EFFECT OF FERMENTED CABBAGE *(Brassica olaeracae)* SUPPLEMENTATION ON BLOOD GLUCOSE LEVEL AND OTHER PHYSIOLOGICAL PARAMETERS IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETIC MALE WISTAR RATS

BY

Muhammad ABDULMALIK
(MBBS 2005, MSc. 2015)
P15MDHP9001

DEPARMENT OF HUMAN PHYSIOLOGY,
FACULTY OF BASIC MEDICAL SCIENCES,
COLLEGE OF MEDICAL SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN HUMAN PHYSIOLOGY

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FACULTY OF BASIC MEDICAL SCIENCES,
COLLEGE OF MEDICAL SCIENCES,
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ZARIA

November, 2021

DECLARATION

I, Muhammad ABDULMALIK declare that the work in this thesis entitled: **Effect of Fermented Cabbage (*Brassica olaeracae*) Supplementation on Blood Glucose and other Physiological Parameters in Streptozotocin-Nicotinamide Induced Diabetic Male Wistar Rats** was carried out by me in the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria,under the supervision of Prof. A. Mohammed, Prof. M.U. Kawu and Dr. Y. Tanko. 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 in this or any other institution.

_____ _____

Muhammad **ABDULMALIK** Date

CERTIFICATION

This thesis titled "**Effect of Cabbage (*Brassica olaeracae*) Supplementation On Blood Glucose Level and other Physiological Parameters in Streptozotocin-Nicotinamide Induced Diabetic Male Wistar Rats"** by MUHAMMAD Abdulmalik, meets the regulation governing the award of Doctorate of Physiology in Human Physiology of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literal presentation.

_____ _____

Prof. A. Mohammed, M.B.B.S., M.Sc., PhD (ABU). Date
Chairman, Supervisory Committee

_____ _____

Prof. M.U. Kawu, D.V.M., M.Sc., PhD (ABU). Date
Member, Supervisory Committee

_____ _____

Dr. Y. Tanko, B.Sc., M.Sc., PhD (ABU). Date
Member, Supervisory Committee

_____ _____

Dr. I.G. Bako B.Sc., M.Sc., PhD (ABU). Date
Head, Department of Human Physiology.
Ahmadu Bello University, Zaria

_____ _____

Prof. S.A. Abdullahi Date
Dean, School of Postgraduate School.
Ahmadu Bello University, Zaria

DEDICATION

This work is dedicated to Almighty Allah, for his guidance and mercy throughout the course of the program and to my beloved parents and family.

ACKNOWLEDGEMENT

I express profound gratitude to Prof. A. Mohammed my supervisor and the Dean, Faculty of Basic Medical Sciences who had always encouraged and inspired me throughout the course of this work. I thank all my teachers and senior professors from various faculties in the University for their comments and suggestions, which enabled me to come up with my work successfully. I also want to thank the entire staff of Human Physiology Department, Ahmadu Bello University, Zaria, especially Malam Bala and Mr. Inuwa who painstakingly assisted in the laboratory work. I am also grateful to Mr. Bamidele of Human Anatomy Department for his tremendous help in carrying out all the haematological analysis for the project and for his understanding. A special thanks to my colleagues, in the Department who worked tirelessly to support me in every aspect of my work. Not forgetting my family, I do not have enough words to express my gratitude to them.

ABSTRACT

Diabetes mellitus (DM) is a global health problem, with evidence suggesting lifestyle modifications in form of diet playing an integral part of its management and reducing the risk of complications. Despite significant progress in the treatment of diabetes using conventional medicines, research in relation to plants in form of fermented cabbage supplementation and its impact on the management of DM is scarce. The aim of this study was to evaluate the effect of fermented cabbage supplementation on blood glucose level and other physiological parameters in streptozotocin-nicotinamide induced diabetic male Wistar rats.This study was carried out on thirty male Wistar rats weighing 150 to 300 g (8-10 weeks old). They were randomly divided into six groups of five rats each. Groups I and II were normal control and diabetic control rats that received distilled water at 1 ml/Kg orally daily. Groups III, IV and V were diabetic rats that received 12.5%, 25%, and 50% fermented cabbage supplementation mixed with 87.5%, 75% and 50% of Vital® feed respectively. Group VI were diabetic control rats treated with metformin at 500 mg/kg/day orally. All treatments lasted for four (4) weeks. Diabetes Mellitus was induced in overnight fasted male Wistar rats (6-8 hours) by administering a single dose of freshly prepared sodium citrate buffer solution of streptozotocin, 65 mg/Kg intraperitoneal (IP) in 50 mm cold citrate buffer (pH 4.5), 15 minutes after IP administration of 120 mg/Kg nicotinamide.The results showed that treatment of diabetic rats with graded doses of fermented cabbage resulted in a significant ($P < 0.05$) steady decrease in blood glucose level and in a dose dependent manner from week 1 to week 4 of treatment when compared with corresponding diabetic untreated group.The serum insulin levels decreased significantly ($P < 0.05$) in the diabetic untreated group following STZ/ Nicotinamide treatment when compared with normal control group. However, administration of graded doses of fermented cabbage to diabetic rats significantly ($P < 0.05$) increased the serum insulin levels respectively, in a dose dependent manner when compared with diabetic control untreated group. The serum insulin receptor levels showed a significantly ($P < 0.05$) increased the serum insulin receptor levels following administration of graded doses of fermented cabbage to diabetic rats. Though not in a dose dependent manner when compared with diabetic untreated group. The liver function tests results obtained showed oral administration of graded doses of fermented cabbage produced a significant ($P < 0.05$) decrease in all the liver enzymes (alkaline phosphatase and both the serum alanine and aspartate aminotransferase) activities when compared with animals in the diabetic control untreated groups. Furthermore, administration of various doses of fermented cabbage produced a significant ($P < 0.05$) increase in serum total protein when compared with animals in the diabetic control untreated groups.The results obtained for serum total cholesterol, triglyceride and LDL-c after oral administration of various doses of fermented cabbage induced a significantly ($P < 0.05$) lower serum cholesterol and LDL-c concentration in a dose dependent manner when compared with rats in the diabetic untreated control group. There was a significantly ($P < 0.05$) higher serum HDL-c level after oral administration of various doses of fermented cabbage in a dose dependent manner when compared with rats in the diabetic untreated control group.Administration of graded doses of fermented cabbage to the diabetic rats significantly ($P < 0.05$) induced higher levels of both T_3 and T_4 respectively, when compared with diabetic untreated control group respectively. However, there was a significantly ($P < 0.05$) lower serum TSH levels when various doses of fermented cabbage was administered, when compared with rats in the diabetic untreated control group.

The red blood cell (RBC) count, haemoglobin (Hb) concentration, and packed cell volume(PCV) significantly increased ($P < 0.05$) followingadministration of graded doses of fermented cabbage to diabetic rats when compared with rats in the diabetic untreated control group.The white blood cell (WBC) count, lymphocytes and neutrophils obtained in diabetic untreated control group were significantly decreased ($P < 0.05$) respectively, when compared to the normal control group respectively. Contrary to the observed trend in the other parameters, the platelet count in the diabetic untreated rats was significantly increased when compared to those obtained in rats of the normal control group. Finally, treatment of diabetic rats with graded doses of fermented cabbage significantly increased ($P < 0.05$) total WBC, lymphocytes and neutrophils count respectively when compared with diabetic untreated control. Conversely, treatment of diabetic rats with fermented cabbage produced a significant decrease ($P < 0.05$) in the platelet counts when compared with the diabetic untreated control group. In conclusion, the results of the present study demonstrated that fermented cabbage supplementation lowered blood glucose levels, serum liver enzymes, serum cholesterol, serum triglyceride and low-density lipoproteins; while serum high density lipoproteins, red blood cell indices, serum total proteins, serum insulin, insulin receptors and thyroid hormones were all increased when compared with the diabetic untreated control rats. These findings may have shown the efficacy of fermented cabbage supplementation as an anti-diabetic supplement.

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LIST OF ABBREVIATIONS

ALP	-	Alkaline Phosphatase
ALT	-	Alanine Aminotransferase
AST	-	Aspartate Aminotransferase
CDM	-	Congenital Diabetes Mellitus
DKA	-	Diabetic Ketoacidosis
DKA	-	Diabetic Ketoacidosis
DM	-	Diabetes Mellitus
DR	-	Diabetic Retinopathy
GDM	-	Gestational Diabetes Mellitus
GLUT	-	Glucose Transporters
HDL	-	High Density Lipoprotein
HHS	-	Hyperosmolar Hyperglycaemic State
HLA	-	Human Leukocyte Antigen
IDF	-	International Diabetes Federation
IRMA	-	Intraretinal Microvascular Abnormalities
IRS-2	-	Insulin Receptor Substrate-2
LDL	-	Low-Density Lipoprotein
NIDDM	-	Non-Insulin Dependent Diabetes Mellitus
NPDR	-	Nonproliferative Diabetic Retinopathy
PLTs	-	Platelets
RBCs	-	Red Blood Cells
T2DM	-	Type 2 diabetes mellitus

TAC	-	Tricarboxylic Acid Cycle
TSH	-	Thyroid Stimulating Hormone
VNTR	-	Variable Number of Tandem Repeat
WBCs	-	White Blood Cells

CHAPTETR ONE

1.0 INTRODUCTION

Diabetes Mellitus (DM) is a non-communicable debilitating disease due to the pancreas not producing adequate insulin or the body not effectively utilizing the insulin produced by the pancreas (WHO, 2016). According to the International Diabetes Federation (IDF), 415 million adults in 2015 were living with diabetes and thenumber isexpected to rise to 642 million by 2040(IDF, 2015). In sub-Saharan Africa, 20 million of the population were predicted to have DM and the number is projected to rise to 41.1 million persons by 2035(Dahiru*et al.,* 2016).It was reported that most developed countries around the world spend up to 10% of their annual health budget on managing and preventing complications from diabetes. This estimate in expenditure in the annual health budget is expected to increase by 2040 (Piroozi*et al.,* 2020).

The most important distinctive feature of DM is an elevated blood glucose concentration, but this abnormality is just one of a number of biochemical and physiological changes that accompany the condition (Olaitan, 2012). Diabetes Mellitus accounts for significantill health and deathdue to its long-time complications. Individuals living with DM in developing countries have high risk of economic distress due to frequent hospital visits and increased cost of drugs at the point of accessing health care (Okoronkwo*et al.,* 2015). This represents a threat to the economy of many countries especially developing ones (Papatheodorou*et al.,* 2016).

The most common form of DM that affects the population, is type 2 diabetes mellitus(T2DM), which usually occurs as a result of the interplay between behavioural, genetic and environmental factors(Baynest, 2015).It is characterized by insulin resistance, lower insulin production and end stage pancreatic beta-cell failure. This may eventually lead to failure in glucose transport to the liver, muscle and fat cells. In a new finding, impaired pancreatic alpha-cell function has been

recognized to be involved in the pathophysiology of T2DM (Olokoba*et al.,* 2012). The management of DM mainly involves lifestyle modification and combinations of antihyperglycaemic drugs in addition to insulin therapy.

Cabbage *(Brassica olaracea)*locally called *Kabeji* in Hausa language and *Akojopa* or *Jaleji* in Yoruba language is an important vegetable consumed either raw or processed in different ways as boiled or fermented (Sami *et al.,* 2013). Cabbage is a cruciferous vegetable, a rich source of beta-carotene, vitamin C and may have the ability to lower the risk of cancers byboosting deoxyribonucleicacid(DNA) repair. It is consumed globally mainly due to its nutritional and medicinal values (Sharma and Rao *et al.,* 2013; Samec*et al.,* 2017).

Fermentation of cabbage plays an important role in food processing and preservation from time immemorial. It improves the quality and functional properties of food by increasing bioavailability of nutrients, and reducing or removing anti-nutritive factors of the particular food (Mukherjee *et al.,* 2016). Fermentation of cabbage as a form of preservation takes a duration of approximately 21-30 days.The slow rate of lactic acidification and salt concentration avert the growth of unwanted organisms during the fermentation (Jagannath *et al.,* 2011).

1.1 STATEMENT OF RESEARCH PROBLEM

Diabetes Mellitus (DM) is a global health problem which affects an estimated 422 million people in 2014 with increased prevalence in developing than developed countries (WHO, 2016).In addition to lifestyle and dietary changes, achieving near-normal glycaemic control still remains the standard for managing DM which significantly decreases the risk of acute and chronic complications(Marin-Penalver*et al.,* 2016; Chaudhury *et al.,* 2017).

The incidence of T2DM amongst adults aged 20-69 years in Nigeria is reported to be 1.7%, affecting all the geopolitical regions with the highest prevalence noted in the south-south zone to be 9.8% compared to the north-west zone which has 3.0%. (Uloko*et al.,* 2018).

Despite drug development and therapeutic interventions, successful treatment of DM still remains a challenge and worldwide research is focused on this aspect (Sawatkar*et al.,* 2015; Shrestha *et al.,* 2017). Conventional antidiabetic medicines include injectable insulin and sulphonylureas. However, their side effects such as dermal reaction; hypoglycaemia and lactic acidosis are of major concern to medical practitioners (Alhadramy, 2016; Yatoo*et al.,* 2017).

1.2 JUSTIFICATION FOR THE STUDY

Dietary management in the form of nutrition plays an integral part of management for T2DMwhich helps to improve glycaemic control and reduce the risk of potential complications (Barclay*et al.,* 2010).Type 2 diabetes mellitus is a global health problem with evidence suggesting lifestyle and some nonpharmacological interventions delay or even prevent its complications over time (Rawal*et al.,* 2012).Lipids play an important role in the pathogenesis of experimentallyinduced diabetes. The level of serum lipids is usuallyelevated in diabetic state which is a risk factor for T2DM associatedcardiovascular diseases (Farook *et al.,* 2011).

There is limited research on food supplementation for the management of DM. In spite of the tremendous efforts made in the treatment of DM through conventional medical management, they have some disadvantages including drug resistance and toxicity (Kooti*et al.,* 2016).

Researches on exploring safe treatments of DM and its complications involve dietary manipulations (Farzaei*et al.,* 2017). In relation to plants as a form of supplementation, most of the studies have not assessed its impact on the course of diabetes. To the best of our knowledge, there is no much work done on therapeutic efficacy of cabbage supplementation in the management of DM. It is therefore necessary to evaluate the ameliorative effects of fermented cabbage supplementation on hyperglycaemia and other related physiological disorders associated with T2DM.

1.3 AIM AND OBJECTIVES

1.3.1 Aim

The aim of the study is to evaluate the effect of fermented cabbage supplementation on blood glucose level and other physiological parameters in streptozotocin – nicotinamide induced diabetic male wistar rats.

1.3.2 The objectives of this study were to determine the effects of:

i. Fermented cabbage supplementation on blood glucose levels in streptozotocin-nicotinamide induced diabetic male wistar rats.

ii. Fermented cabbage supplementation on serum insulin levels in streptozotocin-nicotinamide induced diabetic male wistar rats.

iii. Fermented cabbage supplementation on serum insulin receptor activities in streptozotocin-nicotinamide induced diabetic male wistar rats.

iv. Fermented cabbage supplementation on serum liver enzyme activities: Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total proteins (Albumin/Globulin) in streptozotocin-nicotinamide induced diabetic male wistar rats.

v. Fermented cabbage on lipid profiles: Total serum cholesterol, serum triacylglycerol, high density lipoprotein (HDL) and low-density lipoprotein (LDL) in streptozotocin-nicotinamide induced diabetic male wistar rats.

vi. Fermented cabbage supplementation on thyroid hormones profile: Thyroid Stimulating Hormone (TSH), triiodothyronine (T_3) and thyroxine (T_4) in streptozotocin-nicotinamide induced diabetic male wistar rats.

vii. Fermented cabbage supplementation on Haematological indices: Red blood cells (RBCs), white blood cells (WBCs) and platelets (PLTs) in streptozotocin-nicotinamide induced diabetic male wistar rats.

1.4 HYPOTHESIS

Fermented cabbage supplementation has no effect on blood glucose level and other physiological parameters in streptozotocin-nicotinamide induced diabetic male wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 EPIDEMIOLOGY OF DIABETES MELLITUS

Diabetes mellitus (DM) has been occasionally called a ‘silent’ epidemic. Its clinical features can either be a slow onset, or progressively asymptomatic leading to secondary complications, or rapidly merging symptoms leading to complications such diabetic ketoacidosis (DKA) mostly seen in type 1 diabetes mellitus (T1DM) as well as type 2 diabetes mellitus (T2DM) occasionally and hyperosmolar hyperglycaemic state (HHS) seen in T2DM/or coma (Nwaneri, 2015).

Diabetes mellitus affects carbohydrates, lipids and proteins metabolism. The number of diabetic patients has dramatically increased in recent decades; the prevalence of diabetes was boosted by the increased consumption of refined sugar and processed foods with high calories, along with decreased physical activity (Stephens *et al*., 2006). The prevalence of diabetes is increasing dramatically worldwide and the increase is expected to occur in both developed and developing countries.It was estimated that the prevalent cases of T2DM worldwide will increase by more than 500 millionby the year 2018; along with the consequences of population ageing, increasing urbanization, unhealthy diets, obesity and sedentary lifestyles (Kaiser *et al.,* 2018).

The global prevalence of diabetes was estimated to be 9.3% (463 million people), and may rise to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 with the prevalence higher in developed (10.4%) than undeveloped countries (4.0%)(Saeedi*et al.,* 2019).

Diabetes is a major complication of blindness, kidney failure, heart attacks, stroke and lower limb amputation. There was a 5% increase in premature mortality from DM between the year

2000 and 2016. While in 2019 alone an estimated 1.5 million deaths were directly attributed to DM (WHO, 2021).

There are about 13.6-14.7 million people living with diabetes in Africa (Oputa and Chinenye, 2015). Sub-Saharan Africa counts approximately 7 million people with Nigeria having the highest number (about 1,218,000) of people affected as well as the highest number of people with impaired glucose tolerance (about 3.85 million people). Nigerian South-East 0.25-0.46/1 00016 3:1 male: female prevalence ratio 5-17 years whereas the North-West Nigeria 3.1/1 00017 1:0.6 male: female ratio Age at presentation: 10 ± 4.5 years (Padoa, 2011). Research in 2007 showed increase in the incidence of diabetes mellitus in Federal Medical Centre, Katsina. Older individuals were the most affected. This may be due to the fact that with age, people do not engage themselves in physical activities which lead to accumulation of fats and inadequate burning of sugar in their body (Suleiman, *et al.,* 2011).

Lifestyle modifications such as diet and physical activity; smoking and sedentary lifestyle are the major factors for rapidly rising incidence of DM among developing countries (Sami *et al.,* 2017).Enormous evidence has been shown that both genetic and environmental factors play an important factor in the pathogenesis of T2DM. However, the roles played by genetic factors are still not well understood. However,several studies have shown that obesity (central obesity in particular), physical inactivity, high-fat diet, and diet rich in saturated fatty acids increase the risk of DM (Uusitupa, 2002).

2.2PHYSIOLOGY OF THE PANCREAS

The pancreas is a retroperitoneal organ located in the abdominal cavity. It is connected to the first part of the duodenum through the pancreatic duct. It is an elongated nodular organ measuring about 15-25 cm and approximately 80g in weight(Martini and Nath, 2009). The pancreas is usually referred to as a mixed gland because of its ability to secrete both exocrine (98%) and endocrine (2%) hormones. The exocrine portion secretes mainly digestive enzymes which empties directly through the pancreatic duct (Hug *et al.,* 2003; Chandra and Liddle, 2015; Chu *et al.,* 2006; Amber and Bloom, 2007). The endocrine portion contains approximately 1 million islets supplied with rich capillary nexus conveying the secreted hormones directly into the blood circulating system (Martini and Nath, 2009).

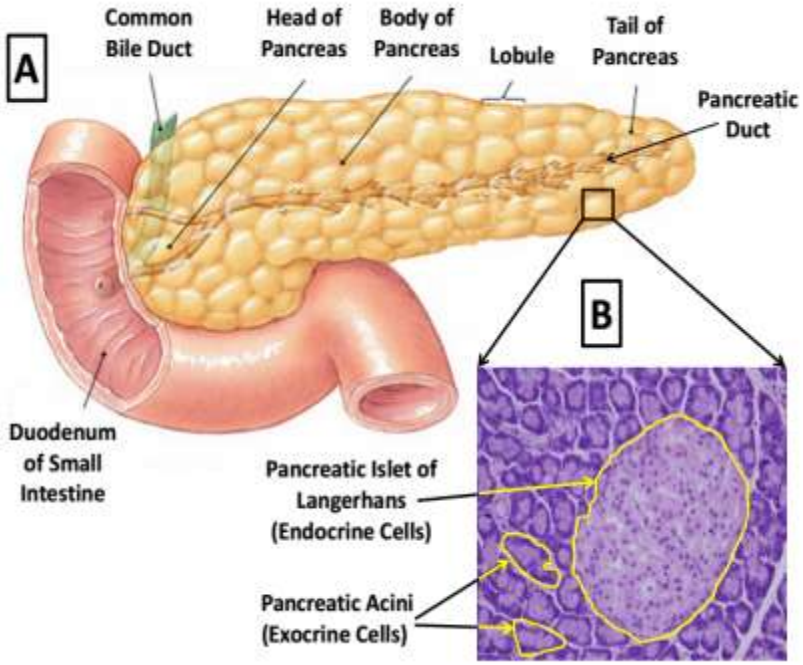

Figure 2.1: Diagrams showing (A) the position of the pancreas and its relationship with the duodenum and (B) a histological picture of the endocrine and exocrine components of the pancreas (Martini and Nath, 2009).

2.2.1 Pancreatic cell types

Beta-cells: These cells secrete insulin and are the most numerous endocrine cells in the pancreas. It constitutes about 70% of the cell population of the islet of Langerhans, concentrated mainly in the central area than the peripheral regions of the islet (Adeghate and Ponery, 2003).

Alpha-cells: Alpha cells are glucagon secreting cells of the endocrine pancreas. They are located on the peripheral part of the islets of Langerhans and they respond to low blood glucose by releasing glucose in to the blood circulation from glycogen stores in the liver (Wendt and Eliasson, 2020).

Delta-cells: Delta cells distributed evenly throughout the pancreas secretes somatostatin and make up an approximate 1-5% of the total islet cells. They carry out their activities by inhibiting pancreatic alpha and Beta cell function and thereby controlling glucose homeostasis (e Drigo*et al.*, 2019).

Pancreatic polypeptide-cells: Pancreatic polypeptide (PP) producing cells are found mainly in the outer area of the pancreatic islets of Langerhans comprising of about 2% of the total islet cells (Adeghate and Ponery, 2003).

2.2.2 Normal beta-cell function

The primary function of beta-cell is the synthesis and secretion of insulin for the purpose of maintaining blood glucose level within normal physiological range. Glucose is known as the main insulin secretagogue even though myriads of other factors such as nutrients, hormones and neurotransmitters play vital roles (McDonald, 2005). The process of insulin secretion is a composite of series of processes instigated by glucose transport into beta cells,by specific glucose transporters known as GLUT 1 and GLUT 2 precisely alongside phosphorylation by

glucokinase, directing metabolic flux through glycolysis. Pyruvate is produced as the terminal product of the pathway (Matschinsky, 1990). This proceeds to the mitochondria where it is decarboxylated to Acetyl-CoA and committed to tricarboxylic acid cycle (TAC). Acetyl-CoA condenses together with oxaloacetate during the TCA to form citrate catalyzed by citrate synthase. Citrate is converted to isocitrate via the action of aconitase. The isocitrate undergoes oxidative decarboxylation to form α-ketoglutarate through the action of NAD-linked isocitrate dehydrogenase. Accordingly, α-ketoglutarate is oxidized to succinyl-CoA in a reaction catalyzed by α-ketoglutarate dehydrogenase(Seino *et al.,* 2010). Succinyl-CoA synthase then catalyze the conversion of succinyl-CoA to succinate with the concomitant phosphorylation of GDP to GTP. Succinate dehydrogenase in other wards catalyzes the oxidation of succinate to fumarate; while fumarase catalyzes the conversion of fumarate to malate and afterward, malate dehydrogenase catalyzes the final step of the tricarboxylic acid cycle, oxidizing malate to oxaloacetate to produce NADH (McDonald,2005; Seino *et al.,* 2010). These series of biochemical processes precipitate an enhanced ratio of ATP to ADP in the cytoplasm, defining the closure of the ATP-sensitive K^+ channels, depolarization of the plasma membrane, influx of extracellular Ca_2^+ and activation of exocytosis which takes place in several stages including recruitment, docking, priming, and fusion of insulin granules to the beta-cell plasma membrane (McDonald *et al.,* 2005; Van Raalte, 2011).

Various researches have shown that high plasma levels of glucose and fatty acids contribute in downregulating pancreatic beta cell function, a phenomenon known as glucolipotoxicity. It is characterized by insulin granules depletion due to excessive and prolonged exposure to secretagogues(Van Raalte, 2011).

