LDL that binds with the receptor, however, mutations in other genes are rare (Rader *et al.,* 2003). According to Harikumar *et al.,* (2013), “primary hyperlipidemia is classified according to the Fredrickson classification which is based on pattern of lipoproteins on electrophoresis or ultracentrifugation. This classification was later adopted by the World Health Organization (WHO). It remains a popular system of classification, but is considered dated by many scholars”.

2.3.2 Secondary Hyperlipidemia (Acquired Hyperlipidemia)

Secondary hyperlipidemia is also called acquired hyperlipidemia due to an underlying cause, some of which include; diabetes, nephrotic syndrome, chronic alcoholism, and hypothyroidism as well as use of drugs like corticosteroids, beta blockers oral contraceptives and myxedema (Harikumar *et al.,* 2013; Norosha *et al.,* 2014), hypothyroidism (Shah and Gohil, 2014) and myxoedema (Onwe *et al.,* 2015). Another cause of hyperlipidemia that is not always included in this category is postprandial hyperlipidemia (Onwe *et al.,* 2015; Ngoc 2015). Secondary hyperlipidemia may mimic the primary forms of hyperlipidemia and together with significant hypertriglyceridemia;it can lead to the development of pancreatitis and premature atherosclerosis (Harikumar *et al.,* 2013).

2.4Pathophysiology of Hyperlipidemia

Hyperlipidemia as the major risk factor of cardiovascular Diseases (CVD) results in various associated complications such as atherosclerosis, stroke,cholilithiasis, metabolic syndrome and corneal arcus (Olorunnisola *et al.,* 2012; Harikumar *et al.,* 2013; Amuamuta 2014).

2.4.1 Pathophysiology of Hypercholesterolemia

Under normal circumstance, low density lipoprotein cholesterol circulates in the body for 2.5 days; it is then bound to the LDL receptor on the liver cells where it will undergo endocytosis and catabolism. The removal of LDL leads to suppressed synthesis of cholesterol in the liver, this process is achieved through inhibition of 3-Hydroxyl-3-Methyl-Glutaryl-CoA reductase (3HMG-CoA reductase) pathway; the rate limiting step denovo in the synthesis of cholesterol. However, in cases of LDL receptor dysfunction as observed in Familial Hyperlipidemia (FH), prolong circulation of LDL for an average duration of 4.5 days occurs. This in turn results in elevation of LDL cholesterol in the blood with normal levels of other lipoproteins. Similar changes are also observed in cases of Apolipoprotein B mutation due to reduced binding of LDL particles to its receptor (Harikumar *et al.,* 2013).Elevation of serum cholesterol and/or triglyceride levels, apolipoprotein B (apoB) levels and increased numbers of small-dense LDL particles are core lipid profile noted in the Familial Combined hyperlipidemia (FCHL)(Amuamuta, 2014). LDL receptor dysfunction may also result from the defect of proprotein convertase subtilisin/kexin type 9 gene (PCSK9) (Austin *et al.,* 2004; Soutar and Naoumova, 2007). PCSK9 completes the catabolism of LDL by binding to LDL receptor, this process occurs after LDL and LDL receptor combination have been ingested (Weinreich and Frishman, 2014). Patients with Familiar Hyperlipidemia may develop accelerated atherosclerosis due to excess LDL in the blood. The degree of atherosclerosis is determined by number of LDL receptors and their functionality (Harikrumah *et al.,* 2013).

2.4.2 Pathophysiology of Hypertriglyceridemia

Excess production of Very Low Density Lipoprotein (VLDL) by the liver in response to increase flow of free fatty acids into the liver and a defect in VLDL triglycerides breakdown and chylomicrons by lipoprotein lipase are major processes involved in the elevation of triglyceride in the blood. Lipoprotein lipase is an enzymes that hydrolysis triglyceride. In this case, there will be elevation of triglycerides as they cannot be hydrolyzed. Metabolism of chylomicrons and VLDL remnants may be delayed which is observed in type III hyperlipidemia (Harikrumah *et al.,* 2013). In addition to Lipoprotein lipase, absence of the surface apoprotein CII31 also elevates plasma triglyceride. Postprandial absorption of chylomicrons from the gastrointestinal tract is said to occur 30 - 60 min after ingestion of fatty meal, such may also result in elevated serum triglycerides for 3 -10 hours(Onwe *et al.,* 2015).

2.5 Risk factors of Hyperlipdemia

There are various risk factors that are associated with the development of hyperlipidemia; changes in life style (Onwe *et al.,* 2015), high fat diets, overweight, alcohol abuse, diabetes, stress and drugs (Rohilla *et al.,* 2012; Amuamuta 2014).

2.5.1 Dietary Causes

High fats dietshave been linked to increase the level of plasma triglyceride (Pejic and Lee, 2006; Onwe *et al.,* 2015). Saturated fat and cholesterol in diets have been reported to raise cholesterol levels in the body especially when it exceeds 40 percent of daily total caloric intake cholesterol intake greater than 300 milligrams (Shattat 2014; Onwe *et al.,* 2015). It has been shown that in humans, saturated fats increase thenumber of VLDL secreted by the liver and the conversionof VLDL to LDL and depress the levels of LDL receptors mRNA in rats (Amuamuta, 2014). Both high carbohydrate diet and intake of fructose increase triglycerides and totaland LDL cholesterols respectively(Mc Carthy, 2004; Zhang *et al.,* 2013).Trans-fat also results in reduced HDL and elevated LDL (Ascherio and Willet, 1997). However, the effect of diet on the level of cholesterol varies depending on individuals(Howell *et al.,* 1997; Mannu *et al.,* 2013). A fall in post prandal hypercholesterolemiacould trigger sudden production of cholesterol by the liverwhich may become high(Nordesgaard *et al.,* 2016).

2.5.2 Obesity and Hyperlipidemia

Obesity is considered to be the largest public health problem globally, especially in industrialized countries (Klop*et al.,* 2013). Both obesity and hyperlipidemia are independently associated with atherosclerosis, non-alcoholic fatty liver disease and insulin resistance (Amuamuta 2014).Obesity in addition to being a risk factor for heart disease also increases the level of cholesterol, triglycerides and Free Fatty Acids. Obesity results in decreased HDL-C with HDL dysfunction and normal or slightly increased LDL-C with increased small dense LDL levels in the body and sometimes increase plasma apolipoprotein B (Rohilla *et al.,* 2012; Klop*et al.,* 2013; Amuamuta 2014). Obesity causes elevation of both fasting and postprandial triglyceride in combination with the preponderance of small dense LDL and low HDL-C. Hypertriglyceridemia in obesity may be the major cause of the other lipid abnormalities dueto the delayed clearance of triglyceride-rich lipoproteins and formation of small dense LDL(Klop*et al.,* 2013).

2.5.3 Diabetes Mellitus

Type II diabetes mellitus is related to a number of lipid and lipoprotein abnormalities (Amuamuta 2014). Impaired action of insulin has been reported to affect not only glucose uptake but also fatty acids (Rohilla *et al.,* 2012). The lipid abnormality most closely associated with insulin resistance and hyperinsulinemia is hypertriglyceridemia and Low HDL concentration (Nikkila *et al.,* 1984; Amuamuta 2014). Insulin resistance increases hydrolysis of intra cellular triglyceride and release fatty acids into circulation, it is also likely to result from interaction of multiple defective genes that increase pressure

on fat cells to store energy (Khan and Flier, 2000). There is a significant increase in the post prandal concentration and time of permanence of TG, chylomicron remnants, VLDL and VLDL remnants in plasma of diabetes patients (Rebolledo and Dato, 2005). Also diabetes mellitus patients have been shown to have low Lipoprotein lipase activity which stimulates more synthesis of VLDL cholesterol by the liver ultimately leading to hyperlipidemia (Onwe *et al.,* 2015). The improvements in glycemic control have been demonstrated to decrease the cholesterol levels in the body (Rohilla *et al.,* 2012).

2.5.4 Genetic Association of Hyperlipidemia

Hyperlipidemia in some cases is hereditary and common generations of families with history of obesity. Some primary lipid disorders can directly result in over production of cholesterol by the body (Amuamuta 2014). Many patients have mutations in the LDLR gene that encodes the LDL receptor protein or mutation of apolipoprotein B (Rader *et al.,* 2003) or proprotein para convertase family known as proprotein convertase subtilisin/kexin type 9 gene (PCSK9)(Austin *et al.,* 2004; Soutar and Naoumova, 2007; Weinreich and Frishman, 2014).

2.5.5 Alcohol and Hyperlipidemia

The effect of moderate alcohol intake on lipid levels have been investigated (Rohilla *et al.,* 2012; Amuamuta 2014) with reports showing that alcoholics have fasting blood triaglyceride above normal and 17% have a concentration greater than 3mmol/L(Amuamuta 2014). Ingestion of alcohol results in increase synthesis of triglyceride and triglyceride rich lipoproteins in the liver. Alcohol also stimulates lipolysis in fatty tissue which increase fatty acids supply to the liver (Van de Weil*et al.,* 2011).Moderate alcohol intake increases HDL-cholesterol (Rohilla *et al.,* 2012), however,increased alcohol intake beyond moderation is associated with increased heart diseasesand alcoholic fatty liver. Substantial alcohol consumption accompanied by high fat intake in patients with fatty liver that are most susceptible to marked hypertriglyceridemia(Pownall*et al.,* 1999). Studies have shown that the pathogenesis of alcohol induced hyperlipidemia and fatty liver occurs as a result both decreased fatty acid oxidation in mitochondria and increase glycerolipid synthesis (Amuamuta 2014).

2.5.6 Age and Hyperlipidemia

Age has been implicated is the development of hyperlipidemia, as cholesterol level is shown to rise as a person gets older. However, before menopause, women tend to have lower TC levels than men of the same age. Levels of LDL tend to rise in post-menopausal women (Rohilla *et al.,* 2012).

2.5.7 Hypothyroidism and Hyperlipidemia

Hypothyroidism is one of the risk factors of hyperlipidemia. This is due to the positive linear correlation reported between thyroid stimulating hormone (TSH) andthe level of total cholesterol, low-density lipoprotein cholesterol and triglyceridesand negative linear correlation between TSH and high-density lipoprotein cholesterollevels. Thyroid hormone activates cholesterol ester transfer protein and lipase. Hypothyroidism results in decreased activity of HMG-CoA reductase which will in turn increase the levels of total cholesterol and low density lipoproteins (Shah and Gohil, 2014).

2.6 Signs and Symptoms of Hyperlipidemia

Hyperlipidemia tends to be discovered during routine examination or evaluation for atherosclerotic cardiovascular disease (Shattat 2014). Some of the symptoms include; xanthoma (deposition of LDL-C in tendons), xanthelasma of eyelid, chest pain, abdominal pain, splenomegaly, hepatomegaly, heart attacks, higher rate of obesity, glucose intolerance, atheromatous plaques in the arteries, arcus senilis, xanthomata and peripheral poly neuropathy as in type V hyperlipidemia (Arun Kumar *et al.,* 2013; Harikumar *et al.,* 2014).

2.6.1 Complications of Hyperlipidemia

2.6.1.1 Atherosclerosis

Atherosclerosis involves an inflammatory process which arises from accumulation of cholesterol in macrophages trapped in the sub-endothelium and extracellular matrix (Ngoc, 2015). Hyperlipidemia is the most important risk factor for atherosclerosis. The response-to-injury hypothesis states that "risk factors such as oxidized LDL, mechanical injury to the endothelium, excessive homocysteine, immunologic attack, or infection-induced changes in endothelial and intimal function lead to endothelial dysfunction and a series of cellular interactions that culminate in atherosclerosis" (Arun Kumar *et al.,* 2013; Onwe *et al.,* 2015). The eventual clinical feature may include; angina, myocardial infarction, arrhythmias, stroke, peripheral arterial disease, abdominal aortic aneurysm, and sudden death(Arun Kumar *et al.,* 2013). Atherosclerotic lesions are thought to arise from transport and retention of plasma LDL through the endothelial cell layer into the extracellular matrix of the subendothelial space. Once in the artery wall, LDL undergoes oxidative modification and non-enzymatic glycation which is due to delayed clearance (Arun Kumar *et al.,* 2013; Ngoc 2015). Mildly oxidized LDL recruits monocytes which becomes macrophages that accelerate oxidation of LDL (Arun Kumar *et al.,* 2013). It has been suggested that plasma LDL must be trapped in some microenvironment with excess oxidants for the modification to occur (Ngoc 2015). The extent of this oxidation and inflammatory response are said to be under genetic control (Arun Kumar *et al.,* 2013). Repeated injury and repair within an atherosclerotic plaque leads to a fibrous cap protecting the underlying core of lipids, collagen, calcium, and inflammatory cells such as T lymphocytes. Maintenance of the fibrous plaque is thus critical for the prevention plaque rupture and subsequent coronary thrombosis (Arun Kumar *et al.,* 2013).

2.6.1.2 Corneal Arcus

Corneal arcus is a whitish, gray or yellowish deposit (1-5mm wide) around the circumference of the cornea known as corneoscleral limbus (Fernandez *et al.,* 2007). The variability of corneal arcus are referred to as; gerontoxon, arcus lipidalis, arcus lipoid cornea (Fernandez *et al.,* 2007).Corneal arcus is associated with high cholesterol in the blood (Amuamuta, 2014), it may occur without any predisposing factor(Fernandez *et al.,* 2007). Retinal vein occlusion is also a concern in individuals with elevated cholesterol. Such individuals are reported to have approximately 2.5-fold higher risk of retinal vein occlusion resulting in visual loss (Amuamuta, 2014).

2.6.1.3 Cholelithiasis

Cholelithiasis is common clinical problems both in Europe and North America with recent estimations indicating that 1% of adults living in these areas have cholelithiasis(Amuamuta 2014). The current concepts of the pathogenesis of cholesterol gallstones is the assumption that super saturation of cholesterol-solubilizing micellar system and consequent precipitation of cholesterol crystals are necessary for such calculi formation(Amuamuta 2014). Efforts have been directed towards determining the circumstances in which super saturation occurs and also ascertaining whether the super saturation is due to a deficiency of lecithin or bile acids, or both or to an excess of cholesterol. Bile acid pool has been found to be low in patients with cholesterol gallstones (Amuamuta 2014).

2.6.1.4 Renal Disease

Progressive renal diseases such as nephritic syndrome is accompanied by lipoprotein abnormalities and transport (i.e. increased VLDL, apo B and pre *β* HDL, low levels of apo A) and high cholesterol level(Trevisan *et al.,* 2006). Abnormal serum lipid levels may contribute to the progression of renal disease with reports showing that circulating lipids bind to and become trapped by extracellular matrix molecules where they undergo oxidation increasing the formation of reactive oxygen species such as superoxide anion and hydrogen peroxide. Hence glomerular cells mimic some characteristics of cells in the atherosclerotic vessel wall with resultant kidney disease (Trevisan *et al.,* 2006).

2.7 Treatment of Hyperlipidemia

2.7.1 Statins

Statins or HMG-CoA reductase inhibitors are majorly implored in the management of hyperlipidemia, especially in the treatment of elevated LDL cholesterol, Total and LDL cholesterol are reduced in a dose-related fashion by 30% or more (Arun Kumar*et al.,* 2013). This class of hypolipidemic drugs include; Lovastatin, Simvastatin, Pravastatin, Fluvastatin, Atorvastatin and Rosuvastatin(Arun Kumar *et al.,* 2013; Shattat 2014). More specifically, this family of agents is considered first line for the treatment of hypercholesterolemia in patients who have failed to respond to dietary therapy (Stein, 1998). Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, interrupting the conversion of HMG-CoA to mevalonate, the rate-limiting step in de novo cholesterol biosynthesis (Arun Kumar *et al.,* 2013). Statins are administered to reduce cardiovascular risk (Mattar and Obeid, 2009), but If the level of triglyceride is markedly elevated, fibrates may be recommended due to their beneficial effects (Luscher, 2011). Some adverse effects of statins include; elevated serum amino transferase levels, elevated creatine kinase levels, constipation and myopathy (Arun Kumar *et al.,* 2013).Other anti hyperlipidemic agents commonly added to statins include ezetimibe, niacin and bile acid sequestrants (Hou and Goldberg, 2009; Arun Kumar *et al.,* 2013). Recentclinical trials have revealed new potential hypolipidemic agents, they include; Acyl-CoA cholesterol acyl transferase inhibitors, Squalene synthase inhibitors, Microsomal triglyceride transfer protein inhibitors and Hydroxymethylglutaryl-CoA synthase inhibitors (Shattat, 2014).

2.7.2 Fibrates

Fibrates which include clofibrate, gemfibrozil, fenofibrate and bezafibrate are class of drugs that cause significant reduction in plasma triglycerides and moderate reduction in LDL cholesterol (Shattat, 2014). Fibrate as a monotherapy, is effective in lowering the level of VLDL in the blood, but may cause a reciprocal increase in the level of LDL. However, total cholesterol level may remain relatively unchanged, while plasma HDL concentrations may rise moderately(10% to 15%) with fibrates (Arun Kumar *et al.,* 2013). The mechanisms involves fibrates acting as ligands for nuclear transcription receptor; PPAR-α. They increase the expression of lipoprotein lipase, apolipoprotein AI and AIIand down-regulate apolipoprotein C-III which is an inhibitor of lipolysis (Shattat, 2014). Fibrates also enhance the production of fatty acid transport protein and acyl-CoA synthetase, which facilitate the uptake of fatty acid by the liver (Shattat, 2014). Fibrates have been reported to cause transient elevations in transaminase and alkaline phosphatase levels (Arun Kumar *et al.,* 2013). Combination treatment of statins and fibrates, though highly effective,have been reported to increase the risk of constipation, elevated creatine kinase levels (Arun Kumar *et al.,* 2013), myopathy and rhabdomyolysis and is hence only done under close supervision (Davidson *et al.,* 2007).

2.7.3 Niacin

Niacin is a water-soluble vitamin of type B which inhibits hormone-sensitive lipase and reduce the fractional clearance of apolipioprotein A-1 (Shattat, 2014). It is an agentused in the treatment of mixed hyperlipidemia, hypercholesterolemia, hypertriglyceridemiaor diabetic dyslipidemia. It reduces the hepatic synthesis of VLDL resulting in the reduction in LDL synthesis. Niacin also increases HDL by reducing its catabolism. Adverse effects of Niacin include; gastro intestinal intolerance elevated liver function test, hyperuricemia and hyperglycemia. It is contra indicated in liver diseases (Arun Kumar *et al.,* 2013).

