EFFECTS OF CLOVE (*SYZYGIUM AROMATICUM*) AND FERMENTED GINGER (*ZINGIBER OFFICINALE*) SUPPLEMENTATIONS ON SOME PHYSIOLOGICAL PARAMETERS IN HIGH FAT DIET-INDUCED TYPE 2 DIABETES IN RABBITS

BY

ABUBAKAR ABDULRAZAK

DEPARTMENT OF HUMAN PHYSIOLOGY
FACULTY OF MEDICINE
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

JULY, 2017

EFFECTS OF CLOVE (*SYZYGIUM AROMATICUM*) AND FERMENTED GINGER (*ZINGIBER OFFICINALE*) SUPPLEMENTATIONS ON SOME PHYSIOLOGICAL PARAMETERS IN HIGH FAT DIET-INDUCED TYPE 2 DIABETES IN RABBITS

BY

ABUBAKAR ABDULRAZAK [BSC, 2009; MSC, 2012 (ABU)]
P13MDHP9004

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

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN HUMAN PHYSIOLOGY

DEPARTMENT OF HUMAN PHYSIOLOGY,
FACULTY OF MEDICINE
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

JULY, 2017

Declaration

I declare that the work in this thesis entitled ‘Effects of clove (*Syzygium aromaticum*) and fermented ginger (*Zingiber officinale*) supplementations on some physiological parameters in high fat diet induced type 2 diabetes in rabbits’ has been carried out by me in the Department of Human Physiology.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

_____	_____	_____
Name of Student	Signature	Date

`

Certification

This Thesis entitled 'EFFECTS OF CLOVE (*SYZYGIUM AROMATICUM*) AND FERMENTED GINGER (*ZINGIBER OFFICINALE*) SUPPLEMENTATIONS ON SOME PHYSIOLOGICAL PARAMETERS IN HIGH FAT DIET INDUCED TYPE 2 DIABETES IN RABBITS'by Abubakar ABDULRAZAK meets the regulations governing the award of the degree of Doctor of Philosophy in Human Physiology of the Ahmadu Bello University,and is approved for its contribution to knowledge and literary presentation.

Prof. Aliyu Mohammed _____ Date _____
Chairman, Supervisory Committee

Dr. Yusuf Tanko _____Date _____
Member, Supervisory Committee

Prof. Abdulaziz Aliyu.U. Dikko_____Date _____
Member, Supervisory Committee

Prof. Aliyu Mohammed_____ Date _____
Head of Department

Prof. S.Z. Abubakar _____ Date _____
Dean, School of Postgraduate Studies

Acknowledgment

First and foremost, I thank Almighty Allah for sparing our life ingood health to date, and to mark the completion of my Ph.D thesis.

I remain indebted to my supervisors: Prof. Aliyu Mohammed, Dr Yusuf Tanko and Prof. Abdulaziz A.U. Dikko for their encouragement, guidance, mentorship and assistance throughout this program, as well for their understanding.

My sincere heart fill gratitude to the entire family of late Malam Abdulrazak Yusuf, especially our father for his effort and sacrifices for our proper upbringing with a solid foundation on discipline,may his gentle soul rest in everlasting peace.I appreciate thesupport and encouragement of my elder brothers and sisters; Mohammed Rabiu, Mohammed Nasir, Dr Mustapha, Salamatu, Sa'adatu, Safiya, Aisha, Maryam and Amina. As well your patient, support and encouragement is immeasurablemy lovely wife, Hafsat Aliyu Shika, and kids (Humaid and Humaida), I say thank you, may Allah bless our family at all time.

My academic adviser from undergraduate, Dr Yusuf Tanko, from whom I learnt a lot especially designing laboratory studies. I want to say thank you again Sir.

The staff of Human Physiology Department, Faculty of Medicine, ABU, Zaria thank you all for the assistance rendered to me during this study.

The staff of Human Physiology Department Faculty of Basic Medical Sciences, College of Medical SciencesKaduna State University Kaduna. All postgraduate students of the department, especially my seniors; Dr Jimoh, Abdulaziz and colleagues. Friends and well-wisher that directly or indirectly contributed to the completion of this work. I appreciate your individual and collective effort to the success of this work.

May Allah Bless and reward you all abundantly, Ameen.

Dedication

This research work is dedicated to the entire family of late Malam Abdulrazak Yusuf.

Abstract

The evaluation of makers for characterized underlined pathologies in diabetes mellitus isuseful in prevention, monitoring and management of the disease. This studyaimed to investigate the effects of clove buds and fermented ginger rhizome supplements onblood glucose, insulin, insulin receptor, leptin levels, Lipid profile, thyroid hormones, thyroid stimulating hormone (TSH) concentrations, oxidative stress and lipid peroxidation biomarkers, liver enzymes and electrolytes levels in high fat diet (HFD) induced type 2 diabetes(T2DM) in rabbits. Thirty male rabbits (8-10 weeks of age) divided into six groups (n=5) were used for the study. Type 2 diabetes was induced by feeding rabbits with a HFD (standard animal feed (SAF) 69% + Cholesterol 1% + Groundnut mill 20% + ground nut oil 10%) for 11 weeks, and animals with blood glucose levels≥ 140 mg/dL were selected as type 2 diabetes animal model (DAM). They were then subjected to experimental protocol and treatments for six weeks as follows.Group I, normal rabbits fed on SAF only (Normal control); Group II, DAM fed on SAF only also(Diabetic control); Group III, DAM fed on SAF + cholestran (0.26 g/kg); Group IV, DAM fed on SAF with clove (12.5%); Group V, DAM fed on SAF with fermented ginger (12.5%) and Group VI, DAM fed on SAF with clove (12.5%) + fermented ginger (12.5%) supplements.Blood glucose was assessedweekly, at the end of six weeks treatments, animals were anesthetized,blood samples were collected via cardiac puncture and the serum was usedfor biochemical assessments. The results revealed a significant($P<0.05$) decrease in blood glucose level at first and fourth week of treatment in all supplement treated groups,adecrease was also noted at fifth week in the clove supplements treated group when compared to the level in diabetic control group. There was also asignificant ($P<0.05$) increase in insulin levels in all DAM groups when compared to normal control group, while leptin

levelssignificantly decreased in clove supplement treated group when compared to the diabetic control group. Triglycerol (TG)level significantly decreased in DAM group treatedwith fermented ginger when compared to diabetic control.Thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) levels significantly decreased in supplement treated groups compared to diabetic control group.The oxidative stress makers, superoxide dismutase (SOD)was significantly increased in DAM group treated on clove + fermented ginger, while catalase (CAT) and (GPx)were significantly increased in the supplement treated groups compared to thediabetic control group.Alsomalondialdehyde (MDA) levelwas significantly decreased in DAM fed on fermented ginger supplement and in the combined supplements(clove + fermented ginger) treated groupscompared to the diabetic control group.Liver enzymes (ALT, AST and ALP)activitieswere significantlydecreased in supplement treatedgroups compared to their levels in diabetic control group. Serum Na^+ level wassignificantlyincreased in DAM groups treated on clove (12.5%) and that treated with fermented ginger (12.5%), compared to that of diabetic control group, while K^+ level wassignificantlydecreased in all supplement treated groups. Bicarbonate ions were increased in clove supplement treated group compared to that of diabetic control. The findings of the present study shows that,supplementation of clove, fermented ginger and their combinationto HFD-induced diabetic rabbits; have hypoglycaemic, hypolipidaemic, antioxidant, hepato-protective and electrolytes normalization activity. This indicate a pronounced ameliorative effectof the studied supplements onthe metabolic derangement observed in HFD-induced diabetic rabbits.Hence, these supplementsshould be considered, amongvalued dietary supplementsintended forrestoration/correction of altered physiological parametersin related condition.

Table of Contents

Title Page ……..……...i

Declaration iii

Certification iv

Acknowledgment v

Dedication..... vi

Abstract vii

Table of Contents..... ix

List of Tables..... xv

List of Figures xvi

List of Plates..... xvii

List of Appendices xviii

Abbreviations..... xix

CHAPTER ONE..... 1

1.0 INTRODUCTION 1

1.1 Background of the Study 1

1.2 Epidemiology and Distribution of Diabetes Mellitus 2

1.3 Statement of Research Problem 4

1.4 Justification of the Study 5

1.5 Aim of the study..... 6

1.6 Objectives of the Study 6

1.7 Research Hypothesis 7

CHAPTER TWO..... 8

2.0 LITRATURE REVIEW..... 8

2.1 Introduction 8

2.1.1 Classification of diabetes mellitus 9
2.1.2 Type1 diabetes mellitus 9
2.1.3. Type 2 diabetes mellitus 10
2.1.4 Gestational diabetes mellitus (GDM) 10
2.1.5 Other specific types of diabetes 11
2.2 Pathophysiology of Diabetes Mellitus 11
2.2.1 Role of insulin in glucose metabolism: 11
2.2.2 Pathophysiology of T2DM 13
2.2.3 Pathophysiology of T1DM 14
2.3 Insulin Resistance 15
2.4 Diagnostic Criteria for Diabetes Mellitus 18
2.4.1 Impaired glucose tolerance and impaired fasting glucose 20
2.5 The Role of lipid in Diabetes Mellitus 20
2.6 Diabetes Mellitus and Oxidative Stress 24
2.6.1 Biomarkers of oxidative Stress in Diabetes Mellitus 29
2.6.2 Superoxide dismutase (SOD) 29
2.6.3 Catalase (CAT) 30
2.6.4 Glutathione (GSH) 31
2.6.5 Lipids Peroxidation 32
2.7 Biomarkers of Liver Injury (liver enzymes) and Type 2 Diabetes Mellitus: ... 34
2.7.1 Alanine transaminase (ALT); 35
2.7.2 Aspartate transaminase (AST); 36
2.7.3 Alkaline phosphatase (ALP); 36
2.7.4 Other Causes of Abnormal Serum Liver Enzymes Levels 36
2.8 Role of Thyroid Hormones in Glucose Metabolism 37

2.8.1 Physiological role of leptin 40

2.9 Serum Electrolytes Levels and Diabetes Mellitus 41

2.9.1 Serum sodium disturbances (hyponatremia and hypernatremia) in DM 42

2.9.2 Serum potassium disturbances (hypokalemia and hyperkalemia) in DM 44

2.9.3 Serum chloride and bicarbonates disturbance in DM 45

2.9.4 Complications of Diabetes Mellitus 47

2.9.5 Managements of Diabetes Mellitus 48

CHAPTER THREE 62

3.0 MATERIALS AND METHODS 62

3.1 Materials 62

3.1.1 Experimental animals 62

3.1.2 Collection and authentication of experimental supplements 62

3.1.3. Drugs and reagents 62

3.1.4 Equipment and other materials 63

3.2 Methods 64

3.2.1 Preparation of high fat diet 64

3.2.2 Preparation of supplement composed diets 64

3.2.2 Induction of type 2 diabetes in rabbits using high fat diet 66

3.4 Experimental Design 66

3.4.1 Animal groupings 66

3.4.2 Determination of blood glucose levels 67

3.4.3 Termination of the study 67

3.5 Laboratory Assessments 68

3.5.1 Estimation of serum insulin 68

3.5.2 Estimation of serum insulin receptor concentration 69

3.5.3 Estimation of serum leptin 70

3.5.4 Estimation of lipid profile 71

3.6 Estimation of Thyroid Hormones (T3, T4), Thyroid Stimulating Hormone ... 75

3.6.1 Estimation of thyroid stimulating hormone (TSH) 75

3.6.2 Estimation of tri-iodothyronine (T_3)..... 76

3.7 Estimation of Oxidative Stress Biomarkers 78

3.7.1 Measurement of superoxide dismutase (SOD)..... 78

3.7.3 Measurement of glutathione peroxidase (GPx)..... 81

3.7.4 Estimation of lipid peroxidation biomarker malondialdehyde (MDA)..... 82

3.8 Estimation of Serum Liver Enzymes Levels 84

3.8.1 Determination of serum alkaline phosphatase..... 84

3.8.2 Serum alanine aminotransferase (ALT) estimation..... 84

3.8.3 Determination of aspartate aminotransferase (AST)..... 85

3.9. Estimation of Serum Electrolytes 86

3.9.1 Estimation of serum sodium ion..... 86

3.9.2 Estimation of serum potassium ion 88

3.9.3 Estimation of serum bicarbonate ion..... 88

3.9.4 Estimation of serum chloride ion 90

3.9 Statistical Analysis..... 91

CHAPTER FOUR 92

4.0 RESULTS..... 92

4.1 Effect of Clove and Fermented Ginger on Blood Glucose Levels in High Fat Diet (HFD) Induced Type 2 Diabetes (T2DM) in Rabbits..... 92

4.2 Effects of Clove and Fermented Ginger Supplements on Serum Insulin, Insulin-Receptor and Leptin Levels in HFD Induced T2DM in Rabbits..... 94

4.3 Effects of Clove and Fermented Ginger Supplements on Serum Lipid Profile in HFD Induce T2DM in Rabbits 96

4.4 Effects of Clove and Fermented Ginger Supplements on Thyroids Hormones and TSH levels in HFD induce T2DM in rabbits 98

4.5 Effects of Clove and Fermented Ginger Supplements on Oxidative Stress Biomarkers and Lipid Peroxidation Maker in HFD Induce T2DM in Rabbits .. 100

4.6 Effects of Clove and Fermented Ginger Supplements on Liver Enzymes Levels in HFD induced T2DM in Rabbits..... 102

4.7 Effects of Clove and Fermented Ginger on Serum Electrolytes Concentrations in HFD Induced T2DM in Rabbits. 104

CHAPTER FIVE..... 106

5.0 DISCUSSION..... 106

5.1 Effects of Clove and Fermented Ginger on Blood Glucose levels..... 106

5.2 Effect of Clove and Fermented Ginger on Serum Insulin and Insulin Receptor Levels 108

5.3 Effect of Clove and Fermented Ginger on Serum Leptin Level 109

5.4 Effect of Clove and Fermented Ginger on Lipid profile..... 109

5.5 Effect of Clove and Ginger Supplements on Serum Thyroid Stimulating Hormone, T_3, and T_4 111

5.6 Effect of Clove and Fermented Ginger on Oxidative Stress Biomarkers 112

5.7 Effect of Clove and Fermented Ginger on Serum Liver Enzymes (ALT, AST, and ALP) 114

5.8 Effect of Clove and Fermented Ginger on Serum Electrolytes..... 115

CHAPTER SIX..... 118

6.0 CONCLUSION, AND RECOMENDATION 118

6.1 Conclusion 118

6.2 Recommendations 118

6.3 Contributions to Knowledge 120

REFERENCES 122

APPENDICES 141

List of Tables

Table 2.1: Index for Euglycaemia and Classification of Hyperglycaemia in Pre-diabetes and Diabetes…… 19

Table 2.2: Cholesterol Levels Recommended for General Diabetes Population…….223

Table 2.3. Active Ingredient and Compounds in Ginger and their Biological Activities……....59

Table 4.1: BloodGlucose Level of High Fat Diet Induced-Hyperglycaemia in Rabbits Treated With Supplemented Diets Clove 12.5%, Fermented Ginger 12.5% And Clove 12.5% + Fermented Ginger 12.5% . …….....93

Table 4.2: Insulin, Leptin And Insulin Receptor Levels Of High Fat Diet Induced-Hyperglycaemic Rabbits Treated With Supplemented Diets Clove 12.5%, Fermented Ginger 12.5% And Clove 12.5% + Fermented Ginger 12.5%.....95

Table 4.3: Lipid Profile Levels Of High Fat Diet Induced-Hyperglycaemic Rabbits Treated With Supplemented Diets Clove 12.5%, Fermented Ginger 12.5% And Clove 12.5% + Fermented Ginger 12.5% . …….....97

Table 4.4: T_3, T_4And TSH Levels of High Fat Diet Induced-Hyperglycaemic Rabbits Treated With Supplemented Diets Clove 12.5%, Fermented Ginger 12.5% And Clove 12.5% + Fermented Ginger 12.5% ……99

Table 4.5: Oxidative Stress And Lipid Peroxidation BiomarkerLevels of High Fat Diet Induced-Hyperglycaemic Rabbits Treated With Supplemented Diets Clove 12.5%, Fermented Ginger 12.5% And Clove 12.5% + Fermented Ginger 12.5% ……..101

Table 4.6: Liver Enzymes Levels of High Fat Diet Induced-Hyperglycaemic Rabbits Treated With Supplemented Diets Clove 12.5%, Fermented Ginger 12.5% And Clove 12.5% + Fermented Ginger 12.5% ……103

Table 4.7: Serum Electrolytes Levels of High Fat Diet Induced-Hyperglycaemic Rabbits Treated With Supplemented Diets Clove 12.5%, Fermented Ginger 12.5% And Clove 12.5% + Fermented Ginger 12.5% ……..……105

List of Figures

Figure2.1: Schematic Diagram of Pathophysiology of Diabetes Mellitus……..……..…17

Figure 2.2: Schematic Diagram of Glucose Induced Cellular Damage Through Oxidative Stress…….....27

List of Plates

Plate I. Clove buds (*Syzygium aromaticum)* ……...55

Plate II. Ginger (Zingiber *officinale*)rhizome **.....**……..…..……...58

List of Appendices

Appendix I: Blood glucose level of high fat diet induced-insulin resistance rabbits treated with clove 12.5%, fermented ginger 12.5% and clove12.5 + fermented ginger 12.5%, and control groups for six weeks ……...…139

Appendix II: Shows insulin, leptin and insulin receptor levels in high fat diet induced-insulin resistance rabbits treated with clove 12.5%, fermented ginger 12.5% and co-administered clove12.5 + fermented ginger 12.5%, and control groups for six weeks …….140

AppendixIII: Lipid Profile of high fat diet induced-insulin resistance rabbits treated with clove 12.5%, fermented ginger 12.5% and co-administered clove12.5 + fermented ginger 12.5%, and control groups for six weeks……..…….. 141

Appendix IV: Serum thyroids hormones and TSH level in control, and high fat diet induced-insulin resistance rabbits treated with clove 12.5%, fermented ginger 12.5% and co-administered clove12.5 + fermented ginger 12.5%, for six weeks ……..…. 142

Appendix V: Oxidative stress biomarkers and lipid peroxidation maker of high fat diet induced-insulin resistance rabbits treated with clove 12.5%, fermented ginger 12.5% and co-administered clove12.5 + fermented ginger 12.5%, and control groups for six weeks ...…….…..143

Appendix VI: Liver Enzymes Activities of high fat diet induced-insulin resistance rabbits treated with clove 12.5%, fermented ginger 12.5% and co-administered clove12.5 + fermented ginger 12.5%, and control groups for six weeks ……...…….……144

Appendix VII: Serum electrolytes and chloride ion levels of high fat diet induced-insulin resistance rabbits treated with clove 12.5%, fermented ginger 12.5% and co-administered clove12.5 + fermented ginger 12.5%, and control groups for six weeks ……..…...……145

Abbreviations

5'NTD	5' nucleotidase
AIDs	Acquired Immune Deficiency Syndrome
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
BUN	Blood Urea Nitrogen
DM	Diabetes Mellitus
GDM	Gestational Diabetes Mellitus
GGT	Gamma Glutamyl Transpeptidase
GPx	Glutathione Peroxidase
HDL	High Density Lipoprotein Cholesterol
HIV	Human Immunodeficiency Virus
IDDM	Insulin Dependent Diabetes Mellitus
INR	International Normalized Ratio
LDH	Lactate Dehydrogenase
LDL	Low Density LipoproteinCholesterol
LFTs	Liver Function Tests
MDA	Malondialdehyde
Na^+	Sodium ion
NIDDM	Non-Insulin Dependent Diabetes Mellitus
OHAs	Oral Hypoglycaemic Drugs
PPAR-ɣ	Peroxisome Proliferator-Activated Receptor gamma
PT	Prothrombin Time

SEM	Standard Error of Mean
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvate Transaminase
SOD	Superoxide Dismutase
T_3	Triiodotyronine
T_4	Thyroxine
TC	Total Cholesterol
TSH	Thyroid Stimulating Hormone
VLDL	Very Low-density lipoprotein- cholesterol
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Diabetes mellitus (DM) is among the fastest-growing health problem in the World, with a rising prevalence in low andmiddle-income countries than in high-income countries over the past decade. In the present day, DM is reaching epidemic proportion in some regions, as a consequence of interaction between change in life-style (lack of exercise, unhealthy diet), obesity,overweight, and individuals' genetic make-up (World Health Organization (WHO), 2016). DM is among the common chronicnon-communicable disease in many populations at present, associated with risk factors that are genetically and environmentallyinclinednot pathogenic agents. It is a pivotal group of metabolic disorder that can secondarily be complicated bymany non-communicable diseasesif not properly and effectively managed (Matters *et al*., 2006).It is characterized by abnormal fuel metabolism, due to defects in insulin secretion, insulin action, or both;which results most notably inhyperglycaemia and dyslipidaemia.DMisfrequently associated with the development of micro and macrovascular complications which include neuropathy, nephropathy, cardiovascular and cerebrovascular diseases (Feldman, 1988 : American Diabetes Association (ADA), 2014). The disease is associated with poor quality of life and increased mortality and morbidity. The long-term hyperglycaemia is an important factor in the development and progression of the micro- and macrovascular health complications in a diabetic (Strojek, 2003). Hence, therapeutic measures for diabetes targets to control blood glucose level is important and this includes dietary therapy, recommended increase in physical activity, and pharmacological therapy in order to control the marked hyperglycaemia (ADA, 2016).

Diabetes has become a pressing health problem for Sub-Saharan Africa (Hall *et al*., 2011). It is however possible to avert much of its complications and burden, if effective, accessible, and affordable interventions with less side effect are implemented in itsprevention and management. Establishing the scientific basis and physiological effect of many traditional plants, herbs, and spices that have not undergone thorough scientific scrutiny;yet many are in-use for prevention and management of several diseases including diabetesacross many communities in Africa,largely due to their easy accessibility, affordability, low costand assumed low side effect (Hall *et al*., 2011).

Extracts of certain herbs and spices have revealedpositive effect on many physiological indices used in management of diabetes; and this includes;ethylacetate and n-butanol fractions of *Ganoderma lucidum* aqueous extract (Mohammed*et al*., 2009), Clove (*Syzygium aromaticum*) flower buds. This has been employed for centuries as food preservatives and as medicinal plants mainly due to its antioxidant and antimicrobial activities (Cortes-Rojas *et al*., 2014).

Ginger, the rhizome of the *Zingiber officinale*plant is a commonly consumed dietary condiment. It isconsidered to be safe and used to cure various diseases. It has also beneficial role in many laboratory disease animal model and potent for many therapeutic activity (Rahmani, *et al*., 2014).

1.2 Epidemiology and Distribution of Diabetes Mellitus

Diabetes Mellitus was considered a rare disease, but risen to global epidemic proportions by the end of twentieth century. This change has been driven by increasing affluence and widespread adoption of "western" lifestyles and diet, among many other factors (Gale, 2014).The worldwide prevalence of diabetes mellitus for all age groups was estimated to be 2.8% in 2000 and it is projected to be 5.4% by 2025, with a prevalence of 347 million in 2013 (Medagama and Bandara, 2014). International Diabetes Federation, 2014 also projected an increase from 21.5 million in 2014 to 41.5 million by the year 2035.

Diabetes mellitus prevalence is increasing globally, and Sub-Saharan Africa is no exception, Type 2 diabetes accounts for over 90% of diabetes in Sub-Saharan Africa. Diabetes exerts a significant burden in this region, and this is expected to increase in future, many diabetic patients face significant challenges accessing diagnosis and treatment, which contributes to the high mortality and prevalence of complications observed (Hall *et al*., 2011).

In 2014, about 387 million people were afflicted with diabetes in the world; 22 million reside in sub-Saharan Africa. Nigeria is the most populated country in Africa and is home to 4 million people with diabetes, or a fifth of all diabetes cases in sub-Saharan Africa in 2014 (Fasanmade and Dagogo-Jack, 2015). The prevalence of diabetes mellitus in Nigeria shows an epidemiological increase in trend from 0.5% in 1960 (Adadevoh, 1960) to 1.6% in parts of Kaduna (Bakari *et al.,* 1999), 2.0% in Zaria (Dahiru *et al*., 2008) and 7.9% in Port Harcourt (Nyewe *et al*., 2003), to about 2.6 million for the whole country population (Hamoudi *et al*., 2012),

The burden of T2DM is disproportionately borne by people of working age in this region, reducing the economic activity of this group through disease and disability, whichaffects both household and national economies (Fasanmade and Dagogo-Jack, 2015). Diabetes therefore not only imposes considerable costs of treatment on families, it also hinders their ability to pay for this treatment through the loss of income of the diabetic member. The burden of DMdue to its increasing prevalence, cost of management, complications and low survival rateamong the economically activeindividuals, will negatively influencethe overall economic status of low and middle-incomedeveloping nations (Fasanmade and Dagogo-Jack, 2015). Hence, it is critical for researchers from all angles to highlight the importance and search for readily accessible and cost effective remedy and interventions for both prevention and management of DM.

1.3 Statement of Research Problem

Diabetes mellitus is one of the common metabolic disorders which 2.8% of the population suffers; andis a major cause of morbidity and mortality worldwide.Among its important complications, include hyperglycaemia (Upendra *et al.*, 2010).

The underlying mechanisms in the onset of diabetes are complex,because hyperglycaemia could result due to the cause-effect relationship of increased oxidative stress (West, 2001). Biomarkers of increased oxidative stress, as measured by indices of lipid peroxidation and protein oxidation are increasedat the onset of both type 1 and type 2 diabetes mellitus, as well as later complications of the disease (Cederberg *et al*., 2001).

Hence, oxidative stress may be implicated and / or mediate the development and progression of micro and macrovascular processes of the disease complications of which leads to neuropathy, nephropathy, cardiovascular and cerebrovascular diseases (Feldman, 1988).

Management of diabetes is still a challenge to the medical profession especially in terms of patient compliance and accessibility to existing pharmacological therapies largely due to inadequate efficacy and serious adverse effects (Srinivasan and Ramarao, 2007).

Paucity of scientific knowledge still exist on the characterized clove and ginger supplements that are assumed beneficial to type 2 diabetic in terms of quantity, quality and their effects on other biological systems, particularly when compared to conventional therapy (Tapsell *et al*., 2006).

1.4 Justification of the Study

- The number of people suffering from DM worldwide is increasing at an alarming rate, an estimation of 366 million people are likely to be diabetic by the year 2030 compared to 191 million estimated in 2000 (Wild *et al.*, 2004).
- Conventional therapies for DM suffers challenges in terms of: high cost, patients compliance, accessibility, affordability and side effects in many population (Srinivasan and Ramarao, 2007).
- Alternative treatments that are not scientifically scrutinized may cause harmful effect rather than the expected beneficial effect on the disease condition.

- Oxidative stress has a pivotal role in DM, Clove and ginger are powerful free-radical scavengers and wide-spectrum antioxidants (Vijayakumar *et al*., 2012).
- Paucity on scientifically based knowledge, on effects of clove and ginger supplements in non-chemical induced-metabolic disorders in animal models, still exist.
- Therefore, ameliorating oxidative stress using antioxidants might be an effective measure for preventionor management of diabetes mellitus and its complications (Giugliano *et al.,* 1996; Mohammed *et al.,* 2009).

1.5 Aim of the study

The aim of this study was to evaluate the effects of clove andfermented ginger supplements on some physiological parameters in high fat diet-inducedtype 2 diabetes in rabbits.

1.6 Objectives of the Study

The specific objectives of this study was to determine the effects of Clove, fermented ginger and their co-supplementation in high fat diet-induced type 2 diabetes in rabbits on some physiological parameters which include:

i. Bloodglucose level
ii. Serum insulin, insulin receptor, and leptin levels
iii. Lipid profile (TC,TAG, HDL-c,and LDL-c)
iv. Serum thyroid hormones (T_3, T_4) and thyroid stimulating hormone(TSH) concentrations
v. Oxidative stress biomarkers (SOD, CAT and GPx), and malondialdehyde (MDA) levels

vi. Serum liver enzymes (ALT, AST and ALP)

vii. Serum electrolytes levels (Na^+, K^+, HCO_3^-) and Cl^- concentration

1.7 Research Hypothesis

Clove, fermented ginger and their combinedsupplementation do not have effects on some physiological parameters in high fat diet-inducedhyperglycaemia in rabbits.

CHAPTER TWO

2.0 LITRATURE REVIEW

2.1 Introduction

Diabetes mellitus (DM) is one of the major health challenges of the 21st century, it results from insulin deficiency, absent or its inactivity (ADA, 2014). The disease arises when beta cells of the islet of Langerhan's in the pancreas produce insufficient insulin, or do not produce insulin at all, or as well due to post receptor defect. Insulin is a hormone that basically aid the absorption of glucose and amino acids into many tissue (adipose tissue, liver and skeletal muscles) cells for storage and utilization. Therefore,insulin deficiency,lack of insulin or its inactivitymay results tohyperglycaemia, and dyslipidaemia, which leads to metabolic disturbances and symptoms of glucosuria, polydipsia and increased urine volume. Diabetes is confirmed and classify after a series of laboratory tests.This condition if not properly manage over a long term period is associated with health complications ranging from dysfunction to derangement of integrity and failure of various organs such as blood vessels, eye, kidneys and nerves, which may finally lead to blindness, renal failure and nerve damage respectively (Bansal *et al*., 2006).

Although genome-wide association studies revealed a genetic contribution in the etiology of diabetes, the environmental risk factors are very likely the most prominent cause of beta cell decline in function in vast majority of cases. Another strong risk factor is lifestyle changes such as lack of physical activity together with improper dieting/adaptation of unhealthy diet which may leads to excessive adipose tissue deposit, contributeddue to chronic elevation of blood glucose and the circulating

plasma saturated free fatty acids (FFAs) levels (WHO, 2006: American Diabetes Association (ADA), 2016).

Chronic intake of saturated free fatty acids or excessively high intake of carbohydrate coupled with lack of physical activity is associated with diabetes and may contribute to the impairment of functional beta cell mass (Brajkovic*et al*., 2016).

The diagnosis of diabetes mellitus is made when people have abnormally high levels of blood glucose. Fasting blood glucose levels should not be higher than 126 mg/dL. Even after eating, blood glucose level should not be higher than 200 mg/dL. In addition, measure of the level of a protein in the blood, haemoglobin A_1C (Glycosylated hemoglobin) can be used to diagnose diabetes (Li *et al.*, 2004).

2.1.1Classification of diabetes mellitus

The American Diabetes Association revised the nomenclature for the major types of diabetes into: Type 1(DM-1), Type 2(DM-2), Gestational and Other specific types of diabetes. This classification of diabetes is based on aetiology,the term "diabetes", without qualification, usually refers to diabetes mellitus. The rare disease diabetes insipidus has similar symptoms as diabetes mellitus, but without disturbances in glucose metabolism (Pittas, and Greenberg, 2003).

2.1.2 Type1 diabetes mellitus

Type1 diabetes mellitus is also referred to insulin dependent diabetes mellitus, childhood-onset diabetes mellitus and juvenile diabetes mellitus. It is characterized by loss of insulin producing beta cells of the islets of Langerhans in the pancreas (ADA,

2014). Type 1 diabetes is further classified into immune-mediated or idiopathic; it is more common among children and young adults (around 20 years). About 5-10% of patients with diabetes have DM-1. Type 1 diabetes affects 3 in 1000 children and its incidence is increasing worldwide both in low and high prevalence populations.

Since it is common among young individuals and insulin hormone is used for treatment, Type 1 diabetes is also referred to as Insulin Dependent Diabetes Mellitus (IDDM) or Juvenile Diabetes (Rother, 2007).

2.1.3. Type 2 diabetes mellitus

This is an array of dysfunctions characterized byhyperglycaemia and resulting from the combination of resistance to insulin action, inadequate insulin secretion and excessive or inappropriate glycogen secretion. The non-responsiveness of the body tissues to insulin mostly involve receptors insufficiency. In type2 diabetes, the predominant abnormality is reduced insulin sensitivity, and the earliest manifestation ishyperglycaemia, which can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver. Type 2 diabetes is the most prevalent form of diabetes, accounting for over 90% of all cases of diabetes (Seuring,*et al*., 2015).

2.1.4 Gestational diabetes mellitus (GDM)

GDM is any degree of glucose intolerance starting in pregnancy or noticed during pregnancy.It is caused by fluctuations of hormonal level during pregnancy. The blood glucose level returns to normal after the gestational period in majority of cases. Gestational diabetes mellitus affects ~ 7% of all pregnancies.The prevalence of GDM

varies in direct proportion to the prevalence of type 2 diabetes for a given ethnic group or population (David and Gardner, 2011).

2.1.5 Other specific types of diabetes

Other types of diabetes occursecondarily to manifestations of a primary pathological or disease condition. Depending on the aetiology, this includes: Maturity onset diabetes of the young (MODY); Genetic Defects in Insulin Action; Diseases of the Exocrine Pancreas; Endocrinopathies; Infections associated; Drug/chemical induced diabetes (Pittas and Greenberg, 2003).

