

EFFECTS OF JAPANESE QUAIL (*Coturnix japonica*) EGG SUPPLEMENTATION ON
POLOXAMER 407-INDUCED HYPERLIPIDEMIC WISTAR RATS

BY

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OCTOBER 2014

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

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE
DEGREE OF MASTER OF SCIENCE IN BIOCHEMISTRY

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

OCTOBER 2014

DECLARATION

I hereby declare that this thesis entitled “**Effects of Japanese Quail (Coturnix japonica) Egg Supplementation on Poloxamer 407-Induced Hyperlipidaemic Wistar Rats**” is a record of my own research work under the supervision of Dr. K. M. Anigo and Prof E Onyike. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented elsewhere for the award of a degree or diploma in any other institution.

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CERTIFICATION

This thesis titled “**Effects of Japanese Quail (*Coturnix japonica*) Egg Supplementation on Poloxamer 407-Induced Hyperlipidaemic Wistar Rats**” by ADENIYI, Oluwamayowa Tolulope meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to God Almighty for his provision, guidance and sustenance of life. Also to my late father Dr J. B. Adeniyi, wish you were here, and to my lovely mother, Prof. (Mrs) E. F. Adeniyi, I say thank you for your love, care, provision and prayers.

ACKNOWLEDGEMENT

I wish to express my profound gratitude and appreciation to God for his love, mercy, protection and provision throughout the period of this research. My gratitude goes to my supervisors, Dr. K.M. Anigo for his thorough supervision, patience, kindness, high level of understanding and advice and also Prof. E. Onyike for her guidance, supervision and advice.

I wish to acknowledge the Head, Department of Biochemistry, A.B.U Zaria, Prof. I. A. Umar and other lecturers like Prof. S.E Atawodi, Prof D.A Ameh, Dr. S.Ibrahim, Mr N. Habila, Mr Abbas, Mr. Y. Apeh, to mention a few for their learned guidance and contribution towards the completion of this work. I wish to thank all other lecturers and staff of the Department of Biochemistry, A.B.U. Zaria for their advice, technical assistance, and provision of needed research materials. With gratitude from my heart, I wish to say a special thanks to Mr U. Ndidi and Dr (Mrs) M. Abdulazeez for their care, unconditional support and constructive suggestion throughout my work, may God reward you. Very special thanks to the staff of Department of Pharmacology and therapeutics, A.B.U. Zaria, Mr J. T. Ose, Alhaji Yau, Mal Nasiru and Mal. Muazu for their assistance and technical support.

I want to say a big thank you to my beloved Ms. Effah Sarah for her love and support throughout this research making it a success, and my siblings Mrs Tomilayo Oladipo and family, Adedayo Adeniyi and Adebayo Adeniyi for your encouragement during the course of this study. To my friends and coursemates, Samuel Atabo, Malik Salman, Aliyu, Oche, Jerry, Tayo, Femi, Chidi, Cj, Kingsley, Bukky, Dumni, Ene Adejor, Ogechi Nkeonye, Oluchi, Stella, Dr James and Mrs Amaya Habila to mention a few. I say thank you for your support.

ABSTRACT

The effects of Japanese quail egg on lipid profile and haematological parameters, antioxidant effect, liver and kidney functions were investigated in poloxamer 407-induced (P407) hyperlipidaemic wistar rats. Sixty rats were grouped into ten. Each group consisting of six rats each of mixed sexes for the purpose of preventive and curative studies. Groups 1, 2, 3 and 4, served as normal control group, two positive control groups, and hyperlipidaemic control group respectively. In the preventive study, groups 5, 6 and 7 were administered Atorvastatin, 0.64ml/kg and 10ml/kg of quail egg respectively for 19 days before the induction of hyperlipidaemia with poloxamer 407 and sacrificed 48 hr later. In the curative study, groups 8, 9 and 10 were induced with hyperlipidaemia before administering Atorvastatin, 0.64ml/kg and 10ml/kg of quail egg respectively and sacrificed 48 hr after. The parameters were determined using standard Methods. In the preventive study, total cholesterol, triacylglycerol, HDL-cholesterol and LDL-cholesterol, total protein, total and conjugated bilirubin, albumin, urea and creatinine level show no significant ($p>0.05$) difference between P407-induced egg treated groups and the P407-induced hyperlipidaemic control group. The atherogenic risk predictor indices (HDL-c/TC, LDL-c/HDL-c), the serum levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) show no significant ($p>0.05$) difference in the P407-induced egg treated group compared to the P407-induced hyperlipidaemic control groups. The activity of catalase (CAT) and superoxide dismutase (SOD) was significant ($p<0.05$) higher in the P407-induced egg treated group compared to the P407-induced hyperlipidaemic control group. The level of malonyl dialdehyde (MDA) was significantly ($p<0.05$) lower in the P407-induced egg treated groups compared to the hyperlipidaemic

control group. In the curative study, total cholesterol, triacylglycerol and HDL-cholesterol, total protein, total and conjugated bilirubin, albumin, urea, creatinine, ALP, AST levels and atherogenic risk predictor indices (HDL-c/TC, LDL-c/HDL-c) were not significantly ($p>0.05$) different between the P407-induced egg treated groups and the P407-induced hyperlipidaemic control group. However, ALT and MDA levels were significantly ($p<0.05$) lower in the P407-induced egg treated groups compared to the P407-induced hyperlipidaemic control group. The activities of SOD and CAT were significantly ($p<0.05$) higher in the P407-induced egg treated groups compared to the P407-induced hyperlipidaemic control group. Therefore, the quail egg possesses properties that prevent lipid peroxidation and may have potential benefits in the management of oxidative stress associated with the hyperlipidaemia.

TABLE OF CONTENTS

Title	Page
Cover Page - - - - -	i
Title Page - - - - -	ii
Declaration - - - - -	iii
Certification - - - - -	iv
Dedication - - - - -	iv
Acknowledgment - - - - -	vi
Abstract - - - - -	vi
Table of Contents - - - - -	viii
List of Table - - - - -	xiv
List of figure - - - - -	xv
Abbreviations, Glossaries and Symbols - - - - -	xvi
CHAPTER ONE - - - - -	1
1.0 Introduction - - - - -	1
1.1 Statement of the Research Problem - - - - -	3
1.2 Justification for the Study - - - - -	3
1.3 Aims and Objectives - - - - -	4
1.3.1 Aim - - - - -	4
1.3.2 Specific objectives - - - - -	4

CHAPTER TWO	-	-	-	-	-	-	-	-	-	6
2.0 Literature Review	-		-	-	-	-	-	-	-	6
2.1 Japanese quail	-	-	-	-	-	-	-	-	-	6
2.2 Quail egg	-	-	-	-	-	-	-	-	-	7
2.3 Claims	-	-	-	-	-	-	-	-	-	8
2.4 Dyslipidaemia	-	-	-	-	-	-	-	-	-	9
2.5 Classification	-	-	-	-	-	-	-	-	-	10
2.5.1 Primary hyperlipidaemia			-	-	-	-	-	-	-	10
2.5.2 Secondary hyperlipidaemia				-	-	-	-	-	-	12
2.6 Signs and symptoms	-	-	-	-	-	-	-	-	-	13
2.7 Management of hyperlipidaemia			-	-	-	-	-	-	-	13
2.8 Hyperlipidaemia and Cardiovascular diseases						-	-	-	-	14
2.9 Artherosclerosis	-	-	-	-	-	-	-	-	-	15
2.10 Poloxamer	-	-	-	-	-	-	-	-	-	17
CHAPTER THREE	-	-	-	-	-	-	-	-	-	20
3.0 MATERIALS AND METHODS			-	-	-	-	-	-	-	20

3.1 Materials	-	-	-	-	-	-	-	-	-	20
3.1.1 Chemical and reagents	-	-	-	-	-	-	-	-	-	20
3.1.2 Sample collection	-	-	-	-	-	-	-	-	-	20
3.1.3 Experimental animals	-	-	-	-	-	-	-	-	-	20
3.1.4 Experimental design	-	-	-	-	-	-	-	-	-	21
3.2 Methodology	-	-	-	-	-	-	-	-	-	22
3.2.1 Egg preparation	-	-	-	-	-	-	-	-	-	22
3.2.2 Induction of hypercholesterolemia	-	-	-	-	-	-	-	-	-	22
3.2.3 Animal grouping and treatment	-	-	-	-	-	-	-	-	-	22
3.2.4 Collection of blood samples and organs	-	-	-	-	-	-	-	-	-	24
3.3 Determination of Biochemical Parameters-	-	-	-	-	-	-	-	-	-	23
3.3.1 Determination of serum total cholesterol (TC)	-	-	-	-	-	-	-	-	-	22
3.3.2 Determination of serum triacylglycerol (TG)	-	-	-	-	-	-	-	-	-	25
3.3.3 Determination of serum high density lipoprotein-cholesterol (HDL-c)	-	-	-	-	-	-	-	-	-	26
3.3.4 Determination of serum low density lipoprotein-cholesterol (LDL-c)	-	-	-	-	-	-	-	-	-	28
3.3.5 Determination of serum alkaline phosphatase (ALP)	-	-	-	-	-	-	-	-	-	28
3.3.6 Determination of serum alanine aminotransferase (ALT)	-	-	-	-	-	-	-	-	-	29

3.3.7	Determination of serum aspartate aminotransferase (AST)	-	-	-	-	-	-	29
3.3. 8	Determination of total protein (TP)	-	-	-	-	-	-	30
3.3.9	Determination of serum albumin (ALB)	-	-	-	-	-	-	31
3.3.10.1	Determination of serum total bilirubin (TB)	-	-	-	-	-	-	32
3.3.10.2	Determination of serum conjugated bilirubin (CB)	-	-	-	-	-	-	33
3.3.11	Determination of serum creatinine	-	-	-	-	-	-	33
3.3.12	Determination of serum urea concentration-	-	-	-	-	-	-	34
3.3.13	Determination of catalase	-	-	-	-	-	-	35
3.3.14	Determination of superoxide dismutase	-	-	-	-	-	-	36
3.3.15	Determination of malondialdehyde (MDA) level	-	-	-	-	-	-	37
3.4	Haematological Assay	-	-	-	-	-	-	38
3.4.1	Determination of packed cell volume (PCV)	-	-	-	-	-	-	38
3.4.2	Differential count determination	-	-	-	-	-	-	38
3.5	Statistical Analysis	-	-	-	-	-	-	39

CHAPTER FOUR	-	-	-	-	-	-	-	-	-	40
4.0 RESULTS	-	-	-	-	-	-	-	-	-	40
4.1.1 Effects of japanese quail egg on lipids profile	-	-	-	-	-	-	-	-	-	40
4.1.2 Effects of japanese quail egg on serum atherogenic risk predictor indices	-	-	-	-	-	-	-	-	-	45
4.1.3 Effects of japanese of quail egg liver damage and function parameters	-	-	-	-	-	-	-	-	-	46
4.1.4 Effects of japanese of quail egg on kidney function parameters	-	-	-	-	-	-	-	-	-	54
4.1.5 Effects of japanese of quail egg on some haematological parameters	-	-	-	-	-	-	-	-	-	56
4.1.6 Effects of japanese of quail egg on anti-oxidant status	-	-	-	-	-	-	-	-	-	58
CHAPTER FIVE	-	-	-	-	-	-	-	-	-	62
5.0 Discussion	-	-	-	-	-	-	-	-	-	62
CHAPTER SIX	-	-	-	-	-	-	-	-	-	69
6.0 SUMMARY, CONCLUSION AND RECOMMENDATION-	-	-	-	-	-	-	-	-	-	69
6.1 Summary	-	-	-	-	-	-	-	-	-	69
6.2 Conclusion	-	-	-	-	-	-	-	-	-	70
6.3 Recommendation-	-	-	-	-	-	-	-	-	-	70
References	-	-	-	-	-	-	-	-	-	71

LIST OF TABLES

Table	Title	Page
Table 4.1:	Effect of Japanese Quail Egg on Lipid Profile of P407-Induced Hyperlipidaemic Wistar Rats - - - - -	44
Table 4.2:	Effect of Japanese Quail Egg on Serum Atherogenic Risk Predictor Indices of P407-Induced Hyperlipidaemic Wistar Rats - -	47
Table 4.3:	Effect of Japanese Quail Egg on Liver Function Parameters P407-Induced Hyperlipidaemic Wistar Rats - - - -	50
Table 4.4:	Effect of Japanese Quail Egg on Liver Function Parameters of P407-Induced Hyperlipidaemic Wistar Rats - - -	53
Table 4.5:	Effect of Japanese Quail Egg on Kidney Function Parameters of P407-Induced Hyperlipidaemic Wistar Rats - - -	56
Table 4.6:	Effect of Japanese Quail egg on Some Haematological Parameters of P407-Induced Hyperlipidaemic Wistar Rats - - -	58
Table 4.7:	Effect of Japanese Quail Egg on Antioxidant Levels of P407-Induced Hyperlipidaemic Wistar Rats - - - - -	61

LIST OF FIGURES

Fig 3.1: Experimental Design	-	-	-	-	-	-	21
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ABBREVIATIONS

ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
CAD	Coronary Artery Disease
CETP	Cholesteryl Ester Transfer Protein
CK	Creatinine kinase
CRP	C-reactive protein
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DPPH	2, 2-diphenyl-1-picrylhydrazyl
FCH	Familial combined hyperlipoproteinemia
FDA	Food and Drug Agency
FFA	Free Fatty Acids
HBP	High Blood Pressure
HDL-c	High-Density Lipoprotein cholesterol

HL	Hepatic Lipase
HLP	Hyperlipoproteinemia
HMG-CoA	3-Hydroxy-3- Methylglutaryl-CoA
IDL	Intermediate Density Lipoprotein
LCAT	Lecithin Cholesterol Acyltransferase
LDL-c	Low-Density Lipoprotein cholesterol
LPL	Lipoprotein Lipase
MI	Myocardial Infarction
NCDs	Non-Communicable Diseases
NCEP	National Cholesterol Education Programme
OGT	O-GlcNAc Transferase
P407	Poloxamer 407
SOD	Superoxide Dismutase
SBP	Systolic Blood Pressure
TAG	Triacylglycerides
TC	Total Cholesterol
TLC	Therapeutic Lifestyle Changes

VLDL Very Low-Density Lipoprotein Cholesterol

WHO World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of death for both men and women among all racial and ethnic groups (Smith, 2004). CVDs including coronary heart disease and stroke are the leading cause of mortality, accounting for nearly 50% of all deaths in the developed world (Mendis *et al.*, 2011). They account for an estimated 12% of all deaths in Nigeria (WHO, 2011). If the current trend continues, the future will even be more challenging considering that globalization and wide spread of western diet to the developing world leading to increase in the rates of obesity and hyperlipidaemia in developing regions (Lim *et al.*, 2012). Therefore, hyperlipidaemia and its associated CVDs represent one of the greatest worldwide economic, social and medical challenges (Olshanky *et al.*, 2005). The elevation of serum total cholesterol and more importantly low density lipoprotein (LDL) cholesterol have been implicated as a primary risk factor for cardiovascular disease (Adebayo *et al.*, 2006).