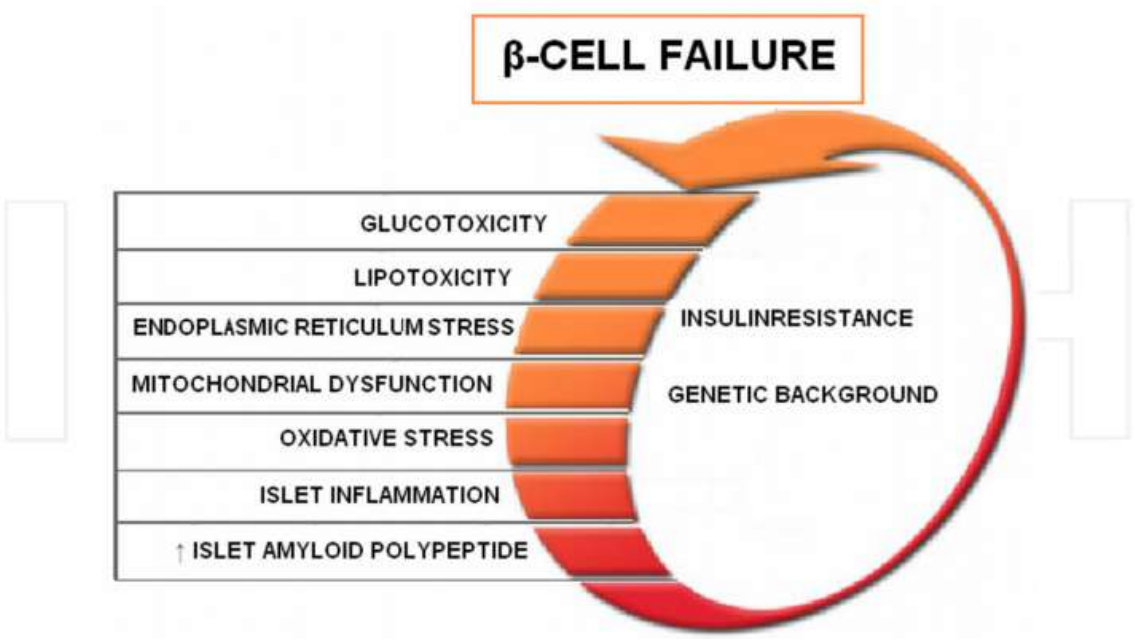

Figure 2.2: Potential mechanism of beta-cell failure (Popa and Mota, 2013).

2.2.3 Insulin

Insulin a polypeptide hormone is synthesized by the beta-cells of the islets of Langerhans both in humans and other mammals.The human insulin is a hormone with a low molecular weight approximately 5,800 KD. It is mainly made up of two polypeptide chains A and B. The A-chain consists of 21 amino acids while the B-chain is made up of 30 amino acidslinked by a disulphide molecule between the cystiene amino acids giving a three-dimensional form to insulin molecule(Weiss, 2009). The most target tissue of insulin actions is the liver, muscle and adipose tissue. Insulin is known to promote the synthesis of carbohydrates, proteins, lipids and nucleic acids. It stimulates glucose transport across muscle and adipocytes, regulates the production of hepatic glycogen as well as the inhibition of glycogenolysis and gluconeogenesis (Piero *et al*., 2012). It also promotes transfer of amino acids across membranes as well as the synthesis of proteins and the inhibition of proteolysis (Cahill and Boston, 1971).

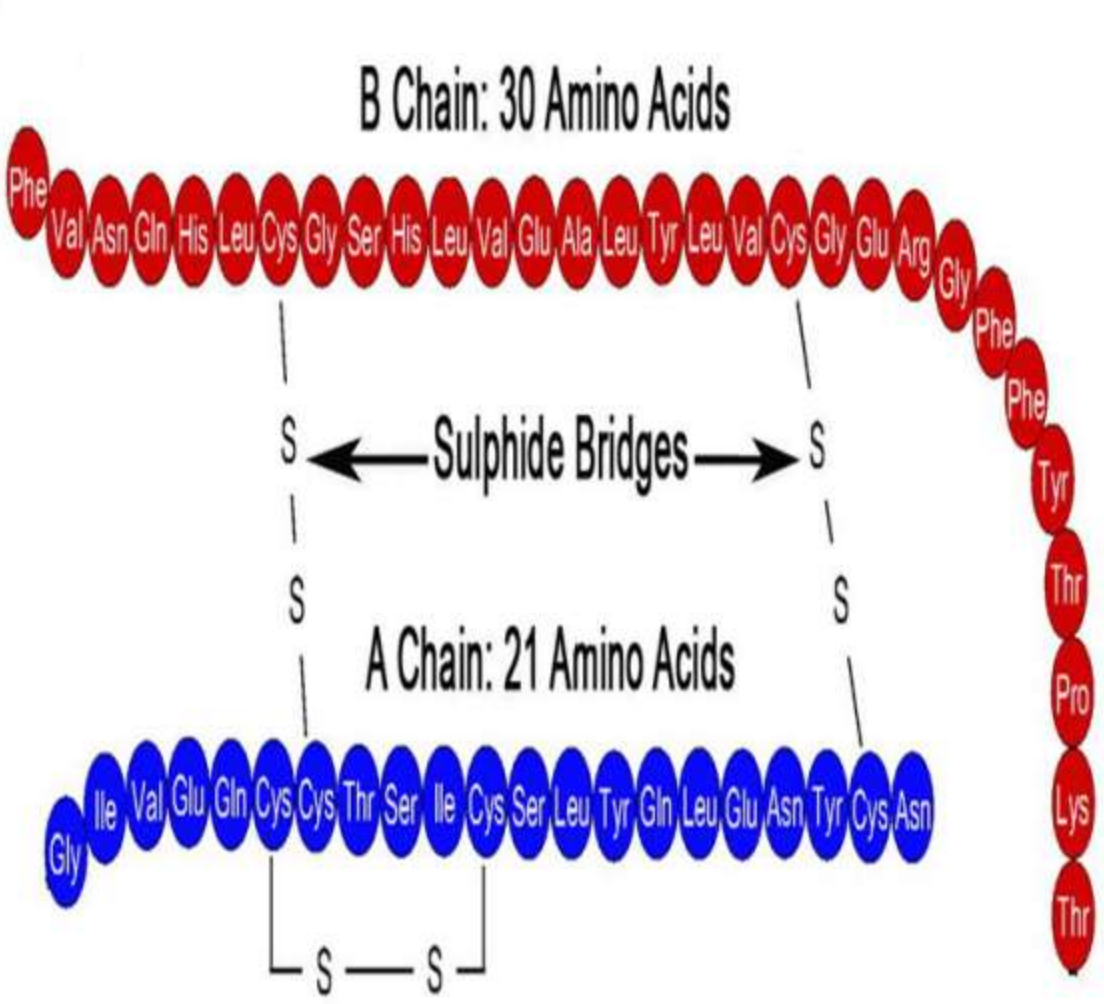

Figure 2.3: Structure of human Insulin Molecule (Montague, 1993).

2.2.3.1 Insulin synthesis

The pancreatic beta cell mainly produces insulin composed of two polypeptide chains formed by a precursor known as pro-insulin (Steiner, 2004). An insulin gene transcription through Ribonucleic acid (RNA) polymerase enzyme forms a messenger Ribonucleic acid (mRNA) which is further processed to a mature mRNA and then translated into a pre-proinsulin in the cytoplasm. The processes involved in the translation of the mRNA into various amino acid sequence of pre-proinsulin takes place in the ribosomes. The ribosomes then attached themselves to the endoplasmic reticulum (ER) to initiate the synthesis of proinsulin. The proinsulin is uniformly packaged into storage granules by the Golgi apparatus (Wicksteed*et al.,* 2001). The stored granules in the Golgi apparatus are transported to the pancreatic beta cells cytoplasm where it finally awaits secretory signals. The secretory beta cell granules contain mixture of mainly insulin and small quantities of proinsulin (Steiner *et al.*, 2005).

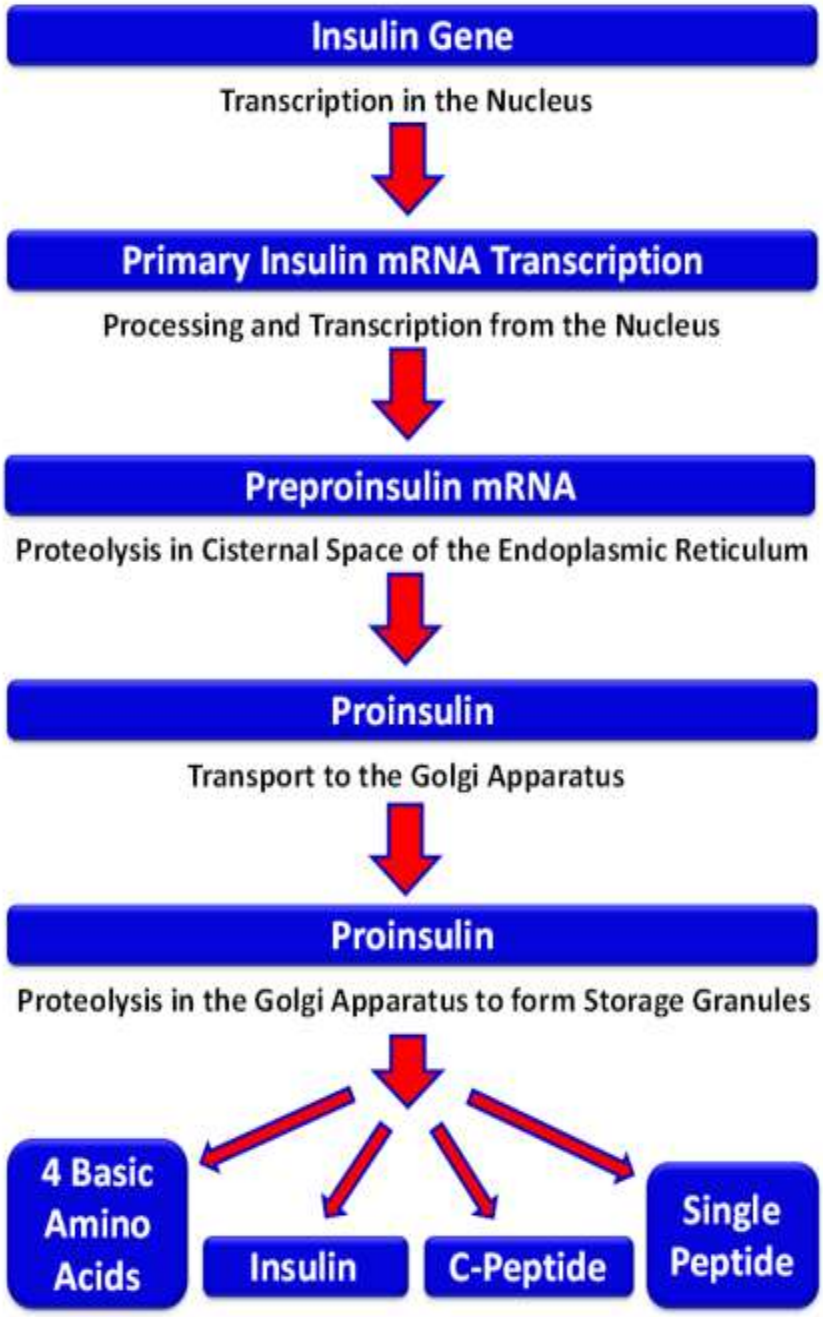

Figure 2.4: Schematic representation of Insulin Synthesis (Montague, 1993).

2.2.3.2. Mechanism of receptor activity

Insulin binds to specific receptors positioned on the plasma membrane. These receptors are saturated which makes both the binding capacity and biological activity of insulin to be maximal at plasma insulin concentration of 20 to 30 μU/ml. Insulin undergoes some conformational changes after binding to its receptor and transmits its signals in the cytoplasm via the action of a second messenger which influence enzymatic processes in the cell(Kibiti, 2006). The two membrane bound enzymes that are associated with insulin actions includes adenyl cyclase (cAMP) and magnesium activated sodium-potassium ATPase systems (Steiner, 1977). In turn, potassium is an important factor in membrane potential and enzymatic regulation while magnesium is involved in the activation of many intracellular enzymes. Intracellular magnesium accumulation is also promoted by insulin action.

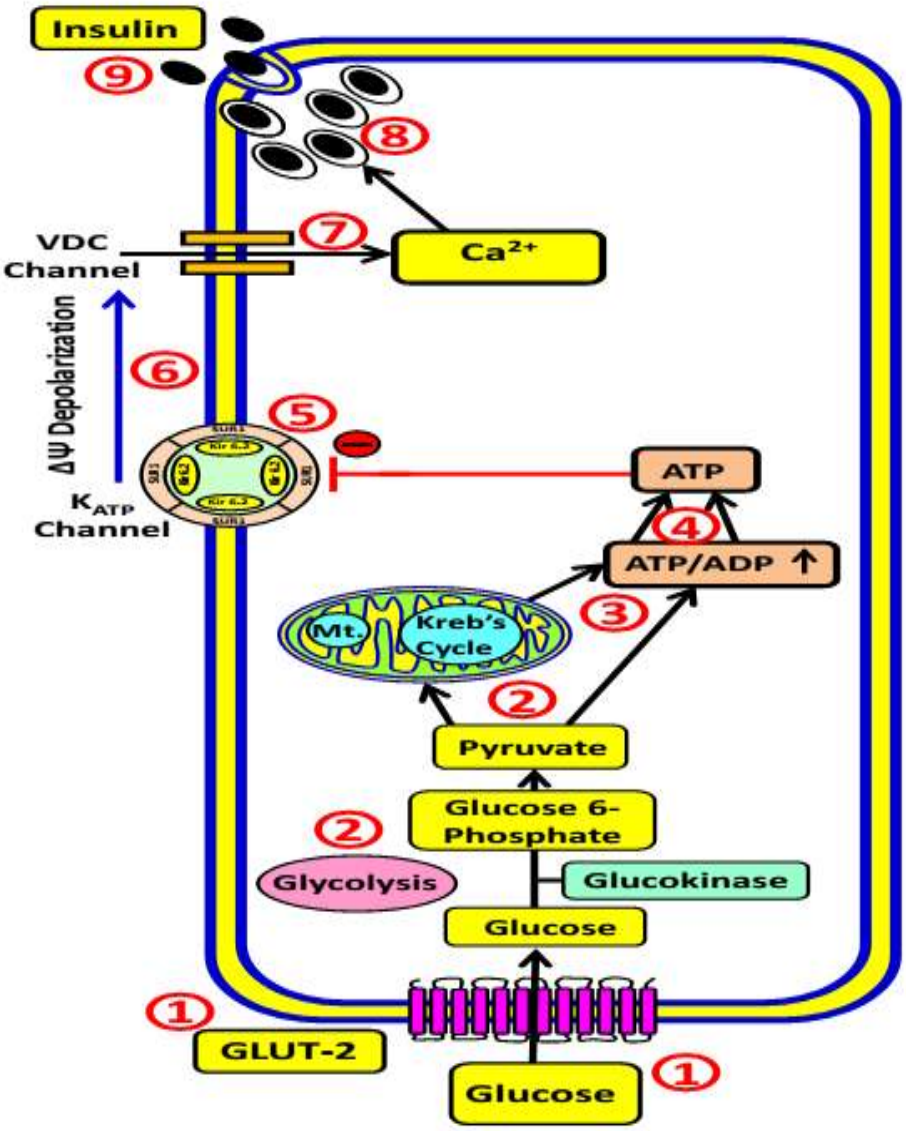

Figure 2.5: Schematic diagram showing the process of glucose-stimulated insulin secretion from an endocrine pancreatic β-cell (Jensen *et al*., 2008)

2.2.3.3 Glucagon

Glucagon is secreted by the alpha cells of the pancreatic islets in much the same manner as insulin. The synthesis of glucagon is inhibited by a negative feedback mechanism when blood glucose is high. Glucagon stimulates the liver to release the glucose stored as glycogen in its

cells into the bloodstream, hence increasing blood glucose to maintain the normal range (Prabhakar, 2014). Imbalance between insulin and glucagon secretory levels causes abnormal blood glucose regulation and results in two critical conditions namely hypoglycaemia and hyperglycaemia.

2.3 CLASSIFICATION OF DIABETES MELLITUS

The resent World Health classification of DM is as follows (WHO, 2019):

i. Type 1 diabetes mellitus

ii. Type 2 diabetes mellitus

iii. Hybrid forms of diabetes mellitus

iv. Other specific types of diabetes mellitus

v. Unclassified diabetes mellitus

vi. Hyperglycaemia first detected during pregnancy

2.3.1 Type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM), also known as autoimmune diabetes, is a chronic metabolic non communicable disease characterized by an absolute insulin deficiency due to pancreatic β-cell loss leading to hyperglycaemia (Katsarou*et al.,* 2017). Various researches have revealed that T1DM is not entirely a disease resulting from dysfunctional immune cells, but associated with different triggers that may provoke an immune response, ranging from the size of the pancreas and β-cell mass to viral infection and metabolic stress (Roep*et al.,* 2020).

The pathophysiology of T1DM appears to result mainly from insulin deficiency caused by autoreactive T-cells of the immune system that destroy the pancreatic beta-cells. The

autoimmune reaction leads to a progressive loss of functional beta-cell mass and declining insulin production (Wu *et al.,* 2019).

Type 1DM is rarely caused by mutational defects in a single gene. These monogenic forms are usually accompanied by multiple other autoimmune conditions due to the disruption of common regulatory pathways. An example is found in the IPEX syndrome (immune dysregulation, poly-endocrinopathy, enteropathy, X-linked), in which mutations in the Foxp3 transcription factor led to the dysfunction of regulatory T cells and wasting multi organ autoimmunity (Van Belle *et al.*, 2011).

The Human Leukocyte Antigen (HLA) region on chromosome 6p21 (commonly termed IDDM1, for insulin-dependent diabetes mellitus locus) is a critical susceptibility locus for many human autoimmune diseases, including T1DM which is one of the most widely studied genetic disorders, accounting for approximately 40 to 50% of the familial aggregation of T1DM. The major genetic determinants of this disease are polymorphisms of class II HLA genes encoding DQ and DR. The DR-DQ haplo-types conferring the highest risk are DR3 and DR4. The risk is much higher for the heterozygote formed by these two haplo-types than for either of the homozygotes (Van Belle *et al.*, 2011; Noble and Valdes, 2011).

The human insulin gene is located on chromosome 11p15.5 was first reported in 1984 to be associated with T1DM in Caucasoid. Gene mapping showed that susceptibility resides in a Variable Number of Tandem Repeat (VNTR) polymorphisms in the promoter region of the insulin gene (Pugliese, 2005).

2.3.2 Type 2 diabetes mellitus

Type 2 Diabetes Mellitus (T2DM) also known as non-insulin dependent diabetes mellitus (NIDDM), is a non-communicable disease characterized by insulin resistance, which could progressively worsen to absolute resistance and cell dysfunction (Artasensi*et al.*, 2020).

The main pathophysiological features of T2DM are insulin resistance in skeletal muscle, liver and adipose tissue, together with impaired insulin secretion. Insulin resistance reduces peripheral glucose uptake and stimulates hepatic glucose output which leads to elevated blood glucose levels. The ensuing hyperglycaemia increases the demand on the beta-cells for a compensatory rise in insulin secretion. This may consequently exhaust the beta-cells and lead to a progressive loss of beta-cell function, resulting in insulin deficiency and subsequent diabetes (Wu *et al.*, 2019).

Obesity is often part of the metabolic syndrome and is accompanied by dyslipidemia and increased circulating leptin and cytokine levels are one of the risk factors of T2DM. These factors have been shown to modulate β-cell function and survival. The influence of dyslipidemia on pancreatic β-cells will depend on specific lipid profile such as the free fatty acids and lipoproteins which have been shown to be pro-apoptotic for the β-cell, others are protective (Donath *et al.*, 2005).

Thetwo principal pathophysiologic abnormalities which underlie most cases of T2DM are insulin resistance and pancreatic β-cell dysfunction (Halban*et al.*, 2014).

Insulin resistance arises as a result of lipid accumulation in the liver and skeletal muscle which triggers pathways that impair insulin signaling, leading to reduced muscle glucose uptake and decreased hepatic glycogen synthesis (Samuel and Shulman, 2016). Increase in hepatocellular

diacylglycerol content activate protein kinase C-ε (PKC), leading to reduced insulin-stimulated tyrosine phosphorylation of Insulin Receptor Substrate-2 (IRS-2). This may lead to reduced mitochondrial function predisposing individuals to intramyocellular lipid accumulation and insulin resistance (Morino*et al.,* 2006).

A decrease in beta-cell number/mass, as a result of genetic or environmental factors, or cell death can reduce beta-cell mass by up to 60% in people with T2DM. In addition, beta-cell exhaustion can arise from oxidative stress due to altered glucose metabolism which can expose beta cells under severe endoplasmic reticulum (ER) stress leading to beta cells failure to secrete insulin despite histologically normal pancreatic beta cells (Wysham and Shubrook, 2020).

Leptin, mainly produced and secreted by the adipose tissue, is an important factor regulating body weight and glucose homeostasis to the amount of body fat. In leptin resistance, high levels of leptin may contribute to the dysregulation of the adipo- insular axis that leads to hyperinsulinemia and promotes T2DM(Maedler*et al.,* 2008). In vitro, chronic exposure of human islets to leptin decreases β- cell production of Interleukin- 1 Receptor antagonist (IL- 1Ra) and induces Interleukin- 1β(IL- 1β) release from islet preparation, leading to impaired β- cell function and apoptosis. Long- term treatment of β- cells with leptin also decreases insulin biosynthesis and secretion (Maedler*et al.,* 2008).

2.3.3 Hybrid forms of diabetes mellitus

Hybrid form of DM is characterized by features of both type 1 and 2 DM having episodes of Diabetic Ketoacidosis (DKA), insulin resistance and obese body type. A large epidemiological study showed that a total of 25.5% of patients suffering from T1DM additionally presented the metabolic syndrome (Khawandanah, 2019).

A slowly evolving form of immune-mediated diabetes has been described and has most frequently present initially with features thought to be T2DM, but have evidence of pancreatic autoantibodies that can react with non-specific cytoplasmic antigens in islet cells, Glutamic Acid Decarboxylase (GAD), protein tyrosine phosphatase IA-2, insulin, or Zinc transporter 8 (ZnT8) (WHO, 2019).

Ketosis prone T2DM can be differentiated from T1DM and T2DM by specific clinical and metabolic features. Glucose toxicity due to hyperglycaemia may play a role in the acute and phasic β-cell failure in ketosis prone T2DM. Restoration of normoglycaemia after insulin therapy is accompanied by a dramatic and prolonged improvement in β-cell insulin secretory function (WHO, 2019).

2.3.4 Other specific types of diabetes mellitus

2.3.4.1Maturity onset diabetes of the young(MODY)

This is a constellation of monogenic disorders characterized by autosomal dominantly inherited T2DM form of diabetes classically presenting in adolescence or young adults before the age of 25 years. It is a rare cause of diabetes (1% of all cases) and is frequently misdiagnosed as T1DM or T2DM (Anık*et al.*, 2015).

2.3.4.2Neonatal diabetes mellitus(NDM)

It is also known as Congenital Diabetes Mellitus (CDM) occurs mostly due to monogenic defects. It is rear in occurrence affecting approximately 1 in 90,000-160,000 live births. Clinical diagnoses are usually at an early stage at about 6 months of age, 80 percent has a known genetic disposition(Aguilar-Bryan and Bryan 2008). Mutations affect the pancreatic beta-cell K-ATP channel. Neonatal hyperglycaemia is more common in the first three to five days after birth, but

can be found in infants up to 10 days of life and it usually resolves within two to three days of onset(Ashcroft and Rorsman, 2012).

2.3.4.3 Wolfram syndrome (WFS)

It's a form of monogenic disorder that classically presents with DM in childhood associated with diabetes insipidus, sensorineural hearing lossand bilateral optic atrophy. It is a rear neurodegenerative disease in Africa even though one case was reported in Brazzaville (Congo). The prevalence of WFS has been estimated between 1 in 770,000 in the United Kingdom and 1 in 100,000 in North America. Additional morbidities include hypogonadism, infertility, hypopituitarism, cerebellar ataxia, peripheral neuropathy, dementia, psychiatric illness, and urinary tract problems (Atipo-Tsiba and Odzili, 2015; Toppings *et al.,* 2018).

There are three well known types of WFS. The first type is due to the mutation of WFS1 (4p16.1) gene. This results in the appearance in the first decade of life of T1DM, diabetes insipidus, sensorineural hearing loss, bilateral optic atrophy and neurological damage signs. The second type is due to the mutation of CISD2 (4q24) gene and differs from the first by the absence of diabetes insipidus. The above two types of WFS are autosomal recessive. The third type (Wolfram-like syndrome) is autosomal dominant, and differs from the first two by its late onset of optic atrophy, T1DM (after adolescence) and hearing impairment is not always present (Atipo-Tsiba and Odzili, 2015).

The pathophysiology of Wolfram syndrome suggests that Wolframin (plays a role in calcium haemostasis) is involved in the survival pathways of neurons and pancreatic beta cells (Padmanabhan *et al.,* 2019).Endoplasmic Reticulum (ER) is a cellular organelle which plays a critical role for cell survival. It helps in Ca^{2+} ions storage and is responsible for the correct

folding and posttranslational modification of secretory proteins, cell surface receptors, and integral membrane proteins. This functional role played by ER can be disrupted by physiological processes, such as post-prandial insulin biosynthesis that requires a great biosynthetic activity from the ER in response to food uptake, or pathological processes like viral infection, toxins, cytokines, and mutant protein expression (Pallotta *et al.,* 2019).

Wolframin, a transmembrane glycoprotein localized primarily to the ER functions to maintain homeostasis in the ER, the cellular organelle responsible for the folding of secretory proteins, such as insulin. Endocrine cells are generally vulnerable to ER stress due to their rapid changes in secretory protein expression levels. When ER homeostasis is disrupted, misfolded and unfolded proteins accumulate, leading to a state of ER stress. The unfolded protein response is a response to ER stress in which cellular apoptosis may be triggered if the stress cannot be relieved (Toppings *et al.,* 2018).

2.3.4.4Alströmsyndrome

Alström Syndrome is a rare autosomal recessive monogenetic disorder caused by mutations in the gene Alström Syndrome protein (ALMS1) chromosome 2p13. The clinical features begin as early as at birth which include cone-rod retinal dystrophy, sensorineural hearing impairment, insulin resistance obesity and in some cases congestive heart failure (CHF) due to dilated cardiomyopathy (DCM) (Marshall *et al.,* 2017).