2.2 Pathophysiology of Diabetes Mellitus

2.2.1 Role of insulin in glucose metabolism:

Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells (primarily muscle and fat cells, but not central nervous system cells). Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus (Matters *et al*., 2006). Humans are capable of digesting some carbohydrates, in particular those most common in food; starch, and some disaccharides such as sucrose and are converted within a few hours to simpler forms, most notably the monosaccharideglucose, the principal carbohydrate energy source used by the body. The rest are passed on for processing by gut flora largely in the colon. Insulin is released into the blood by beta cells (β-cells), found in the islets of Langerhans in the pancreas, in response to rising levels of blood glucose, typically after meal. Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage (Harris, *et. al*., 1995).

Insulin is also the principal control signal for conversion of glucose to glycogen for internal storage in liver and muscle cells. Glucagon is another pancreatic hormone that play an important role in glucose metabolism; it acts in opposite manner to insulin. During lowered glucose levels, there is reduced release of insulin from the β-cells, and in the reverse releasing of glucagon by the α-cells. Glucagon stimulate the conversion of glycogen to glucose in the liver. Therefore glucose produced from internal liver cell stores (as glycogen) re-enters the bloodstream. Normally, this occur when the level of insulin is low, which normally correlates with low levels of blood glucose (David and Brown, 2006).

Higher insulin levels increase some anabolic ("building up") processes, such as cell growth and duplication, protein synthesis, and fat storage. Insulin (or its lack) is the principal signal in converting many of the bidirectional processes of metabolism from a catabolic to an anabolic direction, and *vice versa*. In particular, abnormal insulin functions or lower level leads to intracellular hypoglycaemia, that trigger for alternate cellular metabolic pathways for energy production, especially burning fat as energy source for intracellular activities. Over time the fat-burning metabolic phase may leads to accumulation of ketone bodies in circulation, leading to ketoacidosis. If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or resistance), or if the insulin itself is defective, then glucose will not have its usual effect, so it will not be absorbed properly by those body cells that require it, nor will it be stored appropriately in the liver and muscles. The net effect is persistent high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis (Shisheva and Shechter, 1992). When the glucose concentration in the blood is raised beyond its renal threshold (about 10 mmol/L,

although this may be altered in certain conditions, such as pregnancy), reabsorption of glucose in the proximal renal tubule is incomplete, and part of the glucose remains in the urine (glycosuria). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst (Wolf *et al*., 2009).

2.2.2 Pathophysiology of T2DM

Type 2 diabetes mellitus is characterized by the following three abnormalities: (1) peripheral resistance to insulin, especially in muscle cells; (2) increased production of glucose by the liver; and, (3) altered pancreatic insulin secretion. Increased tissue resistance to insulin generally occurs first and is eventually followed by impaired insulin secretion. The pancreas produces insulin, yet insulin resistance prevents its proper use at the cellular level. Glucose cannot enter target cells and accumulates in the bloodstream, resulting inhyperglycaemia (Brian, 2006). In essence, there is normal production of insulin hormone but the body cells are resistant to insulin. Since the body cells and tissues are non-responsive to insulin, glucose remains in the bloodstream (Eriksson *et al*., 2003). Although the genetic predisposition to type 2 diabetes is strong, no single genetic defect has been found. In addition to genetic influences, acquired risk factors for type 2 diabetes include obesity, advancing age, poor dietary habit (consumption of excess fat) and an inactive lifestyle. Type 2 diabetes usually has a slow onset and may remain undiagnosed for years. Approximately half of those who have type 2 diabetes are unaware of their disease. Unfortunately, the insidious nature of the disease allows prolonged periods of hyperglycaemia to begin exerting negative

effects such as hyperglycaemic-induced oxidative stress on major organ systems. Obesity and oxidative stress are strongly implicated in the onset of T2DM, the disease chronically progress from asymptomatic condition of pre-diabetes to a symptomatic state of clinically defined diabetes. The pre-diabetic state is often refers to metabolic syndrome, which is diagnosed when a patient has 3 out of the following 5 pathologies: obesity, hypertension, fasting hyperglycaemia, elevated serum triglycerides, and decreased high-density lipoprotein. The long-term pathophysiology underlining poorly manage T2DM, is based on associated hyperinsulinemia, hyperglycaemia and dyslipidaemia leading to oxidative stress induce molecular-tissue peroxidation / damage that is highly lethal to the pancreatic tissue. Therefore, hyperinsulinemia may be observed (Heydemann, 2016).

2.2.3 Pathophysiology of T1DM

In Type 1 diabetes, the pancreas cannot synthesize enough amount of insulin hormone as required by the body. The pathophysiology of Type 1 diabetes mellitus suggests that it is an autoimmune disease, in which the body's own immune system generates anti-bodies that attack the beta cells of the pancreas. Consequently, the pancreas secretes little or no insulin (ADA, 2009). The autoimmune beta cell destruction is thought to be triggered by an environmental event, such as a viral infection. Genetically determined susceptibility factors increase the risk of such autoimmune phenomena. The onset of type 1 diabetes is usually abrupt. It generally occurs before the age of 30 years, but may be diagnosed at any age. Type 1 diabetes patient is absolutely dependent on exogenously administered insulin for survival. People with type 1 diabetes are highly susceptible to diabetic ketoacidosis. Because the pancreas produces no insulin, glucose cannot enter cells and remains in the bloodstream. To meet cellular energy needs, fat is

broken down through lipolysis, releasing glycerol and free fatty acids. Glycerol is converted to glucose for cellular use. Fatty acids are converted to ketones, resulting in increased ketone levels in body fluids and decreased hydrogen ion concentration (pH). Ketones are excreted in the urine, accompanied by large amounts of water. The accumulation of ketones in body fluids, decreased pH, electrolyte loss and dehydration from excessive urination, and alterations in the bicarbonate buffer system result in diabetic ketoacidosis (DKA). Untreated DKA can result in coma or death (Brian, 2006).

2.3 Insulin Resistance

Insulin resistance is a pathological state in which insulin action is impaired in target tissues including liver, skeletal muscle, and adipose tissue. Insulin stimulates a signaling network, initiating the activation of the insulin receptor tyrosine kinase and phosphorylation of the insulin receptor substrate (IRS) proteins (e.g., IRS-1 and IRS-2) This pathway is impaired at multiple steps through alterations in the protein levels and activities of the signaling molecules, enzymes, and transcription factors resulting in insulin resistance (Taniguchi *et al*., 2006).

Although the exact causes of insulin resistance are not completely understood, it was postulated that, the major contributors to insulin resistance include excess weight and physical inactivity among other causes such as increased level of counter insulin/insulinemic activity hormones in circulation. Other associations of insulin resistance may include genetic factor, steroid use, old age, sleep problems (sleep apnoea) and cigarette smoking.

In insulin resistant state, though insulin may be available in circulation, insulin receptors may be defective or prevented from binding to insulin due to other biomolecules that compete for the same binding site. This leads to metabolic dysregulation of glucose resulting in hyperglycaemia which leads to many metabolic defects as summarised in figure 2.1. The beta cells in the pancreas response to hyperglycaemia is increased insulin production to meet-up in achieving euglycemia, this leads to continuous increased in insulin production resulting to hyperinsulinemia.

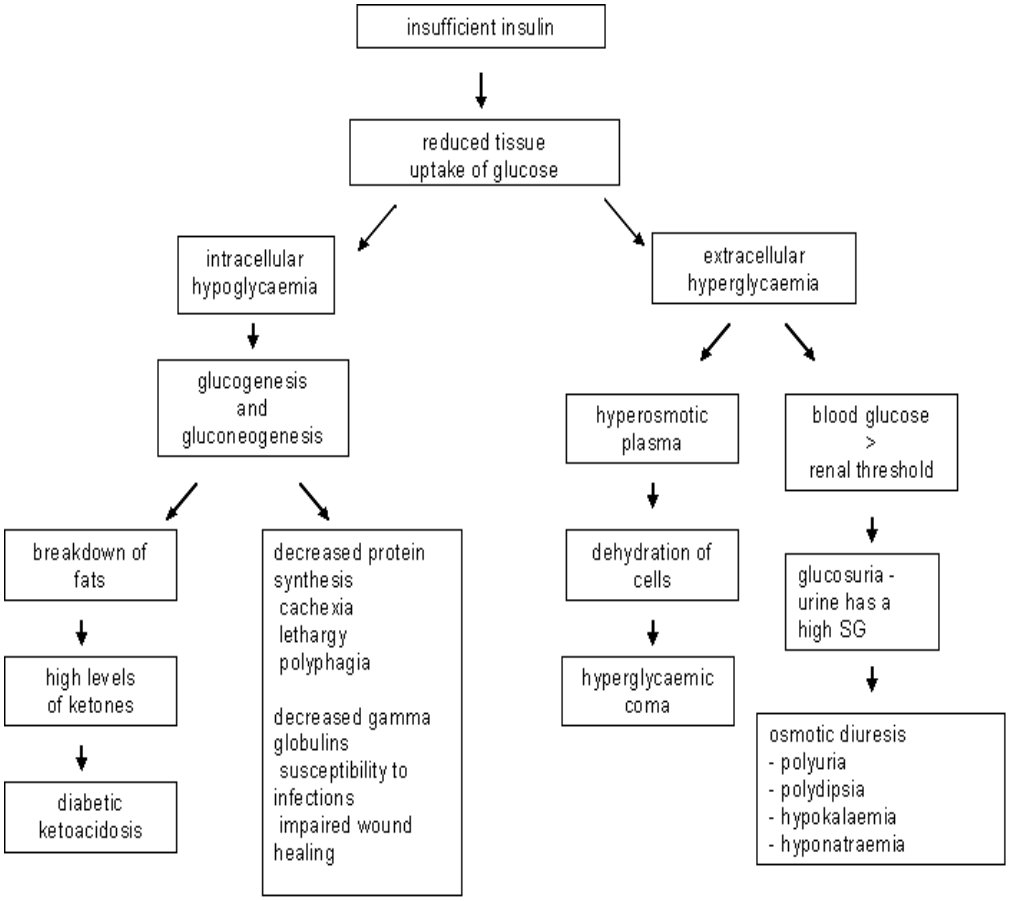


Figure 2.1 Schematic diagram of pathophysiology of type 2 diabetes mellitus, adapted from (Pittas, 2004)

2.4 Diagnostic Criteria for Diabetes Mellitus

Diabetes mellitus is commonly characterized by persistent hyperglycaemia. The physiological blood glucose levels of a healthy person is 60mg/dl to 110mg/dl fasting glucose and 80mg/dl to 160mg/dl in the postprandial state, thus diabetes mellitus can be diagnosed by demonstrating one of the following;(Selvin, 2010)

- Fasting plasma glucose at or above 126mg/dl (>7.0mmol/L)
- Plasma glucose at or above 200mg/dl (11.1mmol/l) two hours after a 75g oral glucose load in a glucose tolerance test.
- Random plasma glucose at or above 200mg/dl (11.1mmol/l)
- Glycated haemoglobin at or above 6.5%.
- Two fasting glucose measurements above 126mg/dl (7.0mmmol/l) or random blood sugar levels greater than 200mg/dl on two occasions is considered diagnostic for diabetes mellitus.
- Patients with fasting glucose between 110mg/dl to 125mg/dl (6.1 to7.0mmmol/l) are considered to have impaired glucose tolerance (Baynest, 2015).

Table 2.1 presents referenced range values of blood glucose and glycosylated haemoglobin for normal, pre-diabetic and diabetes.

Table 2.1 Index for Euglycemia and Classification of Hyperglycemia in Pre-diabetes and Diabetes

	Normal Plasma Glucose Tolerance	Pre-diabetic (IFG and IGT)	Diabetic
FPG	<5.6mmol/L (100mg/dL)	5.6 - 6mmol/L(100-125mg/dL)	>7.0mmol (126mg/dL)
2h PG	<7.8mmol/L (140mg/dL)	7.8 - 11.0mg/dL (140-199mg/dL)	>11.1mmol/L (200mg/dL)
HbA1c	<5.6%	5.7 - 6.4%	≥ 6.5%

(ADA, 2014).

FPG = Fasting plasma glucose, PG = plasma glucose, IFG = Impaired fasting glucose, IGT = impaired glucose tolerance.

2.4.1 Impaired glucose tolerance and impaired fasting glucose

The conditions known as impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) represent metabolic states lying between diabetes and normoglycemia. People with IFG have increased fasting blood glucose levels of 5.6 - 6.9mmo/L (100-126mg/dL) but usually have normal levels of < 5.6mmol/L (100mg/dL) following food consumption. Those with IGT are normoglycemic most of the time, but become hyperglycaemic with plasma glucose levels of 7.8 -11.0mmol/L (140-199mgmg/dL) after 2hours of an oral glucose tolerance tests (ADA, 2014). IGT and IFG are not considered to be clinical entities; rather, they are risk factors for future diabetes. The pathophysiology of IFG and IGT is related primarily to increased insulin resistance whereas endogenous insulin secretion is normal in most patients. Approximately 30 to 40% of individuals with IGT or IFG will develop type 2 diabetes within 10 years after onset (Brian, 2006). Hence, Management of diabetes Mellitus is importantly based on controlling/regulating certain physiological indicators such as blood glucose level, and lipid profile as well.

2.5 The Role of lipid in Diabetes Mellitus

Lipids are body fats synthesized either within cells (endogenous lipids) or derived from dietary fat (exogenous lipids). They are characterized by insolubility in water, and biologically function in cell membranes as important structural units in form of phospholipids, and as a major source of stored energy in adipose tissue in form of triglycerols (TAGs) (Verges, 2015). Lipids components, TAGs, phospholipids, cholesterols and fatty acids, are transported in form of lipoproteins (High-density lipoprotein and low density lipoprotein cholesterols) between sites of synthesis in the

liver and intestine (site of dietary fat absorption) to peripheral tissues, for utilization and storage (John *et al*., 2013).

In type2 diabetes, many factors may affect blood lipid levels, because of interrelationship between carbohydrates and lipid metabolism due to the regulatory role of insulin and it counter hormones on their metabolism. Insulin resistance, absence or inefficiency are primary defects in diabetes, which manifest as distort metabolism of circulatory nutrients especially carbohydrates and lipids (ADA, 2014). Although the exact mechanisms are only partially understood, as postulated elevated concentrations of lipids especially in form of free fatty acids and triglycerides disrupt or modulate the cascade linking insulin receptors with glucose transporters and impair the normal function of the β-cell. Furthermore, they are important modulators of inflammation and may induced subclinical inflammation which also leads to insulin resistance and β-cell dysfunction. Hypertriglyceridemia have been found to worsen glucose metabolism. This results in difficult to control hyperglycaemia in patients with hypertriglyceridemia compared to those with normal triglyceride values (Parhofer, 2015). Therefore, Disorder in carbohydrate metabolism may leads to disorder in lipid metabolism and vice versa. As well in non-diabetic individuals, insulin resistance in combination with hyperinsulinemia has a strong predictive value for future development for T2DM (Dixit, *et al*., 2014).

Clinical recommended lipid profile content are based on indices that include; total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c). Molecules called lipoproteins carry cholesterol in the blood. Two important

kinds of lipoproteins are low-density lipoprotein (LDL) and high-density lipoprotein (HDL). When checking LDL and HDL, triglycerides level is also checked along.

- Total cholesterol measure the total amount of cholesterol in blood and is based on the HDL, LDL, and triglycerides values.
- LDL cholesterol makes up the majority of the body's cholesterol. LDL is known as "bad" cholesterol because; high levels leads to plaque build-up in the arteries which is associated with cardiovascular diseases.
- HDL cholesterol absorbs cholesterol and carries it back to the liver, which metabolized and cleared it from the system. HDL is known as "good" cholesterol, high level of HDL-c is associated with reduced risk for cardiovascular diseases.
- Triglycerides are a type of fat found in the blood that is use/store for energy. The combination of high levels of triglycerides with low HDL cholesterol or high LDL cholesterol has being established to be associated with cardiovascular diseases (CDC, 2011).

Therefore, lipid profile indices are important indicators in monitoring the risk of cardiovascular diseases and other complications associated with diabetes (Verges, 2015).

Normal lipid referenced value for each indices are presented in table 2.2. while dyslipidaemia is a cluster of lipid abnormalities characterized with a high concentration of TG and LDL-c, together with a low concentration of HDL-c. Its results in diabetes due to defective insulin activity which affects; the liver apolipoprotein production, down regulates the enzymatic activity of lipoprotein lipase and cholesterol ester transport protein. Moreover, insulin deficiency reduces the activity of hepatic lipase

and several steps in the production of biologically active lipoprotein lipase. (Ozder, 2014).

Table 2.2 Cholesterol Levels Recommended for Type 2 Diabetes Mellitus Population

NCEP* Guidelines	ADA Guidelines	AACE Guidelines
Total cholesterol	<200 mg/dl	N/A
HDL	>40 mg/dl	>40 mg/dl (men) and >50 mg/dl (women)
LDL	<100 mg/dl	<100 mg/dl
Triglycerides	<150 mg/dl	<150 mg/dl

(The National Cholesterol Education Program (NCEP), Adapted from (NCEP, 2001).

Patients with diabetes and existing cardiovascular disease may have more stringent cholesterol goals. Medical history, gender, age, ethnicity, and even geographic region of origin can affect cholesterol levels. Triglyceride levels can be raised due to kidney and liver diseases as well as alcoholism. High serum cholesterol levels can be triggered by poor dietary habits, pancreatitis, hypothyroidism, genetic lipid disorders, and certain kidney and liver diseases. High levels of LDL reflect an increased risk of coronary artery disease. Diabetes patient are predisposes to high cholesterol and triglyceride levels as well as high blood pressure due to insulin malfunction which play a key role in lipids metabolis, These conditions independently and together with hyperglycaemia increase the risk of heart disease, kidney disease, and other blood vessel complications (NCEP, 2001). Compounds and medication on use to correct lipid abnormalities in diabetic are beneficial in reducing cardiovascular complications.

Distort lipid profile and prolong hyperglycaemia may leads to generation of excess reactive oxygen species (ROS) due to lipotoxicity, auto-oxidation of glucose and hyperglycaemia induced excess generation of reactive oxygen species which are

implicated in causing damage to cellular components in oxidative stress induced peroxidation, this have been implicated in the onset of type2 DM and as well in the long term complications (Pitocco, *et al*., 2013).

2.6 Diabetes Mellitus and Oxidative Stress

Metabolic activities at cellular level results in continuous generation of many metabolic by-products that include free radicals such as reactive oxygen species (ROS), and reactive nitrogen species (RNS) (Bahorun *et al*., 2006). Free radicals are unstable molecules or compounds due to their ionic status of "free" unpaired electron; the transfer of this electron causes the oxidation of certain biochemical cellular products (Pitocco, *et al*., 2013). Although at physiological concentrations, free radicals such as, nitric oxide (NO), superoxide anion, and related reactive oxygen species (ROS) play an important role as regulatory mediators in cellular signalling processes. Many of the ROS-mediated responses actually protect the cells against oxidative stress and re-establish redox homeostasis (Droge, 2002). At higher concentrations ROS deleterious effects is prevented by the body's antioxidant systems comprising of existing endogenous (enzymatic and non-enzymatic) components, along with obtained exogenous antioxidants from diet and other sources, therefore neutralizes the effects of these free radicals to maintain homeostasis (Ceriello, *et al*., 2016). An imbalance between the ROS and antioxidants system in the body due to over production of free radicals or decreased antioxidants capacity leads to a condition known as "oxidative stress" (Tiwari, *et al*., 2013). This plays a pivotal role in the development of pathological condition such as type 2 diabetes mellitus. The inference of oxidative stress in diabetes pathogenesis by the alteration in enzymatic systems, lipid peroxidation, and impaired glutathione metabolism have been revealed (Ullah, et al., 2016). In diabetes mellitus, main sources of oxidative stress are mitochondria. During

oxidative metabolism in the mitochondria, a component of the utilized oxygen is reduced to water, and the remaining oxygen is transformed to oxygen free radical (O), which is an important ROS that is converted to other RS such as ONOO−, OH and H_2O_2 (Moussa, 2008). Chronic hyperglycaemia in diabetes mellitus leads to an upstream of mitochondrial overproduction of ROS, these results in a cascade reaction, which finally causes oxidative induced tissue damage (Giaccoand Brownlee, 2010).

The mechanisms underlying hyperglycaemia-induced diabetic vascular damage (Figure 2.2) could be explain by the following mechanisms: increased flux of glucose through the polyol pathway, increased intracellular formation of advanced glycation end-products (AGEs), increased expression of the receptor for advanced glycation end products and its activating ligands, activation of protein kinase C (PKC) isoforms, and over activity of the hexosamine pathway.

Effects of Increased Polyol Pathway Flux:

The polyol pathway is based on a family of aldo-keto reductase enzymes which utilize a variety of carbonyl compounds as substrates, and reduce them by nicotinic acid adenine dinucleotide phosphate (NADPH) to their respective carbohydrate alcohols (polyols). As postulated, the first glucose is converted to sorbitol by the enzyme aldose reductase, with sorbitol then oxidized to fructose by the enzyme sorbitol dehydrogenase (SDH), with NAD^+ as a cofactor (Giaccoand Brownlee, 2010).

Hyperglycaemia-induced tissues damage via polyol pathway flux could be due to an increased in redox stress caused by consumption of NADPH. NADPH is a cofactor required to regenerate reduced glutathione (GSH), and GSH is an important scavenger of reactive oxygen species (ROS), this could induce or exacerbate intracellular oxidative stress. Indeed, overexpression of aldose reductase have been associated with

increased atherosclerosis in diabetic mice and reduced the expression of genes that regulate regeneration of glutathione. Furthermore, decreased glutathiolation of cellular proteins is related to decrease NO availability in diabetic rats and restoring the NO levels in diabetic animals' increases glutathiolation of cellular proteins which inhibits aldose reductase activity and prevents sorbitol accumulation (Giaccoand Brownlee, 2010).

Effects of Increased Intracellular Advanced Glycation End-products (AGEs) Formation.

Chronic hyperglycaemic state in diabetes mellitus, leads glucose to form covalent adducts with the plasma proteins through a non-enzymatic process known as glycation. This results in protein glycation and formation of advanced glycation end products (AGEs). AGEs interact with plasma membrane localized receptors for AGEs (RAGE) to alter intracellular signaling, gene expression, released of pro-inflammatory molecules and induces free radicals generation (Jaggi *et al*., 2014).

Effects of Protein Kinase C (PKC) Activation:

PKC represent a family of enzymes of at about 11 isoforms widely distributed in mammalian tissues, these enzymes are co-expressed and respond to similar stimulatory agonists in many cell types. The activity of the classic isoforms (cPKC) isoforms (PKC-α, -β1, -β2, and -γ) are activated by phosphatidylserine (PS), calcium, and diacylglycerol (DAG) or phorbol esters (Geraldesand King, 2010).

Excess generated ROS due to hyperglycemia affect nuclear polymerase activity leading to decreased glyceraldehyde 3-phosphate dehydrogenase enzyme expression resulting

in persistent and excessive activation of several PKC isoforms which operates as another pathway mediating tissue injury induced by diabetes-induced ROS. This primarily occur from enhanced *de-novo* synthesis of DAG from glucose via triose phosphate, whose availability is increased because increased ROS inhibit activity of the glycolytic enzyme GAPDH, raising intracellular levels of the DAG precursor triose phosphate. Activity of PKC isoforms could also be enhance as a result from the interaction between AGEs and their cell-surface receptors (Steinberg, 2008).

The Hexosamine Biosynthesis Pathway (HBP):

HBP is a sub-branch of glycolysis. Fructose-6-phosphate is converted to glucosamine-6-phosphate, catalysed by glutamine:fructose-6-phosphate amidotransferase (GFAT). The major end-product is UDP-N-acetylglucosamine (UDP-GlcNAc), along with other amino-glucose generated by HBP. It provides essential building blocks for glycosyl side chains, of proteins and lipids. UDP-GlcNAc regulates flux through HBP by regulating GFAT activity and is the obligatory substrate of O-GlcNAc transferase. O-GlcNAc transferase is a cytosolic and nuclear enzyme, which catalyses a reversible, post-translational protein modification and transferring N-acetylglucosamine in O-linkage (O-GlcNAc) to specific serine/threonine residues of proteins. The metabolic effects of increased flux through HBP are thought to be mediated by increasing O-GlcNAcylation (Buse, 2006). Hence, HBP functions as a cellular nutrient sensor (Kaneto, 2001).

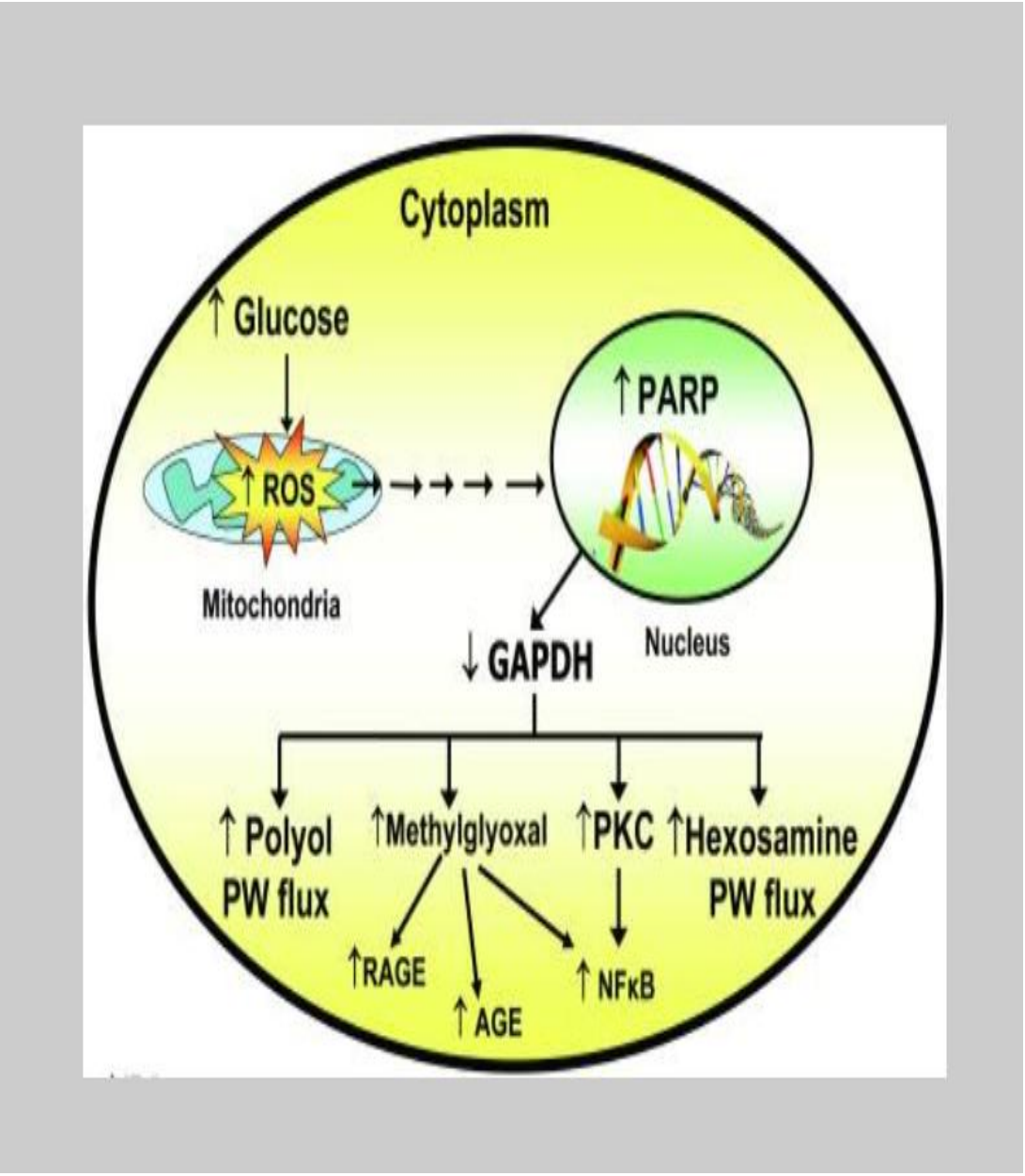


Figure 2.2 Schematic diagram of glucose induced cellular damage through oxidative stress. Adapted from (Giaccoand Brownlee, 2010).

ROS: reactive oxygen species, GAPDH: glyceraldehyde 3-phosphate dehydrogenase, PARP: poly (ADP-ribose) polymerase, peroxisome proliferated activated receptor, PW: pathway, RAGE: receptor of advanced glycation end products, AGE: advanced glycation end products, PKC: protein kinase C.

Figure 2.2 summarized the mechanisms of hyperglycaemia induced cellular damage through oxidative stress. The arrow up (increased); increased glucose concentration (hyperglycaemia) is associated with intracellular increased in ROS production by the mitochondria, this increased poly (ADP-ribose) polymerase (PARP) enzymes activity in the nucleus. The increased in PARP activity in the nucleus will results to increased cytosolic glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Increased GAPDH causes the activation of increased polyol pathway flux, over activity of the hexosamine pathway and increased formation of advanced glycation end products (AGEs), increased expression of the receptor for AGEs and its activating ligands. In addition, as well activation of protein kinase C (PKC).

2.6.1 Biomarkers of oxidative Stress in Diabetes Mellitus

Lipid peroxidation, proteins, Glutathione, catalase and superoxide dismutase activities are various biomarkers that are used to assess status of oxidative stress. Thus, disturbances in their levels in diabetes mellitus signifies a clinical progression of the disease, however if normal levels are maintained or restored by any intervention may as will be beneficial to the patients (Ullah, *et al*., 2016).

2.6.2 Superoxide dismutase (SOD)

Superoxide dismutase (SOD) is the first line of antioxidant enzymes that catalyses the dismutation of superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen, hydrogen peroxide is then converted in to oxygen and water in the presence of other enzymes (Tiwari *et al*., 2013). SOD plays a vital role intracellularly as defender of tissue morphology and is the major extracellular scavenger of superoxide radicals (David, *et al*., 2008). Decline in the level of SOD in diabetes and blood has been

reported, this decline plays a major attributing factor to pathogenesis of many complications (such as diabetic nephropathy) (Geraldes and King, 2010). Over expression of SOD or the supplements of antioxidants including SOD mimetics, targeted to overcome oxidative stress, reduce ROS, and increase antioxidant enzymes, has been shown to prevent diabetes mellitus (Geraldes and King, 2010). It also decrease mitochondrial release of cytochrome C and apoptosis in neurons, prevents diabetes-induced glomerular injury, by reducing oxidative stress, therefore suggesting its role in the regulation of free radical induced tissue damage. SOD exist in about three forms in the mammalian tissue and exhibits compartments specificity in location; Cu-Zn-SOD (SOD 1) is found in the cytosol, Mn-SOD (SOD 2) is found in the mitochondria and EC-SOD (SOD 3) is located in the extracellular space (Fujita, *et al*., 2009;).

2.6.3 Catalase (CAT)

Catalases (CAT) are ubiquitous enzymes that prevent cell oxidative damage from ROS by degrading hydrogen peroxide to water and oxygen ($2H_2O_2 \rightarrow 2\ H_2O + O_2$) with high efficiency (Alfonso-Prieto, *et al*., 2009). They are antioxidant enzyme found in nearly all living organisms exposed to oxygen, and said to be the second in line of action after SOD in mammalian cascade of reactions against free radicals (Chelikani, *et al*., 2004). It is another important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS) (Chelikani, *et al*., 2004). In diabetes mellitus there is increased production of reactive oxygen species, and if not properly manage, oxidative damage may contribute to further development of complications. Due to excessive concentration of hydrogen peroxide, which can cause significantly damage to proteins, DNA, RNA, and lipids (Cojocaru, *et al*., 2004). Deficiency of catalase increases

mitochondrial ROS and fibronectin expression in response to free fatty acids, which were effectively restored by catalase overexpression or N-acetyl cysteine. Low catalase activities can cause methemoglobinaemia and haemolytic anaemia, which are attributed to either deficiency of glucose-6-phosphate dehydrogenase or to other unknown mechanisms. And also may damage heme proteins, cause cell death, and, together with redox active metal ions produce highly toxic hydroxyl radicals. β-cells are rich in mitochondria, and thus a source of ROS which leads to generation of hydrogen peroxide, Catalase protects pancreatic β-cells from damage by hydrogen peroxide through neutralizing it into oxygen and water and thus (Tiwari, *et al*., 2013).

2.6.4 Glutathione (GSH)

Glutathione (GSH) is another important antioxidant system, capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals (Kaneto *et al*., 1996). It is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side chain and the amine group of cysteine, and the carboxyl group of cysteine is attached by normal peptide linkage to a glycine. Glutathione reduces disulfide bonds formed within cytoplasmic proteins to cysteines by serving as an electron donor (Lushchak, 2012). In the process, glutathione is converted to its oxidized form, glutathione disulfide (GSSG), also called L-(–)-glutathione. Once oxidized, reduced glutathione can be regain by the activity of glutathione reductase (GSR) using NADPH as an electron donor. The ratio of reduced glutathione to oxidized glutathione (GSH/GSSG) within cells is often used as a measure of cellular oxidative stress (Couto *et al*., 2013).

Therefore, GSH acts as an antioxidant either directly by interacting with reactive oxygen/nitrogen species (ROS and RNS) and electrophiles or by operating as a cofactor, substrate and co-substrate for various enzymes (Lushchak, 2012).