2.7.4 Bile Acids Resins

Bile Acids resins bind bile acids in the intestinal lumen, with a concurrent interruption of enterohepatic circulation of bile acids. Theresults are decrease bile acid pool size withstimulation of hepatic synthesis of bile acids from cholesterol (Arun Kumar *et al.,* 2013). However,some adverse effect of Bile Acids resins is interruption in the absorption of Vitamins A, D, E and Kand the possible aggravation of hypertriglyceridemia in patients with combined hyperlipidemia (Shattat, 2014).

2.7.5 Ezetimibe

Ezetimibe is well tolerated with approximately 4% of patients experiencing gastrointestinal disturbance, it is used as an adjuvant therapy as it interferes with the absorption of cholesterol from the brush border of the intestine (Arun Kumar *et al.,* 2013). This effect is achieved through blocking of Niemann–Pick C1-like1 protein (NPC1L1), a human sterol transport protein (Shattat, 2014). Common adverse effects of ezetimibe include headache, abdominal pain, diarrhea and tendency to increase liver transaminases(Shattat, 2014).

2.7.6 Dietary supplementation

Fish oil is also used to reduce elevated triglycerides, with the greatest effect occurring in patients with greater severity of hyperlipidemia (Mattar and Obeid, 2009). Fish oil is known to contain three major types of omega-3 fatty acids: eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and alpha-linolenic acid (ALA). Omega-3 fatty acids reduce blood level of triglyceride but have no significant effect on both LDL and HDL cholesterol(Weintrub, 2014; Wu and Parhofer, 2014). However, complications such as thrombocytopenia and bleeding disorders have been recorded, especially with high doses (EPA, 15 to 30 g/day)(Arun Kumar *et al.,* 2013). Polyunsaturated fatty acids in diets helps in lowering the level of LDL cholesterol production and decrease artherosclerosis of coronary arteries as obtained from studies on African green monkeys (Siri-tarino *et al.,* 2010).Intake of diet rich in plant sterols 2 to 3 g daily lowers cholesterol and triglyceride (Arun Kumar *et al.,* 2013).Combining soya foods with prebiotic drinks is reported to increase the colonic fermentation which results in hypocholesterolemic effects of soya (Mannu *et al.*, 2013).

2.7.7 Exercise

Studies have shown that moderate exercise combined with diet interventions decreased the level of TC and LDL by 7 to 18% and 7 to 15%, respectively while TG had 4 to 18% reduction(Varady and Jones, 2005).Gender differences determine the impact ofexercise on cholesterol levels, however,the cause of these differences and underlying mechanism exercise induced decrease in cholesterol is not completely understood (Mannu *et al.*, 2013).The level of HDL is higher in men who exercise and dieted than in women. Exercise attenuates hepatic lipase activity and stimulates lipoprotein lipase (Mannu *et al.*, 2013). The Peak LDL particle diameter increases with exercise and correlates with changesin lipid composition which correlate with age (Mannu *et al.*, 2013).

2.8 Induction of Hyperlipidemia

Hyperlipidemia has been chemically induced using agents such as polychlorinated biphenyls, nicotine, isotretinoin, tumor necrosis factor, copper, estrogen, pertussis toxin, histamine receptor agonists, carbon disulfide in combination with ethanol, Triton WR 1339 or using transgenic models . However, many of these compounds and models are reported to have their limitations. Triton WR 1339 (a polyoxyethylene ether of alkyl phenol) was useful in inducing hyperlipidemia in experimental animals. However, it has a major limitation which is hemolysis from chronic administration. All of these agents had been tested in an attempt to establish a practical animal model for hyperlipidemia and coronary artery diseases (Johnston, 2004).

2.8.1 Poloxamer

Poloxamer are pharmaceutical agents introduced in the 50's as a non-ionic triblock copolymer due to its thermogelling properties. Poloxamer also known as polyethylene-propylene glycol copolymer is non-irritating and non-toxic. Chemically poloxamer is α-Hydro-ω-hydroxypoly (oxyethylene) a poly (oxypropylene)bpoly (oxyethylene) block copolymer with two hydrophilic chains of ethylene oxide chains that sandwiched one hydrophobic propylene oxide chain (Ramya Devi *et al*., 2013). The chemical formular is HO(C2H4O)a(C3H6O)b(C2H4O)aH, where a and b have the values ranging from 10-146. The

varying length of polymer blocks give rise to different polymers which include 124, 188, 237, 338 and 407 with slight difference in their individual properties (Ramya Devi *et al*., 2013). It is commonly represented with the letter 'P' and succeeded by three digits. The first two digits are to be multiplied by 300 which results in the molecular mass of the hydrophobic propylene oxide while the last digit is to be multiplied by ten to determine the content of hydrophilic ethylene oxide in percentage. The common trade name used is Pluronic and the usual copolymer representation denotes its physical appearance (L-Liquid, P-Paste and F-Flake) (Ramya Devi *et al*., 2013).

2.8.2 Poloxamer 407 Induced Hyperlipidemic Models

Poloxamer 407 (P407) is a ubiquitous manmade surfactant and non-ionic detergent with a molecular weight of 12,600. P407 is a liquid at room temperature, assembles into micelles at body temperature, then aggregate into a gel. These temperature-dependent micellization and gelation properties have led to its widespread use in mouthwashes, deodorants, and skin care products and also as an excipient in a variety of pharmaceutical preparations (Dumortier *et al.,* 2006). P407 has a major hyperlipidemic effect as observed in experimental animals (Wout *et al.,* 1992; Johnston and Palmer 1993; Palmer *et al.,* 1998; Johnston 2004).). P-407 has been utilized as a model to induce hyperlipidemia in rodents, due to its convenience, reproducibility, low cost and the lack of undesirable underlying pathological conditions (Hor *et al.,* 2011). The advantage with the p407 model is the production of hyperlipidemia of the hereditary type with reliable phenotypic features associated with the pathogenesis of human atherosclerosis. A high level of circulating lipoprotein cholesterol in the P-407 model partially reproduces familial hypercholesterolemia and familial combined hyperlipidemia (Korolenko *et al.,* 2016). A single intra peritoneal injection of poloxamer-407 between 0.5 g/kg and 1g/kg elevates serum cholesterol and triglyceride in by an order of magnitude or more within 1 to 4 days after. Plasma cholesterol and triglycerides concentrations also return to control levels by 96 to 120 hours following a single injection of P-407 (Wout *et al.,* 1992; Johnston, 2004). This effect is secondary to the inhibition of lipases (hepatic and lipoprotein) and activation of 3-hydroxy-3- methylglutaryl-CoA (HMG-CoA) reductase, lecithin cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP) (Johnston and Palmer 1993; Johnston 2004). Repeated administration of P407 is also associated with the development of early and late onsets of atherosclerotic lesions in rodents (Palmer, *et al.,* 1998; Korolenko *et al.,* 2013). Following parenteral administration, P407 is mostly taken up by the liver (Li *et al.,* 1996). Cogger *et al.* (2006) and Korolenko *et al.* (2013) have both reported changes in the structure of the liver sinusoidal endothelial cells (LSECs) and cytolysis of hepatocytes respectively.

2.9 Camel Milk

The camel is among the animals mentioned in the Quran as a miracle of God as stated in Chapter 88 vs 17. Camel milk has supported bedouin, nomad and pastoral cultures since the domestication of camel started. Herders may for periods survive solely on the milk long distances travel in desert and arid environments(Chakrapany and Chandan, 2014). The Food and Agriculture Organization (FAO) estimation of the total world population of camels to be 22 million as at 2014, 89% of which are one-humped

dromedary camels (*Camelus dromedarius*) and the remaining 11% are the two-humped bactrian camel (*Camelus bactrianus*). The bacterian camels are generally found in the cold deserts of Asia (Chakrapany and Chandan, 2014). Based on historical records, domestication of camels is dated back to 3000years and first occurred in the ArabianPeninsula. However, majority of the world's camel population today is within Africa. The highest concentration of is located in the North East Africa (63%). Kenya precedes Somalia, Sudan, Ethiopia and Mauritania (Chakrapany and Chandan, 2014). Camel is not only an important economic means rural and urban transport, but also serves as a source of milk in some of areas of camel rearing societies (Abbas *et al.,* 2013). Average milk yield of camel ranges 3.5 to 35.0 liter per animal per day with an average lactation yield of 4575 to 20675 liter. The major portion of this milk is utilized in feeding their young ones while the rest of the milk is consumed freshly or in combination with other additives (Khaskheli*et al.,* 2005). The Bedouins of the Middle East believe in its curative powers (Chakrapany and Chandan, 2014), it is also reputed to have anti-infectious, anti-cancerous and anti-diabetic properties (Gizachew *et al.,* 2014). Camel milk helps to treat infectious diseases such as tuberculosis in humans as it was administered on tuberculosis patients in sanitoria in the ancient Russia. In countries such as Kazakhstan, camel milk is used in as an adjunct to chemotherapy for some forms of cancers (Guakhar and Bernard, 2004).

2.9.1 Composition of Camel Milk

Camel milk is opaque white with normal odor; this is due to the presence of fats that are finely homogenized throughout the milk (Yadav *et al.,* 2015). It has a sweet and sharp taste;however, it can be salty sometimes. This generally depends on the type of feed and availability of drinking water. The pH of camel's milk ranges from 6.5 to 6.75 and the density from 1.026 to 1.035. The density and pH of camel milk are lower than those of cow milk. Camel milk also sours very slowly when compared to cow milk and can be kept longer without refrigeration (Jilo and Tegegne, 2016). Camel milk is unique from other ruminant milk in terms of its composition and functionality; it contains high concentration of immune globulins, insulin (Gizachew *et al.,* 2014), vitamins (A, B-2, C and E), sodium, potassium, iron, copper, zinc and magnesium (Al Hashem, 2009) calcium, iron, (Yadav *et al*., 2015) and manganese (Jilo and Tegegne, 2016). The mean values for zinc,
manganese, magnesium, iron, sodium, potassium and calcium in mineral contents in the milk of one humped camel ($100g^{-1}$) are 0.53, 0.05, 10.5, 0.29, 59, 156 and 114 mg respectively(Jilo and Tegegne, 2016).Vitamins present in camel milk have been reported to have antioxidant and hypolipidemic properties (Al Humaid, 2010; Badr *et al.,* 2011). In addition to antioxidant property,vitamin C and important electrolytes like calcium and iron are required for calcium absorption in cases of osteoporosis of which they aresupplied by camel milk due to its rapid absorption in the gastrointestinal tract (Jilo and Tegegne, 2016).Vitamin E represents one of the most fascinating natural resources that have potential influence on wide range of mechanisms predisposing humans to health and disease challenges (Catalgol and Ozer, 2011). It is the principal lipid soluble chain-breaking antioxidant in mitochondria, microsomes, and lipoproteins (Maheswari *et al.*, 2015).The trace elements in camel milk vary as a resultof environmental conditions such as feed and soil. Different breeds of camels have different capacities to deposit minerals in their milk (Wangoh *et al*., 1998).Also genetic and health status of the camel impact on the distribution of these trace elements in the milk (Jilo and Tegegne, 2016). In general the average

amount of components of camel milk is protein 3.4%; fat 3.5%; lactose 4.4%; ash 0.79%, while water covers 87% (Jilo and Tegegne, 2016)

Camel milk contains various proteins impacting on its nutritional value and technological suitability. Milk proteins are a heterogeneous group of compounds that differ in composition and properties (Gizachew *et al.,* 2014). Camel milk has more proteins and whey protein content than cow milk.Casein is the principal protein component in most of the mammalian milk. One humped camel has 1.63 to 2.76 percent casein protein which constitutes 52 to 87 percent of total milk protein (Khaskheli *et al*., 2005).There are four main fractions of casein that have been isolated: αs1-, αs2-, β-, and κ, they have diverse proportion and polymorphism demonstrated in most of the animal species (Barłowska, 2007). Casein from human milk does not contain the αs1-fraction which is the predominant factor causing milk protein allergies (Gizachew *et al.,* 2014). Whey protein is the second biggest fraction of protein of camel milk which covers 20 to 25 percent of camel milk protein. The milk of dromedary camel has a whey protein in range of 0.63 and 0.80 percent(Jilo and Tegegne, 2016).Camel milk's whey protein lacks beta lactoglobulin which is the main serum protein present in milk of other ruminants. Some other whey proteins which have been recognized in camel milk are α lactalbumin, serum albumin, peptidoglycan recognition protein and lactophorin (Abbas *et al.,* 2014). The high level of lysozyme in camel milk is of importance for the storage of milk and inhibition of growth of pathogenic bacteria (Mullaicharam 2014). Besides casein, camel milk also contains lactoferrin and lactoperoxidase(Agrawal *et al*., 2007; Mullaicharam 2014).

Triglycerides of camel milk are accompanied with small amounts of di-and mono-acylglycerols, cholesterols, free fatty acids and phospholipids (Mullaicharam 2014). The milk fat consists mainly of long chained polyunsaturated fatty acid(Mullaicharam 2014) Fat is the major substance that defines milk's energetic value and nutritional properties. Milk fat globules have an average diameter of less than 0.1 μm to approximately
18 μm (El-Zeini, 2006) and consist of a triglyceride core surrounded by a natural biological membrane. The milk fat globules membrane (MFGM) contains
the typical components of any biological membrane such as cholesterol, enzymes, glycoproteins, and glycolipids (Fauquant *et al*., 2007).

2.9.2 Therapeutic Effects of Camel Milk

2.9.2.1 Hypoglycemic Effect

Insulin in camel milk has been reported to possess special properties that make absorption into circulation easier than insulin from other sources or cause resistance to proteolysis. Camel milk insulin is encapsulated in nano particles (lipid vesicles) that make its absorption through the gastrointestinal tract and entry into circulation possible. While some other elements of camel milk make it anti-diabetic, sequence of its insulin and predicted digestion pattern do not suggest differentiability to overcome the mucosal barriers before been degraded and reaching the blood stream (Ajamaluddin *et al.,* 2012; Mullaicharam 2014).

Agrawel *et al.*(2002) studied thea comparison between conventionally treated juvenile diabetes and those drinking camel milk, the results showed that the group drinking camel milk had significantly lowered blood glucoseand Hb levels when compared to the conventionally treated group.

Al Numair (2010) who tested camel milk on type II diabetes rats, concluded that the alterations in plasma insulin, plasma glucose level and lipid profiles during the experimental diabetes was restored to near normal levels by camel milk treatment. He proposed that hypoglycemic and hypolipidemic effect produced by the camel milk may be due to the presence of insulin like protein and high amount of zinc. Zinc is fundamental for living organisms as many enzymes require it for their proper function. It plays a vital in DNA replication, transcription, and protein synthesis, affecting cell division and differentiation(Ibrahim *et al.,* 2017). Zn can also prevent cell damage through activation of the antioxidant systemand non-enzymatic stabilization of biomembrane and biostructures (Dawud *et al.,* 2012).

Sboui *et al.* (2010) conducted a research on the effect of camel milk on blood glucose, cholesterol and total proteins variations in alloxan-induced diabetic dogs. The result showed that there was a significant decrease in blood glucose levels on dogs treated with camel milk. This decrease was statistically illustrated at week 3 by an improvement on glycemic control and a normal range of blood glucose levels. The stability of blood glucose levels was associated to a decline of total proteins and cholesterol concentrations and a steadiness of this state since week 3 and after stopping to drink camel milk.

Abd el Aziz *et al.,*(2012) studied the effect of female camel milk on glycemic control of streptozotocin induced diabetes mellitus in rats. The results showed that camel milkdecreased blood glucose, HbA1C, total cholesterol, triglyceride, low density lipoproteins, very low density lipoprotein and atherogenic index of plasma, creatinine, urea and acetoacetic acid, ketone. Camel milk also increased the level insulin, high density lipoproteins (HDL), pH, bicarbonate and final body weight compared to the disease control.

Isa *et al.* (2013) who also carried out a study on the effect of camel milk supplementation on serum glucose levels, lipid profile and body weight of Alloxan induced diabetic rats reported that there was a significant decrease in total cholesterol, triglyceride, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and a significant increase in high density lipoprotein cholesterol was observed in diabetic rats treated with camel milk group when compared to the diabetic untreated group, also reported was significant change in body weights.

2.9.2.2 Anti-OxidantEffect

Al Hashem (2009) studied the effect of camel milk on oxidative stress biomarkers (MDA, SOD, CAT and GSH) in AluminumChloride ($AlCl_3$) intoxicated rats, the results of his findings revealed that camel milk decreased lipid peroxidation and increased antioxidant enzymes (SOD, CAT and GSH). Such antioxidant property was also confirmed by El Said *et al.,* (2010) in diabetic Rabbits. Al Fartosi *et al.,* (2012) also conducted a research on the role of camel milk against some oxidant-antioxidant markers of male rats treated with carbon tetrachloride (CCl4), the data obtained showed that the rats treated with camel milk had significant decrease in serum MDA along with increased serum albumin and ceruloplasmin. Al

Ayadhi and El Amin (2013) evaluated the effect of camel milk consumption on oxidative stress biomarkers in autistic children, by measuring the plasma levels of glutathione, superoxide dismutase, and myeloperoxidase before and 2 weeks after camel milk consumption, using the ELISA technique. All measured parameters exhibited significant increase after camel milk consumption. They concluded that camel milk could play an important role in decreasing oxidative stress by alteration of antioxidant enzymes and non-enzymatic antioxidant molecules levels, as well as the improvement of autistic behavior as demonstrated by the improved Childhood Autism Rating Scale (CARS).