Multidisciplinary approach is essential for the management of patients with AS and early diagnosis and intervention can help slow the progression of multi-organ dysfunctions and improve quality of life of the patient (Tahini *et al.,* 2020).

2.3.4.5Latent autoimmune diabetes in adults (LADA)

Latent autoimmune diabetes in adults (LADA) commonly referred to as "late onset diabetes" was initially defined as "non-insulin" dependent diabetes which could be a heterogeneous illness that might arise at any age and affects 10% of people older than 35 years and 25% below that age (Banerjee *et al.*, 2019).

Autoimmunity seems to be the first pathological symptom in LADA similar to T1DM. However, patients with LADA often display autoantibodies which is indicative of an autoimmune pathogenesis and the autoimmune process seems to be milder and the progression of beta-cell failure slower. Patients with LADA consistently display higher levels of C-peptide as indicator of insulin secretion and they usually do not require insulin for treatment for some time following diagnosis (Wu *et al.*, 2019).

2.3.4.6Type 3c diabetes mellitus

T3c Diabetes Mellitus (T3cDM) accounts for 5% of diabetics in the US. It is due to chronic pancreatitis leading to the destruction of islet cells. Glucagon and insulin levels are both decreased due to destruction of alpha and beta cells, respectively. This makes the development of diabetic ketoacidosis (DKA) a rare occurrence in these patients, as glucagon is one of the key hormones in the production of ketone bodies (Melki *et al.*, 2019).

The pathophysiology of T3cDM is primarily due to inflammation of the pancreatic beta cells, with associated irreversible fibrosis of islet cell that progresses to islet cell loss. The damage not only involved β-cell mass but also damages the Pancreatic Polypeptide (PP) secreting cells in the early stages of the disease. On the other hand, at the late stage of the disease, it damages the α-cell of islets of the pancreas resulting in a significant reductionof glucagon levels. The

pathogenesis of T3cDM differs significantly from type-2 and type-1 DM where only the β-cell function is compromised and damaged. The pathophysiology of the disease includes functional changes which include insulin deficiency, insulin resistance, pancreatic immune pathogenesis, reduced incretin effect and genetic association with the disease (Bhattamisraa*et al.,* 2019).

2.3.4.7Steroid-induced diabetes

Steroid-diabetes was first coined by Ingle in 1940 to describe hyperglycaemia noted in rats receiving glucocorticoids (Simmons *et al.,* 2012). The effect of glucocorticoids on glucose metabolism is likely due to beta cell dysfunction and insulin resistance in tissues. Glucocorticoids provide a substrate for oxidative stress metabolism increasing lipolysis, proteolysis, and hepatic glucose production(Hwang and Weiss, 2014).Enzymatic activity of 11β-hydroxysteroid dehydrogenase is a well known factor that modifies the biological effects of steroids responsible for glucose intolerance similar to that of T2DM (Tamez-Pérez *et al.,* 2015).Glucocorticoids also impair insulin-mediated glucose uptake by directly interfering with components of the insulin signalling cascade and glucose transporter 4 translocation(GLUT4)(Hwang and Weiss, 2014).

2.3.5Unclassified diabetes mellitus

This describes diabetes that does not clearly fit into other classifications or categories of DM. The classification is used temporarily when there is not a clear-cut diagnostic category especially close to the time of diagnosis (WHO, 2019).

2.3.6Gestational Diabetes Hyperglycaemia first detected during pregnancy

Gestational diabetes mellitus (GDM) is a pregnancy complication, in which women without previously diagnosed DM develop hyperglycaemia during gestation on a background of chronic insulin resistance (Plows *et al.,* 2018).

Studies indicated the occurrence of GDM in sub-Saharan Africa to be 14% and Middle East and North Africa ranged from 8.4 to 24.5%. Research findings also showed that the prevalence of GDM varied to a certain extent with East and West Africa reporting 6 and 14%, respectively (Muche*et al.,* 2019).

Exercise with dietary modifications has been found to lead to improved glycaemic control. Pharmacological intervention is usually employed when conventional therapy of diet and exercise failed (Motha and Dias, 2015).

2.4 PATHOPHYSIOLOGY OF TYPE 2 DIABETES MELLITUS

The key mechanism mainly attributed to the pathophysiology of T2DM depends on the mal functioning of the feedback mechanisms between insulin action and insulin secretion resulting in an abnormal high glucose levels in the blood. In the event of β-cell dysfunction, insulin secretion is drastically reduced, limiting the ability of the body to regulate blood glucose level. Accordingly, it is believed that insulin resistance causes an increase in glucose production in the liver and a decrease glucose uptake in the liver, muscle and adipose tissue. However, it should be noted that both β-cell dysfunction and insulin resistance can occur concurrently leading to a net result of hyperglycaemia, reduced protein synthesis and metabolic derangements (Parveen *et al.,* 2017; Galicia-Garcia *et al.,* 2020).

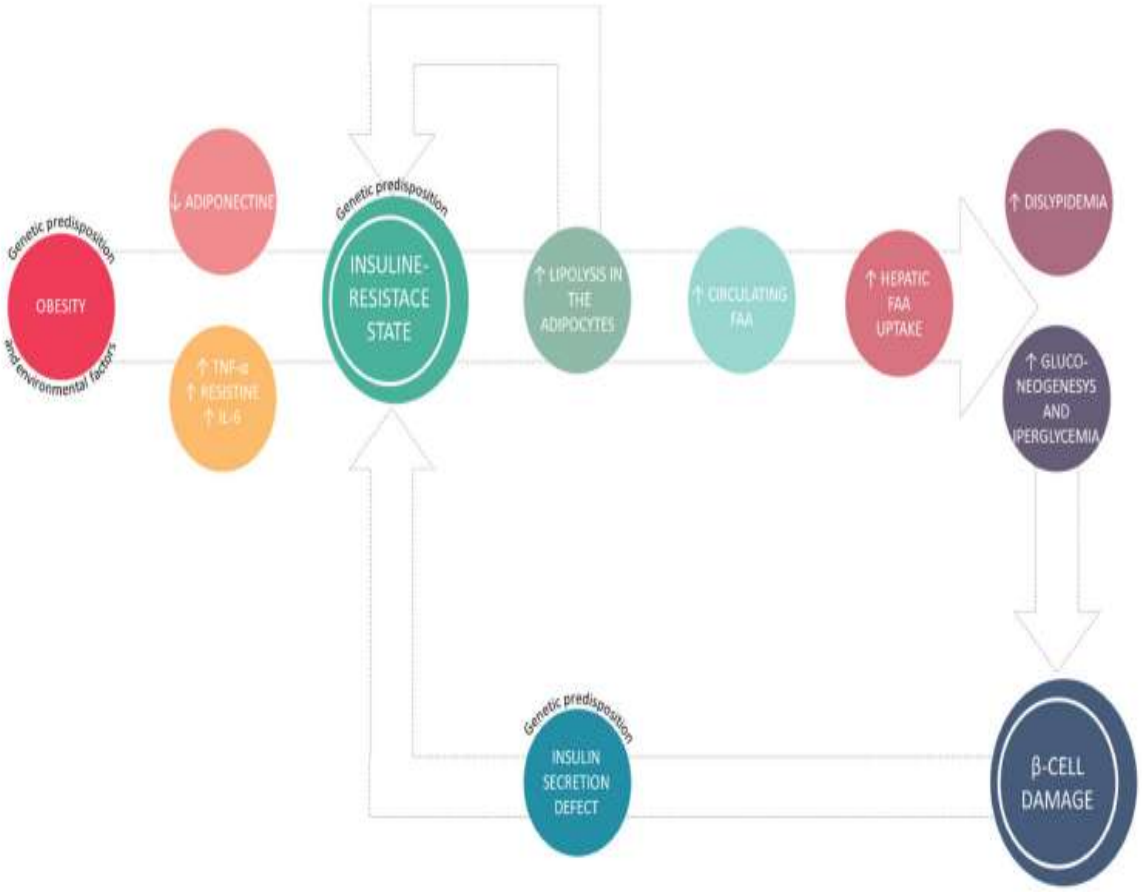

Figure 2.6: Pathophysiology of type 2 diabetes mellitus (T2DM) (Artasensi*et al.*, 2020).

2.5 COMPLICATIONS OF DIABETES MELLITUS

Diabetes mellitus complications are common with T1DM or T2DM which may be deadly if not diagnosed and managed promptly. The complications can be classified as acute or chronic complications.

2.5.1 Acute complications

2.5.1.1 Hypoglycaemia

Hypoglycemia may be defined as an abnormal low plasma glucose concentration that exposes the subject to potential harm with a proposed threshold plasma glucose value <70 mg/dL (Kalra *et al.,* 2013).Hypoglycaemia is common in individuals with diabetes, treated with insulin, sulfonylureas or other insulin secretagogues, because of continuous decrease glucose levels until either insulin boluses or the effect of oral medication are cleared (Heller *et al.*, 2020).

The symptoms of hypoglycaemia can be classified into autonomic and neuroglycopenic. Autonomic symptoms occur at plasma glucose concentrations of approximately 60mg/dL whereas neuroglycopenic symptoms occur at plasma glucose concentrations of approximately 50mg/dL or less (Kittah and Vella 2017).

Autonomic symptoms can further be classified into: adrenergic symptoms that include palpitations, tachycardia, anxiety, tremors; and cholinergic symptoms that include sweating, warmth, nausea and hunger. Neuroglycopenic symptoms include weakness, behavioural changes, visual changes, confusion, dysarthria, dizziness/ lightheadedness, amnesia, lethargy, seizure, loss of consciousness and coma. Brain death has been known to occur in instances when hypoglycemia is protracted (Kittah and Vella, 2017).

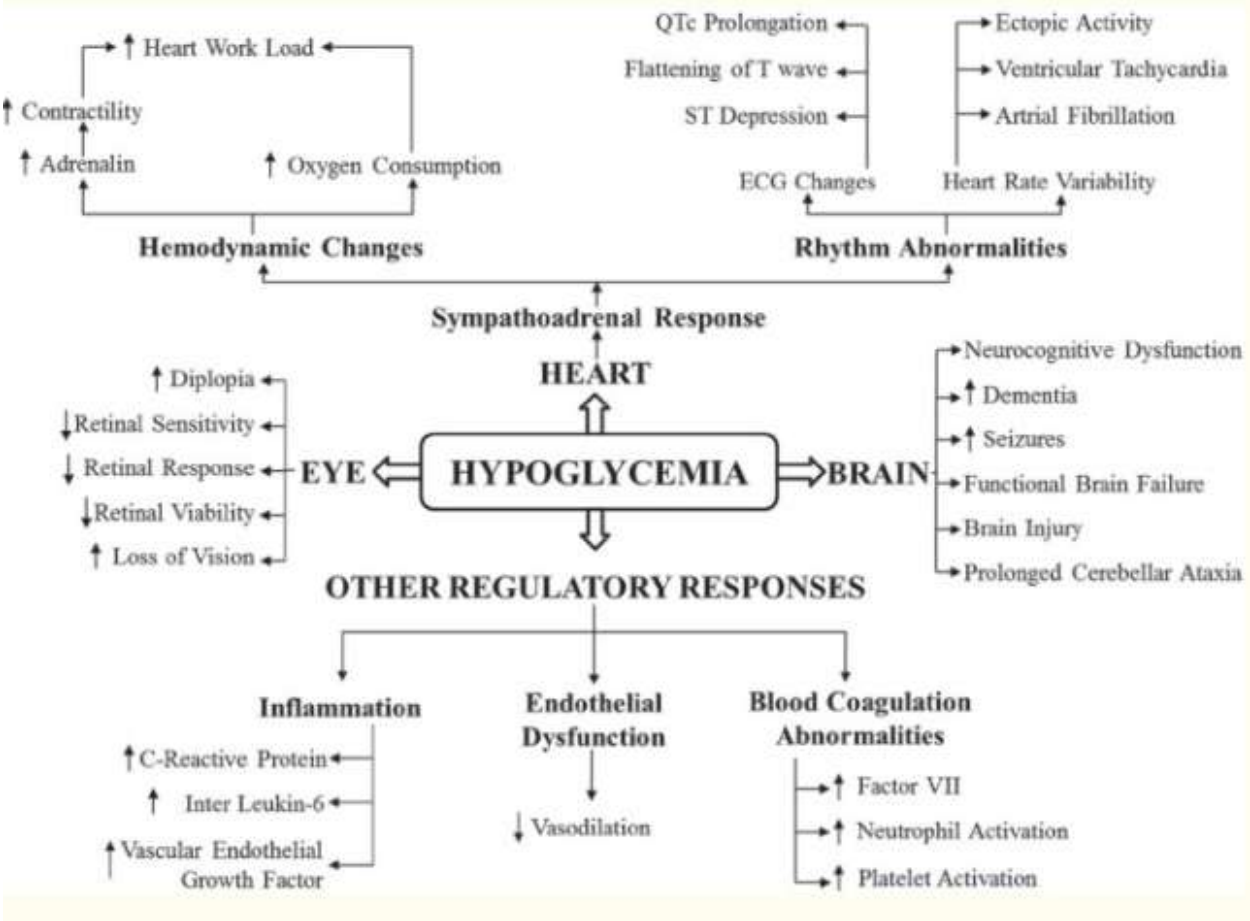

Figure 2.7:Physiological impact of hypoglycaemia on different systems and their counter-regulatory responses (Kalra *et al.*, 2013).

2.5.1.2Diabetic ketoacidosis

Diabetic ketoacidosis (DKA) results commonly from uncontrolled increase in blood sugar level (hyperglycaemia) in T1DM, but may occur in individuals with T2DM. Diabetic ketoacidosis may be associated with a decrease or absence of circulating insulin, cellular starvation, ketogenesis and anion gap metabolic acidosis (Timothy, 2017; Evans, 2019). This may lead to osmotic diuresis with resulting electrolyte loss and volume depletion (Timothy, 2017).

Diabetic ketoacidosis is diagnosed when plasma glucose concentration is above 250 mg/dL, the pH level is less than 7.30, and the bicarbonate level to be 18 mEq per liter or less (Trachtenbarg, 2005).Management of DKA involves prompt fluid replacement, correction of electrolyte imbalance; particularly hypokalaemia, administration of insulin, correction of metabolic acidosis, and treatment of precipitants such as infection, pancreatitis, trauma, and myocardial infarction (Tran *et al.,* 2017).

2.5.1.2 Hyperglycaemic hyperosmolar state

Hyperosmolar hyperglycaemic state (HHS) is a syndrome characterized by severe hyperglycaemia, hyper osmolality, and dehydration in the absence of ketoacidosis. It is the most serious acute hyperglycaemic emergency in patients with T2DM (Pasquel and Umpierrez, 2014).

Hyperglycaemic hyperosmolar state can be precipitated by infections, medications, non-adherence to therapy, undiagnosed diabetes, substance abuse, and coexisting diseases. Laboratory findings in patients with HHS include significant elevations in blood glucose levels greater than 600 mg/dL. The serum osmolarity usually greater than 32 mOsm/L and a pH level greater than 7.30 with mild or absent ketosis (Stoner, 2017).

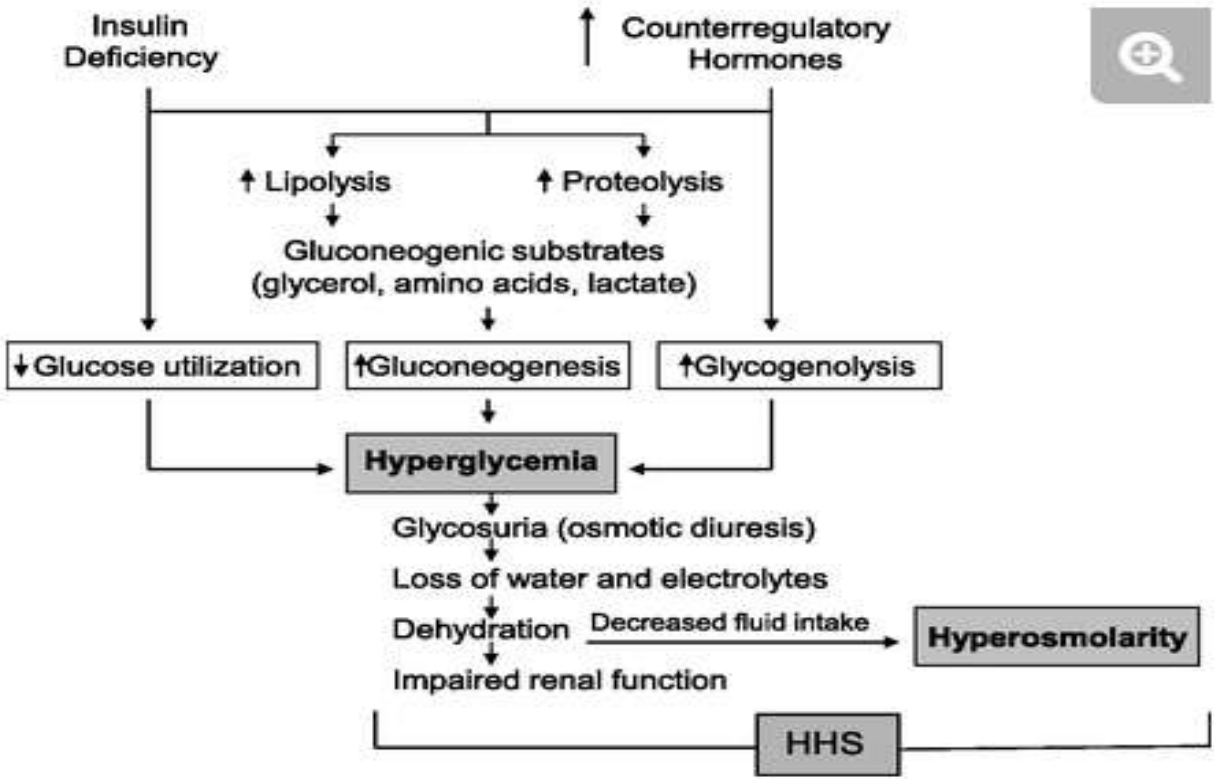

Figure 2.8: Pathogenesis of hyperglycaemic hyperosmolar state (Pasquel and Umpierrez 2014).

2.5.2Chronic complications

2.5.2.1 Diabetic retinopathy

Diabetic retinopathy (DR) is a serious complication of DM and classifiedas a microvascular disease which causes visual impairment and blindness (Hartnett *et al.,* 2017; Wang and Lo, 2018).

Elevated blood glucose levels directly related to hyperglycaemia, are involved in the pathophysiology of DR with inflammation, alteration of retinal blood flow autoregulation, and hemorrheological factors playing an important role in the pathogenesis of DR. Thickening of the

basement membrane, pericyte loss, and disruption of interendothelial tight junctions are characteristic pathophysiological mechanisms in early stages of DR (Corcósteguiet *al.,* 2017).

Diabetic retinopathy is classified in to two broad categories: the earlier stage of nonproliferative diabetic retinopathy (NPDR) and the advanced stage of PDR. The classification of NPDR is based on clinical findings manifested by visible features, including microaneurysms, retinal haemorrhages, intraretinal microvascular abnormalities (IRMA), and venous caliber changes, while PDR is characterized by the hallmark feature of pathologic preretinal neovascularization. An important additional categorization in DR is diabetic macular oedema (DME), which is an important manifestation of DR that occurs across all DR severity levels of both NPDR and PDR and represents the most common cause of vision loss in patients with DR (Duh *et al.,* 2017).

2.5.2.2 Diabetic nephropathy

Diabetic nephropathy (DN) refers to the deterioration of kidney function seen in chronic T1DM and T2DM affecting 20 to 30% of diabetic patients (Shahbazian and Rezaii 2013; Sulaiman, 2019).

Hyperglycaemia is the key of cause of DN with associated production of toxic materials such as advanced glycosylated end product (AGE) and increased activity of aldose reductase (Shahbazian and Rezaii 2013).

The clinical features of DN include albuminuria, which progresses to macroalbuminuria or overt proteinuria over time, microscopic haematuria, which presents only in a small portion of patients, and a low progression rate of renal function. Diabetic nephropathy is divided into 5 stages. The stages 1 and 2 are preclinical stages, characterized by an increase of glomerular filtration rate (GFR), normoalbuminuria (stage 1) or intermittent microalbuminuria (stage 2), and normal blood

pressure. Stage 3 is the onset of clinical stage, characterized by persistent microalbuminuria, mild hypertension, and a normal or slight decline in GFR. Stage 4 is characterized by macroalbuminuria, hypertension, and further decline of GFR. Stage 5 is the end stage of renal disease (Chen *et al.,* 2020).

2.5.2.3 Diabetic neuropathy

Diabetic neuropathy is a chronic complication of both T1DM and T2DM affecting over 90% of diabetic patients. Its pathophysiology is not yet well established but postulated to be due to toxic effects of hyperglycaemia with pain being one of the main symptoms of diabetic neuropathy (Schreiber *et al.,* 2015).

Diabetic neuropathy may present with various types of clinical features with foot ulcer probably being the first presentation. Other symptoms include paresthesia (tingling/pins and needles), numbness and neuropathic pain (often described as burning, lancinating, shooting, or aching) causing great suffering to the individual (Yang *et al.,* 2020).

Prevention and management of diabetic neuropathies focuses on glucose control, pain management and lifestyle modifications (Pop-Busui*et al.,* 2017; Ang *et al.,* 2018).

2.6 STREPTOZOTOCIN

Streptozotocin (STZ) was originally identified in the late 1950's as an antibiotic. It is a naturally occurring compound produced by *bacterium streptomyces achromogene*with a wide spectrum of antibacterial characteristics (Vavra*et al*., 1959; Sharma, 2010). However, its toxic propensity to beta cells of the pancreas which secretes insulin was later discovered and has since been used to create animal models of type 1 diabetes (Mansford and Opie, 1968; Pathak *et al*., 2008). In animal models, it induces hyperglycemic non-ketonic diabetes mellitus synonymous to humans (Bonner-Weir*et al*., 1981). Streptozotocin is known to selectively destroy the insulin producing β-cells via the induction of necrosis (Sharma, 2010). Its action on β-cells is associated with alterations in blood glucose and insulin concentration (Szkudelski, 2001). The presence of glucose moiety in the structure of STZ allows for it transportation through Glucose Transporter 2 (GLUT 2) (Elsner *et al*., 2000). Thus, insulin producing cells which do not express this transporter are resistant to STZ (Lenzen, 2007).

Streptozotocin toxicity in diabetogenesis is attributed to its ability to produce Nitric oxide (NO) which is a bio-regulatory and cytotoxic molecule. This characteristic is thought to be the mechanism through which necrosis is caused in victimized insulin producing cells (Kwon *et al*., 1994). Wada and Yagihashi (2004) reported that nucleic acid alkylation or excessive NO production has been proposed to contribute to STZ-induced beta-cell dysfunction through its inhibition of mitochondrial enzymes. The Deoxyribonucleic Acid (DNA) damage caused by alkylation that is mediated by STZ is being repaired by an excision repair process and requires the activation of the NAD dependent enzyme poly (ADP-ribose) synthetase (Wilson and Leiter, 1990; Sharma, 2010). This process leads to depletion of cellular NAD and ATP and the increased ATP dephosphorylation provides substrate for anthine oxidase which leads to

generation of superoxide radicals and consequently leads to the formation of hydrogen peroxide and hydroxyl radicals (Szkudelski, 2001). STZ has also been proposed to induce apoptosis via the inhibition of the enzyme that is responsible for the reversible intracellular Og1cnacpost-translational modification (Pathak *et al*., 2008; He *et al*., 2009).

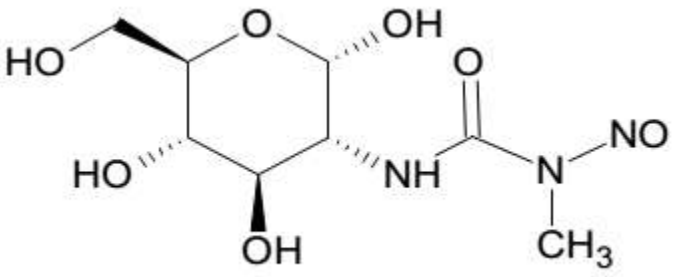

Figure 2.9: Structure of Streptozotocin(Lenzen, 2007).

NICOTINAMIDE

Nicotinamide (Vitamin B_3) is a water soluble pyridine 3 carboxylic acid amide form of niacin. The main source of niacin include meat, liver, green leafy vegetables, wheat, oat, palm kernel oil, legumes, yeast, mushrooms, nuts, milk, fish, tea, and coffee (Bains *et al.*, 2018).Literature showed that induction of rats with streptozotocin/ nicotinamide produced T2DM. Nicotinamide partially protects pancreatic β-cells against streptozotocin by inhibition polymerase-1 of (adenosine diphosphate ribose-ribose) activity and serves as a precursor of nicotinamide adenine

dinucleotide(Pottathil*et al.*, 2020). Nicotinamide is mainly involved in the cellular energy metabolism, DNA repair, and in regulation of transcription process (Bains *et al.*, 2018).

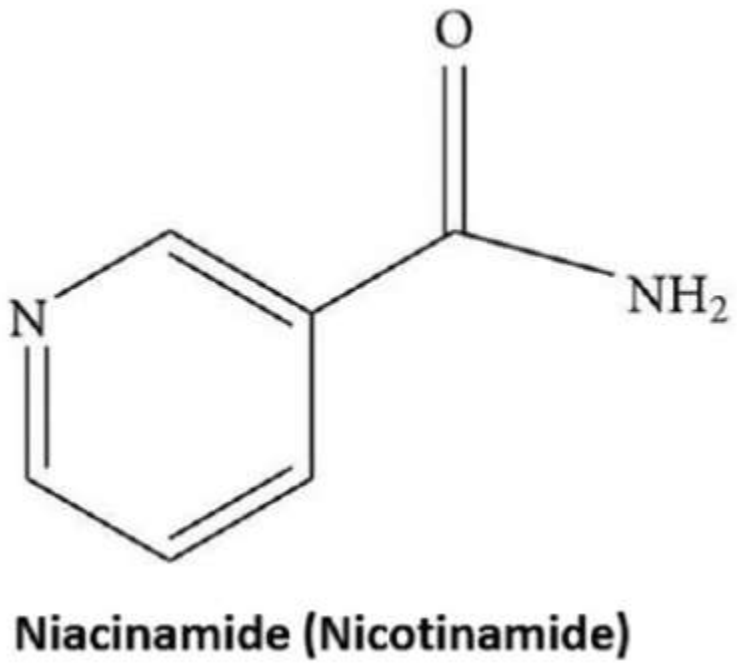

Fig 2.10: Structure of nicotinamide (Bains *et al.*, 2018).