Control of cholesterol levels through therapeutic drugs have significantly reduced the risk of developing atherosclerosis and associated CVDs (Stacy and Egger, 2006; Ray *et al.*, 2006; Khush and Waters, 2006). Notably, statins, a class of cholesterol-lowering drugs inhibiting cholesterol synthesis, have been most widely prescribed for treating hypercholesterolemia and reducing CVDs (Ray *et al.*, 2006; Khush and Waters, 2006). However, adverse effects associated with therapeutic drugs, such as myopathy, liver damage and potential drug-drug interaction have been reported (Trifiro, 2006; Kiotsis *et al.*,

2007). Therefore, development of additional therapies for controlling cholesterol levels is justified, especially for those with better safety profile.

Hypercholesterolemia is the presence of high levels of cholesterol in the blood (Ghatak and Asantha, 1995). It is not a disease but a metabolic derangement that can lead to diseases, especially cardiovascular disease. It is closely related to the term “hyperlipidaemia” that is, elevated levels of lipoproteins in the blood (Ghatak and Asantha, 1995).

The Japanese quail belongs to the order *Galiformes*, family *Phasidae*, genus *Coturnix* and species *japonica*, different from the common quail “*Coturnix coturnix*” which is also known as Coturnix quail, pharaoh's quail, stubble quail and eastern quail (Thear, 1998; Mizutani, 2003). Species or subspecies of the genus *Coturnix* is native to all continents except the Americas (Lee *et al.*, 2000). It is claimed that a Japanese Emperor obtained relief from tuberculosis after eating quail meat, and this led to selection of domestic quail for meat and egg production in Japan in the latter part of the nineteenth century (Howes, 1964). However, this animal finds its true economic and commercial value in its egg production, as domesticated lines of the Japanese quail can lay up to 300 eggs a year at an incredibly efficient feed to egg conversion ratio (Hubrecht and Kirkwood, 2010). The nutritional value of quail eggs is much higher than those offered by other eggs. They are rich sources of antioxidants, minerals, and vitamins, and give a lot of nutrients (Lalwani, 2011). Quail eggs strengthen the immune system, promote memory health, increase brain activity and stabilize the nervous system (Tunsaringkarn, *et al.*, 2013). They help relieve anaemia by increasing the level of haemoglobin in the body while removing toxins and heavy metals (Tunsaringkarn, 2012). The Japanese quail egg have being claimed to improve metabolism,

combat stress, helps in the treatment of obesity, asthma and various forms of allergies (Truffier, 1978).

1.1 Statement of the Research Problem

Cardiovascular disease is the leading cause of death accounting for nearly 50% of all deaths in the western world (Mendis *et al.*, 2011). Hyperlipidaemia contributes significantly in the manifestation and development of atherosclerosis and other coronary heart diseases (CHDs) (Grundy *et al.*, 1999). Due to influx of western diet to the developing world there has been increase in the rates of obesity and hyperlipidaemia in Africa (Lim *et al.*, 2012). High levels of cholesterol particularly total cholesterol, triglycerides and low density lipoprotein cholesterol are mainly responsible for the onset of CHDs (Choudhary *et al.*, 2005). Control of cholesterol levels through therapeutic drugs (notably statins), have significantly reduced the risk of developing cardiovascular disease. However, adverse effects abound with therapeutic drugs, such as myopathy and liver damage (Neuvonen *et al.*, 2006). Therefore, development of additional therapies for controlling cholesterol levels is important, especially for those with better safety profile and lower cost. People are using other remedies such as quail eggs in trying to battle health challenges.

1.2 Justificaton

It has being reported that quail eggs have anti-diabetic, anti-cancer and anti-hyperlipidaemic properties that help to increase appetite, stimulate digestion, and ameliorate constipation, muscle weakness, dizziness, and fatigue (Tunsaringkarn, *et al.*, 2013). Furthermore, the egg also claimed to ameliorate nervous system disorders, improve skin colour and strengthen hair, and improves liver function, diseases of the heart, liver,

kidney, gastro-duodenal ulcer and alopecia. (Truffier, 1978). There is the general belief that consumption of quail egg can help in the management of cardiovascular diseases, but there is no documented work on the consumption of Japanese quail egg on some of the factors that are implicated in the etiology of cardiovascular diseases. With the increasing incidence of cardiovascular diseases in the urban and rural population throughout the world, there is need for development of indigenous, inexpensive sources for anti-hyperlipidaemic crude or purified drugs (Venkatesh *et al.*, 2007). This, therefore, necessitates the need for research into the claims which had led to increase consumption of quail egg in Nigeria in the recent time and the need to find alternative medicine or therapeutic agents that retain therapeutic efficacy, and can be taken for long durations, with minimal or no side effects.

1.3 Aim and Objectives

1.3.1 Aim

The aim of this study was to determine the effect of Japanese quail eggs consumption on haematological and biochemical parameters in poloxamer 407-induced hyperlipidaemic wistar rats.

1.3.2 Specific objectives

- (i) To evaluate the effects of the Japanese quail egg on lipid profile poloxamer 407-induced hyperlipidaemic rats.
- (ii) To determine the effects of the Japanese quail egg on some liver damage indicators (alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) total protein, albumin, total bilirubin and conjugated bilirubin) in poloxamer 407-induced hyperlipidaemic rats.

- (iii) To determine the effects of the Japanese quail egg on some kidney function parameters (creatinine and urea) in poloxamer 407-induced hyperlipidaemic rats.
- (iv) To determine the effect of the Japanese quail egg on some haematological parameters in poloxamer 407-induced hyperlipidaemic rats.
- (v) To assess the effect of the Japanese quail egg on levels of some antioxidants (Superoxide dismutase, catalase) and in poloxamer 407-induced hyperlipidaemic rats.
- (vi) To determine the effect of the Japanese quail egg on lipid peroxidation by measuring the level of Malondialdehyde produced in poloxamer 407-induced hyperlipidaemic rats.

CHAPTER TWO

2.0

LITERATURE REVIEW

2.1 Japanese Quail.

The Japanese quail, also known as Coturnix quail (*Coturnix japonica*) is a species of Old World quail found in East Asia. First considered a sub-species of the Common quail, it was distinguished as its own species in 1983 (Hubrecht, and Kirkwood 2010). The global population size has not been quantified, but the species has been reported to be fairly common (Del Hoyo *et al.*, 1994; Fuller *et al.*, 2000). Populations of the Japanese quail are known to mainly inhabit East Asia and Russia. This includes India, Korea, Japan, and China (Pappas, 2013). This quail has also been found to reside in many parts of Africa, including Tanzania, Malawi, Kenya, Namibia, Madagascar, and the area of the Nile River Valley extending from Kenya to Egypt (Pappas, 2013).

Breeding sites of the Japanese quail are largely localized to East and Central Asia (Barilani *et al.*, 2005) (Purgcerver *et al.*, 2007) in such areas as southeastern Siberia, northern Japan, and the Korean Peninsula. However, it has also been observed to breed in some regions of Europe, as well as Turkey (Pappas, 2013). The earliest records of domesticated Japanese quail populations are from 12th century Japan; however, there is evidence that the species was actually domesticated as early as the 11th century (Hubrecht and Kirkwood, 2010). These birds were originally bred as songbirds, and it is thought that they were regularly used in song contests (Hubrecht and Kirkwood, 2010., Mills *et al.*, 1997). In the early 1900s, Japanese breeders began to selectively breed for increased egg production (Baumgartner, 1994). By 1940, the industry surrounding quail eggs was flourishing.

Unfortunately, the events of World War II led to the complete loss of quail lines bred for their song type, as well as almost all of those bred for egg production. After the war, the few enduring quail left were used to rebuild the industry and all current commercial and laboratory lines today are considered to have originated from this population of quail (Mills *et al.*, 1997).

2.2 The egg

During the 17th century, the Chinese pharmacologist Li Shi Chen discovered, in addition to the nutritional value of the eggs and meat of the Japanese quail, their medicinal value as well (Lin, 2012) Other Japanese and Russian scientists and doctors tasted and confirmed this discovery (Lin, 2012). Because of their extraordinary nutritional and medicinal properties, they are being used with more and more success in Europe and America as well as in the Far East (Dowarah and Sethi, 2014). These eggs are much higher in vitamins and minerals than hen's eggs. They are especially rich in the essential amino acids (methionine, lysine, phenolalanine) (Lalwani, 2011).

Nutritional value of the eggs contain an average of: (per 100 g whole liquid egg) protein 13.1 g, fat 11.2 g, minerals 1.1 g, energy (kcal) 158, calcium 59 mg, phosphorus 220 mg, iron 3.8 mg, vitamin A 300 IU, vitamin B1 0.12 mg, vitamin B2 0.85 mg, niacin 0.10 mg, vitamin B6, biotin, folic and panthotenate Acid, enzymes (peptidase, catalase, glycosidase) (Lalwani, 2011). The average egg weight of a laying flock increases as the birds get older mainly due to physical and physiological changes (Oluyemi and Roberts, 1979).). It has been reported that the hen's age has a constant and significant effect on proportion of egg weight, length and width of the eggs, yolk, egg white and egg shell in total egg mass;

similarly yolk, albumen weight and height increased significantly with age of the bird (Rossi and Pompei, 1995; Danilov, 2000; Luquetti *et al.*, 2004; Akpa *et al.*, 2006 and Egahi *et al.*, 2011). Furthermore there is an increase in repeatability of egg quality traits with linear increase in age of laying Japanese quails (Akpa *et al.*, 2008).

2.3 Claims

Egg consumption is a popular choice for good nutrients which are obtained from varieties of chicken, duck, roe, and caviar. But by a wide margin, the egg most commonly consumed is the chicken egg, typically unfertilized (Applegate, 2000). Besides, a lot of people especially in Asian countries consume quail eggs because they are packed with vitamins and minerals (Tunsaringkarn, *et al.*, 2013). Even with their small size, their nutritional value is three to four times greater than chicken eggs (Tunsaringkarn, *et al.*, 2013). Regular consumption of quail eggs helps fight against many diseases as it is a natural combatant against digestive tract disorders such as stomach ulcers (Truffier, 1978). Quail eggs strengthen the immune system, promote memory health, increase brain activity and stabilize the nervous system (Truffier, 1978). They help with anaemia by increasing the level of haemoglobin in the body while removing toxins and heavy metals (Tunsaringkarn, 2012). Chinese use quail eggs to help treat tuberculosis, asthma, and even diabetes (Ameh, *et al.*, 2013). Quail eggs can help prevent sufferer of kidney, liver, or gallbladder stones and remove these types of stones (Arome, *et al.*, 2013). Quail's eggs are rich sources of antioxidants, minerals, and vitamins, and give a lot of nutrients than many other foods (Lalwani, 2011). There are further claims which include:

1. Quail eggs are used to increase appetite, stimulate digestion, increase vitality and treat constipation, nausea, muscle weakness, dizziness, and fatigue. They also treat insomnia, nervous system disorders, dry mouth, and paleness, muscle pain, peeling skin, hair loss, improves liver function, diseases of the heart, liver, kidney, stomach, thyroid, anaemia (Truffier, 1978).
2. They treat asthma, (Guillery, 1980), heart disease, nervous system disorder and cancerous tumour. Lysozyme contained in eggs is capable of destroying the bacterial cell membrane, which negatively affects the cancerous tumour (Truffier, 1978).
3. In contrast to the chicken, quail eggs do not cause allergies, but rather suppress it (Truffier, 1978). Quail eggs increase haemoglobin, and intensively remove radionuclides from the body (Guillery, 1980), Quail eggs can dissolve and displace stones from the kidney, liver, and gall bladder (Truffier, 1978).
4. The quail meat and eggs can help avoid adverse effects of stress and overwork (Truffier, 1978).
5. Eggs are especially useful for children to enhance development, both physically and mentally (Truffier, 1978). A child that eats 2 quail eggs a day has a better memory, a strong nervous system, sharp vision and is better developed and less sick. It is no wonder the Japanese students eat two quail eggs per day (Truffier, 1978).
6. They give good results in treating infertility, epilepsy, allergies, and all kinds of eczema. (Truffier, 1978).

2.4 Dyslipidemia

Dyslipidemia is an abnormal amount of lipids (e.g. cholesterol and/or fat) in the blood (Pooja *et al.*, 2013). Most dyslipidemias are hyperlipidaemias; that is, an elevation of lipids in the blood. This is often due to diet and lifestyle (Bisht *et al.*, 2012). Prolonged elevation of insulin levels can also lead to dyslipidemia (Bisht *et al.*, 2012). Likewise, increased levels of O-GlcNAc transferase (OGT) may cause dyslipidemia (Fredrickson and Lees, 1965).

2.5 Classification

Hyperlipidaemias are divided into primary and secondary subtypes. Primary hyperlipidaemia is usually due to genetic causes (such as a mutation in the LDL receptor protein or apolipoprotein B (ApoB), which is the part of LDL that binds with the receptor), while secondary hyperlipidaemia arises due to other underlying causes such as diabetes or underlying disorder that leads to alterations in plasma lipid and lipoprotein metabolism (Chait and Brunzell, 1990).

2.5.1 Primary (familial) hyperlipidaemia

Primary (familial) hyperlipidaemias are classified according to the Fredrickson classification which is based on the pattern of lipoproteins on electrophoresis or ultracentrifugation (Fredrickson and Lees, 1965). It was later adopted by the World Health Organization (WHO, 1972). It does not directly account for HDL, and it does not distinguish among the different genes that may be partially responsible for some of these conditions (KishorJain, 2007). The various types of primary hyperlipidaemias include:

(a) Hyperlipoproteinaemia type I

Type I Hyperlipoproteinaemia exists in several forms:

Type 1a, which is due to a deficiency of lipoprotein lipase (LPL) or altered apolipoprotein C2, resulting in elevated chylomicrons, the particles that transfer fatty acids from the digestive tract to the liver.

Type 1b, characterized by a deficiency in Familial apoprotein CII, a condition caused by a lack of lipoprotein lipase activator (Yamamura *et al.*, 1979).

Type 1c, which is characterized by Chylomicronemia due to circulating inhibitor of lipoprotein lipase (James *et al.*, 2006).

(b) Hyperlipoproteinaemia type II

Hyperlipoproteinaemia type II, (HLP II) by far the most common form, is further classified into type IIa and type IIb, depending mainly on whether there is elevation in the triglyceride level in addition to LDL cholesterol (Harikumar *et al.*, 2013).

(b1) Type IIa

This may be sporadic (due to dietary factors), polygenic, or truly familial as a result of a mutation in the LDL receptor gene on chromosome 19 (Nirosha *et al.*, 2014). The familial form is characterized by tendon xanthoma, xanthelasma and premature cardiovascular disease (Shruthi, *et al.*, 2014). HLP IIa is a rare genetic disorder characterized by increased levels of LDL cholesterol in the blood due to the lack of uptake (no Apo B receptors) of LDL particles (Burnett and Hooper, 2008). These individuals may present with a very unique set of physical characteristics such as: Xanthelasma's (yellow deposits of fat

underneath the skin often presenting in the nasal portion of the eye), tendon and tuberous xanthomas, Arcus juvenilis (the graying of the eye often characterized in older individuals), Arterial bruits, claudication, and of course atherosclerosis. (Yamamura, *et al.*, 1979).

