ANTIDIABETIC EFFECT OF METHANOLIC EXTRACTS OF *Laguncularia racemosa* (White Mangrove) IN ALLOXAN INDUCED DIABETIC RATS

BY

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MTech/SLS/2017/7608

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY IN BIOCHEMISTRY

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ABSTRACT

Diabetes mellitus is a metabolic disorder affecting carbohydrates, protein and fat metabolism. The treatment of diabetes mellitus has been confined to the use of oral hypoglycemic agents and insulin, which possess serious side effects. This lead to increasing demand for herbal products having little side effects. The aim of this study is to determine the effects of methanolic extracts of *Laguncularia racemosa* in alloxan induced diabetic rats. Phytochemical screening and antioxidant activity were carried out using standard methods while acute toxicity study was done using up and down method (limit test). Diabetes was induced intraperitoneally in albino rats with 120 mg/kg body weight of alloxan. A total of forty-five rats were treated as follows; Group 1 vehicle (distilled water), Group 2 (induced with no treatment), Group 3 and 4 metformin (standard) at 150 and 300 mg/kg body weight respectively, Group 5 and 6 crude (70 %) methanol extract at 150 and 300 mg/kg body weight, Group 7 and 8 methanol fractions (100 %) 150 and 300 mg/kg body weight, an non-indued group 9 was treated with (70 %) methanol at 300 mg/kg body weight single dose. Dipeptidyl peptidase-4 was assessed using enzyme linked immunoabsorbent assay (ELISA) kits in Euthanized rats. Among the secondary metabolites, Tannis was (8.88 mg/g), flavonoids (1.73 mg/g), phenols (1.68 mg/g), Saponins (1.17 mg/g) and alkaloids (0.23 mg/g). Percentage DPPH scavenging activity at 100 µg/ml was 79.86 % with an IC_{50} value 19.48 µg/ml while percentage inhibition of lipid peroxidation was 63.79 % and ferric antioxidant reducing power assay was 40.65 %. The acute toxicity studies showed that there was no death or lethal effect observed in toxicity study in the anti-diabetic study. Crude and methanol fraction of *Laguncularia racemosa* significant ($p \leq 0.05$) reduce blood glucose level in a dose dependent manner when compared with the diabetic control, crude extract at 300 mg/kg significantly reduced blood glucose level from 20.82 mmoL/l to 5.78 mmoL/l. *Laguncularia racemosa* shows a non-dose dependent activity against DPP-4, The level of DPP4 was 1008.24 pg/ml, compared to normal control group 1095 pg/ml, negative (untreated group) 1828.91 pg/ml. In conclusion, the results of this study showed that the methanol extract of *Laguncularia racemosa* exhibited antidiabetic potential which can be investigated further for the development of oral anti-hyperglycemic drugs.

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ABBREVIATIONS

DM	Diabetes mellitus
DPPH	1,1-diphenyl-2 picrylhydrazyl
FRAP	Ferric Reducing Antioxidants Power Assay
OGTT	Oral Glucose tolerance test
GLP-1	Glucagon-like peptide-1
PPARY	peroxisome proliferation-activated receptor-y
ATP	Adenosine Triphosphate
cAMP	Cyclic Adenine Monophosphate
ADA	American Diabetes Association
HLA	Human Leuckocyte Antigen
HBA1c	Glycosylated Hemoglobin
M_F	Molecular formula
M_w	Molecular weight
RBG	Random Blood Glucose
FBS	Fasting Blood Sugar
NIDDM	Non-insulin dependent diabetes mellitus
IDDM	Insulin dependent diabetes mellitus
GBD	Global Burden of Diseases
GDM	Gestational diabetes mellitus
WHO	World Health Organization
DKA	Diabetic ketoacidosis
MCH	Major histocompatibility complex
MODY	Maturity onset diabetes of the young
ROS	Reactive oxygen species
ESRD	End stage retina diseases
RAAS	Renin angiotensin aldersterone system
DPP4	Dipeptidyl peptidase -4

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Diabetes mellitus is a chronic metabolic disease characterized by raised blood glucose levels, which leads over time to significant impairment to the heart, kidneys, eyes, blood vessels and nerves (WHO, 2016). It is categorized seventh, among the important causes of death globally but it is considered third when its complications are taken into account (Komalavalli *et al.,* 2015; & Trivedi *et al.*, 2016). Diabetes mellitus is associated with wide range of diseases including diabetic nephropathy (Lee *et al.*, 2014; & Bello *et al.,* 2016), liver diseases (Leeds *et al.*, 2009; & Theophine *et al.,* 2017), coronary heart disease and ischaemic stroke (Spencer *et al.*, 2012; & Pingali *et al.,* 2015). Diabetes is a serious condition with potentially devastating complications affecting all age groups worldwide. It is due to the pancreas not producing enough insulin or the cells of the body not responding properly to insulin produced (Ahmed *et al.,* 2008). Diabetes mellitus is categorized into three types but the two major types are type 1 and type 2 widely described as insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) based on aetiology (Zimmet *et al.,* 2004; & Hyeon-Kyu *et al.,* 2015).

Type 1 diabetes is immune mediated and idiopathic forms of β cell dysfunction, which lead to absolute insulin deficiency. It was previously called Insulin-dependent diabetes mellitus or juvenile diabetes. Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency. It can be further classified as immune-mediated or idiopathic. This is an autoimmune mediated disease process, which gives rise to absolute deficiency of insulin and therefore total dependency upon insulin for survival (Zimmet *et al.,* 2004; & Hyeon-Kyu *et al.,* 2015).

Type 2 diabetes is also known as disease of the onset, it is characterized by insulin-resistance, which may be combined with relatively reduced insulin secretion and an insensitivity of the body tissues to insulin so leaving patients with this condition relatively deficient in insulin (Zimmet *et al.,* 2004; & Hyeon-Kyu *et al.,* 2015).

Blood glucose levels are controlled by a complex interaction of multiple chemicals and hormones in the body including the hormone insulin made in the beta cells of the pancreas. Diabetes mellitus consists of a group of syndromes characterized by hyperglycaemia, altered metabolism of lipids, carbohydrates, proteins, and an increased risk of complications from vascular disease. Criteria for the diagnosis of diabetes mellitus have been proposed by several medical organizations (WHO, 2014). The American Diabetes Association criteria include symptoms of diabetes mellitus as polyuria, polydipsia, and unexplained weight loss, a random plasma glucose concentration of greater than 200 mg/dl (11.1 mmole/liter). a fasting plasma glucose concentration of greater than 126 mg/dl (7 mmole/liter), or a plasma glucose concentration of greater than 200 mg/dl (11 mmole/liter) 2 hours after the ingestion of an oral glucose load (Expert Committee on the Diagnoses and Treatment of Diabetes Mellitus, 2015).

Globally, the prevalence of diabetes mellitus continued to increase at an alarming rate (Knowler *et al.,* 2002). This global increase is associated with interplay of risk factors such as genetic and environmental factors, various changes in diets and lifestyle across different cultures, physical inactivity and metabolic factors such as overweight and obesity as reported by the Global Burden of Disease (Roglic *et al.,* 2004) The global estimate of adults living with diabetes was 422 million in 2014 as compared to 108 million in 1980 (WHO, 2016) and estimated to be 642 million by 2040 among adults between the ages of 20 and 70. It has been projected that by the year 2030 over 500 million adults will be affected by. The increase could be as a result of urbanization and aging of the population (Knowler *et al.,* 2002) the projected increase in prevalence is expected to be higher in Africa and Asia where there is rapid

epidemiological transition (King *et al.,* 1998). The prevalence of DM is still lower in traditional rural than urban communities. Previous studies found the prevalence of 1.6 % (Rosenbloom & Silverstein, 2003) in a suburban Northern Nigerian city and a prevalence of 1.4 % is found in a rural population of North Central Nigeria (1.4 %). Most cases of DM in rural and suburban areas remain undiagnosed, and many patients present for the first time with complications (Amos *et al.,* 2010).

Acording to (Andrew *et al.,* 2018), the pooled prevalences of DM in the six geopolitical zones of Nigeria were 3.0 % (95 % CI 1.7–4.3) in the north-west, 5.9 % (95 % CI 2.4–9.4) in the northeast, 3.8 % (95 % CI 2.9–4.7) in the north-central zone, 5.5 % (95 % CI 4.0–7.1) in the south-west, 4.6 % (95 % CI 3.4–5.9) in the south-east, and 9.8 % (95 % CI 7.2–12.4) in the south-south zone. However, the disease has been a major health problem and efforts across the globe have been made to reduce the increase in its prevalence, related complications (Rich, 2006) death from the disease and to increase access to essential antidiabetic agents but despite these great efforts and progresses made in its understanding and management, diabetes and its associated complications are on the increase persistently (Kiswari *et al.,* 2015).The available conventional antidiabetic agents such as the biguanides, sulfonylureas, gliptins and insulin that are used to manage the disease are associated with some undesirable side effects, contraindications and are not readily accessible and affordable to the majority of the affected population. *Laguncularia racemosa* is a dicotyledonous genus in the Family Combretaceae that occurs in mangrove swamps on the Atlantic coasts of the America and West Africa. *L. racemosa* contain various bioactive principles including Tannin, Saponin, Alkaloid, Flavonoid and Phytate. (Wekhe, 2007) reported that *L. racemosa* contain alkaloids and are used for antiparasites, antispasmodic and bacterial antigens. Ahamefule *et al.* (2006) submitted that flavonoid and alkaloid present in *L. racemosa* function in protection against inflammation, allergies, and microbial infestations. However, Kawo, (2009) reported that the

pharmacological activity of phytochemicals in *L. racemosa* plants includes antimicrobial, inflammation inhibiting and cytotoxic activities. The present study aims at finding out the potentials of *L. racemosa* in the prevention and management of Diabetes Mellitus.

1.2 Statement of the Research Problem

In Nigeria, the prevalence Diabetes Mellitus (DM) was estimated at 2.2 % reported by (ADA, 1997). Which has increased to 4.7 % in 2013 estimated by the International Diabetes Federation. Globally, it affects people in both rural and urban settings with rural areas having the lowest percentage (35 %) as compared to 65 % of cases in urban (ADA, 1997).DM is a serious, long term condition with a major impact on the lives and wellbeing of individuals, families, and societies worldwide.it among the top 10 causes of death in adults, and was estimated to have caused four million deaths globally in 2017, globally health expenditure on diabetes was estimated to be USD 727 billion, posing an enormous socioeconomic burden, just under half a billion people are living with diabetes worldwide and the number is projected to increases by 25 % in 2030 and 51 % in 2045. (ADA, 1997). There are a wide variety of medications available for the treatments of diabetes almost 20 % of subjects on metformin or thiazolidenediones had potential contraindications to these medications. Limitations of conventional therapies, and no cure for diabetes a compounding factor, Existing oral diabetes agents have limited in efficacy and suffer an adverse effect, patient underlying health status, medication compliance issues and economic burden to the patients and health care system. A growing body of evidences shows that comorbidities are greatly reduced where adequate glycemic control is archived, Unfortunately, despite the variety of therapies currently available the majority of patients do not achieve normal glycemic goal, these have been a challenge in the management of diabetes.

1.3 Aim and Objectives

The aim of this study was to determine the antidiabetic effect of methanolic extracts of *Laguncularia racemosa* in alloxan induced diabetic rats

The objectives of this study were to:

i. determine the acute oral toxicity of the crude methanolic extracts (hot and cold) of *L. racemosa*
ii. determine the oral glucose tolerance as a priminary tests on the hot and cold methanolic extracts of *L. racemosa*
iii. determine the phytochemical composition of the methanolic crude extract of *L. racemosa*
iv. To investigate the *in vitro* antioxidant properties of methanolic extract of *L. racemosa*
v. evaluate the anti-hyperglycemic activity extracts of *L. racemosa* in alloxan induced diabetic rats.
vi. determine the effect of crude and partially purified extracts of *L. racemosa* on the blood levels of dipepetidyl peptidase - 4 (DPP4) in alloxan induced diabetic rats.

1.4 Justification for the Study

The use of natural products from herbs forms an important component of the health care delivery system in African countries (Cragg & Newman, 2013). The conventional anti-diabetic drugs used are associated with serious side effects which affect the blood, liver, and kidney. (Hellmuth *et al.,* 2000), while some of the drugs are not able to control some complications. These shortcomings have led to the increased patronage of alternative therapies like herbal

medicine. Researches on medicinal plants have led to the discovery of some potential compounds for the development of drugs that function on new or established therapeutic targets (Ramírez-Espinosa *et al.,* 2011). Herbal products are extensively used in the management of diabetes mellitus and recently there is a renaissance of interest in medicinal plants with antihyperglycaemic potential (Mamun-or-Rashid *et al.,* 2014; & Lydia *et al.,* 2016). Hence, the need for more affordable and less expensive and yet easily available and effective medicine for the treatment of diabetes has prompted researchers to explore into nature in search of plants with hypoglycaemic activitivty.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes Mellitus is a group of metabolic diseases characterized by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. Metabolic abnormalities in carbohydrates, lipids, and protein result from the importance of insulin as an anabolic hormone. low level of insulin to achieve adequate response and or insulin resistance of target tissues, mainly skeletal muscles, adipose tissues, and to lesser extent, liver at the level of insulin receptors, signal transduction system, and or effector enzymes or genes are responsible for these metabolic abnormalities. The severity of symptoms is due to the type and duration of diabetes. some of the diabetes patients are asymptomatic especially those with type 2 diabetes during the early year of the disease's others market hyperglycaemia and especially in children with absolute insulin deficiency may suffer from polyuria, polydipsia, polyphagia, weight loss, and blurred vision. Uncontrolled diabetes may lead to stupor, coma, and if not treated death. Due to ketoacidosis or rare from nonketotic hyperosmolar syndrome (craig *et al*., 2009).