Glutathione peroxidase (GPx) is a key important enzyme in cellular cytosolic antioxidant defense systems, its affinity for H_2O_2 is higher than that of catalase. GSH is extensively used as a co-substrate (GSH-enzyme) by glutathione peroxidases in reducing hydrogen peroxide (H_2O_2) or organic peroxides (eg, lipid peroxides) to produced GSSG, water, or alcohols. GPx uses a specific H donor of GSH, for the reduction of non-specific substrates (hydrogen peroxide, lipid and non-lipid hydrogen peroxides). Hence, it detoxifies peroxides as a component of the glutathione cycle (Baker, *et al*., 1993; Lushchak, 2012). With GSH as its co-substrate, GPx activities depend indirectly on the flavoprotein glutathione reductase and cellular NADPH concentration that regulates GSH/GSSG concentrations. Assessment of GPx activities may give a reflection on cellular defensive enzymes activity against muffling effects of peroxides to counter cellularly induced oxidative stress. In DM dietary supplementation with GSH precursor amino acids can restore GSH synthesis and lower oxidative stress and oxidant damage in the face of persistent or poorly control hyperglycaemia (Tiwari, *et al*., 2013).

2.6.5 Lipids Peroxidation

Lipid peroxidation is a free-radical-mediated chain of reactions that, once initiated, results in an oxidative deterioration of polyunsaturated lipids. The most common targets are components of biological membranes. When propagated in biological membranes, these reactions can be initiated or enhanced by a number of toxic products,

including endoperoxides and aldehydes. Free radicals such as hydroperoxides degradation produces a highly toxic hydroxyl radicals, they may also react with transition metals like iron or copper to form stable aldehydes, (such as malondialdehyde (MDA)), that damage cell membranes. Thus, increased level of MDA may summarized the end results of oxidative stress induced tissues damage (Denise, *et al*., 2009). Increased level of MDA in diabetics suggests that peroxidative injury is involved, and this may developed further diabetic complications. The increase in lipid peroxidation is also an indication of decline in defense mechanisms of enzymatic and nonenzymatic antioxidants. Oxidized lipids are able to produce MDA as a decomposition product and the mechanism is thought to involve formation of prostaglandins, like endoperoxides, from polyunsaturated fatty acid (PUFA) with two or more double bonds. Increased MDA level in plasma, serum, and many others tissues has been established in diabetes. Lipid peroxidation in diabetes induced many secondary chronic complications including atherosclerosis and neural disorders. Higher serum lipid peroxidation evaluated in terms of MDA in hyperglycemic mice and was proposed as indicator of increase lipid peroxidation exacerbated the occurrence of myocardial infraction through NADPH oxidase activation (Tiwari, *et al*., 2013). Generally, the antioxidants system in the body plays important protective roles against cellular and histological damages that are produced by ROS and other free radical species.

In DM, poorly control hyperglycaemia and hyperlipidaemia results in oxidative stress due increased generation of free radicals and declined of endogenous antioxidant systems. The free radical mediate cascades of reactions that leads to lipid peroxidation. This can be summarized in the following steps: initiation, propagation, and termination

steps. In the lipid peroxidation initiation step, pro-oxidants like hydroxyl radical abstract the allylic hydrogen forming the carbon-centred lipid radical (L*).

In the propagation phase, lipid radical (L*) rapidly reacts with oxygen to form a lipid peroxy radical (LOO*) which abstracts a hydrogen from another lipid molecule generating a new (L*) and lipid hydroperoxide (LOOH). While in the termination reaction, available antioxidants donate a hydrogen atom to the LOO* species and form a corresponding antioxidant-radical that reacts with another LOO* forming nonradical products, while in absence or decreased capacity of antioxidants to donate hydrogen atom LOO* may reacts will transition metals to form stable aldehydes (toxic products) that damage cell membranes. Once lipid peroxidation is initiated, a propagation of chain reactions will take place until termination products are produced (Ayala *et al*., 2014).

2.7 Biomarkers of Liver Injury (liver enzymes) and Type 2 Diabetes Mellitus:

Biomarkers refer to certain blood parameters which are specifically measured and used as indicators for monitoring physiological processes, disease progression, and responses to treatments (Sattar, 2011). The liver have a multi-task role in regulation of metabolism processes, this makes it more susceptible to diseases especially in patient with metabolic disorder such as diabetes mellitus (Mathur, *et al*., 2016). Liver enzymes are synthesized and stored in hepatocytes and biliary ducts of the liver. They include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase.

Elevated level of liver enzymes in circulation is associated to cases of hepatocytes (liver cells) damage. The occurrence of liver disease and raised liver enzymes is common in poorly manage Type 2 diabetes, and may be multifactorial in origin (Forlani, *et al*., 2008). In this condition, hyperinsulinemia in combination with high free fatty acids (FFA) flux and hyperglycaemia are known to up regulate lipogenic transcription factors, while pathways that decrease free FFA pool are impaired. The increased availability of FFA, glucose and insulin contribute to the increase malonyl-CoA by stimulating Co-a carboxylase that convert acetyl-CoA to malonyl-CoA. The outcome of these events is fatty acid overload and hepatic mitochondrial oxidation system leading to liver disorders such as non-alcoholic fatty liver disease (NAFLD). Serum ALT and AST indicate the concentration of hepatic intracellular enzyme that has leaked into the circulation, and are considered primary markers for hepatocellular injury. Asymptomatic abnormal levels of ALT, AST and ALP are implicated in NAFLD (Mathur *et al*., 2016). In metabolic syndrome and T2DM elevated serum liver levels may as well indicate the end effects or complications of cellular damage induced oxidative stress due to chronic hyperglycaemia, lipotoxicity and overwhelming of the endogenous antioxidant system capacity which is evidence at the onset of the disease and implicated in long term complications of the disease (Welch-White *et al*., 2013).

2.7.1 Alanine transaminase (ALT);

Alanine transaminase (ALT), also called Serum Glutamic Pyruvate Transaminase (SGPT) or Alanine aminotransferase (ALAT) is an enzyme present in hepatocytes. When a hepatocyte is damaged, it leaks this enzyme into the blood, where it is measured. ALT rises dramatically in acute liver damage, such as viral hepatitis or

paracetamol (acetaminophen) overdose. ALT has a physiological range reference value of 7 – 56 U/L (Nabili, 2017).

2.7.2 Aspartate transaminase (AST);

Aspartate transaminase (AST) also called Serum Glutamic Oxaloacetic Transaminase (SGOT) or aspartate aminotransferase (ASAT) is similar to ALT in that it is another enzyme associated with liver parenchymal cells. It is raised in acute liver damage, but is also present in red blood cells, and cardiac and skeletal muscle. The physiological reference value for AST ranges from 10 – 40 U/L (Mengel, and Schwiebert, 2005; Nabili, 2017).

2.7.3 Alkaline phosphatase (ALP);

Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. ALP levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver. With a physiological range of 45 – 115 U/L (Mayo Madical Laboratory test catalog, 2015), ALP is also present in bone and placental tissue and it is found higher in growing children as their bones are being remodelled, (Nyblom *et al.,* (2004); Nyblom *et al*., 2006).

2.7.4 Other Causes of Abnormal Serum Liver Enzymes Levels

The liver is greatly involved in removing toxins from the body. It also makes proteins that aid in the clotting of our blood. When liver enzymes are elevated or decreased, it is a sign of liver impairment that cannot be ignored (Brzoska *et al*., 2003). A variety of conditions elevate liver enzymes. The three major liver disorders/diseases are caused by alcohol consumption: Fatty Liver, Acute Alcoholic Hepatitis and Chronic Alcoholic

Liver Disease. Other conditions that will also cause elevated liver enzymes are varied and include, but are not limited to the following: Cirrhosis, fatty liver, liver injury, cancerous or benign liver tumours, alcoholic liver disease, certain autoimmune disorders, hepatitis, obesity, diabetes, Wilson's disease, biliary obstructions, gallstones, pancreatitis, certain muscle disorders, celiac disease, high cholesterol, mononucleosis, Lupus, leukemia, tuberculosis. Taking certain medications such as acetaminophen, tetracycline and some chemotherapy drugs may also elevate the liver enzymes (Nabili, 2017).

2.8 Role of Thyroid Hormones in Glucose Metabolism

Thyroid hormones, Tri-iodothyronine (T3), and Thyroxine (T4) are synthesized by the thyroid gland whose activity is greatly control centrally, via the hypothalamo-hypophyseal axis by effects of released thyrotropin releasing hormones on anterior pituitary gland to synthesized and release thyroid stimulating hormone (TSH), which in-turn enhance the activity of thyroid gland and thyroid hormones (Yen, 2001). The general effect of thyroid hormones is to activate nuclear transcription of large numbers of genes. The net result is generalized increase in functional activity through regulation of metabolism throughout the body (Guyton and Hall, 2006). Thyroid hormones along with insulin contribute to the regulation of carbohydrate metabolism and pancreatic function in the body, many metabolic disorders and especially diabetes-associated disorders affects thyroid function and its tests to a variable extents. Normal thyroid functions affect glucose and other substrates metabolisms via several mechanisms to maintain normal metabolic rate and energy status in the body. Hyperthyroidism has been recognized to worsen hyperglycaemia (Maxon *et al.*, 1975). The half-life of insulin is reduced in hyperthyroidism most likely secondary to an increased rate of

degradation via increased rate of thermogenesis and enhanced release of biologically inactive insulin precursors (Dimitriadis *et al*., 1985; O'Meara *et al*., 1993). Also in untreated hyperthyroidism, reduced C-peptide to proinsulin occur suggesting an underlying defect in proinsulin processing. These leads to decreased level of active insulin in circulation and impairing its function (Beer *et al*., 1989). Another mechanism explaining the relationship between hyperthyroidism and hyperglycaemia is the increase in glucose absorption from the gut mediated by the excess thyroid hormones (Levin and Smyth, 1963; Matty and Seshadri, 1996) and (Mirella *et al*., 2011).

Thyroid hormones produce an increase in the hepatocyte plasma membrane concentrations of GLUT2 which is the main glucose transporter in the liver, and consequently, the increased levels of GLUT-2 contribute to the increased hepatic glucose output and abnormal glucose metabolism, therefore enhanced endogenous production of glucose in hyperthyroidism condition (Mokuno *et al*., 1999).

Another mechanism through in which hyperthyroidism affects the metabolism of lipids is via increased lipolysis resulting in an increase in free fatty acid (FFA) that stimulates hepatic gluconeogenesis. The increased release of FFA could partially be explained by an enhanced catecholamine-stimulated lipolysis induced by the excess thyroid hormones. Moreover, the non-oxidative glucose disposal in hyperthyroidism is enhanced resulting in an overproduction of lactate that enters the Cori cycle (lactate produced by muscles cells via anaerobic metabolism is converted to glucose in the liver via gluconeogenesis and release back in to the blood circulation, thus closing the cycle) and promotes further hepatic gluconeogenesis (Tazzini, 2016). Hence, diabetic patients

with hyperthyroidism experience worsening of their glycemic control (Sola *et al*., 2002; Bhattacharyya and Wiles, 1999).

In hypothyroidism condition, glucose metabolism is affected as well via several mechanisms, such as a reduced rate of liver glucose production (gluconeogenesis), impaired gut mediated glucose absorption and decreased glucose utilization, which results in overall decreased in hepatic glucose output and decrease in insulin synthesis and finally leading to hypoglycaemia. On the other hand, both clinical and subclinical hypothyroidisms have been recognized as insulin resistant states, due to impaired insulin stimulated glucose utilization in peripheral tissues (Dimitriadis *et al*., 1997; Rochon *et al*., 2003; Dimitriadis *et al*., 2006; Maraton *et al*., 2009) and (Mirella *et al*., 2011).

In patients with metabolic syndrome, higher TSH levels were found compared to that in the non-metabolic group. TSH via its centrally response and regulatory feedback activity on energy homeostasis, could be a useful maker to assess the central regulation of thyroid functions in relation with other hormones that are involve in regulating energy intake, storage and utilization; such as leptin and other adipocytokines. Therefore, TSH may also be useful in monitoring progression of metabolic derangements and associated risk factor for complications relating to cardiovascular disorders in pre-diabetes or in diabetes state. Increasing concentrations or levels of TSH have also been seen to positively correlate in the number of components of the metabolic syndrome in humans (Lai *et al*., 2011; Erdogan *et al*., 2011) and (Qi *et al*., 2017).

Thyroid hormones may influence carbohydrate mechanisms via its interaction with adipocytokines and gut hormones. Adipocytokines are secreted by the adipose tissue, they are involve in energy homeostasis and has important insulin-sensitizing properties. One of these adipocytokines include; Leptin (Mirella *et al*., 2011).

2.8.1 Physiological role of leptin

Leptin is a hormone produced by adipocytes, which specifically regulates energy expenditure and body weight (Martin *et al*., 2010). The name of the adipocytokine leptin, is derived from the Greek word "LEPTOS" meaning thin. It was discovered in 1994 and is produced mainly by adipocytes and in low levels by the gastric fundic epithelium, intestine, placenta, skeletal muscle, mammary epithelium, and brain (Wasim, 2015). The gene (ob gene) is located on chromosome 7q31DNA and has > 15,000 base pairs and there are three exons. The product is a 167 amino acid protein hormone regulating body weight, metabolism and reproductive function (Green *et al*., 1995). Leptin levels in the white adipose tissue and plasma are related to the energy stores, such that leptin increases in obesity and decreases during fasting (Chan *et al*., 2003). Congenital leptin deficiency has been associated with hyperphagia, impaired thermogenesis, insulin resistance, hyperlipidaemia, and central hypogonadism, which can be reversed by leptin treatment (Amita *et al*., 2013). In normal humans, leptin falls along with insulin during fasting and mediates the suppression of thyroid hormone, growth hormone and reproductive hormones. Leptin causes stimulation of appetite and inhibition of sympathetic nerve activity at conditions when the body is short of energy stored (Chan *et al*., 2003). While in availability of excess energy, circulating leptin crosses blood brain barrier to bind with LRb (brain leptin receptor). And mediates its action through the Janus kinase (JAK)–signal transducer and activator of transcription

(STAT) pathway (JAK–STAT3) pathway, as a results in autophosphorylation of JAK1 and 2, tyrosyl-phosphorylation of the cytoplasmic domain of LRb, which leads to phosphorylation and activation of STAT3. Tyrosyl-phosphorylated STAT3 undergoes homodimerization, is translocated to the nucleus, and regulates expression of neuropeptides and other genes to manifest leptin action by central control of energy balance in the brain to cause decrease in food intake and increase energy expenditure (Leshan *et al*., 2006; Martin *et al*., 2010). Therefore, leptin receptor-mediated JAK–STAT signaling is essential for regulation of food intake and body weight. Another mechanism of leptin action is through leptin-stimulated PI3K signaling, this appears to be important for regulation of glucose metabolism. Leptin also limits accumulation of triglycerides in liver and skeletal muscle through a combination of direct activation of adenosine monophosphate activated proteinkinase (AMPK) and indirect actions mediated through central neural pathways, thereby improving insulin sensitivity (Martin *et al*., 2010). Hence, leptin modulates pancreatic β-cells function through direct actions and indirectly through central neural pathways (Kharroubi *et al*., 2003). However, the complex interplay between thyroid hormones and leptin and its possible influence on carbohydrate metabolism remains to be elucidated (Mirella *et al*., 2011) especially in high fat diet induced type 2 diabetes mellitus.

2.9 Serum Electrolytes Levels and Diabetes Mellitus

Serum electrolytes are substance that produces an electrically conducting solution when dissolved in water. They carry a charge and are essential for normal life activities. This include sodium (Na^{+}), potassium (K^{+}), calcium (Ca^{2+}), bicarbonate (HCO_3^{-}), magnesium (Mg^{2+}), chloride ($C1^{-}$), and hydrogen phosphate (HPO_4^{2-}) ions (Wedro, 2016). Diabetics suffer from electrolyte imbalance due to its complications or

as a result of other medications. The imbalance may result due to elevated blood glucose (hyperglycaemia), where the body tries to rid up excess blood glucose by increasing urinary output volume. Increased urination results in water and electrolyte loss, which then upsets the body's balance of electrolytes (Sotirakopoulos, *et al*., 2012; Liamis *et al*., 2014). The balance is especially disturbed between sodium and potassium. Thus, in diabetic conditions the amount of glucose in the blood can off-set the proportion of electrolytes in different compartment of the body. The association between blood glucose and electrolytes is a complex one and is related to a number of other factors such as age and associated condition (Bukonla *et al*., 2012). Electrolytes disturbances include dysnatremias (hyponatremia and hypernatremia), hypokalaemia, and hyperkalaemia (Liamis *et al*., 2014).

2.9.1 Serum sodium disturbances (hyponatremia and hypernatremia) in DM

Measurement of serum sodium is routine in assessing electrolyte, acid-base, and water balance metabolic diseases. Sodium is responsible for approximately 95% of the osmotically active substances in the extracellular compartment, provided the patient is not in renal failure or has severe hyperglycaemia. The reference physiological range for serum sodium is 135-145 mmol/L (Mir, 2013). Hyperglycaemia in patients with poorly controlled diabetes mellitus is a common clinical problem, in which variable changes in the plasma sodium concentration [Na^+] occur (Tiezt, 1990). Glucose penetrates slowly into cells in DM, hence, increased levels of plasma glucose raise the effective plasma osmolality and cause water to move outwards, i.e. from the cells into the extracellular fluid.This leads to a decrease in plasma [Na^+] (Milionis *et al*., 2001). The serum sodium concentration tends to rise during the hypotonic osmotic diuresis induced by glycosuria, and fall during the shift of water from the intracellular to the

extracellular fluid induced by hyperglycaemia (Liamis *et al*., 2014). In this setting, the corrected plasma sodium concentration ([Na^+] corrected) is calculated by increasing [Na^+] by 1.6 mmol/L for every 100 mg/dL increment in the serum glucose levels above normal. If the corrected [Na^+] is above normal, then hypotonic fluid loss has occurred (i.e. water and electrolytes have been lost; water loss has been greater than electrolyte loss). It has been suggested that, hyperglycaemia-induced decrease in sodium concentration is considerably higher than the "standard" correction factor of 1.6 mmol/L Na^+ per 100 mg/dL [5.6mmol/L] glucose), especially when glucose levels are greater than 400 mg/dL (22.4 mmol/L).Moreover, it has been proposed that a correction factor of a 2.4 mmol/L decrease in [Na^+] per 100 mg/dL (5.6 mmol/L) increase in glucose concentration represents a better overall estimate of this association than the "usual" correction factor of 1.6 (Milionis, *et al*., 2001; Liamis *et al*., 2014).

The direct effect of hyperglycaemia on sodium levels is counter balanced by the osmotic diuresis related to glucosuria. Indeed, osmotic diuresis causes water loss in excess of sodium and produces a rise in serum [Na^+] and plasma osmolality (Roscoe *et al*., 1975). Furthermore, decreased water intake and increased insensible losses through the skin and the respiratory tract can aggravate metabolic disturbances. Although, most patients with uncontrolled diabetes mellitus are mildly hyponatraemic, a number of patients with nonketotic hyperglycaemia exhibit hypernatraemia due to excessive free water loss (Kaneko, 2008). In such cases, the profound osmotic diuresis-induced water loss counteracts the hyperglycaemic-induced decrease in serum sodium levels (Liamis *et al*., 2015). Hypernatraemia can also be observed during treatment when the rapid decrease in serum glucose levels and consequently in serum osmolality facilitates water entry into dehydrated cells thereby causing an elevation in extracellular sodium

concentration (Ciarambino *et al*., 2016). It has been suggested that in cases of nonketotic hyperglycemic hyperosmolar syndrome (HHS) altered mental status is predicted best by [Na^+]; serum glucose concentration alone is considered a poor indicator. In fact, there is evidence that hyperglycemic patients with hypertonicity are symptomatic only if hypernatremia is present (Liamis *et al*., 2008).

There is some evidence in animals and man that hypernatremia and hyperosmolarity are associated with impairment of both insulin-mediated glucose metabolism and glucagon-dependent glucose release (Liamis *et al*., 2008).

2.9.2 Serum potassium disturbances (hypokalemia and hyperkalemia) in DM

Potassium is another key electrolyte that facilitates normal nerve conduction, regulation of heartbeat, and muscle contraction, therefore disturbances in its levels is associated with various medical conditions (Chatterjee *et al*., 2011). It also important in maintaining proper fluid balance between cells and body fluids. In diabetes with impaired kidney function, potassium level should be carefully control, as levels can get too high in the body. Hyperkalemia is just as dangerous as hypokalemia (Antonios and Pratab 1987; Amy, 2017). In poorly controlled diabetes, high potassium levels (hyperkalemia) often result from kidney damage, which is usually considered a major complication of diabetes (diabetic nephropathy. Hyperkalaemia can also occur in diabetic ketoacidosis (DKA), a serious metabolic condition more commonly seen in people with Type 1 diabetes (Hamdy, 2017). Hyperkalaemia lead to weakness, paralysis, irregular heartbeat, or even a heart attack (Amy, 2017). The causes of hypokalaemia in diabetes include: redistribution of potassium [K^+] from the extracellular to the intracellular fluid compartment, shift hypokalaemia due to insulin

administration (Liamis *et al*., 2006) gastrointestinal loss of K^+ due to malabsorption syndromes (diabetic-induced motility disorders, bacterial overgrowth, chronic diarrheal states); and renal loss of K^+ (due to osmotic diuresis and/or coexistent hypomagnesemia) (Huang and Kuo, 2007). Hypomagnesemia can cause hypokalaemia possibly because a low intracellular magnesium [Mg^{2+}] concentration activates the renal outer medullary K^+ channel to secrete more K^+ (Liamis *et al*., 2009). Exogenous insulin can induce mild hypokalaemia because it promotes the entry of K^+ into skeletal muscles and hepatic cells by increasing the activity of the Na^+-K^+-ATPase pump (Ebisike, 2011). The increased secretion of epinephrine due to insulin-induced hypoglycaemia may also play a contributory role in hypokalaemia. The major setting in which insulin administration leads to hypokalaemia is during the treatment of severe hyperglycaemia (Appelt, 2015). As mentioned, hyperglycaemia increases serum osmolality resulting in movement of water out of cells. The loss of intracellular water leads to an increased intracellular K^+ concentration, favouring a gradient for K^+ to move out of the cells. Simultaneously, the friction forces between solvent (water) and solute can result in K^+ being carried along with water through the water pores in the cell membrane. In contrast, acidemia probably does not play a major role given that organic acids are much less likely to influence the internal K^+ distribution (Adrogue and Madias, 1981; Liamis *et al*., 2014).

2.9.3 Serum chloride and bicarbonates disturbance in DM

Chloride and bicarbonates ions plays a key role in maintaining acid-base balance in the body. In poorly manage diabetics, distortion of this balance leads to either metabolic acidosis or alkalosis. Metabolic acidosis is the most occurring metabolic derangement pertaining acid base-balance in uncontrolled diabetes mellitus. It is caused by increased

ketoacids and lactate, resulting in an increased anionic gap. While alkalosis occur only occasionally with vomiting being the pronounced clinical symptom resulting in chloride, potassium and hydrogen ions depletion (Koett, *et al*., 1979). Another metabolic complication in poorly controled diabetes is ketoacidosis (DKA) due to the overproduction of β-hydroxybutyric acid and acetoacetic acid (Stojanovic and Ihle, 2011). At physiological pH, both (β-hydroxybutyric acid and acetoacetic acid) ketoacids dissociate completely, and the excess hydrogen ions bind the bicarbonate, resulting in decreased serum bicarbonate levels. Ketone bodies are formed in the liver through ketogenesis (process by which fatty acids are transformed into acetoacetate (AcAc) and 3-hydroxybutyrate (3HB), Fatty acid oxidation in liver mitochondria generates acetyl CoA. Under conditions of low glucose availability, the acetyl CoA cannot be oxidized via the TCA cycle because, in the liver, the oxaloacetate required for the first step (its condensation with acetyl CoA to form citrate), is unavailable being redirected to the process of glucose production (gluconeogenesis). Consequently the acetyl CoA is converted into ketone bodies which are used by non-liver tissues for energy (ATP) production) to compensate energy source in starvation or diabetes for pheriperal tissue cells utilisation (Colin, 2015). Thus, excess ketone bodies generated circulate in the anionic form, which leads to the development of anion gap acidosis that characterizes DKA. The anion gap can be calculated using the following formula: Na+ – (Cl– + HCO3–). The normal anion gap is 12 (standard deviation [SD] 2) mmol/L. In DKA, bicarbonate is replaced by β-hydroxybutyric acid and acetoacetic acid, so that the sum of bicarbonate and chloride concentrations is reduced and the anion gap is thus increased. Despite substantial losses of ketoacids in the urine, the decrease in serum bicarbonate concentration and the increase in the anion gap observed in DKA are about equal (Chiasson, *et al*., 2003). In poorly managed diabetes the liver is continuously

active in fatty acid oxidation and ketone body production and anionic gap is evidence by decrease concentration of bicarbonates with other electrolytes including chloride ions (Colin, 2015). The normal serum range for chloride is 97 to 107 mEq/L. thus, abnormally elevated levels (hyperchloremia) is often comorbid with diabetes or hyponatremia (Cambier, *et al*., 1998).

2.9.4 Complications of Diabetes Mellitus

The common pathology to all types of diabetes mellitus is a sustained high blood glucose level (hyperglycaemia). The disease is characterized by different aetiologies but share similar signs, symptoms and complications. Over a period of time, high glucose level in the bloodstream can lead to severe complications, all forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20), but may be the first symptom in those who have otherwise not received a diagnosis before that time. The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease (Mahler and Adler, 1999). The main "macrovascular" diseases (related to atherosclerosis of larger arteries) are ischemic heart disease (angina and myocardial infarction), stroke and peripheral vascular disease. Diabetes also causes "microvascular" complications—damage to the small blood vessels (Boussageon *et al*., 2011). Diabetic retinopathy is another complication which affects blood vessel formation in the retina of the eye, can lead to visual symptoms, reduced vision, and potentially blindness. In diabetic nephropathy, which result from scarring in the kidney parenchyma can lead to loss of small or progressively larger amounts of protein in the urine, and eventually chronic kidney disease requiring dialysis (Tidy, 2017). Diabetic neuropathy is the impact of diabetes on the nervous system, most commonly causing numbness, tingling and pain in the feet

and also increasing the risk of skin damage due to altered sensation (Schreiber *et al*., 2015). Together with vascular disease in the legs, neuropathy contributes to the risk of diabetes-related foot problems (such as diabetic foot ulcers) that can be difficult to treat and occasionally require amputation (Larsson and Apelqvist, 1995). Damage to the nerves in the autonomic nervous system can lead to paralysis of the stomach (gastroparesis), chronic diarrhoea, and an inability to control heart rate and blood pressure during postural changes (Boussageon, 2011).

Many infections are associated with diabetes, and they are frequently more dangerous in them because the body's normal ability to fight infections is impaired due to hyperglycaemic environment. To compound the problem, infections may worsen glucose control, which further delays recovery from infections (Casqueiro *et al*., 2012).

2.9.5 Managements of Diabetes Mellitus

Diabetes mellitus is a chronic disease, which cannot be cured except in very specific situations. Management concentrates on keeping blood glucose levels as close to normal ("euglycemia") as possible, without causing hypoglycaemia. Depending on the type and stage of the disease, its management can usually be accomplished with Life Style modification, (Diet, and Exercise); and use of appropriate medications (Pharmacological approach) (Nathan *et al*., 2005).

2.9.5.1Life style modification

Life style modification is recommended with the goal of keeping both short and long-term blood glucose levels within acceptable bounds. Patient education, understanding, and participation is vital, since the complications of diabetes are far less common and less severe in people who have well-managed blood glucose levels (Pignone *et al*.,

2010). Attention is also paid to other life style factors that may accelerate the deleterious effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise (Adler *et al*., 2000).

The ideal diet for diabetic patients remains to be determined. Recommendations generally call for low-fat high-carbohydrate diets. The primary purpose of this recommendation is to reduce the risk for coronary heart disease, a major killer of diabetes patients. Some investigators also suggest that high-carbohydrate diets also improve glucose tolerance, in especially patients with type 2 diabetes mellitus (T2DM). Another potential advantage of a low-fat diet (high percentage of carbohydrate) is that it may promote weight reduction. High-fat diets are thought by many investigators to stimulate weight gain. However, if after an adequate trial of this diet, weight reduction is not achieved, this suggests that the patient is consuming large quantities of carbohydrates. Carbohydrates should make up between 50 and 60 percent of a diabetics' caloric intake. Since carbohydrates are rapidly broken down into glucose, therefore carbohydrate heavy meals can cause a sudden rise in blood glucose levels. Ideally, the carbohydrates from vegetables, fruits and whole grains are recommended, as these carbohydrates are broken down more slowly, which helps mitigate the effects of carbohydrates on sudden rise in blood glucose levels (Libby, 2011).

Ideally, the fat should not raise the serum cholesterol level, and hence it should be unsaturated. Monounsaturated fatty acids are preferable to polyunsaturated fatty acids, because polyunsaturated may increase the risk for cancer or promote the oxidation of low-density lipoprotein, another potentially artherogenic change. Many T2DM patients, particularly obese patients in the earlier stages of diabetes, tolerate weight-

maintenance high-carbohydrate diets without deterioration of glucose tolerance. However, as their insulin reserve declines, high-carbohydrate diets may further raise glucose levels, and a lower-carbohydrate diet seems preferable. The same may be true for nonobese patients with T2DM (Scott, 1991).

Exercise is one of the cornerstone in management of DM. Moving toward a more active life is generally inexpensive, convenient, and usually produces great rewards in term of blood glucose control and a general feeling of well-being. Daily moderate intensity aerobic exercise for at least 20 to 30 minutes is recommended for diabetic patient, in order to boost cardiovascular health, keep healthy weight, as well as helps the cells to respond better to insulin. In addition, regular exercise reduces stress, improves concentration, and decreases appetite (Irene *et al*., 2012). Whenever a muscle is actively used, both fatty acids and glucose are burnt during and after the periods of activities. Once the falling glucose level is sensed by the beta cells in the pancreas, insulin output is reduced, giving the cells a rest. This prevents excessive insulin production. In addition, the lower insulin levels signal the liver to empty its glucose reserves (glycogen) into the blood to supply the muscles with needed energy. As physical activities continue, the liver converts amino acids, lactic acid, and fats into glucose to supply the muscles. If activity continues long enough, even the body's fat cells get involved. They compensate for the reduced fatty acid levels in the body by converting their stored triglycerides into fatty acids. Therefore exercise is one of the most effective approach in management of DM, because it lowers blood glucose, fatty acids levels and reduced the workload of pancreas (Lee *et al*., 2015).

2.9.5.2Pharmacological approach;

This involve the use of drugs and other chemical substances termed all together as antidiabetic drugs. These drugs are used to treat diabetes mellitus by lowering glucose levels in the blood. With the exception of insulin, exenatide and pramlintide, all other antidiabetic drugs are administered orally and are thus called, oral antidiabetic agents.There are different classes of oral antidiabetic agents or drugs and their selection depends on the nature of the diabetes, age and co-morbidities. These agents/drugs includes:

- Metformin (Glucophage®, Glumetza®, others). Generally, metformin is the first medication prescribed for type 2 diabetes. It improve the sensitivity of tissues to insulin so that insulin is used more effectively. Metformin also lowers glucose production in the liver.Nausea and diarrhea are possible side effects of metformin.
- Sulfonylureas: These medications help the body secrete more insulin. Examples of medications in this class include glyburide, glipizide and glimepiride. Possible side effects include low blood sugar and weight gain.
- Meglitinides**:** These medications work like sulfonylureas by stimulating the pancreas to secrete more insulin, but they're faster acting, and the duration of their effect in the body is shorter. They also have a risk of causing hypoglycaemia.

  Weight gain is a possibility with this class of medications as well. Examples include repaglinide and nateglinide.
- Thiazolidinediones**:** Like metformin, these medications make the body's tissues more sensitive to insulin. This class of medications has been linked to weight gain and other more-serious side effects, such as an increased risk of heart

failure and fractures. Because of these risks, these medications generally arenot first-choice treatment. Rosiglitazone and pioglitazone are examples of thiazolidinediones.

- DPP-4inhibitors**:** These medications help reduce blood glucose, They don't cause weight gain. Examples of these medications are sitagliptin,saxagliptin and linagliptin.
- GLP-1 receptor agonists: These medications slow digestion and help lower blood glucose levels, though not as much as sulfonylureas. Their use is often associated with some weight loss. Exenatide and liraglutide are examples of GLP-1 receptor agonists. Possible side effects include nausea and an increased risk of pancreatitis.
- SGLT2inhibitors**:** They work by preventing the kidneys from reabsorbing glucose into the blood. Instead, the high quantity is excreted in the urine.Examples include canagliflozin and dapagliflozin. Side effects may include yeast infections and urinary tract infections, increased urination and hypotension (Ripsin *et al*., 2009).
- Insulintherapy**:** Type 1 diabetes is typically treated with insulin, patientwith type 2 diabetes need insulin therapy as well. In the past, insulin therapy was used as a last resort for type 2 diabetes patients, but later on, is often prescribed sooner because of its benefits.

  Normal digestion interferes with insulin taken by mouth, insulin must be injected. Depending on needs, a mixture of insulin types to use throughout the day and night is selected. Often, people with type 2 diabetes start insulin use with long-acting formulated insulin administered at night. Types of

insulininclude:Insulin glulisine, Insulin lispro, Insulin aspart, Insulin glargine, Insulin detemir and Insulin isophane. (Ripsin *et al*., 2009).