(b2) Type IIb

The high VLDL levels are due to over production of substrates, including triglycerides, acetyl CoA, and an increase in B-100 synthesis (Shruthi, *et al.*, 2014). They may also be caused by the decreased clearance of LDL. It is also known as familial combined hyperlipoproteinaemia (FCH) (Dzimiri, 2012), characterized by lysosomal acid lipase deficiency, often called (Cholesteryl ester storage disease)

(c) Hyperlipoproteinaemia type III

This form is due to high chylomicrons and IDL (intermediate density lipoprotein) in the blood. It is also known as *broad beta disease* or *dysbetalipoproteinemia*, the most common cause of this form is a defect in the ApoE E2/E2 genotype synthesis ((Fung *et al.*, 2011).

(d) Hyperlipoproteinaemia type IV

It is also known as familial hypertriacylglycerolemia, an autosomal dominant condition characterized by increased VLDL production and decreased elimination from the blood. It is a common type and can also result from metabolic/endocrine diseases, renal disease, liver diseases, ethanol use/abuse, pregnancy and drug use (Boman *et al.*, 1975).

(e) Hyperlipoproteinaemia type V

Hyperlipoproteinaemia type V is also known as mixed Hyperlipoproteinaemia, familial or mixed hyperlipidaemia, (Anonymous, 2013). It is very similar to type I, but with

high VLDL in addition to chylomicrons (Mattar and Obeid, 2009)

It is also associated with glucose intolerance and hyperuricemia. Combined hyperlipidaemia (also known as "Multiple-type Hyperlipoproteinaemia") is a commonly occurring form of hypercholesterolemia characterised by increased LDL and triglyceride concentrations, often accompanied by decreased HDL. (Mattar and Obeid, 2009)

2.5.2 Acquired (secondary) hyperlipidaemia

Acquired hyperlipidaemias (also called secondary dyslipoproteinemias) often mimic primary forms of hyperlipidaemia and can have similar consequences (Chait, and Brunzell, 1990). They may result in increased risk of premature atherosclerosis or, when associated with marked hypertriglyceridemia, may lead to pancreatitis and other complications of the chylomicronemia syndrome (Chait and Brunzell, 1990). The most common causes of acquired hyperlipidaemia are; diabetes mellitus, use of drugs such as diuretics, beta blockers, and estrogens. Other conditions leading to acquired hyperlipidaemia include: hypothyroidism, renal failure, nephrotic syndrome, alcohol usage, some rare endocrine disorders and metabolic disorders. (Chait and Brunzell, 1990). Another acquired cause of hyperlipidaemia, although not always included in this category, is postprandial hyperlipidaemia, a normal increase following ingestion of food (Pesek, *et al.*, 2011)

2.6 Signs and symptoms of hyperlipidaemia

Hyperlipidaemia usually has no noticeable symptoms and tends to be discovered during routine examination or evaluation for atherosclerotic cardiovascular disease (Nirosha, *et al.*, 2014). However, deposits of cholesterol (known as xanthomas) may form under the skin (especially around the eyes or along the Achilles tendon) in individuals with familial forms of the disorder or in those with very high levels of cholesterol in the blood (Tsouli *et al.*,

2005). Individuals with hypertriglyceridaemia may develop numerous pimple-like lesions across their body (Rader *et al.*, 2003). Extremely high levels of triglycerides may also result in pancreatitis, a severe inflammation of the pancreas that may be life-threatening (Rader *et al.*, 2003).

2.7 Management of hyperlipidaemia

For treatment of type II hyperlipidaemia, dietary modification is the initial approach but many patients require treatment with statins (HMG-CoA reductase inhibitors) to reduce cardiovascular risk (Mattar and Obeid, 2009). If the triglyceride level is markedly raised, fibrates may be preferable due to their beneficial effects (Lüscher, 2011). Combination treatment of statins and fibrates, while highly effective, causes a markedly increased risk of myopathy and rhabdomyolysis and is therefore only done under close supervision (Davidson, *et al.*, 2007). Other agents commonly added to statins are ezetimibe, niacin and bile acid sequestrants (Hou and Goldberg, 2009). Dietary supplementation with fish oil is also used to reduce elevated triglycerides, with the greatest effect occurring in patients with the greatest severity (Mattar and Obeid, 2009). There is some evidence for benefit of plant sterol-containing products and ω_3 -fatty acids (Thompson, 2004).

2.8 Hyperlipidaemia and Cardiovascular Diseases

One of the initiating events of atherosclerotic plaque formation appears to be the entrance of lipoproteins LDL and Lp(a) into the subendothelial space with their oxidatively modified free radicals produced by smooth muscle cells, activated macrophages, and endothelial cells (Bisht, *et al.*, 2012). These oxidatively modified lipoproteins enter macrophages through a scavenger receptor pathway, ultimately yielding lipid-rich foam cells (Libby, *et*

al., 2011). Circulating monocytes are also attracted to smooth muscle and endothelial cells by chemoattractant that is augmented by the oxidatively modified lipoproteins (Israel *et al.*, 1995).

As the macrophage scavenger receptor continues to uptake oxidatively modified lipoproteins, foam cells continue to form and progress to the next level of atherogenesis, which is the formation of the fatty streak (Griendling and Alexander, 1997). At the same time, smooth muscle cells migrate into the subendothelial space and begin proliferating within the intima, contributing to the overall atherogenic process. As the process continues, lesions continue to grow by increased smooth muscle cell proliferation and collagen synthesis (Spagnoli, *et al.*, 1997). At this point, necrosis of the foam cell and formation of an extracellular lipid core occurs, as long as plasma LDL levels are elevated (Spagnoli, *et al.*, 1997). The final phase appears to involve an autoimmune inflammatory response that causes T lymphocyte infiltration of the adventitia (the outermost connective tissue covering of a vessel). This inflammatory response appears to complete the process of plaque formation that is the underlying culprit in CHD (Israel *et al.*, 1995).

2.9 Atherosclerosis

Theories of Atherogenesis

Arteries carry oxygenated blood from the heart to all tissues of the body. The arterial wall is composed of three layers, namely the intima (inner lining), media and adventitia. Atherosclerosis is characterized by lesions in the intima of arteries, seen as raised fibrous plaques ranging in colour from pearly gray to yellowish gray. The cellular components of the plaque include a cell similar to the adjacent endothelial cell, macrophages, fibrinogen

from which fibrin is formed and white blood cells interspersed between dense connective tissues which consist majorly of collagen fibers (Carmena, *et al.*, 2004). Atherosclerosis poses a high risk not just because it can close up an artery, slowing down or entirely restricting blood flow, but may also lead to thrombus formation (Saba, and Oridupa 2012).

A thrombus is a complex aggregation of platelets, red and white blood cells in a fibrin network. Several theories have emanated, suggesting the actual pathogenesis of atherosclerosis. Schoenhagen, (2006) documented the different theories that have been postulated in the course of history. In 1851, Rokitansky suggested the encrustation theory or thrombogenesis which postulates that atherosclerosis began in the intima of arteries with the deposition of thrombus. This is followed by the organisation of the thrombus through infiltration of fibroblast, secondarily followed by deposition of lipid. The German pathologist, Rudolf Virchow postulated the insudation or inflammation theory in 1856. He suggested that infiltration of fatty substances from the blood stream into the arterial wall leads to deposition of cholesterol which acts as an irritant, causing inflammation and the proliferation of cells (Saba, and Oridupa 2012). The cholesterol deposits act as irritant in the arterial intima, initiating inflammatory process as macrophages are incriminated as key role players in the phases of the disease. This theory was further supported by the work of N.N. Anitschkow in 1933 who discovered that a disease resembling human atherosclerosis could be reproduced in rabbits with high serum cholesterol or LDL levels. He thus stated that the phenomenon may be as a result of defects in metabolism of lipids (Schoenhagen, 2006).

The flow theory relates the circulation of blood in vessels to its effect on arterial wall. It states that lesions occurred more often at curved, branching, or bifurcated sites, generally at regions of perturbed blood flow. Other hypotheses that arose from the flow theory include

the stagnation point hypothesis by Fox and Hugh (1966), high wall shear stress hypothesis proposed by Fry (1968), low wall shear stress hypothesis by Caro *et al.*, (1969), diminished lateral pressure hypothesis and the convection-diffusion hypothesis. All these theories were established based on three methods.

Atherosclerotic plaques from autopsy findings from individuals of both sexes, various ages and race with different diseases which included hyperlipidaemia, diabetes and hypertension were considered. Epidemiological studies of factors which promote or prevent development of atherosclerosis, and finally experimental pathology which established the sequence of lesion development or regression were considered. None of these theories entirely explains the pathogenesis of atherosclerosis, but each has explained an aspect of this process (Williams *et al.*, 1998).

2.10 Poloxamer 407

Poloxamers are non-ionic triblock copolymers composed of a central hydrophobic chain of polyoxypropylene (poly (propylene oxide)) flanked by two hydrophilic chains of polyoxyethylene (poly(ethylene oxide)) (Cogger, *et al.*, 2006). The word "poloxamer" was coined by the inventor, Irving Schmolka, who received the patent for these materials in 1973 (Tania, 2007). Poloxamers are also known by the trade names Synperonics, Pluronic and Kolliphor (BASF, 2008). Because the lengths of the polymer blocks can be customized, many different poloxamers exist that have slightly different properties. For the generic term "poloxamer", these copolymers are commonly named with the letter "P" (for poloxamer) followed by three digits, the first two digits x 100 give the approximate molecular mass of the polyoxypropylene core, and the last digit x 10 gives the percentage

polyoxyethylene content. For example, P407 = Poloxamer with a polyoxypropylene molecular mass of 4,000 g/mol and a 70% polyoxyethylene content (Tania, 2007).

For the Pluronic and Synperonic trade names, coding of these copolymers starts with a letter to define its physical form at room temperature (L = liquid, P = paste, F = flake (solid)) followed by two or three digits (Singhare, *et al.*, 2005). The first digit (two digits in a three-digit number) in the numerical designation, multiplied by 300, indicates the approximate molecular weight of the hydrophobe; and the last digit x 10 gives the percentage polyoxyethylene content (e.g., L61 indicates a polyoxypropylene molecular mass of 1,800 g/mol and a 10% polyoxyethylene content). In the example given, poloxamer 181 (P181) = Pluronic L61 and Synperonic PE/L 61. Poloxamer 407 is a triblock copolymer consisting of a central hydrophobic block of polypropylene glycol flanked by two hydrophilic blocks of polyethylene glycol (Dumortier, *et al.*, 2006). The approximate length of the two PEG blocks is 101 repeat units while the approximate length of the propylene glycol block is 56 repeat units (Tania, 2007). This particular compound is also known by the BASF trade name Pluronic F127 or by the Croda trade name Synperonic PE/F 127 (Tania, 2007).

Most of the common uses of poloxamer 407 are related to its surfactant properties. For example, it is widely used in cosmetics for dissolving oily ingredients in water. It can also be found in multi-purpose contact lens cleaning solutions, where its purpose there is to help remove lipid films from the lens. It can also be found in some mouthwashes. There is a research ongoing for using poloxamer 407 for aligning severed blood vessels before gluing them surgically (Anonymous, 2011). Poloxamer 407 (Pluronic RF-127) has been used to induce hyperlipidaemia and hypercholesterolemia in rats, mice and rabbits. It is a

biocompatible, non-ionic surfactant, considered non-toxic and safe during chronic administration for long term studies (Megalli *et al.*, 2005). In rats and mice, the elevations in plasma triacylglycerols appear to be more sensitive to the effects of P-407 when compared to plasma cholesterol. However, to date the mechanism by which P-407 modifies plasma lipid concentrations remains unknown (Zjumira and Wout, 1992).

The accumulation of plasma triacylglycerols following the administration of P-407 has been suggested to be due to inhibition of capillary-bound lipoprotein lipase (LPL) activity (Johnston and palmer, 1993). Other studies (Kishor *et al.*, 2003) have indicated that in rats, the increase in plasma cholesterol following P-407 administration, may be due to inhibition of cholesterol 7 α -hydroxylase, but not stimulation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (Johnston *et al.*, 2001).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and Reagents

All chemicals and reagents used were of analytical grade and purchased from Sigma Chemical Company, St. Louis, U.S.A.

3.1.2 Sample Collection

Quail eggs were obtained from Animal Farm, Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, (ABU) Zaria.

3.1.3 Experimental Animals

A total of 60 adult wistar rats of both sexes weighing 150-200g were obtained from the Department of Pharmacology and Therapeutics, ABU Zaria. The males and females were kept in separate cages of six each per cage. Three males and three females of alternate cages formed a group. The rats were kept in well-aerated laboratory cages and allowed to acclimatize to the laboratory environment for two weeks before the commencement of the experiment. They were fed with growers mash (Vital Feeds Ltd) Zaria, Kaduna State. Water was provided *ad libitum*.

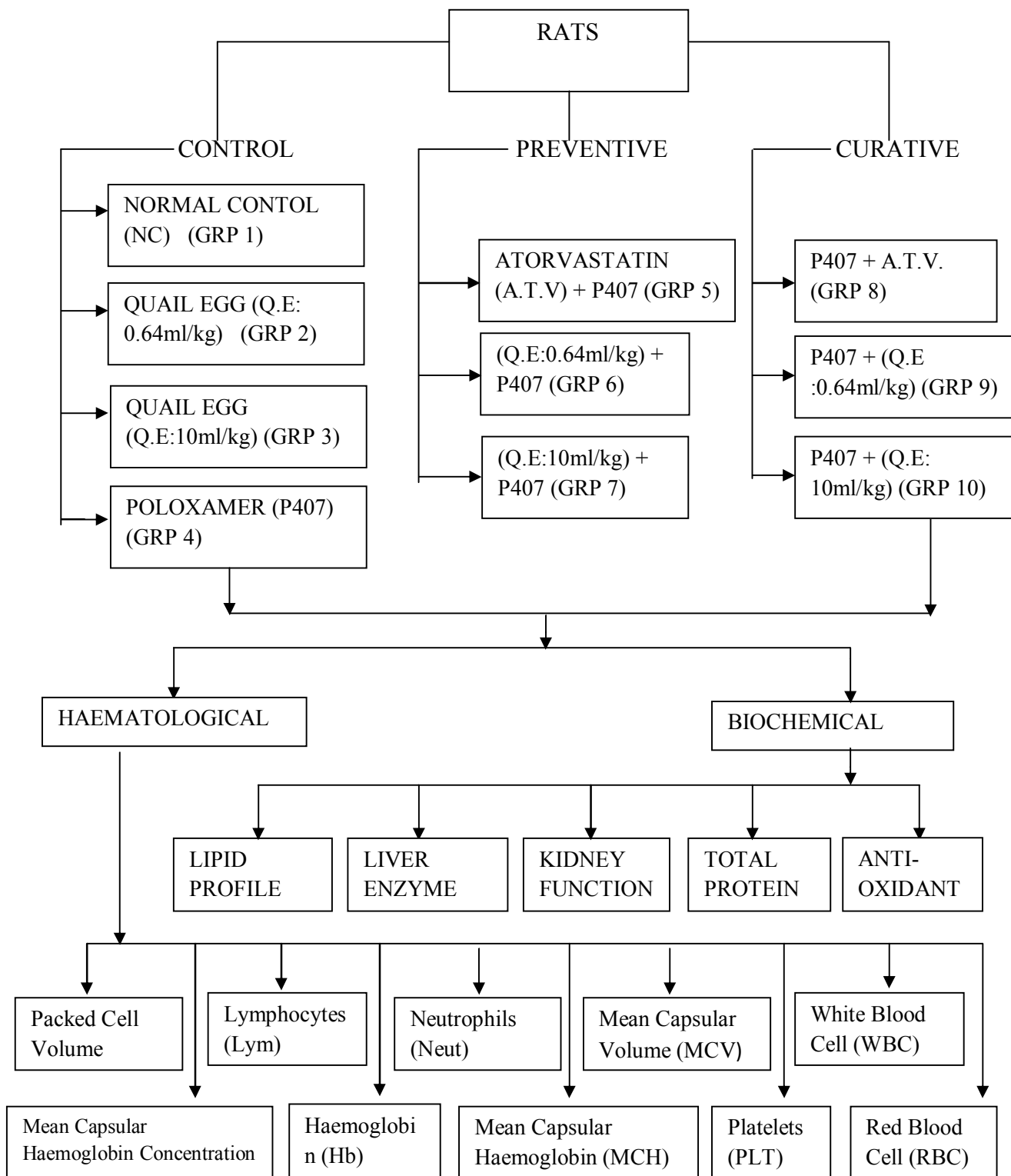


Figure 3.1: Experimental Design for The Effect of Japanese Quail Egg Supplementation on P407-Induced Hyperlipidaemic Wistar Rats

3.2 Methodology

3.2.1 Egg preparation

A day old eggs were obtained daily from the Animal Farm, Department of Animal Science, A.B.U. Zaria. The shells were broken and the content mixed together and given orally to the rats. The amount given was based on the assumption that for a 70kg adult, five eggs are needed per day for 49 days for the treatment of hyperlipidaemia (Truffier, 1978). Therefore, the amount given was then calculated based on the weight of the rats. A quail egg has an average volume of 9 ml.