2.7 CABBAGE

Cabbage *(Brassica olaeracae)* belongs to the genus Brassica which is a monophyletic group of 338 and 3700 known genera and species respectively. It constitutes the world's most cultivated vegetables universally except Antartica Franzke*et al.*,2011). It possesses features which makes it easily distinguishable like flora (cruciform corolla with tetradynamous stamens) and fruit morphology. Some of the major vegetable species belonging to the family *B. olaeracae* includes cauliflower, broccoli, cabbage, kale and Brussels sprouts. The white cabbage belongs to the capita group which is 'head'; a derivative from a Latin root word 'capita'. The leaves are formed into distinct varieties of cabbage heads with variations in colour, shape, size and texture of the

leaves thereby predisposing them to survive under different climatic conditions when cultivated (Björkman*et al*., 2011).

The culture and traditional cuisine of several countries features white cabbage as well as its established usage in traditional medicine. The humans consider it as an important source of dietary phyto-nutrients. Because of the ease in storage and its duration of viability, it is found throughout the year and at affordable cost in the local markets. Although it is often used consumed in fresh salads, it can also be boiled, stir fried as well as consumed in fermented product forms. Some cultures consider consumption of white cabbage to bring good luck as such it receives attention from both the rich and the poor; from Caesars to slaves. It also possesses the ability to facilitate digestion. It has also been reported that philosopher Aristotle ate cabbage before drinking alcohol in an attempt to prevent the alcohol 'from fuddling his prudent academic head'. It has also been implicated throughout history in the treatment of various gastrointestinal problems as well as inflammation and blood purification (Hatfield 2004; Cavender 2006; Passalacqua*et al*., 2007).

Itsphytochemicals play vital roles in health promotion the likes of glucosinolates, phenolic compounds, carotenoids and various vitamins, white cabbage has received attention of researchers all around the world. It is regarded as one of the oldest cultivated plants since ancient times although it's difficult to trace its exact history. However, records like the Sanskrit claim that genus *Brassica* were utilized in India as far back as 3000 BC; but some believe that the ancestors of cabbage were grown along coastal Europe nearly 8000 years ago (Franzke*et al.,*2011).

Figure 2.11:A typical white cabbage (*Brassica olaeracae* var. capitata f. alba) farm(Dunja*et al.*, 2016).

The white cabbage has also been utilized historically in the treatment of different maladies as medicinal herbs with myriads of health benefits. In ancient civilizations, it was consumed as a laxative and the juice used to relieve constipation (Hatfield, 2004). It has also been reportedly used as an antidote for mushroom poisoning as well as to prevent the aftermath of drunkenness. It is also interesting to note that white cabbage is still considered as one of the best traditional remedies to cure hangovers and headaches. It has also been explored in the treatment of fevers and prevention of sunstroke. The leaves have been reportedly used to soothe sore feet and to relieve croup in children. It is also implicated in the reduction of breast engorgement during

breastfeeding (Ayers, 2000). Cavender (2006) reported that the inhabitants in southern Appalachia use cabbage leaves for 'cleaning the blood and bowel' as a main concern for both, treating and preventing different illness. In the traditional knowledge in Lebanon, different Brassica species including wild Brassica *olaeracae* are reported to be against neuralgia and rheumatic diseases (Marc *et al*., 2008). In Italy, white cabbage has been traditionally used for healing contusions, rheumatic pains, and wound as well as against scurvy (Passalacqua*et al*., 2007).

Brassica vegetables are known to possess both antioxidants and anticarcinogenic properties (Liang*et al*., 2006; Chu, *et al*., 2002; Verhoeven, *et al*., 1997). In addition to antioxidant vitamins, carotenoids, and polyphenols, Brassica vegetables provide a large group of glucosinolates, which according to Plumb *et al*. (1996) possess rather low antioxidant activity, but the products of their hydrolysis can protect against cancer (Keum *et al*., 2004). Variation in the antioxidant contents of Brassica vegetables is caused by many factors: variety, maturity at harvest, growing condition, soil state, and condition of post-harvest storage (Jeffery *et al*., 2003; Kurilich*et al*., 1999; Lisiewska and Kmiecik, 1996; Vallejo, *et al*., 2002; van der Berg *et al*., 2000). In addition, Brassica vegetables can be cooked in many ways, while cabbage, broccoli and cauliflower may be eaten raw as the ingredients of different salads.

Cabbage (*Brassicaolaeracae* var. capitata) is characterized by their sulfurous aroma which is produced by thioglucosidase hydrolysis of glucosinolates (Chin and Lindsay, 1993; Chin *et al.*, 1996). High consumption of Brassica vegetables is becoming increasingly popular as glucosinolates are defined as anticarcinogenic compounds (Jahangir *et al.*, 2009). Glucosinolates in cabbage Glucosinolates are thioglucosides containing a cyano and a sulfate group. They are important secondary metabolites in *Brassica* vegetables and derived from amino acid

biosynthesis (Rungapamestry*et al.,* 2006; Jahangir *et al.,* 2009). Twelve distinct glucosinolates are present in *Brassica* family out of 100 previously identified glucosinolates (Stoewsand, 1995). In cabbage, sinigrin, glucoiberin and glucobrassicin are predominant glucosinolates (Jahangir *et al.,* 2009). By enzymatic and non-enzymatic changes, glucosinolates breaks down into numerous bioactive compounds and volatile compounds (Ciska and Kozlowska 2001). Myrosinase hydrolyzes glucosinolates, once cabbage tissue is disrupted by slicing, shredding, cooking or chewing (Chin *et al.,* 1996; Ciska and Kozlowska 2001). Cooked cabbage was analyzed to investigate the effect of cooking time on glucosinolate content. Two aliphatic glucosinolates, sinigrin and glucoiberin, and two indole glucosinolates, glucobrassicin and 4-methoxyglucobrassicin were four major glucosinolates in cabbage. In the first five min of cooking, glucosinolate amount decreased 35 %. After first five min, glucosinolate amount decreased 10-15 % in every five min during 30 min of cooking time. Among all glucosinolates, glucoiberin was found to be more thermolabile, as a higher rate of change was observed for it (Ciska and Kozlowska 2001). In another study 30 min boiling of Brassica vegetables caused a significant decrease, 58-77 %, on glucosinolates (Song and Thornalley 2007). When boiling water was analyzed, it was shown that glucosinolates leached out into the boiling water. The other cooking methods, stir-fry cooking for 0-5 min, steaming for 0-20 min and microwave cooking for 0-3 min did not significantly affect glucosinolate content of Brassica vegetables (Song and Thornalley 2007).

2.8 Fermentation

Fermentare is the latin word from which the word ‘fermentation’ was derived meaning “to leaven.” The practice of fermentation can be traced back to ancient civilizations, deeply rooted in significant number of cultures universally. However, this practice has now gain ascendancy from

household consumption to commercial application in industries around the world aimed at industrial scale production. Although there are various schools of thoughts, one common denominator in the definition of this practice is “transformation of food” which could either be by bacteria, fungi and enzymes activities (Katz, 2012). More so, it is vital to note that fermentation also employ the use of yeast for the transformation of foods and beverages (Giraffa, 2004). Fermented food and beverages taste and smell nice to humans due to the enzymatic alterations carried out by microorganisms (Steinkraus, 1997). It is also important to note that fermentation is also employed to enhance food palatability (Azokpota, 2015) as well as production of variations of food (Rogers, 2008). The different types of food substances prepared via fermentation is predicated upon different cultures as well as the availability of food sources, taste choices, surrounding conditions and the need at the time (Prajapati and Nair, 2008).

Food fermentation is a process that employs the growth and metabolic activity of microorganisms for the stabilization and transformation of food materials. Fermentation was principally developed for the stabilization of perishable agricultural produce(Lavefve*et al.*,2019).Fermentation allows for the generation of scent patterns as well as health benefits peculiar to each product consumed. This process has become vital globally for beverages production. Some of the potential health benefits of fermented foods that have been explored in the recent past were based on an extensive body of anecdotal information, and included such benefits as: antihypertensive activity (Ferreira *et al*., 2007; Nakamura *et al*., 2013; Koyama *et al*., 2014; Ahren*et al*., 2014), blood glucose-lowering benefits (Kamiya*et al*., 2013; Oh *et al*., 2014), antidiarrheal (Kamiya*et al*., 2013; Parvez *et al*., 2006), and antithrombotic properties (Kamiya*et al*., 2013). The comprehensive evaluation of fermented food contents and how they may provide health benefits has led to the targeted identification of certain vitamins, minerals, amino acids,

and phytochemicals (eg, phenolics, fatty acids, and saccharides) that distinguish fermented foods from their nonfermented forms (Rodgers, 2008; Rodriguez *et al*., 2009; Capozzi*et al*., 2012; Sheih*et al*., 2014; Hu *et al*., 2015). Furthermore, the evidence for bioactive components resulting from fermentation of plants and animal products is rapidly increasing with the application of new technologies, such as metabolomics (Lee *et al*., 2009; Yang *et al*., 2009; Kim *et al*., 2012; Liu *et al*., 2014). Notably, the concentrations of bioactive compounds available from fermented products may be dependent on the geographic regions from which the starting product was produced, genetic strains of bacteria utilized, the availability of specific substrates in the fermentation process, the environmental conditions, such as seasonality, and method of preparation or manufacturing process (Nikolopoulou*et al*., 2006; Starr *et al*., 2015). Fermentation may be utilized as a starting process for some food products that will undergo multiple additional steps once the fermentation has been terminated; such as in coffee, chocolate, and sourdough breads processing (Corsetti and Luca, 2007; Crafack*et al*., 2014; Keller *et al*., 2013; Lee *et al*., 2015). Fermentation can also stand strong to signify the final product, as in the case of vinegar-tasting kombucha (Teoh *et al*., 2004) or pungent-smelling blue cheese (Nelson, 1970). One method by which fermented foods may exert beneficial health effects is through bioactive compounds, which are small molecules that confer a biological action, and that may result from chemical changes during the fermentation process (Martins *et al*., 2011). Well-known bioactive components produced and made bioavailable through fermentation include phenolic compounds, which may act as natural antioxidants and immune modulators (Dueñas*et al*., 2005; Martins *et al*., 2011). Fermented foods are known to produce bioactive compounds beyond those found naturally occurring in their unprocessed form (Martins *et al*., 2011). Production of bioactive compounds in fermented foods can differentially occur depending on the specific variety or plant

cultivar. Fermented soybeans, commonly consumed in Korea, China, Japan, Indonesia, and Vietnam, represent an example of a major staple food that has consistently been reported for exhibiting antidiabetic effects. The fermented soybean properties are, in part, due to both quantitative and qualitative changes seen in small molecules following fermentation (Kwon *et al*., 2010). There is a growing body of scientific literature to suggest that fermented foods exhibit promising and sustainable opportunities to target a multitude of disease conditions affecting diverse populations globally. Populations may vary, either equally or preferentially, in the process of furthering information on the health benefits associated with specific types of fermented foods and beverages consumed in the diet.

2.8.1 Benefits of fermentation

The beneficial effects of fermentation were unknown in the past and so many people predominantly use fermentation as a means to preserve foods, boost shelf life and improve flavour (Sanlier*et al.*, 2017).

2.8.1.1 Flavour Enhancement

Fermentation makes food palatable by increasing its aroma and flavour. This property makes fermented food more acceptable and popular than the unfermented foods by consumers (Admassie, 2018).

2.8.1.2 Nutritional quality

Some staple foods such as cereals have poor nutritional values and it appears that fermentation reduces anti-nutritional factors such as phytic acid and tanins in food leading to an increase in the bioavailability of minerals and vitamins (Nkhata, *et al.,*2018).

2.8.1.3 Preservative properties

Fermentation is believed to have been developed in order to preserve foods at the time of scarcity by preserving the food by organic acid and alcohol (Mani, 2018). The preservative activities of lactic acid bacteria (LAB) have been observed in some fermented products such as cereals, and yogurt. Low pH inhibits the growth of pathogenic microorganisms which can cause food spoilage, food poisoning and disease and by doing this, the shelf life of fermented food is prolonged (Admassie, 2018).

2.8.1.4 Antibiotic activities

Lactic acid bacteria (LAB) found commonly in fermented foods applies as a hurdle against non-acid tolerant bacteria, which are ecologically eliminated from the medium due to their sensitivity to acidic environment. Fermentation has been demonstrated to be more effective in the removal of gram negative than the gram-positive bacteria, which are more resistant to fermentation processing(Sharma*et al.*, 2020). In addition, LAB is also known to produce protein antimicrobial agents such as bacteriocins during fermentation. Bacteriocins are peptides that elicit antimicrobial activity against food spoilage organisms and food borne pathogens but do not affect the producing organisms (Mills *et al.*, 2017). It also synthesizes other anti-microbial compounds such as, hydrogen peroxide, reuterin, and reutericyclin. Other antimicrobial activities of fermentation include probiotics production that restores the gut flora in patients suffering from diarrhoea, following usage of antibiotics that destroy the normal flora (Çakır, 2010).

2.9 Liver Enzymes

The liver is known as the primary site of drug metabolism with secondary contributions from other significant tissues such as kidney, brain skin, blood, lungs as well as the gastrointestinal mucosa (Krishna and Klotz, 1994). As a large complex organ, the liver also serves the role of carbohydrate, protein and fat metabolism alongside detoxification activities such as amino acid deamination etc (Burkitt *et al*., 1993). It also maintains blood glucose level via processes such as taking up and storing of glucose as glycogen (glycogenesis), breaking this down to glucose when needed (glycogenolysis) and forming glucose from none carbohydrate sources such as amino acids (gluconeogenesis). Measurement of liver enzymes remains the most practical tool to diagnose liver disease and includes mainly alanine aminotransferase (ALT), an enzyme located inside the hepatocytes, and alkaline phosphatase (ALP), an enzyme in the cells lining the biliary ducts of the liver. Based on elevation of, and ratio between elevations of, these enzymes, hepatocellular, cholestatic or mixed liver injury is diagnosed (Benichou, 1990). There is a growing amount of evidence that indicates that the baseline serum levels of liver enzymes may be associated with the development of a wide range of disease outcomes. Several reports have shown that among these enzymes, elevated baseline levels of gamma-glutamyl transferase (GGT) and alanine transferase (ALT) are each associated with increased risk of future type-2 diabetes mellitus (T2DM) (Kunutsor *et al.,* 2014).

2.9.1 Alkaline phosphatase (ALP)

Alkaline phosphatase originates primarily from two sources: liver and bone. The liver enzymes may be present in a variety of other tissues including the kidney, placenta, intestine and white blood cells. Alkaline phosphatase elevation may be physiological or pathological. The

physiological role of these enzymes is not clear but elevation of its production increases in tissues undergoing metabolic stimulation (Limdi and Hyde, 2003).

Alkaline phosphatase is responsible for the transportation of metabolites across cell membranes. Pathological increase in ALP is usually associated with liver and bone diseases (Gowda *et al*., 2009). This enzyme is also found in mucosal epithelia of small intestine as well as the proximal convoluted tubule of the kidney. In the intestine it plays the role of lipid transport as well as calcification of the bone. Although the serum activity of ALP is from the liver, about 50% is contributed by the bone (Mauro *et al*., 2006). Elevated serum ALP may indicate a positive active bone formation occurring as ALP is a byproduct of osteoblast activity or a disease that affect blood calcium level (hyperparathyroidism), vitamin D deficiency, or damaged liver cells (Sharma *et al.*, 2014).

An extensive evaluation is often not needed in those patients who have only a mild elevation of serum alkaline phsophatase (less than 50% elevation). Such patients may be observed clinically with periodic monitoring of serum liver biochemical tests. Whenever alkaline phosphatase levels are deemed to be abnormally elevated, further evaluation should be done to determine whether the source is hepatic or non-hepatic. Hepatic source for an elevated alkaline phosphatase level is supported by the concomitant elevation of either gamma-glutamyl transpeptidase (GGTP) or 5'-nucleotidase (5NT) (Lowe and John, 2018). Normal serum ALP is 41 to 133U/L. Drug-induced liver injury may present with a cholestatic pattern (preferential increase in ALP or ALT/ALP ratio < 2), although the degree of ALP alteration is variable and may be accompanied by hyperbilirubinemia (Velayudham and Farrell, 2003).

2.9.2 Alanine aminotransferase (ALT)

Alanine transaminase (ALT) is found in blood and many tissues including the kidney, heart, muscle and in higher concentration the liver. Liver cell injury can cause a marked elevation ofALT into the extracellular space and ultimately plasma. Among the liver bio-markers, ALT is considered to be a sensitive and translatable indicator of hepatocellular injury (Gwoda*et al.*, 2009).

Alanine transaminase is purely cytoplasmic catalyzing the transamination reaction (Mauro *et al*., 2006). Normal serum ALT is 7-56 U/ L. Any type of liver cell injury can reasonably increases ALT levels. Elevated values up to 300 U/L are considered nonspecific. Marked elevations of ALT levels have been observed most often in persons with diseases that affect primarily hepatocytes such as viral hepatitis, ischemic liver injury and toxin-induced liver damage(Gwoda*et al.*, 2009). Despite the association between greatly elevated ALT levels and its specificity to hepatocellular diseases, the absolute peak of the ALT elevation does not correlate with the extent of liver cell damage (Kallei*et al*., 1964). In addition, increased ALT level was associated with reduced insulin sensitivity, adiponectin and glucose tolerance as well as increased free fatty acids and triglycerides (James *et al*., 2006). Furthermore, there may be instances of hepatic injury where ALT activity is not elevated due to inhibitory factors such as Vitamin B12 deficiency or interference by the presence of pyridoxal-5′-phosphate inhibitors such as isoniazid or lead. Due to the nonspecificity of ALT to hepatocellular injury, additional tests are often used to help interpret ALT values. In human clinical trials, it is an acceptable and recommended practice to interpret a greater than 3x elevation of ALT above the upper limit of normal (ULN) combined with total bilirubin (TBILI) elevation of greater than 2x above ULN as indicative of severe injury with/without any other evidence (Aulbach and Amuzie, 2017).

2.9.3 Aspartate aminotransferase (AST)

AST catalyze transamination reaction. AST exist in two different isoenzyme forms which are genetically distinct, the mitochondrial and cytoplasmic form. AST is found in highest concentration in heart compared with other tissues of the body such as liver, skeletal muscle and kidney (Mauro *et al*., 2006). Normal serum AST is 0 to 35U/L and elevated mitochondrial AST is seen in extensive tissue necrosis during myocardial infarction and also in chronic liver diseases like liver tissue degeneration and necrosis (Diana, 2007). About 80% of AST activity of the liver is contributed by the mitochondrial isoenzyme, whereas most of the circulating AST activity in normal people is derived from the cytosolic isoenzyme (Aulbach and Amuzie, 2017). However, the ratio of AST/ALT has diagnostic importance in distinguishing between different types of diseases (Panteghini *et al.,* 1983). The relative increase in AST compared with ALT can be useful in supporting a diagnosis of alcohol injury (AST/ALT > 2) versus most other acute liver injuries (AST/ALT ≤ 1), although cirrhosis is also associated with AST/ALT ratio greater than 1(Gwoda*et al.,* 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals/ Reagents

All chemicals used and drugs (streptozotocin, nicotinamide and metformin) were of analytical grade and purchased from Sigma Chemical Company St, Louis USA.

3.1.2 Equipment

Digital glucometer and strips (Accu-check advantage®, Boche Diagnostic Company), spectrophotometer and weighing balance (GF 2000) were used for the experiment.

Assay kits for thyroid hormones,insulin and insulin receptors were purchased from Wuhan Fine Test (China). Analyses were done using Rayto® micro plate reader.

3.1.3 Plant Material

Fresh cabbage was purchased in August 2018 from Kubani farm Zaria, Kaduna State. Confirmation was done by a Taxonomist in the Herbarium section of Department of Botany, Ahmadu Bello University, Zaria and a voucher number (43382) was given for future reference.

3.1.4 Animals

A total of thirty (30) male Wistar rats weighing 150 to 300g (8-10 weeks old) were used for the study. The rats were obtained from the animal house, Department of Human Physiology, Ahmadu Bello University, Zaria. They were kept in well aerated laboratory cages, under room temperature and lightning. Feeds were compounded from grower's mash (Vital Feeds ®Company Plc. Jos) and drinking water was provided *ad libitum*. Ethical clearance was obtained from the

Animal Use and Care Committee, Ahmadu Bello University, Zaria and approval number (ABUCAUC/ 2018/ 068) was given for future reference.

3.2 Methods

3.2.1 Collection and Preparation of Fermented Cabbage

Fresh cabbage was washed, trimmed and the outer dirty leaves were removed. For every 1 kg of cabbage, 30 g of salt was sprinkled in layers and a clean cloth placed above the cabbage with a weight to compress it and fermentation was allowed for 5 to 7 days (Battcock and Azam-Ali, 1998; Enwa, 2014).

The addition of salt during fermentation restricts the growth of gram-negative bacteria and enhances the growth of lactic acid bacteria (LAB).The LAB tolerates high salt concentrations, which gives them an advantage over other less salt-tolerant species and allows the LAB to produce acid that inhibits the growth of undesirable microorganisms and for this reason, initiates the majority of lactic acid fermentations (Mani, 2018). After fermentation the cabbage was then shade dried, weighed and added (moulded) with the animal feed (growers mash) at 12.5 %, 25 % and 50 % proportions made up to 1 g of feed (Muhammad *et al.*, 2016).

3.2.2 Experimental Induction of Type 2 Diabetes Mellitus

Diabetes Mellitus was induced in overnight fasted male Wistar rats(6-8 hours) by administering a single dose of freshly prepared sodium citrate buffer solution of streptozotocin (STZ), 65 mg/Kgintraperitoneal (IP) in 50 mM cold citrate buffer (pH 4.5), 15 minutes after IP administration of 120 mg/Kg Nicotinamide (NA) (Balaji*et al.,* 2020). The animals were continued to be fed and maintained with 10% glucose solution after 6 hours of STZ/ NA injection for the next 24 h to prevent hypoglycaemia(Ghasemi*et al.,* 2014).On day 7 after STZ

/NA treatment, venous blood was collected from tail vein to determine the fasting blood glucose level of the rats (Donovan and Brown, 2006) using One touch basic glucose monitoring machine (Accu-check advantage®, Boche Diagnostic Company). Rats having fasting blood glucose level > 150 mg/dl were used for the study (Furman, 2015).

3.2.3 Experimental Design

The study was carried out on STZ/ NA induced diabetic Wistar rats. The rats were fasted for 6-8 hours with free access to water prior to the induction of diabetes. After induction of DM, the rats were randomly divided into 5 groups of 6rats each as follows:

Group 1: Normal control (NC) rats that receiveddistilled water (1 ml/Kg) orally daily as for four weeks.

Group 2: Diabetic control untreated (DCUT) rats that received distilled water (1 ml/Kg) daily for four weeks.

Group 3: Diabetic control rats treated with metformin (Met) that received 500 mg/kg/day of metformin orally as gavage for four weeks (Rabbani*et al.,* 2009).

Group 4: Diabetic rats that were fed with 12.5 % fermented cabbage supplementation daily for four weeks.

Group 5: Diabetic rats that were fed with 25 % fermented cabbage supplementation daily for four weeks.

Group 6: Diabetic rats that were fed with 50 % fermented cabbage supplementation daily for four weeks (Muhammad *et al.,* 2016).

3.2.4 COLLECTION OF BLOOD SAMPLES AND SERUM PREPARATION

At the end of four weeks of experiment, all animals were sacrificed after an overnight fast of 6-8 hours. They were euthanized using a cocktail of diazepam 5mg/Kg and Ketamine 75mg/Kg (Green*et al.*, 1981). Blood samples (5ml) were collected from all the animals through cardiac puncture. Three millilitres (3ml) of the collected blood sample collected was put in to ethylenediaminetetraacetic acid (EDTA) bottle for haematological analysis. The remaining two millilitres (2ml) of blood was put into plain bottles and allowed to clot and the serum separated by centrifugation (using Denley BS400 centrifuge; England) at 3,000 *g* for 10 minutes. The sera were decanted stored in plain tubes at 4ºC (Nwosu *et al.*, 2009) and used to for the determination of other biochemical parameters.

3.2.5 Determination of blood glucoseconcentration

Blood glucose levels were determined during treatment at week 0, 1, 2, 3 and 4 respectively. The blood samples were collected from the rat's tail veins after an overnight fast (6 to 8 hours). The blood glucose levels were determined using a digital glucometer (Accu-Check Advantage, Roche Diagnostic®, Germany); and the results was expressed in mg/dL (Rheney and Kirk, 2000).

3.2.6 Estimation of serum insulin levels

Serum insulin was estimated using rat serum insulin ELISA kit (Wuhan Fine Biotech Co., Ltd. China)in accordance to the manufacturers manual guide lines.

3.2.6.1 Principle:

This ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on insulin molecules. During incubation, insulin in the sample reacts with anti-insulin antibodies bound to the micro titration well and with horseradish peroxidise HRP-

streptavidin conjugated anti-insulin antibodies. A washing step removes unbound enzyme labelled antibody. The bound conjugate is detected by reaction with 3, 3', 5, 5'-tetramethylbenzidine (TMB, a frequently used chromogenic in ELISAs). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically at absorbance wave length of 450 nm using microplate reader.