2.1.1 Incidence and prevalence of diabetes mellitus

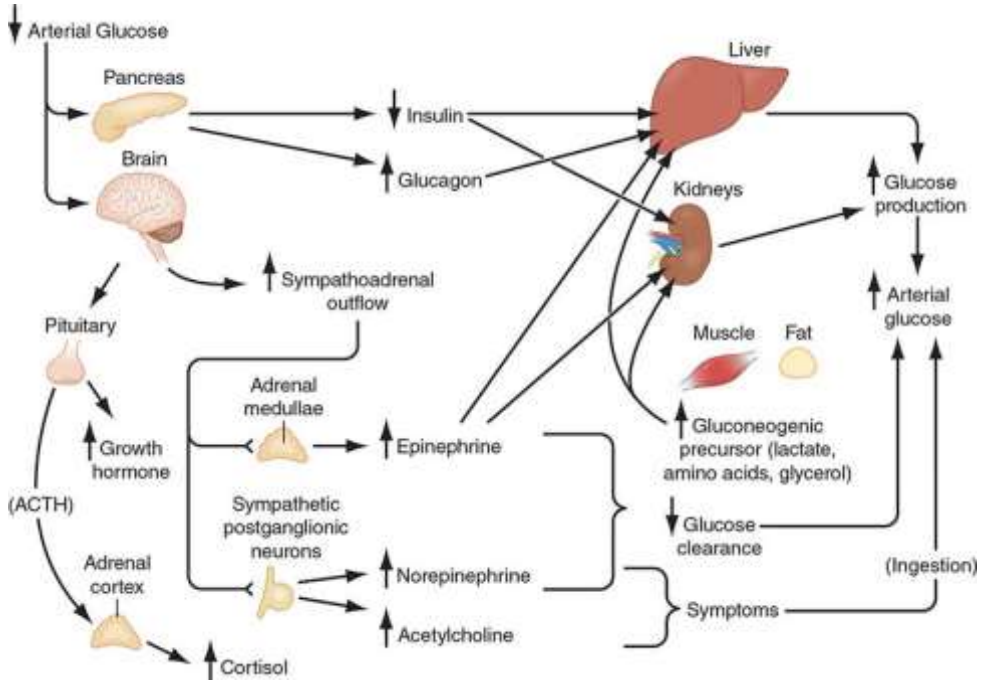
The application form epidemiology to the research of diabetes mellitus has produced important ideas, on the diseases records, livelihood, prevalence, morbidity and mortality in many different neighborhoods across the world. Recognition of illness triggers and probable eliminations practices that could be applied to halt to wait distributes of the condition, that has achieved crisis dimensions in equally produce and building places (shapiro *et al.,* 2002) regrettably, changes in personal diabetic outcomes haven't translated into commensurate community wellness advantages.

The high prevelences in diabetes is becoming alarming all over the world and it projected to be 366 million persons internationally, with form 2 diabetes more than 90 % of instances, (Patlak, 2002). Based on literature evaluation, there are several figures on the prevalence of form 2

diabetes mellitus in Africa as a whole, according to study guide; diabetes mellitus influenced 32 % of Africans, with 40 % apptoximately (2.0 percent) residing in ethopia.

2.1.2 Causes of diabetes mellitus

The deficiency in the production or action of insulin is the cause of diabetes mellitus. There is frequent coexistence in the same patient of impairment of insulin secretion and defects in insulin action, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycaemia, Lack of insulin due to the destruction of insulin-producing beta cells in the pancrease results to type 1 diabetes. In type 2 diabetes, the body's immune system attacks and destroys the beta cells (Akah *et al.,* 2002).



Figure, 2.1 Physiology of Glucose Counter Regulation: Mechanisms that Normally Prevent or Rapidly Correct Hypoglycaemia

Source: Cryer *et al*., (2009).

Normally, the immune system protects the body from infection by identifying and destroying bacteria, viruses, and other potentially harmful foreign substances. In autoimmune diseases, the immune system attacks the body's own cells (NDIC, 2011). Combination of factors like

hereditary including insulin resistance results to type 2 diabetes most common form of diabetes mellitus. It develops when the body can no longer produce enough insulin to compensate for the impaired ability to use insulin. It is becoming more common in overweight, obese children and adolescents. Scientists think genetic susceptibility and environmental factors are the most likely triggers of type 2 diabetes (Cryer *et al*., 2009). Physical inactivity and obesity are strongly associated with the development of type 2 diabetes. People who are genetically susceptible to type 2 diabetes are more vulnerable when these risk factors are present.

2.1.3 Classificaiton of diabetes mellitus

A major requirement for orderly epidemiologic and clinical research on and for the management of diabetes mellitus is an appropriate classification. Furthermore, the process of understanding the etiology of a disease and studying its natural history involves the ability to identify and differentiate between its various forms and place them into a rational etiopathologic framework (Harris & Zimmet, 1997). The contemporary classification of diabetes and other categories of glucose intolerance, based on research on this heterogeneous syndrome, were developed in 1979 by the National Diabetes Data Group. Two major forms of diabetes are recognized in Western countries; insulin dependent diabetes mellitus (IDDM, type I diabetes) and non-insulin dependent diabetes (NIDDM, type II diabetes). The evidence of this heterogeneity is overwhelming and includes the following: a) there are many distinct disorders, most of which are individually rare, in which glucose intolerance is a feature; b) there are large differences in the prevalence of the major forms of diabetes among various racial or ethnic groups world-wide; c) glucose tolerance presents variable clinical features, for example, the differences between thin ketosis-prone, insulin dependent diabetes and obese, non-ketotic insulin resistant diabetes; d) genetic, immunologic and clinical studies show that in

Western countries, the forms of diabetes with their onset primarily in youth or in adulthood are distinct entities; e) the type of non-insulin requiring diabetes in young people, which is inherited in an autosomal dominant fashion is clearly different from the classic acute diabetes of juveniles; and f) in tropical countries, several clinical presentations occur, including fibrocalcific pancreatitis and malnutrition-related diabetes. This and other collective evidence have been used to divide diabetes mellitus into four distinct types namely;

i. insulin dependant diabetes,
ii. non-insulin dependant diabetes,
iii. malnutrition-related diabetes,
iv. Other types of diabetes.

The classification highlights the marked heterogeneity of the diabetic syndrome. Such heterogeneity has important implications not only for clinical management of diabetes but also for biomedical research (Harris & Zimmet, 2015). In this study the focus was mainly on type II diabetes while type I diabetes was discussed briefly to point out the differences between the two types of diabetes.

2.1.3.1 *Insulin dependent diabetes mellitus (IDDM)*

The subclass of diabetes, type I diabetes, is generally characterized by the abrupt onset of severe symptoms, dependence on exogenous insulin to sustain life and proneness to ketosis even in the basal state, all of which is caused by absolute insulin deficiency. IDDM is the most prevalent type of diabetes among children and young adults in developing countries, and was formally termed juvenile diabetes (Harris & Zimmet, 2015). It is a catabolic disorder in which circulating insulin is virtually absent, plasma glucagon is elevated, and the pancreatic B cells fail to respond to all insulinogenic stimuli (Nolte & Karam, 2015).

Type I diabetes is thought to result from an infectious or toxic environmental contingency in people whose immune systems are genetically predisposed to develop a vigorous autoimmune

response against pancreatic B cell antigens. Extrinsic factors that might affect B cell functioning include damage caused by viruses such as the mumps virus and coxsackie virus B4, by chemical agents, or by destructive cytotoxins and antibodies released from sensitized immunocytes. An underlying genetic defect relating to pancreatic B cell replication or function may predispose a person to the development of B cell failure after viral infections. In addition, specific HLA genes may increase susceptibility to a diabetogenic virus or may be linked to certain immune response genes that predispose patients to a destructive autoimmune response against their own islet cells (auto aggression).

Observations that pancreatic B cell damage appears to be lessened when immunosuppressive drugs such as cyclosporine or azathioprine are given at the initial

Occurance of type I diabetes support the importance of auto-aggression by the immune system as a major factor in the pathogenesis of this type of diabetes (Nolte & Karam, 2015).

2.1.3.2 *Non-insulin dependent diabetes mellitus (NIDDM)*

Type II diabetes greatly out numbers all other forms of diabetes. Patients with NIDDM are not dependant on exogenous insulin for prevention of ketonuria and are not prone to ketosis (Frati *et al*., 1990). However, they may require insulin for the correction of fasting hyperglycaemia if this cannot be achieved with the use of diet or oral agents, and they may develop ketosis under special circumstances such as severe stress precipitated by infections or trauma (Harris & Zimmet, 2015). The pathogenesis in type II diabetes is that the pancreas produces insulin but the body does not utilize the insulin correctly. This is primarily due to peripheral tissue insulin resistance where insulin-receptors or other intermediates in the insulin signaling pathways within body cells are insensitive to insulin and consequently glucose does not readily enter the tissue leading to hyperglycaemia or elevated blood glucose concentrations (Ahmed *et al.,* 2018).

Obesity, which generally results in impaired insulin action, is a common risk factor for this type of diabetes, and most patients with type II diabetes are obese (Nolte & Karan, 2015) and will ultimately require mu ltiple anti-diabetic agents to maintain adequate glycaemic control (Gerich, 2001).

2.1.4.3 *Gestational diabetes mellitus*

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2 %-5 % of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable but requires careful medical supervision throughout the pregnancy. About 20 %-50 % of affected women develop type 2 diabetes later in life (Lawrence *et al.,* 2005).

Even though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformations. A 2008 study completed in the United State found that the more American women are entering pregnancy with pre-existing diabetes (Lyssenko *et al.,* 2008). In fact, the rate of diabetes in expectant mothers has more than double in the past 6 years (Lawrence *et al.,* 2005). This is particularly problematic as diabetes raises the risk of complication during pregnancy as well as increasing the potential that the children of diabetic mothers will also become diabetic in future.

2.2 Clinical Features of Diabetic Mellitus

2.2.1 Sign and symptoms

The classical triad of diabetes symptoms include polyuria, polydipsia, and polyphagia, which are, respectively, frequent urination, increased thirst and consequent increased fluid intake, and increase appetite symptoms may develop quite rapidly (weeks or months) in type I

diabetes particularly in children. However, in type 2 diabetes symptoms usually develop much more slowly and may be subtle or completely absent. Type I diabetes may also cause a rapid and significant weight loss (despite normal or even increased eating) and irreducible fatigue. All of these symptoms except weight loss can also manifest in type 2 diabetes in patients whose diabetes is poorly controlled (Santaguida *et al.,* 2008). When the glucose concentration in the blood is raised beyond its renal threshold, reabsorption of glucose in the proximal renaltubule is incomplete, and part of the glucose remains in the urine (glycosuria). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased thirst (Tarnow *et al.,* 2008).

Prolonged high blood glucose leads to changes in the shape of the lenses of the eyes, resulting in vision changes; sustained sensible glucose control usually returns the lens to its original shape. Blurred vision is a common complaint leading to a diabetes diagnosis. Type 1 DM should always be suspected in cases of rapid vision change (Theodore *et al,* 2008). Patients (usually with type 1 diabetes) may also initially present with diabetes ketoacidosis (DKA), an extreme state of metabolic dysregulation characterized by the smell of acetone on the patient's breath; a rapid, deep breathing known as Kussmaul breathing; polyuria, nausea, vomiting and abdominal pain, and any of many altered states of consciousness or arousal (such as hostility and mania or, equally, confused and lethargy). In severe DKA, coma may follow, progressing to death. Diabetic ketocidosis is a medical emergency and required immediate hospitalization. A rarer but equally severe possibility is hyperosmolar nonketotic state which in more common in type 2 diabetes and is mainly the result of dehydration due to loss of body water. Often, the patient has been drinking extreme amounts of sugar-containing drinks, leading to a vicious circle in regard to the water loss (Genuth, 2006; & Sniderman *et al.,* 2007).

2.2.2 Genetics of diabetes mellitus

Over 20 regions in the human genome are associated with Type 1 diabetes, but make little contribution to overall susceptibility to Type 1 diabetes (Davies *et al.,* 1994 and Concannon *et al.,* 1998). The strongest linkage with Type 1 diabetes is shown by the human leucocyte antigen (*HLA*) gene cluster in the major histocompatibility complex (*MHC*) located on chromosome 6p 21 (Ghosh & Schork, 1996). HLA antigens are cell-surface glycoproteins that play a crucial role in presenting auto antigen peptide fragments to T lymphocytes and thus initiate an auto immune response (Nerup *et al.,* 1997). They comprised of two classes, class I and class II, which are encoded by different genes within the HLA region and thus differ fundamentally in structure. Class I molecules comprise the HLA A, B, C while class II molecules comprise HLA DP, DQ and DR and are coded by their respective genes (Nerup *et al.,* 1997). The HLA class II molecules are central to the human immune response because they present peptide antigens to T-helper (CD 4 positive) cells. There are two types of class II genes: those encoding polypeptides and those encoding polypeptides which together form the functional class II heterodimer. This results in a variety of genes (Yamagata *et al.,* 1996).