3.2.2 Induction of Hyperlipidaemia

Poloxamer 407 (Lutrol F127; BASF, Ludwigshafen, Germany), a non-ionic surfactant was used as hyperlipidaemia inducing agent (Johnston and Palmer, 1993). 300 mg/kg body weight of Poloxamer 407 was administered once intraperitoneally. Total cholesterol and triacylglycerol concentrations of some randomly selected induced rats were determined 2 hours after induction using standard diagnostic kits, using the Randox[®] kit (Randox Laboratories Limited UK). Rats with total cholesterol and triacylglycerol concentrations above 200 mg/dl and 160 mg/dl respectively were considered hyperlipidaemic (Delvin *et al.*, 2006).

3.2.3 Animal Grouping and Treatment

The rats were randomly divided into 10 groups of 6 rats each.

CONTROL GROUP

Group 1 Rats were fed normal chow *ad libitum* and served as normal control.

Group 2 Rats were given quail egg (Q.E. 0.64 ml/kg body weight) and served as positive control.

Group 3 Rats were given quail egg (Q.E. 10 ml/kg body weight) and served as positive control.

Group 4 Rats were given Poloxamer 407 (300 mg/kg body weight) and served as hyperlipidaemic control.

PREVENTIVE STUDY GROUP: Group 5 served as standard drug control while groups (6 and 7) were given quail egg (0.64 ml/kg and 10 ml/kg) respectively from day 1 to day 19 before induction of hyperlipidaemia with P407 and sacrificed on the 21st day.

Group 5 Rats were given atorvastatin (10 mg/kg b.wt) + Poloxamer 407 (300 mg/kg b.wt). This served as standard drug control

Group 6 Rats were given quail egg (0.64 ml/kg) + poloxamer 407 (300 mg/kg b.wt).

Group 7 Rats were given quail egg (10 ml/kg) + poloxamer 407 (300 mg/kg b.wt).

CURATIVE STUDY GROUP: These groups were induced with P407 on day 1 and groups 9 and 10 treated with quail egg (0.64 ml/kg and 10 ml/kg) respectively after the induction of hyperlipidaemia had being confirmed (2 hours after induction). They were also fed with quail egg the next day before they were sacrificed 48hrs after induction of hyperlipidaemia.

Group 8 served as standard drug control.

Group 8 Rats were given Poloxamer 407 (300 mg/kg b.wt) + atorvastatin (10 mg/kg b.wt). This served as standard drug control.

Group 9 Rats were given poloxamer 407 (300 mg/kg b.wt) + quail egg (0.64 ml/kg b.wt)

Group 10 Rats were given poloxamer 407 (300 mg/kg b.wt) + quail egg (10 ml/kg b.wt)

3.2.5 Collection of Serum Sample

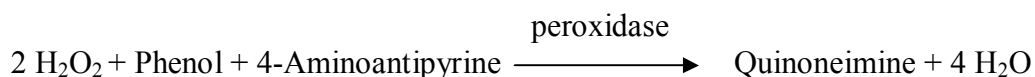
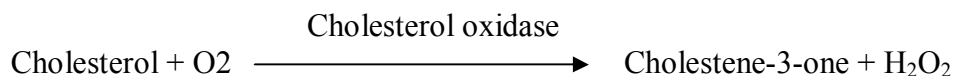
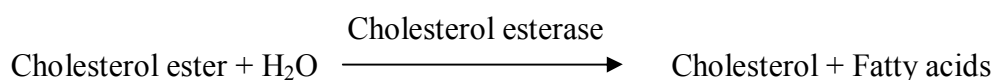
On the 21st day of the experiment, chloroform-inhalation anesthesia was performed on all experimental rats for the preventive group, while the rats in the curative group were sacrifice 48 hr after induction. The anesthetized rats were bled by cardiac puncture; the blood samples collected were centrifuged at a speed of 3000g for 15 minutes and supernatant collected for further analysis.

3.3 Determination of Biochemical Parameters

3.3.1 Determination of serum total cholesterol (TC) concentration

The serum level of TC was quantified by spectrophotometric methods as described by Stein (1987) using the Randox[®] kit (Randox Laboratories Limited UK).

Principle: Cholesterol in blood is majorly in the form of cholesteryl esters, which is hydrolysed by cholesterol esterase into cholesterol. Cholesterol is then oxidized by cholesterol oxidase to yield H₂O₂. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase which can be detected colorimetrically.



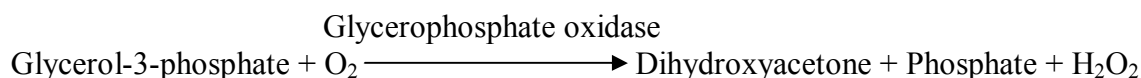
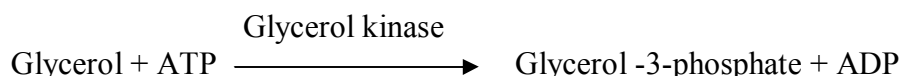
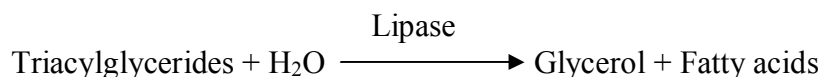
Procedure: Ten microlitres of distilled water, 10 µl of cholesterol standard and 10 µl of test serum were dispensed into the test tubes labelled “reagent blank”, “standard” and “sample” respectively. One millilitre of the cholesterol reagent provided was then added to each of the tubes and mixed. The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

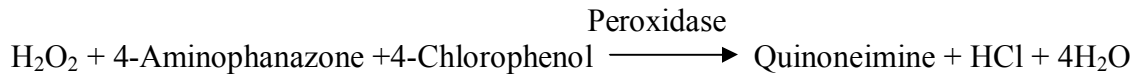
$$\text{Total cholesterol (mg/dl)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{conc. of standard}$$

3.3.2 Determination of serum triacylglycerides (TG) concentration

The serum level of TG was determined by enzymatic method described by Stein (1987) using Randox[®] kit (Randox Laboratories Limited UK).

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





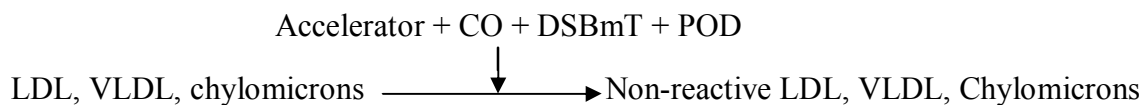
Procedure: One millilitre of the radox TG enzyme reagent (containing a mixture of lipases, glycerol-kinase, ATP, 4-aminophenazone, 4-chlorophenol, peroxidise, glycerol-3-phosphate oxidase and magnesium ions) was dispensed into three separately labelled test tubes: “standard”, “sample” and “reagent blank”.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

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

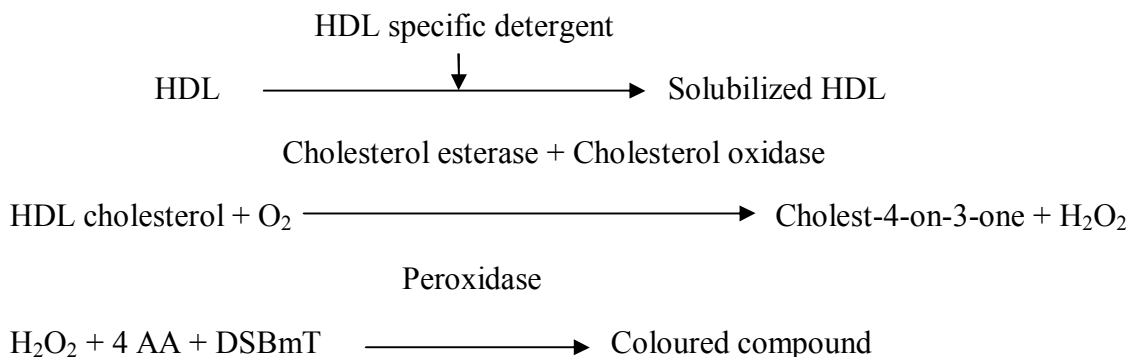
3.3.3 Determination of Serum high density lipoprotein cholesterol (HDL-c) concentration

HDL-c level was determined using the ELITech[®] kit (ELITech Clinical Systems, France) based on the method described by Naito (2003).

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



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



AA: Amino antipyrine

DSBmT: 4-N-bis(4-sulphobutyl)-*m*-toludine-disodium)

POD: Peroxidase

CO: Cholesterol oxidase

Procedure: Exactly 240 μl of reagent 1 (cholesterol oxidase, peroxidase, ascorbate oxidase and 4-N-bis(4-sulphobutyl)-*m*-toludine-disodium) was dispensed into three separate test tubes labelled “blank”(containing 2.4 μl distilled water), “standard” (containing 2.4 μl of HDL-c calibrator) and “sample” (containing 2.4 μl of test serum). The mixture was incubated at 37°C for 4 minutes 40 seconds in a water bath and the absorbance (A_1) measured using a colorimeter at 578 nm against the blank. Exactly 80 μl of Reagent R₂ (4-amino-Antipyrine, cholesterol esterase) was then added to each of the tubes and the resultant mixture incubated at 37°C for 4 minutes in a water bath and the absorbance (A_2) measured using a colorimeter at 578 nm against the blank. The concentration of HDL-c was then calculated using the formula:

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

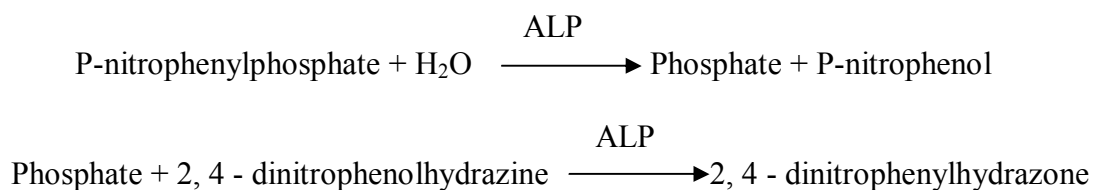
3.3.4 Determination of serum low density lipoprotein- cholesterol (LDL-c) concentration

The serum level of LDL-c was calculated according to the protocol of Friedewald (1972) using the equation: $LDL-c \text{ (mg/dl)} = TC - TG/5 - HDL-c$

3.3.5 Determination of serum alkaline phosphatase (ALP) activity

The ALP activity was determined by the standard method described by Haussament (1977) using the Randox[®] kit (Randox Laboratories Limited UK).

Principle: Alkaline phosphatase is measured by monitoring the concentration of phosphate hydrazone formed when phosphate reacts with 2, 4-dinitrophenylhydrazine at 37⁰C.



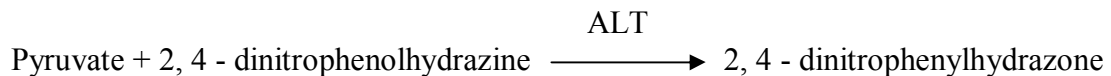
Procedure: Exactly 0.01ml of test serum was dispensed into a 1 ml cuvette followed by the addition of 0.05 ml of the reagent (prethanolamine buffer, MgCl₃, p-nitrophenylphosphate). The absorbance of the mixture was immediately taken using a colorimeter at 405 nm, and the timer started simultaneously as the initial absorbance was recorded. The absorbance was read again at 1, 2, and 3 minutes at 405 nm. The initial absorbance reading was subtracted from the absorbance readings at 1, 2 and 3 minutes (i.e. $A_{1/2/3} - A_{\text{initial}}$). The average of the three values obtained was then computed and used to calculate the ALP activity using the formula

$$ALP \text{ (U/I)} = 2760 \times \Delta A \text{ at } 405 \text{ nm/min}$$

3.3.6 Determination of serum alanine aminotransferase (ALT) activity

The ALT activity was determined using the Randox[®] kit (Randox Laboratories Limited UK) based on the method described by Reitman and Frankel (1957) and expressed in U/l.

Principle: Alanine aminotrasferase is measured by monitoring the concentration of pyruvate hydrazone formed when pyruvate reacts with 2, 4-dinitrophenylhydrazine at 37°C.



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

3.3.7 Determination of serum aspartate aminotransferase (AST) activity

The AST activity was determined using the Randox[®] kit (Randox Laboratories Limited UK) based on the method described by Reitman and Frankel (1957) and expressed in U/l

Principle: AST was measured by monitoring the concentration of oxaloacetate hydrazone formed when reacted with 2, 4-dinitrophenylhydrazine at 540 nm and 37°C.



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-dinitrophenylhydrazine) to each of the test tubes and the mixture allowed to stand in a water bath at 25°C for 20 minutes. At the end of the 20 minutes, 5 ml of sodium hydroxide (0.4 mol/l) was then dispensed into each of the tubes and the absorbance of the “sample” (A_{sample}) was read against the “reagent blank” using a colorimeter at 546 nm. The activity of AST in the serum was obtained by extrapolating the corresponding absorbance from the plot of the standard calibration curve for the enzymes provided in the manual of the kit.

3.3.8 Determination of serum total protein (TP) concentration

The serum TP concentration was determined using the Randox[®] kit (Randox Laboratories Limited UK) based on the method described by Fine (1935).

Principle: The protein assay is based on a two-step reaction of protein with an alkaline copper tartarate solution and Folin reagent leading to colour development. First is the reaction between protein and copper at alkaline pH, and subsequently, the reduction of Folin reagent by the copper-treated protein. Colour formation is due to amino acids that reduce the Folin reagent, yielding reduced species that impart a characteristic blue colour.

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

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

3.3.9 Determination of serum albumin (ALB) concentration

The serum ALB concentration was determined using the Randox[®] kit (Randox Laboratories Limited UK) based on the method described by Doumas *et al.*, (1971).