3.2.6.2 Procedure

The standard, test samples and control (blank) wells where set on the pre-coated plate and their positions recorded respectively. The plates where washed twice before adding standard, sample and control (blank) wells.

100 µL of standard aliquot(anti insulin antibodies) was added to zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube, 6th tube and control (blank) tube into the standard wells.100 µL of properly diluted sample was then added to the test sample (sera) wells. The plate was then sealed with a cover and incubates at 37°C for 90 minutes. The cover was removed after incubation and the plate content was discarded, and wash plate 2 times with wash buffer. Not allowing the wells to dry completely at any time.

100 µL of biotin-labelled antibody solution was added into above wells (standard, test sample and blank wells at the bottom of each well without touching the sidewall. The plate was covered again and incubated at 37°C for 60 minutes. After incubation, the plate cover was removed and the plate washed 3 times with wash buffer, allowing the wash buffer to stay in the wells for at least 1-2 minutes each time.

100 µL of HRP-streptavidin conjugate was now added into each well; the plate was covered and allowed to incubate at 37°C for 30 minutes. After which the cover was removed and the plate

washed 5 times with wash buffer, and leaving the wash buffer stay in the wells for at least 1-2 minutes each time. 90 µL of 3, 3', 5, 5'- tetramethylbenzidine (TMB) substrate was then added into each well, then covering the plate and incubating at 37°C in dark within 10-20 minutes. 50 µL stop solution was finally added into each well and the colour will turn yellow immediately. The density of yellow colour is proportional to the target amount of sample captured in plate and read spectrophotometrically at absorbance wavelength of 450 nm using a microplate reader.

3.2.7 Estimation of serum insulin receptors

Serum insulin receptor was estimated using rat ISR (insulin receptor) ELISA kit (Wuhan Fine Biotech Co., Ltd. China) in accordance to the manufacturers manual guide lines.

Principle:

This ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on insulin receptor molecules. During incubation, insulin in the sample reacts with anti-insulin antibodies bound to the micro titration well and with HRP-streptavidin conjugated anti-insulin antibodies. A washing step removes unbound enzyme labelled antibody. The bound conjugate is detected by reaction with 3, 3', 5, 5'- tetramethylbenzidine (TMB, a frequently used chromogenic in ELISAs). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically at absorbance wave length of 450 nm using microplate reader.

3.2.7.1 Procedure:

The standard, test samples and control (blank) wells where set on the pre-coated plate and their positions recorded respectively. The plates where washed twice before adding standard, sample and control (blank) wells.

100 µL of standard aliquot(anti insulin receptor antibodies) was added to zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube, 6th tube and control (blank) tube into the standard wells.100 µL of properly diluted sample was then added to the test sample (sera) wells. The plate was then sealed with a cover and incubates at 37°C for 90 minutes. The cover was removed after incubation and the plate content was discarded, and wash plate 2 times with wash buffer. Not allowing the wells to dry completely at any time.

100 µL of biotin-labelled antibody solution was added into above wells (standard, test sample and blank wells at the bottom of each well without touching the sidewall. The plate was covered again and incubated at 37°C for 60 minutes. After incubation, the plate cover was removed and the plate washed 3 times with wash buffer, allowing the wash buffer to stay in the wells for at least 1-2 minutes each time.

100 µL of HRP-streptavidin conjugate was now added into each well; the plate was covered and allowed to incubate at 37°C for 30 minutes. After which the cover was removed and the plate washed 5 times with wash buffer, and leaving the wash buffer stay in the wells for at least 1-2 minutes each time. 90 µL of 3, 3', 5, 5'- tetramethylbenzidine (TMB) substrate was then added into each well, then covering the plate and incubating at 37°C in dark within 10-20 minutes. 50 µL stop solution was finally added into each well and the colour will turn yellow immediately. The density of yellow colour is proportional to the target amount of sample captured in plate and read spectrophotometrically at absorbance wavelength of 450 nm using a microplate reader.

3.2.8 Determination of serum liver enzyme activities

3.2.8.1 Serum Alanine aminotransferase (ALT)

Principle:

Alanine aminotransferase was estimated by the method described by Reitman and Frankel (1957).Alanine aminotransferase (ALT) catalyzes the transfer of the amino group from alanine to oxaloglutarate with the formation of glutmate and pyruvate. The latter is reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD+, proportional to the activity of ALT present in the sample.

L-Alanine + 2-oxoglutarate $\xrightarrow{AST}$ L-Glutamate + Pyruvate

Pyruvate + NADH + H^+ $\xrightarrow{MDH}$ Lactate + NAD^+

Procedure:

1000 µL of the reagent (ALT substrate + ALT coenzyme) was added to 100 µL of the sample, mixed and the incubated for 37ºC for one minute. The change in absorbance of the sample was measured per minute at a wavelength of 340 nm as follows:

Total ALT activity (µL) = ΔA/min x 3333

(ΔA/min) =Average change in absorbance per minute

3.2.8.2 Serum Aspartate aminotransferase (AST)

Principle:

Aspartate aminotransferase (AST) was estimated by the method described by Reitman and Frankel (1957).Aspartate aminotransferase (AST) catalyses the reversible transfer of an amino

group aspartate to α-ketoglutarate forming glutamate and oxalacetate. The oxalacetate produced is reduced to malate by malate dehydrogenase (MDH) and NADH:

L-Aspartate + α-Ketoglutarate $\xrightarrow{AST}$ Glutamate + Oxaloacetate

Oxalacetate + NADH + H^+ $\xrightarrow{MDH}$ Malate + NAD^+

Procedure:

1000 µL of the reagent (buffer + substrate) was be added to 100 µL of the sample, mixed and the incubated for 37ºC for one minute. The change in absorbance of the sample was measured per minute at a wavelength of 340 nm as follows:

Total AST activity (µL) = ΔA/min x 1750

(ΔA/min) =Average change in absorbance per minute

3.2.8.3 Serum Alkaline phosphatase (ALP)

Principle:

Alkaline phosphatase was estimated by method described by Bowersand Mc Comb (1966)Alkaline phosphatases (ALP) catalyze the hydrolysis of a wide variety of physiologic and non-physiologic phosphoric acid esters in alkaline medium. Alkaline phosphatase (ALP) hydrolyzes the colourless p-nitrophenyl phosphate to p-nitrophenol and phosphate in the presence of magnesium ions. The product of enzyme hydrolysis p-nitrophenol, has a yellow color at the pH of the reaction. The rate of p-nitrophenol formation is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance at 405 nm.

p-nitrophenylphosphate + H_2O $\xrightarrow[Mg^{++}]{ALP}$ p-nitrophenol + Phosphate

Procedure:

1000 µL of the reagent(Diethanolamine buffer/ Mg^{++}+ p-Nitrophenyl phosphate) wasadded to 100 µL of the sample mixed and incubated at 37ºC for 10minutes. The change inabsorbance of the sample was measured per minutespectrophotometerically at wavelength of 590nm asfollows:

Absorbance of sample/Absorbance standard × Valueof standard (µ/L)

3.2.8.4 Serum Total Proteins (Albumin/ Globulin)

Principle:

The serum total proteins were determined by Biuret method of Reinhold (1953). Proteins give an intensive violet-blue complex with copper salts in an alkaline medium. Iodide is included as an antioxidant. The intensity of the colour formed is proportional to the total protein concentration in sample.

Procedure:

1000 µL of the reagent (Biuret + Bovine albumin) was added to100 µLof the sample and standard. This was incubated for 5 mins at 37ºC after mixing and the absorbance of the sample (A_{sample}), standard ($A_{standard}$) and blank (A_{blank}) was then be measured against the reagent blank within 30 mins.

$$\text{Total protein in sample (g/dl)} = \frac{\text{(A) Sample - (A) Blank) X (Conc. of Standard)}}{\text{(A) Standard - (A) Blank}}$$

3.2.9 Determination of Lipid Profiles

3.2.9.1 Serum total cholesterol

Principle:

The serum total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by the method of Knight *et al.,* (1972).

Unsaturated lipids react with sulphuric acid to form carbonium ions. In a second step the carbonium ions react with phosphovainilline to give a pink colour. The intensity of the colour formed is proportional to the total lipids concentration in the sample.

Procedure:

1000 µL of the reagent (Phosphovainilline + Phosphoric acid) was added to100 µL of the sample and standard. This was incubated for 15 mins at 37ºC after mixing and the absorbance of the sample (A_{sample}), standard ($A_{standard}$) and blank (A_{blank}) was then be measured against the reagent blank within 1 hour at 520 nm.

$$\text{Total Lipid in sample (mg/dl)} = \frac{\text{(A) Sample - (A) Blank) X (Conc. of Standard)}}{\text{(A) Standard - (A) Blank}}$$

3.2.9.2 Serum triglyceride

Principle:

The serum triglyceride was determined as described by the method of Tietz (1990). Sample triglycerides incubated with lipoproteinlipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). In the last reaction, hydrogen peroxide (H_2O_2) reacts with 4- aminophenazone (4-AP) and p-chlorophenol in presence of peroxidase (POD) to give a red coloured dye:

Triglycerides + $H_2O \xrightarrow{LPL}$ Glycerol + free fatty acids

Glycerol + ATP $\xrightarrow{\text{Glycerol kinase}}$ G3P + ADP

G3P + O_2 $\xrightarrow{\text{GPO}}$ DAP + H_2O_2

H_2O_2 + P - 4AP+ P - Chlorophenol $\xrightarrow{\text{GPO}}$ Quinone + H_2O

The intensity of the colour formed is proportional to the triglycerides concentration in sample.

Procedure:

1000 µL of the reagent (R_1: LPL, GK, GPO, POD and 4-AP + R_2: Triglyceride standard) was added to100 µL of the sample and standard. This was incubated for 15 mins at 37ºC after mixing and the absorbance of the sample (A_{sample}), standard ($A_{standard}$) and blank (A_{blank}) was then be measured against the reagent blank within 1 hour at 505 nm.

Triglyceride in sample (mg/dl) = $\frac{\text{(A) Sample - (A) Blank) X (Conc. of Standard)}}{\text{(A) Standard - (A) Blank}}$

3.2.9.3 Serum High-Density Lipoprotein Cholesterol (HDL-C)

Principle:

The serum triglyceride was determined as described by the method of Tietz (1990). The method depends on the properties of a detergent which solubilises only the HDL so that the HDL-c is released to react with the cholesterol esterase, cholesterol oxidase and chromogens to give colour. The non HDL lipoproteins LDL,VLDL and chylomicrons are inhibited from reacting with the enzymes due to absorption of the detergents on their surfaces. The intensity of the colour formed is proportional to the HDL-c concentration in the sample.

Procedure:

1000 µL of the reagent (R1: cholesterol oxidase, DSBmT + R2: cholesterol esterase, 4-aminoantipyrine, detergent, ascorbic oxidase, peroxidase) was added to100 µL of the sample and standard. This was incubated for 15 mins at 37ºC after mixing and the absorbance of the sample (A_{sample}), standard ($A_{standard}$) and blank (A_{blank}) was then be measured against the reagent blank within 1 hour at 505 nm.

HDLcin sample (mg/dl) = (A) Sample - (A) Blank) X (Conc. of Standard)
(A) Standard - (A) Blank

3.2.9.4 Serum Low Density Lipoprotein Cholesterol (LDL-C)

Principle:

The serum LDL-C level was determined according to the protocol of Friedewald*et al.*, (1972).The assay takes place in two steps.

1. Elimination of lipoprotein:

Cholesterol esters $\xrightarrow{CHE}$ Cholesterol + fatty acids

Cholesterol + O_2 $\xrightarrow{CHOD}$ DAP + H_2O_2

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

2. Measurement of LDLc

Cholesterol esters $\xrightarrow{CHE}$ Cholesterol + fatty acids

Cholesterol + O_2 $\xrightarrow{CHOD}$ DAP + H_2O_2

$2H_2O_2$ +TOOS + 4-AA $\xrightarrow{POD}$ Quinone Pigment

The intensity of the colour formed is proportional to the LDLc concentration in the sample.

Procedure:

1000 µL of the reagent (R_1: Cholesterol esterase, cholesterol oxidase, Catalase, TOOS + R_2: 4-Aminoantipyrine, Peroxidase) was added to100 µL of the sample and standard. This was incubated for 15 mins at 37ºC after mixing and the absorbance of the sample (A_{sample}), standard ($A_{standard}$) and blank (A_{blank}) was then be measured against the reagent blank within 1 hour at 600 nm.

$$\text{LDLcin sample (mg/dl)} = \frac{\text{(A) Sample - (A) Blank) X (Conc. of Standard)}}{\text{(A) Standard - (A) Blank}}$$

3.2.10 Estimation of Thyroid function tests

Serum Triiodothyronine (T_3),Thyroxin (T_4) and Thyroid Stimulating Hormone (TSH) analysis were carried out according to the manufacture's(Wuhan Fine Biotech Co., Ltd. China) manualusing ELISA kit.

3.2.10.1 Principle (T_3)

This ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on T_3 molecules. During incubation, T_3in the sample reacts with anti-T_3antibodies bound to the micro titration well and with HRP-streptavidin conjugated anti-T_3antibodies. A washing step removes unbound enzyme labelled antibody. The bound conjugate is detected by reaction with 3, 3', 5, 5'- tetramethylbenzidine (TMB, a frequently used chromogenic in ELISAs). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically at absorbance wave length of 450 nm using microplate reader.

3.2.10.2 Procedure T_3

The standard, test samples and control (blank) wells where set on the pre-coated plate and their positions recorded respectively. The plates where washed twice before adding standard, sample and control (blank) wells.

100 µL of standard aliquot(anti-T_3 antibodies) was added to zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube, 6th tube and control (blank) tube into the standard wells.100 µL of properly diluted sample was then added to the test sample (sera) wells. The plate was then sealed with a cover and incubates at 37°C for 90 minutes. The cover was removed after incubation and the plate content was discarded, and wash plate 2 times with wash buffer. Not allowing the wells to dry completely at any time.

100 µL of biotin-labelled antibody solution was added into above wells (standard, test sample and blank wells at the bottom of each well without touching the sidewall. The plate was covered again and incubated at 37°C for 60 minutes. After incubation, the plate cover was removed and the plate washed 3 times with wash buffer, allowing the wash buffer to stay in the wells for at least 1-2 minutes each time.

100 µL of HRP-streptavidin conjugate was now added into each well; the plate was covered and allowed to incubate at 37°C for 30 minutes. After which the cover was removed and the plate washed 5 times with wash buffer, and leaving the wash buffer stay in the wells for at least 1-2 minutes each time. 90 µL of 3, 3', 5, 5'- tetramethylbenzidine (TMB) substrate was then added into each well, then covering the plate and incubating at 37°C in dark within 10-20 minutes. 50 µL stop solution was finally added into each well and the colour will turn yellow immediately. The density of yellow colour is proportional to the target amount of sample captured in plate and read spectrophotometrically at absorbance wavelength of 450 nm using a microplate reader.

3.2.10.3 Principle (T_4)

This ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on T_4molecules. During incubation, T_4 in the sample reacts with anti-T_4 antibodies bound to the micro titration well and with HRP-streptavidin conjugated anti-T_4 antibodies. A washing step removes unbound enzyme labelled antibody. The bound conjugate is detected by reaction with 3, 3', 5, 5'- tetramethylbenzidine (TMB, a frequently used chromogenic in ELISAs). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically at absorbance wave length of 450 nm using microplate reader.

3.2.10.4 Procedure T_4

The standard, test samples and control (blank) wells where set on the pre-coated plate and their positions recorded respectively. The plates where washed twice before adding standard, sample and control (blank) wells.

100 µL of standard aliquot(anti T_4 antibodies) was added to zero tube, 1^{st} tube, 2^{nd} tube, 3^{rd} tube, 4^{th} tube, 5^{th} tube, 6^{th} tube and control (blank) tube into the standard wells.100 µL of properly diluted sample was then added to the test sample (sera) wells. The plate was then sealed with a cover and incubates at 37°C for 90 minutes. The cover was removed after incubation and the plate content was discarded, and wash plate 2 times with wash buffer. Not allowing the wells to dry completely at any time.

100 µL of biotin-labelled antibody solution was added into above wells (standard, test sample and blank wells at the bottom of each well without touching the sidewall. The plate was covered again and incubated at 37°C for 60 minutes. After incubation, the plate cover was removed and

the plate washed 3 times with wash buffer, allowing the wash buffer to stay in the wells for at least 1-2 minutes each time.

100 µL of HRP-streptavidin conjugate was now added into each well; the plate was covered and allowed to incubate at 37°C for 30 minutes. After which the cover was removed and the plate washed 5 times with wash buffer, and leaving the wash buffer stay in the wells for at least 1-2 minutes each time. 90 µL of 3, 3', 5, 5'- tetramethylbenzidine (TMB) substrate was then added into each well, then covering the plate and incubating at 37°C in dark within 10-20 minutes. 50 µL stop solution was finally added into each well and the colour will turn yellow immediately. The density of yellow colour is proportional to the target amount of sample captured in plate and read spectrophotometrically at absorbance wavelength of 450 nm using a microplate reader.

3.2.10.5 Principle (TSH)

This ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on TSH molecules. During incubation, TSH in the sample reacts with anti-T_4 antibodies bound to the micro titration well and with HRP-Streptavidin conjugated anti-T_4 antibodies. A washing step removes unbound enzyme labelled antibody. The bound conjugate is detected by reaction with 3, 3', 5, 5'- tetramethylbenzidine (TMB, a frequently used chromogenic in ELISAs). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically at absorbance wave length of 450 nm using microplate reader.

3.2.10.5 Procedure TSH

The standard, test samples and control (blank) wells where set on the pre-coated plate and their positions recorded respectively. The plates where washed twice before adding standard, sample and control (blank) wells.

100 µL of standard aliquot(anti TSH antibodies) was added to zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube, 6th tube and control (blank) tube into the standard wells.100 µL of properly diluted sample was then added to the test sample (sera) wells. The plate was then sealed with a cover and incubates at 37°C for 90 minutes. The cover was removed after incubation and the plate content was discarded, and wash plate 2 times with wash buffer. Not allowing the wells to dry completely at any time.

100 µL of biotin-labelled antibody solution was added into above wells (standard, test sample and blank wells at the bottom of each well without touching the sidewall. The plate was covered again and incubated at 37°C for 60 minutes. After incubation, the plate cover was removed and the plate washed 3 times with wash buffer, allowing the wash buffer to stay in the wells for at least 1-2 minutes each time.

100 µL of HRP-streptavidin conjugate was now added into each well; the plate was covered and allowed to incubate at 37°C for 30 minutes. After which the cover was removed and the plate washed 5 times with wash buffer, and leaving the wash buffer stay in the wells for at least 1-2 minutes each time. 90 µL of 3, 3', 5, 5'- tetramethylbenzidine (TMB) substrate was then added into each well, then covering the plate and incubating at 37°C in dark within 10-20 minutes. 50 µL stop solution was finally added into each well and the colour will turn yellow immediately. The density of yellow colour is proportional to the target amount of sample captured in plate and read spectrophotometrically at absorbance wavelength of 450 nm using a microplate reader.

3.2.11 Determination of haematological indices

Haematological indices (Red blood cell count, Haemoglobin concentrations, Packed cell volume, White blood cell count, Differential white blood cell count and Platelet count) was analyzed using automated haematology analyzer (Systmex model k21N, USA), at Department of Haematology and Blood Transfusion, Ahmadu Bello University, Zaria.

2.2.12 Dataanalysis

Data obtained from each group was expressed as mean ± SEM. The data was statically analysed using one-way ANOVA and *Tukey's post-hoc* test was used to compare the level of significance between the control and experimental groups. All data were evaluated using SPSS version 21.0 software. The value of $p<0.05$ was considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of Fermented Cabbage Supplementation on Fasting Blood Glucose Level inStreptozotocin/Nicotinamide induced Diabetic Male Wistar Rats

Figure 4.1 shows the effect of four-week oral administration of graded doses of fermented cabbage supplementation on fasting blood glucose level in streptozotocin/nicotinamideinduced diabetic male Wistar rats. Treatment of diabetic animals with graded doses of fermented cabbage (12.5%, 25% and 50%) resulted in a significant ($P < 0.05$) steady decrease in blood glucose level and in a dose dependent manner from week 0of treatment (228±14.98, 224.25±8.91 and 239.25±20.79 mg/dl);week 1 (190.75±6.7, 150.75±15.46 and171.75±16.65mg/dl);week 2 (198.75±4.9, 179.5±8.9 and150±17.49mg/dl);week 3 (188.5±4.73, 175.5±9.6 and151.25±17.9mg/dl) and week 4 (193±2.3, 169.75±8.2 and162±15.05mg/dl)respectively, when compared with corresponding diabetic untreated control group(224.1±8.43, 218±7.8, 221.75±1.37, 231.75±3.8mg/dl).

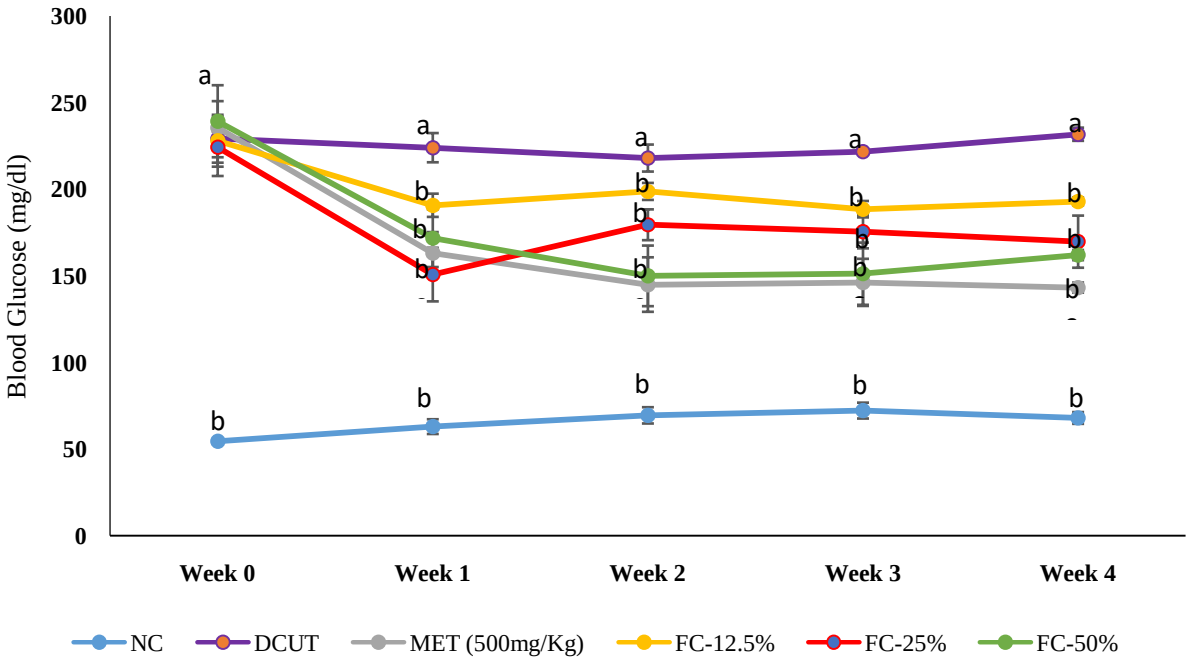

Figure 4.1: Effect of Fermented Cabbage Supplementation on Fasting Blood Glucose Level inStreptozotocin/ Nicotinamide induced Diabetic Male Wistar Rats

a: significant difference compared to normal control

b:significant difference compared to diabetic untreated group

NC= Normal control; DCUT= Diabetic untreated control; FC-12.5%= Diabetic rats treated with 12.5% of fermented cabbage; FC-25%= Diabetic rats treated with 25% of fermented cabbage; FC-50%= Diabetic rats treated with 50% of fermented cabbage, Metformin= Diabetic rats treated with metformin (500 mg/Kg).

4.2 Effect of Fermented CabbageSupplementation on Serum Insulin Level in Streptozotocin/Nicotinamideinduced Diabetic Male Wistar Rats

The serum insulin levels decreased significantly ($P < 0.05$) in the diabetic untreated group (3.5±1.0 pmol/L) following STZ/nicotimide treatment when comparedwith normal control (8.6±1.8pmol/L). However, administration of graded doses (12.5%, 25% and 50%) of fermented cabbage to diabetic rats significantly ($P < 0.05$) increasedthe serum insulin levels (6.8±0.5, 8.1±0.4 and 8.4±0.6 pmol/L), respectively and in a dose dependent manner when compared with diabetic untreated control group(3.5 ± 1.0 pmol/L). Treatment of diabetic rats with the standard drug (metformin500 mg/kg) produced a significantly ($P < 0.05$) elevated serum insulin level (9.6±0.3pmol/L), when compared with fermented cabbage treated groups at various graded dosage of 12.5%, 25% and 50% (6.8 ± 0.5, 8.1 ± 0.4 and 8.4 ± 0.6 pmol/L), respectively.