2.9.5.3 Complementary and alternative remedy for diabetes

Complementary and alternative treatments for diabetes include herbs, supplements of spices or micronutrients and dieting. Some herbs and supplements have been popular therapies for different diseases, therapies with antihyperglycaemic effects are increasingly sought by patient with diabetes across populations. The use of alternative treatments may be only partially alleviated by conventional treatment because of it widely (Pandey *et al*., 2011). Extracts of Aloe vera, ginseng, ginger, cinnamon, clove, curry and garlic have been shown to exert antioxidant, anti-inflammatory property in animal disease model, especially in diseases associated with oxidative stress including diabetes. Oxidative stress play a pivotal role in the onset and progress of end organs complications in diabetesmellitus (Ullah, *et al*., 2016).

Cloves:

Cloves are commonly use as spice and remedy for certain illness. They are "flower buds" from evergreen rain-forest tree native to Indonesia. They belong to the family of *Myrtaceae* of the genus; *Syzygium*, and scientifically named as *Syzygium aromaticum.* (Dorenburg and Page, 2003). Plate I shows clove buds, structurally each bud consists of a long calyx terminating in four Spreading sepals, and four unopened petals, which form a small ball (dome) at the center. The active principles in clove are known to have antioxidant, anti-septic, local anaesthetic, anti-inflammatory, rubefacient (warming and soothing), carminative and anti-flatulent propertiesThe essential oil, eugenol in this spice has been in therapeutic use in dentistry as a local-anaesthetic and antiseptic for teeth and gum disease (Kamatou *et al*., 2012).



Plate I. Clove buds (*Syzygium aromaticum)*, (A. Abdulrazak, personal photograph, July 15, 2017).

The spice also contains a good amount of minerals like potassium, manganese, iron, selenium and magnesium. Potassium is an important electrolyte of cell and body fluids that helps control heart rate and blood pressure. Manganese is used by the body as a co-factor for the antioxidant enzyme, superoxide dismutase(Park *et al*., 2011). In addition, the spice bud contain vitamin A and beta-carotene. These compounds are known to have antioxidant properties. Vitamin A is also required by the body for maintaining healthy mucus membranes and skin in addition to being essential for vision (Zheng *et al*., 1992).

Additionally, this spice is a good source of Vitamin-K, vitamin-B6 (pyridoxine), thiamine (vitamin B-1), Vitamin-C and riboflavin. Vitamin C helps the body develop resistance against infectious agents and scavenge harmful oxygen-free radicals (Park *et al*., 2011).

The essential volatile oils (eugenol, eugenol acetate and β-cariofileno) functions as a rubefacient, meaning that it irritates the skin and expands the blood vessels, increasing the flow of blood to make the skin feel warmer, making it a popular home remedy for arthritis and sore muscles, used for poultice or in hot baths. Clove oil is also used in aromatherapy (Cortes-Rojas, *et al*., 2014). These numerous health beneficial properties of clove made it important for further detailed studies to establish its scientific benefits especially as supplement alone or in combination with other supplements:

Ginger:

This refers to thick-knotted brown underground stem (rhizome) (see plate II) of the plant *Zingiber officinale* belonging to the family Zingiberaceae that has been used for centuries in Asian cuisine and medicine (Abdel Halim, 2008). It is native to Africa, India, China, Australia and Jamaica, commonly used as a spice or flavouring agent in cooking, or as an alternative 'herbal' treatment for various ailments such as nausea and indigestion, as well as fragrance in soaps and cosmetics (Megan, 2016).



Plate 2. Ginger rhizome, (A. Abdulrazak, personal photograph, July 15, 2017).

Ginger rhizome can be used fresh, dried and powdered, or as a juice or oil. It has a pungent and sharp aroma and adds a strong spicy flavour to food and drink. It is one of the most widely consumed spices worldwide. It has a long history of use as herbal medicine to treat a variety of diseases including nausea and vomiting, constipation, indigestion (dyspepsia), pain, and cold induced syndromes (Abdel Halim, 2008). More recently, it was reported that ginger extract also possesses anti-cancer, anti-clotting, anti-inflammatory, and anti-oxidative characteristics, since it can scavenge superoxide anion and hydroxyl radicals (Elshater *et al*., 2009). Ginger is known to contain a number of potentially bioactive substances, mainly gingerols and their related dehydration products, the shogaols, as well as volatile oils including sesquiterpenes, such as beta-bisabolene and zingiberene, and monoterpenes, mainly geranial and neral (Al-Amin *et al*., 2006). In addition, phytochemical reports have shown that the main constituents of ginger are gingerol, shogaol, zingerone and paradol. It was reported that 6-gingerol and 6-shogaol are the major gingerol and shogaol present in the rhizome (Li *et al*., 2012). Table 2.2 summarise active compounds found in ginger and their biological activities.

Table 2.3 Active Compounds in Ginger and their Biological Activities

S/NO	Active Compound in Ginger	Biological Activities of the Active compounds
1	Gingerol and gingerol related compound	Antioxidant
		Hepatoprotective
		Anti-microbial
		Anti-inflammation and anti-analgesic
		Anti-tumor
2	Paradol	Anti-oxidant
		Anti-microbial
		Anti-cancerous effect
3	Ginger flavonoids	Anti-oxidant, Neuroprotective effect
4	Shogaol	Potent inhibitors of nitric oxide (NO) synthesis in activated macrophages.
	6-Dehydroshogaol,	Antioxidant properties
	6-shogaol and 1-dehydro-6-gingerdione	Anti-ulcerative effects
		Anti-cancerous
5	Zingerone	Anti-oxidant
		Anti-inflammation
6	Zerumbone	Antitumorigenic effect
		Anti-cancerous
7	Terpenoids	Anticancer activity, anti-inflammation, anti-oxidant
8	1- Dehydro-(10)gingedione	Anti-inflammatory
9	Phenolic compounds	Anti-inflammatory
		Antioxidative properties
		Anticarcinogenic and antimutagenic activities
		Neuroprotective effect
10	Galanolactone and Diterpenoid	Anti-emetic effect

(Rahmani *et al*., 2014; Hsu*et al*., 2015; Prasad, S., and Tyagi, A. K. 2015)

2.9.2 Molecular mechanism of high fat diet-induced type 2 diabetes animal model

Feeding rodent a high fat diet containing 20 to 60% fat content over certain period of time have been suggested to produce a perfect model of type 2 diabetes animal. Approximately 40% fat content diet fed to rodents for eleven weeks led to metabolic syndrome, hyperglycaemia, insulin resistance and subsequently type 2 DM (Heydemann, 2016). Although the molecular mechanisms underlying HFD-induced insulin resistance and T2DM have not been fully elucidated, but experimental evidence suggested that, chronic intake of high fatdiet is associated with excess fatty acids in circulation, increased lipid content, hyperglycaemia, increased lipid deposit in tissues and enlargement of fat storage leading to increased released of inflammation makers. Adipose tissue, liver and skeletal muscle are primary sites for fat storage and processing. Excess lipid accumulation in these tissues lead to distort metabolic process along certain metabolic pathway especially insulin signalling pathway, affecting normal post receptor response which results in insulin resistance and hyperglycaemia (Hong *et al*., 2016). Enlargement of adipose tissue due to excess fat accumulation is associated with inflammation. One of the major pathways that involved inflammation inducedinsulin resistance (IR) is via IkB kinase-β (IKKβ) or nuclear factorκB (NFκb) activity. Cytokines release from inflamed tissues activate IKKβ, which in turn leads to phosphorylation of IκBα. IκBα phosphorylation leads to protein ubiquitination and degradation, which releases NFκb to translocate into the nucleus and induce the gene expression of inflammatory mediators. IKKβ promotes serine phosphorylation of IRS-1, inhibiting insulin signalling, which causes IR. Moreover, cytokines activate the c-JUN N-terminal kinase (JNK)/AP-1 pathway. JNK, similar to IKK β, phosphorylates IRS-1 at a serine residue, impairing insulin signalling. In the liver, inflammation begins due to chronic stimulation of lipid accumulation within hepatocytes (steatosis). This

event leads to the presence of inflammatory cytokines, inflammation may induce gluconeogenesis as an attempt to protect the liver from steatosis by stimulating the conversion of fatty acids (FAs) into glucose. Hepatic inflammation and related IR are linked to overexpression of IKK-β. Therefore this linked lipid accumulation (lipotoxicity) and IR, as FAs inhibit insulin-stimulated glucose uptake. Upon FA entrance into cells, the FAs are esterified by coenzyme-A into acyl-CoAs, which are transferred to a glycerol origin, forming monoacylglycerol (MAG), diacylglycerol (DAG) and triacylglycerol (TAG). The FAs also esterify with sphingosine to form ceramides. Skeletal muscle cells are protected from the deleterious effect of lipids by storing them as TAG, but with limited capacity. Therefore, a great influx of lipidspromotes the formation of other molecules that function as intracellular signalling molecules, interfering with normal insulin signalling such as DAG and ceramides (Barbosa-Da-Silva *et al*., 2014). Both animal and plant derived high fat diets induce changes in insulin action in insulin sensitive tissues, which causes stimulation of glucose uptake to reduced in brown and white adipose tissues. Therefore,decreases insulin receptor autophosphorylation and activation of glycogen synthase kinase-3lead to intracellular hypoglycaemia and extracellular hyperglycaemia. Due to intracellular energy depth, insulin counter regulatory hormones such as glucagon stimulates gluconeogenesis to compensate cellular energy demand. This further worsen the energy balance and metabolism leading to dyslipidaemia, metabolic syndrome, insulin resistance and development of type 2 diabetes (Buettner *et al*., 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1Experimental animals

Thirty (30) male rabbitsof different crossbreeds (New Zealand and local breed) of age range 8 -10 weeks weighing between 280 – 300g were used for the study. Animals were housed in the Departmental animal house of Human Physiology Department, Faculty of Medicine, Ahmadu Bello University Zaria, in well-aerated laboratory cages and were fed with growers' and staters' mash from (Vital feed Company Jos Plateau, Nigeria) and they had access to water *ad libitum*. Animal care was in accordance with the guide for the care and use of Laboratory Animals, Institute for Laboratory Animal Research, National Institute of Health (NIH Publication No.80-23; 1996).

3.1.2Collection and authentication of experimental supplements

Clove (*Syzygiumaromaticum*) buds and Ginger (*Zingiber officinale*) rhizome were purchased at Tudun-Wada Market Zaria, Kaduna, Nigeria. They were presented to the herbarium unit of the Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria, where they were authenticated with previously deposited voucher number of 900127 and 2261 respectively.

3.1.3. Drugs and reagents

All chemicals used were of analytical grades; these included:

i. Cholesterol (powder) was purchased from KEM LIGHT Laboratory PVT. LTD, Mumbai India. (CAS: 57-88-5, mw: 386.67, lot no.: 100814).

ii. Cholestran powder (PHARCO Pharmaceuticals, Alexandria, Egypt) purchased from Amira Pharmacy, Tudun Wada Zaria.

iii. Assay kits for assessment of liver enzymes (Randox Laboratory Ltd, United Kingdom, North West Life Science Specialities, Vancouver, Canada), ALT (Randox AL100). AST (Randox AS 101) and ALP (Biolabo, Ref 92214). Purchased through Kayomeg Medical Diagnostic Laboratory, Kaduna.

iv. Methylated spirit, cotton wool, disposable pipette tips

v. Sodium and potassium reagent set (colorimetric method), (TECO Diagnostics, 1268 N. Lakeview Avenue, Anaheim), CA 92807: 1-800-2222-9880

vi. Rabbit ELISA Kit: LEP (No.GA-E0017RB), INS(No.GA-E0015RB), ISR (No.GA-E0016RB), SOD (No.GA-E0018RB), CAT (No.GA-E0019RB), GPx (No.GA-E0020RB), MDA (No.GA-E0021RB), T_3 (No.GA-E0022RB), T_4 ELISA Kit (No.GA-E0023RB), TSH (No.GA-E0023RB) LOT 20151012, GenAsia Biotech Co., Ltd. Shanghai, China.

3.1.4Equipment and other materials

i. Digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany)

ii. Glucose- test strips for assessment of plasma glucose levels, manufacture by Accu-check Advantage II, Roche Diagnostics GmbH Germany.

iii. Animal feed (Vital feed, km 17, Zawan Roundabout P.O. box 13462, Jos, Plateau state Nigeria.),

iv. Groundnut mill, and ground nut oil were purchased in Samaru-Zaria market, Kaduna Nigeria

v. Dissecting Set

vi. Digital weighing scale, Model EK 3052 balance (England)

vii. Test tubes/rack

viii. Syringe and needle (5ml)

ix. Microplate reader (RT-2100C, Rayto)

x. 800D Table Toplow Speed centrifuge (Mehco)

xi. Automated and graded micropipette (Perfect tm)

3.2Methods

3.2.1 Preparation of high fat diet

The high fat diet was constituted in the Departmental Animal house, Human Physiology Department as follows:

A bag of Vital feed weighing 25 kg with original composition of; Crude protein 13%, fat 8%, crude fibre 15 %, calcium 0.9% was reconstituted as follows:

- 8 kg (32 %) of feed was weighed and removed out of the 25 kg of the animal feed. Cholesterol powder weighing 0.25kg, Groundnut mill 5kg, and ground nut oil 2.25kg were accordingly added into the remaining 17 kg of the feed in a large bowland mixed thoroughly. The new reconstituted diet (high fat diet) composed of approximately: 40% fat, Crude protein <14%, crude fibre <15 %, calcium <0.9%, metabolised energy <2550 Kcal/Kg min, as described by (Kolawole *et al.*, 2012) with slight modification.

3.2.2 Preparation of supplement composed diets

The supplements containing diet were reconstituted in the Animal house of the Human Physiology Department, ABU, Zaria.

3.2.2.1Fermented ginger (12.5%) supplemented diet

Fresh ginger rhizome was purchased from Tudun Wada Zaria market, it wassliced and shade/air dry.The dried ginger wasmacerated in a bowl in tap water and covered for 48hrs, then the water was drained and the fermented ginger was spread to dry again. This was then reduced to powder state using a grinding machine.

Out of a bag weighting 25kg of normal animal feed (vital feed), 3.125kg (12.5% of 25) of the feed was weight and removed. Thereafter fermented ginger powder weighing 3.125kg was then added into the remaining 21.875kg of the feed and mixed thoroughly. This gave a 25kg bag of feed containingfermented ginger 3.125kg (12.5 % of the total feed) supplement.

3.2.2.2Clove (12.5%) supplemented diet

Dry Clove buds was purchased from Tudun Wada Zaria market, it was spread under shadefor 24hours. The dried clove was then reduced to powder state using a grinding machine.

Out of another bag of normal animal feed (vital feed) weighing 25kg, 3.125kg (i.e 12.5% of 25) of the feed was removed.

Thereafter clove buds powder weighing 3.125kg was then added into the remaining 21.875kg of the initial full bag feed and mixed thoroughly to give a 25kg bag weight of feed, which contained clove buds powder 3.125kg (12.5 % of the total feed) supplement.

3.2.2.3Fermented ginger (12.5%) + clove (12.5%) supplemented diet

From another bag weighing 25kg of standard animal feed, 6.25kg of feed was removed,to give 18.75kg.Then 3.125kg of fermented ginger powder and 3.125kg of clove powder were weighed and added each at a time into the 18.75kg of the feed,in a big bowland mixed thoroughly to give a mixture of supplemented dietof total weight 25kg, which contained: animal feed 18.75kg (75%),fermented ginger 3.125kg (12.5 %)and clove 3.125kg (12.5%) supplements.

3.2.2 Induction of type 2 diabetes in rabbits using high fat diet

Twenty-five (25) animals were feed with high fat diet for eleven (11) weeks to induce type 2 diabetes, after which each animal was bled from the marginal vein on the ear lobe, usingtest strip with a digital glucometer.Glycemic level wasmeasured and recorded for each animal. Rabbits having glucose levels greater than or equal to 140mg/dL(7.7mmol/L) were considered hyperglycaemic and selected for the study(Srinivasan and Ramarao, 2007; Heydemann, 2016; Tanko *et al*., 2016).

3.4 Experimental Design

The study was carried out using high fat diet induced-type 2 diabetes in rabbits, then animals were treated with clove and fermented ginger supplemented diets for six weeks.

3.4.1Animal groupings

In the study, 30 rabbits were used, each group comprised five rabbits (n=5), with 5 groups of diabetic model and a normal control group:

Group I: Normal rabbits fed on standard animal feed only

Group II: Diabeticrabbits fed onstandard animal feed only

Group III: Diabetic rabbits fed on standard animal feed and administered cholestran (0.26g/kg body weight)(Adaramoye *et al*., 2008).

Group IV: Diabetic rabbits fed on12.5% clove supplement.

Group V: Diabetic rabbits fed on 12.5% fermented ginger supplement.

Group VI: Diabetic rabbits fed on12.5 % clove andfermented ginger 12.5% supplements co-administered.

Animals in all groups were fed according to their group protocols for six (6) weeks.

3.4.2Determination of blood glucose levels

During the experimental period, blood glucose levels were determined at weekly intervals, with a digital glucometer using the glucose oxidase principle (Beach and Turner, 1958). Rabbits were bled through marginal vein of the ear lobe by the use of disposable needle, the result were recorded in mg/dL(Rheney and Kirk, 2000).

3.4.3Termination of the study

Animals were subjected to light anaesthesia using chloroform vapour in an air-tight chamber, at the end of six weeks period of treatment. Blood samples were collected via cardiac puncture using syringe and needle and released into blood tubes (Plain tubes). Blood samples collected were centrifuged at 2000**g* for 10minutes using a bench centrifuge to get serum, which was refrigerated in plain bottles and later used for laboratory analysis and assay, which were performed at Kayomeg Medical Diagnostic Laboratory, 4 Ribadu Road, off Kofar Gamji, Kaduna.

3.5 Laboratory Assessments

3.5.1 Estimation of serum insulin

Serum insulin was estimated using rabbit INS (insulin) ELISA kit in accordance to the manufacturers guide.

Procedure:

The wells were labelled using a masking tape starting with the controls down to test wells. Beginning with serial dilution of the standard solution; from theoriginal standard solution (160mU/L),120ul and 120ulstandard diluents were pipetted using automated graded pipette, and added to the first control well (CW). This was mixed, and 120ul from the CW solution and 120ul standard diluents were added to the second control well (CW2) and mixed. Then 120ul from CW2 and 120ul standard diluents were added to the third control well (CW3). Another 120ul from CW3 and 120ul standard diluents were added in to the fourth control well (CW4) and mixed. Finally 120ul from CW4 and 120ul standard diluents were added in to the fifth control well (CW5) and mixed. This give a 50% downgraded serial dilution of the original standard solution concentration down to the fifth control well.

Thereafter, 50ul standard andstreptavidin-Horse Radish Peroxidase (HRP) 50ul were added in to standard solution well.Then into to the remaining test wells: 40ul of test serum, 10ul INS antibodies and 50ul streptavidin-HRP were added each at a time.All wells were shake gently to mixed, and then incubated at 37°C for 60minutes. After which the wells were washed five times with a washing solution to removed unbound enzymes. Then, wells are dried and substrate 50ul chromogen solution A was added to each well, followed with 50ul chromogen solution B to each well. This was shake gently and incubated for 10 minutesat 37°C.Then, stop solution was added to each

well,colour change from blue to yellow was observed. Then, the whole plate of wells was inserted into the microplate reader, and the absorbance under 450nm wavelength was read as displayed on the screen of the machine immediately and were recorded. A graph of concentration of standard against their absorbance was plotted, then, the absorbance of each sample was traced on that of the standard axis, and its concentration is gotten on the standard's concentration axis and recorded.

3.5.2 Estimation of serum insulin receptor concentration

Serum insulin receptor was estimated using rabbit ISR (insulin receptor) ELISA kit in accordance to the manufacturers guide.

Procedure:

First, the wells are labelled using a masking tape starting with the controls down to test wells, then followed with a serial dilution in the first five control wells as follows, from the original standard solution [120mU/L] 120ul and 120ul standard diluents were pipetted using automated graded pipette, and added to the first control well (CW1). This was mixed, and 120ul from the CW1 solution and 120ul standard diluents were added to the second control well (CW2) and mixed. Then 120ul from CW2 and 120ul standard diluents were added to the third control well (CW3). Another 120ul from CW3 and 120ul standard diluents were added in to the fourth control well (CW4) and mixed. Finally 120ul from CW4 and 120ul standard diluents were added in to the fifth control well (CW5) and mixed. This give a 50% downgraded serial dilution of the original standard solution concentration from CW1 down to the fifth control well.

Thereafter, 50ul standard and streptavidin-HRP 50ul were added in to standard solution well. Then into to the remaining test wells: 40ul of test serum, 10ul ISR antibodies and

50ul streptavidin-HRP were added each at a time. All wells were shake gently to mixed, and then incubated at 37°C for 60 minutes. After which the wells were washed five times with a washing solution to removed unbound enzymes. Then, wells are dried and substrate 50ul chromogen solution A was added to each well, followed with 50ul chromogen solution B to each well. This was shake gently and incubated for 10 minutes at 37°C. Then, stop solution was added to each well, colour change from blue to yellow was observed. Then, the whole plate of wells was inserted into the microplate reader, and the absorbance under 450nm wavelength was read as displayed on the screen of the machine immediately and were recorded. A graph of concentration of standard against their absorbance was plotted, then, the absorbance of each sample was traced on that of the standard axis, and its concentration is gotten on the standard's concentration axis and recorded.

3.5.3 Estimation of serum leptin

Serum leptin was estimated using rabbit LEP (leptin) ELISA kit in accordance to the manufacturers guide.

Procedure:

First, the wells are labelled using a masking tape starting with the controls down to test wells, then followed with a serial dilution in the first five control wells as follows, from the original standard solution [80ng/mL] 120ul and 120ul standard diluents were pipetted using automated graded pipette, and added to the first control well (CW1). This was mixed, and 120ul from the CW1 solution and 120ul standard diluents were added to the second control well (CW2) and mixed. Then 120ul from CW2 and 120ul standard diluents were added to the third control well (CW3). Another 120ul from CW3 and 120ul standard diluents were added in to the fourth control well (CW4) and

mixed. Finally 120ul from CW4 and 120ul standard diluents were added in to the fifth control well (CW5) and mixed. This give a 50% downgraded serial dilution of the original standard solution concentration from CW1 down to the fifth control well.

Thereafter, 50ul standard and streptavidin-HRP 50ul were added in to standard solution well. Then into to the remaining test wells: 40ul of test serum, 10ul LEP antibodies and 50ul streptavidin-HRP were added each at a time. All wells were shake gently to mixed, and then incubated at 37°C for 60 minutes. After which the wells were washed five times with a washing solution to removed unbound enzymes. Then, wells are dried and substrate 50ul chromogen solution A was added to each well, followed with 50ul chromogen solution B to each well. This was shake gently and incubated for 10 minutes at 37°C. Then, stop solution was added to each well, colour change from blue to yellow was observed. Then, the whole plate of wells was inserted into the microplate reader, and the absorbance under 450nm wavelength was read as displayed on the screen of the machine immediately and were recorded. A graph of concentration of standard against their absorbance was plotted, then, the absorbance of each sample was traced on that of the standard axis, and its concentration is gotten on the standard's concentration axis and recorded.

Serum insulin, insulin receptor and leptin levels were assessed based on the principle described by Schmidt and Schmidt, (1976).

3.5.4Estimation of lipid profile

3.5.4.1Determination of total cholesterol(TC)

- Total serum cholesterol level was estimated by the enzymaticmethod of Watson (1960).

Principle;

The cholesterol I determine after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

Cholesterol ester +H_2O (cholesterol esterase) → cholesterol +fatty acids

Cholesterol + O_2(cholesterol oxidase) →cholestene-3-one +H_2O_2

$2H_2O_2$ + phenol + 4-Aminoantipyrine (peroxidase) → quinoneimine +$4H_2O$

Reagent

Reagent (R1. reagent) composition; 4-Aminoantipyrine 0.03mmol/l; phenol 6mmol/l; peroxidase≥0.5U/ml; cholesterol esterase ≥ 0.15U/ml; cholesterol oxidase ≥0.1U/ml; pipes buffer 80mmol/l, at pH 6.8.

Procedure:

Using fresh ddH_2O, a new Gain Calibration in cuvette mode was performed. CHOL was select in the Run Test screen to carry out a water blank as instructed.

Pipette into a cuvette:

Reagent blank SO= ddH_2O 10ul

Standard S1= standard10ul

Sample= sample 10ul

Then, reagent 1000ul, 1000ul and 1.00ml into reagent blank SO, Standard S1, and sample respectively. All test samples were mix with R1 and incubate for 10 minutes at 20-25°C, and absorbance of the sample (A_{sample}) were measure against the reagent blank within 60 minutes at wavelength of 500nm.

Manual calculation;

Concentration of cholesterol in sample= $\frac{Asample}{Astandard} * con.of\ standard$

Using a standard;Concentration of standard (38.7), results were gotten and recorded for each sample.

3.5.4.2 Determination of high-density lipoprotein cholesterol (HDL-c).

- Serum high density lipoprotein cholesterol (HDL-C) was estimated by the method reported by Freidewald *et al*., (1972),

Principle:

In presence of magnesium ions, the very low density and low density lipoproteins are precipitated from serum by phosphotugstate, after removed by centrifugation the clear supernatant containing high density lipoproteins is used for the determination of HDL-c

Reagent:

Reagent are ready to use as supplied

Reagent I; HDL-C precipitating reagent

HDL-C standard; 50mg/dl

Procedure:

- Equal amount of serum and HDL-C precipitating reagent were mix in the glass tube and mix vigorously. (eg, serum 0.2ml+HDL precipitating reagent).
- Mixture is centrifuge for 10 minutes at 1500-2000*g*. to

Separate supernatant from precipitate, the supernatant fraction contains HDL.

- Cholesterol reagent and photometer were maintained at 37°C using a water bath.
- Then, pipette into the following cuvette; Blank containing cholesterol reagent 1000ul: Standard containing Std 50ul and cholesterol reagent 1000ul: and

samples supernatant containing 50ul of sample supernatant and cholesterol reagent 1000ul.

- Cuvette were mixed and incubated for 5min at 37°C, then the absorbance of sample and standard were read at 505nm against reagent blank.
- Results are calculated as follows:

  HDL Chol (mg/dl)= = $\frac{(Abs)\ of\ sample}{(Abs) of\ standard} * standard\ value * 2$

  Where 2 is the serum dilution factor

3.5.4.3Determination of serum triglycerol (TG)

- Serum triglycerol (TG) was estimated by the method of Fossati *et. al*., (1982).
- While serum low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) werecalculated according to the Friedewald formula:

LDL-C=TC –(HDL-C+TG/5)

and VLDL-C=TG/5 (Ahmadi, *et al*., 2008; Friedewald *et. al* ., 1972)

where:

LDL-C = Low density lipoprotein cholesterol

VLDL-C = very low density lipoprotein cholesterol

HDL-C = high density lipoprotein cholesterol.

TC = total cholesterol

TG = serum triglyceride

3.6 Estimation of Thyroid Hormones (T3, T4), Thyroid Stimulating Hormone

3.6.1 Estimation of thyroid stimulating hormone (TSH)

Thyroid stimulating hormone (TSH) was determine using rabbit TSH ELISA kit in accordance to the manufacturers guide.

Principle;

The kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay rabbit TSH.

Procedure:

First, the wells are labelled using a masking tape starting with the controls down to test wells, then followed with a serial dilution in the first five control wells as follows, from the original standard solution [128mU/L] 120ul and 120ul standard diluents were pipetted using automated graded pipette, and added to the first control well (CW1). This was mixed, and 120ul from the CW1 solution and 120ul standard diluents were added to the second control well (CW2) and mixed. Then 120ul from CW2 and 120ul standard diluents were added to the third control well (CW3). Another 120ul from CW3 and 120ul standard diluents were added in to the fourth control well (CW4) and mixed. Finally 120ul from CW4 and 120ul standard diluents were added in to the fifth control well (CW5) and mixed. This give a 50% downgraded serial dilution of the original standard solution concentration from CW1 down to the fifth control well.

Thereafter, 50ul standard and streptavidin-HRP 50ul were added in to standard solution well. Then into to the remaining test wells: 40ul of test serum, 10ul TSH antibodies and 50ul streptavidin-HRP were added each at a time. All wells were shake gently to mixed, and then incubated at 37°C for 60 minutes. After which the wells were washed five times with a washing solution to removed unbound enzymes. Then, wells are dried and substrate 50ul chromogen solution A was added to each well, followed with 50ul

chromogen solution B to each well. This was shake gently and incubated for 10 minutes at 37°C. Then, a stop solution was added to each well, colour change from blue to yellow was observed. Then, the whole plate of wells was inserted into the microplate reader, and the absorbance under 450nm wavelength was read as displayed on the screen of the machine immediately and were recorded. A graph of concentration of standard against their absorbance was plotted, then, the absorbance of each sample was traced on that of the standard axis, and its concentration is gotten on the standard's concentration axis and recorded.

3.6.2 Estimation of tri-iodothyronine (T_3)

T_3 was determine using rabbit Tri-iodothyronine (T_3) ELISA kit in accordance to the manufacturers guide.

First, the wells are labelled using a masking tape starting with the controls down to test wells, then followed with a serial dilution in the first five control wells as follows, from the original standard solution [14.4ng/ml] 120ul and 120ul standard diluents were pipetted using automated graded pipette, and added to the first control well (CW1). This was mixed, and 120ul from the CW1 solution and 120ul standard diluents were added to the second control well (CW2) and mixed. Then 120ul from CW2 and 120ul standard diluents were added to the third control well (CW3). Another 120ul from CW3 and 120ul standard diluents were added in to the fourth control well (CW4) and mixed. Finally 120ul from CW4 and 120ul standard diluents were added in to the fifth control well (CW5) and mixed. This give a 50% downgraded serial dilution of the original standard solution concentration from CW1 down to the fifth control well.

Thereafter, 50ul standard and streptavidin-HRP 50ul were added in to standard solution well. Then into to the remaining test wells: 40ul of test serum, 10ul T_3 antibodies and

50ul streptavidin-HRP were added each at a time. All wells were shake gently to mixed, and then incubated at 37°C for 60 minutes. After which the wells were washed five times with a washing solution to removed unbound enzymes. Then, wells are dried and substrate 50ul chromogen solution A was added to each well, followed with 50ul chromogen solution B to each well. This was shake gently and incubated for 10 minutes at 37°C. Then, a stop solution was added to each well, colour change from blue to yellow was observed. Then, the whole plate of wells was inserted into the microplate reader, and the absorbance under 450nm wavelength was read as displayed on the screen of the machine immediately and were recorded. A graph of concentration of standard against their absorbance was plotted, then, the absorbance of each sample was traced on that of the standard axis, and its concentration is gotten on the standard's concentration axis and recorded.

3.6.3 Estimation of thyroxine (T_4)

Thyroxine (T_4) was estimated using rabbit T_4 ELISA kit in accordance to the manufacturers guide.

Briefly:

First, the wells are labelled using a masking tape starting with the controls down to test wells, then followed with a serial dilution in the first five control wells as follows, from the original standard solution [192nmol/L], 120ul and 120ul from standard diluents were pipetted using automated graded pipette, and added to the first control well (CW1). This was mixed, and 120ul from the CW1 solution and 120ul standard diluents were added to the second control well (CW2) and mixed. Then 120ul from CW2 and 120ul standard diluents were added to the third control well (CW3). Another 120ul from CW3 and 120ul standard diluents were added in to the fourth control well (CW4)

and mixed. Finally 120ul from CW4 and 120ul standard diluents were added in to the fifth control well (CW5) and mixed. This give a 50% downgraded serial dilution of the original standard solution concentration from CW1 down to the fifth control well.

Thereafter, 50ul standard and streptavidin-HRP 50ul were added in to standard solution well. Then into to the remaining test wells: 40ul of test serum, 10ul T_4 antibodies and 50ul streptavidin-HRP were added each at a time. All wells were shake gently to mixed, and then incubated at 37°C for 60 minutes. After which the wells were washed five times with a washing solution to removed unbound enzymes. Then, wells are dried and substrate 50ul chromogen solution A was added to each well, followed with 50ul chromogen solution B to each well. This was shake gently and incubated for 10 minutes at 37°C. Then, a stop solution was added to each well, colour change from blue to yellow was observed. Then, the whole plate of wells was inserted into the microplate reader, and the absorbance under 450nm wavelength was read as displayed on the screen of the machine immediately and were recorded. A graph of concentration of standard against their absorbance was plotted, then, the absorbance of each sample was traced on that of the standard axis, and its concentration is gotten on the standard's concentration axis and recorded.

3.7 Estimation of Oxidative Stress Biomarkers

3.7.1 Measurement of superoxide dismutase (SOD)

The plasma SOD was determine using rabbit Superoxide Dismutase (SOD) ELISA kit in accordance to the manufacturers guide, this is based on the method described by Martin *et al*.,(1987).