Type 2 diabetes shows a clear familial aggregation but it does not segregate in a classical Mendelian fashion. It is polygenic, with different combinations of gene defects. Genetic and environmental factors may affect insulin biosynthesis, insulin secretion and insulin action. The complex interactions between genes and the environment complicate the task of identifying any single genetic susceptibility factor for Type 2 diabetes (Walley *et al.,* 2006). The maintenance of normal glucose homeostasis depends on a precisely balanced and dynamic interaction between tissue sensitivity to insulin (especially in muscle and liver) and insulin secretion. The molecular circuitry that maintains glucose homeostasis depends on the result of several combined gene defects, or from the simultaneous action of several susceptible alleles, or else from combinations of frequent variants at several loci that may have deleterious effects when predisposing environmental factors are present. It is generally accepted that insulin

resistance (IR) precedes the failure of insulin secretion and exacerbates this by imposing an increased secretory burden on the cells (Ferrannini, 1998). However, subtle abnormalities in cell function have been demonstrated early in the course of Type 2 diabetes mellitus (Vionnet *et al.,* 1992), and even in first degree relatives of individuals with Type 2 diabetes mellitus - suggesting a possible basis for an inherited component for cell failure (Kalsilamdrof & Tentouris, 2003). A prospective study in Pima Indians showed that the progression from normal to IGT and finally to Type 2 diabetes was accompanied by a progressive decline in cell secretory capacity (Weyer *et al.,* 1999). The mechanisms underlying cell failure in Type 2 diabetes however remain elusive. Type 2 diabetes being an extremely heterogenous disorder, phenotypically, and pathogenetically, is polygenic in nature. This means that multiple genes (polymorphism), each insufficient in themselves, must be present in order to cause diabetes. Such genes might affect cell apoptosis, regeneration, glucose sensing, glucose metabolism, ion channels, energy transduction, and other islet proteins necessary for synthesis, packaging, movement and release of secretory granules (Barret, 2008). Many rare forms of defective glucose metabolism have been shown to be caused by gene defects involving the cell and the insulin receptor (Defronzo & Prato, 1996). Of these the most common and important form is the maturity onset diabetes of the young (MODY).

2.2.3 Diagnosis of diabetes mellitus

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following: (WHO, 2014)

i. Fasting plasma glucose level ≥ 7.0 mmol/L (126 mg/dL).
ii. Plasma glucose ≥ 11.1 mmol/L (200 mg/dL) two hours after a 75 g oral glucose load as in a glucose tolerance test.
iii. Hyperglycemia and casual plasma glucose ≥ 11.1 mmol/L (200 mg/dL).
iv. Glycated hemoglobin (Hb A1C) ≥ 6.5 %.

A positive result, in the absence of unequivocal hyperglycemia, should be confirmed by a repeat of any of the above-listed methods on a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test (Seydah *et al.,* 2001). According to the current definition, two fasting glucose measurements above 126 mg/dL (7.0 mmol/L) are considered diagnostic for diabetes mellitus. People with fasting glucose levels from 100 to 125 mg/dL (5.6 to 6.9 mmol/L) are considered to have impaired fasting glucose (Bhakti, 2013). Patients with plasma glucose at or above 140 mg/dL (7.8 mmol/L), but not over 200 mg/dL (11.1 mmol/L), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two pre-diabetic states, the latter in particular is a major risk factor for progression to fullblown diabetes mellitus as well as cardiovascular disease (Mathew, 1998).

2.2.4 Diagnostic tests

Some of the commonly employed tests in the diagnosis of diabetes mellitus include oral glucose tolerance test (OGTT) and in some cases, fasting blood glucose (FBS). A. Oral glucose tolerance test (OGTT). This is the most accepted and widely applied test for the diagnosis of diabetes mellitus. In this test, the patients must fast for 14 hours and should discontinue glucose-altering medication at least 3 days prior to test. The patients must not smoke cigarette or drink alcohol or coffee just before and during the test, and patient must not be carbohydrate depleted 3 days prior to the test (George, 1992). After fasting for about 14 hours, the patient is given an oral glucose load of 75-100 g and blood sample is withdrawn every 30 minutes for the next 2 hours since in 2 hours the blood glucose level (<200 mg %) in a non-diabetic patient is expected to have normalized. But in diabetic, the plasma glucose level is higher than 200 mg (Aguwa & Omole, 2004), and returns to the baseline more slowly than it

does in normal or non-diabetics (Ganong, 1999). B. Fasting blood glucose (FBG) Blood sample is collected and analyzed after the patient has fasted over night, after a period of unimpaired carbohydrate intake. The normal range of fasting blood glucose is 70-110 mg% when collected from the venous blood (Aguwa & Omole, 2004).

2.2.4.1 *Monitoring test*

This method is mainly employed to monitor the therapeutic outcomes in management of diabetes mellitus and to enable the health personnel to choose the right drugs especially in ambulatory patients, Person that should be screened include those with strong family history of diabetes mellitus, persons severely obese, mothers with babies above 3.7 kg body weight at birth and patients scheduled for surgical operations (George, 1992).

2.2.4.2 *Postprandial glood glucose test*

This involves withdrawing of blood sample form the patient 2 hours after feeding heavy carbohydrate meal or being fed on 100 g glucose load. In a non-diabetic patient, the blood glucose level returns to normal 70- 110 mg after 2 hours while in diabetics, hyperglycaemia becomes apparent after 2 hours (Aguwa & Omole, 2004).

2.2.4.3 *Urine test*

Testing of early morning urine with clinistix or clinitest strips to determine the presence of glucose in urine can be done with as little as 0.25 % glucose in the urine. Clinitix (glucose oxidase) is glucose specific and the best quantitative estimation method for urine glucose level while the clinitest (copper reduction method) is non-specific for glucose only. The urine test method is gradually being relegated for the more concise and glucose specific automated electronic devices (Aguwa & Omole, 2004).

2.2.4.4 *Random blood glucose test (RBG)*

Blood sample is withdrawn from the patient and analyzed at any time of the day irrespective of meal that was taken when the blood glucose level is above 250 mg %. The patient is further tested with a method for diagnosis. (Cathy & Michelle, 2013).

2.2.4.5 *Glycosylated heamoglobin*

Glucose has been found to bind to proteins irreversibly and nonenzymatically thus causes chemical alteration in the proteins. The nonenzymatic glycosylation of the proteins occur by direct reaction between the aldehyde groups of the reducing sugars and primary amino groups in proteins to form Schiff bases that is rearranged to form stable protein ketoamine derivatives. This contributes to diabetes complications because it is an oxidative process (Odukoya & Ogbeche, 2002). In normal individual, the glycosylated haemoglobin ($HBA1_C$) is between 3-6 % while in a diabetic patient, the level may be as high as 18-20 %. It is used to monitor therapy compliance in diabetics considering their blood glucose control.

2.3 Complication in Diabetes

Hyperglycemia triggers damage to the vasculature and thus, leads to the failure of various organs including kidney, heart, retina of eyes and nerves; usually develop after many years of diabetes. This gives rise to the development of vascular complications which are categorized into micro- and macrovascular complications.

Microvascular disease or microangiopathy is actually the thickening of walls of small blood vessels so that they bleed and leakage of protein occurs. This narrowing of blood vessels results in decreased blood flow and impairment of oxygen flow throughout the body which leads to the damage of tissues or organs that are extremely sensitive to oxygen levels i.e., kidney cells, nerve cells and retina

On the other hand, macrovascular disease or macroangiopathy is the disease of large blood vessels due to clot formations that further results in the decreased blood flow all through the

body. This may cause heart diseases, peripheral vascular diseases or stroke. Both micro- and macrovascular complications are the result of hyperglycemia and it seems that they both may be interconnected but who precedes whom or whether they progress together, it is not clear. Complications of T2DM keep on increasing due to increasing burden of diabetes (Aristides & Rayas, 2007).

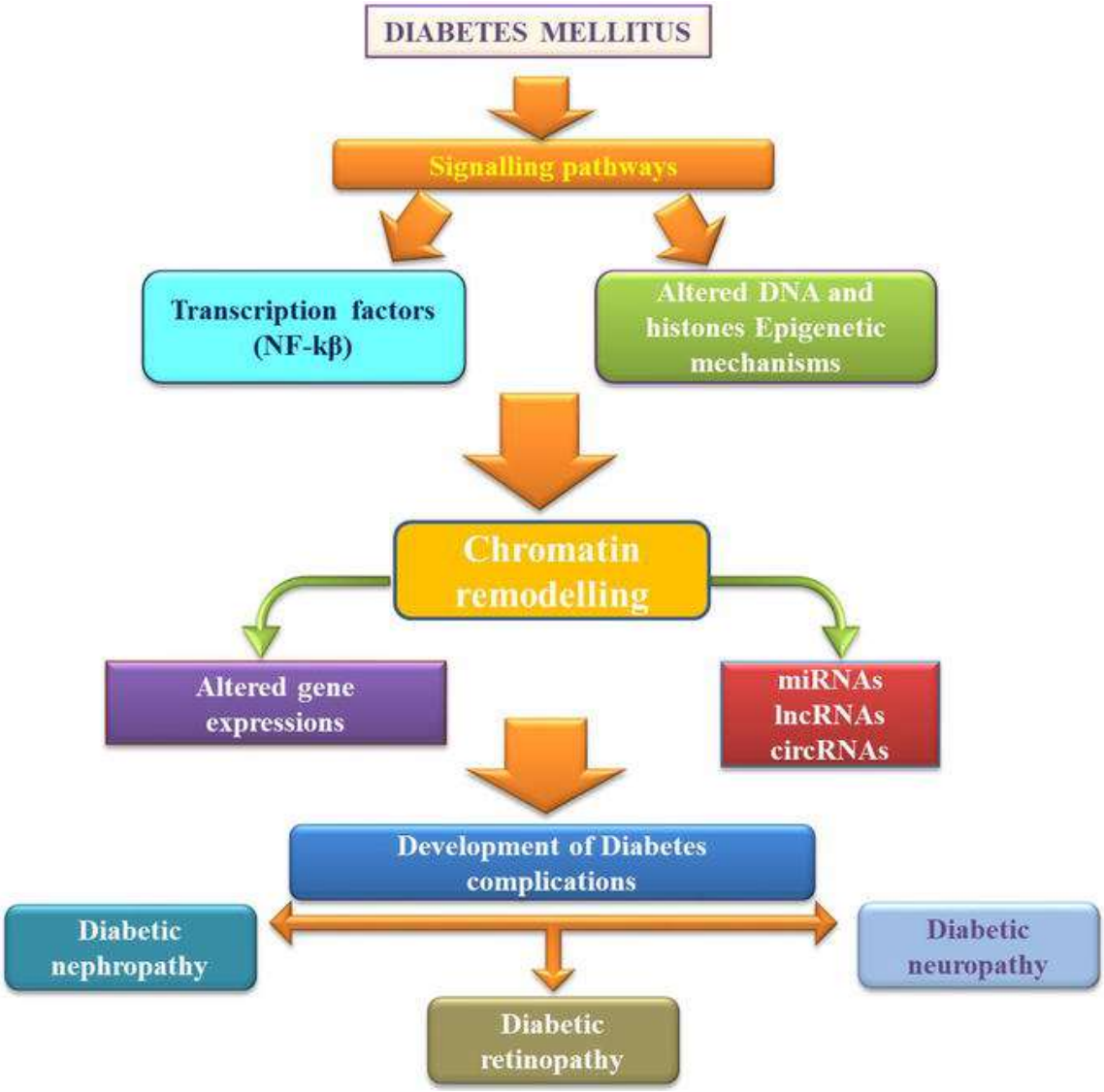


Fig.2.2 Pathways Preceding the Development of Microvascular Complications of Diabetes.

Source: Cryer *et al*., (2009)

2.3.1 Diabetic nephropathy (DN)

Is the major microvascular complication of diabetes affecting 20–30 % of patients with type 2 diabetes mellitus (Shabazian & Rezaii, 2013). Which weaken the quality of life leading to increased morbidity and mortality. Symptoms of DN are less evident in the early years of diabetes, usually develops after many years of diabetes. In India approximately 48 % cases of CKD are caused by diabetes (Rajput *et al.*, 2017). DN is defined as a clinical syndrome characterized by persistent proteinuria, a moderate deterioration of eGFR and an increasing arterial blood pressure (Jensen *et al.*, 1987). Being the foremost cause of end-stage renal disease (ESRD), it results in considerable morbidity and mortality and incurs massive burden of cost on patient and the society as well. Pathways, specifically renin-angiotensin-aldosterone system (RAAS), have been known to play a central role in the development and progression of nephropathy which eventually triggers numerous inflammatory factors directing to the development of fibrosis in the kidney, hypertension/hyperfilteration in the glomerulus and increased permeability to macromolecules leading to proteinuria (Seidegard, *et al.*,1988). It has been seen that some patients with good glycemic control may develop DN at later stages and patients with poor glycemic control may not always develop DN. This may partly be due to genetic predisposition among various ethnic populations. Presence of diabetic nephropathy within families and the large differences in its incidence among diabetic populations with different ethnicity suggests the contribution of several genetic and epigenetic factors in the development and progression of DN.