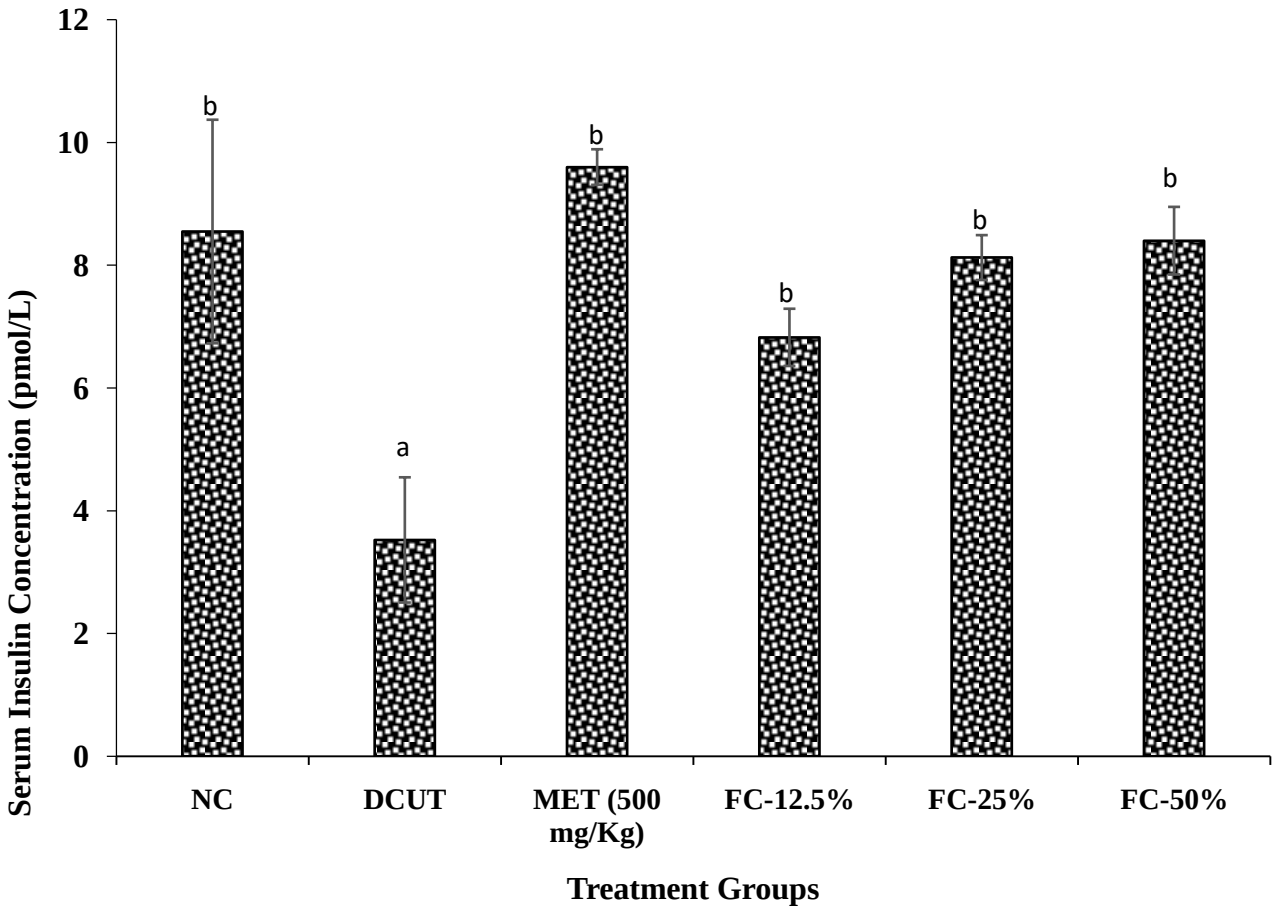

Figure 4.2:**Serum Insulin Levels in Streptozotocin/nicotinamide-induced Diabetic Male Wistar Rats Treated with Fermented CabbageSupplementation for four weeks.**

Bars with different letters (a,b) are statistically significant ($p<0.05$)

NC= Normal control; DCUT= Diabetic untreated control; FC-12.5%= Diabetic rats treated with 12.5% of fermented cabbage; FC-25%= Diabetic rats treated with 25% of fermented cabbage; FC-50%= Diabetic rats treated with 50% of fermented cabbage, Metformin= Diabetic rats treated with metformin (500 mg/Kg).

4.3 Effectof Fermented CabbageSupplementation on Serum Insulin Receptor LevelinStreptozotocin/Nicotinamide inducedDiabetic Male Wistar Rats

The serum insulin receptor levels decreased significantly ($P < 0.05$) in the diabetic untreated group (3.5± 0.9 pmol/L) following STZ/nicotinamide treatment when comparedwith normal control (8.2±1.4pmol/L). However, administration of graded doses (12.5%,25% and 50%) of fermented cabbageto diabetic rats significantly ($P < 0.05$) increasedthe serum insulin receptor levels (6.9±0.5, 6.2±0.2and 7.0 ±0.8 pmol/L), respectively though not in a dose dependent manner compared with the diabetic control untreated group.

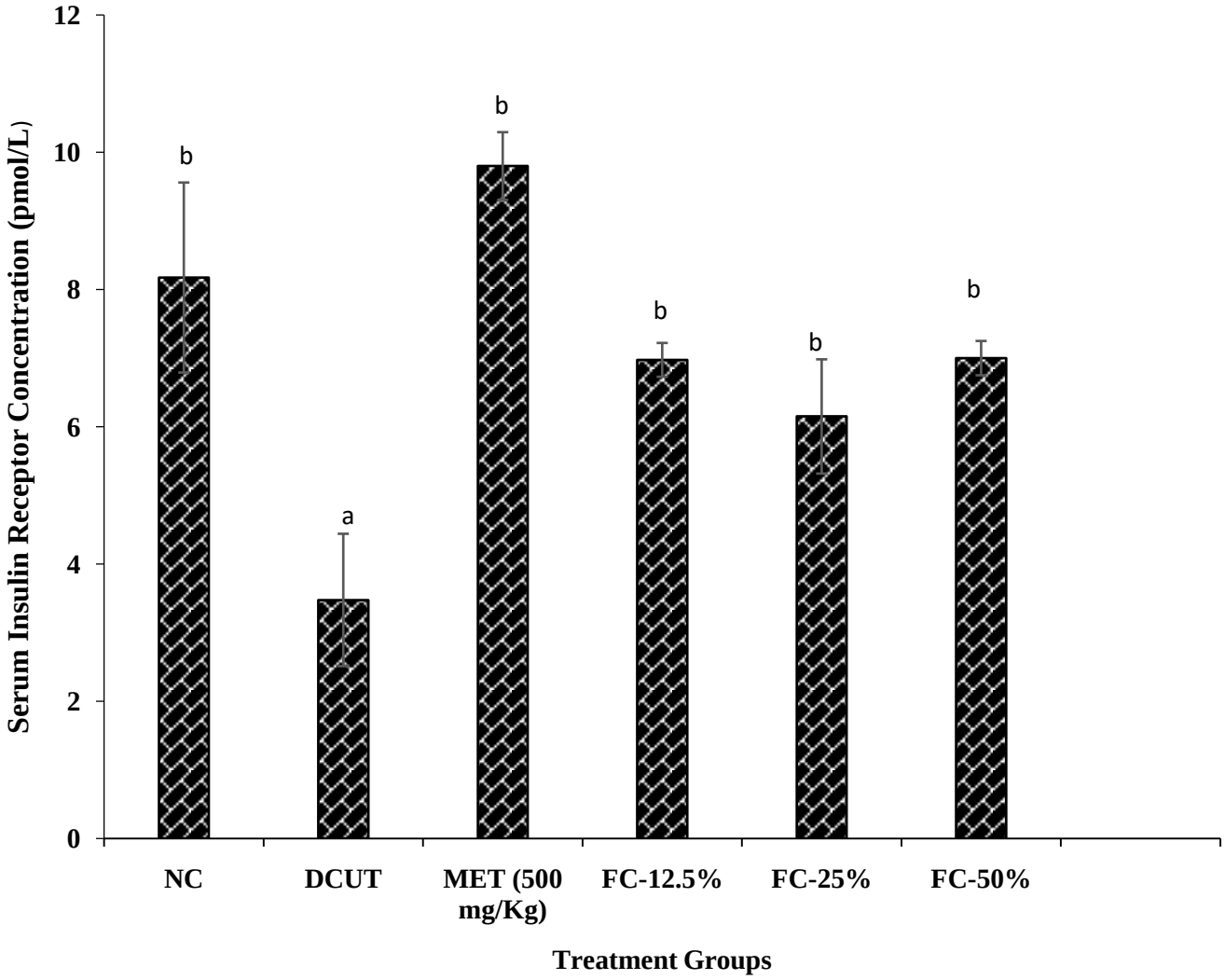

Figure 4.3:**Serum Insulin Receptor Level in Streptozotocin/Nicotinamideinduced Diabetic Male Wistar Rats Treated with Fermented CabbageSupplementation for four weeks.**

Bars with different letters (a, b) arestatistically significant ($P < 0.05$)

NC= Normal control; DCUT= Diabetic untreated control; FC-12.5%= Diabetic rats treated with 12.5% of fermented cabbage; FC-25%= Diabetic rats treated with 25% of fermented cabbage; FC-50%= Diabetic rats treated with 50% of fermented cabbage; MET= Diabetic rats treated with metformin (500 mg/Kg).

4.4 Effectof Fermented CabbageSupplementation on Serum Liver Enzymes Activity inStreptozotocin/NicotinamideinducedDiabetic Male Wistar Rats

4.4.1 Effectof Fermented CabbageSupplementation on Serum Alanine Aminotransferase (ALT)Activity inStreptozotocin/NicotinamideinducedDiabetic Male Wistar Rats

The results obtained showed that the diabetic untreated control rats had significantly ($P < 0.05$) higher ALT activity(74.8±1.7 IU/L), when compared with rats in the normal control group (59.5±0.3IU/L). Oral administration of various doses (12.5%, 25% and 50%) of fermented cabbage produced a significant ($P < 0.05$) lower concentrations of ALT (58.3±2.8, 60.3±2.5 and58.5±1.1 IU/L) when compared with rats in the diabetic untreated control group.

4.4.2 Effectof Fermented CabbageSupplementation on Serum AspartateAminotransferase (AST) Activity inStreptozotocin/NicotinamideinducedDiabetic Male Wistar Rats

The results obtained showed that the diabetic untreated control rats had significantly ($P < 0.05$) higher AST activity(177.5±3.8IU/L), when compared with the rats in the normal control group (134.8±1.3IU/L). Oral administration of various doses (12.5%, 25% and 50%) of fermented cabbage produced significantly ($P < 0.05$) lower AST activities (132.3±4.7, 133±2.1 and134±1.9IU/L) respectively, when compared with rats in the diabetic control untreated group.

4.4.3 Effectof Fermented CabbageSupplementation on Serum Alkaline Phosphatase(ALP) Activity inStreptozotocin/NicotinamideinducedDiabetic Male Wistar Rats

The results obtained showed that the diabetic untreated control rats had significantly ($P < 0.05$) higher ALP activity(82.8±2.4IU/L), when compared with the rats in the normal control group (51.5±0.3IU/L). Oral administration of various doses (12.5%, 25% and 50%) of fermented cabbage produced significantly ($P < 0.05$) lower ALP activities(53.3±2.6, 53.5±1.7 and56±2.6IU/L) respectively, when compared with rats in the diabetic untreated control group.

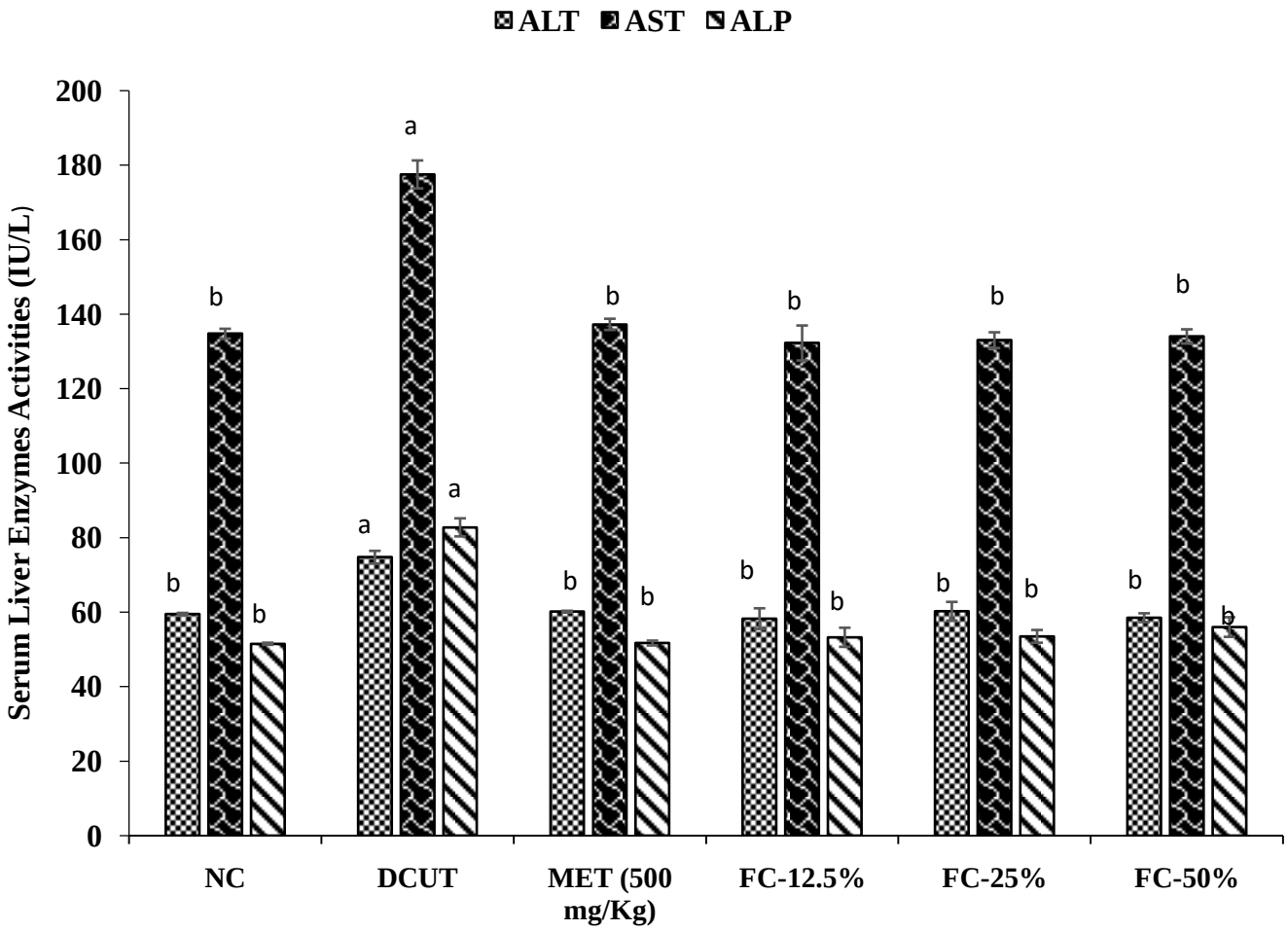

Figure 4.4: Serum Liver Enzymes Activity in Streptozotocin/Nicotinamideinduced Diabetic Male Wistar RatsTreated with Fermented CabbageSupplementation for four weeks.

Bars with different superscript letters (a, b) differ significantly ($P < 0.05$) compared with the control groups (normal and diabetic untreated control); while bars with the same superscript letters were not significantly different ($P > 0.05$) compared with the control groups.

NC= Normal control; DCUT= Diabetic control untreated; FC-12.5%= Diabetic rats treated with 12.5% of fermented cabbage; FC-25%= Diabetic rats treated with 25% of fermented cabbage; FC-50%= Diabetic rats treated with 50% of fermented cabbage; MET= Diabetic rats treated with metformin (500 mg/Kg).

4.5Effect of Fermented CabbageSupplementation on Serum Total Proteinin Streptozotocin/Nicotinamideinduced Diabetic Male Wistar Rats

The results obtained showed that the diabetic untreated control rats had significantly ($P < 0.05$) lowerserum total protein concentration(49.2±1.5g/L), when compared with the rats in the normal control group (61.8±0.6g/L). Oral administration of various doses (12.5%, 25% and 50%) of fermented cabbage produced a significantly ($P < 0.05$) higher serum total protein (64.2±1.7, 69.1±1.4 and64.5±2.1g/L) respectively, when compared with rats in the diabetic control untreated group.

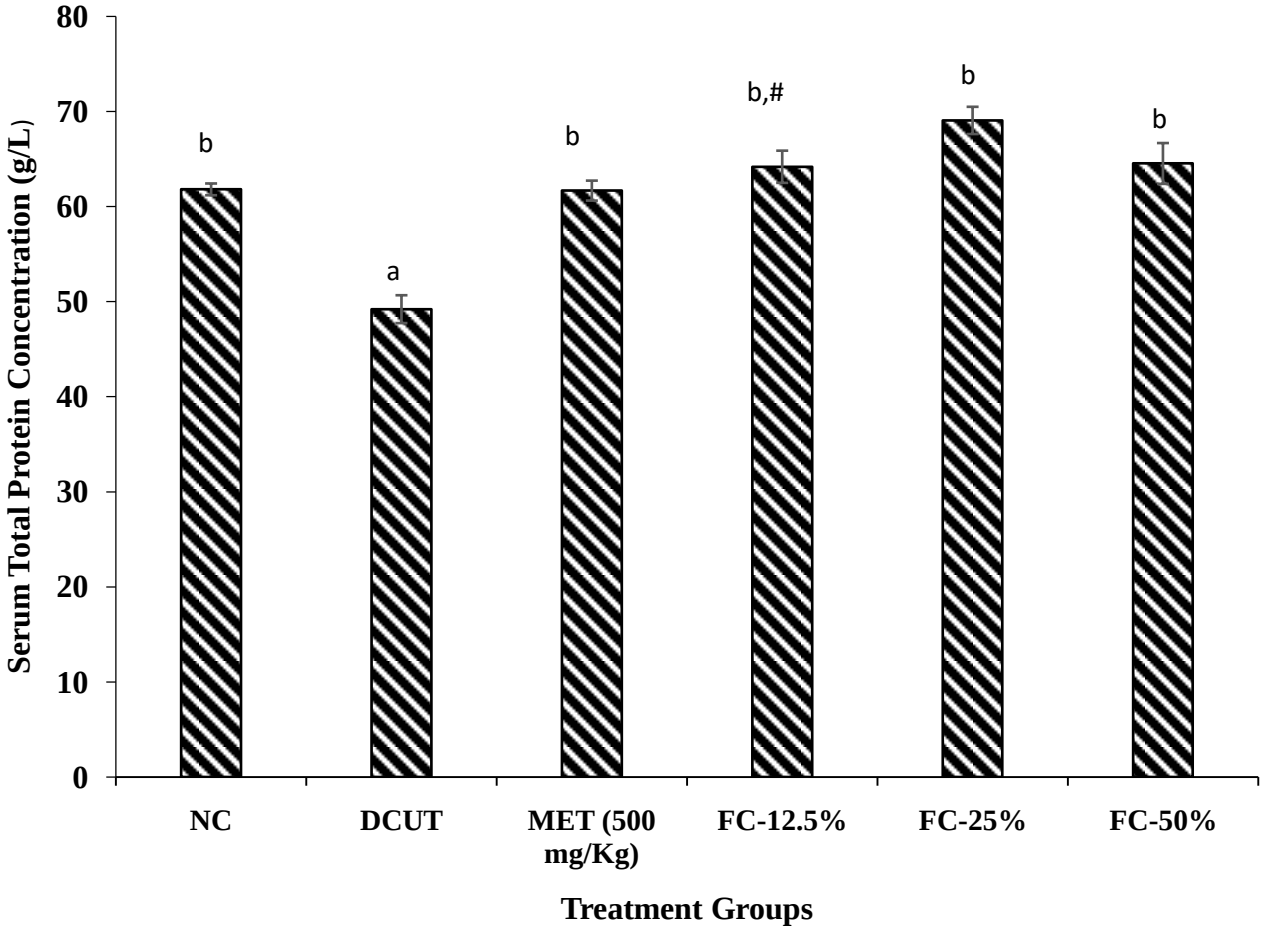

Figure 4.5: Serum Total Protein Concentration in Streptozotocin/Nicotinamideinduced Diabetic Male Wistar Rats Treated with Fermented CabbageSupplementation for four weeks.

Bars with different letters (a, b) differ significantly ($P < 0.05$) compared with the control groups (normal and diabetic untreated control).

#: Differ significantly ($P < 0.05$) compared with standard control (metformin)

NC= Normal control; DCUT= Diabetic control untreated; FC-12.5%= Diabetic rats treated with 12.5% of fermented cabbage; FC-25%= Diabetic rats treated with 25% of fermented cabbage; FC-50%= Diabetic rats treated with 50% of fermented cabbage; MET= Diabetic rats treated with metformin (500 mg/Kg).

4.6 Effect ofFermented Cabbage Supplementation on Serum Lipid ProfileinStreptozotocin/NicotinamideInduced Diabetic Male Wistar Rats

4.6.1Effect on Serum Total Cholesterol (TC)

Figure 4.6:The results obtained showed that the diabetic control untreated rats had a significant ($P < 0.05$) higher serum total cholesterol (186.3±1.4mg/dl), when compared with the rats in the normal control group (83.95±0.3mg/dl). However,oral administration of various doses (12.5%, 25% and 50%) of fermented cabbage induced a significantly ($P < 0.05$) lower serum cholesterol concentrationin a dose dependent manner (128.43±1.0, 116.83±2.0 and112.4±3.9mg/dl) respectively, when compared with rats in the diabetic untreated control group.

4.6.2Effect on Serum Triglyceride (TG)

Fig 4.5: The results obtained showed that the diabetic untreated control rats had a significantly ($P < 0.05$) higher serum triglyceride level(229.68±4.2mg/dl), when compared with the animals in the normal control group (105.07±0.5mg/dl). However,oral administration of various doses (12.5%, 25% and 50%) of fermented cabbage showed a significantly ($P < 0.05$) lower serum level cholesterol in a dose dependent manner (186.23±8.6, 129.83±7.0 and186.05±5.6mg/dl) respectively, when compared with rats in the diabetic untreated control group.

4.6.3Effect on Serum Low Density Lipoprotein-cholesterol (LDL-c)

The results obtained showed that the diabetic untreated control rats had a significantly ($P < 0.05$) higher serum LDL-c level(54.98±2.0mg/dl), when compared with the rats in the normal control group (39.10 ±0.3mg/dl). However,oral administration of various doses (12.5%, 25% and 50%) of fermented cabbage showed a significantly ($P < 0.05$) lower serum LDL-clevel in a dose dependent manner (37.83±2.3, 41.43±2.7 and41.75±1.1mg/dl) respectively, when compared with rats in the diabetic untreated control group.

4.6.4Effect on Serum High Density Lipoprotein-cholesterol (HDL-c)

The results obtained showed that the diabetic untreated rats had a significantly ($P < 0.05$)lower serum HDL-c level(24.87±1.4mg/dl), when compared with the rats in the normal control group (45.95±0.5mg/dl). However,oral administration of various doses (12.5%, 25% and 50%) of fermented cabbage and metformin (500 mg/kg) produced a significantly ($P < 0.05$) higher serumHDL-c level(40.85±0.8, 36.93±4.0, 41.15±1.5mg/dl) and (45.80±1.0mg/dl) respectively, when compared with rats in the diabetic untreated control group.

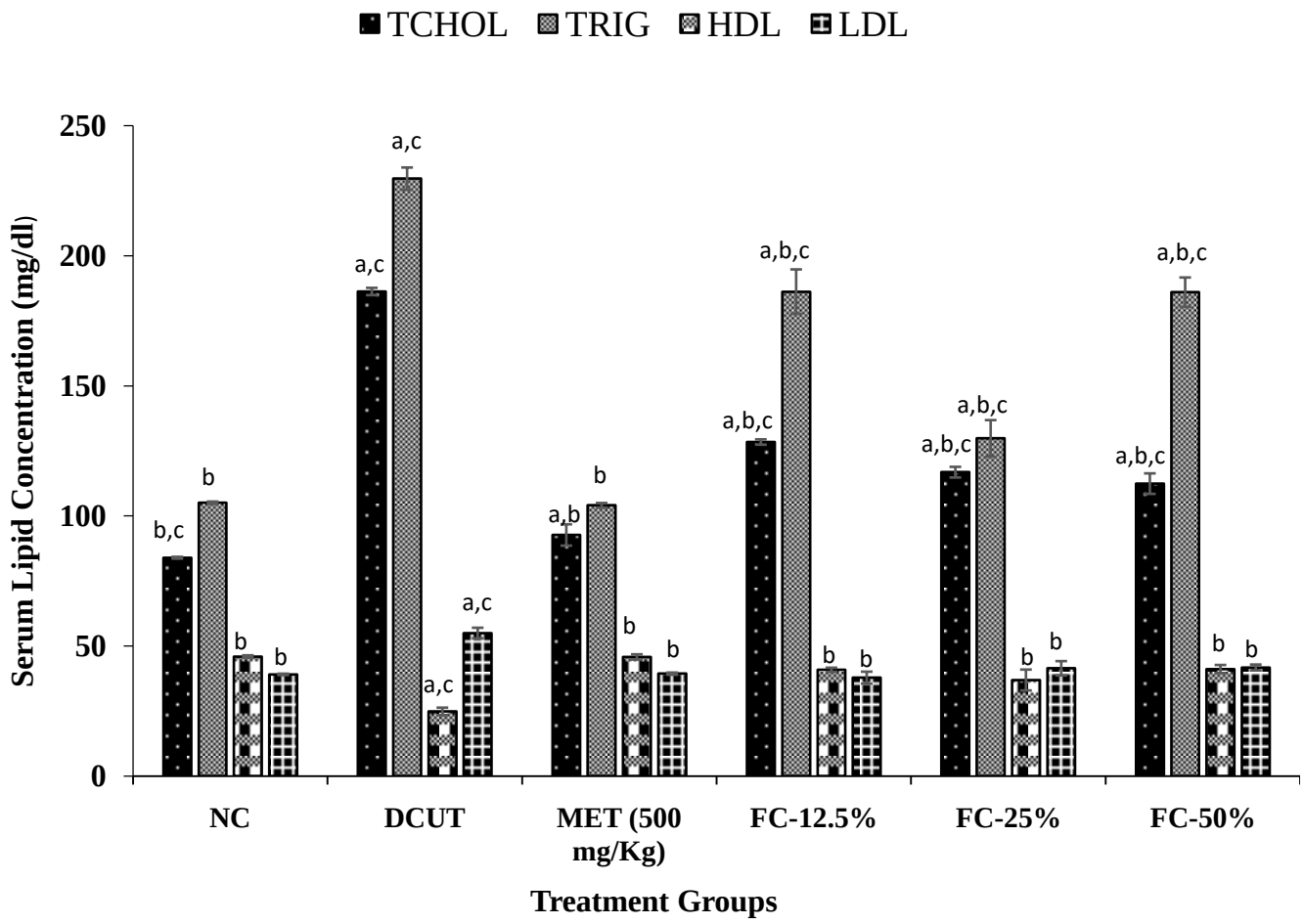

Figure 4.6: Serum Lipid Profile in Streptozotocin/Nicotinamideinduced Diabetic Male Wistar Rats Treated with Fermented CabbageSupplementation for four weeks.