Procedure in brief:

First, the wells were labelled using a masking tape starting with the controls down to test wells, then followed with a serial dilution in the first five control wells as follows, from the original standard solution [320ng/ml] 120ul and 120ul standard diluents were pipetted using automated graded pipette, and added to the first control well (CW1). This was mixed, and 120ul from the CW1 solution and 120ul standard diluents were added to the second control well (CW2) and mixed. Then 120ul from CW2 and 120ul standard diluents were added to the third control well (CW3). Another 120ul from CW3 and 120ul standard diluents were added in to the fourth control well (CW4) and mixed. Finally 120ul from CW4 and 120ul standard diluents were added in to the fifth control well (CW5) and mixed. This give a 50% downgraded serial dilution of the original standard solution concentration from CW1 down to the fifth control well.

Thereafter, 50ul standard and streptavidin-HRP 50ul were added in to standard solution well. Then into to the remaining test wells: 40ul of test serum, 10ul SOD antibodies and 50ul streptavidin-HRP were added each at a time. All wells were shake gently to mixed, and then incubated at 37°C for 60 minutes. After which the wells were washed five times with a washing solution to removed unbound enzymes. Then, wells are dried and substrate 50ul chromogen solution A was added to each well, followed with 50ul chromogen solution B to each well. This was shake gently and incubated for 10 minutes at 37°C. Then, a stop solution was added to each well, colour change from blue to yellow was observed. Then, the whole plate of wells was inserted into the microplate reader, and the absorbance under 450nm wavelength was read as displayed on the screen of the machine immediately and were recorded. A graph of concentration of standard against their absorbance was plotted, then, the absorbance of each sample

was traced on that of the standard axis, and its concentration is gotten on the standard's concentration axis and recorded.

3.6.2 Measurement of catalase (CAT) activity

The plasma Catalase (CAT) was determine using rabbit Catalase (CAT) ELISA kit in accordance to the manufacturers guide, which is based on the method described by Aebi, (1984).

Procedure in brief:

First, the wells were labelled using a masking tape starting with the controls down to test wells, then followed with a serial dilution in the first five control wells as follows, from the original standard solution [240ng/ml] 120ul and 120ul standard diluents were pipetted using automated graded pipette, and added to the first control well (CW1). This was mixed, and 120ul from the CW1 solution and 120ul standard diluents were added to the second control well (CW2) and mixed. Then 120ul from CW2 and 120ul standard diluents were added to the third control well (CW3). Another 120ul from CW3 and 120ul standard diluents were added in to the fourth control well (CW4) and mixed. Finally 120ul from CW4 and 120ul standard diluents were added in to the fifth control well (CW5) and mixed. This give a 50% downgraded serial dilution of the original standard solution concentration from CW1 down to the fifth control well.

Thereafter, 50ul standard and streptavidin-HRP 50ul were added in to standard solution well. Then into to the remaining test wells: 40ul of test serum, 10ul CAT antibodies and 50ul streptavidin-HRP were added each at a time. All wells were shake gently to mixed, and then incubated at 37°C for 60 minutes. After which the wells were washed five times with a washing solution to removed unbound enzymes. Then, wells are dried and substrate 50ul chromogen solution A was added to each well, followed with 50ul

chromogen solution B to each well. This was shake gently and incubated for 10 minutes at 37°C. Then, a stop solution was added to each well, colour change from blue to yellow was observed. Then, the whole plate of wells was inserted into the microplate reader, and the absorbance under 450nm wavelength was read as displayed on the screen of the machine immediately and were recorded. A graph of concentration of standard against their absorbance was plotted, then, the absorbance of each sample was traced on that of the standard axis, and its concentration is gotten on the standard's concentration axis and recorded.

3.7.3 Measurement of glutathione peroxidase (GPx)

Glutathione peroxidase (GPx) was determine basedon the method of Beutler and Kelly (1963). The technique involves protein precipitation by meta-phosphoric acid.

Rabbit glutathione peroxidase (GPx) ELISA kit was used in accordance to the manufacturers guide to assessed GPx.

Procedure in brief:

First, the wells were labelled using a masking tape starting with the controls down to test wells, then followed with a serial dilution in the first five control wells as follows, from the original standard solution [160ng/ml] 120ul and 120ul standard diluents were pipetted using automated graded pipette, and added to the first control well (CW1). This was mixed, and 120ul from the CW1 solution and 120ul standard diluents were added to the second control well (CW2) and mixed. Then 120ul from CW2 and 120ul standard diluents were added to the third control well (CW3). Another 120ul from CW3 and 120ul standard diluents were added in to the fourth control well (CW4) and mixed. Finally 120ul from CW4 and 120ul standard diluents were added in to the fifth

control well (CW5) and mixed. This give a 50% downgraded serial dilution of the original standard solution concentration from CW1 down to the fifth control well.

Thereafter, 50ul standard and streptavidin-HRP 50ul were added in to standard solution well. Then into to the remaining test wells: 40ul of test serum, 10ul GPx antibodies and 50ul streptavidin-HRP were added each at a time. All wells were shake gently to mixed, and then incubated at 37°C for 60 minutes. After which the wells were washed five times with a washing solution to removed unbound enzymes. Then, wells are dried and substrate 50ul chromogen solution A was added to each well, followed with 50ul chromogen solution B to each well. This was shake gently and incubated for 10 minutes at 37°C. Then, a stop solution was added to each well, colour change from blue to yellow was observed. Then, the whole plate of wells was inserted into the microplate reader, and the absorbance under 450nm wavelength was read as displayed on the screen of the machine immediately and were recorded. A graph of concentration of standard against their absorbance was plotted, then, the absorbance of each sample was traced on that of the standard axis, and its concentration is gotten on the standard's concentration axis and recorded.

3.7.4 Estimation of lipid peroxidation biomarker malondialdehyde (MDA)

The plasma Malondialdehyde (MDA) was determine using rabbit MDA ELISA kit in accordance to the manufacturers guide.

Procedure in brief:

First, the wells were labelled using a masking tape starting with the controls down to test wells, then followed with a serial dilution in the first five control wells as follows, from the original standard solution [32nmol/ml] 120ul and 120ul standard diluents were pipetted using automated graded pipette, and added to the first control well

(CW1). This was mixed, and 120ul from the CW1 solution and 120ul standard diluents were added to the second control well (CW2) and mixed. Then 120ul from CW2 and 120ul standard diluents were added to the third control well (CW3). Another 120ul from CW3 and 120ul standard diluents were added in to the fourth control well (CW4) and mixed. Finally 120ul from CW4 and 120ul standard diluents were added in to the fifth control well (CW5) and mixed. This give a 50% downgraded serial dilution of the original standard solution concentration from CW1 down to the fifth control well.

Thereafter, 50ul standard and streptavidin-HRP 50ul were added in to standard solution well. Then into to the remaining test wells: 40ul of test serum, 10ul MDA antibodies and 50ul streptavidin-HRP were added each at a time. All wells were shake gently to mixed, and then incubated at 37°C for 60 minutes. After which the wells were washed five times with a washing solution to removed unbound enzymes. Then, wells are dried and substrate 50ul chromogen solution A was added to each well, followed with 50ul chromogen solution B to each well. This was shake gently and incubated for 10 minutes at 37°C. Then, a stop solution was added to each well, colour change from blue to yellow was observed. Then, the whole plate of wells was inserted into the microplate reader, and the absorbance under 450nm wavelength was read as displayed on the screen of the machine immediately and were recorded. A graph of concentration of standard against their absorbance was plotted, then, the absorbance of each sample was traced on that of the standard axis, and its concentration is gotten on the standard's concentration axis and recorded.

3.8Estimation of Serum Liver Enzymes Levels

3.8.1 Determination of serum alkaline phosphatase

Serum alkaline phosphatase (ALP) activity was assayed by the method of Bassey *et al*., (1946).

Principle: alkaline phosphatase (ALP) catalyses the hydrolysis of p-nitrophenyl phosphatase at alkaline pH, liberating p-nitrophenol and phosphatase, the rate of p-Nitrophenol formation, measured photometrically is proportional to the catalytic concentration of alkaline phosphatase present in the sample.

Reagent:

reagent I: buffer reagent

reagent II: substrate reagent

reagent preparation; working reagent was prepared mixing four parts of buffer reagent with one part of substrate reagent.

- Assay Procedure; 20ul of the sample and 1000ul 0f working reagent were pipette into test tubes for all test samples,
- Mixture were mixed well and incubated at 37°C for 60 secs.
- Absorbance read at 405nm and Increased absorbance was measured every 30 secs for 2 min and change in Abs/min (≙A/min) was determine.
- ALP concentration was calculated as (U/l) =≙A/min * 2720

  Where, 2720 is a specified factor for the test.

3.8.2 Serum alanine aminotransferase (ALT) estimation

Serum alanine aminotransferase activity was assayed by the method of Reitman andFrankel (1957).

Principle; α-oxoglutarate+ L-alanine (GPT)→L-glutamate+ pyruvate

Alanine aminotransferase is measure by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenylhydrazine.

Reagent composition; R1. buffer (phosphate buffer 100mmol/l, pH 7.4: l-alnine200mmol/l, α-oxoglutarate2.0mmol/l), and R2. 2,4-dinitrophenylhydrazine 2.0mmol/l. other material used are sodium hydroxide solution (0.4mmol/l) and Randox Reitman and Frankel ALT/AST level 2 control (Cat. No. SC 2643)

Procedure;

The following were pipette in to test tube;

Sample blank (Test tube), contains sample solution R1 0.5ml only, and in to test tube sample test, serum 0.1ml+ 0.5mlsolution R1.

These were mixed and incubate for 30 min at 37°C.

Then, solution R2 0.5ml was added to both test tubes and 0.1ml of serum to sample blank test tube, these were mixed and allow to stand for 20min at 20-25°C.

Then, sodium hydroxide 5.0ml was added to both test tubes, mixtures were mix again and absorbance is read of the sample against the sample blank after 5 min at a wavelength Hg 546nm and cuvette of 1cm light path.

Finally, the activity of ALT is obtain from a standard table of absorbance with corresponding activity, and values were recorded (Rietman and Frankel, 1957: Schmidt and Schmidt, 1963).

3.8.3 Determination of aspartate aminotransferase (AST)

Serum Aspartate Aminotransferase activity was assayed by the method of Reitman and Frankel (1957).

Principle; α-oxoglutarate+ L-aspartate (GOT) → L-glutamate+ Oxaloacetate

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

Reagent composition; R1. buffer (phosphate buffer 100mmol/l, pH 7.4: l

L-aspartate 100mmol/l, α-oxoglutarate 2.0mmol/l), and R2. 2,4-dinitrophenylhydrazine 2.0mmol/l. other material used are sodium hydroxide solution (0.4mmol/l) and Randox Reitman and Frankel ALT/AST level 2 control (Cat. No. SC 2643)

Procedure;

The following were pipette in to test tube;

Sample blank (Test tube), contains sample solution R1 0.5ml only, and in to test tube sample test, serum 0.1ml+ 0.5mlsolution R1.

These were mix and incubate for 30 min at 37°C.

Then, solution R2 0.5ml was added to both test tubes and 0.1ml of serum to sample blank test tube, these were mixed and allow to stand for 20min at 20-25°C.

Then, sodium hydroxide 5.0ml was added to both test tubes, mixtures were mix again and absorbance is read of the sample against the sample blank after 5 min at a wavelength Hg 546nm and cuvette of 1cm light path.

Finally the activity of AST is obtain from a standard table of absorbance with corresponding activity, and values were recorded (Reitman and Frankel, 1957: Schmidt and Schmidt, 1963).

3.9.Estimation of Serum Electrolytes

3.9.1 Estimation of serum sodium ion

Principle: Serum sodium was estimated based on the modification of methods first describe by Maruna(1958)andTrinder (1951) in which sodium is precipitated as the triple salt, sodium magnesium uranyl acetate. With excess uranium then being reacted

with ferrocyanide producing a chromophore whose absorbance varies inversely as concentration of sodium in the test specimen.

Reagent composition

Filtrate reagent; Uranyl Acetate 2.1 mM and magnesium acetate 20nM in ethyl alcohol,

Acid reagent; A diluted acetic acid

Sodium color reagent; potassium ferrocyanide, non-reactive stabilizers, and fillers

Sodium standard; sodium chloride solution (150 mEq/L).

Procedure

Filtrate preparation;

Test tubes were labeled as follows; blank, standard, control, test1-test 30.

1.0 ml of filtrate reagent was pipette in to all test tubes,

50ul of sample was added in to all test tubes and distilled water to the blank,

Tubes were shaked vigorously and mix continuously for about 3 minutes

Tubes were centrifuge at 1500g for 10 minutes and a supernatant fluid ready for test is gotten,

A second set of test tubes were labeled corresponding to the first labelled

1.0ml of acid reagent was pipette to all test tubes

50ul of supernatant was added to respective tubes and mix

50ul of color reagent was added to all tubes and mix,

Spectrophotometer is zero with distilled water at 550 nm and finally absorbance of all tubes were read and recorded.

Conc. Sodium (mEq/L) = $\frac{(Abs\ of\ blank - Abs\ of\ sample)}{(Abs\ of\ blank - Abs\ of\ standard} * conc.of\ standard\ (mEq/L)$

3.9.2 Estimation of serum potassium ion

Principle;

The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension (Terri and Sesin, 1958), the turbididty of which is proportional to potassium concentration in range of 2-7 mEq/L.

Reagent contents;

Potassium reagent; sodium tetraphenylboron 2.1nm. Preservatives and thickening agents.

Potassium standard; equivalent to 4mEq/L.

Procedure

Test tubes were labeled as follows; blank, standard, control, test 1- test 30

1.0ml of potassium reagent is pipette in toall tubes

0.01ml (10ul) of sample was added to tube and mix allow to stand at room temperature for 3minute,

Then, spectrophotometer was zero with reagent blank at wave length of 500nm, and absorbance of all tubes are read and recorded.

Potassium conc. (mEq/L) = $\frac{(Abs)\ of\ sample}{(Abs) of\ standard} * conc. of\ standard\ (mEq/L)$

(Vogel,1960).

3.9.3 Estimation of serum bicarbonate ion

This was determined using the enzymatic method for determining total CO_2 as bicarbonates and dissolved gases,

HCO_3^- + phosphoenoppyruvate → Oxaloacetate + H_2PO_4

Oxaloacetate + NADH → malate + NAD^+

The decrease in absorbance due to the oxidation of NADH in NAD^+ is directly proportional to the amount of total CO_2 in the specimen and is measured at 380nm

Procedure

Working Reagents include;

Vial R1 (content; phosphoenolpyruvate 8.0mmol/L, NADH 1.6mmol/L, PEPC (phosphoenolpyruvate carboxidase), MDH (malate dehydrogenase), and Buffer pH20%).

Vial R2 standard, sodium bicarbonate 27mmol/L.

Fisrtly,

Test tubes were labeled as follows; blank, standard, control, assay test 1- test 30

1.0ml of Vial R1 was pipette in to all tubes,

Then, 10ul of distil water into blank test tube, and Vial R2 into standard containing test tube.

Then 10uL of working specimen was added into assay test tubes.

Test tubes content were mix and incuvatte for about 5 minutes at 37°C.

Then absorbance was read at 380nmm of the assay test against that of blank test tube in a 1cm length cuvette.

Results were calculated as

Total $CO_2 = \frac{(Abs)\ of\ blank - Abs\ sample}{(Abs) of Blank - Abs\ of\ standard} * conc.\ of\ standard$

Total CO_2 is approximately 10% higher than bicarbonates.

This is based on the principle reported by Norris, *et al.*, (1975) and Forrester, *et al.*, (1976).

3.9.4 Estimation of serum chloride ion

This was done based on themodified Thiocyanate method of Volhard's as described by Ernest *et al*., (1950).

Reagent composition;

Chloride Reagent 2 x 30 mL,

Mercuric (II) thiocyanate 2 mmol/L

Nitric acid 29 mmol/L

Ferric Nitrate 20 mmol/L

Procedure

First, Test tubes were labelled as follows; blank, standard, control, assay test 1- test 30

1.0ml of Vial R1 was pipette in to all tubes,

Then, 10ul of distil water into blank test tube, and Vial R2 into standard containing test tube.

Then 10uL of working specimen was added into assay test tubes.

Test tubes content were mix and in cuvatte for about 5 minutes at 37°C.

Then absorbance was read at 380nmm of the assay test against that of blank test tube in a 1cm length cuvette.

Results were calculated as

Total Cl_2^-= ((Abs) of blank-Abs sample)/((Abs)ofBlank-Abs of standard)*conc.of standard (Schonfed and Lowellen, 1964).

3.9Statistical Analysis

Data obtained were expressed as mean ± SEM, statistically analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc test was used to compare the level of significance between test groups and negative control, $P < 0.5$ value was taken to be statistically significant. SPSS version 20.0 software was used for the analysis (Duncan *et al*., 1977).

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of Clove and Fermented Ginger on Blood Glucose Levels in High Fat Diet (HFD) Induced Type 2 Diabetes (T2DM) in Rabbits.

The results of blood glucose level (Table 4.1) revealed a significant ($P < 0.05$) decrease in blood glucose level at the second and fourth week of treatment in the diabetic model rabbits groups treated with clove 12.5%, fermented ginger 12.5%, and the combined clove 12.5% + fermented ginger 12.5% supplements, compared to diabetic model rabbits group fed on standard feed. While at the fifth week only, the group on clove 12.5% supplement showed a significant decrease in blood glucose level compared to that of diabetic model rabbits group fed on standard feed ($P < 0.05$).

Table 4.1: Blood Glucose Level in High Fat Diet (HFD) Induced- type 2 Diabetes (T2DM) in Rabbits Fed on Diets Containing Clove 12.5%, Fermented Ginger 12.5% and Clove12.5 + Fermented Ginger 12.5% Supplements and Control Groups.

Groups	BGL Week 0 (mg/dL)	BGL Week 1 (mg/dL)	BGL Week 2 (mg/dL)	BGL Week 3 (mg/dL)	BGL Week 4 (mg/dL)	BGL Week 5 (mg/dL)
Normal Control (NC)	109.80 ± 3.14	103.20 ± 4.62	101.80 ± 2.71	108.00 ± 1.92	95.20 ± 3.95	97.60 ± 2.04
Diabetic Control (DC)	140.00 ± 0.84[a]	119.00 ± 2.63[a]	123.40 ± 2.46[a]	140.80 ± 1.59[a]	125.40 ± 2.94[a]	114.00 ± 2.24[a]
Diabetic + Chl. (0.26g/kg)	140.80 ± 1.53[a]	155.40 ± 2.87[a,b]	140.80 ± 0.86[a,b]	159.60 ± 2.84[a,b]	122.60 ± 0.98[a]	126.40 ± 1.17[a,b]
Diabetic + CLV (12.5%)	149.40 ± 2.04[a]	117.20 ± 1.59[a,]	128.00 ± 0.71[a,b]	162.60 ± 2.20[a,b]	104.60 ± 1.94[a,b]	105.20 ± 0.58[b]
Diabetic + Fg (12.5%)	149.20 ± 1.50[a]	132.80 ± 3.57[a,b]	134.00 ± 2.30[a,b]	141.80 ± 2.48[a]	112.20 ± 1.36[a,b]	132.00 ± 2.68[a,b]
Diabetic + CLV (12.5%) + (Fg 12.5%)	149.40 ± 2.62[a]	143.20 ± 3.32[a,b]	136.60 ± 1.36[a,b]	168.00 ± 2.55[a,b]	112.20 ± 2.31[a,b]	127.80 ± 2.06[a,b]

Values are presented as mean ± SEM; n=5, P< 0.05 = significant.
Values with superscripts: **[a]** = significant different in comparison to NC group, and **[b]** = significant different when compared to DC group.

Group 1 = Normal control; Group 2 = Diabetic control; Group 3 = Diabetic received Chl. (cholestran);Group 4 = Diabetic fed on CLV (clove) supplement; Group 5 = Diabetic fed on Fg (fermented ginger) supplement; Group 6 = Diabetic fed on CLV (clove) + Fg (fermented ginger) supplement.

4.2 Effects of Clove and Fermented Ginger Supplements on Serum Insulin, Insulin-Receptor and Leptin Levels in HFD Induced T2DM in Rabbits.

The results (Table 4.2) revealed a statistically significant ($P < 0.05$) increase in serum insulin level of all the HFD induced diabetic animal groups (groups 2-6), compared to the normal control group (group1). Moreover, a significant ($P < 0.05$) increase in group 5 (clove 12.5% supplement treated group), compared to serum level of insulin in diabetic rabbits fed on standard feed (group 2) was also recorded. Serum leptin level also showed a significant decrease in clove 12.5% supplement treated group (group 4) compared to the level seen in group 2 ($P < 0.05$). While a significant ($P < 0.05$) increase in serum insulin receptor was also seen in all HFD induced diabetic animals, compared to that of NC group.

Table 4.2: Insulin, Leptin and Insulin-receptor Levels in High-fat Diet Induced Type 2 DM in Rabbits treated with Clove 12.5%, Fermented ginger 12.5% and co-administered clove12.5 + fermented ginger 12.5%, and control groups After six weeks treatment period.

Experimental Groups	Insulin (ng/ml)	Leptin (ng/ml)	Insulin Receptor (ng/ml)
Normal Control (NC)	9.00 ± 0.58	12.50 ± 1.44	15.00 ± 5.51
Diabetic Control (DC)	26.00 ± 2.51[a]	14.60 ± 1.96	29.40 ± 4.12 [a]
Diabetic + Chl. (0.26g/kg)	25.60 ± 9.44[a]	12.60 ± 1.18	32.20 ± 4.05 [a]
Diabetic + CLV (12.5%)	31.20 ± 3.56 [a]	10.60 ± 1.60 [a,b]	30.40 ± 3.64 [a]
Diabetic + Fg (12.5%)	37.20 ± 3.44[a,b]	14.20 ± 0.86	27.80 ± 2.58 [a]
Diabetic + CLV (12.5%) + (Fg 12.5%)	26.40 ± 6.74 [a]	12.70 ± 2.63	28.60 ± 5.53 [a]

Values are presented as mean ± SEM; n=5: P< 0.05 = significant.
Superscripts: **[a]** = significant different compared to NC group; and **[b]** = significant difference when compared to DC group.

Group 1 = Normal control; Group 2 = Diabetic control; Group 3 = Diabetic received Chl. (cholestran);Group 4 = Diabetic fed on CLV (clove) supplement; Group 5 = Diabetic fed on Fg (fermented ginger) supplement; Group 6 = Diabetic fed on CLV (clove) + Fg (fermented ginger) supplement.

4.3 Effects of Clove and Fermented Ginger Supplements on Serum Lipid Profile in HFD Induce T2DM in Rabbits.

The results of lipid profile, revealed a significantly ($P < 0.05$) decrease in total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) in diabetic animal treated with fermented ginger supplement (group 5), when compared to that of diabetic animal fed on normal feed (group 2). HDL-c levels also show a significant ($P< 0.05$) decrease in diabetic animals treated with clove when compared to that of group 2, but with no significant difference to that of NC group. While in the diabetic animals treated with combined (fermented ginger + clove) supplements group (group 6), a significant ($P< 0.05$) decrease in HDL-c level was seen when compared to the level in group 2, but with significant difference to that of NC group.

Table 4.3: Lipid Profile of High Fat Diet Induced- T2DM in Rabbits Treated with Clove 12.5%, Fermented Ginger 12.5% and Co-Administered Clove12.5 + Fermented Ginger 12.5%, and Control Groups for Six Weeks.

GROUPS	**TC (mg/dL)**	**TG (mg/dL)**	**HDL-c (mg/dL)**	**LDL-c (mg/dL)**
Normal Control (NC)	144.4±3.63	88.6±11.5	20.33±2.70	39.4±14.25
Diabetic Control (DC)	180.7±14.74[a]	111.8±7.1[a]	26.90±2.72[a]	47.31±9.81
Diabetic + Chl (0.26g/kg)	184.3±11.32 [a]	79.8±10.9	23.11±2.10	18.6±7.52[a,b]
Diabetic + CLV (12.5%)	180.15±15.17[a]	117.5±16.24[a]	20.81±1.4[b]	61.37±13.07
Diabetic + Fgg (12.5%)	143.06±23.47[b]	80.45±5.8[b]	17.20±3.72[b]	39.5±6.02[a,b]
Diabetic + CLV (12.5%) + Fgg (12.5%)	178.80±28.10[a]	125.91±21.42[a]	14.95±1.06[a,b]	69.71±10.55[a]

Values are presented as mean ± SEM; n=5: P< 0.05 = significant.
Superscripts: **[a]** = significant different when compared to NC group, and **[b]** = significant different when compared to DC group.

Group 1 = Normal control; Group 2 = Diabetic control; Group 3 = Diabetic received Chl. (cholestran); Group 4 = Diabetic fed on CLV (clove) supplement; Group 5 = Diabetic fed on Fg (fermented ginger) supplement; Group 6 = Diabetic fed on CLV (clove) + Fg (fermented ginger) supplement.

4.4 Effects of Clove and Fermented Ginger Supplements on Thyroids Hormones and TSH levels in HFD induce T2DM in rabbits.

The results reveal a statistically significant ($P < 0.05$) decrease in serum T_3 level in diabetic animals treated with fermented ginger (12.5%) supplements and in the combined clove + fermented ginger supplemented group, when compared to that of diabetic group on normal feed. T_4 levels level were seen to significantly ($P < 0.05$) decrease in all supplemented diet treated groups (clove 12.5%, ginger 12.5% and combined clove 12.5% + ginger 12.5% treatments groups), when compared to that of the diabetic rabbits group fed on normal feed. while the results on serum TSH shows a statistically significant ($P < 0.05$) increase in groups 5, when compared to that of diabetic control group.

Table 4.4: Serum Thyroids Hormones and TSH Level in Control and in High Fat Diet induced- T2DM in Rabbits Treated with Clove 12.5%, Fermented Ginger 12.5% and Co-administered Clove12.5% + Fermented Ginger 12.5% Supplements for six weeks.

GROUPS	T_3 (ng/ml)	T_4 (ng/ml)	TSH (ng/ml)
Normal Control (NC)	1.8±0.12	74.33±3.5	1.3±0.12
Diabetic Control (DC)	2.1±0.071[a]	79.4±1.5	0.8±0.093 [a]
Diabetic + Chl. (0.26g/kg)	1.84±0.93	61.2±0.6[a,**b**]	1.00±0.071
Diabetic + CLV (12.5%)	1.72±0.06	47.4±1.36[a,b]	0.94±0.07
Diabetic + Fg (12.5%)	1.6±0.14[b]	49.8±2.9[a,b]	1.64±0.093[b]
Diabetic + CLV (12.5%) + (Fg 12.5%)	1.5±0.93[b]	58.00±2.02[a,b]	1.1±0.071

Values are presented as mean ± SEM; n=5: P< 0.05 = significant.
Values with superscripts: **[a]** = significant different when compared to NC group, and **[b]** = significant different when compared to DC group.

Group 1 = Normal control; Group 2 = Diabetic control; Group 3 = Diabetic received Chl. (cholestran); Group 4 = Diabetic fed on CLV (clove) supplement; Group 5 = Diabetic fed on Fg (fermented ginger) supplement; Group 6 = Diabetic fed on CLV (clove) + Fg (fermented ginger) supplement.

4.5 Effects of Clove and Fermented Ginger Supplements on Oxidative Stress Biomarkers and Lipid Peroxidation Maker in HFD Induce T2DM in Rabbits.

The results (Table 4.5) show, a significant ($P < 0.05$) decrease of serum superoxide dismutase (SOD) activity and a significant increase in malondialdehyde (MDA) levels in diabetic animal fed on normal diet, when compared to that of normal control. This same trend was noticed in diabetic animal treated with cholestran group. clove 12.5% supplement, fermented ginger 12.5% supplement and clove 12.5% + fermented ginger 12.5% co-administered groups revealed a significant ($P < 0.05$) increase in CAT activities, when compared to the level of activities in diabetic control group. And a significant ($P < 0.05$) decrease in MDA levels in group 5 and 6, when compared to that of group 2. Group 6 (diabetic animals treated on clove 12.5% + fermented ginger 12.5% supplements) shows a significant ($P < 0.05$) increase in SOD with a significant decrease in MDA levels when compared to that of group 2. And as well a significant ($P < 0.05$) increase in CAT and GPx activities in group 6, when compared to their activities in both control groups.

Table 4.5: Oxidative Stress Biomarkers and Lipid Peroxidation Maker of HFD induced- T2DM in Rabbits Treated with Clove 12.5%, Fermented Ginger 12.5% and Co-administered Clove12.5 + Fermented Ginger 12.5%, and in Control Groups after Six Weeks of Treatment.

GROUPS	SOD (IU/L)	CAT (IU/L)	GPx (IU/L)	MDA(nmol/L)
Normal Control (NC)	2.03±0.09	38.7±1.5	43.00±1.53	1.17±0.09
Diabetic Control (DC)	1.60±0.071[a]	37.6±0.67	42.8±0.9	1.54±0.051[a]
Diabetic + Chl. (0.26g/kg)	1.52±0.73 [a]	40.8±0.6	45.4±0.75	1.5±0.051[a]
Diabetic + CLV (12.5%)	1.78±0.66	42.00±0.9[b]	45.20±0.6	1.4±0.071[a]
Diabetic + Fg (12.5%)	1.68±0.06	41.4±1.21[a,b]	45.6±1.12	1.3±0.077[b]
Diabetic + CLV (12.5%) + (Fg 12.5%)	2.1±0.071[b]	45.8±O.73[a,b]	49.4±0.51[a,b]	1.1±0.071[b]

Values are presented as mean ± SEM; n=5: P< 0.05 = significant.
Values with superscripts: **[a]** = significant different when compared to NC group, and **[b]** = significant different when compared to DC group.

Group 1 = Normal control; Group 2 = Diabetic control; Group 3 = Diabetic received Chl. (cholestran); Group 4 = Diabetic fed on CLV (clove) supplement; Group 5 = Diabetic fed on Fg (fermented ginger) supplement; Group 6 = Diabetic fed on CLV (clove) + Fg (fermented ginger) supplement.

4.6 Effects of Clove and Fermented Ginger Supplements on Liver Enzymes Levels in HFD induced T2DM in Rabbits

The results on liver enzymes (table 4.6), revealed a significant ($P < 0.05$) increase in aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities in group 2 (diabetic animals fed on normal feed), when compared to normal control group (group1). A similar trend to the result in group 2 was seen in group 3 (diabetic group administered cholestran). A significant ($P < 0.05$) decrease in alanine aminotransferase (ALT) was seen in group 4 (diabetic group treated with clove 12.5% supplement) when compared to that of group 2, while AST activities significantly ($P < 0.05$) decrease in group 4 when compared to that levels of group 1 and group 2. Group 5 (fermented ginger 12.5% supplement) results reveal a significant ($P < 0.05$) decrease in serum ALT, AST and ALP levels, when compared to diabetic rabbits group on normal feed (group 2). Group 6 (diabetic animals treated with clove 12.5% + fermented ginger 12.5% supplement) revealed a significant ($P < 0.05$) decrease in AST level when compared to that of group 2, and a significant increase in ALP level when compared to the level in group 1.

Table 4.6: Liver Enzymes Activities of HFD induced T2DM in Rabbits treated with clove 12.5%, fermented ginger 12.5% and co-administered clove12.5 + fermented ginger 12.5%, and in control groups

GROUPS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Normal Control (NC)	53.00±20.84	32.7±11.84	1.33±0.11
Diabetic Control (DC)	227.00±60.2[a]	32.00±7.75	3.70±0.53[a]
Diabetic + Chl. (0.26g/kg)	194.6±25.83[a]	26.6±2.94	4.11±0.4[a]
Diabetic + CLV (12.5%)	56.00±13.08[b]	7.8±1.60 [a,b]	2.63±0.44[a]
Diabetic + Fg (12.5%)	14.2±3.40 [a,b]	13.2±2.63[a,b]	2.1±0.244[a]
Diabetic + CLV (12.5%) + (Fg 12.5%)	33.6±5.1[b]	22.6±3.20	2.55±0.62[a]

Values are presented as mean ± SEM; n=5: P< 0.05 = significant.
Values with superscripts: **[a]** = significant different when compared to NC group, and **[b]** = significant different when compared to DC group.

Group 1 = Normal control; Group 2 = Diabetic control; Group 3 = Diabetic received Chl. (cholestran); Group 4 = Diabetic fed on CLV (clove) supplement; Group 5 = Diabetic fed on Fg (fermented ginger) supplement; Group 6 = Diabetic fed on CLV (clove) + Fg (fermented ginger) supplement.

4.7 Effects of Clove and Fermented Ginger on Serum Electrolytes Concentrations in HFD Induced T2DM in Rabbits.

Table 4.7 shows the results of serum electrolytes; a significant ($P < 0.05$) decrease in sodium ion (Na^+) and bicarbonate ion (HCO_3^-) concentrations were seen in group 2 (diabetic group fed on normal feed) compared to their levels in group 1 (normal control group). A significant ($P < 0.05$) increase in serum sodium (Na^+) ions in groups 4 and 5 (groups fed on clove 12.5%, and fermented ginger 12.5% supplemented diets) compared to that of group 2 (diabetic control group). Potassium (K^+) ion concentration reveal a significantly ($P < 0.05$) decrease in all supplements fed groups (clove 12.5%, fermented ginger 12.5% and combined clove 12.5% + fermented ginger 12.5% supplemented diets), compared with that of diabetic control group (group 2), while the results of chloride (Cl^-) ion concentration shows a significant ($P < 0.05$) decrease in diabetic rabbits group fed on clove 12.5% (group 4) supplemented diet also when compared to that of group 2. Finally the results of bicarbonate (HCO_3^-) ion shows a statistically significant ($P < 0.05$) increase in group 4 animals compared with that of diabetic control group (group 2).