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

Procedure: Three millilitres of the Bromocresol green (BCG) were dispensed into each of the tubes labelled “reagent blank”, “standard” and “sample” containing 0.01 ml distilled water, 0.01 ml standard albumin and 0.01 ml test serum respectively. The mixture was then incubated for 5 minutes at 25°C in a water bath. After the incubation, the absorbance of the sample (A_{sample}) and that of the standard (A_{standard}) against the “reagent blank” was read

using a colorimeter at 578 nm. The values obtained were used to calculate the albumin concentration using the formula:

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

3.3.10 Determination of serum bilirubin concentrations

3.3.10.1 Determination of serum total bilirubin (TB) concentration

The serum TB concentration was determined using the Randox[®] kit (Randox Laboratories Limited UK) based on the method described by Jendrassik and Grof (1938) and Sherlock (1951).

Principle: Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Procedure: Exactly 200 µl of reagent 1 (sulphanilic acid) were dispensed each into two different test tubes labelled “sample blank” and “sample” followed by the addition of 1 drop (50 µl) of reagent 2 (nitrite) and the 1000 µl of reagent 3 (caffeine). Two hundred microlitre of the test serum were then dispensed into each of the tubes and the mixture incubated in a water bath for 10 minutes at 25°C. This was followed by the addition of 1000 µl of reagent 4 (tartrate) and the mixture incubated again at 25°C for 10 minutes. The absorbance of the sample (A_{TB}) was then read against the sample blank using a colorimeter at 578 nm. The total bilirubin concentration was then calculated using the formula:

$$\text{Total bilirubin (mg/dl)} = 10.8 \times A_{\text{TB}} (578\text{nm})$$

3.3.10.2 Determination of serum direct/conjugated bilirubin (DB/CB) concentration

The serum CB concentration was determined using the Randox[®] kit (Randox Laboratories Limited UK) based on the method described by Jendrassik and Grof (1938) and Sherlock (1951).

Principle: Direct/Conjugated bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex.

Procedure: Exactly 200 µl of reagent 1 (sulphanilic acid) were dispensed each into two different test tubes labelled “sample blank” and “sample” followed by the addition of 1 drop (50 µl) of reagent 2 (nitrite) and the 2000 µl of 0.9% sodium chloride. Two hundred microlitres of the test serum were then dispensed into each of the tubes and the mixture incubated in a water bath for 10 minutes at 25°C. The absorbance of the sample (A_{DB}) was then read against the sample blank using a colorimeter at 546 nm. The direct bilirubin concentration was then calculated using the formula:

$$\text{Conjugated bilirubin (mg/dl)} = 14.4 \times A_{DB} (546\text{nm})$$

A_{DB} : Absorbance of direct bilirubin

3.3.11 Determination of serum creatinine concentration

The serum creatinine concentration was determined using the Randox[®] kit (Randox Laboratories Limited UK) based on the method described by Bartels and Bohmer (1972).

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

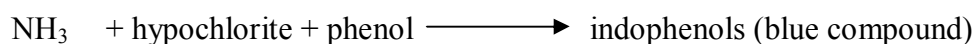
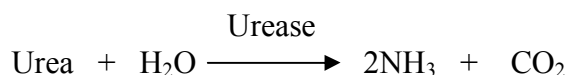
Procedure: Exactly 0.1 ml of creatinine standard solution and 0.1 ml of test serum were dispensed into separate cuvettes labelled standard and sample respectively. This was followed by addition of 1.0 ml of the working reagent (picric acid + NaOH). The absorbances of the mixtures were then taken after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

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

3.3.12 Determination of serum urea concentration

The serum urea concentration was determined using the Randox[®] kit (Randox Laboratories Limited UK) based on the method of Berthelot's reaction (Fawcett and Scout, 1960).

Principle: Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia formed is then measured photometrically.



Procedure: Exactly, 100 μl of reagent 1 (sodium nitroprusside [6 mmol/l] + urease [1g/l]) were dispensed into three separately labelled test tubes: reagent blank, standard and sample containing 10 μl of distilled water, 10 μl urea standard and 10 μl test serum

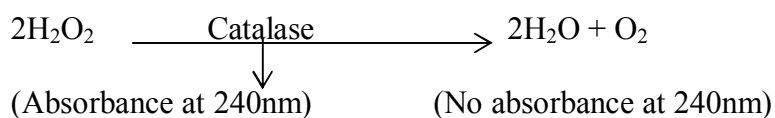
respectively. The mixture was then incubated at 37°C for 10 minutes in a water bath. After the period of incubation 2.5 ml of reagent 2 (phenol [120 mmol/l]) was added to the contents of each test tube followed by 2.5 ml of reagent 3 (Sodium hypochlorite [27 mmol/l]). The mixture was then incubated for 15 minutes at 37°C in a water bath. The absorbance of the sample (A_{sample}) and that of the standard (A_{standard}) were then read against the “reagent blank” using a colorimeter at 546 nm and the readings obtained were used for the calculation of the serum urea concentration with the aid of the formula:

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

3.3.13 Determination of Catalase

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

Principle: Catalase enzyme activity can be measured by monitoring the consumption of H_2O_2 substrate at 240 nm.



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

Procedure: Assay Protocol:

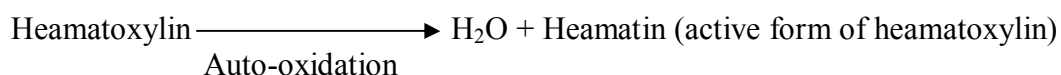
Cuvette Assay:

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

3.3.14 Superoxide dismutase (SOD)

Superoxide Dismutase activity was measured using the method described by Martin *et al.*, (1987).

Principle: Auto-oxidation of heamatoxylin (with increase in absorbance at 560 nm) is inhibited by SOD activity at the assay pH 7.8; the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range. SOD activity in the sample was determined by measuring the amount of heamatin at 560 nm.



Procedure: Assay Protocol:

Microplate Assay

Sample/standard layout was recorded. To each well used for testing, 230 μL of Assay Buffer were added. Next 10 μL of assay buffer (for blank) or 10 μL sample were added. It

was shaken to mix then incubated for 2 minutes. Afterwards, 10 μ L of hematoxylin reagent was added to begin reaction. A multi-channel pipette was used. The mixture was mixed quickly using the instrument's shaker function and immediately began recording the absorbance at 560 nm every 10 seconds.

3.3.15 Estimation of Lipid Peroxidation by Measuring Malondialdehyde (MDA) level (Varshney and Kale, 1990).

Principle: Malondialdehyde (MDA) is a low molecular weight end-product of lipid hydroperoxide decomposition and is the most often measured as an index of lipid peroxidation. The assay is based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA adduct which absorbs strongly at 532 nm.

Procedure:

Samples were incubated with 0.01 ml butylated hydroxytoluene (BHT) in ethanol, 0.025 ml phosphoric acid, in phosphate buffer (pH 7.0) with EDTA and 0.025 ml thiobarbituric acid solution. The samples were shaken vigorously and heated at 60°C for 60 minutes, and then centrifuged at 10,000g for 3 minutes. The red adduct formed in the supernatant fractions were estimated by measuring the absorbance at 532 nm.

Calculations

A calibration curve was prepared with an MDA standard (North West Life Science Specialties™, Vancouver, USA).

3.4. Haematological Assay

3.4.1 Determination of Packed Cell Volume (PCV)

PCV is the volume of red blood cells (RBC) expressed as a fraction of the total volume of the blood. The microhaematocrit method was used (Cheesbrough, 2000).

Principle: The red blood cells are heavier than plasma with specific gravity of 1090 and 1030 respectively. When blood is placed in a capillary tube and centrifuged, they settle and pack together because of the centrifugal force acting on them. The volume occupied by the cells is measured and its ratio with that of the volume of the whole blood is calculated.

Procedure: Blood from the rat's tail was collected into the capillary tube via capillary action, one end of the tube was sealed by flaming and centrifuged at a speed of 650g for 10 minutes. The PCV values were read directly from the microhaematocrit reader and the reading was expressed as a percentage erythrocytes.

3.4.2 Differential count determination

Platelet, neutrophils, lymphocytes, Haemoglobin (Hb), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Red Blood Cell Count (RBC) and White Blood Cell Count (WBC) were determined using the Sysmex Haematological Autoanalyzer (XE-5000)

Principle: The Sysmex Automated Haematology System (XE-5000) combines the fluorescent flow cytometry and hydrodynamic technologies to analyze blood samples. The fluorescent flow cytometry utilizes the ability of polymethin dye to stain the different

population of cells differently. The diode laser bench detects the fluorescence of nucleic acid, nucleus to plasma ratio of the stained cells and the volume of the cell as they pass through the aperture to discriminate between cells.

The hydrodynamic technology utilizes direct current to count cells as they pass through a dedicated aperture of known dimension. As the cells pass through this aperture, they create an electrical resistance which is detected as electronic pulse. The intensity of the electronic pulse from each cell is proportional to the cell volume.

The system, then, uses complex algorithm to automatically discriminate and separate the different cell populations.

3.5 STATISTICAL ANALYSIS

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

CHAPTER FOUR

4.0

RESULTS

4.1 Biochemical Parameters

4.1.1: Effects of Japanese Quail Egg on Lipid Profile of P407-Induced Hyperlipidaemic Wistar Rats

The effects of administration of Japanese quail egg on lipid profile of P407-induced hyperlipidaemic rats are presented in Table 4.1. From the preventive studies;

4.1.1.1: Effect on Total Cholesterol level

The total cholesterol level in rats in the normal control group was (59.89.08 \pm 6.45) as shown in table 4.1. After induction with P407, cholesterol level in P407-induced hyperlipidaemic control group had a significantly ($p > 0.05$) higher value (232.89 \pm 20.67) than the normal control group. Treatment with 0.64ml/kg and 10ml/kg of quail egg had values of (231.64 \pm 34.36 and 244.12 \pm 11.70) respectively. There was no significant ($p < 0.05$) difference between the egg treated group and the P407-induced hyperlipidaemic control group.

4.1.1.2: Effect on Triacylglycerol level

The triacylglycerol level in rats in the normal control group was (58.12 \pm 16.31) as shown in table 4.1. After inducing hyperlipidaemia, triacylglycerol level was significantly ($p > 0.05$) higher in P407 induced hyperlipidaemic control group (202.93 \pm 2.29) compared to the normal control group. Treatment with 0.64ml/kg and 10ml/kg of quail egg had values of (190.18 \pm 32.25 and 200.90 \pm 2.17) respectively. There was no significant ($p < 0.05$)

difference in the egg treated groups compared to the P07-induced hyperlipidaemic control group.

4.1.1.3: Effect on High Density Lipoprotein Cholesterol level

Table 4.1 shows an HDL-cholesterol level of (37.18±5.88) in the normal control group. After induction of hyperlipidaemia, a higher value of (54.92±7.70) was obtained. Treatment with 0.64ml/kg and 10ml/kg of quail egg had values of (52.56±16.01 and 55.51±5.77) respectively. There was no significant ($p < 0.05$) difference in the egg treated groups compared to the P407-induced hyperlipidaemic control group.

4.1.1.4: Effect on Low Density Lipoprotein Cholesterol level

The effect on LDL- cholesterol is presented in Table 4.1. The LDL- cholesterol level in the P407-induced hyperlipidaemic control group was significantly ($p > 0.05$) higher (128.91±4.58) compared to normal control group (45.63±4.35). Treatment with 0.64ml/kg and 10ml/kg of quail egg had values of (128.63±17.30 and 137.41±13.83) respectively, indicating no significant ($p < 0.05$) difference in the egg treated groups compared to the hyperlipidaemic control group.

The curative effect of Japanese quail egg on lipid profile P407-induced hyperlipidaemic rats after 48hrs of induction is shown in Table 4.1.

4.2.1: Effect on Total Cholesterol level

The total cholesterol level in rats in the normal control group was (59.89±6.45) as shown in table 4.1. After induction with P407, cholesterol level in P407 induced group had a

significantly ($p > 0.05$) higher value (232.89 ± 20.67) than the normal control group. Treatment with 0.64ml/kg and 10ml/kg of quail egg had values of (259.71 ± 13.16 and 251.60 ± 26.70) respectively. There was no significant ($p < 0.05$) difference between the egg treated group and the hyperlipidaemic control group.

4.2.2: Effect on Triacylglycerol level

The triacylglycerol level in rats in the control group was (58.12 ± 16.31) as shown in table 4.1. After inducing hyperlipidaemia, triacylglycerol level was significantly ($p > 0.05$) higher in P407 induced hyperlipidaemic control group (202.93 ± 2.29) compared to the normal control group. Treatment with 0.64ml/kg and 10ml/kg of quail egg had values of (201.35 ± 4.42 and 202.23 ± 10.46) respectively. There was no significant ($p < 0.05$) difference in the egg treated groups compared to the P407-induced hyperlipidaemic control group.

4.2.3: Effect on High Density Lipoprotein Cholesterol level

Table 4.1 shows an HDL-cholesterol level of (37.18 ± 5.88) in the normal control group. After induction of hyperlipidaemia, a higher value of (54.92 ± 7.70) was obtained. Treatment with 0.64ml/kg and 10ml/kg of quail egg had values of (67.77 ± 26.92 and 66.51 ± 3.29) respectively. There was no significant ($p < 0.05$) difference in the egg treated groups compared to the P407-induced hyperlipidaemic control group.

4.2.4: Effect on Low Density Lipoprotein Cholesterol level

The effect on LDL- cholesterol is presented in Table 4.1. The LDL- cholesterol level in the P407-induced hyperlipidaemic control group was significantly ($p > 0.05$) higher (128.91 ± 4.58) compared to normal control group (45.63 ± 4.35). Treatment with 0.64ml/kg and 10ml/kg of quail egg had values of (168.77 ± 7.38 and 162.28 ± 2.76) respectively. The egg treated groups show a significantly ($p > 0.05$) higher value compared to the P407-induced hyperlipidaemic control group.

Table 4.1: Effects of Japanese Quail Egg on Lipid Profile of P407-Induced Hyperlipidaemic Wistar Rats

GROUP (n=6)	TC(mg/dl)	TG(mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
GROUP 1	59.89 ± 6.45 ^a	58.12 ± 16.31 ^a	37.18 ± 5.88 ^{abc}	45.63 ± 4.35 ^a
GROUP 2	68.98 ± 2.36 ^a	67.07 ± 15.03 ^a	35.90 ± 2.22 ^{ab}	52.39 ± 1.22 ^a
GROUP 3	76.46 ± 4.33 ^a	64.15 ± 12.74 ^a	44.87 ± 15.55 ^{abc}	48.10 ± 2.76 ^a
GROUP 4	232.89 ± 20.67 ^c	202.93 ± 2.29 ^c	54.92 ± 7.70 ^{cd}	128.91 ± 4.58 ^d
GROUP 5	130.77 ± 2.38 ^b	114.10 ± 3.30 ^b	33.33 ± 2.23 ^a	74.38 ± 2.77 ^b
GROUP 6	231.64 ± 34.36 ^c	190.18 ± 32.25 ^c	52.56 ± 16.01 ^{bcd}	128.63 ± 17.30 ^d
GROUP 7	244.12 ± 11.70 ^{cd}	200.90 ± 2.17 ^c	55.51 ± 5.77 ^{bcd}	137.41 ± 13.83 ^d
GROUP 8	150.18 ± 6.79 ^b	144.19 ± 4.67 ^b	39.67 ± 4.52 ^{abc}	94.37 ± 13.18 ^c
GROUP 9	259.71 ± 13.16 ^{cd}	201.35 ± 4.42 ^c	67.77 ± 26.92 ^d	168.77 ± 7.38 ^e
GROUP 10	251.60 ± 26.70 ^{cd}	202.23 ± 10.46 ^c	66.51 ± 3.29 ^d	162.28 ± 2.76 ^e

Values are means ± Standard deviation

Values with different superscripts down the column are significantly different ($P < 0.05$)

Group 1: Normal Control Rats, Group 2: Rats administered quail egg (0.64mls/Kg body weight), Group 3: Rats administered quail egg (10ml/kg body weight) Group 4: Poloxamer 407-Induced Rats, Group 5: 10mg of Atorvastatin+P407-Induced Hyperlipidaemic Rats, Group 6: 0.64ml of quail egg/kg body weight+P407-Induced Hyperlipidaemic Rats, Group 7: 10ml of quail egg/kg body weight+P407 Induced Hyperlipidaemic Rats, Group 8: P407-Induced Hyperlipidaemic Rats+10mg of Atorvastatin, Group 9: P407-Induced Hyperlipidaemic Rats+0.64ml of quail egg/kg body weight, Group 10: P407-Induced Hyperlipidaemic Rats+10ml/kg body weight of quail egg.