Bars with letters (a, b, c) differ significantly ($P < 0.05$) compared with the control groups (normal, diabetic untreated control and standard) respectively.

NC= Normal control; DCUT= Diabetic control untreated; FC-12.5%= Diabetic rats treated with 12.5% of fermented cabbage; FC-25%= Diabetic rats treated with 25% of fermented cabbage; FC-50%= Diabetic rats treated with 50% of fermented cabbage;MET = Diabetic rats treated with metformin (500 mg/Kg).

4.7Effect of Fermented Cabbage Supplementation on Serum Triiodothyronine (T_3) in Streptozotocin/NicotinamideInduced Diabetic Male Wistar Rats

The serum T_3level wassignificantlylower ($P < 0.05$) in the diabetic untreated group (78.62±3.8ng/dl) when compared with normal control (112.38±11.3ng/dl). However, administration of graded doses (12.5%, 25% and 50%) of fermented cabbage significantly ($P < 0.05$) increase serumT_3level (103.50±5.9, 121.75±1.1and 125.66±4.3ng/dl) respectively, in a dose dependent manner when compared with diabetic untreated control group (78.62±3.8ng/dl).

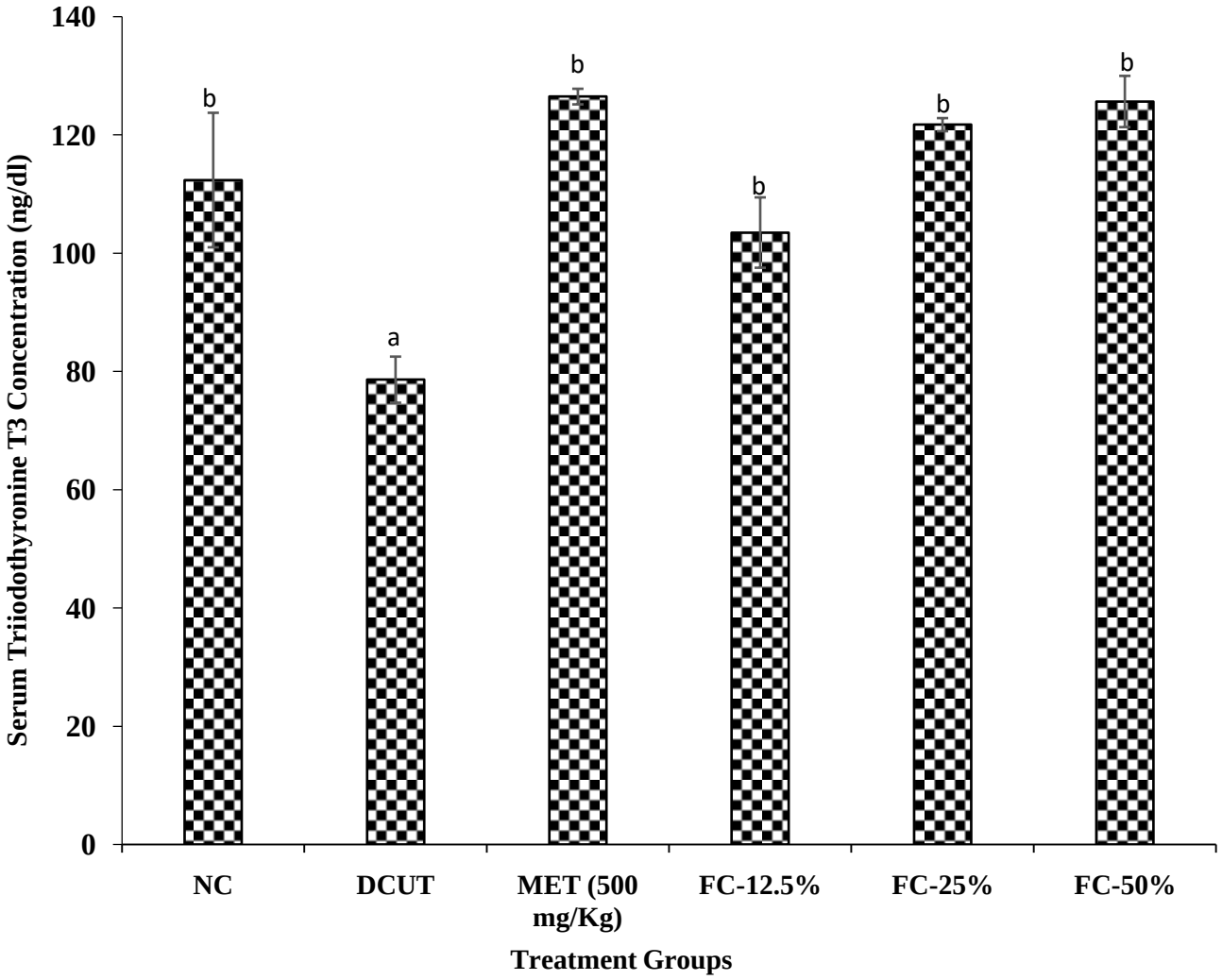

Figure 4.7: Serum Triiodothyronine (T_3) in Streptozotocin/Nicotinamideinduced Diabetic Male Wistar Rats Treated with Fermented CabbageSupplementation for four weeks.

Bars with different letters (a, b) differ significantly ($P < 0.05$) compared with the control groups (normal and diabetic untreated control)

NC= Normal control; DCUT= Diabetic control untreated; FC-12.5%= Diabetic rats treated with 12.5% of fermented cabbage; FC-25%= Diabetic rats treated with 25% of fermented cabbage; FC-50%= Diabetic rats treated with 50% of fermented cabbage; MET= Diabetic rats treated with metformin (500 mg/Kg).

4.8Effect of Fermented Cabbage Supplementation on Serum Thyroxine (T_4) inStreptozotocin/Nicotinamide induced Diabetic Male Wistar Rats

The results obtained showed that the diabetic control untreated rats had a significantly ($P < 0.05$)lower T_4level(5.67±0.7µg/dl), when compared with the rats in the normal control group (7.15±0.4µg/dl). However,oral administration of various doses (12.5%, 25% and 50%) of fermented cabbage induced a significantly ($P < 0.05$) higher serum T_4 levels(7.85±0.3, 8.27±0.1 and7.96±0.2µg/dl) respectively, when compared with rats in the diabetic untreated control group.

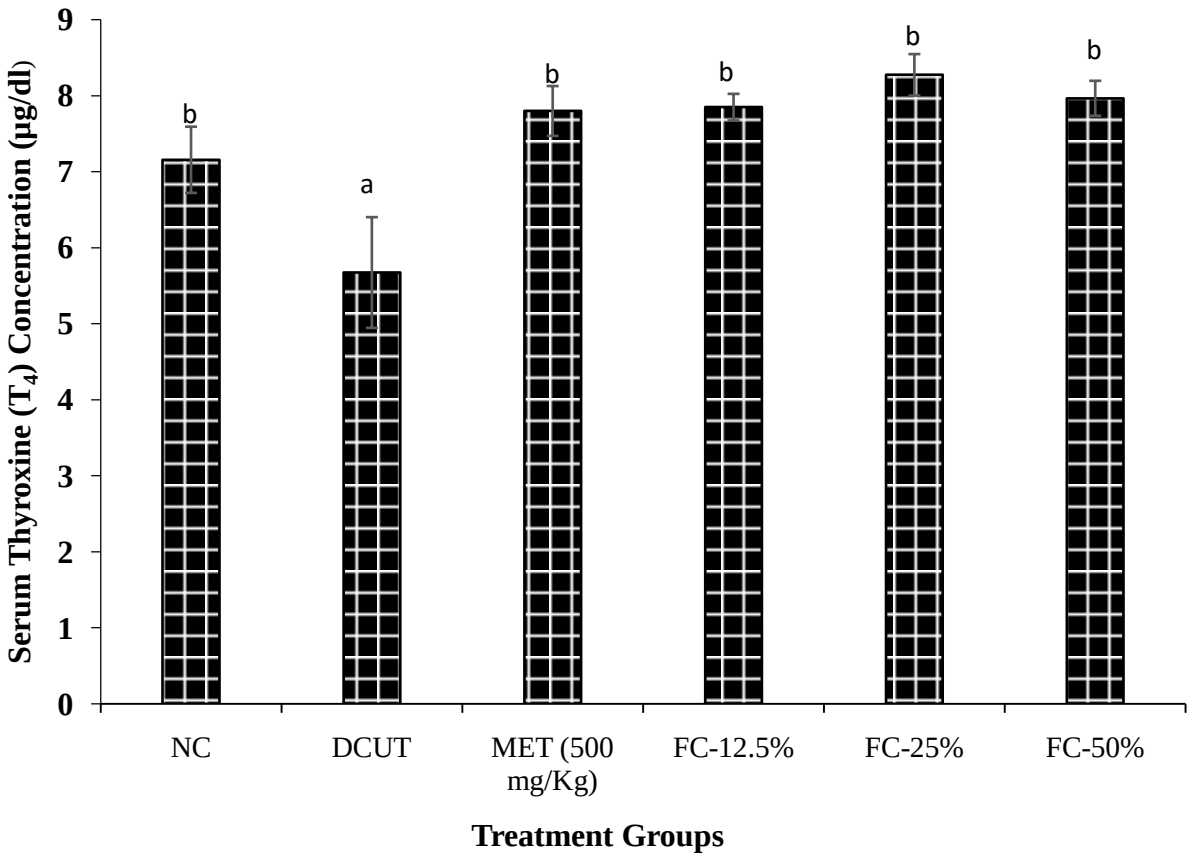

Figure 4.8: Serum Thyroxine (T_4) in Streptozotocin/Nicotinamideinduced Diabetic Male Wistar Rats Treated with Fermented CabbageSupplementation for four weeks.

Bars with different letters (a, b) differ significantly ($P < 0.05$) compared with the control groups (normal and diabetic untreated control)

NC= Normal control; DCUT= Diabetic control untreated; FC-12.5%= Diabetic rats treated with 12.5% of fermented cabbage; FC-25%= Diabetic rats treated with 25% of fermented cabbage; FC-50%= Diabetic rats treated with 50% of fermented cabbage; MET= Diabetic rats treated with metformin (500 mg/Kg).

4.9Effect of Fermented Cabbage Supplementation on Serum Thyroid Stimulating Hormone (TSH) inStreptozotocin/Nicotinamide induced Diabetic Male Wistar Rats

The results obtained showed that the diabetic untreated control rats had a significantly ($P < 0.05$) higher TSH level(3.96±0.4µIU/dl), when compared with the rats in the normal control group (2.81±0.5µIU/dl). However,oral administration of various doses (12.5%, 25% and 50%) of fermented cabbage induced significantly ($P < 0.05$) lower serum TSH levels(2.45±0.2, 2.37±0.2 and2.0±0.4µIU/dl) respectively,when compared with rats in the diabetic untreated control group.

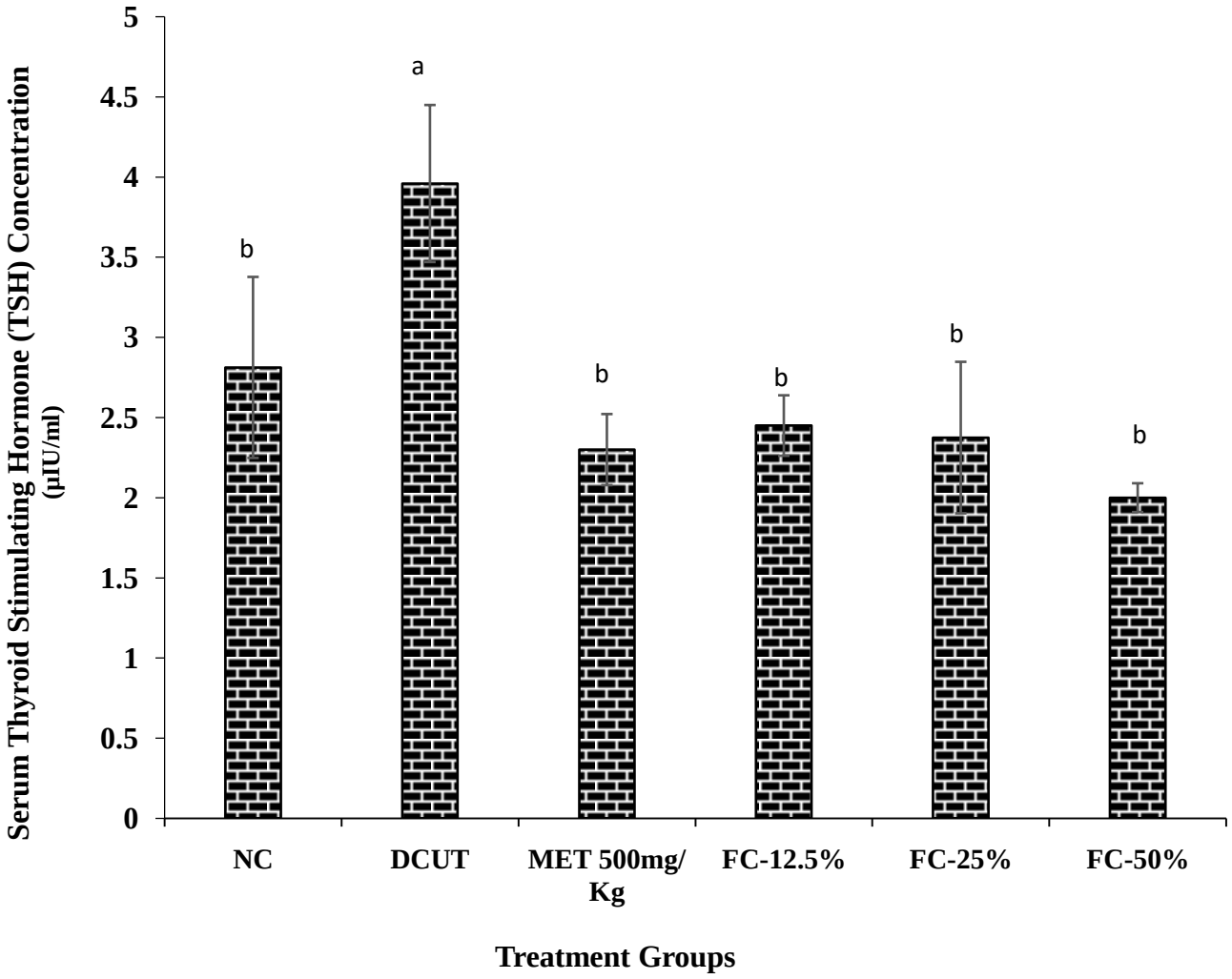

Figure 4.9: Serum Thyroid Stimulating Hormone (TSH) in Streptozotocin/Nicotinamideinduced Diabetic Male Wistar Rats Treated with Fermented CabbageSupplementation for four weeks.

Bars with different letters (a, b) differ significantly ($P < 0.05$) compared with the control groups (normal and diabetic untreated control)

NC= Normal control; DCUT= Diabetic control untreated; FC-12.5%= Diabetic rats treated with 12.5% of fermented cabbage; FC-25%= Diabetic rats treated with 25% of fermented cabbage; FC-50%= Diabetic rats treated with 50% of fermented cabbage; MET= Diabetic rats treated with metformin (500 mg/Kg).

4.10 Effect of Fermented Cabbage Supplementation on Hematological Indices inStreptozotocin/Nicotinamide induced Diabetic Male Wistar Rats

4.10.1 The Effect of Fermented Cabbage on Erythrocytic Indices in Diabetic Rats

The red blood cell count (RBC), haemoglobin(Hb) concentration, packed cell volume(PCV),mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration MCHC of the untreated diabetic controlrats were significantly ($P < 0.05$) decreased ($4.28 \pm 0.14 \times 10^{12}$/L), (8.13± 0.7 g/dL),(24.42 ± 0.67 %), (45.21 ± 4.8 fl), (11.11 ± 1.5 Pg) and (25.4 ± 0.9 g/dL) when compared to the normal control group ($6.66 \pm 0.14 \times 10^{12}$/L), (11.98 ± 0.3 g/dL), (37.49 ± 0.4 %), (56.4 ± 1.8 fl), (17.98 ± 0.3 Pg) and (31.98 ± 1.1 g/dL).However, administration of graded doses of fermented cabbage (12.5%, 25% and 50%) significantly increase ($P < 0.05$) RBC count (5.88 ± 0.27, 5.38 ± 0.27 and 5.34 ± 0.23 x 10^{12}/L); Hb concentration (13.15 ± 0.8g/dL, 13.95 ± 1.0g/dL and 12.25 ± 0.6 g/dL) andPCV (39.85 ± 2.6%, 40.42 ± 2.0% and40.25 ± 1.3 %)respectively. In addition, MCV values following supplementation with fermented cabbage(12.5%, 25% and 50%) were significantly increase($P < 0.05$)(68.52 ± 6.6fl, 75.94 ±6.2fl and 67.74 ±2.3fl); MCH (22.59 ± 2.0Pg, 25.6 ± 2.2Pg and22.99 ± 0.9 Pg) and MCHC (33.03±0.2g/dL, 33.7 ± 1.2g/dL and 33.93 ± 0.6 g/dL) and (36.75 ± 1.3 g/dL) when compared with the diabetic untreated control group.

4.10.2 The Effect of Fermented Cabbage on Leucocytes and Platelets Indices in Diabetic Rats

The total white blood cell (WBC) count, lymphocytes and neutrophils in the diabetic untreatedcontrol group were significantly lower ($P < 0.05$) (6.6 ± 0.54 $x10^9$/L, 4.51 ± 0.1$x10^9$/Land 1.70 ± 0.06 $x10^9$/L),when compared to the normal control group (14.44 ±0.09 $x10^9$/L, 11.35±0.25 $x10^9$/L and 3.56 ± 0.06 $x10^9$/L) respectively. Contrary to the observed trend in the other parameters, the platelets count in the diabetic untreated rats were significantly increased($P < 0.05$)when compared to those obtained in rats of the normal control group (891.2 ± 1.8 $x10^9$/L vs 380.55 ± 8.3 $x10^9$/L). Treatment of diabetic rats with graded doses of fermented cabbage significantly increase ($P < 0.05$) the WBC count(10.0 ±0.87 $x10^9$/L, 11.3 ±0.7 $x10^9$/L and10.47 ± 0.48 $x10^9$/L); lymphocytes count(9.05 ± 0.26 $x10^9$/L, 9.08 ± 0.27 $x10^9$/L, 10.5 ± 0.36 $x10^9$/L) and neutrophils count (2.8± 0.17 $x10^9$/L, 3.28 ± 0.22 $x10^9$/L, 2.5 ± 0.28 $x10^9$/L) when compared with diabetic untreated control group. Conversely, treatment of diabetic rats with fermented cabbage produced a significant decrease ($P < 0.05$) in the platelets counts (472.13 ± 8.4 $x10^9$/L, 544.10 ± 10.9 $x10^9$/L and 573.78 ± 26.8 $x10^9$/L) when compared with the diabeticuntreated control group.

Table4.1: Effects of Fermented Cabbage on Erythrocyte Indices in Streptozotocin/ Nicotinamideinduced Diabetic Male Wistar Rats

GROUPS	RBC ($x10^{12}$/L)	Hb (g/dl)	PCV (%)	MCV (fL)	MCH (pg)	MCHC(g/dl)
NC	6.66±0.14^{b}	11.98±0.3^{b}	37.49±0.4^{b}	56.4±1.8^{b}	17.98±0.3^{b}	31.98±1.1^{b}
DCUT	4.28±0.14^{a}	8.13±0.7^{a}	24.42±0.67^{a}	45.21±4.8^{a}	11.11±1.5^{a}	25.4±0.9^{a}
MET	6.0±0.9^{b}	11.25±0.4^{b}	33.75±1.4^{b}	73.0±3.2^{ab}	23.75±1.2^{b}	36.75±1.3$^{a\,b}$
FC-12.5%	7.88 ± 0.27^{b}	13.15 ± 0.8^{b}	39.85 ± 2.6^{b}	68.52±6.6^{ab}	22.59±2.0^{b}	33.03±0.2^{b}
FC-25%	7.38±0.27^{b}	13.95±1.0^{b}	40.42±2.0^{ab}	75.94±6^{ab}	25.6±2.2^{b}	33.7±1.2^{b}
FC-50%	7.34±0.23^{b}	12.25±0.6^{b}	40.25±1.3^{ab}	67.74±2.3^{b}	22.99±0.9^{b}	33.93±0.6^{b}

a,b = Means on the same column with different superscript letters differ significantly ($P < 0.05$)

NC= Normal control; DCUT= Diabetic control untreated; FC-12.5%= Diabetic rats treated with 12.5% of fermented cabbage; FC-25%b= Diabetic rats treated with 25% of fermented cabbage; FC-50%= Diabetic rats treated with 50% of fermented cabbage; MET= Diabetic rats treated with metformin (500 mg/Kg).

RBC = Red blood cell;Hb = Haemoglobin concentration; PCV = Packed cell volume; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration.

Table 4.2: Effects of Fermented CabbageSupplementationon Leucocytes and Platelets inStreptozotocin/ Nicotinamideinduced Diabetic Male Wistar Rats

GROUPS	WBC($x10^9$/L)	Neutrophil($x10^9$/L)	Lymphocyte($x10^9$/L)	Platelet($x10^9$/L)
NC	14.44±0.09*	3.56±0.065*	11.35±0.25*	380.55±8.3*
DCUT	6.60±0.54	1.7±0.07	4.51±0.10	891.20±1.8
MET	11.85±0.5*	3.3±0.47*	12.63±0.74*	635.55±37.2*
FC-12.5%	10±0.87*	2.8±0.17	9.05±0.26*	472.13±8.4*
FC-25%	11.3±0.7*	3.28±0.22*	9.08±0.27*	544.00±10.9*
FC-50%	10.47±0.48*	2.5±0.28	10.5±0.36*	573.78±26.8*

* = Statistically significant ($P < 0.05$) compared with the Diabetic untreated groups

NC= Normal control; DCUT= Diabetic control untreated; FC-12.5%= Diabetic rats treated with 12.5% of fermented cabbage; FC-25%= Diabetic rats treated with 25% of fermented cabbage; FC-50%= Diabetic rats treated with 50% of fermented cabbage; MET= Diabetic rats treated with metformin (500 mg/Kg); WBC=White blood cell count.

CHAPTER FIVE

5.0 DISCUSSION

Type 2 diabetes Mellitus(T2DM) is a heterogeneous disorder characterized mainly by insulinresistance coupled with ß-cell dysfunction (Shaw *et al.,* 2010). Streptozotocin/ Nicotinamide(STZ/ NA)induced type 2diabetic rat model is being increasingly used due to its close similarityto non-obese human T2DM(Ananda *et al.,* 2012).

Administration of graded doses of fermented cabbage to STZ/ NA diabetic group of ratssignificantly reversedhyperglycaemia revealing theantidiabetic potential of fermented cabbage. Fermented cabbage may have reducedblood glucose levels via some bio-molecules such as saponins that stimulate pancreatic beta cells to secrete insulin in to the blood leading to improved glucose metabolism(Asadujjaman *et al.,* 2011).

Another possible mechanism involved in the hypoglycaemic action of fermented cabbage may be via stimulation of insulin secretion and up-regulation of insulin receptor as observed in the present study from the pancreas;and thereby enhanced insulin sensitivity in various organs especially the muscles, promoting glucose uptake and metabolism and possibly inhibiting hepatic glycogenolysis and gluconeogenesis.This could be also due to the ability of fermented cabbage to bind to receptors on the surface of pancreatic β-cells; as a result, the cell membrane creates an influx of calcium ions and a subsequent release of insulin. The decrease may be also due to the effect ofdifferent polyphenolic compounds present in cabbage such as flavonoids, alkaloids and anthrocyanin which have been reported to have anti-diabeticproperties. Our findings also agreed with that of (Alsuhaibani, 2013) who demonstrated the hypoglycaemic activities of red cabbage andmanganese for the treatment of diabetes in rats. Similarly, Nizamutdinova*et al.* (2009),had

earlier reported that the administration of anthocyanins(also found in cabbage) markedly decreased glucoselevels and increase utilization of glucose by tissuesin diabetic rats.

In addition, another possible mechanism through which fermented cabbage (FC) was able to reduce blood glucose level could be via the effect of lactic acid bacteria that is present in FC as a result of the fermentation process. Lactic acid bacteria could have improved the glycaemic control by acting on gut bacteria to produce insulino-tropic polypeptides and GLP-1 (glucagon-like peptide-l) and glucose-dependent insulino-tropic polypeptide [GIP]; thereby, increasing glucose uptake by muscles, stimulating the liver absorption of blood glucose, and increase in the amount of insulin released from the β cells of the isletsand suppression of hepatic glucose production(Yao *et al.,* 2017).