Table 4.7: Serum Electrolytes Concentrations in HFD Induced T2DM in Rabbits Treated for Six Weeks with Clove 12.5%, Fermented Ginger 12.5% and Co-administered Clove 12.5 + Fermented Ginger 12.5%, and in Control Groups

GROUPS	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	HCO_3^- (mEq/L)
Normal Control (NC)	123.9±13.3	9.43±0.83	94.33±1.2	30.00±1.53
Diabetic Control (DC)	68.7±9.8[a]	10.82±0.5	89.2±2.8	25.4±0.81[a]
Diabetic + Chl. (0.26g/kg)	121.7±15.12	10.4±0.6	85.6±1.9[a]	27.6±1.8
Diabetic + CLV (12.5%)	144.97±3.8[b]	4.05±0.14[b]	83.6±1.81[b]	31.8±1.8[b]
Diabetic + Fg (12.5%)	111.6±11.5[b]	3.31±0.22[b]	91.00±2.43	27.2±0.9
Diabetic + CLV (12.5%) + (Fg 12.5%)	68.2±5.7[a]	5.1±0.81[b]	87.4±1.63	27.00±0.071

Values are presented as mean ± SEM; n=5: P< 0.05 = significant.
Superscripts: **a** = significant different when compared to NC group, and **b** = significant different when compared to DC group.

Group 1 = Normal control; Group 2 = Diabetic control; Group 3 = Diabetic received Chl. (cholestran); Group 4 = Diabetic fed on CLV (clove) supplement; Group 5 = Diabetic fed on Fg (fermented ginger) supplement; Group 6 = Diabetic fed on CLV (clove) + Fg (fermented ginger) supplement.

CHAPTER FIVE

5.0 DISCUSSION

Type 2 diabetes mellitus (T2DM) is now a global health threat, which results from interaction between genetic, environmental, and behavioural risk factors (Baynest, 2015). It characterized metabolic disorder by the presence of chronic hyperglycaemia accompanied by impairment in the metabolism of carbohydrates, lipids, proteins and excess generation of reactive oxygen species, that leads to metabolic dysfunctions.

In the present study, HFD was fed to rabbits for eleven weeks to induced T2DM model, this was ascertain by hyperglycaemia, the animal were grouped and subjected to clove and fermented ginger supplements treatments with a disease control group untreated and a normal control as reference groups. Hyperglycaemia, hyperlipidaemia, elevated insulin, leptin and liver enzymes among other distorted physiological indices were observed in the disease control group compared to values of the same indices of the normal control group. The results gotten in the induction of hyperglycaemia and metabolic dysregulation of parameters that play different roles in metabolic processes is in line with the work of Jimoh *et al*. (2015), who reported that consumptions of cholesterol diet causes distortion along metabolic pathways leading to hyperglycaemia, lipidaemia and excessive body weight gain. HFD also lead to obesity and type-2 diabetes mellitus in laboratory animals (Kolawole *et al*., 2012; Jimoh *et al*., 2015; Heydemann, 2016).

5.1 Effects of Clove and Fermented Ginger on Blood Glucose levels

From the study, the rabbits group that received 12.5% supplement of clove had a significant decrease in blood glucose level, compared to that of diabetic rabbits group fed on standard feed. This is also seen in the other supplements treated groups. This

observation could be explained to be due to the effects of certain active constituents such as dehydrodieugenol and dehydrodieugenol B in clove, as well as Gingerol and shogaol present in ginger supplements which affect different level of insulin signalling cascade pathway or post insulin-receptor complex by increasing expression of glucose transporters and other molecular modulators of insulin activity. This result in increased glucose intake by muscle cells. (Arablou, *et al*., 2014; Hyun, *et al*., 2014). These mechanisms leads to increased muscular and hepatic tissues glucose uptake activities, which will result in increased rate of clearing blood glucose and maintain a physiological level of circulatory glucose. (Arablou *et al*., 2014). Another mechanism of action of ginger that may explain this action could be from inhibition of oxidative stress. Gingerol and shogaol which are active ingredient present in ginger may have contributed to the hypoglycemic effect observed in this study. The effects observed could be as a result of protein expression regulation at cellular level (Rahmani, *et al*., 2014). The findings of the present study are similar with the work of Nafiseh *et al*. (2015), in which it was demonstrated that treatment with ginger significantly decreased blood glucose and other parameters tested in diabetic patients. Similarly Mozaffari-Khosravi, *et al*. (2014) demonstrated also that daily consumption of one-gram capsules of ginger powder for 8 weeks is useful for patients with type 2 diabetes as it reduced fasting blood glucose and glycated hemoglobin (HbA1c). This work is also in agreement with that of Khan *et al*. (2006), in which capsule of cloves were found to improve the function of insulin and to lower blood glucose and concluded that consumption of 1-3g of clove improve glucose level in diabetic patients. However, this work do not agree with the report of Ashade, *et al*. (2014), where ginger peel do not lower blood glucose levels in cat fish. This difference may be as a result of use of the rhizome of ginger not the peel. The pronounced hypoglycaemic activity of the clove

supplement may probably be also attributed to the activities of dehydrodieugenol and dehydrodieugenol B that had potent PPAR-γ ligand-binding activity, therefore causing increased insulin sensitivity via muffling the effect of lipotoxicity along insulin signalling pathway (Kuroda *et al*. 2012).

5.2 Effect of Clove and Fermented Ginger on Serum Insulin and Insulin Receptor Levels

The result of this study revealed a significant increase in serum insulin level in high fat diet induced diabetic rabbits when compared to the normal control rabbits. Insulin activity may be low in this animals (diabetic control) due to the presence of excess FFAs in circulation that inhibit insulin signalling pathways. Consequent to this, higher plasma nutrients will be present in circulation that further leads to secretion of counter regulatory hormones such as glucagon and GLP1, which stimulates further secretion and release of insulin in to the circulation, resulting in hyperinsulinemia over time (Han *et al*., 2009). Treatment with fermented ginger also reveal a significant increase in insulin levels when compared to the level in diabetic control, fermented ginger may have exert insulin mimetic effects thereby sparing serum insulin or may have attributes to increase insulin secretion and release.

In the present study, the result of serum the insulin receptor level showed an increase similar to what was observe with insulin. The high fat diet, could have induced insulin resistance as a underline mechanism leading to metabolic dysregulations in the animal model, resulting in the up-regulation of the insulin receptor, being a physiological response to sustained hyperglycaemia seen in pre-diabetic state or in diabetes with insulin resistance (Hyun *et al*., 2014). This findings could be explain by the proposed

mechanism underlining the hypoglycaemic effect of ginger through increasing uptake of glucose into muscle cells without using insulin via regulating insulin inhibitory and stimulatory receptor proteins gene expressions (Arablou *et al*., 2014).

5.3 Effect of Clove and Fermented Ginger on Serum Leptin Level

Serum leptin level was significantly decreased in clove 12.5% supplemented diet treated group, when compared to hyperglycaemic rabbits fed on normal feed. Leptin is produced by adipocytes and regulates energy expenditure, feeding behaviour and body weight. Abnormal higher levels of leptin are associated to hyperinsulinemia and insulin resistance, leptin have a direct effect on glucose level independent of body weight and food intake (Kamohara *et al*., 1997). Nevertheless, leptin level is an indicator of better energy balance and control, it exerts an important role in regulation of glucose homeostasis, the mechanism of this effect may be at different level including glucose absorption, and as well secretion of insulin and down regulate counter regulatory hormones such as glucagon (Gabriela *et al*., 2015). So, the clove supplemented group may have an enhanced sensitivity effect on circulatory leptin which complements the down regulatory effect of the supplement on blood glucose level. Hence, the hypoglycaemic activity of the supplement may also be attributable to the physiological adaptive leptin effect on glucose homeostasis.

5.4 Effect of Clove and Fermented Ginger on Lipid profile

The result of the lipid profile in the present study revealed a significant decrease in total cholesterol, and triglycerides in fermented ginger supplemented group, when compared to that of the control. However, high-density lipoprotein cholesterol level were significantly lower in other treated group compared to the control. This suggest a high

lipid content of the high fat diet rabbits group fed on standard feed, while supplementation of 12.5% fermented ginger treatment for six weeks decrease the lipid content. This observed positive effect may be associated with decrease risk of cardiovascular disease and result from enhancing rate of hepatic lipid metabolism or decreasing lipid absorption in the gastrointestinal tract. Result of high-density lipoprotein (HDL) cholesterol showed a significant decrease in all supplement groups. While treatment with fermented ginger for six week revealed a decrease in LDL cholesterol content when compared to that of the diabetic fed on standard animal feed. This result is similar with the work of Ashade *et al*. (2014), and Khan*et al*., (2006). Both reported a significant decrease effect of clove and ginger peel in total serum cholesterol, triglycerides, and low-density lipoprotein not on HDL-cholesterol. Abnormal lipid content is usually associated with high plasma triglyceride, low HDL cholesterol and increased LDL cholesterol particle concentrations which attribute to increased free fatty acid flux (lipogenesis) and this can lead to insulin resistance, hyperlipidaemia which can damage beta cells and attributes to insulin dysfunction as seen to occur in diabetic dyslipidaemia. Furthermore, abnormalities in lipid content is related to increased risk of cardiovascular related disease (CVDs) and high mortality rate associated with CVDs in diabetes mellitus (Mahler and Adler, 1999). In this study, intake of high fat diet for eleven weeks leads to dyslipidaemia as observed in the disease control group fed on standard feed. This could suggest present of excessive fat which is an attributing factor to insulin resistance that characterized, metabolic disturbances featuring dyslipidaemia and hyperglycaemia in the animals due to insulin dysfunction (Madkor *et al*., 2011).

The result of the effect of the fermented ginger supplement is similar to a related study aimed to investigate the potential effect of fresh ginger extracts on serum lipid profile in alloxan-induced diabetes and propylthiouracil-induced hypothyroidism in rats. The study reported a decrease in the level of total cholesterol (TC), and low density lipoprotein (LDL) in the serum of rats that were treated by ginger extracts (Al-noory *et al*., 2013). The exact mechanism of action of ginger to regulate dyslipidemia in diabetes control is not fully understood, however, it is suggested to result from the effect of 6-dehydroshogaol, 6-shogaol and 1-dehydro-6-gingerdione that act via the inhibition of oxidative stress and anti-inflammatory processes (Rahmani *et al*. 2014). While the effects of cloves observed may also be attributable to its ability to modulate lipid metabolism via it potent cholesteryl ester transfer protein (CETP) inhibitory activity (Khan *et al*. 2006).

5.5 Effect of Clove and Ginger Supplements on Serum Thyroid Stimulating Hormone, T_3, and T_4

Thyroid hormones (T_3, and T_4)were observed to have been significantly decreased in all supplement treated groups, compared to the control group, except for T_3 in group 4. Thyroid hormones affect glucose metabolism via several mechanisms. High level of thyroid hormones have been recognized to worsen hyperglycaemia due to their promotive activity on hepatic glucose production, increased glucose gut absorption and increased rate of degradation on insulin (Maxon *et al*., 1975). In the present study, the high level of thyroid hormones seen in the diabetic control group may be attributed to the co-existence of hyperglycaemia and higher insulin levels observed in the diabetic control group. Thyroid hormones are insulin antagonists, both insulin and thyroid hormones are involved in cellular metabolism. Excess or deficit of any one can result in

functional derangement of the other, which may leads to metabolic dysregulations. Excess circulatory thyroid hormones leads to reduced half-life of insulin (Dimitriadis, *et al*., 1985; O'Meara *et al*., 1993). As well, higher levels of thyroid hormones increases glucose gut absorption (Levin and Smyth, 1963; Matty and Seshadri, 1965).

Endogenous production of glucose is mediated by higher levels of thyroid hormones via: increased in the hepatocyte plasma membrane concentrations of GLUT2, which is the main glucose transporter in the liver, and consequently, contribute to the increased hepatic glucose output and resulting hyperglycaemia (Dimitriadis *et al*., 2006; Cettour-Rose *et al*., 2005). Therefore the results of the effects observed in supplements treated group may indicate the ameliorative effects of the supplements observed on the underlined pathologies (hyperglycemia, hyperlipidemia and leptinemia) in diabetes. This could be among the attributing factors that causes no significant decrease on blood glucose level in the high fat diet induced animals on normal diet group and may explain the distorted lipid profile observed in this group. This agree with the suggested that diabetic subjects with high level of thyroid hormones experience worsening of their glycaemic control (Solá *et al*., 2002; Bhattacharyya and Wiles, 1999).

5.6 Effect of Clove and Fermented Ginger on Oxidative Stress Biomarkers

Superoxide dismutase, catalase, and glutathione peroxidase activity were all increased significantly. However MDA concentration was reduced especially after the six weeks of treatment with the supplements. This observed effect may indicate antioxidant activity-like effects exerted by the supplements use in the treatment group especially the decrease MDA levels which may have resulted from suppressed lipid peroxidation cellular damage. SOD offers the first line of enzymatic defence against ROS, it convert

super oxides to hydrogen peroxides while CAT and GPx further degrade the peroxides to oxygen and water. SOD, CAT and GPx were seen to be up regulated and this may indicate a pronounce antioxidant effect of the treatment given to the group. Hyperglycaemia is associated with increased production of ROS due to alternate pathway for glucose metabolism. This occur via DAG formation and PKC activation, in which it increases DAG synthesis resulting from free fatty acids and glycerol-3-p conjugation, and this leads to excessive increase in PKC activity. This leads to decreased insulin signalling and thus, induced or exacerbate insulin resistance (Ahmad, 2015). The findings of this study on the pronounced antioxidant activity of fermented ginger supplement alone and when combined with clove, could be due the presence of 6-dehydroshogaol, 6-shogaol and 1-dehydro-6-gingerdione which are potent inhibitors of nitric oxide (NO) synthesis in activated macrophages, hence associated with potent antioxidant properties (Rahmani *et al*., 2014). The combined supplements gave a better result which may indicate a synergistic effect of the clove supplement with the ginger due to presence of phenolic substances, found in abundance in the clove. In addition both clove and ginger have certain minerals (such as, iron, calcium, potassium, and magnesium,) that act as co-factor to enhances enzymatic reactions especially antioxidant enzymes (Kurado *et al*., 2012). The result is similar to the work of El-Akabawy and El-Kholy, 2014, and the study of Nafiseh *et al*. (2015) that reported the potential of ginger in reducing diabetes-induced oxidative stress markers (Ahmadi *et al*., 2013).

5.7 Effect of Clove and Fermented Ginger on Serum Liver Enzymes (ALT, AST, and ALP)

Intracellular hepatocytes enzymes plays a vital role in numerous metabolic activities of the liver, small levels of these enzymes are acceptable in the blood, but higher levels in circulation may indicate structural damage and as well functional defects to hepatocytes. The result of this study revealed a significant decrease in serum ALT, AST, and ALP level in all the supplement treated groups compared to controls. Liver enzymes are cytoplasmic proteins of hepatocytes and cells lining the biliary ducts, they perform a variety of functions in the body such as filtration and cleansing of the blood, excretion and metabolism (Levinthal and Tavill, 2017). When liver enzymes are elevated or decreased beyond physiological range, it could be a sign of liver impairment (Brzoska *et al*., 2003). Other reasons for distorted liver enzymes may include toxins, drugs, degenerative disease conditions, and nutrient (macro and micro) deficiencies (McClatchey, 2002). Furthermore, Type 2 diabetes (T2DM) is associated with a clinical spectrum of liver abnormalities collectively known as non-alcoholic fatty liver disease (NAFLD), in which there is a high incidence of abnormal higher ALT levels in newly diagnosed patient with T2DM, which is associated with features of the metabolic syndrome especially lipid abnormalities (Shreyas *et al*., 2012).

Treatment with clove and fermented ginger supplements revealed to have a hepatoprotective effect in the high fat diet induced diabetic rabbits. This effect of clove and fermented ginger could probably be due to the presence of eugenol and phenolic compounds as well as gingerol and other related compounds (Rahmani *et al*., 2014). The findings of this study on elevated liver enzymes in the untreated experimental

group may be attributes of oxidative stress resulting from lower antioxidant enzymes and elevated MDA levels observed in the diabetic control group.

5.8 Effect of Clove and Fermented Ginger on Serum Electrolytes

Serum electrolytes have vital functions in regulation of water distribution between different bodily compartments and maintenance of pH within physiological range, other regulatory activity is along electrolytic reactions that occur within and across many biological membranes (Shah *et al*., 2011). Altered electrolytes balance is secondary to poorly control hyperglycaemia, hyperlipidaemia and associated pathologies in T2DM. Elevated levels are associated to dehydration, while decreased levels are seen in condition such as salt losing nephritis, and diabetic acidosis. Diabetes ketoacidosis is a major cause of death in poorly manage DM (Schonfed and Lowellen, 1964). The association between blood glucose and serum electrolytes is a complex one and is related to a number of factors and associated conditions (Shah *et al*., 2011; Liamis *et al*., 2014). The findings of the present study reveal an increase in the sodium level in the groups treated with clove and fermented ginger supplements as compared to the diabetic control on normal diet group, but no significant difference compared to the normal control group. This indicate normalization of the sodium level in these treatments groups, this may reflect the hypoglycaemic activity of these supplements earlier recorded, sodium ions level is restored along the process of recovery from hyperglycaemia. Hyperglycaemia leads to dysnatremia, through either a decrease in sodium concentration caused by hyperglycaemia induced water movement from intracellular to extracellular compartment or increased serum sodium ion concentration caused by glucosuria induced osmotic diuresis. Therefore the sodium level normalization could be secondary to ameliorative effect of the supplements on

hyperglycaemia in the groups. While the result seen in the diabetic control could be as a result of the existence of hyperglycaemia and hyperlipidaemia as attributes in the disease animals (Liamis *et al*., 2014).

The Potassium ion concentration was significantly decreased in all the supplement treated groups as compared with to the disease control on normal diet. This is similar with other studies in which insulin therapy for was observed to lowers serum K^+ concentration, by driving K^+ into cells (both directly and indirectly) to decrease blood glucose (Liamis, *et al*., 2014). The hypoglycaemic activity of the supplements may have resulted in this observation. Therefore the trend on the findings of this study shows an improvement in Na^+/ K^+ concentration in all diabetic rabbits treated with the experimental supplements which is beneficial in management of associated pathologies related to this electrolytes imbalance.

Furthermore, clove supplements for six weeks in this study revealed a significant increase in bicarbonate and a decrease in chloride ions, when compared with that of diabetic control group on normal diet. But with no significance difference when compared to their levels in normal rabbits on normal feed. The findings of this study indicate the restoration of bicarbonate and as well a better chloride ion levels in the clove supplemented group. This indicates an improved acid-base buffering system status that is important in prevention of disturbed metabolic induced acid-base balance disorders in T2DM. The findings of this study concerning sodium and potassium indicate a fluid shift corrective activity of the supplements, while the findings on chloride ion and bicarbonates reveal a positive activity of clove supplements in the maintenance of homeostasis concerning acid-base balance in the treated group. The activity of these supplements observed on serum electrolyte could also be probably due

to the abundance in minerals and ionic content of the used supplements, whereby these supplements continuously supplying the body with replacement of the lost electrolyte due to the underlined pathologies in the experimental animals. And as well fermentation of the ginger could have facilitates better absorption of the ions from the gastro intestinal tract likewise.

CHAPTER SIX

6.0 CONCLUSION, AND RECOMENDATION

6.1 Conclusion

In conclusion, the findings of the present study demonstrated that high-fat diet induced T2DM rabbit model is associated with hyperglycaemia, dyslipidaemia, oxidative stress, elevated liver enzymes and electrolytes disturbance, which were ameliorated by clove and fermented ginger dietary supplementation. The effect observed could be as a results of interactions between supplements biological active constituents' and endogenous antioxidant system in the experimental animal model.

6.2 Recommendations

Based on the findings of the present study on the effects of clove and fermented ginger supplementations in high fat diet induced hyperglycaemic rabbits, the following are recommended, that:

i. Clove and fermented ginger should be consider among the dietary supplement formulation with possible intention of reversing the effects of high fat cholesterol containing diet on numerous physiological parameters studied.
ii. Further, the anti-hyperglycaemic effect of clove and fermented ginger is to be assessed in a more detailed research design for a longer period that may aim on exploring further, effects of the supplements on other physiological indices that are useful in monitoring other regulatory molecules of glucose metabolism including insulin counter regulatory hormones such as glucagon, and intracellular regulators of insulin receptor sensitivity in diabetes animal model
iii. The beneficial effects of clove and fermented ginger supplement such as; hypoglycaemic, lipid lowering, and anti-oxidant activities in diabetes should be

assessed in a wider range of different percentages other than that use in this study. Based on the positive improvement effect of clove and fermented ginger seen in some assessed parameters in the present study, the beneficial effects of clove and fermented ginger supplement on insulin and insulin receptor level should be further researched for a longer period.

iv. The intake of clove and fermented ginger supplements should be with caution in hypothyroidism, due to their observed down-regulatory activities on thyroid hormones in this study.

6.3 Contributions to Knowledge

From the findings of this study, the following are the contributions to knowledge:

1. Clove 12.5%, fermented ginger 12.5% and the co-supplementation (Clove 12.5% + fermented ginger 12.5%) significantly ($P<0.05$) decreased blood glucose level to (104.6 ± 1.94; 112.2 ± 1.3;112.2± 2.31 (mg/dL), respectively), when compared to the level (125.4 ± 2.94 mg/dL) in diabetic control group fed on standard feed at fourth week of treatment in high-fat diet induced type 2 diabetic rabbits.
2. Serum insulin level was significantly ($P < 0.05$) increased in high-fat diet induced diabetic rabbits treated with Clove 12.5% to (31.2 ±3.56 ng/mL) and fermented ginger 12.5% (37.2 ± 3.44 ng/mL) supplementations when compared with the levels in diabetic control group (26.0 ± 2.51).
3. Leptin level (10.60 ± 1.60 ng/mL) significantly ($P< 0.05$) decreased in clove 12.5% treated group of high fat diet induced type 2 diabetic rabbits when compared to the diabetic control group levels (14.6 ± 1.96 ng/mL).
4. Level of total cholesterol (TC) and triglycerides (TG) in the high fat diet induced diabetic rabbits treated with fermented ginger 12.5% for six weeks (TC = 143.06 ± 23.47, and TG = 80.45 ± 5.8 (mg/dL)), supplements were significantly ($P < 0.05$) decreased when compared to the levels (TC = 180.7 ± 14.75, and TG = 111.8 ± 7.1 (mg/dL)) in diabetic animals on normal feed.
5. T_3 and T_4 were significantly ($P<0.05$) decreased in high-fat diet induced diabetic rabbits due to the six weeks supplementations of clove 12.5% (T_3 = 1.72 ± 0.06 and T_4 = 47.4 ± 1.36 (ng/dL)), fermented ginger 12.5% (T_3 = 1.6 ± 0.14 and T_4 = 49.8 ± 2.9 (ng/dL)) and the co-supplementation (Clove 12.5% + fermented ginger 12.5%) group (T_3 = 1.5 ± 0.93 and T_4 = 58.0 ± 2.02 (ng/dL)) when

compared to the levels (T_3 = 2.1 ± 0.07 and T_4 = 79.4 ± 1.5 (ng/dL)) in diabetic control rabbits.

6. Lipid peroxidation was decreased in high-fat diet induced diabetic rabbits after six weeks supplementation of fermented ginger, as well as co-supplementation with clove + fermented ginger, as evidenced, by a significant ($P < 0.05$) decrease in MDA concentration in the groups treated with fermented ginger 12.5% (MDA=1.3 ± 0.07 nmol/L), and the co-supplemented clove 12.5% + fermented ginger 12.5% group (MDA=1.1 ± 0.71 nmol/L), when compared to the level in diabetic control group (MDA=1.54 ± 0.051 nmol/L)
7. Co-supplementation of clove 12.5% + fermented ginger 12.5% for six weeks showed a boost in activity of circulatory endogenous antioxidant enzymes as evidenced by a significant ($P < 0.05$) increase in SOD (2.1 ± 0.071 IU/L), CAT (45.8 ± 0.73 IU/L), and GPx (49.4 ± 0.51 IU/L) levels when compared to the levels in diabetic control rabbits SOD (1.6 ± 0.071 IU/L), CAT (37.6 ± 0.67 IU/L), and GPx (42.8 ± 0.9 IU/L).
8. Dietary supplementations for six weeks significantly ($P < 0.05$) decreased the levels of liver enzymes in; clove (ALT=7.8 ± 1.6 and AST= 56.0.0 ± 13.08 (IU/L)), fermented ginger (ALT=13.2 ± 2.63, AST= 14.2 ± 3.40, and ALP= 2.1 ± 0.24 (IU/L)), and the combined clove + fermented ginger (AST= 33.6 ± 60.2 IU/L) diabetic supplemented groups when compared to the levels (ALT=32.0 ± 7.75, AST= 227.0 ± 60.2, and ALP= 3.7± 0.53 (IU/L)) in diabetic control.
9. Six weeks of treatment with clove 12.5% supplements to the diabetic rabbits (high-fat diet induced), normalized the derangements in serum electrolytes when compared to their concentrations in the diabetic control group, Na^+ = from 68.70 ± 9.80 to 144.97 ± 3.80; K^+ =from 10.82 ± 0.50, to 4.05 ± 0.14; Cl^- = from 89.20

± 2.80 to 83.60 ± 1.81 and HCO_3^- = from 25.40 ± 0.81 to 31.80 ±1.80. while in the fermented ginger treated group, Na^+ = from 68.70 ± 9.80 to 111.60 ± 11.50; K^+ from 10.82 ± 0.50, to 3.31 ± 0.22. And for the combined clove + fermented ginger supplement, K^+ = from 10.82 ± 0.50, to 5.10 ± 0.81, all with $P < 0.05$.

REFERENCES

Abdel Halim, A. S. (2008). Efficacy of Zingiber officinale on third stage larvae and adult fecundity of Musca domestica and Anopheles pharoensis. *Journal of Egyptial Society of Parasitology*, 38(2):385-392.

Adadevoh, B. K. (1968) "Temporary diabetes" in adult Nigerians. Transactions of the *Royal Society of Tropical medicine and Hygiene*, 62:528–530.

Adaramoye, O.A., Akintayo, O., Achem, J., and Fafunso. M.A. (2008), Lipid-lowering effects of methanolic extract of Vernonia amygdalina leaves in rats fed on high cholesterol diet, *Vascular Health Risk Management*, 4(1): 235–241.

Adler, A. I., Stratton, I. M. and Neil, H. A. (2000). Association of Systolic Blood Pressure with Macrovascular and Microvascular Complications of type 2 Diabetes (UKPDS 36): prospective observational study. *British Medical Journal*321 (7258): 412–9.

Adrogué, H. J. and Madias, N. E. (1981). Changes in plasma potassium concentration during acute acid-base disturbances. *American Journal of Medicine*, 71: 456–467.

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

Ahmad, N. K., Rahmat, A. K., Mushtaq, A. and Nadia M. (2015), Role of Antioxidant in oxidative stress and Diabetes Mellitus,*Journal of Pharmacognosy and Phytochemistry*, 3(6): 217-220.

Ahmadi, S. A., Boroumand, M. A., Gohari-Moghaddam, K., Tajik, P. and Dibaj, S.M. (2008), The impact of low serum triglyceride on LDL-cholesterol estimation, *Archive of Iranian Medicine*. 11(3): 318-21.

Al-Amin, Z. M., Thomson, M., Al-Qattan, K. K., Peltonen-Shalaby, R. and Ali, M. (2006). Anti- diabetic and hypolipidemic properties of ginger (*Zingiber officinale*) in streptozotocin-induced diabetic rats. *British Journal of Nutrition*; 96: 660–666.

Al-Azhary, D. B. (2011). Ginger enhances antioxidant activity and attenuates atherogenesis in diabetic cholesterol-fed rats. *Australian Journal of Basic and Applied Sciences*; 5: 2150–2158.

Alfonso-Prieto, M., Biarnés, X., Vidossich, P. and Rovira, C. (2009). The Molecular Mechanism of the Catalase Reaction. *Journal of American Chemical Society*, 131 (33): 11751–11761

Al-Noory, A. S., Amreen, A. N., Hymoor, S., (2013). Antihyperlipidemic effects of ginger extracts in alloxan-induced diabetes and propylthiouracil-induced hypothyroidism in (rats). *Pharmacological Research*, 5: 157-61

American Diabetes Association (2014). Diagnosis and Classification of Diabetes Mellitus, *Diabetes Care* 37; 1.

American Diabetes Association (2016). Standards of medical care in diabetes—2016. *Diabetes Care*, 39(1), 1-106.

American Diabetes Association, (2009). Standards of Medical Care in Diabetes, *Diabetes Care*. 32:13-61.

Amit Kumar Dixit, Ranjit Dey, Aela Suresh, Siddhartha Chaudhuri, Ashok Kumar Panda, Achintya Mitra and Jayram Hazra (2014). The prevalence of dyslipidemia in patients with diabetes mellitus of ayurveda Hospital, *Journal of Diabetes and Metabolic Disorders*, 3:58

Amita, Y., Megha, A. K., Vandana, S. and Anil Y. (2013), Role of leptin and adiponectin in insulin resistance. *Clinical Chemica Acta*, 417; 80-84.

Amy, C. (2017), Diabetes Self Management; the power of potassium, www.diabetesselfmanagement.com, retrieved on 29/04/2017.

Antonios, H. T. and Pratap, S. A. (1987), Serum Potassium Concentration in Hyperglycemia of Diabetes Mellitus With Long-term Dialysis; *West Journal of Medicine*, 146(5): 571–575.

Arablou, T., Aryaeian, N., Valizadeh, M., Sharifi, F., Hosseini, A. and Djalali, M. (2014), The effect of ginger consumption on glycemic status, lipid profile and some inflammatory markers in patients with type 2 diabetes mellitus. *International Journal of Food Science and Nutrition*, 65(4): 515-20.

Ashade, O., Adelusi O. E. and Ligali, N. A. (2013). Histopathological effects of untreated ginger peel (zingiber officinale) fish meal on the intestinal tissue profiling of African Cat fish (Clarias grariepinus), *International journal of fisheries and aquatic studies*; 2(2): 95-98.

Ayala, A., Muñoz, M. F., and Argüelles, S. (2014). Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal, *Oxidative Medicine and Cellular Longevity;* 2014 (2014), 360438, 31.

Bakari, A. G., Onyemelukwe, G. C., Sani, B. G., Hassan, S. S. and Aliyu, T. M. (1999), Prevalence of diabetes in suburban Northern Nigeria: Results of a public screening survey. *Diabetes International*, 9:59-60.

Baker, R. D., Baker, S. S., LaRosa, K., Whitney, C. and Newburger, P. E. (1993). Selenium regulation of glutathione peroxidase in human hepatoma cell line Hep3B. *Archieve of Biochemistry Biophysics*, 304(1): 53-57.

Bansal, S., Chawla, D., Siddarth, M., Banerjee, B. D., Madhu, S. V., and Tripathi, A. K., (2013). A study on serum advanced glycation end products and its association with oxidative stress and paraoxonase activity in type 2 diabetic patients with vascular complications, *Clinical Biochemistry,* 46(1-2): 109–114.

Bansal, V., Kalita, J. and Misra, U. K (2006), Diabetic neuropathy; *Postgraduate Medical Journal*, 82(964): 95-100.

Barbosa-Da-Silva, S., Sarmento, I. B., Bargut, T. C. L., Souza-Mello, V., Aguila, M. B. and Mandarim-De-Lacerda, C. A. (2014). Animal models of nutritional

induction of type 2 diabetes mellitus. *International Journal of Morphology*, 32(1): 279-293.

Bassey, O. A., Lowry, O. H. and Brock, M. J. (1946). A method for the rapid determination of alkaline phosphatase with five cubic millimetres of serum. *Journal of Biological Chemistry*, 164: 321-325.

Baynest, W. H. (2015). Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus. *Baynes Journal of Diabetes and Metabolism*, 6:541.

Beach, E. F. and Turner, J. J. (1958).An Enzymatic method for glucose determination in body fluids. Clinical Chemistry, 46, 4:468.

Beer, S. F., Parr, J. H., Temple, R. C. and Hales, C. N. (1989). "The effect of thyroid disease on proinsulin and C-peptide levels," *Clinical Endocrinology*, 30(4): 379–383.

Bhattacharyya, A. and Wiles, P. G. (1999). "Diabetic ketoacidosis precipitated by thyrotoxicosis," *Postgraduate Medical Journal*, 75(883): 291–292.

Boussageon, R., Bejan-Angoulvant, T. and Saadatian-Elahi, M., (2011). "Effect of intensive glucose lowering treatment on all cause mortality, cardiovascular death, and microvascular events in type 2 diabetes: meta-analysis of randomised controlled trials". *British Medical Journal*343: 4169.