TC: Total cholesterol, TG: Triglycerides, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol.

Groups 5, 6 and 7: preventive studies. Groups 8, 9 and 10: curative studies.

4.1.2: Effects of Japanese Quail Egg on Serum Atherogenic Risk Predictor Indices of P407-Induced Hyperlipidaemic Wistar Rats

Preventive effect of Japanese quail egg before induction of hyperlipidaemia with P407 on serum atherogenic risk predictor indices is shown in Table 4.2. The result shows no significant ($p \leq 0.05$) difference in HDL-c/TC, ratios in the P407-induced egg treated groups (0.64ml/kg and 10ml/kg of quail egg had values of (0.24 ± 0.04) and (0.28 ± 0.03)) compared to the P407-induced hyperlipidaemic control group having a value of (0.24 ± 0.03) , which had HDL-c/TC ratio less than 0.30. The result of the LDL-c/HDL-c shows no significant ($p \leq 0.05$) difference in ratios of P407-induced egg treated groups (0.64ml/kg and 10ml/kg of quail egg had values of (2.23 ± 0.51) and (2.57 ± 0.60)) compared to the P407-induced hyperlipidaemic control group with (2.38 ± 0.22) , with values of LDL-c/HDL-c ratio greater than 2.3. while Log TG/HDL-c ratios shows no significant ($p \leq 0.05$) difference in P407-induced egg treated groups (0.64ml/kg and 10ml/kg of quail egg had values of (0.53 ± 0.04) and (0.54 ± 0.05)) compared to the P407-induced hyperlipidaemic control group having (0.57 ± 0.04) .

Table 4.2 shows the curative effect of Japanese quail egg on serum atherogenic risk predictor indices of P407-induced hyperlipidaemic rats. The HDL-c/TC ratio had no significant ($p > 0.05$) increase in the egg treated groups (groups 9 and 10) with values 0.27 ± 0.09 and 0.26 ± 0.03 respectively compared to the P407-induced hyperlipidaemic control group which was 0.24 ± 0.04 . The LDL-c/HDL-c value of the P407-induced hyperlipidaemic control group was 2.38 ± 0.02 and shows no significant ($p > 0.05$) difference compared to the egg treated groups (groups 9 and 10) with values of 2.49 ± 0.92

and 2.49 ± 0.17 respectively. Also, log (TG/HDL-c) ratio shows a significantly ($p < 0.05$) lower value in the egg treated groups (9 and 10) with values 0.47 ± 0.05 and 0.49 ± 0.02 respectively compared to the P407-induced hyperlipidaemic control group which was 0.57 ± 0.04 , with values of HDL-c/TC less than 0.3 and the LDL-c/HDL-c ratio greater than 2.3.

Table 4.2: Effects of Japanese Quail Egg on Serum Atherogenic Risk Predictor Indices of P407-Induced Hyperlipidaemic Wistar Rats

GROUP (n=6)	HDL-c/TC	LDL-c/HDL-c	Log(TG/HDL-c)
GROUP 1	0.62 ± 0.03 ^b	1.23 ± 0.94 ^{ab}	0.19 ± 0.08 ^a
GROUP 2	0.52 ± 0.01 ^b	1.46 ± 0.17 ^b	0.27 ± 0.03 ^a
GROUP 3	0.59 ± 0.09 ^b	1.07 ± 1.17 ^a	0.15 ± 0.14 ^a
GROUP 4	0.24 ± 0.03 ^a	2.38 ± 0.22 ^{cd}	0.57 ± 0.04 ^c
GROUP 5	0.35 ± 0.01 ^a	2.23 ± 0.51 ^c	0.53 ± 0.04 ^{bc}
GROUP 6	0.24 ± 0.04 ^a	2.57 ± 0.60 ^d	0.54 ± 0.05 ^{bc}
GROUP 7	0.28 ± 0.03 ^a	2.47 ± 0.27 ^{cd}	0.46 ± 0.02 ^b
GROUP 8	0.26 ± 0.04 ^a	2.38 ± 0.36 ^c	0.56 ± 0.02 ^{bc}
GROUP 9	0.27 ± 0.09 ^a	2.49 ± 0.92 ^{cd}	0.47 ± 0.05 ^b
GROUP 10	0.26 ± 0.03 ^a	2.49 ± 0.17 ^{cd}	0.49 ± 0.02 ^b

Values are means ± Standard deviation

Values with different superscripts down the column are significantly different ($P < 0.05$)

Values of HDL-c/TC ratio < 0.30 are atherogenic and undesirable, values of LDL-c/HDL-c ratio > 2.3 are atherogenic and undesirable (Ojiakor and Nwanjo, 2005)

Group 1: Normal Control Rats, Group 2: Rats administered quail egg (0.64mls/Kg body weight), Group 3: Rats administered quail egg (10ml/kg body weight) Group 4: Poloxamer 407-Induced Rats, Group 5: 10mg of Atorvastatin+P407-Induced Hyperlipidaemic Rats, Group 6: 0.64ml of quail egg/kg body weight+P407-Induced Hyperlipidaemic Rats, Group 7: 10ml of quail egg/kg body weight+P407 Induced Hyperlipidaemic Rats, Group 8: P407-Induced Hyperlipidaemic Rats+10mg of Atorvastatin, Group 9: P407-Induced Hyperlipidaemic Rats+0.64ml of quail egg/kg body weight, Group 10: P407-Induced Hyperlipidaemic Rats+10ml/kg body weight of quail egg.

TC: Total cholesterol, TG: Triglycerides, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol

Groups 5, 6 and 7: preventive studies. Groups 8, 9 and 10: curative studies.

4.1.3: Effects of Japanese Quail Egg on Serum Liver Damage Indicators and Liver Function Parameters of P407-Induced Hyperlipidaemic Wistar Rats

4.1.3.1 Serum Liver Damage Indicators

The preventive effect of Japanese quail egg on serum liver damage indicators of P407-induced hyperlipidaemic rats is presented in Table 4.3. There was a significantly ($p < 0.05$) higher value (123.61 ± 3.45) in alanine aminotransferase (ALT) activity in P407-induced hyperlipidaemic control group compared to the normal control group (33.98 ± 8.22). There was no significant ($p > 0.05$) difference in the egg treated groups (6 and 7) with values 126 ± 4.72 and 116 ± 14.73 compared to the P407-induced hyperlipidaemic control group. The aspartate aminotransferase (AST) activity, shows no significant ($p > 0.05$) difference in the egg treated groups (6 and 7) 88.45 ± 0.26 and 89.19 ± 1.09 respectively compared with the P407-induced hyperlipidaemic control group with a value of 88.53 ± 0.53 . The alkaline phosphatase (ALP) levels in P407-induced hyperlipidaemic control group shows no significant ($p > 0.05$) difference with a value of 38.10 ± 26.49 compared to the egg treated groups (6 and 7) with values of 45.40 ± 40.58 and 55.30 ± 16.80 respectively.

The curative study in Table 4.3 shows that serum ALP activity has no significant ($p > 0.05$) difference in the egg treated groups (9 and 10) with values 42.55 ± 11.50 and 44.20 ± 30.72 compared to the P407-induced hyperlipidaemic control group with a value of 38.10 ± 26.49 . The serum AST activity shows no significant ($p > 0.05$) difference in the P407-induced egg treated groups (9 and 10) with values of 90.37 ± 1.13 and 88.53 ± 0.53 respectively compared to hyperlipidaemic control group of 88.53 ± 0.53 . However, the level of ALT activity was

significantly ($P < 0.05$) lower in the egg treated groups (9 and 10) with values 110.87 ± 13.98 and 92.48 ± 6.62 compared with the P407-induced hyperlipidaemic control group.

Table 4.3: Effects of Japanese Quail Egg on Serum Liver Damage Indicators of P407-Induced Hyperlipidaemic Wistar Rats

GROUP (n=6)	AST(U/I/l)	ALT(U/I/l)	ALP(U/I/l)
GROUP 1	89.71 ± 1.51 ^{bc}	33.98 ± 8.22 ^a	39.07 ± 32.31 ^a
GROUP 2	88.79 ± 0.75 ^{abc}	27.38 ± 2.67 ^a	42.41 ± 5.31 ^a
GROUP 3	87.74 ± 1.77 ^a	26.91 ± 1.81 ^a	45.00 ± 5.31 ^a
GROUP 4	88.53 ± 0.53 ^{ab}	123.61 ± 3.45 ^c	38.10 ± 26.49 ^a
GROUP 5	89.19 ± 1.09 ^{abc}	33.03 ± 5.98 ^a	35.90 ± 24.20 ^a
GROUP 6	89.45 ± 0.26 ^{bc}	126.91 ± 4.72 ^c	45.40 ± 40.58 ^a
GROUP 7	89.19 ± 1.09 ^{abc}	116.15 ± 14.73 ^{de}	55.30 ± 16.80 ^a
GROUP 8	89.58 ± 0.30 ^{bc}	75.76 ± 5.97 ^b	40.57 ± 24.21 ^a
GROUP 9	90.37 ± 1.13 ^{bc}	110.87 ± 13.98 ^d	42.55 ± 11.50 ^a
GROUP 10	88.53 ± 0.53 ^{ab}	92.48 ± 6.62 ^c	44.20 ± 30.72 ^a

Values are means ± Standard deviation

Values with different superscripts down the column are significantly different ($P < 0.05$)

Group 1: Normal Control Rats, Group 2: Rats administered quail egg (0.64mls/Kg body weight), Group 3: Rats administered quail egg (10ml/kg body weight) Group 4: Poloxamer 407-Induced Rats, Group 5: 10mg of Atorvastatin+P407-Induced Hyperlipidaemic Rats, Group 6: 0.64ml of quail egg/kg body weight+P407-Induced Hyperlipidaemic Rats, Group 7: 10ml of quail egg/kg body weight+P407 Induced Hyperlipidaemic Rats, Group 8: P407-Induced Hyperlipidaemic Rats+10mg of Atorvastatin, Group 9: P407-Induced Hyperlipidaemic Rats+0.64ml of quail egg/kg body weight, Group 10: P407-Induced Hyperlipidaemic Rats+10ml/kg body weight of quail egg.

ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

Groups 5, 6 and 7: preventive studies. Groups 8, 9 and 10: curative studies.

4.1.3.2 Liver Damage Parameters

The preventive effect of Japanese quail egg on liver damage parameters is presented on table 4.4. Serum concentrations of total protein (TP) shows no significant ($p > 0.05$) difference in the egg treated groups (6 and 7) with values of 6.48 ± 0.12 and 6.67 ± 0.37 respectively compared to the P407-induced hyperlipidaemic control group having a value of 6.40 ± 0.26 . The serum albumin (ALB) concentration shows no significant ($p > 0.05$) difference in the egg treated groups (6 and 7) with values of 4.28 ± 0.03 and 4.24 ± 0.05 respectively compared to the P407-induced hyperlipidaemic control group of 4.32 ± 0.08 . The total bilirubin (TB) concentration, values of the egg treated groups (6 and 7) were 0.81 ± 0.90 and 0.71 ± 0.19 respectively; compared to the P407-induced hyperlipidaemic control group with a value of 0.52 ± 0.29 shows no significant ($p > 0.05$) difference. The conjugated bilirubin (CB) concentration shows no significant ($p > 0.05$) difference in the egg treated groups (6 and 7) with values of 1.65 ± 0.92 and 0.98 ± 0.35 respectively compared to the P407-induced hyperlipidaemic control group of 1.55 ± 0.83 .

In the curative study, Serum concentrations of total protein (TP) shows no significant ($p > 0.05$) difference in the egg treated groups (9 and 10) with values of 6.98 ± 0.48 and 6.62 ± 0.11 respectively compared to the P407-induced hyperlipidaemic control group having a value of 6.40 ± 0.26 . The serum albumin (ALB) concentration shows no significant ($p > 0.05$) difference in the egg treated groups (9 and 10) with values of 4.30 ± 0.02 and 4.28 ± 0.08 respectively compared to the P407-induced hyperlipidaemic control group of 4.32 ± 0.08 . The total bilirubin (TB) concentration, values of the egg treated groups (9 and 10) were 0.76 ± 0.78 and 0.62 ± 0.95 respectively; compared to the P407-induced hyperlipidaemic control group with a value of 0.52 ± 0.29 shows no significant ($p > 0.05$)

difference. The conjugated bilirubin (CB) concentration shows no significant ($p > 0.05$) difference in the egg treated groups (9 and 10) with values of 1.56 ± 0.71 and 1.23 ± 0.70 respectively compared to the P407-induced hyperlipidaemic control group of 1.55 ± 0.83 .

Table 4.4: Effects of Japanese Quail Egg on Liver Function Parameters of P407-Induced Hyperlipidaemic Wistar Rats

GROUP (n=6)	TP(g/dl)	ALB(g/dl)	TB(Mg/dl)	CB(Mg/dl)
GROUP 1	6.55 ± 1.40 ^a	4.14 ± 0.07 ^a	0.57 ± 0.77 ^a	1.43 ± 0.69 ^{abc}
GROUP 2	6.41 ± 0.10 ^a	4.42 ± 0.15 ^c	0.29 ± 0.11 ^a	1.30 ± 0.66 ^{abc}
GROUP 3	6.45 ± 0.08 ^a	4.31 ± 0.03 ^b	0.43 ± 0.18 ^{ab}	0.67 ± 0.24 ^a
GROUP 4	6.40 ± 0.26 ^a	4.32 ± 0.08 ^{bc}	0.52 ± 0.29 ^a	1.55 ± 0.83 ^{abc}
GROUP 5	6.58 ± 0.12 ^a	4.15 ± 0.06 ^a	0.43 ± 0.10 ^a	1.02 ± 0.48 ^{abc}
GROUP 6	6.48 ± 0.12 ^a	4.28 ± 0.03 ^b	0.81 ± 0.90 ^a	1.65 ± 0.92 ^{bc}
GROUP 7	6.67 ± 0.37 ^{ab}	4.24 ± 0.05 ^{ab}	0.71 ± 0.19 ^a	0.98 ± 0.35 ^{ab}
GROUP 8	6.49 ± 0.11 ^a	4.24 ± 0.02 ^{ab}	0.48 ± 0.24 ^a	1.33 ± 0.24 ^{abc}
GROUP 9	6.98 ± 0.48 ^{ab}	4.30 ± 0.02 ^b	0.76 ± 0.78 ^a	1.56 ± 0.71 ^c
GROUP 10	6.62 ± 0.11 ^{ab}	4.28 ± 0.08 ^b	0.62 ± 0.95 ^a	1.23 ± 0.70 ^{abc}

Values are means ± Standard deviation

Values with different superscripts down the column are significantly different ($P < 0.05$)

Group 1: Normal Control Rats, Group 2: Rats administered quail egg (0.64mls/Kg body weight), Group 3: Rats administered quail egg (10ml/kg body weight) Group 4: Poloxamer 407-Induced Rats, Group 5: 10mg of Atorvastatin+P407-Induced Hyperlipidaemic Rats, Group 6: 0.64ml of quail egg/kg body weight+P407-Induced Hyperlipidaemic Rats, Group 7: 10ml of quail egg/kg body weight+P407 Induced Hyperlipidaemic Rats, Group 8: P407-Induced Hyperlipidaemic Rats+10mg of Atorvastatin, Group 9: P407-Induced Hyperlipidaemic Rats+0.64ml of quail egg/kg body weight, Group 10: P407-Induced Hyperlipidaemic Rats+10ml/kg body weight of quail egg.