2.9.2.3 Hepatoprotective Effect

Camel milk was shown to improve liver enzymes, total protein, albumin, globulin, lactate dehydrogenase, urea and creatinine level of rats treated with Aluminum Chloride (Al Hashem, 2009). A research conducted to evaluate the effect of camel milk supplementation on blood parameters and liver function of hepatitis patients demonstrated that camel milk improves total protein, albumin, platelets and lymphocytes counts in the patients. On the other hand, camel milk also has the ability to reduce the elevated level of bilirubin, globulin and granulocytes (Abbas *et al.,* 2014)

2.9.2.4 Other medicinal uses of camel milk

Raw camel milk as well as its fermented goods is used to manage constipation, diarrhea, stomach ulcers, and wounds. It is also employed to improve ovulation of female ovaries. Camel milk is full of evenly balanced in terms of nutritional constituents with good a influence in growth and development of particular body organs, metabolic responses towards nutrients absorption, digestion and fight against diseases (Gizachew *et al.,* 2014).


Plate I: A brown one humped camel from Kaura Namoda, Zamfara state (Snapped by the candidate, April 2017).



Plate II: A bottle of raw camel milk collected from one humped camel at Kaura Namoda, Zamfara State(Snapped by the candidate, April 2017).

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials

Weighing Balance (Salter, Homedics Group Ltd, Production No. IB-1066-1011-03).
Atorvastatin Calcium Tablet 20mg (Strovas Tablet 20mg/kg, Ranbaxy Laboratory Ltd, Paonta Sahib Distribution, Sirmour. H.P. – 173025 India, NAFDAC Reg No: MNB/95/2).
Poloxamer 407 ((Lutrol F127; BASF, Ludwigshafen, Germany, Sigma Aldrich Company).
Disposable syringes (2ml).
Oral cannular.
Cotton wool.
Camel milk (3 litre).
Dissection Kits and Dissection Board.
Distilled water.
Heparinized Capillary Tubes (Alere EDTA Capillary Tubes).
Plain Test tubes (10 packs).
Mortar and Piston.
Formalin
Chloroform
Surgical gloves

3.2 Experimental Design

3.2.1 Experimental Animals

Forty two (42)apparently healthy male Wistar rats weighing 150-200gm were obtained from the animal house of Faculty of Pharmaceutical Sciences of Ahmadu Bello University Zaria, all the rats were kept in the same animal house. Housing was in well-ventilated plastic cages (5 rats per cage) with normal photoperiod. All the animals were allowed access to water *ad libitum*. They were also fed the same type of food elements (vital feeds) during the experiment. The rats were allowed to acclimatize under laboratory conditions for two weeks before commencement of the experiment.

3.2.2 Camel Milk Collection

Milk collection was done every day from camel herds *(Camelus dromedarius)* in Kaura Namoda Farms, Zamfara state. The milk was stored in screwed bottles under ice. The milk was transferred and refrigerated at 3-5ºC (37-41ºF) in the Department of Pharmacology Laboratory Ahmadu Bello University Zaria.

3.2.3 Proximate Analysis of Camel Milk

Proximate analysis of the milk was carried out at National Animal Production Research Institute (NAPRI) Zaria and NARICT before commencement of administration. PH of the milk was carried out at

Department of Pharmaceutical Chemistry, Ahmadu Bello University Zaria. Determination of all constituents was based on the guidelines outlined by the Association of Analytical Chemist (AOAC), (2005).

3.2.4 Acute Toxicity Study of Camel Milk

A total of twelve rats were used to calculate the Median Lethal Dose (LD_{50}). The $LD_{50}$of camel milk determination was conducted using the method of Lorke (1983) with slight modification. This study was conducted in 2 stages. In the first phase, rats were divided into 3 groups with 3 rats in each. The animals were treated with camel milk as follows; 0.01ml/kg,0.1ml/kg and1ml/kg orally. In the second phase, rats were grouped into three, each group were treated at 1.6ml/kg, 2.9ml/kg and 5.0ml/kg respectively. The administration was done orally. Clinical signs of toxicity in each group of rats were observed for 24hrs after treatment and recorded. The final LD_{50} was calculated.

3.2.4.1 Determinationof Camel Milk concentration

A beaker was placed on the gallen metter balance.10mls of the milk was equivalent 10 g after weighing. Hence, 1ml of camel milk is equivalent to 1g.

3.2.5 Induction of Hyperlipidemia

Poloxamer 407 (Lutrol F127; BASF, Ludwigshafen, Germany) was used to induce hyperlipidemia. Administration of Poloxamer 407 was at a dose of 500mg/kg intra peritoneally twice a week for 21 days (Woo *et al.,* 2010; Korolenko *et al.,* 2013; Victor *et al.,* 2014). Prior to the administration, Poloxamer 407 was dissolved in distilled water and refrigerated overnight to facilitate its dissolution (Johnston and Palmer, 1993). Needles and syringes used for administration were cooled to prevent gelation within the syringe during injection as described by (Johnston and Palmer, 1993). Levels of total cholesterol and triglyceride concentrations above 200mg/dl and 160mg/dl respectively were considered hyperlipidemic (Delvin, 2006)

3.2.6 Preparation of Standard Drug

Atorvastatin calcium was purchased in a tablet form at strength 20 mg. Tablets were dissolved in distilled water and administered orally once daily.

3.2.7 Grouping of Animals

Animals were divided into six groups with five in each group as follows:

Group 1: Normal control orally administered distilled water 1 ml/kg/dayfor 21days.

Group II: Hyperlipidemic negative control.

Group III: Hyperlipidemic positive control treated with Atorvastatin tablet (AT) orally at 20mg/kg/day for 21 days.

Groups IV: Hyperlipidemic group treated with camel milk at a dose of 0.25ml/kg/dayorally for 21 days.

Group V: Hyperlipidemic group treated with camel milk at a dose of 0.50ml/kg/day orally for 21 days.

Group VI: Hyperlipidemic group treated with camel milk at a dose of 1ml/kg/day orally for 21 days.

3.2.8 Collection of Blood samples

At the end of the 21 days experimental period, the animals were fasted overnight.All the animals were anaesthetized under chloroform vapor in an anesthetic box with lid cover Blood samples were collected via cardiac puncture into anticoagulant free tubes and allowed to coagulate. The samples werecentrifuged at a speed of3000 g for 15 minutes.The resultant serum was harvested into plain sample bottles for biochemical analysis.

3.3 Biochemical Assays

3.3.1 Estimation of Serum lipid profile markers

Lipid profiles were determined using Agappe Diagnostics Reagents (Agappe Diagnostics, Switzerland, GmbH). The procedures are according to manufacturer's manual and explained below;

3.3.1.1 Determination of Serum Total cholesterol (TC)

Serum TC was determined by the method of Allain(1974) using Agappe diagnostics cholesterol reagent.

Reagent Composition

Cholesterol R1	
Pipes Buffer (PH 6.9)	50mmol/L
Phenol	24mmol/L
Sodium Cholate	0.5mmol/L
Choleterol R2	
Cholesterol Esterase	>200U/L
Cholesterol Oxidase	>250U/L
Peroxidase	>1000U/L
4-Aminoantipyrine	0.5mmol/L

Principle

The principle is based on enzymatic colorimetric determination of total cholesterol according to the following reactions:

Cholesterol ester + H_20 $\xrightarrow{\text{cholesterol esterase}}$ Cholesterol + Fatty Acids

Cholesterol ester + 0_2 $\xrightarrow{\text{cholesterol esterase}}$ Cholesterol + 4-Cholesten-3-one + H_20_2

$2H_20_2$ + Phenol + 4 Aminoantipyrine $\xrightarrow{\text{Peroxidase}}$ Red Quinone + $4H_2O$

Procedure

Firstly, Cholesterol reagent 1 (R1) was mixed with reagent 2 (R2) as indicated in the vial label to for a working reagent. 1000µL of theworking reagent was added to each of the sample and standard. This was incubated for 5minutes at 37ºC after mixing and the absorbance of the sample (A_{sample}) and ($A_{standard}$) were measured against the reagent blank within 30 minutes at 630nm. The value of TC present in serum was expressed in mg/dL.

TC Concentration (mg/dL) = A *sample* / A *standard x* 200.

3.3.1.2 Determination of Serum Triglycerides Level (TG)

Serum TG was determined method of Jacobs and Van demark (1960)using Agappe diagnostics triglyceride reagent.

Reagent Composition

Pipes Buffer (PH 7.0)	5mmol/L
TOPS	5.3mmol/L
Potassium Ferrocyanate	10mmol/L
Magnesium salt	17mmol/L
4-Aminoantipyrine	0.5mmol/L
ATP	3.15mmol/L
Lipoprotein Lipase	>1800U/L
Glycerol Kinase	>450U/L
Glycerol-3-Phosphate Oxidase	>3500U/L
Peroxidase	>450U/L

Princple

The principle is based on enzymatic colorimetric determination of triglyceride according to the following reactions:

$TG + H_20 \xrightarrow{\text{Lipoprotein Lipase}}$ Glycerol + Fatty Acid

Glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol-3-Phosphate + ADP

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

$4H_2O_2 +$ 4- Aminoantipyrine + TOPS $\xrightarrow{\text{Peroxidase}}$ Violet Colored Complex

Procedure

1000µL of the reagent was added to each of the sample and standard. This was incubated for 5minutes at 37ºC after mixing and the absorbance of the sample (A_{sample}) and ($A_{standard}$) were measured against the reagent blank within 30 minutes at 546nm. The value of TG present in serum was expressed in mg/dL.

TG Concentration = A *sample* / A *standard x* 200 mg/dL.

3.3.1.3 Determination of Serum High Density Lipoprotein (HDL)

Determination of Serum High Density Lipoprotein Cholesterol (HDL) was estimated by the method of Assmann (1979) using Agappe diagnostics HDL reagent.

Reagent

HDL Cholesterol R1

Phospotungstate	14mmol/L
Magnesium Chloride	1mmol/L

Preservative

Principle

Low Density Lipoproteins (LDL) and Very Low Density Lipoproteins (VLDL) and chylomicrons fractions were precipitated quantitatively by addition of phosphaturic acid in the presence of magnesium ions*.* After centrifugation of the supernatant, HDL is in the supernatant

Procedure

300µL of the reagent was added to each of the sample and standard. The mixture was allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes at 4000rpm. The supernatant represents the HDL fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant,was determined. To determine the level of HDL Cholesterol, 1000µL of the reagent was added to each of the sample and standard. This was incubated for 5minutes at 37ºC after mixing and the absorbance of the sample (A_{sample}) and ($A_{standard}$) were measured against the reagent blank within 30 minutes at 630nm. The value of HDL present in serum was expressed in mg/dL.

HDL Cholesterol Concentration (mg/dL) = A *sample* / A *standard* xN x 2.

Where, 2= Dilution Factor of the sample

N= Standard Concentration (50mg/dL)

3.3.1.4 Determination of Serum Low Density Lipoproteins cholesterol (LDL-C)

Serum LDL level was calculated according to the formula developed by Friedewald *et al.* (1972) as follows:

Serum LDL-C = TC– (HDL-C + TG/5).

3.3.2Determination of Oxidative Stress Biomarkers

3.3.2.1 Evaluation of Malondialdehyde (MDA)

Thiobarbituric acids (TBA) content, a measure of lipid peroxidation, was assayed in the form of Thiobarbituric Acid Reacting Substances (TBARS) according to Fraga *et al.* (1988).

Principle

The formation of Malondealdehyde (MDA) is the basis for the well-known Thiobarbituric acids (TBA) method used for evaluating the extent of lipid peroxidation. A low pH of 2-3 and a high temperature (100ºC), malondialehyde binds Thiobarbituric acids to form a pink complex which absorbs maximally at 532nm.

Procedure

0.5ml of the sample, 0.5ml of normal saline and 1.0ml of 10% TCA were mixed together. 0.25ml of 0.1M of Thiobarbituric acids was also added to the mixture and incubated for 95ºC, cooled and centrifuged at 300rpm for 20minutes. The absorbance of the pink color produced was read at 535nm

3.3.2.2 Evaluation of Glutathione Peroxidase (GPx)

Glutathione peroxidase was measured using by the method of Rotruck *et al.* (1973).The reaction was made up of 0.2ml of 0.4M Phosphate Buffer at pH 7.0, 10mM of Sodium azide,).2ml serum, 0.2ml reduced Gluthathione and 0.1ml hydrogen Peroxide. The content was then incubated for 10 to 15mins at 37º. After incubation,0.4 10% TCA was added and centrifuged at 3200 g for 20mins.he spernatant was assayed for Gluthathione content using Ethman's reagent(19.8mg 5.5-thiobunitrobenzoic acid[DTNB] in 100ml 0.1% sodium Nitrate

3.3.2.3 Determination of Superoxide Dismutase Activity (SOD)

Superoxide dismutase was examined using the described by Martin *et al.* (1987). It is based on the principle of superoxide inhibition of autoxidation of hematoxylin at pH 7.8 (with increase of absorbance oat 560nm); the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range. SOD activity in the sample will be determined by measuring the amount of haematin at 560nm

Haematoxylin $\xrightarrow{\text{Autoxidatiion}}$ H_2O Haematin (active form of haematoxylin)

Procedure

To 920 µL of phosphate buffer (0.05M, pH 7.8) was added to 40 µL of sample. A reagent test was prepared by replacing the sample with 40 µL of sample dilution buffer (0.85 %NaCl). This mixture was incubated for 2 minutes at 25 ºC before addition of 40 µL of hematoxylin. After addition of 40 µL of hematoxylin, absorbance of the sample test and reagent test was read at 560nm immediately and after 5 minutes against the sample blank which is distilled water.

3.3.2.4 Determination of Catalase Activity (CAT)

Catalase enzyme activity was measured based on the method of Aebi (1984). Catalase scavenges hydrogen peroxide converting it to water and molecular oxygen. The activity of catalase in the ample was determined by following the the rate of decrease in absorbance at 240nm.

$2H_2O_2 \xrightarrow{Catalase} 2H_2O_2 + O_2$

Procedure

10 µL of sample serum was added to 2.8ml of 50Mm potassium phosphate buffer (pH 7.0) in 3ml cuvette. The reaction was initiated by addition of 0.1ml of fresh 30Mm H_2O_2 and the decomposition rate of H_2O_2 was measured at 240nm for 5minutes on a spectrophotometer. A molarextinction coefficient of $0.041mM^{-1}c^{-1}$ was used to measure catalase activity which was expressed an nmole H_2O_2 decreased/min/mgof protein.

3.3.3Estimation of Serum Proteins

3.3.3.1 Estimation of Total Protein

Total protein was measured by biuret reaction (copper salt in an alkaline medium), as described by Gomall*et al.*(1949), using Agappe diagnostics total protein reagent (Agappe Diagnostics Switzerland, GmbH).

eagent Composition

Potassium Iodide	6mmol/L
Potassium Sodium Tartarate	21mmol/L
Copper Sulphate	6mmol/L
Sodium Hydroxide	58mmol/L

Principle

The principle involves protein in serum sample forming a blue colored complex when treated with cupric ions in alkaline solution.

Procedure

1000µL of the reagent was added to each of the sample and standard. This was incubated for 10minutes at 37ºC after mixing and the absorbance of the sample (A_{sample}) and ($A_{standard}$) were measured against the reagent blank within 30 minutes at 546nm. The value of C present in serum was expressed in g/dL.

Total Protein (TP) Concentration (g/dL) = A *sample* / A *standard x* 6.

3.3.3.2 Estimation of Plasma Albumin

Albumin was measured by colorimetric estimation as described by Doumas*et al.* (1971) using the Agappe diagnostics albumin reagent (Agappe Diagnostics, Switzerland, GmbH) which contained bromocresol green (BCG).

Reagent Composition

Succinate Buffer (PH 4.20)	75mmol/L
Bromocresol green	0.1g/L

Principle

The reaction between albumin from serum and the dye bromocresol green produces changes in color that is proportional to albumin concentration.

Procedure

1000µL of the reagent was added to each of the sample and standard. This was incubated for 1 minute at room temperature after mixing and the absorbance of the sample (A_{sample}) and ($A_{standard}$) were measured against the reagent blank within 30 minutes at 630nm. The value of albumin present in serum was expressed in g/dL.

Albumin (Alb) Concentration (g/dL) = A *sample* / A *standard x* 3.

3.3.3.3 Estimation of Plasma Globulin (Glb)

Globulin was measured from the difference between total protein and albumin (Olorunnisola *et al.,* 2012).

3.3.4Determination of Liver Enzymes

3.3.4.1 Determination of serum Alkaline Phosphatase (ALP) activity

The ALP activity was determined by a standard method based on German Society of Clinical Chemistry and Scandinavian Society of Clinical Chemistry Recommendations using Biolabo ALP reagent (Biolabo SA, 02160,Maizy, France).

Reagent Composition

Vial 1(Buffer): 1 mol/L of Diethanolamine Buffer (DEA) pH 10, 0.5mol/L magnesium Chloride

Vial 2(Substrate): 10mol/L of P-Nitrophenyl Phospate

Principle

Alkaline phosphate catalyses the hydrolysis of P-Nitrophenyl phospate into P-Nitrophenol and Phospate in alkaline solution,. The rate of formation of P-Nitrophenol proportional to the ALP activity is measured at 405nm.

Procedure

The content of Vial 1(Buffer) was mixed gently with Vial 2(Substrate)for about two minutes for complete resolution. At room temperature 1ml of the reagent was pipette into a thermostated cuvette, 10µL of the serum was added to the reagent and thoroughly mixed at 37ºC.Initial absorbance after one minute was recorded. Successive absorbance was recorded every minute during 3minutes duration. The average of the three values obtained was then computed and used to calculate the ALP activity using the formula provided by the manufacturer as shown below:

ALP (U/L) = 5450 × ΔA at 405nm/min

Where ΔA = Changes in absorbance

ALP Activity = $\frac{\Delta Abs/min\ assay}{\Delta Abs/min\ Calibrator}$ x Calibrator Concentration

3.3.4.2 Determination of Alanine Aminotransferase (ALT)

Alanine Aminotransferase was measured based on the method described by Reitman and Frankel (1957) using ALT assay kits of Randox Laboratories Limited United Kingdom. Assay was according to manufacturer's manual.

Reagent Composition

R1 (Buffer)

Phosphate Buffer (PH 7.4)	100mmol/L
L-Alanine	200mmol/L
α-oxoglutarate	2.00mmol/L

R2

2,4-Dinitrophenyl Hydrazine	2.0mmol/L

Principle

ALT is measured by monitoring the concentration of Pyruvate Hydrazone formed with 2, 4-dinitrophenylhydrazine.