Brajkovic, S., Ferdaoussi, M., Pawlowski, V., Ezanno, H., Plaisance, V., Zmuda, E., Hai, T., Annicotte, J.,Waeber, G. and Abderrahmani, A. (2016). Islet Brain 1 Protects Insulin Producing Cells against Lipotoxicity; *Journal of Diabetes Research*; 15(85), 62-69.

Brian, L. M. (2006). Diabetes Pathophysiology: Armenian Medical Network, Retrieved from, www.health.am. Inc. 2015.

Brzoska, M. M., Moniuszko-Jakoniuk, J., Piłat-Marcinkiewicz, B. and Sawicki. B. (2003). Liver and kidney function and histology in rats exposed to cadmium and ethanol, *Alcohol and Alcoholism*, 38(1): 2–10

Bukonla, A., Benson, O. K., Akinsola, A. R., Aribigbola, C., Adesola, A. and Seyi, A. (2012). Effect of Type 1 Diabetes On Serum Electrolytes (Sodium and Potassium) Levels and Testosterone Hormone in Human Male Subjects. *Journal of Biochemistry*, 3: 9.

Buse, M. G. (2006). Hexosamines, insulin resistance and the complications of diabetes: current status. Am J Physiol Endocrinol Metab. 2006 Jan; 290(1): E1–E8. doi: 10.1152/ajpendo.00329.2005.

Cambier, C., Detry, B., Beerens, D., Florquin, S., Ansay, M., Frans, A., Clerbaux, T., Gustin, P. (1998). Effects of hyperchloremia on blood oxygen binding in healthy calves. *Journal of Applied Physiology*. 85 (4): 1267–1272.

Casqueiro, J., Casqueiro, J. and Alves. C. (2012). Infections in patients with diabetes mellitus: A review of pathogenesis, *Indian J Endocrinology and Metabolism*, 16(1): 27–36.

Cederberg, J., Basu, S., Eriksson, U. J. (2001). Increased rate of lipid peroxidation and protein carbonylation in experimental diabetic pregnancy. *Diabetologia*, 44(6):766-74.

Center for Disease Control, (2011). Vital signs: prevalence, treatment, and control of high levels of low-density lipoprotein cholesterol. United States, 1999–2002 and 2005–2008. *MMWR*, 60(4):109–114.

Ceriello. A., Testa, R. and Genovese, S. (2016).Clinical implications of oxidative stress and potential role of natural antioxidants in diabetic vascular complications, *Nutrition, Metabolism and Cardiovascular Diseases*, 26 (4); 285-292.

Cettour-Rose, P., Theander-Carrillo, C., Asensio, C., Klein, M., Visser, T. J., Burger, A. G., Meier, C. A. and Rohner-Jeanrenaud, F. (2005). "Hypothyroidism in rats decreases peripheral glucose utilisation, a defect partially corrected by central leptin infusion," *Diabetologia*, 48(4): 624–633,

Chan J., Heist, K., Depaoli, A. M., Veldhuis, J. D. and Mantzoros, C. S. (2003), The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short term starvation in healthy men, *The journal of clinical investigation*, 111(9): 1409-1421.

Chatterjee, R., Yeh, H., Edelman, D. and Brancati, F. (2011). Potassium and risk of Type 2 diabetes, Expert Review Endocrinology and Metabolism 6(5): 665–672.

Chelikani, P., Fita, I. and Loewen, P.C. (2004). Diversity of structures and properties among catalases. *Cellular and Molecular Life Sciences*. 61 (2): 192–208.

Chiasson, J. L., Aris-Jilwan, N., Bélanger, R., Bertrand, S., Beauregard, H., Ekoé, J. M., Fournier, H. and Havrankova, J. (2003). Diagnosis and treatment of diabetic ketoacidosis and the hyperglycemic hyperosmolar state. *Canadian Medical Association Journal*, 168: 859–866.

Ciarambino, T., Castellino, P., Malatino, L., Di Somma, S., Biolo, G., Paolisso, G., and Adinolfi, L.E. (2016), Prolonged hypernatremia triggered by hyperglycemic hyperosmolar state. Italian Journal Of Medicine,10(2): 26.

Cojocaru, I. M., Cojocaru, M., Muşuroi, C., Botezat, M., Lazăr, L. and Drută, A. (2004). Lipid peroxidation and catalase in diabetes mellitus with and without ischemic stroke. *Romanian Journal of Internal Medicine*, 42(2): 423-9.

Colin, W. (2015). Ketone body metabolism [internet]. 2015 Nov 18; *Diapedia* 51040851169 rev. no. 29. Retrieved from: https://doi.org/10.14496/dia.51040851169.29

Coppari, R. and Bjoraek, C. (2012), The Potential of Leptin for Treating Diabetes and Its Mechanism of Action, *Nature Review on Drug Discovery*. 11(9): 692–708.

Cortés-Rojas, D. F., Fernandes de Souza, C. A. and Oliveira, W. P (2014). Clove (*aromaticum*): a precious spice, *Asian Pacific Journal of Tropical Biomedicine*; 4(2): 90–96.

Couto, N., Malys, N., Gaskell, S. and Barber, J. (2013). "Partition and Turnover of Glutathione Reductase from Saccharomyces cerevisiae: a Proteomic Approach". *Journal of Proteome Research*. 12 (6): 2885–2894.

Dahiru, T., Jibo, A, Hassan, A. A., and Mande, A. T. (2008). Prevalence of Diabetes in a Semi-Urban Community in Northern Nigeria, *Nigerian Journal of Medicine*, 17(4), 414-416.

David, G. and Gardner, D (2011). Greenspan's basic and clinical endocrinology (9th ed.). New York: McGraw-Hill Medical. 17: P333.

David, S. S., Michael D., M., David P. B., Greg, L. H., Hitomi, C., Berglund, J. A Elizabeth D. G., Cary, S. C. and Tainer, J. A.(2009), Superoxide Dismutase Structures, Stability, Mechanism and Insights into the Human Disease Amyotrophic Lateral Sclerosis from Eukaryotic Thermophile Alvinella pompejana, *Journal of Molecular Biology*, 385(5): 1534–1555.

Davis, F.B. and Davis, P. J. (1981). Water metabolism in diabetes mellitus. American *Journal of Medicine*; 70: 210–214.

Davis, M. J. and Brown, S. A. (2006). Carbohydrates, Hormones, And Endurance Performance, *Sports Science Exchange*, 80(4) -1

Denise, G., Lucas, S. M., Juliana, V., Clóvis, P., Gabriela, S., Solange, C. G., Valdeci, J. P., João, B. T. and Rocha, M. F. (2009). Importance of the lipid peroxidation biomarkers and methodological aspects for malondialdehyde quantification. *Quím. Nova*, 32: 1, Retrieved from; http://dx.doi.org/10.1590/S0100-40422009000100032.

Dimitriadis, G., Baker, B. and Marsh, H. (1985), "Effect of thyroid hormone excess on action, secretion, and metabolism of insulin in humans," *The American journal of physiology*, 248(5): E593–E601,

Dimitriadis, G., Mitrou, P., and Lambadiari, V., (2006), "Insulin action in adipose tissue and muscle in hypothyroidism," *Journal of Clinical Endocrinology and Metabolism*, 91(12): 4930–4937,

Dimitriadis, G., Parry-Billings, M. and Bevan, S. (1997), "The effects of insulin on transport and metabolism of glucose in skeletal muscle from hyperthyroid and hypothyroid rats," *European Journal of Clinical Investigation*, 27(6): 475–483.

Dixit, K. A., Dey, R., Suresh, A., Chaudhuri, S., Panda, K. A., Mitra, A. and Hazra, J. (2014). The prevalence of dyslipidemia in patients with diabetes mellitus of ayurveda Hospital. *Journal of Diabetes and Metabolic Disorder*,13: 58.

Dorenburg, A. and Page, K. (2003). *The New American Chef: Cooking with the Best Flavors and Techniques from Around the World*, John Wiley and Sons Inc.

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Review*, 82(1): 47-95.

Duncan, R.C., Knapp. R. G., and Miller, M. C. (1977), Test of hypothesis in Population Means. In: *Introductory Biostatistics for the Health Sciences*, John Wiley and SonsInc. NY: 71-97

Ebisike, C. (2011). Hyperkalemia and diabetes, Livestrong. Com.ww.livestrong.com/article/477274-hypokalemia-diabetes/

El-Akabawy, G. and El-Kholy, W. (2014). Neuroprotective effect of ginger in the brain of streptozotocin-induced diabetic rats. *Annals of Anatomy*. 196(2-3):119-128.

Elshater, A-E. A., Muhammad, M. A., Salman, M. and Moussa, M. A. (2009). Effect of ginger extract consumption on levels of blood glucose, lipid profile and kidney functions in Alloxan induced-diabetic rats. *Egypt Academic Journal of Biology Science*; 2: 153–162.

Erdogan, M. (2011). Metabolic syndrome prevelance in subclinic and overt hypothyroid patients and the relation among metabolic syndrome parameters, *Journal of Endocrinological Investigation*.

Eriksson, J. G., Mannelin, M., Rastas, M., Salminen, V., Eriksson, J., and Tuomilehto, J. (2003). The Finnish Diabetes Prevention Study. Annals of Nutrition and Metabolism, 46:1465–1473

Fasanmed, A. O., and Dagogo-jack, S. (2015). Diabetes Care in Nigeria, *Annals of Global Health*, 81(6), 821-829.

Feldman, J. M., (1988). In Diabetes Mellitus, 9th ed. Indianapolis, Eli Lilly & Co., 28-42.

Forlani, G., Di-Bonito, P., Mannucci, E., Capaldo, B., Genovese, S., Orrasch, M., Scaldaferri, L., Di Bartolo, P., Melandri, P., Dei Cas, A., Zavaroni, I. and Marchesini, G. (2008), Prevalence of elevated liver enzymes in Type 2 diabetes mellitus and its association with the metabolic syndrome, *Journal of Endocrinology Investigations*; 31(2): 146-52.

Forrester, R. L., Wataji, J. J., Silverman, D. A. and Pierre, J. K. (1976). Enzymatic methods for estimation of bicarbonates, *Journal of Clinical Chemistry*. 22, 243-245.

Fossati, P. and Prencipe, L (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry*, 28(10): 2077-2080.

Freitas, M., Gomes, A., Porto, G. and Fernandes, E. (2010). "Nickel induces oxidative burst, NF-κB activation and interleukin-8 production in human neutrophils," *Journal of Biological Inorganic Chemistry*, 15(8): 1275–1283.

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

Fujita, H., Fujishima, H., Chida, S., Takahashi, K., Qi, Z., Kanetsuna, Y., Breyer, M. D., Harris, R. C.,Yamada, Y., and Takahashi, T. (2009). Reduction of Renal Superoxide Dismutase in Progressive Diabetic Nephropathy. J*ournal of American society of nephrology*, 20(6); 1303-1313.

Gabriela, F., Sonia, P., David, G., Carlos, D. and Sulay, T. (2015). Leptin, 20 years of searching for glucose homeostasis, *Life Science*, http:/ dx.doi.org/10.1016/.1fs.2015.02.008

Gaede, P., Vedel, P., Larsen, N., Jensen, G.V., Parving, H. H. and Pedersen, O. (2003). Multifactorial Intervention and Cardiovascular Disease in Patients with type 2 diabetes. *New England Journal of Medicine*. 348: 383-393.

Gale, E. A. M (2014). The living Text book of Diabetes; Epidemiology of Diabetes Mellitus [internet], Diapedia, 11040851147; 26. Available from https://doi.org/10.14496/dia.11040851147.26.

Geraldes, P. and King, G. L. (2010). Activation of Protein Kinase C Isoforms & Its Impact on Diabetic Complications, *CircRes*. 106(8): 1319–1331.

Geraldes, P., and King, G. L. (2010).Activation of Protein Kinase C Isoforms & Its Impact on Diabetic Complications**,** *Circ Res.* 106(8): 1319–1331.

Giacco, F. and Brownlee, M. (2010), Oxidative stress and diabetic complications, Circ Res. 2010 Oct 29; 107(9): 1058–1070.

Giugliano, D., Ceriello, A. and Paolisso, G. (1995). Diabetes mellitus, hypertension, and cardiovascular disease: which role for oxidative stress? *Metabolism*, 44(3): 363–368.

Goth., L. and Vitai, M. (1996). "Hypocatalasemia in hospital patients," *Clinical Chemistry*, 42(2): 341–342.

Gradinaru, D., Borsa, C., Ionescu, C. and Margina, D. (2013). "Advanced oxidative and glycoxidative protein damage markers in the elderly with type 2 diabetes," *Journal of Proteomics*, 13: 181–184.

Gray, R. S., Irvine, W. J. and Clarke, B. F. (1979). "Screening for thyroid dysfunction in diabetics," *British Medical Journal*, 2(6202): 1439.

Green ED, Maffei M, Braden VV, Proenca R, Desilva U, Zhang Y, et al 91995). The human obese (oB) gene : RNA expression pattern and mapping on the physical, cytogenetic maps of chromosomes 7, *Genome Research*, 5(1): 5-12

Guyton, A. C., and Hall, J. E. (2006), Text book of medical physiology; Thyroid metabolic hormones, eleventh edition, Elsevier. Inc. 1600 John F. Kennedy Blvd., Suite 1800 Philadelphia, Pennysvania 19103-2899, international edition. Pp 931-938.

Hall, V., Thomsen, R. W.,Henriksen, O.and Lohse, N. (2011). Diabetes in Sub Saharan Africa 1999-2011: Epidemiology and public health implications. a systematic review *BMC Public Health*, 11: 564.

Hamdy, O. (2017). Diabetic Ketoacidosis, *Medscape*, Retrieved from emedicine.medscape.com/article/48361-overview.

Hamoudi, M. N., Al Ayoubi, D. I.,Vanama, J., Yahaya, J. and Usman, F. U. (2012). Assessment of Knowledge and Awareness of Diabetic and Non-Diabetic Population Towards Diabetes Mellitus in Kaduna, Nigeria. *Journal of Advanced Scientific Research*, 3(3): 46-50.

Han, D., Hancock, C., Jung, S., and Holloszy, O. J. (2009). Is "fat-induced" muscle insulin resistance rapidly reversible?. *American Journal of Physiology-Endocrinology and Metabolism*, 297(1): 236-241

Harris, M. I., Couric, C. C., Reiber, G., Boyko, E., Stern, M. and Bennet, P. (1995). Diabetes in America, 2nd ed. Washington DC: U.S. Printing Office; *NIH Publication,* 95–1468.

Heydemann, A. (2016). An overview of murine high fat diet as a model for type 2 Diabetes Mellitus, *Journal of Diabetes Research,* 2902351, 14.

Hisalkar, P. J., Patne, A. B., Karnik, A. C., Fawade, M. M. and Mumbare, S. S. (2012). Ferric reducing ability of plasma with lipid peroxidation in type 2 diabetes, *International Journal of Pharmacy and Biological Sciences*, 2(2): 53–56.

Hsu, Y., Hung, J., Tsai, Y., Tsai, E., Huang, M., Hou, M., and Kuo, P. (2015). 6-Shogaol, an Active Constituent of Dietary Ginger, Impairs Cancer Development and Lung Metastasis by Inhibiting the Secretion of CC-Chemokine Ligand 2 (CCL2) in Tumor-Associated Dendritic Cells, *Journal of Agriculture and Food Chemistry*, 63 (6), 1730–1738.

Huang, C. and Kuo, E. (2007). Mehcanism of hypokalemia in magnesium deficiency. *Journal of the American Society of Nephrology*, (18)10: 2649-2652.

Hyun, J. H., Wonyoung, K., Dae, H. L., Youngjae, L. and Chang-Hoon, H. (2014). Effects of resveratrol on the insulin signaling pathway of obese mice. *Journal of Veterinary Science*, 15(2): 179-185.

Iglesias, P., Alvarez, F. F., Codoceo, R., and Díez, J. J., (2003). Serum concentrations of adipocytokines in patients with hyperthyroidism and hypothyroidism before and after control of thyroid function, *Clinical Endocrinology*, 59(5): 621–629.

Irene, S., Belinda, E. and Norbert S. (2012). Type 2 Diabetes Self-Management: Role of Diet Self-Efficacy, *Canadian Journal of Diabetes*. 36(2012): 337-344.

Jaggi, S. A., Singh, P. V., Bali, A., and Singh, N. (2014). Advanced Glycation End Products and Diabetic Complications. *The Korean Journal of Physiology and Pharmacology*, 18(1): 1–14.

Jílková, Z. M., Pavelka, S., Flachs, P., Hensler, M., Kůs, V., and Kopecký, J., (2010). Modulation of type I iodothyronine 5'-deiodinase activity in white adipose tissue by nutrition: possible involvement of leptin, *Physiological Research*, 59(4): 561–569.

Jimoh, A., Tanko, Y., Ahmed, A., Mohammed, A. and Ayo, J. O. (2015), Protective Effect of Resveratrol Co-Administration with Cholesterol Diet on Erythrocyte Osmotic Fragility and Malondialdehyde Concentration in Rabbits, *British Journal of Pharmaceutical Research,* 6(1): 14-21.

John, D., Griffin, M. S. and Alice, H., Lichtenstein, D. Sc. (2013). Dietary Cholesterol and Plasma Lipoprotein Profiles: Randomized-Controlled Trials, *Current Nutritional Report*, 2(4), 274–282.

Kamatou, G. P., Vermaak, I. and Viljoen, A. M. (2012). Eugenol--from the remote Maluku Islands to the international market place: a review of a remarkable and versatile molecule. *Molecules*, 17(6), 6953–6981.

Kamohara , S., Burcelin,R., Halaas, J.I., Friedman, J.M., and Charron M.J., (1997), Acute stimulation of glucose metabolism in mice by leptin treatment, *Nature* 389 (6649): 374-377.

Kaneko, J. J. (2008), Carbohydrate metabolism and its diseases, Clinical Biochemistry of Domestic Animals six edition, *Academic Press*, 8: 45-80. Retrieved from Sciencedirect.com/topics/agricultural-and-biological-science/glycosuria.

Kaneto H., Fujii J., Myint T., Miyazawa N., Islam K.N., Kawasaki Y., Suzuki K., Makamura M., Tatsumi H., and Yamasaki Y. (1996). Reducing sugars trigger oxidative modification and apoptosis in pancreatic β-cells by provoking oxidative stress through the glycation reaction. *Biochemistry Journal*. 320: 855–863.

Kaneto, H., Xu, G., Song, K., Suzuma, K., Bonner-Weir, S., Sharma, A. and Weir, G. C. (2001).Activation of the Hexosamine Pathway Leads to Deterioration of Pancreatic β-Cell Function through the Induction of Oxidative Stress,*The Journal of Biological Chemistry* 276, 31099-31104.

Khan, A., Qadir, S. S., Khattak, K. N. D. and Anderson, R. A. (2006), Clove improve glucose, cholesterol and triglycerides of people with type 2 diabetes mellitus, *Federation of American Society of experimental biology journal,* 20: A990.

Kharroubi, L., Rass, C. J., Eizirik, D. L. and Cnop, M. (2003), Expression of adiponectin receptors in pancreaticβ cells; *Biochemical Biophysics research communication*, 312; 1118-1122.

Koett, J., Howell, J., Steinberg, J. and Wolf, J., (1979). Diabetes Ketoalkalosis, *Clinical Chemistry*, 25(7): 1329-1330.

Kolawale, O. T., Kolawale, S. O., Ayankunle, A. A and Olaniran, I. O. (2012). Methanolic leaf extract of persea Americana protects rats against cholesterol-induced hyperglycemia. *British Journal of Medicine and Medical Research*, 2(2): 235-242

Kuroda, M., Mimaki, Y., Ohtomo, T., Yamada, J., Nishiyama, T., Mae, T., Kishida, H., and Kawada, T., (2012), Hypoglycemic effects of clove (aromaticum flower buds) on genetically diabetic KK-Ay mice and identification of the active ingredients. *Journal of Natural Medicine*, 66(2): 394-399.

Lai, Y., Wang, J. and Jiang, F. (2011), "The relationship between serum thyrotropin and components of metabolic syndrome," *Endocrine Journal*, 58(11): 23–30.

Larsson, J. and Apelqvist, J. (1995). Towards less amputations in diabetic patients: Incidence, causes, cost, treatment, and prevention (review). Acta Orthopaedica Scandinavica, 66(2): 181-192

Lee, S. F., Pei D., Chi, M. J. and Jeng, C. (2015). An investigation and comparison of the effectiveness of different exercise programmes in improving glucose metabolism and pancreatic β cell function of type 2 diabetes patients. *International Journal of Clinical Practice*. 69(10):1159-1170.

Leonhardt, U., Ritzel, U., Schäfer, G., Becker, W., and Ramadori, G., (1998). Serum leptin levels in hypo- and hyperthyroidism," *Journal of Endocrinology*, 157(1): 75–79.

Leshan, R, L., Bjorholm, M. M. and Myers, M.G., (2006), Leptin receptor signaling and action in the central nervous system, *Obesity*, 14 (5): 2085-2125.

Levin, R. J., and Smyth, D. H., (1963). "The effect of the thyroid gland on intestinal absorption of hexoses," *The Journal of physiology*, 169, 755–769.

Levinthal, N. G., and Tavill, S. A. (2017). Liver Disease and Diabetes Mellitus, *Clinical Diabetes*, 17(2) 1999.

Li, W. L., Zhen, H. C., Bukuru, J. And DeKimpe, N. (2004); Natural medicines used in Chinese medical system for therapy of Diabetes Mellitus, *Journal of Ethnopharmacology*, 92: 1-21

Li, Y., Tran, V. H., Duke, C. C. and Roufogalis, B. D. (2012). Gingerols of Zingiber officinale enhance glucose uptake by increasing cell surface GLUT4 in cultured L6 myotubes. *Journal of Planta. Medica.*; 78: 1549–1

Liamis, G., Kalogirou, M., Saugos, V. and Elisaf, M. (2006). Therapeutic approach in patients with dysnatraemias. *Nephrology Dial Transplant*, 21: 1564–1569.

Liamis, G., Liberopoulos, E., Barkas, F. and Elisaf, M. (2014), Diabetes mellitus and electrolyte disorders, *World Journal Clinical Cases*. 2(10): 488–496.

Liamis, G., Liberopoulos, E., Barkas, F. and Elisaf, M. (2013). Spurious electrolyte disorders: a diagnostic challenge for clinicians. *American Journal of Nephrology*, 38: 50–57.

Liamis, G., Milionis, H. and Elisaf, M. (2008). A review of drug-induced hyponatremia. *American Journal of Kidney Diseases*, 52: 144–153.

Liamis, G., Milionis, H. and Elisaf, M. (2008). Blood pressure drug therapy and electrolyte disturbances. *International Journal of Clinical Practice*, 62: 1572–1580.

Liamis, G., Milionis, H. J. and Elisaf, M. (2009). A review of drug-induced hypocalcemia. *Journal of Bone Mineral Metabolism*, 27: 635–642.

Liamis, G., Tsimihodimos V, and Elisaf, M. (2015). Hyponatremia in Diabetes Mellitus: Clues to Diagnosis and Treatment. Journal of Diabetes and Metabolism 6 (6): 560.

Libby, S. W. (2011). Diabetes Diet, Retrieved from; *MayoClinic.com.* 2016.

Likidlilid, A., Patchanans, N., Poldee, S. and Peerapatdit, T. (2007). Glutathione and glutathione peroxidase in type 1 diabetic patients. *Journal of Medical Association of Thailand*, 90(9): 1759-67.

Lushchak, V. I. (2012).Glutathione Homeostasis and Functions: Potential Targets for Medical Interventions; *Journal of Amino Acids*, 2012 (2012), 736837, 26

Madkor, H. R., Mansour, S. W. and Ramadan, G. (2011). Modulatory effects of garlic, ginger, turmeric and their mixture on hyperglycemia, dyslipidemia and oxidative stress in streptozotocin-nicotinamide diabetic rats. *British Journal of Nutrition*,105: 1210–1217.

Mahler, J. R. and Adler, L. M. (1999), Type 2 Diabetes Mellitus: Update on Diagnosis, Pathophysiology, and Treatment, *The Journal of Clinical Endocrinology and Metabolism*, 84(4), 1165–1171

Maratou, E., Hadjidakis, D. J. and Kollias, A. (2009) Studies of insulin resistance in patients with clinical and subclinical hypothyroidism, *European Journal of Endocrinology*, 160(5): 785–790.

Martin, G. M., Rudolph, L. L., Randy, J. S. and Michael, W. S.(2010). Obesity and Leptin Resistance: Distinguishing Cause from Effect Trends *Endocrinology and Metabolism*. 21(11), 643–651.

Maruna, R. F. L. (1958). Colorimetric determination of sodium. *Clinical Chemistry Acta*, 2:581.

Mathers C. D. and Loncar, D. (2006). Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Medicine*, 3(11):442.

Mathur, S., Mehta, K. D., Kapoor, Y. S. (2016). Liver Functions in Type-2 Diabetes Mellitus Patients; *International Journal of Scientific Study*. 3(10), 43-47.

Matty, A. J. and Seshadri, B. (1965). Effect of thyroxine on the isolated rat intestine, *Gut*, (6) 200–202,

Maxon, H. R., Kreines, K. W., Goldsmith, R. E. and Knowles, H. C. (1975). Long-term observations of glucose tolerance in thyrotoxic patients, *Archives of Internal Medicine*, 135(11): 1477–1480,

Mayo Medical Laboratories test catalog. ALP. http://www.mayomedicallaboratories.com/testcatalog/Clinical+and+Interpretive/8340. Accessed June 30, 2015.

Mazaki-Tovi, M., Feuermann, Y. and Segev, G. (2010). Increased serum leptin and insulin concentrations in canine hypothyroidism, *Veterinary Journal*, 183(1): 109–114.

McClatchey, K. D. (2002). Clinical laboratory medicine. (McClatchey: Clinical laboratory medicine) 2nd Edition, Lippincott Williams and Wilkins. P 288.

Medagama, B. A. and Bandara, R. (2014). The use of Complementary and Alternative Medicines (CAMs) in the treatment of diabetes mellitus: is continued use safe and effective? *Nutrition Journal*. 13(1), 102.

Megan, W. RDN. (2016). Ginger: Health Benefits. Medical News Today, Facts Research, retrieved from http://www.loveyourselftohealth.info/nutrition/ginger-can-boost-health/.

Mengel, M. B. and Schwiebert, L. P. (2005). Family medicine: ambulatory care and prevention. *McGraw-Hill Professional*. 268.

Milionis, J. H., Liamis, L. G. and Elisaf, M. S (2001). Plasma sodium changes in the hyperglycaemic state: Clinical aspects of pathophysiology and management, *International Journal of Diabetes and Metabolism*, 9(3): 60-66.

Mir, F. (2013). Serum sodium, Medscape. emedicine.medscape.com/article/2099065_overview.

Mirella, H. M., Zantout, S. and Sami, T. A. (2011). Thyroid Disorders and Diabetes Mellitus; *Journal of Thyroid Research*, 2011: 7.

Mohammed, A., Adelaiye1, A. B., Bakari, A. G., and Mabrouk, M. A., (2009). Anti-diabetic and some haematological effects of ethylacetate and n-butanol fractions of Ganoderma lucidum aqueous extract in alloxan - induced diabetic wistar rats, *International Journal of Medicine and Medical Sciences* , 1(12): 530-535.

Mokuno, T., Uchimura, K. and Hayashi R. (1999), Glucose transporter 2 concentrations in hyper- and hypothyroid rat livers," *Journal of Endocrinology*, 160(2): 285–289.

Morris, D., Khurasany, M., Nguyen, T., Kim, J., Guilford, F., and Mehta, R., (2013). "Glutathione and infection," *Biochimica et Biophysica Acta*, 1830(5): 3329–3349.

Moussa, S. A. (2008). Oxidative stress in diabetes mellitus. *Romanian Journal Biophysics*, 18(3): 225-236.

Mozaffari-Khosravi, H., Talaei, B., Jalali, B. A., Najarzadeh, A. and Mozayan, M. R. (2014). The effect of ginger powder supplementation on insulin resistance and

glycemic indices in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. *Complementary Therapies in Medicine*, 22(1):9-16.

Multinational, multicenter study of the Mediterranean Group for the Study of Diabetes (MGSD), (2003). Dietary Fat Intake as Risk Factor for the Development of Diabetes: *Diabetes Care,* (26)*:* 302-307.

Nabili. N. S. (2017). Liver blood tests. Medscape. www.emedicinehealth.com.liver_blood_tests/article_em.htm.

Nafiseh, K., Farzad, S., Asadolla, R., Tayebeh, R., Payam, H. and Mohsen m T. (2015). the effects of ginger on fasting blood sugar, hemoglobin A1c, Apolipoprotein B, Apolipoprotein A-1 and malondialdehyde in type 2 diabetes patients. *Iranian Journal of Pharmaceutical Research,* 14(1): 131-140.

Nathan, D. M., Cleary, P. A. and Backlund, J. Y. (2005). "Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes". *The New England Journal of Medicine* 353(25): 2643–53.

National Cholesterol Education Program, National Heart, Lung, and Blood Institute, (2001). *Third Report of the National Cholesterol Education Program*. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. 01-3670.

National Institute of Health (NIH), (1996). Guide for the Care and Use of Laboratory Animals. *NIH publication,* 80-123.

Norris, K. A., Atkinson, A. R., and Smith, W. G. (1975). *Journal of Clinical Chemistry*, 21: 1093

Nyblom, H., Berggren, U., Balldin, J., and Olsson, R. (2004). "High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking" Alcohol, *Alcohol*. 39(4): 336–339.

Nyblom, H., Björnsson, E., Simrén, M., Aldenborg, F., Almer, S., and Olsson, R. (2006). The AST/ALT ratio as an indicator of cirrhosis in patients with PBC. *Liver International*. 26(7): 840–845.

Nyenwe, E. A., Odia, O. J., Ihekwaba, A. E., Ojule, A., and Babatunde, S. (2003) Type 2 diabetes in adult Nigerians: a study of its prevalence and risk factors in Port Harcourt, Nigeria. *Diabetes Research and Clinical Practice*, 62:177-185.

O'Meara, N. M., Blackman, J. D., Sturis, J. and Polonsky, K. S. (1993). Alterations in the kinetics of C-peptide and insulin secretion in hyperthyroidism, *Journal of Clinical Endocrinology and Metabolism*, 76(1): 79–84.

Ozder, A (2014). Lipid profile abnormalities seen in T2DM patients in primary healthcare in Turkey: a cross-sectional study, *Lipids in Health and Disease*,13: 183.

Pandey, A., Tripathi, P., Pandey, R., Srivatava, R. and Goswami, S. (2011). Alternative therapies useful in the management of diabetes: A systematic review. Journal of *Pharmacy and Bioallied Science*, 3(4): 504–512.

Parhofer, G. K. (2015). Interaction between Glucose and Lipid Metabolism: More than Diabetic Dyslipidemia, *Diabetes and Metabolism Journal*. 39(5): 353–362.

Park, Y., Hong, Y. N., Weyers, A., Kim, Y. S. and Linhardt, R. J. (2011). Polysaccharides and phytochemicals: a natural reservoir for the green synthesis of gold and silver nanoparticles. IET *Nanobiotechnology*, 5:69–78.

Phillips, M., Cataneo, R. N., Cheema, T., and Greenberg, J. (2004), Increased breath biomarkers of oxidative stress in diabetes mellitus, *Clinica Chimica Acta*, 344 (1-2): 189–194.

Pignone, M., Alberts, M. J, and Colwell, J. A. (2010). Aspirin for primary prevention of cardiovascular events in people with diabetes: a position statement of the American Diabetes Association, a scientific statement of the American Heart Association, and an expert consensus document of the American College of Cardiology Foundation. *Diabetes Care* 33 (6): 1395–402.

Pinkney, J. H., Goodrick, S. J. and Katz, J. (1998). Leptin and the pituitary-thyroid axis: a comparative study in lean, obese, hypothyroid and hyperthyroid subjects, *Clinical Endocrinology*, 49(5): 583–588.

Pitocco,D., Tesauro, M., Alessandro, R., Ghirlanda, G. and Cardillo, C. (2013), Oxidative Stress in Diabetes: Implications for Vascular and Other Complications, *International Journal of Molecular Science*. 14(11), 21525–21550.

Pittas, A. G. and Greenberg, A. S. (2003). Contemporary Diagnosis and Management of Diabetes. Handbooks in Health Care Co, Newtown, PA. 2003.2. Retrieved from, http://ocw.tufts.edu/Content/14/lecturenotes/265878. 2015.

Prasad, S., and Tyagi, A. K. (2015). Ginger and Its Constituents: Role in Prevention and Treatment of Gastrointestinal Cancer, *Gastroenterology Research and Practice.* 2015 (2015),11.http://dx.doi.org/10.1155/2015/142979

Qi, Q., Zhang, Q. M., Li, C. J., Dong, R. N., Li, J. J., Shi, J. Y., Yu, D. M. and Zhang, J. Y. (2017). Association of Thyroid-Stimulating Hormone Levels with Microvascular Complications in Type 2 Diabetes Patients. *Medical Science Monitor*: 4(23), 2715-2720.

Rahmani, A. H., Al-shabrmi, F. M. and Aly, S. M (2014). Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities, International Journal of Physiology, Pathophysiology and Pharmacology; 6(2): 125–136.