TP: Total protein, ALB: Albumin, TB: Total bilirubin, CB: Conjugated bilirubin.

Groups 5, 6 and 7: preventive studies. Groups 8, 9 and 10: curative studies.

4.1.4: Effects of Japanese quail Egg on Kidney Function Parameters of P407-Induced Hyperlipidaemic Wistar Rats

The preventive effect of Japanese quail egg on Serum creatinine and urea concentrations is presented in Table 4.5. The serum creatinine concentration shows no significant ($p > 0.05$) difference in the egg treated groups (6 and 7) having values of 0.52 ± 0.21 and 0.65 ± 0.64 respectively compared to the P407-induced hyperlipidaemic control group of value 0.71 ± 0.37 . Also, the serum urea concentration shows no significant ($p > 0.05$) difference in the egg treated groups (6 and 7) with values of 57.85 ± 0.92 and 60.48 ± 2.07 respectively, compared to the P407-induced hyperlipidaemic control group of 60.49 ± 2.07 creatinine and urea concentrations of the P407-induced hyperlipidaemic egg treated groups compared to the hyperlipidermic control.

The kidney function parameters of curative study are presented in Table 4.5. The serum creatinine concentration shows no significant ($p > 0.05$) difference in the egg treated groups (9 and 10) having values of 0.39 ± 0.26 and 0.45 ± 0.25 respectively compared to the P407-induced hyperlipidaemic control group of value 0.71 ± 0.37 . Also, the serum urea concentration shows no significant ($p > 0.05$) difference in the egg treated groups (9 and 10) with values of 62.77 ± 5.79 and 59.53 ± 3.33 respectively, compared to the P407-induced hyperlipidaemic control group of 60.49 ± 2.07 creatinine and urea concentrations of the P407-induced hyperlipidaemic egg treated groups compared to the hyperlipidermic control.

Table 4.5: Effects of Japanese Quail Egg on Kidney Function Parameters of P407-Induced Hyperlipidaemic Wistar Rats

GROUP (n=6)	UREA(mg/dl)	CREATININE(mg/dl)
GROUP 1	55.45 ± 2.64 ^{ab}	0.32 ± 0.32 ^a
GROUP 2	56.41 ± 1.64 ^{abc}	0.71 ± 0.24 ^{ab}
GROUP 3	54.49 ± 0.92 ^{ab}	0.84 ± 0.44 ^{ab}
GROUP 4	60.49 ± 2.07 ^{bc}	0.71 ± 0.37 ^{ab}
GROUP 5	53.77 ± 0.78 ^{ab}	0.39 ± 0.15 ^{ab}
GROUP 6	57.85 ± 0.92 ^{bc}	0.52 ± 0.21 ^{ab}
GROUP 7	60.48 ± 2.07 ^{abc}	0.65 ± 0.64 ^{ab}
GROUP 8	57.61 ± 1.92 ^{abc}	0.55 ± 0.37 ^{ab}
GROUP 9	62.77 ± 5.79 ^{bcd}	0.39 ± 0.26 ^{ab}
GROUP 10	59.53 ± 3.33 ^{bc}	0.45 ± 0.25 ^{ab}

Values are means ± Standard deviation

Values with different superscripts down the column are significantly different ($P < 0.05$)

Group 1: Normal Control Rats, Group 2: Rats administered quail egg (0.64mls/Kg body weight), Group 3: Rats administered quail egg (10ml/kg body weight) Group 4: Poloxamer 407-Induced Rats, Group 5: 10mg of Atorvastatin+P407-Induced Hyperlipidaemic Rats, Group 6: 0.64ml of quail egg/kg body weight+P407-Induced Hyperlipidaemic Rats, Group 7: 10ml of quail egg/kg body weight+P407 Induced Hyperlipidaemic Rats, Group 8: P407-Induced Hyperlipidaemic Rats+10mg of Atorvastatin, Group 9: P407-Induced Hyperlipidaemic Rats+0.64ml of quail egg/kg body weight, Group 10: P407-Induced Hyperlipidaemic Rats+10ml/kg body weight of quail egg. Groups 5, 6 and 7: preventive studies. Groups 8, 9 and 10: curative studies.

4.1.5 Effects effect of Japanese Quail egg on Some Haematological Parameters of P407-Induced Hyperlipidaemic Wistar Rats

Table 4.6 shows the preventive effect of oral administration of Japanese quail egg on some haematological parameters of P407-induced hyperlipidaemic rats. The result shows no significant ($p > 0.05$) difference in the levels of haemoglobin (Hb), lymphocytes, white blood cell (WBC), neutrophils, platelets, Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Packed Cell Volume (PCV) and Red Blood Cell Count (RBC) in the egg treated group (6 and 7) compared to the P407-induced hyperlipidaemic control group. (Group 4)

The curative effect of Japanese quail egg on some haematological parameters of P407-induced hyperlipidaemic rats is presented in Table 4.6. The result shows no significant ($p > 0.05$) difference in the levels of Hb, lymphocytes, neutrophils, platelets MCH, MCHC, MCV, WBC, RBC and PCV in P407 in the egg treated group (9 and 10) compared to the P407-induced hyperlipidermic control group. (Group 4).

Table 4.6: Effects of Japanese Quail egg on Some Haematological Parameters of P407 Induced Hyperlipidaemic Wistar Rats

Group (n=6)	Hb (mg/dl)	Lym (%)	Neut (%)	MCH (pg)	MCHC (g/dl)	MCV (fl)	PCV (%)	PLT ($\times 10^3$/ul)	RBC ($\times 10^6$/ul)	WBC ($\times 10^3$/ul)
GROUP 1	13.00 \pm 1.61 ^a	82.33 \pm 6.94 ^{ab}	17.68 \pm 6.94 ^{ab}	18.98 \pm 1.28 ^b	32.00 \pm 0.47 ^{ab}	59.40 \pm 4.90 ^{ab}	40.63 \pm 5.20 ^a	772.00 \pm 193.29 ^{ab}	6.87 \pm 0.93 ^a	12.43 \pm 4.71 ^{ab}
GROUP 2	13.00 \pm 0.42 ^a	87.78 \pm 3.06 ^b	12.23 \pm 3.06 ^a	19.03 \pm 0.92 ^b	32.60 \pm 0.74 ^b	58.25 \pm 2.24 ^{ab}	39.90 \pm 2.05 ^a	834.50 \pm 78.76 ^b	6.86 \pm 0.42 ^a	12.73 \pm 2.88 ^{ab}
GROUP 3	14.30 \pm 0.37 ^{ab}	84.65 \pm 3.03 ^b	15.35 \pm 3.03 ^a	17.73 \pm 0.83 ^{ab}	31.70 \pm 0.97 ^{ab}	59.50 \pm 1.92 ^{ab}	45.15 \pm 1.63 ^a	718.75 \pm 58.23 ^{ab}	8.08 \pm 0.34 ^{ab}	17.13 \pm 2.47 ^b
GROUP 4	12.10 \pm 0.27 ^a	76.35 \pm 16.36 ^{ab}	23.65 \pm 16.36 ^{ab}	19.08 \pm 0.78 ^b	32.03 \pm 0.71 ^{ab}	59.50 \pm 1.92 ^{ab}	37.80 \pm 0.99 ^a	809.75 \pm 119.34 ^b	6.36 \pm 0.26 ^a	13.33 \pm 3.50 ^{ab}
GROUP 5	13.63 \pm 0.68 ^a	82.18 \pm 2.11 ^{ab}	19.45 \pm 1.94 ^{ab}	19.51 \pm 0.80 ^b	32.60 \pm 0.54 ^b	59.57 \pm 1.58 ^{ab}	40.52 \pm 0.75 ^a	764.75 \pm 93.55 ^{ab}	6.70 \pm 0.57 ^a	16.13 \pm 1.59 ^b
GROUP 6	12.83 \pm 0.30 ^a	77.23 \pm 15.34 ^{ab}	22.78 \pm 15.34 ^{ab}	20.00 \pm 0.90 ^b	32.68 \pm 0.47 ^b	61.15 \pm 2.21 ^{ab}	39.28 \pm 1.24 ^a	843.00 \pm 93.59 ^b	6.43 \pm 0.37 ^a	16.90 \pm 5.91 ^b
GROUP 7	11.10 \pm 6.84 ^a	65.23 \pm 37.39 ^{ab}	24.78 \pm 37.39 ^{ab}	15.55 \pm 4.89 ^{ab}	27.15 \pm 8.04 ^{ab}	57.03 \pm 2.68 ^{ab}	36.13 \pm 20.56 ^a	769.00 \pm 291.99 ^{ab}	6.29 \pm 3.58 ^a	12.13 \pm 4.96 ^{ab}
GROUP 8	13.21 \pm 0.54 ^a	83.49 \pm 4.48 ^{ab}	18.57 \pm 4.76 ^{ab}	19.02 \pm 0.87 ^b	32.71 \pm 0.57 ^b	59.31 \pm 2.13 ^{ab}	39.16 \pm 2.46 ^a	796.75 \pm 112.82 ^{ab}	6.93 \pm 0.83 ^a	13.03 \pm 3.18 ^{ab}
GROUP 9	13.90 \pm 1.13 ^{ab}	83.93 \pm 4.13 ^{ab}	16.58 \pm 7.57 ^a	19.80 \pm 0.92 ^b	33.38 \pm 1.97 ^b	58.66 \pm 1.73 ^{ab}	41.43 \pm 5.58 ^a	716.75 \pm 134.31 ^{ab}	7.05 \pm 0.84 ^a	13.78 \pm 5.25 ^{ab}
GROUP 10	13.23 \pm 0.49 ^{ab}	88.35 \pm 4.67 ^b	11.65 \pm 4.67 ^a	18.63 \pm 0.82 ^{ab}	31.00 \pm 0.47 ^{ab}	59.95 \pm 2.40 ^{ab}	42.65 \pm 2.11 ^a	716.50 \pm 160.89 ^{ab}	7.12 \pm 0.35 ^a	20.3 \pm 7.67 ^b

Values are means \pm Standard deviations

Values with different superscripts down the column are significantly different ($P < 0.05$)

Group 1: Normal Control Rats, Group 2: Rats administered quail egg (0.64mls/Kg body weight), Group 3: Rats administered quail egg (10ml/kg body weight) Group 4: Poloxamer 407-Induced Rats, Group 5: 10mg of Atorvastatin+P407-Induced Hyperlipidaemic Rats, Group 6: 0.64ml of quail egg/kg body weight+P407-Induced Hyperlipidaemic Rats, Group 7: 10ml of quail egg/kg body weight+P407 Induced Hyperlipidaemic Rats, Group 8: P407-Induced Hyperlipidaemic Rats+10mg of Atorvastatin, Group 9: P407-Induced Hyperlipidaemic Rats+0.64ml of quail egg/kg body weight, Group 10: P407-Induced Hyperlipidaemic Rats+10ml/kg body weight of quail egg.

Hb: Haemoglobin, Lym: Lymphocytes, Neut: Neutrophils, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, MCV: Mean corpuscular volume, PCV: Packed Cell Volume, PLT: Platelet, RBC: Red Blood Cell Count, WBC: White Blood Cell Count.

Groups 5, 6 and 7: preventive studies. Groups 8, 9 and 10: curative studies.

4.1.6: Effects of Japanese Quail Egg on Serum Antioxidant Enzymes and Lipid Peroxidation of P407-Induced Hyperlipidaemic Wistar Rats

The effect of oral administration of Japanese quail egg on some serum antioxidant enzymes and lipid peroxidation in P407-induced hyperlipidaemic rats in the preventive study is presented in Table 4.7. Induction of hyperlipidemia significantly ($p < 0.05$) lowered the activities of catalase (CAT) and superoxide dismutase (SOD) in the serum, while it increased the level of malondialdehyde (MDA) compared to normal control group. The egg treated groups (6 and 7) shows a significantly ($p < 0.05$) lowered levels of malondialdehyde (MDA) with values of 1.77 ± 0.15 and 1.77 ± 0.06 compared to the P407-induced hyperlipidaemic control group with a value of 2.43 ± 0.06 . The CAT activity shows a significantly ($p < 0.05$) higher values in the egg treated groups (6 and 7) of 68.00 ± 2.00 and 68.33 ± 1.53 respectively compared to the P407-induced hyperlipidaemic control group with a value of 60.67 ± 2.08 . The superoxide dismutase (SOD) activity shows a significantly ($p < 0.05$) higher values of 2.33 ± 0.25 and 1.87 ± 0.10 in the egg treated groups (6 and 7) compared to the P407-induced hyperlipidaemic control group with a value of 1.53 ± 0.06 .

The curative effect of Japanese quail egg on some serum antioxidant enzymes and lipid peroxidation also showed a similar pattern as the preventive study as is shown in Table 4.7. The result shows a significantly ($p < 0.05$) lower level of MDA in the egg treated groups (9 and 10) with values of 1.60 ± 0.20 and 1.27 ± 0.25 compared to the P407-induced hyperlipidaemic control group with a value of 2.43 ± 0.06 . The CAT activity shows a significantly ($p < 0.05$) higher values in the egg treated groups (9 and 10) 68.33 ± 1.53 and 69.67 ± 2.08 respectively compared to the P407-induced hyperlipidaemic control group with a value of 60.67 ± 0.58 . The superoxide dismutase (SOD) activity shows a significantly

($p < 0.05$) higher values of 2.00 ± 0.10 and 2.23 ± 0.15 in the egg treated groups (9 and 10) compared to the P407-induced hyperlipidaemic control group with a value of 1.53 ± 0.06 .