2.3.2 Diabetic retinopathy (DR)

Is a medical condition where damage to retina, as a result of high glucose, it is the most frequent cause of blindness in patients with diabetes (Group ER, 2000). Patients with DR usually does not develop any major symptoms at an early stage but during later stages physiological and metabolic abnormalities can appear leading to blindness, if left untreated. The risk factors associated with DR includes high blood glucose duration and type of diabetes (klein, *et al*., 1984), high Blood Pressure (Matthews *et al*., 2004) and, lipids (Ferris *et al*., 1996).

Presently it is being diagnosed with the identification of microvascular lesions in the retina. It has been differentiated clinically in 2 categories on the basis of ophthalmic observation: proliferative DR (PDR), the advance stage and; non-proliferative DR (NPDR), the early stage. NPDR can be identified by fundus where hard exudates, microaneurysms or haemorrhages are seen. NPDR is further categorized into mild, moderate and severe NPDR. On the other hand, detection of retinal neovascularization confirms PDR. The risk of progression of DR can be reduced by early detection, but it is difficult to achieve as there is little or no symptoms at early stages. Several molecular mechanisms are thought to involve in the development and progression of DR including polyol pathway, enhanced expression of vascular endothelial growth factor (VEGF), production of advance glycation end products (AGEs), activation of RAAS, hemodynamic alterations, etc. Current treatment involves conventional laser therapy and anti-VEGF or other anti-angiogenic, anti-inflammatory, non-steroidal anti-inflammatory drugs (NSAIDs) treatment. Despite this, reading is also difficult in patients with severe retina loss. Some treatments are precise but they are associated with high cost or side effects. Hence, the discovery of fundamental molecular mechanisms involved is required for the development of more specific interventions.

2.3.3 Diabetic neuropathy

Is nerve damage from high blood glucose (sugar) levels in people with diabetes Nerves throughout the body can suffer damage. People with poor glucose control and who have had diabetes for a long time are at highest risk for nerve damage. Diabetic neuropathy, a life-threatening complication involves both peripheral and autonomic nerves, affecting almost half of the diabetic population. The risk of development of diabetic neuropathy is directly proportional to both the duration and magnitude of hyperglycaemia. In addition, some individuals may also possess genetic facets that influence their predisposition in developing such complications (Granberg *et al.,* 2005). The prevalence of diabetic neuropathy varies from

country to country although the precise nature of the injury to the peripheral nerves from hyperglycaemia is not yet certain, the mechanisms of hyperglycaemia-induced polyol pathway, injury from AGEs, and enhanced oxidative stress have been implicated in its pathogenesis. The damage to peripheral nerves may be mediated by effects on nerve tissue or by endothelial injury or vascular dysfunction. Peripheral neuropathy in diabetes appears in several forms depending on the site, manifesting as sensory, focal/multifocal, and autonomic neuropathies. Diabetic neuropathy has resulted in more than 80% amputations after foot ulceration or injury (Berger *et al.,* 2009).

2.3.4 Macrovascular complications

Macrovascular complications are terms associated with the diabetes condition that occurs due to damage in large blood vessels. The damage is meant to occur when the blood sugar level is on a surge. Macrovascular complications include cardiovascular conditions like cardiac arrest, heart failure, stroke, numbness due to low blood supply to legs. But these complications can be snubbed through a proper diet, quality sleep, exercise, and keeping away from a stressful life. A scientific study proves that through a random sampling it was clear that having a better rate of metabolism will slow down the complications caused by Type 1 Diabetes and Type 2 Diabetes (Ferris, *et al.,* 1996) (Bailey & Day, 1989).

2.3.5 Atherosclerosis

This condition is a coronary artery disease that occurs when the blood vessel walls become thick and inelastic due to plaque caused by fat built up towards blockage. These cholesterol creating substances settle around the artery walls making it more difficult for the transportation of oxygen and other nutrients from the heart to the arteries, veins, and all other parts of the body. This also lowers that amount of blood flow to the organs and tissues of the body. In some cases, it restricts the transportation of blood to the legs or hands, making it numb (Centofani, 1995).

2.3.6 Transient ischemic attack (Stroke)

In this condition, there will be a temporary shutdown or slowdown of blood flow to the brain that will result in a stroke or TIA condition (Klein, *et al.,* 1984). The condition will be unnoticed and comes when things are going out of your control. Some of the signs that you are about to be attacked by the stroke condition include numbness on the face that is one-sided, trouble speaking and understanding, unexpected blurred vision, uninvited and extreme headache, and dizziness. Other complications include coronary heart diseases, peripheral artery diseases, chronic kidney disease etc.

2.4 Pathophysiology of Diabetes Mellitus

2.4.1 The endocrine pancreas

The human pancreas is basically composed of two types of secretory cells that are both involved in nutrient handling: 98 % of the cells- the exocrine type, secretes a food processing enzyme bicarbonate mixture into the duodenum, while the remaining 2 % the endocrine type, have a metabolic function and secrete a mixture of nutrient-generated hormones into the portal vein. This small endocrine part is of vital importance in maintaining glucose homeostasis through the action of the 51-amino acid peptide insulin. Four endocrine cell types can be distinguished: A cells (alpha), B cells (beta), D cells (delta) and PP cells (pancreatic polypeptide) (Klöppel & In't Veld, 1997). These endocrine cells are distributed throughout the pancreas in areas known as islets.

2.4.2 Diabetes-related islet changes

The islet changes, from a morphological point of view, associated with various types of diabetes can be divided into those with and without severe beta-cell loss. Severe beta-cell loss is found in type I diabetes and some uncommon forms of diabetes such as virus-related

diabetes and congenital diabetes. Islets without severe loss of beta-cells are encountered in type II diabetes and in the secondary forms of diabetes (Klöppel & In't Veld, 1997).

2.4.3 Mechanism of insulin

Insulin is a protein composed of 2 polypeptide chains A and B linked by disulphide bridges. Banting and Best discovered insulin in 1921. The chain A and B have 21 and 30 amino acids respectively. The molecular mass of insulin is 5.734 KDa. A chain contains an intra-chain disulphide bridge linking residue 6 and 11. Insulin synthesized in the form of preproinsulin occurs in beta cell of pancreas. Preproinsulin is the ultimate precursor (Bliss, 2000). After synthesis, it released into cisternal space of rough endoplasmic reticulum cleaved into proinsulin by proteolytic enzymes. Proinsulin with a C (connecting) chain linking A and B chains, is then transported by microvesicles to the Golgi apparatus. Proinsulin is converted to insulin, which continues in maturing granules through the action of prohormone convertase 2 and 3 and carboxyl peptidase. Secretion of insulin occurs in response to various stimuli like glucose, arginine, and sulphonylureas though physiologically glucose is the major determinant.

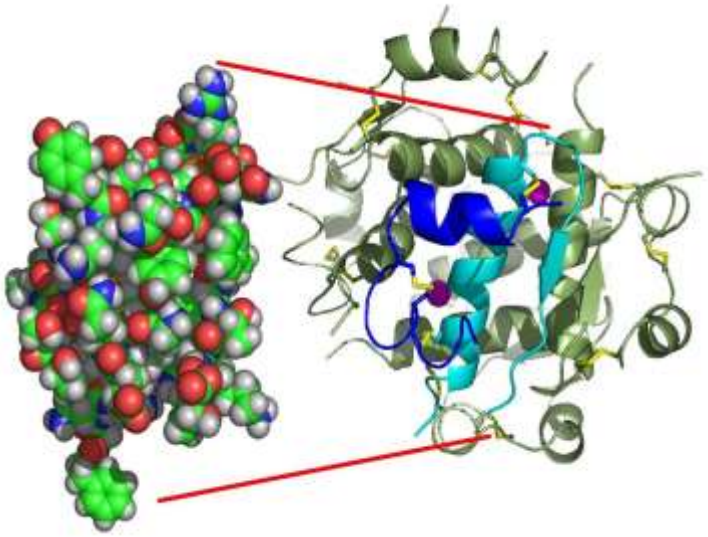


Figure 2.3: Structure of Human Insulin

Source: Weiss, M. *et al., (2000).*

2.4.3.1 *Effect of insulin on carbohydrate metabolism*

When there is inhibition of glucose consumption in the peripheral tissues it contributes to elevated blood glucose levels and equally increase glucose formation in the liver. In the absence of insulin, the insulin antagonistic hormone glucagon and epinephrine dominate metabolic regulation. This will increase gluconeogenesis, increase of glucose by the liver and stops glycolysis which effects are mediated by the intracellular second messenger cAMP and fructose-2,6-bisphosphate. Similarly, glycogen synthesis is inhibited and glycogen degradation accelerated by way of intracellular cascades that result in the phosphorylation of both glycogen synthase, which becomes inactive and phosphorylation, which becomes active. Increase level of glucose to a great distance blends by the derailment of muscle metabolism. The muscle no longer able to take up glucose switches to protein breakdown for its needs of ATP. Glutamine and alanine generated from disposal of nitrogen from degraded amino acids transported to the liver where they supply the urea cycle and gluconeogenesis with substrate (Michael *et al.,* 2016).

2.4.3.2 *Effect of insulin deficiency on protein metabolism*

In type 1 diabetes, individuals are in a catabolic state without insulin replacement. During insulin deficiency, it been demonstrated that there are increases in protein breakdown and protein synthesis in whole body. From regional studies, protein breakdown and protein synthesis inhibit when insulin is replaced in splanchnic tissue but only protein breakdown inhibits in skeletal muscle. This is because increase in protein synthesis in splanchnic tissue is greater than the increase in protein breakdown. In contrast, when insulin is deficient there is a net increase in protein breakdown in skeletal muscle resulting in a net release of amino acids (Subramanian & Chait, 2014).

2.4.3.3 *Effect of insulin deficiency on lipid metabolism*

Insulin deficiency leads to dis-inhibition of hormone sensitive lipase in fat tissue. This dis-inhibition of hormone sensitive lipase induces the breakdown of triacylglycerol to glycerol and fatty acids, released into the circulation. Fatty acids may also enter the liver and be degraded to acetyl-coA. Excess of blood glucose increases acetyl CoA in the liver, which increases the intracellular glucose level (note that the liver can take up glucose without insulin). This drives the synthesis of ketone bodies, triacylglycerol and cholesterol; the levels increase in the blood of diabetic patients. The increased blood fat increases the incidence and severity of atherosclerosis in diabetic patients (Michael, 2008).

2.4.4 Alloxan synthesis

Alloxan is a derivative of urea, which causes selective modification of β-cells of pancreatic islets (Iranloye *et al.,* 2011). Insulin-producing beta cells found in the pancreas is destroyed selectively by alloxan. It has an underlying mechanism, which involves the uptake of the compound due to its similarity to glucose, and high efficient uptake mechanism of the pancreatic beta cells.

2.4.4.1 *Mechanism of action of alloxan*

Alloxan also known as (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidine tetrone) causes diabetes when it is administered parenterally: intravenously, intraperitoneally or subcutaneously. The action of alloxan in the pancreas is preceded by its quick uptake by the beta cells. This quick uptake by insulin-secreting cells has been proposed to be one of the important features of determining alloxan diabetogenicity. The dose administration is specie dependent. It has been shown by several experimental studies that alloxan evoke a sudden rise in insulin secretion in the presence or absence of glucose which appeared just after alloxan treatment (Viswanathaswany *et al.,* 2011).

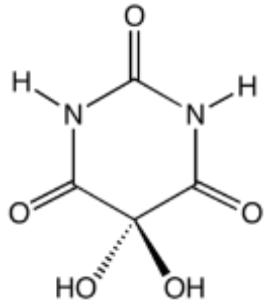


Figure 2.4: Chemical Structure of Alloxan

Source: Tipson, R.S., (1953)**.**

2.5 Pharmacological Managements of Diabetes Mellitus

While lifestyle modifications and metformin are the cornerstone of the initial management of type 2 diabetes mellitus, there is an increasing array of second and third-line pharmacological agents for this condition. These include sulphonylureas, insulin, thiazolidinediones and alpha-glucosidase inhibitors, with the more recent addition of glucagon- like peptide-1 agonists, dipeptidyl peptidase-IV inhibitors and pramlintide. Moreover, insulin analogues that better simulate endogenous insulin secretion have been developed. (Aristides & Rayas, 2007).

2.5.1 Oral hypoglycemic agents

History In contrast to the systematic studies that led to the isolation of insulin, the sulfonylurea was discovered accidentally. Jaben and colleagues noted that some sulfonamides caused hypoglycemia in experimental animals. Soon thereafter, I butyl -3-sulfonylurea (carbutamide) became the first clinically useful sulfonylurea for the treatment of diabetes. Although later withdrawn because of adverse effects on the bone marrow, this compound led to the development of the entire class of sulfonylareas. The sulfonylureas are divided into two groups or generations of agent. The first group includes tolbutamide, acetohexamide, tolazamide, and chlorpropamide.

Second, more potent ones include glyburide (glibenclamide), glipizide, gliclazide, and glimepride. In 1997, repaglimide, the first number of a new class of oral insulin secretagogues called meglitinide was approved for clinical use. This agent has gained acceptance as a fast-acting premeal therapy to limit postprandial hyperglycemia. Another example of the class is Nateglamide.The goat's plant (*Galega officinalis*), used to treat diabetes in Europe in medieval times was found in the early twentieth century to contain guanidine. Guanidine has hypoglycemic properties but was too toxic for clinical use. During the 1920s, biguanides were investigated for use in diabetes, but they were overshadowed by the discovery of insulin (Defronzo & Goodman, 1996). Shortly after the introduction of the sulfenylureas the first biguanides became available for clinical use which includes phenformin and Metformin. Phenformin was withdrawn from the market because of an increased frequency of lactic acidosis associated with its use, Metformin has been used. Thiazolidinedions is the second major class of insulin sensitizers." These agents bind to peroxisome proliferator activated receptors (principally PPARY), resulting in increased glucose uptake in muscle and reduced endogenous glucose production. The first of this agent troglitazone was withdrawn from use in the United States in 2000 because of an association with hepatic toxicity.