Procedure

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

The value of ALT present in serum is expressed in U/L.

Alanine Aminotransferase (ALT) Concentration U/L) = A *sample* / A *standard*.

3.3.4.3 Determination of Aspartate Aminotransferase (AST)

Aspartate Aminotransferase was measured based on the method described by Reitman and Frankel (1957) using AST assay kits of Randox Laboratories Limited United Kingdom. Assay was according to manufacturer's manual.

Reagent Composition

R1 (Buffer)

Phosphate Buffer (PH 7.4)	100mmol/L
L-Aspartate	100mmol/L
α -oxoglutarate	2.00mmol/L

R2

2, 4-Dinitrophenyl Hydrazine	2.0mmol/L

Principle

ALT is measured by monitoring the concentration of Oxaloacetate Hydrazone formed with 2, 4-dinitrophenylhydrazine.

Procedure

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

The value of albumin present in serum was expressed I U/L.

Aspartate Aminotransferase (AST) Concentration (U/L) = A *sample* / A *standard*.

3.3.5 Determination of Serum Electrolytes

3.3.5.1 Determination of Sodium ion (Na^+)

Na^+ was estimated using Agappe diagnostics Na^+reagent (Agappe Diagnostics Switzerland, GmbH). Na^+ was estimated by colorimetric method based on the method of Trinder (1951).

Principle

Sodium and proteins are precipitated together by magnesium uranyl acetate as Uranyl magnesium sodium acetate salt. Excess of uranyl salt react with potassium ferrocyanide to produce brownish colour which is inversely proportional to sodium concentration. It is measured photometrically at 530nm.

Reagent Composition

Sodium (Precipitating Reagent) R1

Uranyl Acetate	19mmol/L
Magnesium Acetate	140mmol/L

Sodium (Color Reagent) R2

Ammonium Thioglycolate	550mmol/L

Ammonia 550mmol/L

Procedure

1000µL of precipitating reagent was added to each of the sample and standard. This was incubated for 5minutes at room temperature after mixing vigorously and centrifuged at3000 g for 2 minutes to obtain a clear supernatant. The supernatants were transferred into another test tubes and 1000µL of coloring reagent was added to each of the sample and standard. Then it was mixed and allowed to stand for 5 minutes at room temperature. The absorbance of the sample (A_{sample}) and ($A_{standard}$) were measured against the reagent blank within 30 minutes at 546nm. The value of Na^+ present in serum is expressed in mmol/L.

Na^+ Concentration = A (*blank)* – A (*sample*) / A (*blank*) – A (*standard*) *x* Standard Concentration.

3.3.5.2 Determination of Potassium ion (K^+)

Potassium ionwas estimated by turbidometric method as described by Henry *et al.*(1974)using Agappe diagnostics K^+reagent (Agappe Diagnostics Switzerland, GmbH).

Reagent Composition

Potassium R1

Sodium Tetraphenylboron 0.2mmol/L

Principle

The principle is based on the extent of turbidity being proportional to the K^+ concentration. It is measured photometrically at578nm.

Procedure

1000µL of the reagent was added to each of the sample and standard. It was incubated at room temperature for 5minutes after mixing. The absorbance of the standard and the test sample were measured against distilled water at 578nm for 10minutes. K^+ present in serum was expressed in mmol/L

K^+ Concentration = A (*sample*) / A (*standard*) *x* Standard Concentration.

3.3.5.3 Determination of Chloride ion

Chloride was measured by the method of Schonfield and Lowellen (1964) using Agappe diagnostics chloride reagent (Agappe Diagnostics Switzerland, GmbH).

Reagent Composition

Mercuric Chloride	2mmol/L
Nitric Acid	29mmol/L
Ferric Nitrate	20mmol/L

Principle

In an acid medium, chloride ions and mercury-II-thiocyanate form thiocyanate ions. These ions react with HNO_3 and Fe^{3+} and effect a red color. The intensity of the color is directly proportional to the level of chloride ions.

Procedure

1000µL of the reagent was added to each of the sample and standard. This was incubated for 1minutes after mixing and the absorbance of the sample (A_{sample}) and ($A_{standard}$) were measured against the reagent blank at 505nm. The value of Cl^- present in serum was expressed in mEq/L.

Cl^- Concentration (mEq/L) = A *sample* / A *standard x* 100.

3.3.5.4 Determination of Bicarbonate

Determination of bicarbonate was done using the enzymatic method of Forrester *et al.* (1976).Biolabo Bicarbonate Reagent was utilized for this procedure.

Principle

In this procedure bicarbonate ($HCO3^-$) and phosphoenolpyruvate (PEP) are converted to oxaloacetate and phosphate in the reaction catalyzed by phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (MD) catalyzes the reduction of oxaloacetate to malate with the concomitant oxidation of reduced nicotinamide adenine dinucleotide (NADH). This oxidation of NADH results in a decrease in absorbance of the reaction mixture measured bichromatically at 380proportional to the Bicarbonate content of the sample.

$$PEP + HCO_3^- \xrightarrow{PEPC} Oxaloacetate + H_2PO_4$$

Magnesium

$$Oxaloacetate + NADH + H^+ \xrightarrow{MD} Malate + NAD^+$$

Reagents Composition

Final concentration of reactive ingredients:

MD	>200 IU/L
PEPC	>1000 IU/L
PEP	8.0 mmol/L
NADH	1.6 mmol/L
Buffer PH (20ºC) 8.0±0.1	66mmol/L
Stabilizers	

Procedure

1ml of the reagent was added to each of the sample and standard. This was incubated for 5minutes at 37ºC after mixing and the absorbance of the sample (A_{sample}) and ($A_{standard}$) were measured against the reagent blank at 380nm. The value of Cl^- present in serum was expressed in mEq/L.

Total CO_2 Concentration (mEq/L) = A *blank* - A *sample* / A *blank* - A *standard x* Standard Concentration.

3.4 Statistical Analysis

Data obtained was expressed as mean (±SEM). The result was analysed using one way analysis of Variance(ANOVA), followed by Tukey's Post hoc Test to compare the level of significance between groups using Statcato software version 0.9.12. Values of p ≤ 0.05 were considered significant.

CHAPTER FOUR

4.0 Results

4.1 Quantitative analysis of camel milk

The camel milk constituents as determined from proximate analysis are shown in table 4.1. Camel milk revealed the following constituents; ash, carbohydrates, fats, fatty acids, lactic acid, nitrogen, moisture and glycoside. Camel milk also proved the presence of vitamins A, B2, C and E at various wavelengths.

Table 4.1: Quantitative analysis of milk from one humped camelof kaura namoda farm

S/N	Compound	Composition(%, mg/dL)
01	Total solid	12.30%
02	Ash	0.61%
03	Moisture	87.70%
04	Carbohydrate	3.91%
05	Fats	3.70%
06	Lactic acid	0.30%
07	Solid non fat	8.60%
08	Nitrogen	0.64%
09	Crude Protein	4.08%
10	Cardiac glycoside	Positive
11	Free fatty acids	3.23mg/dL
12	Vitamin A	1.167 mg/dL
13	Vitamin B2	6.942 mg/dL
14	Vitamin C	0.329 mg/dL
15	Vitamin E	6.075 mg/dL

4.2 Results for Median Lethal Dose (LD_{50}) of Camel Milk in Wistar Rats

Camel milk showed no toxic effect in Wistar rats after oral administration. Thisimplies that camel milk has a median lethal dose that is greater than 5ml/kg.

Table 4.2 (a):Result for LD_{50} of Oral Administration of Camel Milk on Wistar Rats during Phase 1

Group(N=1)	Dose(ml/kg)	Clinical Signs	Number of Death	% Mortality
1	0.01None	0/1	0	
2	0.10None	0/1	0	
3	1.00	None	0/1	0

Table 4.2(b):Result for LD_{50} of Oral Administration of Camel Milk on Wistar Rats during Phase 2

Group(N=1)	Dose(ml/kg)	Clinical Signs	Number of Death	% Mortality
1	1.6None 0/1	0		
2	2.9None0/1 0			
3	5.0None 0/10			

LD_{50} = √ (highest non-lethal dose) × (lowest lethal dose)

4.3 Effect of Camel Milk on Total Cholesterol and Triglyceride of P407 Induced Hyperlipidemic Wistar Rats

4.3.1 Effect of camel milk on Total Cholesterol

The mean total cholesterol level in rats in the normal control group was (74.28 ±5.70 mg/dL) as shown in Figure 4.3. After induction with P407, cholesterol level in the hyperlipidemic negative control group was significantly ($p<0.05$) higher (727.24 ±126.59 mg/dL) than the normal control group (74.28 ±5.70 mg/dL). Treatment with camel milk at 1ml/kg orally resulted in significant($p<0.05$) reduction in cholesterol level (174.68 ±46.92 mg/dL) more than atorvastatin at 20 mg/kg (215.39 ±32.68 mg/dL).

4.3.2 Effect of camel milk on Triglyceride

The triglyceride level in rats of the normal control group was (38.97±1.51 mg/dL) as shown in Figure 4.3. There wassignificant increase ($p < 0.05$) in triglycerideof the hyperlipidemic negative control group (203.16 ± 27.64mg/dL) compared to normal control group (38.97±1.51 mg/dL). Treatment with Atorvastatin at a dose of 20mg/kg decreased TG to 38.74±2.97mg/dLandalso all treatment

groups;0.25ml/kg(63.57±6.34mg/dL),0.5ml/kg(45.07±3.13mg/dL),1ml/kg(91.38±5.52mg/dL) significantly ($p<0.05$) reduced high triglyceride level induced by P407 when compared to hyperlipidemic negative control group. Camel milk at a dose of 0.5 ml/kg had the highest reduction (45.073 ±3.138 mg/dL) followed by atorvastatin 20mg/kg (46.40 ±7.99 mg/dL) compared to the hyperlipidemic negative control group.

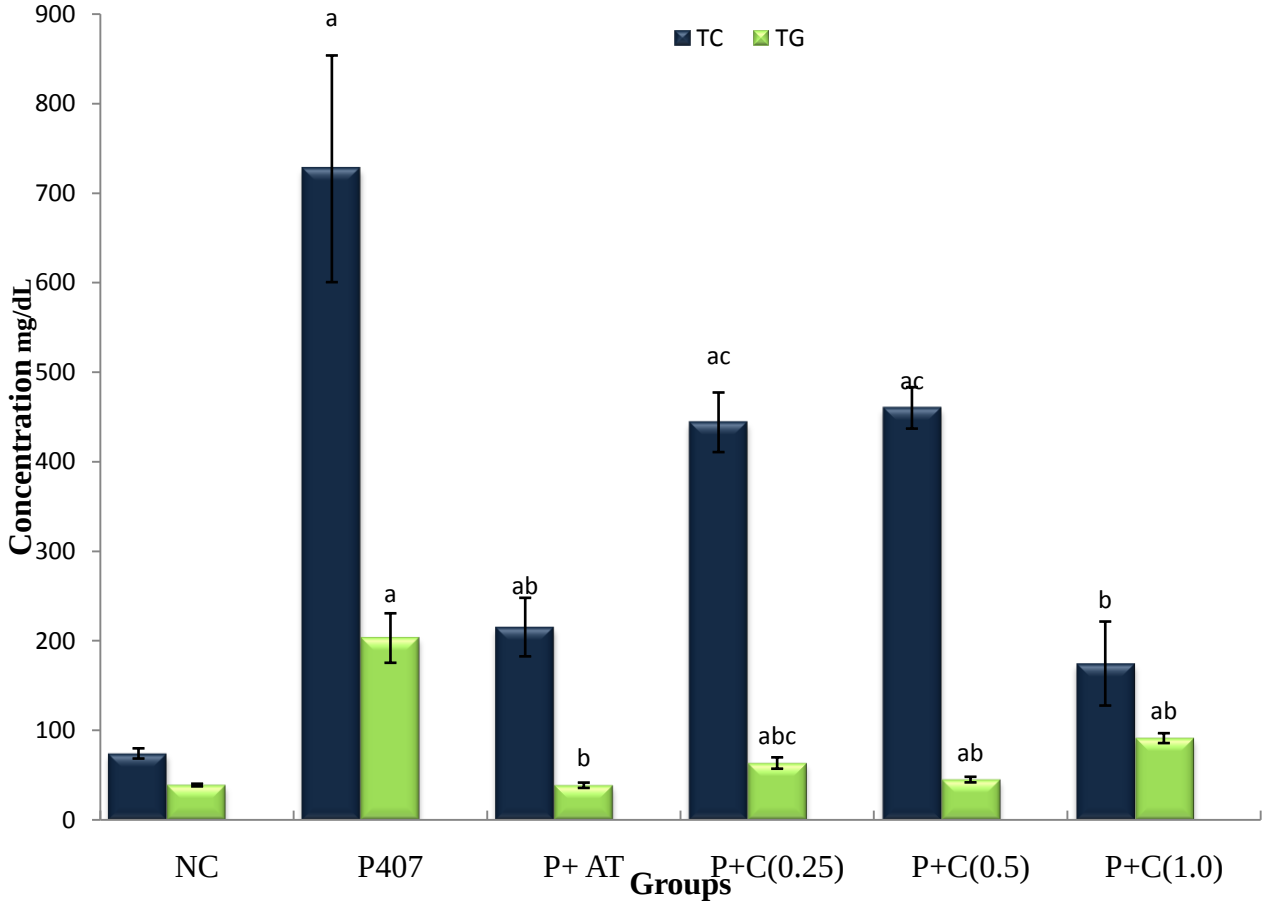


Figure 4.3: Effect of Camel Milk on Total Cholesterol (TC) and Triglyceride (TG) on P407 Induced Hyperlipidemic Wistar Rats.Results are expressed as mean ± standard error of mean.Values with superscripts are statistically significant ($P<0.05$); a = when compared to Group NC, b = when compared to Group P407, c = when compared to Group P+AT.

NC: Normal control.
P407: Hyperlipidemic negative control.
P+AT: P407+AT (20mg/kg).
P+C(0.25):P407+Camelmilk(0.25ml/kg).
P+C(0.5):P407+Camel milk (0.5ml/kg).

P+C(1.0): P407+ Camel milk(1.0ml/kg).

4.4 Effect of Camel Milk on High Density Lipoprotein and Low Density Lipoprotein on P407 Induced Hyperlipidemic Wistar Rats

4.4.1 Effect camel milk on High Density Lipoprotein

Figure 4.4 showed serum concentration of HDL-cholesterol. The HDL-cholesterol level in the normal control group was 35.97 ± 2.43mg/dL. There was a significant ($p< 0.05$) increase between the camel milk treatment group at 0.25ml/kg (208.72±7.88 mg/dL) and the normal control group. There was also significant ($p<0.05$) decrease between all camel milk treatment groups(208.72±7.88 mg/dL, 56.05±9.80mg/dL and 41.28±2.47mg/dL)respectively compared to the hyperlipidemic negative control group.

4.4.2 Effect of camel milk on Low Density Lipoprotein

The effect on LDL-cholesterol is also shown in Figure 4.4. The LDL- cholesterol level in the hyperlipidemic negative control group showed significant increase ($p<0.05$) (414.09±25.96mg/dL) compared to the normal control group (29.74±5.85mg/dL). The camel milk treated group at0.25ml/kg (214.15±21.72mg/dL), 1ml/kg (114.75±42.83mg/dL) and atorvastatin treated group (177.59±30.36mg/dL) showed significant ($p<0.05$) decrease compared to the hyperlipidemic negative controlgroup.

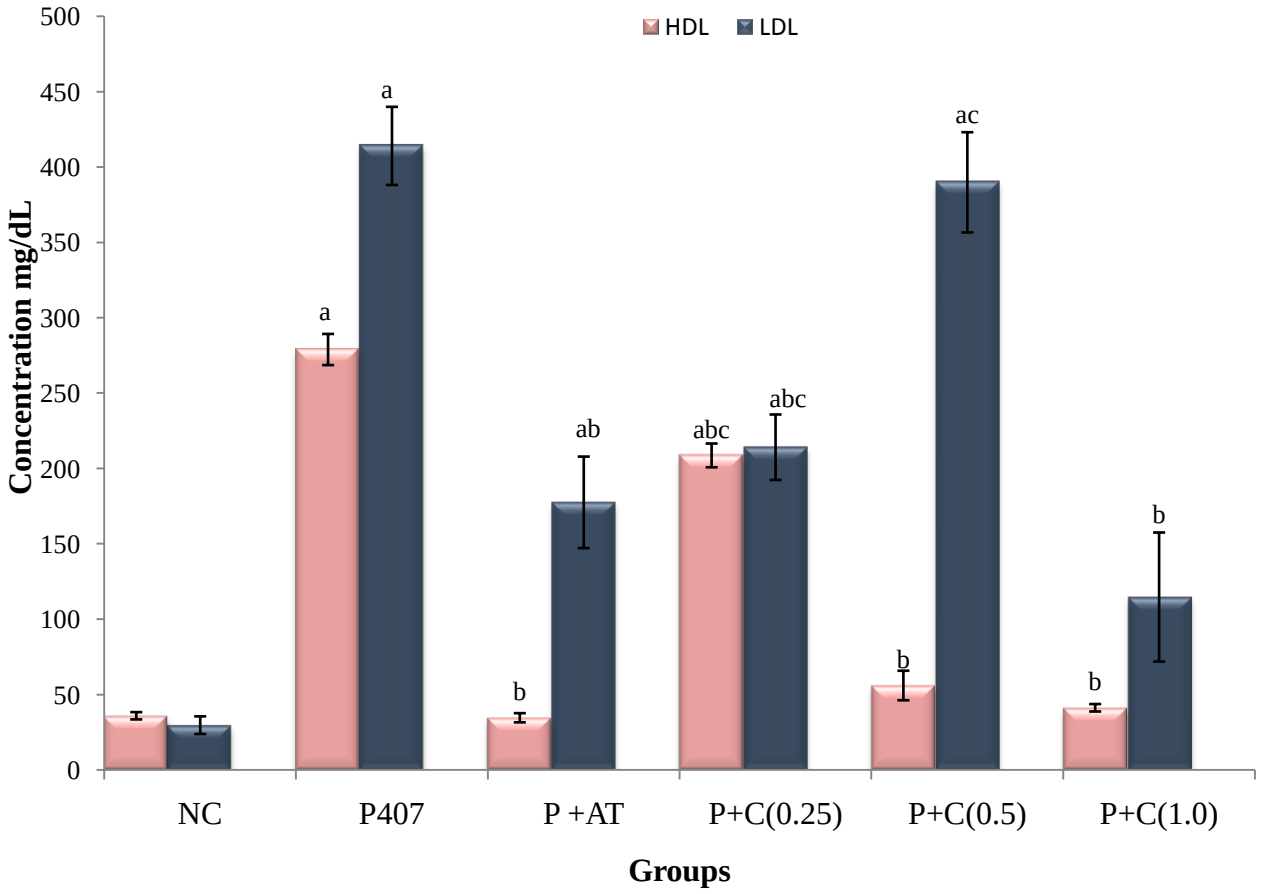


Figure 4.4.: Effect of Camel Milk on High Density Lipoprotein and Low Density Lipoprotein on P407 Induced Hyperlipidemic Wistar Rats. Results are expressed as mean ± standard error of mean. Values with superscripts are statistically significant (P<0.05); a = when compared to Group NC, b = when compared to Group P407, c = when compared to Group P+AT.