Table 4.7: Effects of Japanese Quail Egg on Antioxidant Levels in P407-Induced Hyperlipidaemic Wistar Rats

GROUP (n=6)	MDA(umol/l)	SOD(U/I/l)	CAT(U/I/l)
GROUP 1	2.00 ± 0.20 ^{cd}	2.40 ± 0.30 ^d	67.67 ± 2.52 ^b
GROUP 2	2.00 ± 0.10 ^{cd}	2.10 ± 0.10 ^{bcd}	67.00 ± 2.00 ^b
GROUP 3	2.10 ± 0.10 ^d	2.26 ± 0.05 ^{cd}	67.67 ± 0.58 ^b
GROUP 4	2.43 ± 0.06 ^c	1.53 ± 0.06 ^a	60.67 ± 2.08 ^a
GROUP 5	1.93 ± 0.21 ^{cd}	2.33 ± 0.25 ^{cd}	67.33 ± 1.52 ^b
GROUP 6	1.77 ± 0.15 ^{bc}	1.87 ± 0.10 ^b	68.00 ± 2.00 ^b
GROUP 7	1.77 ± 0.06 ^{bc}	1.90 ± 0.06 ^b	68.33 ± 1.53 ^b
GROUP 8	2.10 ± 0.27 ^d	2.30 ± 0.30 ^{cd}	67.67 ± 1.16 ^b
GROUP 9	1.60 ± 0.20 ^b	2.00 ± 0.10 ^{bc}	68.33 ± 1.53 ^b
GROUP 10	1.27 ± 0.25 ^a	2.23 ± 0.15 ^{cd}	69.67 ± 2.08 ^b

Values are means ± Standard deviation

Values with different superscripts down the column are significantly different ($P < 0.05$)

Group 1: Normal Control Rats, Group 2: Rats administered quail egg (0.64mls/Kg body weight), Group 3: Rats administered quail egg (10ml/kg body weight) Group 4: Poloxamer 407-Induced Rats, Group 5: 10mg of Atorvastatin+P407-Induced Hyperlipidaemic Rats, Group 6: 0.64ml of quail egg/kg body weight+P407-Induced Hyperlipidaemic Rats, Group 7: 10ml of quail egg/kg body weight+P407 Induced Hyperlipidaemic Rats, Group 8: P407-Induced Hyperlipidaemic Rats+10mg of Atorvastatin, Group 9: P407-Induced Hyperlipidaemic Rats+0.64ml of quail egg/kg body weight, Group 10: P407-Induced Hyperlipidaemic Rats+10ml/kg body weight of quail egg.

SOD: Superoxide Dismutase, CAT: Catalase, MDA: Malondialdehyde.

Groups 5, 6 and 7: preventive studies. Groups 8, 9 and 10: curative studies.

CHAPTER FIVE

5.0

DISCUSSION

Animals and products derived from different organs of their bodies have constituted part of the inventory of medicinal substances used in various cultures since ancient times (Jennings and Kaiser 1998). The healing of human ailments by using therapeutics based on medicines obtained from animals or ultimately derived from them is known as zootherapy (Kurien, 1998). The use of the Japanese quail (*Coturnix japonica*) egg in Nigeria in the treatment of CVDs has not been documented. Eggs are known to be rich in fats, maternal antibodies, protein, bioactive nutrients and lysozyme (Schaafsma *et al.*, 2000). Egg components such as lysozyme, leutein, phosvitin and other biochemical substances are beneficial to human well-being (Spark, 2006)

Hyperlipidemia is responsible for the onset and progression of atherosclerosis (Poss *et al.*, 2011), a major risk factor in the development of coronary heart diseases (CHDs) such as ischaemic heart disease, myocardial infarction and stroke (Vaziri and Norris, 2011). CHDs are responsible for about 17 million deaths in the world (Boutayeb, 2006).

In the present study, Poloxamer 407 (P407) was the hyperlipidaemic inducing agent used (Johnston, 2004). P407 has been utilized in the hyperlipidaemic model due to its convenience, reproducibility and the lack of undesirable underlying pathological conditions (Wout *et al.*, 1992). A 300 mg injection of P407 produces a significant ($P < 0.05$) increase in triacylglyceride (TG), total cholesterol (TC), LDL-c and HDL-c levels (Wout *et al.*, 1992). This study agrees with the work of Megalli *et al.* (2005) who used extract of *Gynostemma pentaphyllum* in the treatment of P407-induced hyperlipidemia; which caused increased levels of triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol.

In clinical practice, effective and intensive lipid-lowering action is important in order to reduce and prevent CHDs (Abdulazeez, 2011). In this study, the high levels of TC, TG and LDL-c suggest the quail egg does not possess anti-hyperlipidaemic properties.

The elevated level of TC in rats in this study is in line with the work of Ramya *et al.*, (2012) suggesting that the quail egg could not alter the inhibitory property of P407 which inhibits cholesterol 7 α -hydroxylase, but not necessarily stimulating 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase (Johnston *et al.*, 2001).

Increase in TG concentration following P407 i.p. injection results primarily from an inhibition of TG degradation (Abdulazeez, 2011). P407 directly inhibits capillary lipoprotein lipase (LPL) responsible for plasma TG hydrolysis (Johnston, 2004). In this study, the quail egg was not able to reduce the level of TG therefore; suggesting that it could not activate endothelium bound lipoprotein lipase which hydrolyses triacylglycerol into fatty acid (Sikarwar and Patil (2011)).

LDL (low density lipoprotein) is responsible for transporting cholesterol to the body cells (Beckmann *et al.*, 2009). It transports about 60-70% of total cholesterol (Al-mahmood *et al.*, 2014). Therefore, an increase in TC level consequently increases LDL-c. The increased LDL-c which is not removed in the process of lipid metabolism is likely to flow into the sub-endothelial space, as well as to undergo oxidation (Joo *et al.*, 2010). The oxidized LDL is phagocytized by the scavengers of macrophages and the fat-laden macrophage is left with the lipid core filled with cholesterol after necrocytosis and then arteriosclerosis is initiated (Beckmann *et al.*, 2009). In this study, quail egg showed an increase in LDL-c levels this is in line with the work of Ramya *et al.*, (2012) suggesting that there might be some addition of dietary cholesterol that cannot be hydrolyzed because of inactivation of cholesterol 7 α -hydroxylase.

HDL-c acts as cholesterol scavenger. HDL-c binds and transports excess cholesterol and cholesterol esters from the blood and peripheral tissues to the liver where it is synthesized to bile acids (Brewer, 2004). It plays an important role in reducing blood and peripheral cholesterol concentrations and inhibits formation of atherosclerotic plaque in the aorta (Karmarkar, 2008; Kim *et al.*, 2008). Hence it is known as the protective cholesterol.

Atherogenic risk predictor indices (HDL-c/TC, LDL-c/HDL-c and log (TG/HDL-c)) are mathematical relationships between TC, TG, LDL-c and HDL-c that have been successfully used as markers of assessing atherosclerosis development (Kastelein *et al.*, 2008) and extent of CHDs. HDL-c/TC ratio greater than 0.3 and LDL-c/HDL-c ratio less than 2.3 indicate a reduced risk of peripheral arterial disease (Ojiakor and Nwanjo, 2005). However, log (TG/HDL-c) has been considered the most accurate in determining the extent of atherosclerosis and the risk of myocardial infarction (Dobiasova *et al.*, 2005). It has been suggested that log (TG/HDL-c) values of -0.3 to 0.1 are associated with low, 0.1 to 0.24 with medium and above 0.24 with high cardiovascular disease risk (Dobiasova, 2006).

According to these ranges provided by Ojiakor and Nwanjo (2005) for HDL-c/TC and LDL-c/HDL-c ratios and Dobiasova (2006) for log (TG/HDL-c), for the predictor of atherogenic risk index, in both the preventive and curative studies, all induced animals were at high risk of cardiovascular disease after administration of 300mg/kg body weight of P407, which is in line with the work of Ramya *et al.*, (2012) suggesting that the quail egg has no anti-atherogenic activity.

Alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are enzymes localized in the liver (Mohan *et al.*, 2007) with ALT being the most specific liver injury marker (Sherlock and Dooley, 2002) and a more selective liver parenchymal enzyme (Afzal *et al.*, 2013). Elevated serum activity of these enzymes has been

reported to be indicators of calculated risk of cardiovascular disease (Otunola *et al.*, 2010). According to Pincus and Schaffner (1996), serum transaminases (AST and ALT) and alkaline phosphatase (ALP) have long been considered as sensitive indicators of hepatic injury (Molander *et al.*, 1955), Injury to the hepatocytes alters their transport functions and membrane permeability, leading to the leakage of enzymes from their cells (Krishna *et al.*, 2007). This leakage causes an increase in levels of serum ALT, AST and ALP (Zimmerman and Seeff, 1970).

There has been conflicting reports on the effect of poloxamer 407-induced hyperlipidemia on biochemical parameters related to hepatic functions (ALP, AST and ALT). 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). Although, Ameh *et al.*, (2013) found no effect on ALT except on AST, while Johnston *et al.*, (1999) reported that P407 does not cause hepatic injury or damage. The discrepancy in the serum levels of the enzymes could probably be attributed to the levels and duration of hyperlipidemia (Lu *et al.*, 2007). In these studies, the elevated levels of ALT observed in the serum of hyperlipidaemic group may be due to injuries inflicted to the liver due to the accumulation of triglycerides and other fats in the liver cells and these conforms with the work of Hyeung *et al.*, 2006) who worked with the extract of *Picrorrhizarhizoma* in the treatment of hyperlipidemia.

The result of this work in the curative study showed a ($P < 0.05$) decrease in the liver marker enzyme levels in the induced treated groups on administration of the quail egg and atorvastatin, which is in line with the work of Sheneni *et al.*, (2014). The reversal of this liver marker enzyme towards normalcy by the egg observed in this study may be due to the prevention of the leakage of intracellular enzymes by the presence of bioactive nutrients and

their membrane stabilizing activity (Muthu *et al.*, 2008, Chavan, *et al.*, 2012). This is in agreement with the work of (Chavan *et al.*, 2012) that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes.

Serum levels of total protein (TP), albumin (ALB), total bilirubin (TB), conjugated bilirubin (CB) and direct bilirubin (DB) are indices used to assess liver function as well as disease progression (Saad *et al.*, 2006; Uthandi and Ramasamy, 2011). The levels of these liver function parameters were affected as a result of fatty liver injuries inflicted by hyperlipidemia (Sheneni *et al.*, 2014). Bilirubin is excreted by the liver and as such interference with the normal liver function which in turn affects its rate of conjugation or excretion. Thus a high level of total protein and bilirubin is used as indices for liver function and bile excretion status (Usha *et al.*, 2008). The present study showed that the quail egg have no effect on the liver function parameters.

The kidney plays a very important role in removal of metabolic wastes from the blood stream. It also helps in maintaining homeostasis by reabsorbing important material and excreting waste products (James *et al.*, 2010). Its functionality is assessed by determining the serum concentration of excretory constituents (Spencer *et al.*, 2011). Urea is the main end product of protein catabolism; it varies directly with protein intake and inversely with the rate of excretion (Ranjna, 1999). Renal diseases which diminish the glomerular filtration lead to urea retention and decrease in urea is seen in severe liver disease with destruction of cells leading to impairment of the urea cycle (Ranjna, 1999). Creatinine is a waste product formed in muscle by creatinine metabolism (Allen, 2012). Creatinine which is synthesized in the liver passes into the circulation and is taken up almost entirely by skeletal muscle (Wurochekke *et al.*, 2008). Its retention in the blood is evidence of kidney impairment

(Spencer *et al.*, 2011). In the present study, administration of P407 and quail had no impairment on the kidney function.

Haematological parameters are a frequently used laboratory tests performed to support the diagnosis of several diseases such as; anaemia, certain cancers, infections, acute hemorrhagic states, allergies and immunodeficiency disorders or used in periodic health examination and preoperative evaluations (George and Parker, 2003). The functional state of the blood systems changes dynamically according to the nature, strength and duration of exposure to external factors (Dimitrova *et al.*, 2010). Assessment of haematological parameters can be used not only to determine the extent of deleterious effect of a disease on the blood of an animal, but also to explain the functions of a plant part or its extract in relation to blood (Yakubu *et al.*, 2007). In this study, the administration of P407 and quail egg had no deleterious effect on haematological parameters.

An extensive range of antioxidant defense both endogenous and exogenous is present to protect cellular components from radical-induced damage (Prior and Cao 1999). These defenses include antioxidant enzymes like SOD and CAT. Malondialdehyde (MDA) is a product of lipid peroxidation (Devaki *et al.*, 2004). An increase in the serum MDA level indicates elevated level of lipid peroxidation (Trible and Jones, 1987). In this study, quail egg reduced the level of MDA produced which is in accordance with the findings of Mathew and Blessing (2007), who used extract of *Nauclea latifolia* in the treatment of hyperlipidemia, suggesting that quail's egg may possess the natural antioxidants necessary for protection against free radical damage since marked decrease in the levels of lipid peroxides was recorded in the rats pretreated with quail egg. This antioxidative effects can be attributed to the presence of natural antioxidants e.g. carotenoids, present in the egg (Gwerm *et al.*, 2003). The increase in SOD and CAT found in this study is in line with the work of Mahfouz *et al.*,

(1997) suggesting that the egg possesses antioxidant property that helps to scavenge free radicals.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1. Summary

The outcome of this study is summarized as follows:

- (i) Japanese quail egg supplementation had no significant effect ($p>0.05$) on the levels of total cholesterol (TC), triacylglycerol (TG) and low density lipoprotein (LDL-c) in wistar rats.
- (ii) The atherogenic risk predictor indices of the preventive and curative studies shows that all treated groups were at risk of developing atherosclerosis.
- (iii) The effect of Japanese quail egg supplementation on liver damage indicators (AST, and ALP) showed that the egg had no significant effect ($p>0.05$) on the P407-induced hyperlipidaemic rats. However, in the curative study, the egg had significantly ($p<0.05$) lowered ALT which might indicate some form of ability to prevent liver damage. The Japanese egg had no significant effect ($p>0.05$) on the level of albumin and total protein.
- (iv) The effect of Japanese quail egg supplementation on kidney function parameters show no significant effect ($p>0.05$) on the levels of creatinine and urea in the P407-induced hyperlipidaemic induced rats.
- (v) In both studies, Japanese quail egg supplementation significantly ($p<0.05$) lowered the level of malondialdehyde an indicator of lipid peroxidation in P407-induced hyperlipidaemic treated groups. There was also a significant increase ($p<0.05$) in the activities of SOD and CAT indicators of oxidative stress in P407-induced hyperlipidaemic treated groups.

6.2 Conclusion

Japanese quail egg supplementation had no effect on the serum concentrations of total cholesterol, triacylglycerol and high density lipoprotein cholesterol in the P407-induced hyperlipidaemic rats. However, there was an increase in the concentration of low density lipoprotein cholesterol. Supplementation of wistar rats with Japanese quail egg showed no effect on some liver damage parameters which includes, AST, ALP, total protein, bilirubin, urea, creatinine and albumin concentrations in the hyperlipidaemic induced rats.

The Japanese quail egg caused an increase in the activities of superoxide dismutase and catalase and decrease in malondialdehyde levels in hyperlipidaemic induced rats which indicate that it possesses some antioxidant properties. These suggest that the egg does not possess any property that may reduce the level of hyperlipidaemia but could have some anti-oxidant property. The outcome of this suggests that consumption of Japanese quail egg may not have hypolipidaemic benefits as claimed.

6.3 Recommendations

- Further work should be carried out to identify and isolate the bioactive compound that contributed to the anti-oxidative properties of Japanese quail egg.
- There is need for studies on the hyperlipidaemic effect of excess consumption of the egg since the consumption of the Japanese quail egg is on the increase following recommendations that are made in order to enhance the health of the Nigerian populace.
- There is need to enlighten the public of the false claims that Japanese quail egg consumption possesses hypolipidemic properties.

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