2.5.1.1 Sulfonylureas

Sulfonylureas cause hypoglycemia by stimulating insulin release from pancreatic B-cells. It binds to the SUR1subunits and blocks the ATPsensitive Potassium ion channel (Aguilar-Bryan *et al.,* 1995). The drugs thus resemble physiological secretagogues (e.g glucose, leucine), which also lower the conductance of this channel. Reduced Potassium ion conductance causes membrane depolarization and influx of Calcium ion through voltage-sensitive Calcium ion channels. Sulfonylureas also may further increase insulin levels by reducing hepatic clearance of the hormone. Sulfonylureas also stimulate release of somatostation, and they may suppress the secretion of glucagons slightly (Philipson & Steiner, 1995). Although extrapanreatic effects

of sulfonylureas can be demonstrated, they are of main clinical significant in the treatment type 2 DM Patients Repaglimide Meglitimides: Like sulfonylurea, repaglimide stimulates insulin release by closing ATP- dependent potassium channels in pancreatic β-cells.

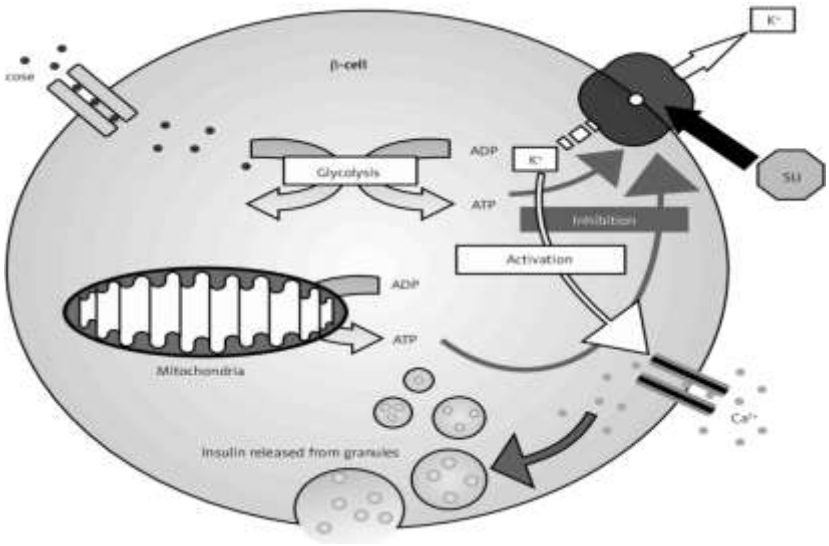


Figure 2.5: Mechanism of Action of Sulfonylureas

Source: Daniele *et al*., (2015).

The drug is absorbed rapidly from the gastrointestinal tract, and peak blood levels are obtained within 1 hour. The half –life of the drug is about 1hour. These features of the drug allow for multiple preprandial uses as compared with the classical once-or twice-daily dosing of sulfonylureas. Biguanides (metformin): Metformin is antihyperglycemic, not hypoglycemic (Bailey, 1992). It does not cause insulin release from the pancreas and generally does not cause hypoglycemia, even in large doses. Metformin has no significant effects in the secretion of glucagons, cortisol, growth hormone, or somatostatin. Metformin reduces glucose levels primarily by decreasing hepatic glucose production and by increasing insulin action in muscle and fat. The mechanism by which metformin reduces hepatic glucose production is controversial, but most data indicate an effect on reducing gluconeogenesis (Stumvoll *et al.,*

1995). Metformin also may decrease plasma glucose by reducing the absorption of glucose from the intestine, but this action has not been shown to have clinical relevance. Thazolidinediones: They are selective agents for nuclear peroxisome proliferation-activated receptor-y (PPARY). These drugs bind to PPARY, which activates insulin-responsive genes that regulate carbohydrate and lipid metabolism. It requires insulin to be present for their action thiazolidinediones exert their principal effects by increasing insulin sensitivity in peripheral tissue but also may lower glucose production by the liver. Thiazolidinediones increase glucose transport into muscle and adipose tissue by enhancing the synthesis and translocation of specific forms of the glucose transporter. Although muscle is a major insulin-sensitive tissue, PPARY is vertically absent in skeletal muscle. The first-generation sulfonylureas vary considerably in their half-lives and extent of metabolism. The half-life of acetohexamide is short but the drug is reduced to an active compound whose half-life is similar to those of tolbutanide and tolazamide 4-7hrs, it may be necessary to take these drugs in divided daily dose. Chlorpropamide has a long half-life (24 to 48 hrs). The second-generation agents are approximate 100 times more potent than those in the first group. Although their half-lives are short (3 to 5 hours), their hypoglycemic effects are evident for 12 to 24 hrs, and they often can be administered once daily. The reason for the discrepancies between their half-lives and duration of action is not clear. All the sulfonylurea is metabolized by the liver, and the metabolites are excreted in the Urine. Metabolism of chlopropamide is incomplete, and about 20% of the drug is excreted unchanged. Thus, sulfonylureas should be administered with caution to patients with either renal or hepatic insufficiency. Therapeutic uses Sulfonylureas are used do control hyperglycemia in type 2 DM patients who cannot achieve appropriate control with changes in diet alone. In all patients, continued dietary restrictions are essential to maximize the efficacy of the sulfonylureas. Contraindication to the use of these drugs include type 1 DM, pregnancy, lactation, and for the older preparations, significant hepatic or renal insufficiency.

2.5.1.2 *Thiazolidinediones*

Three thiazolidinediones have been used in clinical practice (tioglitazone, rosiglitazone, and pioglitazone) however, troglitazone was withdrawn from use because it was associated with severe hepatic toxicity. Rosiglitazone and pioglitazone can lower haemoglobin A1c levels by 1 % to 1.5 % in patients with type 2 DM. The thiazolidone tend to increase highdensity lipoprotein (HDL) cholesterol but have variable effects on triglyceredinediones and low-density lipoprotem (LDL) cholesterol. Hence thiazolidinediones have been reported to cause anemia, weight gain, edema and plasma volume expansion (Ruderman & Prentki, 2004).

2.5.1.3 *Glucagon-like peptides (incretins)*

Over some decades ago, reported that oral as compared with intraveneus delivery of glucose produced a greater release of insulin. Subsequent work identified two hormone-glucose-dependent insulinotropic polypeptide gastric inhibitory polypeptide and glucagon-like peptide-1 (GLP -1)- that are released from the upper and lower bowel that augment glucose dependent insulin secretion (Mayo *et al.,* 2003).

These hormones are termed incretins. The two incretins deferentially stimulate insulin secreting. GLP has little effect on increasing insulin secretion in type 2 DM, whereas GLP-1 significantly augments glucose dependent insulin secretion. Consequently, GLP-1 has become an attractive target for therapeutic development in type 2 DM. GLP-1 also reduces glucagon secretion, slow gastric emptying, and decrease appetite. Incretins are inactivated by dipeptidyl peptidase IV enzyme (DPP-IV) within (1-2 minutes) of its release in. Consequently, considerable work has been performed to produce GLP-1 reception agonist that maintain the physiologic effects of the native incretin but are resistant to actions of DPP-IV i.e incretin mimetics. The GLP receptor is expressed in the pancreatic islet, as well as, the gut, adipose tissue, heart, pituitary, adrenal cortex and the brain (Usdai *et al.,* 1993). Incretins are hormones produced from the gastrointestinal tract that act to enhance the usual release of insulin after the oral

ingestion of carbohydrates (Nauck *et al.,* 1986; & Drucker, 2003). They also slow the gastric absorption of nutrients and act to promote a feeling of satisfy that can lead to weight loss in overweight individuals. These agents work to lower glucose levels without causing hypoglycemia, but with gradual weight loss due to decrease in caloric intake. Exenatide augment the hypoglycemic effects of sulphonylureas when co-administered, but on its own, will not course hypoglycemia, and do not when used in combination with metformin. Liraglutide is a human long –acting form of glucagon-like peptide-1 (GLP-1) that is similar in action to extinitide.

2.5.1.4 *Alpha- glucosidase inhibitors*

Alpha-glucosidase inhibitors act by inhibiting the enzyme alpha glucosidase found in the brush border cells that line the small intestine, which cleaves more complex carbohydrates like starch, dextrin, and disaccharides into sugar (Davis *et al.,* 1996). Because they inhibit the breakdowns and subsequent absorption of carbohydrates from the gut following meals, these drugs impact on postprandial hyperglycemia more reasonably and modestly on fasting plasma glucose level. α-glucosidase inhibitors do not stimulate insulin release and therefore do not result in hypoglycemia. They can significantly improve hemoglobin A1c levels in severely hyperglycemia type 2 diabetes mellitus patients. The serious side effect observed with these agents is gastrointestinal effects such as abdominal discomfort, bloating, flatulence and diarrhea. Examples include Acarbose (PRECOSE) and migletol (glyset). Acarbose treatment has been linked to elevation in serum transaminase level and the use of this agent is contraindicated in patients with liver cirrhosis (Chiasson *et al.,* 2002).

2.5.2 Mechanism of action of anti-diabetic drugs

The present treatment of diabetes is focused on controlling and lowering blood glucose to a normal level. The mechanisms of both western medicines and the Traditional medicines to lower blood glucose are to:

i. stimulate B. cell of pancreatic islet to release insulin
ii. resist the hormones which rise blood glucose
iii. increase the number or rise the appetency and sensitivity of insulin receptor site to insulin,
iv. decrease the leading-out of glycogen
v. enhance the use of glucose in the tissue and organ
vi. clear away free radicals, resist lipid peroxidation and convert the metabolic disorder of lipid and protein,
vii. Improve microcirculation in the body.

Based on the above-mentioned mechanisms, the drugs clinically used to treat diabetes can be mainly divided into insulin, insulin secretagogues, insulin sensitivity improvement factor, insulin like growth factor, aldose reductase inhibitors, α- glucosidase inhibitors, protein glycation inhibitors, almost all of which are chemical and biochemical drugs (Zhao, 1999). The effect of these drugs is only aimed to lower the level of blood glucose. Moreover, in most cases, side effect such as hypoglycemia, lactic acid intoxication and gastrointestinal upset appear after patients took these medicines.

2.5.3 Role of medicinal plants in the management of diabetes mellitus

Medicinal plants have a vast potential in the treatment of various ailments due to the presence of therapeutically important phytochemicals and several marketed medications are available to alleviate the symptoms of diabetes. However, these over the counter drugs are expensive and associated with several complications. Herbal medicines are gaining importance as they are cost-effective and also display improved therapeutic effects with lesser side effects.

Medicinal plants are used in the treatment of various illness due to the presence of therapeutically important phytochemicals Sometimes in patients with diabetes mellitus, the levels of antioxidant parameters are found to decrease, hence in many studies phytochemicals are suggested to improve the insulin sensitivity Some phyto compounds such as flavonoids, prophenyl phenols, are also found effective in the complications of diabetes (Van *et al.,* 2008).

2.5.3.1 *Phytochemicals Constituents*

Phytochemicals are non- nutritive natural occurring plant chemicals that have protective or disease preventing potentials. Some are responsible for the organoleptic properties Such as deep purple of blue berries and the smell of garlic. Plants are known to produce these chemicals to protect themselves but have been shown to protect human against disease and pest. Human beings have been utilizing plants for basic preventive and the curative health care since time immemorial. The green plant represents a reservoir of effective chemo-therapeutants and can provide valuable source of medicine for treatment of various aliment (Azubuogu, 2012). Phytochemicals are found in plant based food like fruit vegetables, beans, and grains. When ingested by animals phytochemical elicit varied biochemical and pharmacological action. There are some evidence that diet rich in fruit vegetable beans and grain reduce the risk of certain type of cancer and other disease. Researchers are looking for specific compounds in this food that may account for this healthful effect in humans. Available scientific evidence does not support claims that taking phytochemicals supplement are as goods for long – terms health as consuming the fruit, vegetable beans and grains from which they are taken. some scientist speculate that potential health benefits of Phytochemicals may best be derived from consumption of whole food (Azubuogu, 2012) Different plant species contain different combinations of phytochemicals.

2.5.3.2 *Type of phytochemicals*

Scientist estimated that there may be as many as 10,000 different phytochemicals having potentials to effect disease such as cancers, stroke, metabolic syndrome however only a relatively small percent of this phytochemicals has been analysed these Phytochemicals include steroid, terpenoid alkaloid, flavonoid anthraquinone tannins, saponins, e.t.c

2.5.3.3 ***Alkaloids contents***

Alkaloids are group of naturally occurring organic compound that contain at least one nitrogen atoms. This group also includes some related compound with neutral and even weakly acidic properties. Some synthetic compounds of similar structure are also attributed to alkaloid (Robert, 2008). In addition to Carbon Hydrogen and Nitrogen, alkaloid may also contain Chlorine, Bromine and Phosphorus. Many alkaloids can be purified from crude extract by acid – base extraction. Alkaloids often have pharmacological effect and used as medication, as recreational drugs or in entheogenic rituals, e.g. the local anesthetic and stimulant cocaine, the psychedelic psilocin, the stimulant caffeine and nicotine. Alkaloids have a range of antimicrobial and insecticidal properties including anti-feedant and toxic properties and have potentials for commercial development as wood treatment agent (Mao & Henderson, 2007). Although alkaloids act on diversity of metabolic system in human other animals they almost uniformly invoke a bitter a taste (Rhoades, 2007).