NC: Normal control.
P407: Hyperlipidemic negative control.
P+AT: P407+AT (20mg/kg).
P+C (0.25):P407+Camel milk (0.25ml/kg).
P+C (0.5):P407+Camel milk (0.5ml/kg).
P+C (1.0): P407+ Camel milk (1.0ml/kg).

4.5Effect of Camel Milk on Serum Oxidative Stress Biomarkers of P407 Induced Hyperlipidemic Wistar Rats

4.5.1 Effect of camel milk on Catalase

The effect of P407 on catalase activity showed a significant ($p<0.05$) decrease (3.26 ± 0.47 U/ml) in the hyperlipidemic negative control group compared to the normal control group (7.25 ± 0.69U/ml) as shown in Table 4.5. The mean values of all camel milk treated groups at 0.5ml/kg and 1ml/kg) (3.62 ± 0.72U/ml and 3.88 ±0.33U/ml) are higher though not statistically significant ($p > 0.05$) while the 0.25ml/kg (3.04±0.36U/ml) was lower($p<0.05$) compared to the hyperlipidemic negative control group (3.26 ± 0.47 U/ml).

4.5.2 Effect of camel on Superoxide Dismutase

There was a significant ($p<0.05$) decrease in superoxide dismutase in the hyperlipidemic control group(3.25±1.05U/ml)compared to normal control group (7.25±0.69 U/ml) as shown in Table 4.5. Camel milk treatment groups 0.25ml/kg and 0.5ml/kg (9.25±0.51 U/ml and 11.04± 1.14 U/ml) showed significant increase ($p<0.05$) compared to hyperlipidemic negative control group (3.25±1.05U/ml.

4.5.3 Effect of camel milk on Malondialdehyde

Serum Malondialdehyde level showed significant ($p<0.05$) increase in the hyperlipidemic negative control group (180.18±18.66nMols/ml) compared to normal control group (119.23±0.97 nMols/ml) as shown in Table 4.5. Camel milk treated groups at 0.25ml/kg, 0.5ml/kg and 1ml/kg (130.00±9.05nMols/ml 125.82±9.77nMols/ml and165.82 ±16.72nMols/ml)were all decreased though not statistically significant ($p>0.05$) difference compared to the normal control group.

4.5.4 Effect of camel milk on Gluthathione Peroxidase

There was increase in GPx at 0.25ml/kg (32.81 ± 5.18 U/ml), 0. 5ml/kg (47.60 ± 5.60U/ml) and 1ml/kg (36.82 ± 3.85U/ml) treatment with camel milk but showed nostatistical significance ($p>0.05$) compared to the hyperlipidemic negative control group (25.32 ±0.60 U/ml) as shown in Table 4.5.

Table 4.5: Effect of Camel Milk on Oxidative Stress Biomarkers of Poloxamer 407 Induced Hyperlipidemic Wistar Rats

Group (n=5)	CAT (U/ml)	SOD (U/ml)	GPx (U/ml)	MDA(nMols/ml)
NC	7.25 ± 0.69	11.23 ± 0.97	31.97 ± 3.70	119.23±20.70
P407	3.26 ± 0.47[a]	3.25 ± 1.05[a]	25.32 ±0.60	180.18±18.66[a]
P +AT	5.59 ± 1.64	5.68 ± 0.65[a]	31.03 ± 6.00	173.27±14.07[a]
P+C(0.25)	3.04 ± 0.36[a]	9.25 ± 0.51[bc]	32.81 ± 5.18	130.00 ± 9.05[c]
P+C(0.5)	3.62 ± 0.72[a]	11.04 ± 1.14[bc]	47.60 ± 5.60	125.82 ± 9.77[c]
P+C(1.0)	3.88 ± 0.33[a]	7.58 ± 1.70	36.82 ± 3.85	165.82 ±16.72

* Results are expressed as mean ± standard error of mean. Values with superscripts are statistically significant (P<0.05); a = when compared to Group NC, b = when compared to Group P407, c = when compared to Group P+AT.

NC: Normal control.
P407: Hyperlipidemic negative control.
P+AT: P407+AT (20mg/kg).
P+C (0.25):P407+Camel milk (0.25ml/kg).
P+C (0.5):P407+Camel milk (0.5ml/kg).
P+C (1.0): P407+ Camel milk (1.0ml/kg).

4.6 Effect of Camel Milk on Serum Total Protein, Albumin and Globulin Ratio of Poloxamer 407 Induced Hyperlipidemic Wistar Rats

4.6.1 Effect of camel milkon Total Protein

Serum concentration of total protein (TP) is shown in Figure 4.6.1. There was significant (p<0.05) increase in the concentrations of serum TP between normal control group (5.56±0.26g/dL) and hyperlipidemic negative control group (11.23±1.25g/dL). There wassignificant (p<0.05) decrease between thehyperlipidemic negative control group and camel milk treated groups at 0.25ml/kg(5.55±0.56g/dL) and 1ml/kg (6.42±0.53g/dL) respectively.

4.6.2 Effect of camel milkon Albumin

Serum concentration of albumin (Alb) is shown in Table 4.6.1.There was no significant (P>0.05) difference in the serum albumin concentration between the normal control group(3.15±0.10g/dL) and the hyperlipidemic negative control group (3.27±0.05g/dL), atorvastatin treated groups(3.48±0.11g/dL). Camel milk treatment groupsat 0.25ml/kg, 0.5ml/kg and 1ml/kg (3.14±0.39g/dL, 2.94±0.04g/dL and 2.98±0.05g/dL) showed no significant difference compared to the hyperlipidemic negative control group (3.27±0.05g/dL).

4.6.3 Effect of camel milkon Globulin

Serum concentration of Globulin (Glb) is shown in Figure 4.6.1.There was significant ($p<0.05$) increase in the concentrations of serum globulin between normal control group (2.41±0.17g/dL) and hyperlipidemic negative control group (7.75±1.28g/dL). There was significant ($p<0.05$) decrease between thehyperlipidemic negative control group compared to camel milk at a dose of0.25ml/kg(2.41±0.51mg/dL) and 1ml/kg(3.43±0.60g/dL) respectively.

4.6.4 Effect of camel milkon Albumin/Globulin Ratio

Serum albumin/globulin ratio is shown in Figure 4.6.2. There was significant ($p<0.05$) decrease in the albumin/globulin Ratio between the normal control group (1.32±0.06), hyperlipidemic negative control group (0.49±0.08). All Camel milk treated groups showed significant ($p<0.05$) increase compared to the hyperlipidemic negative controlgroup; .0.25ml/kg(1.33±0.11), 0.50ml/kg (0.87±0.11) and 1ml/kg (0.94±0.11) respectively.

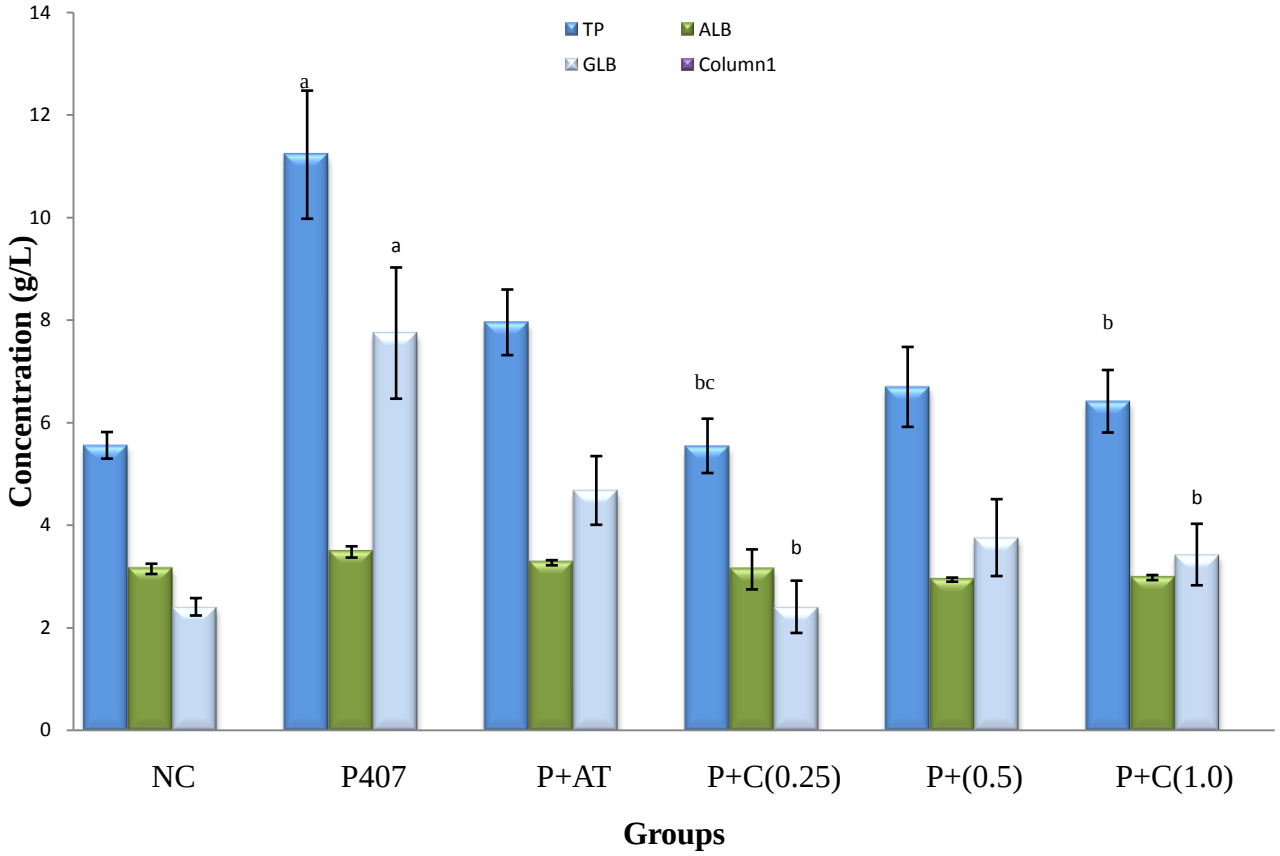


Figure 4.6.1: Effect of Camel Milk on Serum Total Protein, Albumin and Globulin of Poloxamer 407 Induced Hyperlipidemic Wistar Rat. Results are expressed as mean ± standard error of mean. Values with superscripts are statistically significant (P<0.05); a = when compared to Group NC, b = when compared to Group P407, c = when compared to Group P+AT.

NC: Normal control.
P407: Hyperlipidemic negative control.
P+AT: P407+AT (20mg/kg).
P+C (0.25):P407+Camel milk (0.25ml/kg).
P+C (0.5):P407+Camel milk (0.5ml/kg).
P+C (1.0): P407+ Camel milk (1.0ml/kg).

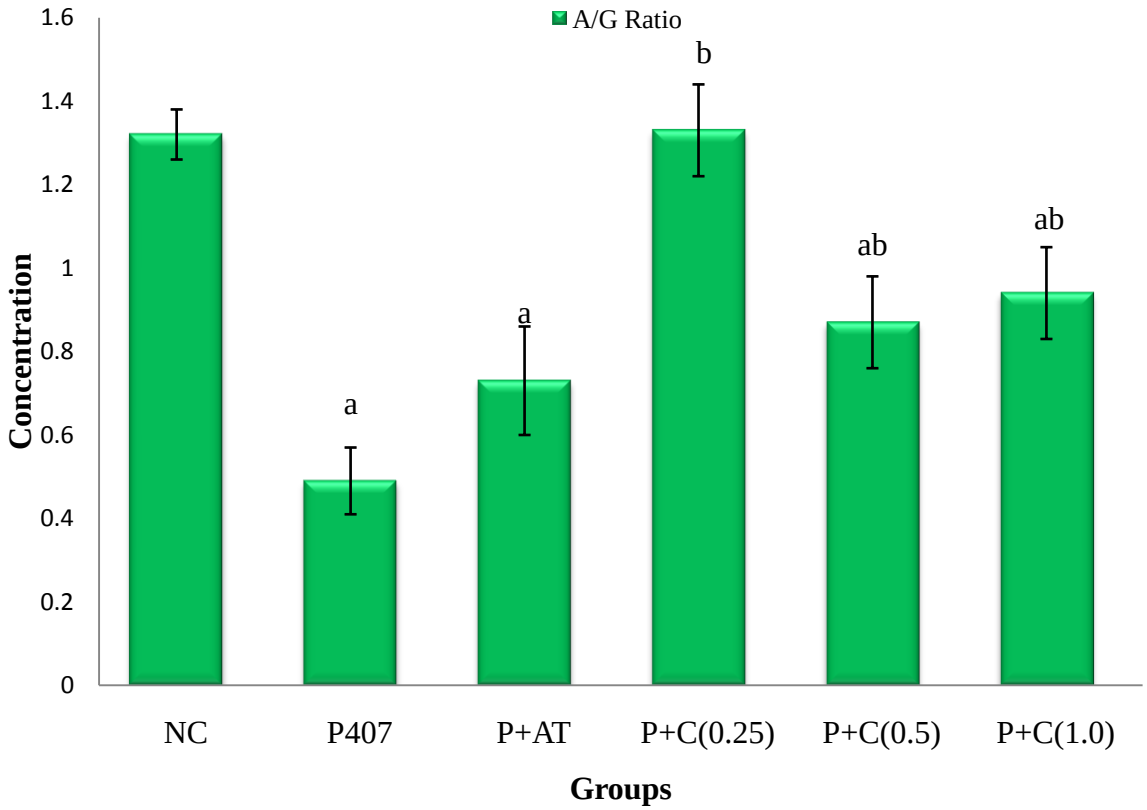


Figure 4.6.2: Effect of Camel Milk on Serum Albumin/Globulin Ratio of Poloxamer 407 Induced Hyperlipidemic Wistar Rats. Values with superscripts are statistically significant (P<0.05); a = when compared to Group NC, b = when compared to Group P407, c = when compared to Group P+AT.

NC: Normal control.
P407: Hyperlipidemic negative control.
P+AT: P407+AT (20mg/kg).
P+C (0.25):P407+Camel milk (0.25ml/kg).
P+C (0.5):P407+Camel milk (0.5ml/kg).
P+C (1.0): P407+ Camel milk (1.0ml/kg).

4.7 Effect of camel milk on liver enzymes of poloxamer 407 induced hyperlipidemic Rats

4.7.1 Effect of camel milkon Alkaline Phospatase (ALP)

The effect of camel milk on serum ALP in P407 induced hyperlipidemic wistar rats is presented in Figure 4.7. ALP showed decrease in level but not statistically significant (p> 0.05) in activity between the hyperlipidemic negative control group and all groups treated with camel milk.

4.7.2 Effect of camel milkon Alanine Aminotransferase (ALT)

The serum ALT activity showed significant increase ($p< 0.05$) between the normal control group (4.0 ± 0.70U/L) and the hyperlipidemic negative control group (42.50± 4.54U/L). There was significant difference ($p<0.05$) in reduction between all camel milk (6.40±1.03U/L, 10.20± 2.26U/L and 11.20± 1.39U/L) treated groupscompared to the hyperlipidemic negative control group(42.50± 4.54U/L).

4.7.3 Effect of camel milkon Aspartate Aminotransferase (AST)

There was significant increasein AST ($p<0.05$) between normal control group (11.40± 1.05U/L) and Hyperlipidemic negative control group (122.75±22.45U/L) as shown in figure 4.7. There was significant decrease ($p<0.05$) between all camel milk treatment group (0.25ml/kg, 0.5ml/kg and 1ml/kg),(20.00±3.17U/L, 28.40± 4.51U/L and 33.20± 5.51U/L) respectively compared to the hyperlipidemic negative contrl group.