Lactic acid bacteria could have also improved the glycaemic control by modulation of intestinal microbiotic composition which improve intestinal barrier function and diminish the translocation of micro-organisms and their derivatives, from the gut to the systemic circulation, thereby reducing the concomitant release of pro-inflammatory cytokines. Moreover, antioxidant properties of lactic acid bacteria have been shown in previous studies (Mathews *et al.,* 2002; Kobyliak*et al.,* 2018). Therefore,modulating inflammation and oxidative stress has been demonstrated as the possible mechanism by which lactic acid bacteria improve of glycaemic response in previous research.

The serum insulin and insulin receptor level in this studywas observed to have markedly increase. The STZ/NAcould have induced insulin resistance as an underlining mechanism leading to metabolic dysregulation in the experimental rats, resulting in the up-regulation of the

insulin receptors, being a physiological response to sustained hyperglycaemia seen in diabetes with insulin resistance (Hyun *et al*., 2014).

Aspartate transaminase (AST) and alanine transaminase (AST) are important markers of liver damage; however, ALT is more specific than AST. In other word, alkaline phosphatase (ALP) levels are highly increased in hepartobiliary and bone disorders (Esani, 2014). It was observed that treatment with fermented cabbage decreased the activities of the elevated liver enzymes. This is suggestive of hepato-cellular protective function of fermented cabbage.

The result on serum total protein (TP) shows a significant decrease in the diabetic untreated control group when compared with normal control rats. This is in agreement with the work of Mohammed *et al,* (2015), Indira *et al*. (2016), and Zaidi *et al.* (2019), who found out that diabeticssuffer from significant decrease in serum TP which is as a result of chronically elevated blood glucose. After treatment with graded doses of fermented cabbage, there was a significant increase in serum total protein. This increase could be due to the ability of FC to increase insulin level in the treated groups which will in turn increase synthesis of protein by the liver.

Diabetic rats orally administered graded doses of fermented cabbage produced a significant decrease in serum lipid profile.This could be due to the ability of fermented cabbage ability toinhibit lipogenesis and/or increase LPL activity; and also,possibly inhibition of cholesterol biosynthetic activity and better faecal bile acid excretion.

Interestingly, all doses of fermented cabbagecaused significant elevation in serum HDL-C. This is a valuable advantage since HDL-C ishelpful in transporting excess cholesterol from peripheral tissues to theliver for excretion in the bile. Thus, fermented cabbage may have thepotential to

delay or prevent the development of atherosclerosis andcoronary heart disease (CHD) as secondary complications of severediabetes mellitus (Shariq *et al.,* 2015).

The ability of fermented cabbage to reduce plasma cholesterol and triglycerides in diabetic rats could be explained by the insulin increasing capacity of fermented cabbage observed in this study. Insulin isrequired for the inhibition of hormone-sensitive lipasethus, increasing the utilization of glucose and decreasing the mobilization of free fatty acids from fat depots (Ramesh and Pugalendi, 2005).

Furthermore, the hypocholesterolaemic effect of fermented cabbage observed in this study may also be attributable to its antioxidant property. This may have occurred through decreased activity of 3-hydroxyl-3-methyl-glutaryl co-enzyme A (HMG CoA) reductase, which is the key regulatory enzyme in cholesterol biosynthesis (Olooto*et al.,* 2014);resulting in reduction in cholesterol absorption by the intestinal wall and/or reduction of LDL-receptors within the peripheral tissue (Danesh and Kanwar, 2004; Olooto*et al.,* 2014). This observation,suggest a cardio-protective property of fermented cabbage. The effect of fermented cabbage on the lipid profile may also be secondary to the glycaemic control observed in the present study. In addition, the significantly lower LDL-C cholesterol level recorded in the current study may have contributed to the higher serum HDL lipoprotein cholesterolin the experimental rats.

James *et al*. (2010) reported that about 30% of blood cholesterol is carried in the form of HDL-C. The HDL-C function to remove cholesterol atheroma within arteries and transport it back to the liver for its excretion or reutilization; thus, high level of HDL-C protects against cardiovascular disease. The observed increase in the serum HDL-C level in rats treated with various doses of fermented cabbage indicates that the fermented cabbagehas HDL-C

enhancingeffect. More so, the stabilization of serum triglyceride and cholesterol levels in rats by fermented cabbage may be attributed to enhancedglucose utilization, and hence depressed mobilization and utilization of fat (Momo *et al*., 2006; Iweala and Oludare, 2011). This finding suggests that fermented cabbage may be useful in reducing the complications of hyperlipidemia and hypercholesterolemia often seen in diabetics.

Thyroid hormones(THs) constitute important mediators of body metabolismand affect various metabolic aspects involving glucose andinsulin metabolism, through a variety of mechanisms. The decrease observed in T_3 and T_4 levelsin treated ratsindicate a state ofhyperthyroidism, possibly due to the goitrogenic effect of cyanide contained in the fermented cabbage.

Furthermore, the result of the present studyagreed with the finding of Peppa *et al.*,(2010) who demonstrated thefrequency of thyroid dysfunction among diabetes patients.Thyroid hormones are insulin antagonists, while both insulin andthyroid hormones are involved in cellular metabolism. Excessor deficit of any one can result in functional derangement ofthe other.

Therefore, the observed increase in serum levels of T_3 and T_4 in fermented cabbage treated groups indicates ameliorative effectof FC against derangement of thyroid functional parameters in diabetic state. This ability of FC to improve thyroid functional indices in diabetic rats could be either due to its ability to directly stimulate the thyroid gland to release T_3 and T_4 or inhibit the release of thyroid stimulating hormone (TSH) as evident in the decreased level of TSH seen in this study.

Diabetes Mellitus has been reported to be accompanied by increased production of free radicals (Baynesand Thorpe 1999; Mohammed *et al.,* 2013), which cause damage to the cellular proteins,

membrane lipids and nucleic acids, and eventually cell death (Arun and Ramesh, 2002;Olszewska*et al.,* 2012).

However, treatment of diabetic rats with fermented cabbage significantly improved the levels of red cell count, haemoglobin concentration and packed cell volume, with up to 50% improvement in the FC group when compared with the diabetic control group.

The probable mechanism by which FC exerts its haemopoietic effect may be linked to increase protein synthesis or mobilization from the liver as well as possible increase in erythropoietin from the bone marrow cells of the animals. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells (Ohlsson and Aher, 2006; Oyedemi*et al.,* 2011).

The stimulation of this hormone enhances rapid synthesis of red blood cells which is reflected in increases in levels of RBC, Hb and PCV. It also often leads to improved level of MCH and MCHC (Abu-Zaiton, 2010).Hence, the haemopoietic effect of FC may probably be due to its ability to stimulate erythropoietin, thrombopoietin and granulopoietin production in respect to anaemia.Thrombocytopenia and leucopenia was observed in diabetic untreated control group in the current study.

In like manner, the MCV, MCH and MCHC were significantly decreased in the diabetic control rats when compared with rats of the normal control group. The diabetic induced alterations in MCV, MCH and MCHC have been reported to be due to direct or feedback responses of structural damage to RBC membranes resulting in haemolysis and impairment in haemoglobin synthesis; and stress-related release of RBCs from the spleen and hypoxia (Marei *et al.,* 1998; Shah, 2006). In addition, the significant decrease in MCV suggests that the diabetic rats had

tendency towards microcytosis, a type of anaemia caused by iron deficiency resulting in low RBC volume.

In this study, treatment of diabetic rats with FC significantly restored changes in MCV, MCH and MCHC values when compared to the diabetic untreated control and normal control groups. The MCH and MCHC are used to define the concentration of haemoglobin present in a single RBC (Hall, 2016); and to suggest the restoration of oxygen-carrying capacity of the blood. Streptozotocin is a well-known chemical that suppresses the immune system by damaging WBC and the pancreas (Fagbohun*et al.*, 2020). The present study showed a significant reduction total WBC count and its differentials such as lymphocytes and neutrophils. The reduction of these parameters could be linked to the suppression of leucocytes from the bone marrow which may caused by the STZ used for the induction of diabetes.

Treatment of diabetic rats with FC produced an ameliorative effect as reflected by significant decrease in WBC, neutrophils and lymphocytes counts respectively, when compared with the diabetic untreated control group. This is an indication of increased cell mediated immune responsein the diabetic rats that were administered with fermented cabbage.

Platelets play a role in blood clotting which is mediated through meshwork of fibrin fibres. The fibres usually adhere to any vascular opening and hence prevent further blood loss. It plays a crucial role in reducing blood loss and repair of vascular injury (Oyedemi*et al.*, 2011). Treatment diabetic rats with graded doses of FC showed a significant decrease in platelet count. The decrease may be linked to FC having some phytochemical elements that have the potential to affect platelet function.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

This study has established that:

i. Fermented cabbage supplementation caused a steady decrease ($P < 0.05$) in blood glucose levels in a dose dependent manner from week 1-4 of treatment: week 1 (190.75 ± 6.7, 150.75 ± 15.46, 171.75 ± 16.65 and 163.25 ± 12.03 mg/dl); week 2 (198.75 ± 4.9, 179.5±8.9, 150 ± 17.49 and 145 ± 15.73 mg/dl); week 3 (188.5 ± 4.73, 175.5 ±9.6, 151.25 ± 17.9 and 146.25±13.6 mg/dl) and week 4 (193 ± 2.3, 169.75 ± 8.2, 162 ± 15.05 and 143.25 ± 2.92 mg/dl) respectively; when compared with corresponding diabetic untreated control group (224.1 ± 8.43, 218 ± 7.8, 221.75 ± 1.37, 231.75 ± 3.8 mg/dl).

ii. Administration of graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation to diabetic rats significantly ($P < 0.05$) increased the serum insulin levels 6.8 ± 0.5, 8.1 ± 0.4 and 8.4 ± 0.6 pmol/L; respectively and in a dose dependent manner when compared with diabetic untreated control group (3.5 ± 1.0 pmol/L).

iii. The serum insulin receptor levels decreased significantly ($P < 0.05$) in the diabetic untreated group (3.5 ± 0.9 pmol/L) following STZ/ nicotinamide treatment when compared with normal control (8.2 ± 1.4 pmol/L). However, administration of graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation to diabetic rats significantly ($P < 0.05$) increased the serum insulin receptor levels 6.9 ± 0.5, 6.2 ± 0.2 and 7.0 ± 0.8 pmol/L respectively; though not in a dose dependent manner when compared with the diabetic control group (3.5 ± 0.9 pmol/L).

iv. Fermented cabbage supplementation significantly decreased ($P < 0.05$) liver enzymes activities as follows:

a. Serum ALT level was significantly ($P < 0.05$) decreased (58.3 ± 2.8, 60.3 ± 2.5, 58.5 ± 1.1 IU/L) with graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation respectively; when compared to the diabetic untreated control group (74.8 ± 1.7 IU/L).

b. The serum AST level was significantly ($P < 0.05$) lowered (132.3 ± 4.7, 133 ± 2.1, 134 ± 1.9 IU/L) with graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation respectively, when compared to diabetic untreated control group (177.5 ± 3.8 IU/L).

c. The serum ALP level was significantly ($P < 0.05$) lowered (53.3 ± 2.6, 53.5 ± 1.7, 56 ± 2.6 IU/L) with graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation respectively, when compared to diabetic untreated control group (82.8 ± 2.4 IU/L).

v. The results of the study showed that oral administration of various doses (12.5%, 25% and 50%) of fermented cabbage supplementation produced a significantly ($P < 0.05$) higher serum total protein 64.2±1.7, 69.1±1.4, 64.5±2.1 g/L respectively; when compared to the diabetic untreated group control (49.2 ± 1.5 g/L).

vi. The effect of fermented cabbage supplementation on serum lipid profile were as follows:

a. The serum total cholesterol was significantly ($P < 0.05$) decreased (128.43 ± 1.0, 116.83 ± 2.0, 112.43 ± 3.9 mg/dl) with graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation respectively, when compared to the diabetic untreated group (186.3 ± 1.4 mg/dl).

b. Serum triglyceride level was significantly ($P < 0.05$) reduced (186.23 ± 8.6, 129.83 ± 7.0, 186.05 ± 5.6 mg/dl) with graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation respectively; when compared to the diabetic untreated group (229.68 ± 4.2 mg/dl).

c. The serum low density lipoprotein level was significantly ($P < 0.05$) reduced (37.83 ± 2.3, 41.43 ± 2.7, 41.75 ± 1.1 mg/dl) with graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation respectively, when compared to the diabetic untreated group (54.98 ± 2.0 mg/dl).

d. The serum high density lipoprotein level was significantly ($P < 0.05$) higher (40.85 ± 0.8, 36.93 ± 4.0, 41.15 ± 1.5 mg/dl) with graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation respectively; when compared to the diabetic untreated group (24.87 ± 1.4 mg/dl).

vii. Fermented cabbage supplementation significantly ($P < 0.05$) increased thyroid hormones (T_3 and T_4), and significantly decreased TSH level compared to the diabetic control group as follows:

a. Serum T_3 level was significantly ($P < 0.05$) increased (103.50 ± 5.9, 121.75 ± 1.1 and 125.66 ± 4.3 ng/dl) with graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation respectively; when compared to diabetic untreated control group (78.62 ± 3.8 ng/dl).

b. Serum T_4 level was significantly ($P < 0.05$) higher (7.85 ± 0.3, 8.27 ± 0.1, 7.96 ± 0.2 µg/dl) with graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation respectively; when compared to diabetic untreated control group (5.67 ± 0.7 µg/dl).

c. Serum TSH level was significantly ($P < 0.05$) decreased (2.45 ± 0.2, 2.37 ± 0.2, 2.0 ± 0.4 µIU/dl) with graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation respectively; when compared to diabetic untreated control group (3.96 ± 0.4 µIU/dl).

viii. Findings on haematological indicesfrom this study showed that:

a. The RBC count, Hb concentration, and PCV levels were significantly ($P < 0.05$) increased (5.88 ± 0.27, 5.38 ± 0.27, $5.34 \pm 0.23 \times 10^{12}$/L), ($13.15 \pm 0.8$ g/dL, 13.95 ± 1.0 g/dL, 12.25 ± 0.6 g/dL) and (39.85 ± 2.6 %, 40.42 ± 2.0 %, 40.25 ± 1.3 %) with graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation respectively; when compared to diabetic untreated control group ($4.28 \pm 0.14 \times 10^{12}$/L), ($8.13 \pm 0.7$ g/dL) and (24.42 ± 0.67 %).

b. The MCV, MCH and MCHC levels were significantly ($P < 0.05$) increased (68.52 ± 6.6 fl, 75.94 ± 6.2 fl, 67.74 ± 2.3 fl); (22.59 ± 2.0 Pg, 25.6 ± 2.2 Pg, 22.99 ± 0.9 Pg) and (33.03 ± 0.2 g/dL, 33.7 ± 1.2 g/dL, 33.93 ± 0.6 g/dL) with graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation respectively; when compared to diabetic untreated control group (45.21 ± 4.8 fl), (11.11 ± 1.5 Pg) and (25.4 ± 0.9 g/dL).

c. The WBC levels were significantly ($P < 0.05$) increased ($10 \pm 0.87 \times 10^9$/L, $11.3 \pm 0.7 \times 10^9$/L, $10.47 \pm 0.48 \times 10^9$/L) with graded doses (12.5%, 25% and 50%) of fermented cabbage supplementation respectively; when compared to diabetic untreated control group ($6.6 \pm 0.54 \times 10^9$/L, $4.51 \pm 0.1 \times 10^9$/L and $1.70 \pm 0.06 \times 10^9$/L).

d. The platelet level was significant decreased ($P < 0.05$) ($472.13 \pm 8.4 \times 10^9$/L, $544.10 \pm 10.9 \times 10^9$/L, $573.78 \pm 26.8 \times 10^9$/L) with graded doses (12.5%, 25% and 50%) of

fermented cabbage supplementation respectively; when compared to diabetic untreated control group ($891.2 \pm 1.8 \times 10^9$/L).

6.2 Conclusion

The results of the present study demonstrated that fermented cabbage supplementation lowered blood glucose levels, serum liver enzymes, serum cholesterol, serum triglyceride and low-density lipoproteins; while serum high density lipoproteins, red blood cell indices, serum total proteins, serum insulin, insulin receptors and thyroid hormoneswere all increased when compared with the diabetic untreated control rats.These findings show the efficacy of fermented cabbage supplementation as an anti-diabetic supplement.

6.3 Recommendations

Based on the findings of this study, the following recommendations were made:

i. Fermented cabbage could be incorporated into the dietary management of diabetic patients.
ii. Studies should be carried out on the effect of fermented cabbage supplementation on inflammatory biomarkers on diabetic animals such as tumour necrosis factor alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6); as elevations of these inflammatory biomarkers plays critical role in several complications of diabetes.
iii. Further studies should also be carried out to investigate the precise molecular mechanism(s)/ glucose transporters involved in the hypoglycaemic effect of fermented cabbage supplementationon blood glucose level.
iv. Finally, studies should be carried out on specific gene expression and epigenetic mechanisms involved in the anti-diabetic properties of fermented cabbage in order

toascertain its exact mechanism of action. This is with particular reference to its pro-insulin and insulin receptor activities.

6.4 Contributions to knowledge

i. Fermented cabbage supplementation decreases blood glucose level significantly in a dose depended manner (12.5%, 25% and 50%) from week 1of treatment (190.75 ± 6.7, 150.75 ± 15.46 and 171.75 ± 16.65 mg/dL); week 2 (198.75 ± 4.9, 179.5 ± 8.9 and 150.0 ± 17.49 mg/dL); week 3 (188.5 ± 4.73, 175.5 ± 9.6 and 151.25 ± 17.9 mg/dL) and week 4 (193.0 ± 2.3, 169.75 ± 8.2 and 162.0 ± 15.05 mg/dL), in streptozotocin/ nicotinamide induced diabetic Wistar rats in a dose dependent manner.

ii. Fermented cabbage supplementation at graded doses (12.5%, 25% and 50%)significantly increasedserum insulin level(6.8 ± 0.5, 8.1 ± 0.4 and 8.4 ± 0.6pmol/L) in streptozotocin/nicotinamide induced diabetic Wistar rats in a dose dependent manner.

iii. Administration of graded doses of fermented cabbage supplementation (12.5%, 25% and 50%) significantly increased serum insulin receptor levels (6.9 ± 0.5, 6.2 ± 0.2 and 7.0 ± 0.8 pmol/L), respectively but in none dose dependent manner.

iv. Fermented cabbage supplementation significantly lowered the liver enzymes (ALT, AST and ALP) activities (58.3 ± 2.8, 60.3 ± 2.5 and 58.5 ± 1.1 IU/L); (132.3 ± 4.7, 133.0 ± 2.1 and 134.0 ± 1.9 IU/L) and (53.3 ± 2.6, 53.5 ± 1.7 and 56.0 ± 2.6 IU/L), respectively in streptozotocin/ nicotinamide induced diabetic Wistar rats.

v. Fermented cabbage supplementation at graded doses (12.5%, 25% and 50%) increased significantly serum level of total protein (64.2 ± 1.7, 69.1 ± 1.4 and 64.5 ± 2.1 g/L) in streptozotocin/ nicotinamide induced diabetic Wistar rats.

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APPENDIX I: Effect of Fermented Cabbage Supplementation on Blood Glucose Level in Streptozotocin/ Nicotinamide Induced Diabetic Male Wistar Rats

	Week0	Week1	Week2	Week3	Week4
NC	54.5±1.32[b]	63±4.26[b]	69.5±4.7[b]	72.25±4.58[b]	68±3.4[b]
DCUT	229.25±21.6[a]	224±8.43[a]	218±7.8[a]	221.75±1.[37a]	231.75±3.8[a]
Met	235.5±6.27[b]	163.3±12.03[b]	145.2±15.73[b]	146.25±13.63[b]	143.25±2.92[b]
FC-12.5%	228±14.9[b]	190.75±6.7[b]	198.75±4.9[b]	188.5±4.73[b]	193±2.3[b]
FC-25%	224.25±8.91[b]	150.8±16.46[b]	149.5±8.9[b]	175.5±9.6[b]	169.75±8.2[b]
FC-50%	239.3±20.79[b]	171.3±16.65[b]	150±17.49[b]	151.25±17.96[b]	162±15.05[b]

APPENDIX II: Effect of Fermented Cabbage Supplementation on Serum Insulin Level in Streptozotocin/ Nicotinamide Induced Diabetic Male Wistar Rats

GROUP	INS (pmol/L)
NC	8.6 ± 1.8^{bcde}
DCUT	3.5 ± 1.0^{a}
METFORMIN	9.6 ± 0.3^{bcde}
FC-12.5%	6.8 ± 0.5^{b}
FC-25%	8.1 ± 0.4^{b}
FC-50%	8.4 ± 0.6^{b}
F-value	48.6
p-value	<0.0001

APPENDIX III: Effect of Fermented Cabbage Supplementation on Insulin Receptor Level in Streptozotocin/ Nicotinamide Induced Diabetic Male Wistar Rats

GROUP	ISR(pmol/L)
NC	8.2 ± 1.4^{bcde}
DCUT	3.5 ± 0.9^{a}
METFORMIN	9.8 ± 0.3^{bcde}
FC-12.5%	6.9 ± 0.5^{b}
FC-25%	6.2 ± 0.2^{b}
FC-50%	7.0 ± 0.9^{b}
F-value	30.7
p-value	<0.0001

APPENDIX IV: Effect of Fermented Cabbage Supplementation on Liver Enzymes in Streptozotocin/ Nicotinamide Induced Diabetic Male Wistar Rats

GROUPS	ALT (IU/L)	AST(IU/L)	ALP(IU/L)
NC	59.5 ± 0.3^{b}	134.8 ± 1.3^{b}	51.5 ± 0.3^{b}
DCUT	74.8 ± 1.7^{a}	177.5 ± 3.8^{a}	82.8 ± 2.4^{a}
METFORMIN	60.12 ± 0.2^{b}	137.2 ± 1.5^{b}	51.7 ± 0.6^{b}
FC-12.5%	58.3 ± 2.8^{b}	132.3 ± 4.7^{b}	53.3 ± 2.6^{b}
FC-25%	60.3 ± 2.5^{b}	133.0 ± 2.1^{b}	$53.5 \pm .1.7^{b}$
FC-50%	58.50 ± 1.1^{b}	134.0 ± 1.9^{b}	56.0 ± 2.6^{b}
F-value	12.63	38.93	40.11
p-value	<0.0001	<0.0001	<0.0001

APPENDIX V: Effect of Fermented Cabbage Supplementation on Total Protein Level in Streptozotocin/ Nicotinamide Induced Diabetic Male Wistar Rats

GROUPS	TP (g/L)
NC	61.8 ± 0.6^{b}
DCUT	49.2 ± 1.5^{a}
METFORMIN	61.67 ± 1.1^{b}
FC-12.5%	64.2 ± 1.76^{b}
FC-25%	$69.1\pm1.4^{ab\#}$
FC-50%	64.5 ± 2.1^{b}
F-value	20.4
p-value	<0.0001

APPENDIX VI: Effect of Fermented Cabbage Supplementation on Lipid Profile in Streptozotocin/ Nicotinamide Induced Diabetic Male Wistar Rats

GROUP	TCHOL (mg/dl)	TRIG(mg/dl)	HDL(mg/dl)	LDL(mg/dl)
NC	83.95 ± 0.3^{bcde}	105.07 ± 0.5^{bcde}	45.95 ± 0.5^{bd}	39 ± 0.3^{b}
DCUT	186.3 ± 1.4^{a}	229.68 ± 4.2^{a}	24.87 ± 1.4^{a}	54.98 ± 2.0^{a}
METFORMIN	92.70±4.1b*	104.1 ± 0.08^{bcde}	45.80 ± 1.0^{bd}	39.37 ± 0.4^{b}
FC-25%	116.83 ± 2.0^{b}	129.83 ± 7.0^{bce}	36.93 ± 4.0^{b}	41.43 ± 2.7^{b}
FC-50%	112.43 ± 3.9^{bd}	186.05 ± 5.6^{b}	41.15 ± 1.5^{b}	41.75 ± 1.1^{b}
F-value	195.8	91.26	16.3	12.90
p-value	<0.000	<0.000	<0.000	<0.000

APPENDIX VII: Effect of Fermented Cabbage Supplementation on Thyroid Hormones in Streptozotocin/ Nicotinamide Induced Diabetic Male Wistar Rats

GROUP	T3 (ng/dl)	T4 (µg/dl)	TSH (µIU/dl)
NC	112.38±11.3[b]	7.15±0.4[b]	2.81±0.5[b]
METFORMIN	126.50 ±1.32[b]	7.80 ±0.23[b]	2.3±0.09[b]
FC-12.5%	103.50±5.9[b]	7.85±0.3[b]	2.45±0.2[b]
FC-25%	121.75±1.1[b]	8.27±0.1[b]	2.37±0.2[b]
FC-50%	125.66±4.3[b]	7.96±0.2[b]	2.0±0.4[b]
F-value	71.5	14.8	25.9
p-value	<0.0001	<0.0001	<0.0001

APPENDIX VIII: PHYTOCHEMICAL SCREENING OF FERMENTED CABBAGE *(Brassica olaeracae)*

S/ No.	Phytocostituent	Observation
1	Carbohydrate	+ +
2	Anthraquinones	+ +
3	Triterpens	+ +
4	Cardiac glycosides	+
5	Saponin	+
6	Tannin	+
7	Flavonoid	+
8	Alkaloid	+

+ = Presence

- = Absence

APPENDIX IX: AQUANTITATIVE ANALYSIS RESULT

Phytochemicals	Mean ± SD (%)
Soluble Carbohydrate	3.42 ± 0.0025
Tannin	2.75 ± 0.0038
Cyanide	1.61 ± 0.0028
Reducing sugars	3.37 ± 0.0041
Phenol	7.24 ± 0.0021
Terpenoid	0.87 ± 0.0032
Saponin	0.95 ± 0.0005