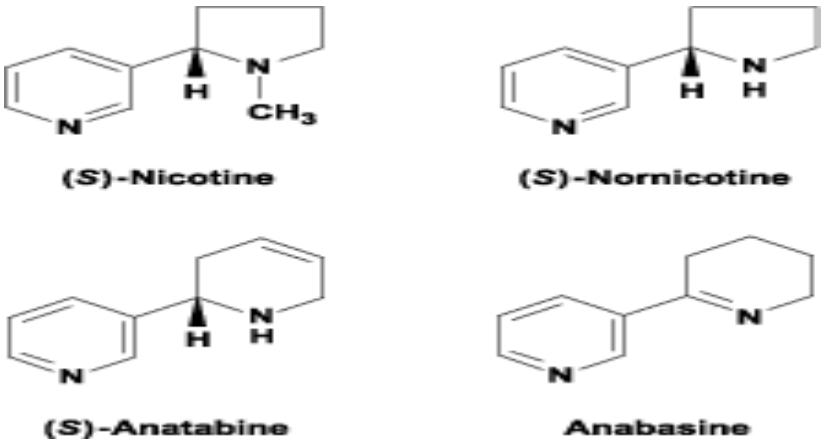


Figure 2.6: Structure of Nicotine and other Alkaloid

Source: M .C craw-hill science and Technology, (2005).

2.5.3.4 ***Saponins contents***

Saponins are naturally glycoside with distinctive foaming characteristic. They are found in any plant but get their name from the soapwort plant (saponaria) the root of which was use historically as soap. Saponin are amphiphatic glycoside grouped in terms of phenomenology by the soap – like foaming they produce when shaken in aqueous solution and in term of structure by their composition of I or more hydrophilic glycoside``e moieties combined with lipophilic triterpene derivatives. They consist of polycyclic aglycone that is either a choline steroid or triterpenoid attached through C3 and an ether bond to a sugar side chain (Gao & wang, 2006). The aglycone referred to as sapogenin and stereoid saponin is called saraponin. Some saponins reduce the feed intake and growth rate of non-ruminant animals while others are not very harmful, e.g. the saponin found in oats and spinach increase the body ability to absorbed calcium and silicon, thus aiding digestion. Certain pasture weed contain substantial quantities of dangerous saponin and result in life threatening toxicities for certain animal's species (Magalheas *et al*., 2003).

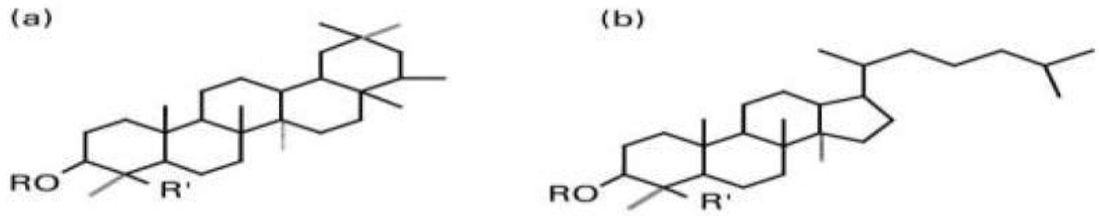


Figure 2.7: Basic Structure of Sapogenin (a) Triterpenoid (b) Steroid

Source: Magalheas *et al*., (2003).

2.5.3.5 ***Tannin contents***

Tannins are naturally occurring plant polyphenol with the empirical formula of $C_{14}H_{14}O_{11}$. They have a yellow-white to brown colour that deepen when expose to light and also have a faint

characteristic odour. They are commonly found in fruits chocolate, legumes forages legumes trees and in greases and bind to precipitate protein. The precipitating properties are use in clarifying or cleaning of wines and beers. Tannins are also use in tanning of leathers and are valuable as an external medicine because it is astringent and styptic. Tannins have been reported to be responsible for decrease in feed intake, growth rate, feed efficiency, net–metabolizable energy, and protein digestibility in experimental animals. Therefore, food rich low in tannin is considered to be of low nutritional value. However recent finding indicate that the major effect of tannin was not due to their inhibition on food consumption or digestion but rather the decrease efficiency in converting the absorbed nutrient to new body substrate (Chung *et al*., 2008; & Wang *et al*., 2011).

2.5.3.6 *Anthraquinones constituents*

Anthraquinone are aromatic organic compound also known as 9, 10-anthracenedione, anthradione, 9,10-anthrachinon, anthracene, 9-10 quinone, 9-10 dihydro 9, 10-dioxoan thracene and trade names hoelite, moket, corbit and others. Anthraquinone occur naturally in some plant (like alone, senna,) fungi, lichen and insects where it serves as a basic skeleton for their pigment. Natural anthraquinone derivatives tend to have laxative effect. Anthraquinone is insoluble in water, alcohol but dissolve in nitrobenzene and aniline it is chemically fairly stable under normal condition. Anthraquinone can be obtained by oxidation of anthracene, condensation of benzene with phthalic anhydride in the presence of ALC_3 and by diel –alder reaction (From anthraquinone and 1,3- dien) Anthraquinone is used in production of dye and are use as catalyst in production of wood pulp and paper industry. 2-ethlanthraquionone is used to provide H_2O_2 (Li *et al*., 2011).

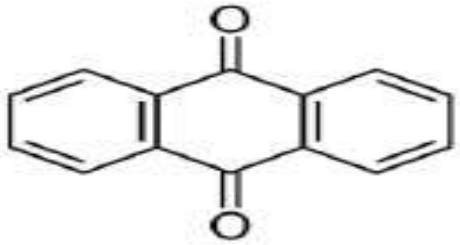


Figure 2.8: Anthraquinones

Source: Iwanete *et al*., (2012)

2.5.3.7 *Flavonoid contents*

Flavonoids are most commonly known for their antioxidant activity and they have strong ability to modify the body's reaction to allergies, carcinogenesis and viruses. They have been referred to as native biological response modifier in experiments. Flavonoids have shown anti allergic, anti-inflammatory, anti-microbial and anti-cancer activity and therefore interest in their preventive role in cancer and cardiovascular disease has increased (Lakhampal & Rai, 2007).

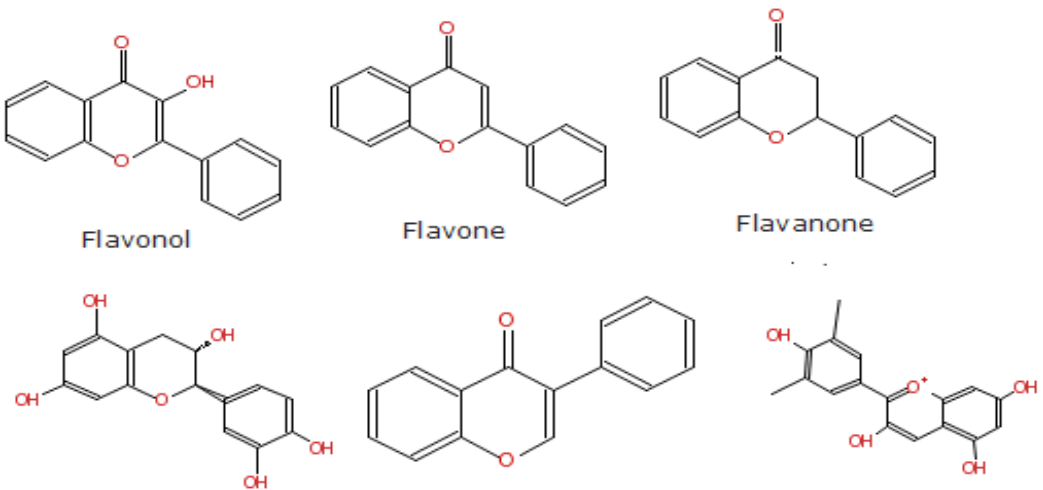


Figure 2.9: Structure of Major Class of Flavonoids

Source: Lakhampal and Rai (2017).

2.5.3.8 *Glycosides contents*

Glycosides are natural organic chemicals, that can be hydrolysed to sugars (glycone) β_form and a non-sugar (aglycone) or called genin and are said to be water soluble and bitter in taste (Brito-Arias, 2007). Glycosides occur in both plants and animals. They play vital roles within plant of which some protects the plant from bacteria and diseases (Edeoga *et al*., 2005). Plants containing glycosides are well known for their pharmacological activity. Arbutine, salicine and anthraglucosides are all examples of plant-derived glycosides exhibiting therapeutic (healing) properties whilst other important glycosides are digitalis glycosides (e.g. digoxin, digitoxin and gitoxin), ouabain, k-strophanthin, scillaren A, B, convollosides that are essential for good heart activity (Ekaeta *et al*., 2013). Among other therapeutic effects, glycosides have also been reported to exhibit antimalarial activity. A mode of action proposed this biological activity is the inhibition of haemoglobin proteases such as plasmepsin II (Dell-Agli *et al*., 2003).

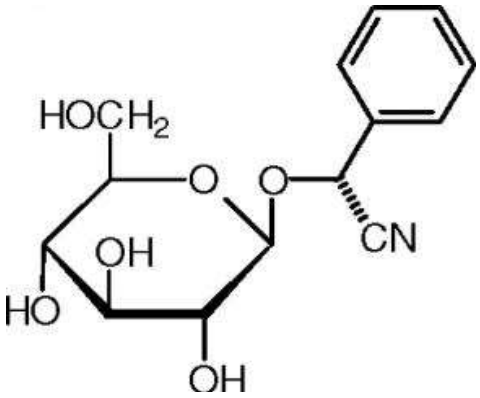


Figure 2.10: Structure of Glycoside

Source: Iwanette, (2012).

2.5.4 Use of medicinal plants in the treatments of diabetes

Medicinal plants have been used by the population since the beginning as a tool to prevent and fight diseases. In addition, to strengthening local culture and offering cheaper labor, this practice is an alternative to conventional therapies. The use of these plants in metabolic disorders has been studied worldwide. It has shown promising results in reducing glycemic levels and profiles related to diabetes, such as LDL lipid profile, total cholesterol, triglycerides, and glycated hemoglobin (HbA1c). These results can be potential sources for pharmacological treatments, through the production of medicines, as well as the implementation of these plants in the diet, dispensing with the use of commercialized drugs. (Srinivasan, 2006).

2.6 *Laguncularia racemosa*

The common name, “white mangrove,” is based on the white salt deposits that are expelled from the leaves and form surface deposits. Others speculate that the name is based on the white flowers (Michael *et al.,* 2016). This native evergreen tree grows in the coastal areas of south Florida, the Caribbean, and Central America. It is generally found further upland than black (*Avicennia germinans*) and red (*Rhizophora mangle*) mangroves, and can reach heights of 30 to 40 feet in full sun (Michael *et al.,* 2016). Leaves are simple, opposite, and between 1 and

3 inches long. The tops and undersides of the leaves are light green with a thick, leathery, and smooth exterior (Michael *et al.,* 2016). One distinguishing characteristic of the white mangrove is the presence of two glands on the petiole just below the leaf base, where excess salt is excreted. The bark is light brown with vertical ridges and can grow a single- or multi-stemmed trunk. Inconspicuous and fragrant white flowers bloom almost year round, occurring as spikes in leaf axils or on the tips of branches. Oblong fruit pods are green to brownish and about ¾ inches in length. Each pod contains one seed and the fruit ripens in the fall (Michael *et al.,* 2016).

Taxonomic classification of *Laguncularia racemosa* (White mangrove)

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Myrtales
Family	Cumbretacea
Genus	*Laguncularia*
Species	*Laguncularia racemosa (Michael et al., 2016).*

Figure 2.11: *Laguncularia racemosa* Leaf (White mangrove)

Source: (Michael *et al.,* 2016).