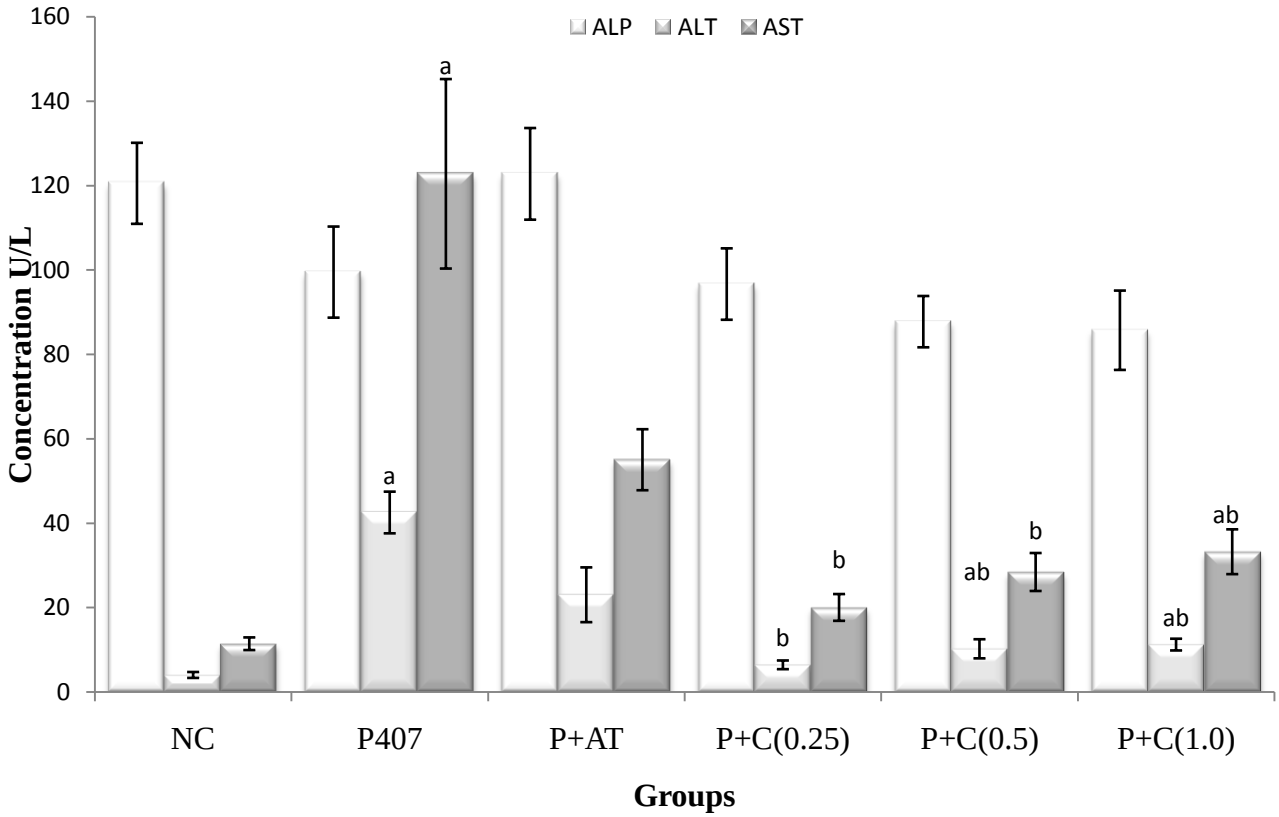


Figure 4.7.1: Effect of camel milk on serum liver enzymes of poloxamer 407 induced hyperlipidemic

Wistar Rats. Results are expressed as mean ± standard error of mean. Values with superscripts are statistically significant (P<0.05); a = when compared to Group NC, b = when compared to Group P407, c = when compared to Group P+AT.

NC: Normal control.
P407: Hyperlipidemic negative control.
P+AT: P407+AT (20mg/kg).
P+C (0.25):P407+Camel milk (0.25ml/kg).
P+C (0.5):P407+Camel milk (0.5ml/kg).
P+C (1.0): P407+ Camel milk (1.0ml/kg).

4.8Effect of camel milk on Serum Electrolytes of P407 Induced Hyperlipidemic Wistar Rats

4.8.1 Effect of camel milkon Sodium and Potassium

The result showed no statistical significant (p>0.05) difference in sodium and potassium concentrations of all the groups as shown in table 4.8.

4.8.2 Effectof camel milk on Chloride

The serum level of chloride ion as shown in table 4.8. was increased in the hyperlipidemic negative control group (116.50 ±3.61mmol/L) compared to the normal control group (102.50±6.69mmol/L) but not statistically significance (p>0.05).There was significant (p<0.05) decrease of chloride in all camel milk treated groups (102.22±2.22 mEq/L, 93.40±2.65 mEq/Land 90.00±2.04 mEq/L) compared to the hyperlipidemic negative control group (116.50 ±3.61mmol/L).

4.8.3 Effect of camel milkon Bicarbonate

There was significant increase (p<0.05) in the normal control group(25.50±2.10 mmol/L) and hyperlipidemic negative control group(40.50±0.50mmol/L) as shown in table 4.8.All camel milk treated groups(24.40±1.80 mmol/L, 26.8±1.68 mmol/L and 26.00±0.70 mmol/L) showed signifcant (p>0.05) reduction compared to the hyperlipidemic negative control group.

Table 4.8: Effect of Camel Milk on Serum Electrolytes of Poloxamer 407 Induced Hyperlipidemic Wistar Rats

Group (n=5)	Na^+ (mmol/L)	K^+(mmol/L)	Cl^-(mEq/L)	HCO_3^-(mEq/L)
NC	86.16 ± 4.69	4.07±0.36	102.50±6.69	25.50± 2.10
P407	86.27 ± 3.47	4.08± 0.14	116.50±3.61	40.50± 0.50[a]

P+AT	90.27 ± 2.97	3.85 ± 0.08	90.66±2.33[b]	35.33 ± 2.18
P+C(0.25)	93.82 ± 5.31	3.93 ± 0.19	102.22±2.22[bc]	24.4 ± 1.80[bc]
P+C(0.5)	94.06 ± 2.98	3.86 ± 0.12	93.40±2.65[b]	26.80± 1.68[bc]
P+C(1.0)	90.14 ± 5.38	4.16 ± 0.26	90.00±2.04[b]	26.00 ± 0.70[bc]

* Results are expressed as mean ± standard error of mean. Values with superscripts are statistically significant (P<0.05); a = when compared to Group NC, b = when compared to Group P407, c = when compared to Group P+AT.

NC: Normal control.
P407: Hyperlipidemic negative control.
P+AT: P407+AT (20mg/kg).
P+C (0.25):P407+Camel milk (0.25ml/kg).
P+C (0.5):P407+Camel milk (0.5ml/kg).
P+C (1.0): P407+ Camel milk (1.0ml/kg).

CHAPTER FIVE

5.0 Discussion

In the present study, Wistar rats treated with P-407 were characterized by higher serum lipid profiles after repeated administration for 3 weeks.There was a significant increase in the level of TC, TG, HDL-C and LDL-C in the hyperlipidemic group compared with the normal control group. All camel milktreated groups had significant decrease in TG compared to the hyperlipidemic group. Camel milk significantly reduced serum TC and LDL at 1ml/kg but had significant increase in HDL at 0.25ml/kg. These findings are in agreement with the results of Al Numair (2010), Abd el aziz *et al.*(2012) and Isa *et al.*(2013). Camel milk differs from other ruminant milk due to its low cholesterol, sugar and protein, but high sodium, potassium, iron, copper, zinc and magnesium, vitamins, insulin and immunoglobulins (Al-Hashem, 2009; Kamal *et al.,* 2007). Camel milk contains vitamins C and E as shown in table 4.1; both of which have been reported to decrease TC TG and LDL (Badr *et al.*, 2011).Vitamin C and Emay have inhibited3-the activity of HMG-CoA reductase, thereby decreasing the synthesis of mevalonate from acetyl-Co-A andthe absorption of cholesterol from the intestine to form complexes with compounds such as glycosides (table 4.1b) orstimulatedcholesterol-7-alpha-hydroxylase which to convert more cholesterol into bile acids (James *et al.,* 2010). Ibrahim *et al.* (2017) proposed from their study that the reduction in TC and LDL cholesterol by camel milk could as well be due to the inhibition of cholesterol acyl transferase enzyme, increase binding of LDL to LDL receptor and/or through an indirect effect on thyroid hormones. The decrease in TG by camel milk is due to high amount of zinc and insulin present in the milk (Al Numair 2010; Abd el aziz *et al.,*2012). It was previously shown that zinc supplementation facilitates insulin secretory activity in pancreatic islets of the ob/ob mouse (Begin-Heick*et et al.,* 1985). Extracellular ATP and zinc activate multiple P2X purinergic receptor channelsexpressed by islet beta-cells to potentiate insulin secretion (Richards *et al*., 2008). Insulin stimulates lipoprotein lipase activity resulting in the hydrolysis of TG to monoacylglycerol and fatty acids (Arkilla *et al.,* 2001, Klop *et al.,* 2013).The significant increase in HDL at 0.25ml/kg implies that oral administration of camel milk have potential ability of reducing the risk of atherosclerosis. High density lipoproteins carry cholesterol and cholesterol esters from the peripheral tissues and cells to the liver where cholesterol is metabolized into bile acids. An elevated level of HDL-C also prevents artherosclerosis by inhibiting the oxidative modification of LDL-C (Yoon *et al.,* 2008) and suppression of cytokines-induced endothelial adhesion molecules function (Thalia *et al.,* 2015). This pathway plays a very important role in decreasing cholesterol levels in the blood and peripheral tissues and inhibiting atherosclerotic plaque formation in the aorta (Kim *et al.,* 2008; Imafidon 2010). ..

Measurement of thiobarbituric acid (TBARS) has been used as an indicatorto determine lipid peroxidation and indirectly, oxidative stress both in vitro and in vivo (Fraga *et al.,* 1988; Beltowski *et al.,* 2000). Lipid peroxidation is initiated by peroxidative decomposition of membrane polyunsaturated fatty acids by free radicals, leading to their transformation and fragmentation to alkanes and reactive aldehyde compounds such as aldehydes and 4-hydroxy-2, 3-nonenals (HNE)(Olorunnisola *et al.,* 2012; Ngoc 2015). The effect of Poloxamer 407 in this study, showed a significant increase in MDA levels in serumcompared to the normal control group. The observed increase in lipid peroxidationis consistent

with other studies (Dutta and Bishayi 2009; Olorunnisola *et al.,* 2012 Woo *et al.,* 2012). Co adminstration of Poloxamer 407 with camel milkat doses of 0.25ml/kg and0.50ml/kg significantly reduced MDA concentration compared to Atorvastatin treatment. The result of our findings agrees with the works ofAl Hashem (2009) and Al Fartosi*et al.*(2012). The decrease in lipid peroxidation in the present study may be due to the free radical scavenging properties of vitamins and zinc present in the milk as reported by Ibrahim *et al.* (2017). Vitamin C is a scavenger of free oxygen radicals which are toxic by product of various metabolic processes (Dawud *et al.,* 2012). Vitamin E inhibits the process of lipid peroxidation through its radical chain-breaking antioxidant properties (Bjelakovic *et al*., 2007). Both Vitamins C and E also work synergistically to quench the activities of free radicals and regenerate the reduced form of vitamin E (Chuong *et al.,* 2008). Also, the zinc content of camel milk may have induced the production of metallothionein, an effective scavenger of hydroxyl radicals (Sahin and Kucuk, 2003).

Serum antioxidant enzymes (SOD and CAT) activitywassignificantly decreased in thehyperlipidemic rats compared to normal control group. The decrease in the activities of these enzymes during hyperlipidemia is attributed previously to the excessive utilization of these enzymes in inactivating the free radicals generated due to the hyperlipidemia (Ma *et al.,* 2011; Olorunnisola *et al.,* 2012). Co-administration of camel milk to P407 induced hyperlipidemic rats at doses0.25ml/kg, and 0.50ml/kg significantly increased the level of SOD which is consistent with the findings of Al Hashem (2009) and El Said*et al.* (2010) but differs with respect to reduced catalase activity. This difference may be attributed to the percentage composition of each elements,genetic status of the camel or geographical location where the milk is found(Jilo and Tegegne 2016).SOD converts superoxide anion radicals produced in the body to hydrogen peroxide thereby reducing a likelihood of superoxide anion interacting with nitric oxide to form reactive metabolites (Knott and Bossy-Wetzel, 2009). Our results indicate that camel milk had a free radical scavenging property which probably provides tissue protection from hyperlipidemia.

Hyperlipidemia is one factor that isinjurious to the liver. It sometimes results into fatty infiltration of the liver leading to non-alcoholic fatty liver (Assy *et al.,* 2000) and if not treated,it will lead to inflammation of the liver. It is characterized by varying degree of liver injury ranging from steatosis to steatohepatitis, fibrosis and necrosis (Day and James, 1998).Cholesterol activates liver x receptor resulting in negative balance between storage and oxidation of fatty acids (Schuppan and Schattenberg, 2013). The increase in both TC and TG elevatescirculating fatty acids which undergo incomplete beta oxidation to form non-triglyceride metabolites such as diacylglycerol (DG) and lysophosphatidyl choline(Schuppan and Schattenberg, 2013). Such metabolites stimulate reactive oxygen species, lipid peroxidation and necro inflammatory cytokines such as interleukin 1β (IL- β), interleukin 6 (IL-6)and tumour necrosing factor alpha (TNF-α)to cause lipotoxic injury of hepatocytes (Korish and Arafah, 2013; Schuppan and Schattenberg, 2013). Total protein is the measurement of all proteins in the blood serum. Majority of the proteins found in the blood are produced by theliver. By evaluating the total amount of protein in the blood, liver function in generating proteins and disease progression can be understood (Saad *et al.,* 2006; Abbas *et al.,* 2014). Total protein produced by the liver includes mainly globulin and albumin (Abbas *et al.,* 2014). The results of our findings showed a significant increase in total protein of the hyperlipidemiccontrol group compared to the normal control group. The increase total protein from our resultsdiffer from the works of Olorunnisola *et al.*(2012) and Victor *et al.,*(2014)

but are consistent with the works of Korolenko *et al.* (2016).Camel milk significantly reduced the level of total protein of P407 induced hyperlipidemic Wistar rats which is in agreement with the work of Abbas *et al.* (2014). Albumin is important as nonspecific transport mechanism for many physiologic substances as well as drugs, ions, amino acids and hormones. Albumin also serves as a precursor for tissue proteins and in nutrition, proteins play a small part in maintaining the plasma pH. They are negatively charged at body pH and therefore act as bases accepting hydrogen ions. Albumin is important plasma antioxidant activity; it primarily binds free fatty acids, divalentcations and hydrogen oxychloride(HOCl) (Akinbinu *et al.,* 2008). In chronic liver disease, the levels of albumin usually remain within normal limit until majority of the liver is damaged (Kaneco *et al.,* 2008; Abbas *et al.,* 2014). There was no significant change in albumin between all the groups. Globulin is elevatedin chronic infection and liver disease such as necrosis (Abbas *et al.,* 2014). Our findings showed significant increase in globulin level of hyperlipidemic control group compared to the normal control group.Increase in serum globulin level may have resulted from inflammation or necrosis effect of P407 induced hyperlipidemia on the liver(Korolenko *et al.,* 2016). Camel milk significantlyreduced the level of globulin of P407 induced hyperlipidemic Wistar rats which is in agreement with the work of Abbas *et al.* (2014). This protective effect may be attributed to the high level of proteins and immunoglobulin present in camel milk (Kamal *et al.,* 2007; Al-Hashem, 2009) or due to regeneration and maintenance of hepatocytes integrity by the milk (Ibrahim *et al.,*2017). There was significant decrease of serum A/G ratio in all treated groups except the group administered camel milk at a dose of 0.25ml/kg. However all camel milk treated group showed significant increase in A/G ratio compared to the hyperlipidemic negative control group.

An elevated serum level of ALT and AST is indicative of liver disease as this enzymes are present in large quantities in the liver and increases in the serum. Serum ALT is thought to be more specific for hepatic degeneration (Giboney, 2005). Following hepatocellular damage, hepatocytes alter their transport functions and membrane permeability, leading to the leakage of these enzymes from their cell (Mohan *et al.,* 2007; Al Hashem 2009). ALP is a sensitive biomarker of liver disease, it is a membranebound enzyme related to the transport of various metabolites. It is dependent on energy metabolism and processes in the body and a decrease in its activity may indicate impaired cellular energy processing (Al Hashem, 2009). There have been conflicting reports on the effect of poloxamer 407 induced hyperlipidemia on liver transaminases. Report on the effects of poloxamer 407 induced hyperlipidemia on serum levels of the above enzymes showed that hyperlipidemia elevated serum levels of ALT and AST (Hyeung *et al.,*2006). However, Ameh *et al.* (2013) found no effect on ALT except on AST,while Johnston *et al.* (1999) reported thatP407 does not cause hepatic injury. Moreover, Victor *et al.,* (2014) showed elevation of all the three liver enzymes after induction of Wistar rats with P407. The discrepancies in the serum levels of the enzymes was attributed to the level and duration of hyperlipidemia (Lu *et al.,* 2007). In the present studies, there was significant elevation in the serum levels of ALT and AST but not ALP of hyperlipidemic control group when compared to the normal control group. This may be due to hepatic damageas a result of accumulation of triglycerides and other fats in the hepatocytes and this is in conformity with the work of Hyeung *et al.* (2006). There was a significant reduction of ALT and ASTin all the camel milk treated groups on administration, which is consistent with findings of Al Hashem (2009) and Abbas *et al.* (2014) but differs with the work of Helal *et al.* (2012).The reversal of these liver enzymes towards normalcy by the milk

observed in this study maybe due to membrane stabilizing activity to prevent leakage of intracellular enzymes. This is in agreement with the work of Chavan *et al.* (2012) and Ibrahim *et al.* (2017) that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes. It thereforeclearly manifests of the hepatoprotective effects of camel milk.

Normal balance of electrolytes is essential for the normal function of cells and organs. Common electrolytes that are measured with blood testing include sodium, potassium, chloride, and bicarbonate. Abnormal concentration of sodium and chloride in the blood can affect the osmotic pressureof the body fluid therefore altering blood pressure (Cheesbrough, 2002). Potassium is the major component for cardiac function and abnormality in potassium level may result in cardiac dysfunction sincehypokalemia is associated with abnormal cardiac rhythm (Ozbek *et al.,* 2000).Bicarbonate is produced by the pancreas and liver to neutralize the acidic pH produce by acids in the gastrointestinal tract and maintain the acid-base buffering system of the blood. Significant decrease in bicarbonate concentrations in the blood could lead to disturbances known as acidosis where as an increase in the level of bicarbonates results in alkalosis (Abubakar and Sule, 2010; Guyton 2010).From our study, we did not observeany significant change in the level of electrolytes in all groups except for bicarbonate which had significantincrease in the hyperlipidemic control group. This implies that P407 induced hyperlipidemia can result in alkalosis in male Wistar rats. All camel milk treated groups significantly reduced the level of bicarbonate in P407 induced hyperlipidemic male Wistar rats. This neutralizing effect by camel milk could be attributed to acidic content of the milk such as vitamin C and lactic acid (table 4.1), thus highlighting the protective role of camel milk in the maintenance of electrolyte balance during hyperlipidemia state in Wistar rats.