Reitman, S. and Frankel, S. (1957), Colorimetry method for the determination of serum glutamic oxalacetic and glutamicpyruvic transaminases. *American journal of clinical pathology*, 28: 56.

Rheney, C. C. and Kurk, K. K. (2000). Performance of three blood glucose meter. *Annals of Pharmacotherapy*, 34(3), 317-321.

Ripsin, C.M., Kang, H, and Urban, R.J. (2009), Management of blood glucose in type 2 diabetes mellitus. *American family physician*, 79 (1): 29–36.

Rochon, C., Tauveron, I., and Dejax, C. (2003). "Response of glucose disposal to hyperinsulinaemia in human hypothyroidism and hyperthyroidism," *Clinical Science*, 104(1): 7–15.

Roscoe, J. M., Halperin, M. L., Rolleston, F. S. and Goldstein, M. B. (1975). Hyperglycemia-induced hyponatremia: metabolic considerations in calculation of serum sodium depression. *Canadian Medical Association Journal*; 112(4): 452–453.

Rother K.I., (2007). "Diabetes treatment—bridging the divide". *The New England Journal of Medicine*, 356(15): 1499–1501.

Sattar, N. (2012). Biomarkers for Diabetes prediction, pathogenesis or pharmacotherapy guidance? Past, Present and future possibilities. *Diabetes Medicine.* 29, 5-13.

Schales, O., and Schales, S. S. (1941). A simple and accurate method for the determination of chloride in biological fluids. *Journal of Biological chemistry*, 140: 879-884.

Schmidt, E. and Schmidt, F. W. (1976). Clinical enzymology. *FEBS Letters,* 4(62):62-79.

Schonfed, R. G. and Lewellen C. J. (1964). A colorimetric method for the determination of serum chloride. *Journal of Clinical Chemistry*. 10, 533–539.

Schreiber, K. A., Nones, F. M. C., Reis, C. R., Chichorro, G. J. and Cunha, M. J. (2015). Diabetic neuropathic pain: Physiopathology and treatment. *World Journal of Diabetes*, 6(3): 432–444.

Scott, M. G, (1991). Dietary Therapy in Diabetes Mellitus: Is There a Single Best Diet?; *Diabetes Care ,* 14 (9): 796-801.

Selvin, E., Steffes M. W. and Zhu, H. (2010). Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. *The New England Journal of Medicine,* 362 (9): 800–11.

Seuring, T., Archangelidi, O. and Suhrcke, M. (2015). The Economic Costs of Type 2 Diabetes: A Global Systematic Review, *Pharmacoeconomics*; 33(8): 811–831.

Shah, D., Nanm, d. S., Jaishankar, G. B., Chilakala, S., and Wang, K. (2011) Pre-Term Exposure Patterns in Neonatal intensive care Unit Alter Immunological Outcome in Neonates. *Journal of Aller Ther,* 2: 106.

Shin, D. S., Ro, S. Y., Song, J. K., Kim, J. Y., Eui Jung Lee, E. J., Lee, J. Y., Ahn, O. K. and Hong, J. K. (2016). Risk of Diabetes Mellitus on Incidence of Out-of-Hospital Cardiac Arrests: A Case-Control Study. *PLoS One*, 11(4): 0154245.

Shisheva, A. and Shechter, Y. (1992). Quercetin selectively inhibits insulin receptor function in vitro and the bioresponses of insulin and insulinomimetic agents in rat adipocytes. *Biochemistry*, 31(34), 8059–8063.

Shreyas, S. Elizabeth, J. W.and Michael, G. M. (2012), Raised liver enzymes in newly diagnosed Type 2 diabetes are associated with weight and lipids, but not glycaemic control, *Indian Journal of Endocrinology and Metabolism* ; 16(6): 1012–1014.

Sivitz, W. I. and Yorek, M. A. (2010). Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities, *Antioxidants and Redox Signaling*, 12;4: 537–577.

Skyler, J. S. (1998). Insulin Therapy in type 2 Diabetes Mellitus, .In: DeFronzo RA, ed. Current therapy of diabetes mellitus. St. Louis: *Mosby,*108: 16.

Solá, E., Morillas, C., Garzón, S., Gómez-Balaguer, M.. and Hernández-Mijares, A. (2002). "Association between diabetic ketoacidosis and thyrotoxicosis," *Acta Diabetologica*, 39; 4: 235–237.

Sotirakopoulos, N., Kalogiannidou, I., Tersi, M., Armentzioiou, K., Sivridis, D., and Mavromatidis, K. (2012). Acid-base and electrolyte disorders in patients with diabetes mellitus. *Saudi Journal of Kidney Disease and Transplantation*, 23(1): 58-62.

Srinivasan, K. and Ramarao, P. (2007), Animal Model in Type2 Diabetes Research An Overview, *Indian journal of Medical Research*. 125, 451-472.

Steinberg, S. F. (2008). Structural basis of protein kinase C isoform function, *Physiology Review.*;88(4):1341-78. doi: 10.1152/physrev.00034.2007.

Stojanovic, V. and Ihle, S. (2011). Role of beta-hydroxybutyric acid in diabetic ketoacidosis: A review, *Canadian Veterinary Journal*. 52(4): 426–430.

Strojek, K., (2003). Features of macrovascular complications in type 2 diabetic patients. *Acta Diabetologica*, 40; 334–337.

Taniguchi, C. M., Emanuelli, B. and Kahn, C. R. (2006), Critical nodes in signaling pathways, insights into Insulin action. *Nat. Rev. molecular cell Biology*. 7; 85-96

Tanko, Y., Jimoh, A., Ahmed, A., Adam, A., Ejeh, L., Mohammed, A. and Ayo, J. O. (2016). Effects of Selenium Yeast on Blood Glucose and Antioxidant Biomarkers in Cholesterol Fed Diet Induced Type 2 Diabetes Mellitus in Wistar Rats. Nigerian Journal of Physiological Society. 31(2016) 147-152.

Tanko, Y., Jimoh, A., Goji, A. D. T., Mohammed, A. and Musa, K. Y (2011). Hypoglycemic effects of the methanolic extract of Aerial part of chysanthellum-indicum in rats. Journal *of Natural Product and* Plant Resources. 1(2): 1-7.

Tapsell, L. C., Hemphill, I., Cobiac, L., Patch, C. S., Sullivan, D. R., Fenech, M., Roodenrys, S., Keogh, J. B., Clifton, P. M., Williams, P. G., Fazio, V. A., Inge, K. E. (2006). Health benefits of herbs and spices: the past, the present, the future. *Medical Journal of Australia*. 185(4), 4-24.

Tazzini, N. (2016). Carbohydrates; Cori cycle: Definition, function, Biochemistry, Involved Tissues, *Biochemistry and Nutrition*. Retrieved from, www.tuscany-dietnet/2016/12/18/coricycle.

Tiezt, N. W. (1990). Clinical Guide to Laboratory Test, Second Edition W.B. Saunders Company, Philadelphia, U.S.A. 554-556.

Tiwari, B. K., Pandey, K. B., Abidi, A. B., and Rizvi1, S. I. (2013). Markers of Oxidative Stress during Diabetes Mellitus, *Journal of Biomarkers*, Article ID 378790, Retrieved from; http://dx.doi.org/10.1155/2013/378790.

Trinder, P. (1951). Colorimetric Determination of Sodium. *Analyst*, 76:596.

UKPDS (1998). A randomized trial of efficacy of early addition of metformin in sulfonylurea-treated type 2 diabetes. *Diabetes Care*. 28 (21): 87-92.

Ullah, A., Khan, A., and Khan, I. (2016), Diabetes mellitus and oxidative stress—A concise review, *Saudi Pharmaceutical Journal* 24(5): 547–553.

Upendra, R. M., Sreenivasulu M., Chengaiah B., Jaganmohan. R. K. and Madhusudhana C. (2010). Herbal Medicines for Diabetes Mellitus: *International journal of pharm tech research,* 2(3): 1883-1892;

Valcavi, R., Zini, M., Peino, R., Casanueva, F. F. and Dieguez, C. (1997). "Influence of thyroid status on serum immunoreactive leptin levels," *Journal of Clinical Endocrinology and Metabolism*, 82(5): 1632–1634.

Verges, B. (2015). Pathophysiology of diabetic dyslipidaemia: where are we? *Diabetologia*. 58(5): 886–899.

Wang, X., Tao, L., and Hai, C. X., (2012). "Redox-regulating role of insulin: the essence of insulin effect," *Molecular and Cellular Endocrinology*, 349(2): 111–127.

Wasim, M (2015). Role of Leptin in Obesity, *Journal of obesity and weight loss therapy*. 5:258.

Watson, D. (1960). A simple method for the determination of serum cholesterol. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, 5: 637-643.

Wedro, B. (2016). Electrolytes, Electrolyte article, emedicinehealth.com/electrolytes/article_em.htm

Welch-White, V., Dawkins, N., Graham, T. and Pace, R. (2013).The impact of high fat diets on physiological changes in euthyroid and thyroid altered rats. *Lipids in Health and Disease*.12:100

West, I.C. (2000). Radicals and oxidative stress in diabetes. *Diabetic Medicine Journal*, 17: 171-180.

Wild, S., Rogic, G.,Green, A.,Sicree, R., and King H., (2004); Global prevalence of diabetes; estimates for the year 2000 and projections for 2030; *Diabetes care*; 27 (5): 1047-1053.

Wolf, S., Rave, K., Heinemann, L., and Roggen, K. (2009). Renal glucose excretion and tubular reabsorption rate related to blood glucose in subjects with type 2 diabetes with a critical reappraisal of the "renal glucose threshold" model. *Hormone Metabolism Research*. 41(8):600-604.

World Health Organization (WHO) (2006). Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia. *Report of WHO/IDF Consultation*, WK810, WHO Document Production Services Geneva, Switzerland.

World Health Organization (WHO) (2015). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Geneva, (WHO/NCD/NCS/99.2). *Diabetes Fact Sheet* N°312.

World Health Organization (WHO), (2016). Global Report on Diabetes. Retrieved from www.who.int/diabetes/publications/grd-2016/en/

Yen, M. P. (2001). Physiological and Molecular Basis of Thyroid Hormone action. *Physiological Review*, 81(3), 1097-1142.

Zheng, G. Q., Kenny, P. M. and Lam, K. T. (1992). Sesquiterpenes from clove (Eugenia caryophyllata) as potential anticarcinogenic agents. *Journal of Natural Products*, 55: 999–1003.

APPENDICES

APPENDIX I

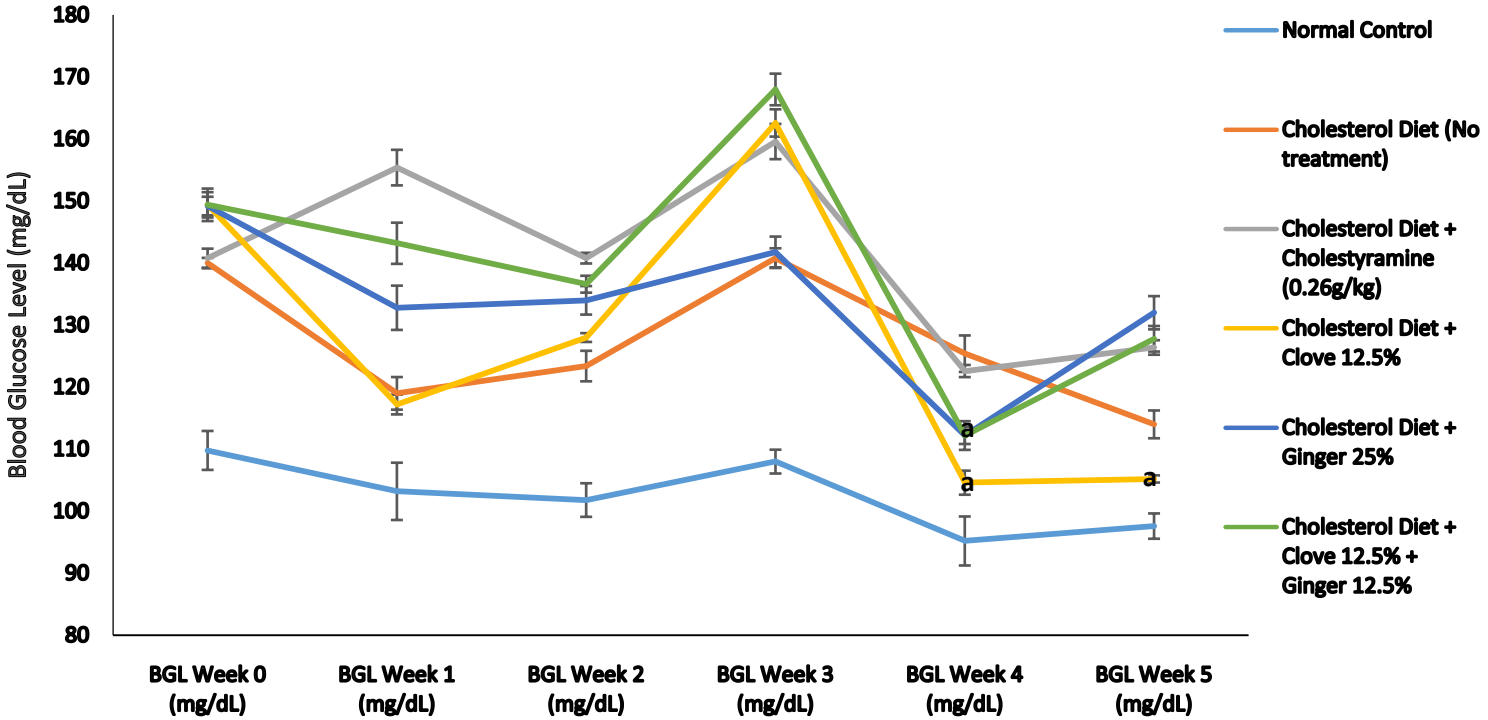


Figure 1: Blood glucose levels (BGL) in control groups, and high fat diet induced-hyperglycemic rabbit's groups treated with clove (12.5%), fermented ginger (12.5%), and co-administered clove (12.5%) and fermented ginger (12.5%) supplemented diet.

Significant at P< 0.05 value: n=5; a=significant difference when compared to cholesterol diet fed group no treatment

APPENDIX 2

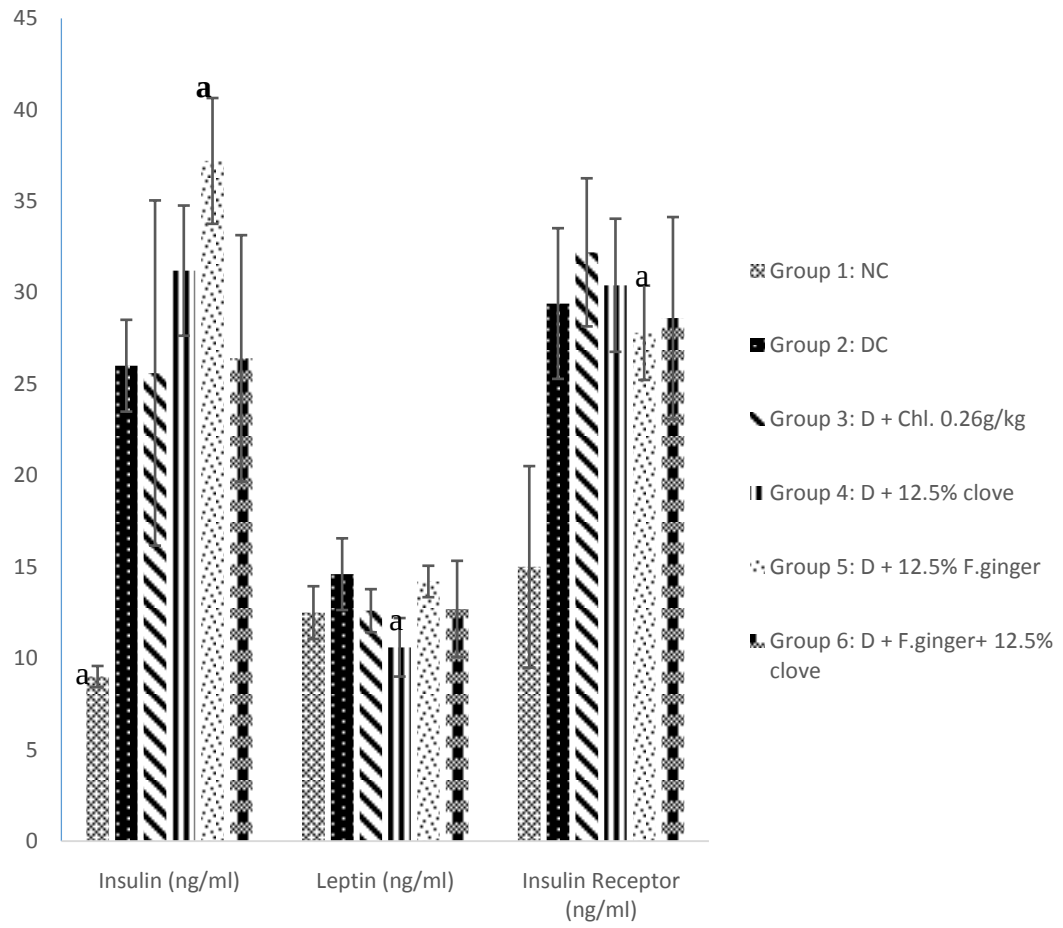


Figure 2: shows serum levels of insulin, leptin and insulin receptor of experimental animal groups;
(NC = Normal control; DC = Diabetic control; D+Chl = Diabetic administered cholestran; D + CLV = Diabetic fed on clove supplement; D + Fg = Diabetic fed on fermented ginger supplement).

Note: Significant at P< 0.05 value: n=5; a=significant difference when compared DC group.

APPENDIX 3

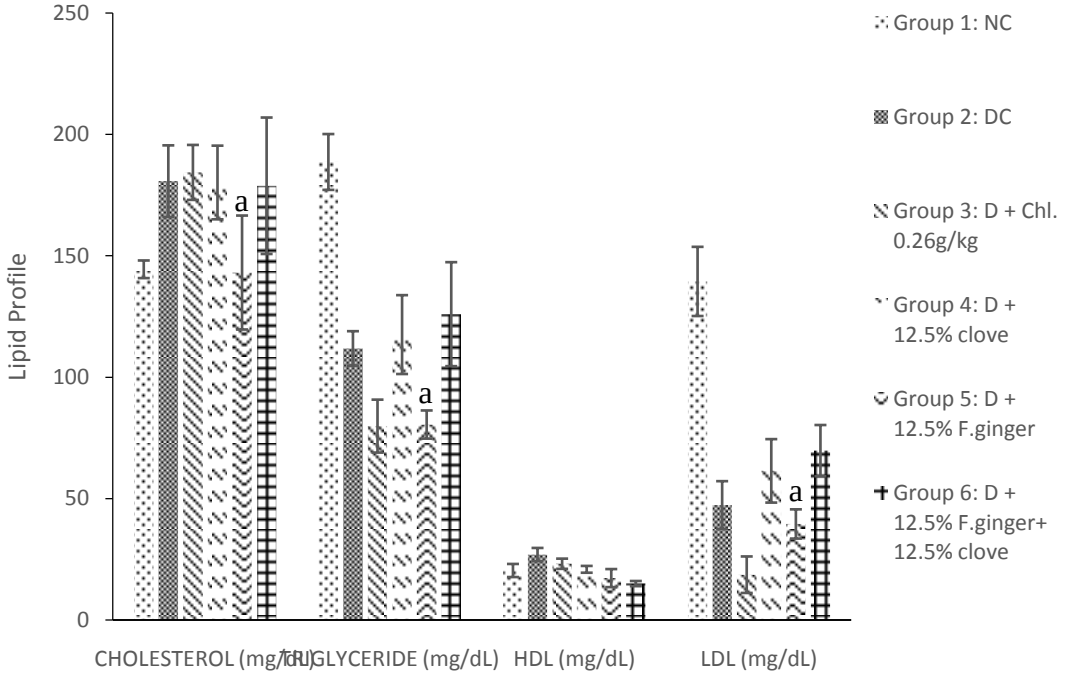


Figure 3;Shows Lipid profile of experimental animal groups;
(NC = Normal control; DC = Diabetic control; D+Chl = Diabetic administered cholestran; D + CLV = Diabetic fed on clove supplement; D + Fg = Diabetic fed on fermented ginger supplement)

Note: Significant at P< 0.05 value: n=5; a=significant difference compared to DC group.

APPENDIX 4

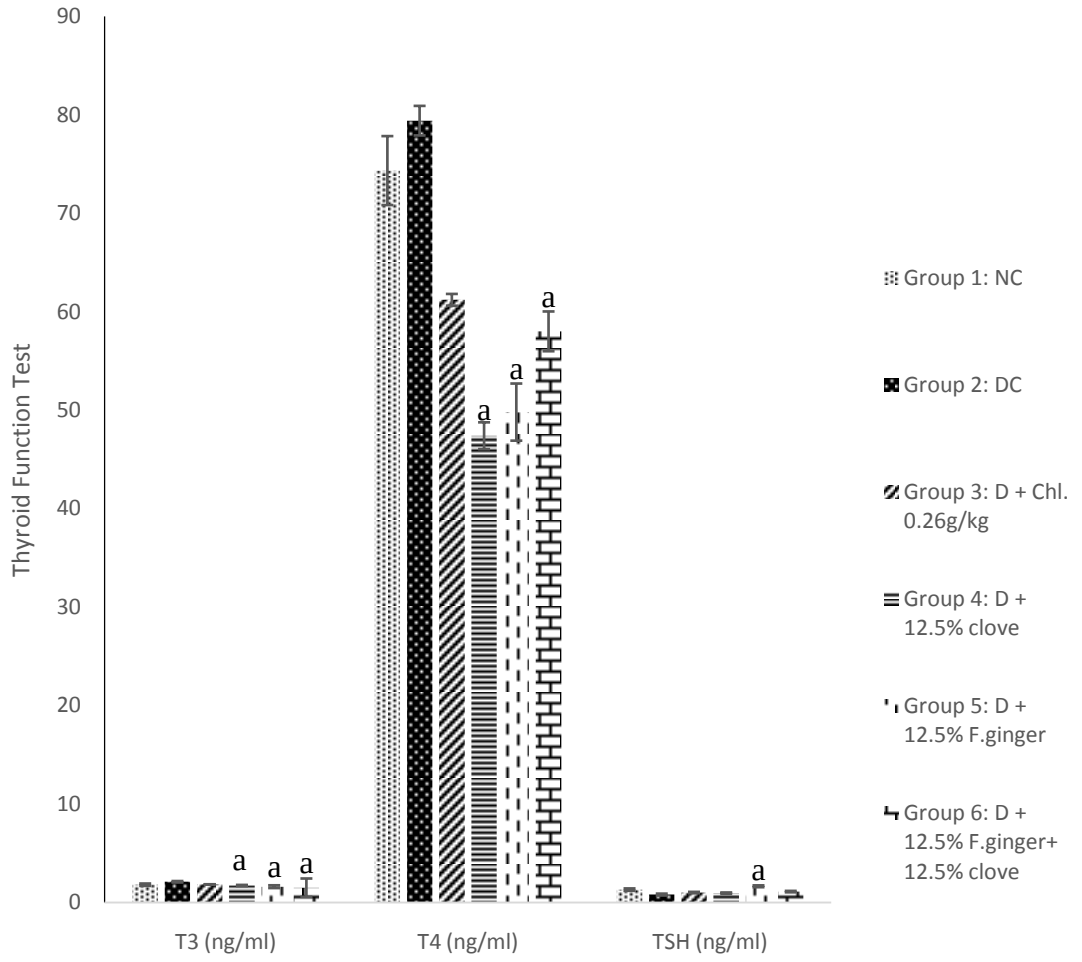


Figure 4: shows thyroids hormones (T3= Triiodothronine, T4= thyroxine, and TSH= Thyroid stimulating hormone) levels of experimental animal groups;

(NC = Normal control; DC = Diabetic control; D+Chl = Diabetic administered cholestran; D + CLV = Diabetic fed on clove supplement; D + Fg = Diabetic fed on fermented ginger supplement)

Note: Significant at $P< 0.05$ value: n=5; a=significant difference when compared to DC group.

APPENDIX 5

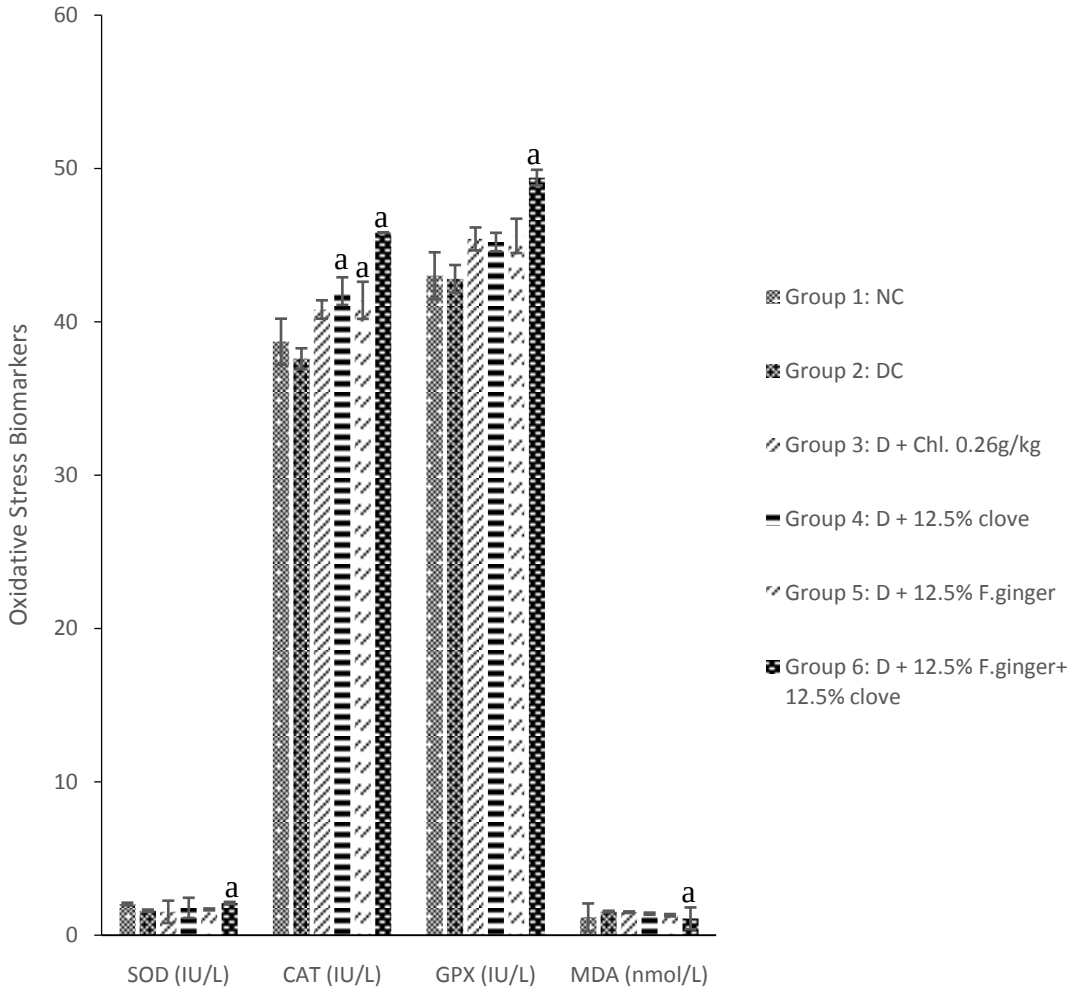


Figure 5;Oxidative Stress Biomakers and MDA levels in experimental animals groups;

(NC = Normal control; DC = Diabetic control; D+Chl = Diabetic administered cholestran; D + CLV = Diabetic fed on clove supplement; D + Fg = Diabetic fed on fermented ginger supplement).

Note: Significant at P< 0.05 value: n=5; a=significant difference.

APPENDIX 6

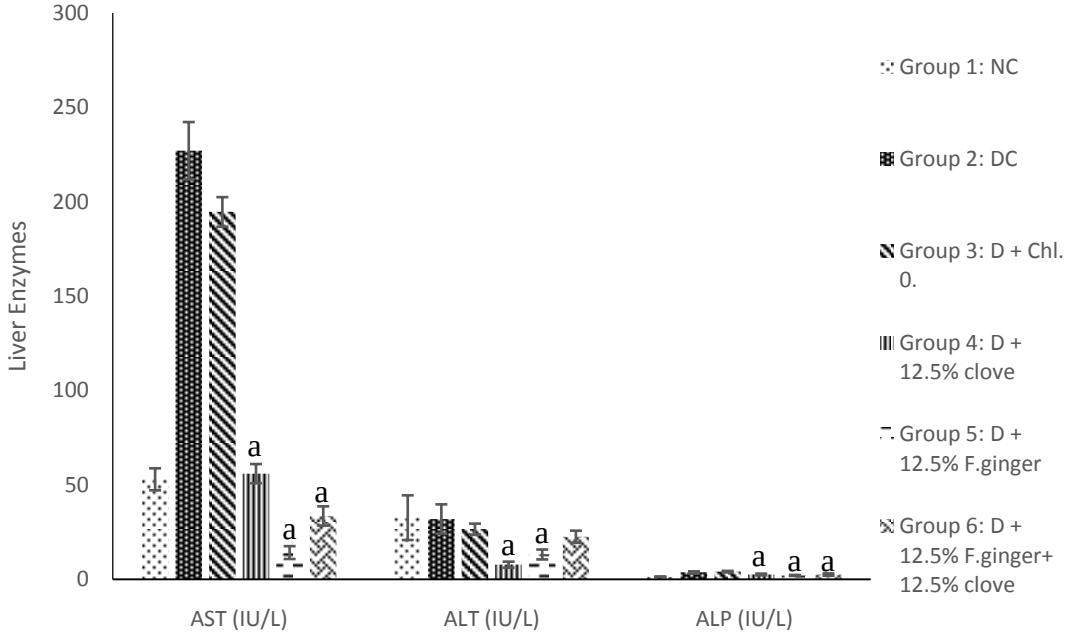


Figure 6. Results of serum liver enzymes activities in experimental animal groups;

(NC = Normal control; DC = Diabetic control; D+Chl = Diabetic administered cholestran; D + CLV = Diabetic fed on clove supplement; D + Fg = Diabetic fed on fermented ginger supplement)

Note: Significant at P< 0.05 value: n=5; a=significant difference

APPENDIX 7

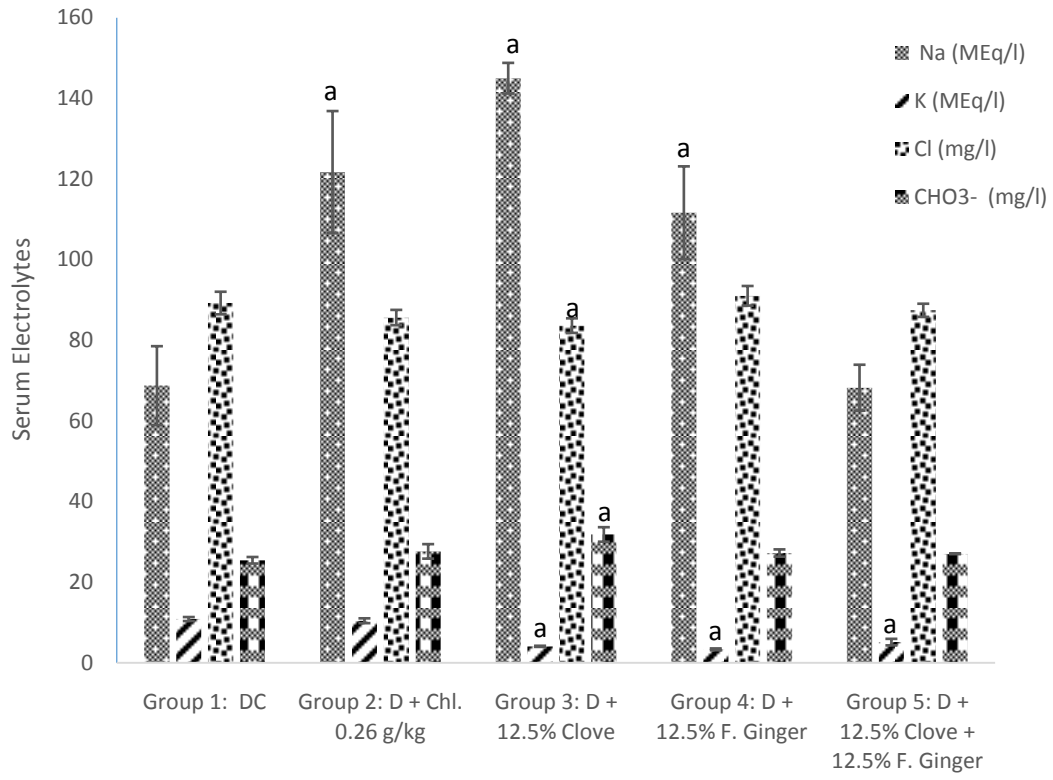


Figure 7:The results of serum electrolytes concentrations of experimental animal groups; (NC = Normal control; DC = Diabetic control; D+Chl = Diabetic administered cholestran; D + CLV = Diabetic fed on clove supplement; D + Fg = Diabetic fed on fermented ginger supplement)

Note: Significant at P< 0.05 value: n=5; a=significant difference compared to DC group.