A chemical and pharmacological survey of mangrove plants in the Australia region revealed that several species of mangroves plants leaves possess antiviral activity and healing properties in popular folk medicine that are attributed to Rhizophora trees (Red mangrove) (Bandaranayake, 2002). Similarly, the root, leaf and stem extracts of Rhizophora trees have inhibitory properties affecting the growth of various human pathogenic organisms and among these are bacteria, fungi and viruses, (Bandaranayake, 2002). It has been reported that mangrove plant cured throat cancer with gargles of mangrove bark (Bandaranayake, 1998). Bark of red mangrove trees have been used in folk remedy for a wide array of diseases. More

recently, (Mulholand *et al.,* 2004) reported that extracts of Rhizophora mangle (Black mangrove) had anti-diabetic and antihyperglycemic properties. (Prasad *et al.,* 2009) asserted that Avicennia plants, especially *Laguncularia racemosa* leaves are used in traditional medicine that might serve as lead for the development of drugs. The influence of mangroves trees on reproductive health and their performance enhancement attributes in human and animal has been reported to be due to the following phytochemicals: Alkaloids, Lignins, Flavonoids, Lipids, Benzernoids, Steroids, Alkanes, Tanin and Saponins. The use of such phytochemical extracts by herbalists to improve the reproductive hormones and the overall performance of animals and man was associated to its phytochemical (aphrodisiac) properties (Sofowora, 1993). The results of the phytochemicals analysis of the forest plant revealed that it contains favorable phytochemicals of Tannin, Saponin, Alkaloid, Flavonoid and Phytate agreed with the work of (Bandaaranyake, 2002) who reported that, mangrove plants are rich sources of steroids, triterpens, saponins, flavonoids, alkaloids and tannins. (Wekhe, 2007) reported that alkaloids are used as antiparasites, antispasmodic and bacterial antigens. (Ahamefule *et al.,* 2006) submitted that flavonoid and alkaloid present in some mangrove plants such as *Langucularia racemosa* function in protection against inflammation, allergies, and microbial infestations. (Kawo *et al.,* 2009) reported the pharmacological activities of phytochemicals in plants to include antimicrobial, inflammation inhibiting and cytotoxic activities. Thus, livestock farmers are encouraged to use this mangrove plant due to its pharmacological benefits thereby saving funds meant for drugs (antibiotics), since most of these phytochemicals are present in this analyzed *Laguncularia racemosa* leaves. (Bandaranayake, 2002) reviewed the role of flavonoids, alkaloid and saponin as therapeutic agents and have implicated the flavonoids components in forest plants such as mangrove leaves in enhancing aphrodisiac properties and indirectly influencing the production of estrogen/testosterone in animals and Man. Animals that consumes this forest leaves are likely to experience high libido and significant increase in reproductive performance. Flavonoid is the most common widely distributed groups of

phytochemicals in forest plants (phenolics) as reported by (Ahamefule *et al.,* 2006). Its biological function includes protection against allegies, platelet aggregation, microbes, ulcer and tumors, he further stated that several biological activities such as cytotoxic, anti-neoplastic, antibacterial, ant herpetic, steroidal, and anthelminthic are reported to influence defense against invading parasites. Therefore, it's in line to state that the use of mangrove leaves in animal feeds manipulation will assist in improving reproductive efficiency and also help in enhancing body immunity against the possible various diseases.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and reagent

All chemical used in this work were of analytical grade and they include ; Methanol was purchased from E. Merck (Germany), N-haxene and ethyl acetate, JDHChemicals (Bangladesh ltd), distilled water, metformin (Standard drugs), petroleum Ether, alloxan monohydrate, Explicit chemicals (indian), citrate buffer, filter paper (WhatmanNo4), glucose and plant samples (*L. recemosa*), Mayers reagents, Dragendroffs reagent, Folins-denis reagent, Folin-Ciocalteu reagent and sulfuric acid were products of sigma chemical Co.

3.1.1.2 *Enzyme kits*

Dipeptidyl peptidase 4 assay kits were purchased from my biosource USA.

3.1.2 Equipment

The equipment used for this study include; Soxhet extractor, test tubes, measuring cylinder, weighing balance, Fine-test Glucometer, Hot air oven, mortar and pestle, cage, analytical balance, refrigerator, calibrated syringe, insulin syringe, measuring cylinder, hand gloves, conical flask, volumetric flask, blender, beaker, foil paper, sterile container, medical blade, spatula, funnel, sieve, and filter paper and heating bath.

3.1.3 Plants materials

The fresh leaves of *Laguncularia racemosa* were obtained from its natural habitat around fresh water swamp area in Lagos. *Laguncularia racemosa* leaves were obtained in the month of June

2019. Taxonomic authentication of the plant was conducted at the Institute of oceanography and marine research (NIOMR) Lagos State.

3.2 Methods

3.2.1 Sample preparation and extraction

The fresh leaves of *Laguncularia racemosa* were dried at room temperature for two weeks. The dried leaves were then blended to powder using electronic blending machine, the powder was weighed and collected into clean a container and labelled.

3.2.2 Extraction

Fifty grams (50 g) of the powdered dried leaves of *Laguncularia racemosa* was weighed and extracted 70 % methanol was used as the solvents for hot (reflux for two hours) and cold extraction for 72 hours. The plants extracts were sieved using filter paper, concentrated in a rotary evaporator (RE-6000) and the yield was calculated.

The percentage yield of the extract of *Laguncularia racemosa was* determined by weighing the coarse sample before extraction and the *Laguncularia racemosa* extracts after concentration and then calculated using the formula.

$$\text{Percentage yield (\%)} = \frac{\text{Weight (g) of the concentrated extract}}{\text{Weight (g) of the } \textit{Laguncularia racemosa} \text{ leaves}} \times 100$$

3.2.3 Oral glucose tolerance test

Oral glucose tolerances test was conducted, in brief a single dose of 150 mg/kg body weight of 70 % methanolic extracts of *L. racemosa* was administered to the glucose 2 g/kg body weight induced hypoglycemic rats the blood glucose level was monitored over a course of 90

minutes 0,30,60,90 minutes respectively (Augusti *et al.,* 1994). and expressed as mean ± standard deviation.

3.2.4 Quantitative phytochemical screening

Crude extracts of *L. recemosa* was screened for the presence of secondary metabolites thus, test for flavonoids, alkaloids, saponins, tannins and phenols were performed using standard procedures (Sofowora, 2008).

3.2.4.1 *Test for saponin*

One gram of the extracts was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously.the formation of stable foam was taken as an indication for the presense of saponins.

3.2.4.2 *Test for flavonoids (alkaline reagent test)*

One gram of the extract was treated with few drops of 20 % sodium hydroxide solution formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presences of flavonoids.

3.2.4.3 *Test for tannins (braymers test)*

One gram of the extracts was treated with 10 % ferric chloride and observed for formation of blue or greenish colour solution.

3.2.4.4 *Test for alkaloids (Wagner's reagents)*

One gram of extracts was mixed with 2 ml of 1 % HCl and heated gently. wagners reagent was then addede to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids**.**

3.2.4.5 *Test for phenol (ferric chloride test)*

One gram of the extract was treated with aqueous 5 % ferric chloride and observed for formation of deep blue or black colour.

3.2.5. Quantitative phytochemical screening of methanolic extracts of *L. recemosa*

3.2.5.1. *Determinationof saponins*

A 0.5 g of the sample was added to 20 ml of 1M of HCl and was boiled for 4 hrs. this was filtered after cooling and 50 ml of petroleum ether was added to the filterate to obtain ether layer which was evapourated to dryness. About 5 ml of acetone –ethanol (1:1) was added to residue 0.4 ml each was taken into 3 different test tubes, 6 ml of Fe_2SO_4 reagent was added into them followed by 2 ml of concentrated H_2SO_4.These were thoroughly mixed and the absorbance was taken at 490 nm after 10 min (Oloyede, 2005). Standard saponins were used to establish the calibration curve.

3.2.5.2 *Determination of total flavonoid*

Total flavonoid was determined using aluminium chloride colometric method. Quercetin was used to established the calibration curve. Exaclty 0.5 ml of the diluted sample was added into test tube containing 1.5 ml of CH_3OH (methanol), 0.1 ml of 10 % $ALCL_3$ solution and 0.1 ml sodium acetate ($NaCH_3COO^-$) were added, followed by 2.8 ml of distilled water. After incubation at room temperature (23^{o}C) for 30 min, the absorbance of the reaction mixture was measured at 415 nm (Chang *et al*., 2002). The amount of 10 % $ALCl_3$ was substituted by the same amount of disttiled water in blank.

3.2.5.3 *Determination of tannin*

Exactly 0.2 g of sample was measured into a 50 ml beaker, 20 ml of 50 % methanol was added and covered with para film and placed in a water bath at 80^{o}C for 1 hr. This was shaken thoroughly to ensure a uniform mixture. The extract was quantitavely filtered using a double layered Whatman No 41 filter paper into a 100 ml volumetric flask, 20 ml distilled water

was added, 2.5 ml Folin-Denis and 10 ml of Na_2CO_3 were added and mixed properly.The mixture was made up to mark with distilled water,mixed well and allowed to stand for 20 min for the development of a bluish-green colour. The absorbances of the tannic acid solutions as well as samples were read after colour development at a wavelength of 760 nm (AOAC, 1984).

3.2.5.4 *Determination of total alkaloids*

A 0.5 g of the sample was dissolved in 96 % ethanol and 20 % H_2SO_4 in the ratio (1:1). 1 ml of the filtrate was added to 5 ml of 60 % H_2SO_4 and allowed to stand for 5 min. 5 ml of 0.5 % formaldehyde was added and allowed to stand for 3 hours. Absorbance was read at 565 nm (Harborne, 1976). The extinction coefficient (E296, ethanol ETOH=15136 MCM) of vincristine was used as reference alkaloid.

3.2.5.5 *Determination of total phenol content (TPC)*

The total phenolic content of extract was determined using the Folin –Ciocalteu method. An aliquot of 300 µl was extract was dispensed into test tube (in triplicates), 1.5 ml of Folin –Ciocalteu reagent (diluted 10 times) followed by Na_2CO_3 solution (7.7 w/v) were added. The reaction mixture was mixed, allowed to stand for 30 minutes at room temperature and the absorbance was measured at 765 nm against a blank prepared by dispensing 300 µl of distilled water instead of sample extract (Chang *et al.,* 2002). Total phenolic content was expressed as gallic acid equivalent in mg/g material. The calibration equation for gallic acid was Y= 0.0645 x -0.0034 (Regression coefficient =0.999).

3.3 Determination of *in Vitro* Antioxidant Activity

The antioxidant acivity of plant extract was determined by three methods which are 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, ferric reducing antioxidant power assay methods, and inhibition of lipid peroxidation. All the assays were carried out in triplicates and statistical results were obtained for the data.

3.3.1 1,1-diphenyl -2-picrylhydrazl (DPPH) free radical scavenging activity

The free radical scavenging capacity of the methanol extract of *L. racemosa* was determined by the method of (Gyanmfi *et al.,* 1999). The DPPH solution (0.004 % w/v) was prepared in 95 % methanol. 0.025 g of methanol extract of *L. racemosa* was mixed with 100 ml of 95 % methanol to prepare the stock solution (250 ug/mL). From this stock solution 0.2, 0.4, 0.6, 0.8 and 1.0 ml were taken in five test tubes and by serial dilution with same solvent each test tube were made up to 1.0 ml whose concentration was then 50, 100, 150, 200 and 250 ugml respectively. One milliliter of freshly prepared DPPH solution (0.004 % w/v) was added in each of these test tubes containing *L.recemosa* methanolic extract (50, 100, 150, 200, and 250 ugml).the mixture was left in the dark for 30 minutes and the absorbances was taken aat 516 nm using a spectrophotometer.control sample was prepared containing the same volume without any extracts,methanol was used as blank .percentage scavenging of the DPPH free radical was measured using the following equation:

$$\text{DPPH radical scavenging (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

3.3.2 Determination feric reducing antioxidant power assay (FARP)

For the measurement of the reductive ability; transformation of ferric (Fe^{3+}) to ferrous (Fe^{2+}) was investigated in the presences of methanol extract of *L. recemosa* following the standard method of Oyaizu, (1986). Aloquot (2.5 ml) of the extract was mixed with 2.5 ml of 200 mM of sodium phosphate buffer (pH 6.6) and 2.5 ml of 1 % potsssium ferricynaide. The mixture was incubated at 500C for 20 minutes and then 2.5 ml of 10 % trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $Fecl_3$ (0.5 ml, 0.1 %) and the absorbance was measure at 700 nm. Ascorbic acid was used as the standard. The higher

the absorbance, the higher the reducing ability of the sample. A graph of absorbance against concentration of extract was plotted and compared with that of the standard.

3.3.3 Inhibition of lipid peroxidation

Different dilution of the plant extract (12.5-100 µg/ml) each and 50 µl of 10 % egg yolk were mixed together in a test tube. Distilled water (10 µl) was used as the control and ascorbic acid was used as the positive control. Acetic acid (20 % solution, 150 µl) and 0.8 % thiobarbituric acid (TBA, 150 µl) were added to each test tube. Total volume was adjusted to 400 µl by adding distilled water. These mixtures were vortexed for 5s and kept in a water bath at 95 °C for 60 min. Butanol (1 ml) was added to each test tube and vortexed for 5 s. After centrifuging at 1500 g for 5 min, butanol layer was separated. Absorbance values were measured at 532 nm (Chang *et al.,* 2009).

Antioxidant index (AI) was calculated using the following equation:

$AI = (1-T/C) \times 100$

T = absorbance of test sample C = absorbance of fully oxidized control.

3.4 *In Vivo* Studies

3.4.1 Experimental animals

Healthy albino rats of average weight (120-185) g were obtained and put in cages and house in the Department of Biochemistry, Federal University of Technology Minna, Niger state. The animals were kept in a well-ventilated condition, at temperature of 27 ± 2 ^{o}C, 70% relative humidity and 12h light and 12h darkness cycle. They were Fed on standard feeds (Ewu feed mills, edo state, Nigeria) and also had access to constant water They were then allowed to acclimatize to the laboratory conditions for the periods of two weeks before the commencement of the experiments the study was carried out according to the Guide for the

Care and the Use of Laboratory Animals of the institute of Laboratory Resources, Commission of Life Sciences, Natural Research Council, USA.

3.4.2 Acute toxicity study of the methanolic extracts of *L. racemosa*

The median lethal dose (LD_{50}) of the methanolic extract of *L.recemosa* was determined by the up and down method (Bruce ,1985).involves sequential dosing of single animals with the test substance within a time interval of 48 hours.after the administration of the first dose, the next is determined by the outcome of the subsequent dose administerd.if the animal survives the subsequent dose the dose is adjusted upward ,but when mortality is recorded at subsequent dose, it is adjusted downward. the adjustment of dose either upward or downward is by a constant factor. testing is terminated when the upper limit (2000-5000 mg/kg) have been reached without mortality or when the LD_{50} have been established from the test.