CHAPTER SIX

6.0 Conclusion, Recommendations, Contributions to Knowledge and Limitations

6.1 Conclusion

Theresults of our findings demonstrated that camel milk reduced alteredlipid profile, oxidative stress, total protein, A/G ratio,liver enzymes and bicarbonate level of Poloxamer 407 induced hyperlipidemic Wistar rats. This suggests that consumption of camel milk could be beneficial in alleviating health hazards associated with hyperlipidemia.

6.2 Recommendations

i. Camel milk may be recommended as an adjuvant therapy in treatment of hyperlipidemia.

ii. Further studies are required to assess the most effective dose of camel milk needed to be taken to control hyperlipidemia state.

iii. Further studies are required to assess the effect of camel milk on total, direct and indirect bilirubin in poloxamer 407 induced hyperlipidemic wistar rats

6.3 Contributions to Knowledge

i. Camel milk at a dose of 1ml/kg reduced total cholesterol (174.68±46.92 mg/dL) triglyceride (91.38±5.52mg/dL) and low density lipoproteins (114.75±42.83mg/dL) compared to the hyperlipidemic negative control group (727.24 ±126.59 mg/dL, 203.16 ± 27.64mg/dL and 414.09±25.96mg/dL) respectively.

ii. Administration of camel milk at doses of 0.25ml/kg and 0.5ml/kg significantly increased superoxide dismutase (9.25±0.51 U/ml and 11.04± 1.14 U/ml)compared to the hyperlipidemic negative control group (3.25 ± 1.05U/ml)

iii. All Camel milk treated groups showed significant increase A/G ratio (1.33±0.11, 0.87±0.11 and 0.94±0.11) compared to the hyperlipidemic negative control group(0.49±0.08) respectively.

iv. All Camel milk treated groups showed significant decrease in bicarbonate level(24.40±1.80 mmol/L, 26.8±1.68 mmol/L and 26.00±0.70 mmol/L) respectively compared to the hyperlipidemic negative control group (40.50±0.50mmol/L).

6.4 Limitations

i. Collection of camel milk was done during the month of February and March which may have impacted on the distribution of constituents in the milk.

ii. Transportation of the milk from Kaura Namoda local government area of Zamfara state takes over 250km to cover.

REFERENCES

Abbas, S., Ashraf, H., Nazir, A., and Safraz, L. (2013).Physico-chemical analysis and chemical composition of camel milk. *International Researchers*, 2(2):84-98.

Abbas, S., Imran, R., Nazir, A., Qamar, M.F. and Sarfraz L. (2014). Effect of camel milk supplementation on blood parameters and liver function of hepatitis patients.*American Journal of Ethnomedicine,* 1(3): 129-146.

Abdelaziz,A. D., Ali, K. A., Ahmed, A. H., Mansour, H. Z.and Mohamed M.S.(2012). A study on the effect of female camel (*Camelus Dromedarius*) milk on glycemic control of streptozotocin (STZ) induced diabetes mellitus in rats. *Journal of American Science,* 8(4):459-465.

Abubakar, S. M. and Sule, M. S. (2010). Effect of oral administration of aqueous extract of *Cassia occidentalis L.* seeds on serum electrolytes concentration in rats.*Bayero Journal Pure and Applied Science,* 3 (1): 183 – 187.

Adekunle, A.S., Adedeji, A.L., Oyewo, E.O., Adedosun O.T. and Omotoso A.T. (2013).Hyperlipidemia induced by atherogenic diet enhanced oxidative stress in the Kidney and inflammatory responses: An in-vivo study. *Asian Journal of Natural and Applied Sciences*, 2(1): 82-93.

Aebi, H. (1984). Catalase in vitro.*Method Enzymology*, 105: 121-126.

Agrawel, R. P., Swami, S. C., Beniwal, R., Kochar, D. K. and Kothari, R.P. (2002). Effect of camel milk on glycemic control, risk factors and diabetes quality of life in type 1 diabetes: A randomized prospective controlled study. *International Journal of Diabetes in Developing Countries*, 22: 70-74.

Ajamaluddin, M., Abdulrahman, A., Ewa, S. and Jerzy, J. (2012.A study of the anti-diabetic agents of camel milk. *International Journal of Molecular Medicine,* 30:585-92.

Akinbinu, O.M., Ogunyemi, O.E., Arinola, O.G., Adenaike, A.F. and Adegoke, O.D. (2008).Assessment of antioxidants and nutritional status of pulmonary tuberculosis patients in Nigeria.*European Journal of General Medicine,* 5:208–211.

AL-Ayadhi L.Y. and Elamin, N.E. (2013). Camel Milk as a Potential Therapy as an Antioxidant in Autism Spectrum Disorder (ASD).*Evidence Based Complement Alternative Medicine*, (602834):1-8.

Al-Fartosi, K.G., Majid, A., Auda, M.A. and Hussein M.H. (2012). The Role of Camel's Milk against Some Oxidant-Antioxidant markers of male rats treated with CCl_4.*International Journal of Research in Pharmaceutical and Biomedical Sciences*,3(1): 385- 389.

AlHashem, F. (2009). Camel milk protects against aluminium chloride-induced toxicity in the liver and kidney of white albino rats. *American Journal of Biochemistry and Biotechnology,* 5: 98-108.

Al Humaid, A.I., Mousa, H.M., El Mergawi, R.A. and Abdel-salam A.M. (2010).Chemical composition and antioxidant activity of dates and dates-camel-milk mixtures as a protective meal against lipid peroxidation activity in rats. *Journal of Food Technology*, 5: 22-30.

Al-Numair K.S. (2010). Type II diabetic rats and the hypolipidemic effect of camel milk.*Journal of Food, Agriculture and Environment*, 8 (2): 77-81.

Allain, C.C. (1974). Enzymatic determination of total serum cholesterol.*Clinical Chemistry* 20: 470–475.

Ameh, D.A., Adejo, E.B., James D.B., (2013). Effect of *Garcinal kola* biflavonoid fractions on some biochemical parameters of P407-induced hyperlipidemia rats: A phytopreventive and phytotheraupeutic studies.*Comparative Clinical Pathology,*24(1): 109-115.

Amuamuta, A. (2014). A Review on Risk Factors/Indicators and Effects of Hyperlipidemia.*Middle-East Journal of Scientific Research,* 22(6): 886-893.

Arkkila, P.E., Koskinen, P.J. and Kantola, I.M. (2001). Diabetic complications are associated with liver enzyme activities in people with type I diabetes. *Diabetes Research in Clinical Practice*, 52:113-118.

Arun, Kumar., Dhaliya S. A. Surya, A. S., Dawn, V. T., Carla, B. and Sunil, C. (2013). A Review of Hyperlipidemia and Medicinal Plants.*International Journal of Applied Pharmaceutical Science and Biomedical Science*, 2(4): 219-237.

Ascherio, A. and Willet, W.C. (1997). Health effects of trans fatty acids. *American Journal of Clinical Nutrition,* 66(4): 1006s-1010s.

Assmann, G. (1979). A fully enzymatic colorimetric determination of HDL-cholesterol in the serum.*Internist*, 20:559.

Association of Analytical Chemist,(2005). Official Method of Analysis.18th Edition.Association of Analytical Chemist, Maryland, USA.

Assy, N., Kaita, K., Mymin, D., Levy, C., Rosser B. and Minuk, G. (2000). Fatty infiltration of liver in hyperlipidemic condition.*Digestive Disease Science,* 45(10): 1929-1934.

Austin, M.A., Hutter, C.M., Zimmern, R.L. and Humphries, S.E. (2004). Genetic Causes of Monogenic HeterozygousFamilial Hypercholesterolemia: A HuGE Prevalence Review. *American Journal of Epidemiology,* 160:407–420.

Badr, G., Bashandy, S., Ebaid, H., Mohany, M. and Sayed, D. (2011). Vitamin C supplementation reconstitutes polyfunctional T cells in streptozocin induced diabetic rats. *European Journal of Nutrition,* 25: 1-11.

Barłowska, J., Litwi, C. Z., Kedzierska-Matysek, M.and Litwi, C. A.(2007).Non polymorphism of caprinemilk αs1-casein in relation to performance of fourpolish goat breeds.*Poland Journal of Veterinary Sciences*, 10:159–64.

Bansal M.P. and Jaswal S. (2009). Hypercholesterolemia induced oxidative stress is reduced in rats with diets enriched with supplement from *Dunaliella salina algae*.*American Journal of Biomedical Science.* 1(3):196-204.

Begin-Heick,N., Dalpe-Scott, M., Rowe, J. and Heick, H.M. (1985). Zinc supplementation attenuates insulin secretory activity in pancreatic islets of the ob/ob mouse. *Diabetes*34:179-184.

Beltowski, J., Wójcicka, G., Górny, D. and Marciniak, A. (2000).The effect of dietary-induced obesity on lipid peroxidation, antioxidant enzymes and total plasma antioxidant capacity.*Journal of Physiology and Pharmacology*, 51, 883–896.

Bjelakovic, G.,Nikolova, L.L., Gluud S. and Gluud C. (2007). Mortality in randomized trials of antioxidant supplements for primary and secondary prevention. *Journal of American Medical Association,*297(8): 842-857.

Catalgol, B. and Ozer N.K (2011). Protective effects of vitamin E against hypercholesterolemia- induced age-related disease. *Genes Nutr,* 1-8. doi 10.1007/s12263-011-0235-9.

Chakrapany, S. and Chandan, S. (2014). Therapeutic Value of Camel Milk: A Review. *Advance Journal of Pharmacy and Life science Research*, 2(3):7-13.

Chavan, S.D., Patil, S.B. and Naikwade N.S. (2012). Biochemical and histopathological Studies of *Butea monosperma(Lam)* taub leaves on paracetamol induced hepatotoxicity in Albino rats. *Journal of Pharmacy Research*, 5(8):4006-4008.

Cheesbrough, M. (2002).*District Laboratory Practice in Tropical Countries*: Part 1. Cambridge University Press, Cambridge, U.K, pp 229-370.

Chuong P.H., Lien, A.P., and Hua, H. (2008). Free Radicals, Antioxidants in disease and health. *International Journal of Biomedical Science,* 4(2): 89-96.

Cogger, V. C., Hilmer, S. N., Sullivan, D., Muller, M., Fraser, R. and Le Couteur, D. G. (2006). Hyperlipidemia and surfactants: The liver sieve is a link. *Atherosclerosis* 189: 273–81.

Dashty, M (2014). A quick look at biochemistry: lipid metabolism. *Journal of Diabetes and Metabolism* 2 (324): 1-19.

Davidson, M. H., Armani, A., McKenney, J. M., and Jacobson, T. A. (2007) Safety considerations with fibrate therapy.*American Journal of Cardiology*, 99:3C-18C

Davidson, M.H. and Tooth, P.P. (2004). Comparative effect of lipid lowering therapies.*Progress in Cardiovascular Disease*, 47:173-204.

Dawud, F.A., Eze, E.D., Ardja, A.A., Isa, A.S., Jimoh, A., Bashiru M. and Malgwi, I.S. (2012). Ameliorative effects of vitamin C and zinc in Alloxan-induced diabetes and oxidative stress in Wistar rats, *Current Research Journal of Biological Sciences,* 4(2): 123-129.

Day, C.P. and James, O.F. (1998). Steatohepatitis: a tale of two “hits”. *Gastroenterology*, 114(4): 842-845.

Delvin, E. (2006). Overproduction of intestinal lipoprotein containing apolipoprotein B-48 in Psammomys obesus: impact of dietary n-3 fatty acids. *Diabetologia*, 49:1937–45

Dumortier, G., Grossiord, J. L., Agnely, F. and Chaumeil, J. C. (2006).A review of poloxamer 407 pharmaceutical and pharmacological characteristics.*Pharmaceutical Research* 23: 2709–2.

Doumas, B.T., Watson, W. A. and Biggs, H.G., (1971).Albumin standards and the measurement of serum albumin with bromocresol green. *Clinical Chemistry Acta*, 31: 87-96.

Dutta, K. and Bishayi, B.(2009). Escherichia colilipopolysaccharide administration alters antioxidant profile during hypercholesterolemia. *Indian Journal of Clinical Biochemistry*, 24:179–183.

El-Said, E., El-Said, G., Ramadan, E. and Esraa, T. (2010). Effect of camel milk on oxidative stresses in experimentally induced diabetic Rabbits. *Veterinary Research Forum,* 1: 30-43.

El-Zeini, H. (2006).Microstructure rheological and geometrical properties of fat globules of milk from different animal species.*Poland Journal of Food Nutritional Sciences*, 56:147– 154.

Fauquant, C., Briard-Bion, V., Leconte, N., Guichardant, M.and Michalski, M. (2007). Membrane phospholipids and sterols in micro-filtered milk fat Globules. *European Journal of Lipid Science and Technology*, 12:1167–73.

Fernandez, A., Sorokin, A. and Thompson, P.D. (2007). Corneal arcus as coronary artery disease risk factor. *Atherosclerosis,* 193: 235–240.

Forrester, R.L., L.J. Wataji, Silverman, D.A. and Pierre, K.J.(1976).Enzymatic method for determination of CO_2 in serum.*Clinical Chemistry*, 22: 243-245.

Fraga, C.G., Leibovitz, B.E. and Toppel, A.L. (1988). Lipid peroxidation measured as TBARS in tissue slices: Charaterization and comparison with homogenates and microsomes. *Free Radical Biology Medicine*, 4: 155-161.

Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972).Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge.*Clinical Chemistry*, 18(6): 499-502.

Gibony, P.T. (2005). Mildly elevated liver transaminase in asymptomatic patients.*American Family Physician,* 71(6): 1105-1110.

Gizachew, A., Teha, J. and Birhanu, T. (2014). Review on Medicinal and Nutritional values of camel milk. *Nature and Science* 12(12):35-41.

Gomall, A.G., Bardawill, C.S. and David, M.M. (1949).Determination of serum proteins by means of the biuret reaction.*Journal of biological chemistr*y, (177):751-766.

Guakhar and Bernand, (2004). A better knowledge of milk quality parameters: A preliminary step for improving the camel milk market opportunity in a transition economy- the case of Kazakhstan. Saving the Camel

and Peoples' Livelihoods, Building a Multi stockholder Platform for the Conservation of the Camel in Rajasthan *In:International conference*, Sadri, Rajasthan, India. pp. 28-36.

Guyton A. C. and Hall J.E. (2006).*Textbook of Medical Physiology.*11th Edition, Elsevier Inc. pp 829-1104.

Harikumar, K., Abdul Althaf, S., Kishore kumar, B., Ramunaik, M. and Suvarna, C.H. (2013). A Review on Hyperlipidemic. *International Journal of Novel Trends in Pharmaceutical Sciences,* 3(4): 59-75.

Hassan, A. I. and Bayoumi, M. M. (2010).Efficiency of Camel Milk and Honey Bee in alleviation of Diabetes in Rats.*Nature and Science*, 8(10): 333-341.

Helal, E.G.E., Abd-Elwahab, S.M. and Mohammad, A.A. (2012).Effect of Camel Milk on Alloxan-Induced Diabetic Rats.*The Egyptian Journal of Hospital Medicine*, 49: 539– 55.

Henry, R.J., Cannon, D.C. and Winkelman, J.W. (1974).*ClinicalChemistry, Principles and Techniques*.2nd edition, Harper and Row, pp 525.

Hor, S.Y., Farsi, E., Yam, M.F., Nuyah N.M. and Asmawi, M.Z. (2011). Lipid-lowering effects of Coriolus versicolor extract in poloxamer 407-induced hypercholesterolaemic rats and high cholesterol-fed rats. *Journal of Medicinal Plants Research*, 5(11):2261-2266.

Hou, R. and Goldberg, A.C. (2009). Lipoprotein cholesterol: statins, ezetimibe, bile acidsequestrants and combinations: comparative efficacy and safety. *EndocrinologyMetabolism of Clinic of North America,* 38:79-97.

Howell W.H., Mc Namara, D.j., Tosca, M.A., Smith, B.T. and Gaines J.A. (1997). Plasma lipids and lipoprotein responses to dietary fat and cholesterol: A meta-analysis. *American Journal of Clinical Nutrition,* 65(6):1747-1764.

Hyeung, S.L., Hyo, C.A. and Sae, K.K. (2006). Hypolipemic effect of water extracts of Picrorrhizarhizoma in PX-407 induced hyperlipemic ICR mouse model with hepatoprotective effects: A prevention study. *Journal of Ethnopharmacology,* 105: 380–386.

Ibrahim, M.A., Wani, F.A. and Rahiman,S. (2017). Hepatoprotective effect of olive oil and camel milk on acetaminophen-induced liver toxicity in mice, *International Journal of Medical Science and Public Health,* 6:186-194.

Imafidon, K. E. (2010). Tissue Lipid Profile of Rats Administered Aqueous Extract of Hibiscus Rosa-Sinensis. Linn.*Journal of Basic and Applied Sciences*, 2 6(1):1-3.

Isa, S.A., Ibrahim, K.G. and Abubakar, I. (2013). Effect of camel milk's supplementation on serum glucose levels, lipid profile and body weight of Alloxan-induced diabetic rats. *Nigerian Journal of Basic and Applied Science,* 21(3): 187-191.

Jacobs, N.J. and Van demark, P.J.(1960). The purification and properties of the alpha glycerol phosphate-oxidizing enzyme of Streptococcus faecalis 10C1.*Archives of Biochemistry and Biophysics,* 88:250-5.

James, D.B., Elebo, N., Sanusi A.M. and Odoemene L. (2010).Some Biochemical Effect of Intra peritoneal Administration of Phyllanthus amarus Aquoeus Extacts on Normaglycemic Albino Rats. *Asian Journal of Medical Sciences*, 2(1):7-10.

Jilo, T.K. and Tegegne, D. (2016).Chemical composition and medicinal value of camel milk.*International Journal of Research Studies in Biosciences,*4(4): 13-25.

Johnston, T. P. (2004). The P-407-induced murine model of dose-controlled hyperlipidemia and atherosclerosis: A review of findings to date. *Journal of Cardiovascular Pharmacology*, 43: 595–606.

Johnston, T.P. and Palmer, W.K. (1993).Mechanism of poloxamer 407-induced hypertriglyceridemia in the rat.*Biochemical Pharmacology,* 46:1037–42.

Johnston, T. P., Baker, J. C., Jamal A. S., Hall, D. D., Emeson, E. E., and Palmer, W. K. (1999) Potential down regulation of HMG-CoA reductase after prolonged administration of P-407 in C57BL/6 mice. *Journal of Cardiovascular Pharmacology* 34: 831–842.

Kamal, A.M., Salama, O.A. and El-Saied, K.M. (2007). Changes in amino acids profile of camel milk protein during the early lactation. *International Journal of Dairy Science*, 2: 226-234.

Kaneko, J.J., Harvey, J.W. and Bruss, M.L. (2008).*Clinical biochemistryof domestic animals.* 6th Edition, Elsevier Academic, Amsterdam, Pp 356-365.

Khan, B.B. and Flier J.S. (2000).Obesity and Insulin Resistance.*Journal of Clinical Investigation,*106:473-481.

Khaskheli, M., M. A. Arain, S. Chaudhry, A. H. Soomro and Qureshi.T.A (2005).Physico chemical quality of camelmilk.*Journal of Agriculture and Social Sciences,* 2: 164-166.

Kim, H., Jeong, D., Jung, H., Yokozawa, T. and Choi J, J. (2008).Hypolipidemic Effects of Sophora flavescens and Its Constituents in Poloxamer 407-Induced Hyperlipidemic and Cholesterol-Fed Rats.*Biology and Pharmacy Bulletin,* 31(1):73-78.

Klop, B., Elte J.W.F. and Cabezas C. M. (2013). Dyslipidemia in Obesity: Mechanisms and Potential Targets. *Nutrients*, 5: 1218-1240.

Knott A.B. and Bossy-Wetzel, E. (2009).Nitric oxide in health and disease of the nervous system.*Antioxidant and Redox Signaling,* 11: 541-553..

Kolawole, O.T., Kolawole, S.O., Ayankunl, A.A. and Olaniran I.O. (2012). Methanol Leaf Extract of Persea americana protects Rats against Cholesterol-Induced Hyperlipidemia. *British Journal of Medicine and Medical Research,* 2(2): 235-242.

Korish, A.A and Arafah, M.A. (2013). Camel milk ameliorates steatohepatitis, insulin resistance and lipid peroxidation in experimentalnon-alcoholic fatty liver disease. *BMC Complementary and Alternative Medicine*,13(264): 1-12.

Korolenko, T.A., Johnston, T.P., Dubrovina, N.I., Kisarova, Y.A., Zhanaeva, S.Y., Cherkanova, M.S., Filjushina, E.E.,Alexeenko, T.V., Eva Machova, E. and Zhukova, N.A. (2013). Effect of poloxamer 407 administration on the serum lipids profile, anxiety level and protease activity in the heart and liver of mice.*Interdisciplinary Toxicology*, 6(1): 18–25.

Korolenko, T.A., Johnston, T.P., Tuzikov, F.V., Tuzikova, N.A., Pupyshev, A.B., Spiridonov, V.K., Goncharova., N.V., Maiborodin, I.V. and Zhukova N.A. (2016). Early-stage atherosclerosi in poloxamer 407-induced hyperlipidemic mice:pathological features and changes in the lipid composition of serum lipoprotein fractions and subfractions. *Lipids in Health and Disease,* 15(16): 1-13.

Li, C., Palmer, W. K. and Johnston, T. P. (1996). Disposition of poloxamer407 in rats following a single intraperitoneal injection assessed using a simplified colorimetric assay. *Journal of Pharmaceutical Biometrics Anals*14: 659–65.

Lim, S., S, Vos, T., Flaxman, A., D, Danaei, G., Shibuya, K., Adair-Rohani, H. (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet,* 2012.

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

Lu, L.S., Wu, C.C., Hung, L.M., Chiang, M.T., Lin, C.T., Lin, C.W., and Su, M.J. (2007). Apocynin alleviated hepatic oxidative burden and reduced liver injury in hypercholesterolaemia. *Liver International*27: 529-537.

Luscher, T. F. (2011). Triglyceride rich lipoprotein and high density lipoprotein cholesterol in patients at high risk of cardiovascular evident and guidance for management. *European Heart Journal*. Published on behalf of European society of cardiology.http://dx.doi.org/10-1093/euroheart/eh112

Ma, J., Qiao, Z. and Xiang, X. (2011). Aqueous extract of *Astragalus mongholicus* ameliorates high cholesterol diet induced oxidative injury in experimental rat's models. *Journal of Plants Research*, 5: 855–858.

Maheswari, E., Saraswathy, R.G. and Santhranii, T. (2014).Hepatoprotective and antioxidant activity of *N*-acetyl cysteine in carbamazepine-administered rats.Indian Journal of Pharmacology, 46(2): 211–215.

Mannu, G.S., Zaman, M.J.,Gupter, A., Rehman, H.U. and Myint, P.K. (2013).Evidence of lifestyle modification in the management of hypercholesterolemia.*Current Cardiology Reviews,*9(1):2-14.

Martin, J.P., Dailey, M. and Sugarman, E. (1987). Negative and positive assays superoxide dismutase based on hematoxylin autoxidation. *Archives of Biochemistry and Biophysics*, 255: 329-336.

Mattar, M.and Obeid, O. (2009) Fish oil and the management of hypertriglyceridemia. *Journal of Nutritional Health*: 20(1):41-9.

Mattes, R.D. (2005).Fat taste and lipid metabolism in humans.*Physiology of Behaviour,* 86: 691–697.

Mc Carthy, M.F. (2004). An evaluation of triglyceride reflecting decreased triglyceride clearance may not be pathogenic- relevance to high carbohydrate diet.*Medical Hypothesis* 63(6): 1065-1073.

Mohan, K.G., Pallavi, E., Kumar, E.R., Ramesh, M. and Venkatesh, S. (2007).Hepatoprotective activity of *Ficus carica Linn*.leafextract against carbon tetrachloride-induced hepatotoxicity in rats. *DARU Journal of Pharnaceutical Science,* 15(3): 162-166.

Mullaicharam A.R. (2014). A review on medicinal properties of camel milk.*World Journal of Pharmaceutical Sciences,* 2(3): 237-242.

Ngoc, A. L. (2015). Lipoprotein-associated oxidative stress: A new twist to the postprandial hypothesis. *International Journal of Molecular Science,* 16: 401-419.

Nikkila, E.A. (1984). Plasma lipid and lipoprotein abnormalities in diabetes.*Diabetes and HeartDisease,*133-167.

Nirosha, K., Divya , M., Vamsi, M. and Mohemmed, S. (2014). A review on hyperlipidemia.*International Journal of Novel Trends in Pharmaceutical Science,* 4(5):81-92.

Nordestgaard, B.G., Langsted, A., Mora, S., Kolovou, G., Baum, H., Bruckert, E., Eatts, G.F., Sypniewska, G. and Wiklund, O. (2016). Fasting is not routinely required for determination of lipidprofile; Clinical and laboratory implications including flagging at desirable concentration cut- point.Consensus statement from European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine.*European Heart Journal,* 37(25) 1944-1958.

Oguejiofor O.O., Onwukwe C.H. and Odenigbo C.U. (2012). Dyslipidemia in Nigeria: Prevalence and Pattern, *Annals of African Medicine,*11(4): 197-202.

Olorunnisola, S.O., Graeme, B. and Anthony, J.A. (2012). Protective Effect of *T. violacea Rhizome* extract against hypercholesterolemia-induced oxidative stress in Wistar rats. *Molecules*,17: 6033-6045.

Onwe, P.E., Folawiyo M.A., Anyigor –Ogah, C.S., Umahi, G., Okorocha, A.E. and Afoke, A.O. (2015).Hyperlipidemia: Etiology and Possible Control. *IOSR Journal of Dental and Medical Sciences,*14 (10): 93-100.

Ooi, L.G. and Liong M.T. (2010). Cholesterol-lowering effects of probiotics and prebiotics: a review of in vivo and in vitro findings. *International Journal of Molecular Sciences,* 11: 2499-2522.

Ozbek, E., Turkoz, Y., Sahna, E., Ozugurlu, F., Mizrak, B. and Ozbek, M., (2000). Melatonin administration prevents the nephrotoxicity induced by gentamicin. *BJU International*, 85 (6): 742-746.

Palmer, W. K., Emeson, E. E. and Johnston, T. P. (1998).Poloxamer 407-induced atherogenesis in the C57BL/6 mouse.*Atherosclerosis*136:115–23.

Pejic, R.N. and Lee, D.T. (2006).Hypertriglyceridemia.*Journal of American Board of Family Medicine,* 19(3): 310-316.

Pownall, H.J., Ballantyne, C.M., Kimball, K.T., Simpson, S.L., Yeshurun, D. and Gotto, A.M.Jr. (1999). Effect of moderate alcohol consumption on hypertriglyceridemia: a study in the fasting state. *Archives of Internal Medicine,* 159:981-987.

Rader, D.J., Cohen, J., and Hobbs, H.H. (2003). Monogenic hypercholesterolemia: new insights in pathogenesis and treatment. *Journal of Clinical Investment,* 111(12): 1795–803

Ramya Devi, D., Sandhya, P. and Vedha Hari, B.N. (2013). Poloxamer: A novel functional molecule for drug delivery and gene therapy. *Journal of Pharmaceutical Science and Research,* 5(8):159 – 165.

Recommendations of German Society for Clinical and Laboratory investigations (1972).*Clinical Chemistry Clinical Biochemistry,* 10: 290-291.

Redwan, R.M., Larsen, N.L. and Wilson I.A (2003). Simplified procedure for elimination of co-purified contaminant proteins from human colostrum IgA1. *Journal of Egypt-German Society of Zoology,* 40A: 251-60.

Reitman, S. and Frankel, S. (1957).A colourimetric method for the determination of serum glutamate-oxaloacetate and pyruvate transaminases. *American Journal of Clinical Pathology*, 28: 56-63.

Rebolledo, O.R. and Dato, S.M.A. (2005). Postprandial hyperglycemia and hyperlipidemiagenerated glycoxidative stress: its contribution to the pathogenesis of diabetes complications, *European Review for Medical and Pharmacological Sciences,* 9: 191-208.

Rhodes, C.M., Stryer, L. and Tasker, R. (1995).*Biochemistry*.4th Edition. WH Freeman. San Fransisco, pp 280-703.

Richards, W.C., Contreras, J.L., Berecek, K.H. and Schwiebert, E.M. (2008). Extracellular ATP and zinc are co secreted with insulin and activate multiple P2X purinergic receptor channels expressed by islets beta-cells to potentiate insulin secretion. *Purinergic signal* 4:393-405.

Rohilla, A., Gadar, N., Rohilla, S., Dahiyya, A. and Kushnoor, A. (2012).Hyperlipidemia- A deadly pathological condition.*International Journal of Current Pharmaceutical Research,* 4(2): 15-18.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hoekstra, W.G. (1973). Selenium: biochemical role as a component of glutathione Peroxidase. *Science*, 179: 588- 590.

Saad, B., Azaizeh H., Abu-Hijleh, G. and Said S. (2006). Safety of traditional Arab herbal medicine.*Evidence Based ComplementAlternative Medicine,* (3): 433–439.

Sahin, A. and Kucuk, O. (2003). Zinc supplementation alleviates heat stress in laying Japanese quail. *Journal of Nutrition,* 133: 2808-28111.

Sboui, A., Djegham M., Khorchani, T., Hammadi, M.,Barhoumi, K. and Belhadj, O. (2010). Effect of camel milk on blood glucose, cholesterol and total proteins variations in alloxan-induced diabetic dogs.*International Journal Diabetes and Metabolism*, 18:5-11.

Scandinavian Journal of Clinical and Laboratory investigations (1974). 33: 291-306.

Schönfeld, R. G. and Lowellen, C. S. (1964).*Clinical Chemistry and Biochemistry,* 10: 533.

Schuppan, D. and Schattenberg, J.M (2013).Non-alcoholic steatohepatitis: Pathogenesis and novel therapeutic approaches,*Journal of Gastroenterology and Hepatology*,**28** (1): 68–76.

Sefi, M., Fetoui, H., Makni, M. and Zeghal, N. (2010). Mitigating effects of antioxidant properties of *Artemisia campestris* leaf extract on hyperlipidemia,advanced glycation end products and oxidativestress in alloxan-induced diabetic rats, *Food and Chemical Toxicology*, 48(7):1986-1993.

Shah K.N. and Gohil P.V.(2014). Hypothyroidism and Atherosclerosis: From Etiology to Pathophysiology. *Scholars Academic Journal of Pharmacy,* 3(1): 89-96.

Shattat, G.F. (2014). A Review Article on Hyperlipidemia: Types, Treatments and New Drug Targets. *Biomedical and Pharmacology Journal,* 7(2):399-409.

Siri-tarino, P.W., Sun, Q., Hu, F.B. and Krauss R.M. (2010).Saturated fats,carbohydrate and cardiovascular disease.*American Journal of Clinical Nutrition,* 91:502-509.

Smith, S.C. (2004).Heart diseases prevention must get more attention: World Health Federation. *Circulation,* 1: 2.

Sodipo, O.A., Abdulrahman, F. and Sandabe, U. (2012). Effects of the aqueous fruit extract of *Solanum macrocarpum Linn*. on hematological Pparameters of chronic Triton-induced hyperlipidemic rats. *Journal Physiology and Pharmacology Advances,* 2(2): 122-132.

Soutar, K.A. and Naoumova, P.S. (2007).Mechanism of Diseases;Genetic cause of familial hyperlipidemia.*Nature Clinical Practice Cardiovascular Medicine*, 4:214-225.

Stein, E.A. (1998). Cerivastatin in Primary Hyperlipidemia: A Multicenter Analysis of Efficacy and Safety. *American Journal of Cardiology* 82:40J-46J.

Teramoto, T., Sasaki, J., Ishibashi, S., Birou, S., Daida, H., Dohi, S., Egusa, G., Hiro, T., Hirobe, K., Lida, M., Kihara, S., Kinoshita, M., Maruyama, C., Ohta, T., Okamura, T.,Yamashita, S., Yokode, M. and Yokote, K., (2013).Familial Hypercholesterolemia; Executive Summary of the Japan Artherosclerotic Society Guidelinesfor the diagnosis and Prevention of Cardiovascular Diseases. *Journal of Artherosclerosis and Thrombosis,* (21)1:6-10

Thalia M. T., Amanda S. S., Luana A. C., Gabriela V. M. M.A., Leda F., Patricia, F.L.(2015). Oxidative stress in the pathophysiology of metabolic syndrome: which mechanisms are involved?.*Brazil Journal of Pathology and Medical Laboratory,* 51(4): 231-239.

Thomas, S and Rich, M.W. (2007).Epidemiology, Pathophysiology, and prognosis of heart failure in the elderly.*Clinical Geriatric Medicine*, 23(1): 1-10.

Trevisan, R., Dodesini A.r. and Lepore, G. (2006). Lipids and Renal Disease.*Journal of the American Society of Nephrology*, 17: S145–S147.

Trinder, P. (1951). A rapid method for the determination of sodium in serum. *Analyst,* 76: 596-599.

Van de Wiel, A. Bessembinders, K. and Wielders, J. (2011). Severe Hypertriglyceridemia influenced by Alcohol. *Alcohol and Alcoholism,* 46(2):113-116.

Varady K.A. and Jones, P.J.H. (2005).Combination Diet and Exercise Interventions for the Treatment of Dyslipidemia: an Effective Preliminary Strategy to Lower Cholesterol Levels?.*The Journal of Nutrition*, 1829-1835.

Victor, D. S., Dorcas, B. J. and Sunday E. A. (2014). Ethanolic extracts of *Vitex doniana* possesses hepatocuractive property in Poloxamer induced hyperlipidemia. *Science Research*, 2(3): 49-54.

Wangoh, J., Farah, Z. and Puhan, Z. (1998).Composition of milk from three camel (*Camelus dromedarius*) breeds in Kenya during lactation.*Milchwissenschaft*, 53:136–139.

Weinreich, M. and Frishman, W.H. (2014).Antihyperlipidemic therapies targeting PCSK9.*Cardiology in Review*, 22(3):140-146.

Weintrub, H.S. (2014). Overview of prescription of omega-3 fatty acid products for hypertriglyceridemia.*Postgraduate Medicine,* 126(7): 7-18.

Woo, J., Jae, H.R. and Han, J.O. (2010). The Influence of Sam-Chil-Geun (Panax Notoginseng) on the serum lipid levels and inflammations of rats with hyperlipidemia induced by Poloxamer-407. *Yonsei Medical Journal*, 51(4):504-510.

Woo, S.J., Yoon, S.K. and In, C.S. (2012). Antioxidant and lipid-lowering effects of *Artemisia capillaris* on a rat model of hyperlipidemia. *Journal of Korean Oriental Medicine,* 33(2):11-14.

Wu, L. and Parhofer, K.G.(2014). Diabetic dyslipidemia.*Metabolism:clinical and experimental,* 63(12): 1469-1479.

Wout, Z.G., Pec, E.A., Maggiore, J., *et al.* (1992). Poloxamer 407-mediated changes in plasma cholesterol and triglycerides following intraperitoneal injection to rats.*Journal of Parenteral Science Technology*, 46:192–200.

Yadav, A.K., Kumar, R., Priyadarshini, L. and Singh, J. (2015). Composition and medicinal properties of camel milk: A Review. *Asian Journal of Dairy and Food Research,* 34(2): 83-91.

Yateem, A., Balba, M.T., Al-Surrayai, T., AlMutairi, B. and Al-Daher, R.(2008). Isolation of lactic acid bacteria with perobiotic protential from camel milk.*International Journal of Dairy Science* 3: 194-199.

Yoon, N.Y., Kim, H.R., Chung, H.Y. and Choi, J.S. (2008). Anti-hyperlipidemic effect of an edible brown algae, *Ecklonia stolonifera*, and its constituents on poloxamer 407-induced hyperlipidemic and cholesterol-fed rats. *Archives of Pharmaceutical Research,* 31:1564-71.

Zhang, Y.H., An, T., Zhang, R.C., Zhou, Q., Huang, Y. and Zhang J. (2013). Very high fructose intake increases serum LDL cholesterol and total cholesterol: a meta-analysis of controlled feeding trials. *The Journal of Nutrition,* 143(9): 1391-1398.