3.4.3. Antidiabetic studies of the extract of *L. racemosa*

3.4.3.1 *Induction of diabetes*

The blood glucose concentration (mg/dl) of the rats were tested before induction with alloxan, A fresly prepare solution of alloxan monohydrates (120mg/kg) was injected inter-peritonally in overnight fasted rats.diabetes status was monitored with blood samples obtaibed from the tail vein puncture using an automated glucose sensor machine Glucometer Analyser (AccuChek Active).for each rat in all the groups at intervals of days 0,7,14, and 21 days food were withdrawn from the rats but allowed access to water.

Principle: this method is based on the reaction of glucose and oxygen in the presences of glucose oxidase to yield gluconic acid and hydrogen peroxide (H_2O_2). The hydrogen peroxide formed subsequently reacts under catalysid of oeroxidise,with phenol and 4-aminophenazone to for a red-violet quinoneimine dye as indicator.in other words, it oxidizes the dyes in a reaction mediated by peroxidise producing a blue coloured product.the intensity of the colour

which is propotional to the glucose concentration in the sample was read from the Accu -chek active glucometer.fasting blood glucose level of rats were checked regularly up to the stable hyperglycaemia stage. Animals with marked hyperglycaemia with blood glucose levels above 200 mg/dL (≥11.1 mmol/L), were considered diabetic and suitable for use in the study (Etuk *et al*., 2010).

3.4.3.2. *Experimental design*

Total of 45 animals divided into nine (9) groups 5 animals per group were used in the study.

The groups were labelled as follows:

i. Group 1: normal control (non - diabetic normal saline).
ii. Group 2: negative control (induced + no treatment)
iii. Group 3: standard group (induced + standard drug (metformin) 150 mg/kg body weight).
iv. Group 4: standard group (induced + standard drug (metformin) 300 mg/kg body weight).
v. Group 5: crude extract (induced + crude extract (70 % methanolic extract) 150 mg/kg body.
vi. Group 6: crude extract (induced + crude extract (70 % methanolic extract) 300 mg/kg body weight).
vii. Group 7: methanol partition (induced + methanol partition (100 % methanol partition) 150 mg/kg body weight).
viii. Group 8: methanol partition (induced + methanol partition (100 % methanol partition 300 mg/kg body weight).

The fasting blood glucose was monitored for 21days at 7 days interval (day 0, day 7, day 14 and day 21). (Frode and Medeiros, 2008).

3.5 Determination of DPP-4 Acivity of methanolic extract of *L. recemosa*

In addition to the groups listed above, five animals (group 9) were set aside for single day treatment using crude extract (300 mg/kg body weight), All reagents were prepared before the commencement of assay.

i. standard solution (50 µl) was added to six wells on the microplate
ii. sample (10 µl) was added to the well followed by 40 µl of diluent to the sample well.
iii. HRP (100 µl)- conjugate was added to each well (standard and sample wells). After which it was covered with a seal plate membrane, gently shaken to mix and incubated for 60 minutes at 37 ºC.
iv. The wash solution which was diluted with 20X distilled water was poured into the microplate washer (DNX-9620). Thereafter, the microplate was inserted after removing the seal and allowed to wash for 1 minute (5X)
v. For colour development, 50 µl of chromogene solution A is added to each well, followed by 50 µl of chromogene solution B. the plate is slightly shaken to mix the solution and incubated for 15 minutes at 37 ºC, away from light.
vi. Stop solution (50 µl) is added to each well to stop the reaction.
vii. Blank well contains all constituents except the analyte.
viii. The microplate was inserted into the microplate reader and absorbance was taken for each well (twice), the mean absorbance was calculated.
ix. A standard curve was plotted using the values obtained from the standard solution. The corresponding values for the samples were determined by plotting their absorbance on the standard curve. (My biosource, USA).

3.6 Data Analysis

Data obtained in this study were analysed using the statistical software SPSS 18.0 2008. Numerical data were presented as mean ± standard Error.The signifance of the mean

differnces between two independent groups was determined using one way analysis of variance (ANOVA) while multiple comparison were used when comparing more than two groups. The level of significance was set at p≤0.005.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Percentage yield of methanolic extracts of *L. racemosa.*

The percentage yields of 70 % methanolic extracts of *L. racemosa* (hot and cold) is presented in table 4.1. The percentage yields of the extracts were 41.0 % and 26.2 % respectively.

Table 4.1: Percentage yields of 70 % methanol crude extracts of *L. racemosa* hot and cold

Plant sample	Powdered weight (g)	% yield
L. racemosa hot crude extract	50 g	41.0
L. racemosa cold crude extract	50 g	26.2

4.1.2 Acute toxicity testing

The result of safe dose determination of methanol extracts of *L. racemosa* (hot and cold) are shown in Table 4.2 there was no mortality within 24 hours after oral administration (up and

down method). No mortality was recorded at 5000 mg/kg bodyweight. Little signs of toxicity such as licking the body and momentary immobility were however observed.

Table 4.2: Acute toxicity testing of *L. racemosa* (hot and cold) methanol extracts

Treatment	Dose(mg/kgbw)	No of Animals	Mortality	Sign of Toxicity
L.racemosa cold extract	5000	5	0/5	Licking of the body, Momentary immobility.
L.racemosa hot extract	5000	5	0/5	Licking of the body, Momentary immobility

4.1.3 Phytochemicals analysis of methanol extract of *L. racemosa*

4.1.3.1 *Quantitative phytochemical analysis of methanol extract of L. racemosa*

The quantitative phytochemical screening of methanol extract from *L. racemosa*, detected the presences of alkaloids, tannis, saponins, flavonoids and phenols.the results of quantitative phytochemical screening of methanol extract of *L. racemosa* are given in Table 4.3. The concentration of phytochemicals detected were; The extract was found to contain more of Tannins (8.88 ± 0.63 mg/g) than any other screened phytochemicals followed by flavonoids, saponins and Phenols, alkaloids (0.23 mg/g) was found to have the least quantity.

Table 4.3: Quantitative phytochemical analysis of methanol extract of *Laguncularia racemosa*

Phytochemical	Concentration (mg/g)
Tannins	8.88±0.63
Saponins	1.17±0.31
Alkaloids	0.23±0.02
Flavonoids	1.73±0.21
Phenol	1.68±0.30

Data are presented in mean ± Standard Error of Mean of triplicate determinations at ($p \leq 0.05$).

4.1.4 *In vitro* Antioxidant Activity of Methanol Extract of *L. racemosa*

4.1.4.1 *DPPH free radical scavenging activity of methanol extracts of L. racemosa*

The effect of *L. racemosa* is shown in Figure 4.4 which shows the effect of methanol extract of *Laguncularia racemosa* on 1,1-diphenyl-2-picrylhydrazl (DPPH). There was an increase DPPH radical scavenging acitivity of the methanol extracts of *L. racemosa* in a dose-dependent manner which is comparable with the standard drug (Gallic acid) which significantly different ($p<0.05$) with Garlic acid (Table 4.4).

Table 4.4: Percentage inhibition of DPPH radical scavenging activity of *Laguncularia racemosa* extract

Extracts Concentration (µg/mL)	Gallic acid (standard)	*Laguncularia racemosa* Extracts Activity
100	86.89 ± 0.76^{b}	79.86 ± 1.51^{a}
50	77.43 ± 0.44^{b}	52.18 ± 0.41^{a}
25	66.15 ± 0.42^{b}	42.75 ± 1.13^{b}
12.5	62.27 ± 0.79^{b}	22.15 ± 1.08^{b}
IC_{50}	48.14 ± 2.05^{b}	19.48 ± 3.45^{a}

Values are expressed in mean ± standard error of mean. Values with same superscript on the same row have no significant difference at $p<0.05$

4.1.4.2 *Ferric reducing activity of methanol extract of Laguncularia racemosa*

Figure 4.5 shows the ferric reducing ability of methanol extract of *L. racemosa* in a dose dependent manner which is significantly different ($p<0.05$) with the standard (Gallic acid) as shown in Table 4.5.

Table 4.5: Ferric reducing acitivity of methanol extract of *Laguncularia racemosa*

Extract Concentration (µg/mL)	Gallic acid (standard)	*Laguncularia racemosa* (Extract Activity)
100	86.21±0.00[b]	40.65±0.20[a]
50	75.92±0.01[b]	40.1±0.00[a]
25	64.1±0.16[b]	38.2±0.08[a]

12.5	58.2±0.00[b]	27.00±0.25[a]
IC_{50}	12.45±6.33[a]	163.36±20.45[b]

Values are expressed in mean ± standard error of mean. Values with same superscript on the same row have no significant difference at $p<0.05$.

4.1.4.3 *Inhibition of lipid peroxidation*

The extract shows a higher inhibition of lipid peroxidation which was significantly different ($p<0.05$) when compared with Garlic acid (standard) as shown in Table 4.6 *L. racemosa* shows a dose – dependent, with highest activity observed at 100 µg/ml (63.79 ± 0.02) and the least activity observed at 12.5 µg /ml (17.37 ± 0.16).

Table 4.6: Inhibition of lipid peroxidation on methanolic extracts of *Laguncularia racemosa*

Extract Concentration (µg/mL)	Gallic acid(standard)	*Laguncularia racemosa* (extract acivity*)*
100	72.65±0.98[b]	63.79 ± 0.02[a]
50	59.66 ± 0.59[a]	61.48 ± 0.08[a]
25	46.73 ± 0.20[b]	31.18 ± 0.32[a]
12.5	37.62 ± 0. 36[b]	17.37 ± 0.16[a]
IC_{50}	32.85±6.82[a]	59.64±5.33[b]

Values are expressed in mean ± standard error of mean. Values with same superscript on the same row have no significant difference at $p<0.05$

4.1.5 Oral glucose tolerance test

Figure 4.7 shows the oral glucose tolerance test of methanol extract (hot and cold) of *L. racemosa,* cold extracts shows a better suppression of blood glucose as compared to the hot there is a constant rise in the level of blood glucose in the negative (untreated) group were as there was no effect on the blood glucose as seen in the (normal) group.

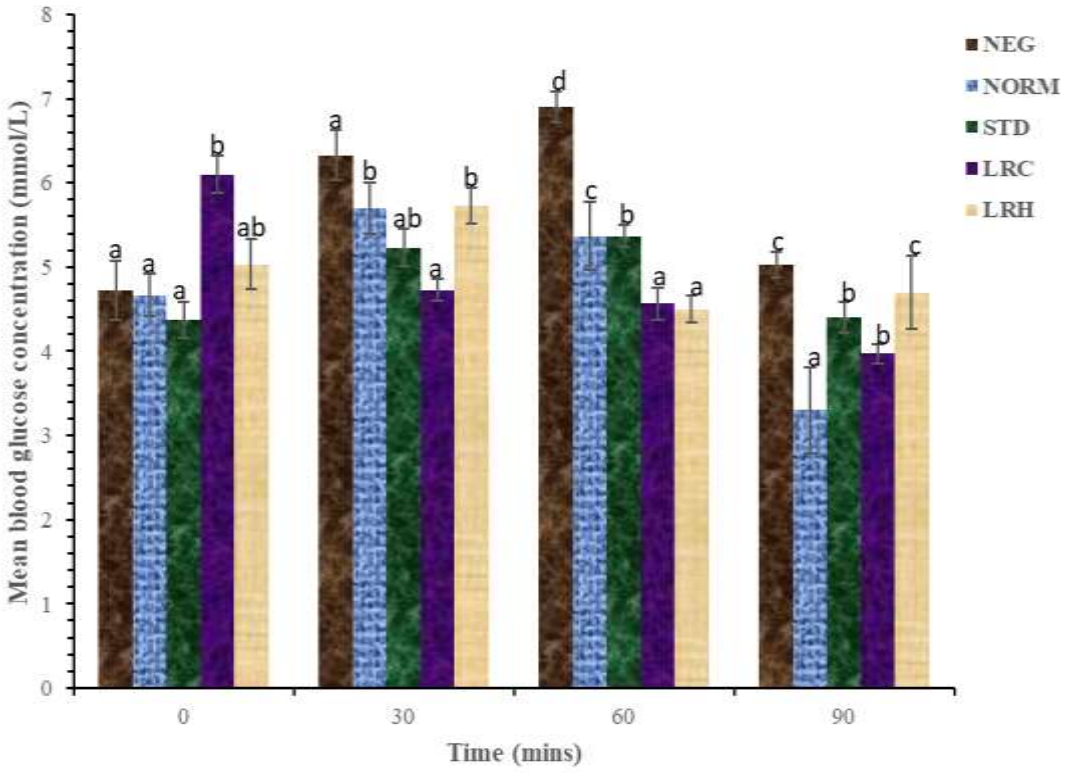


Figure 4.1: Oral glucose tolerance test of methanol extracts (hot and cold) of *L. racemosa*

Key; NEG= negative control (untreated), NORM= normal control, STD= standard drug s(metformin). LRC= *L. racemosa* cold, LRH= *L. recemosa* hot.

4.1.6 Antidiabetic activity of methanol extract and fractions of *L. racemosa* on the blood glucose level of alloxan-induced diabetic rats

Figure 4.7 shows the antidiabetic activity of *L. racemosa* in alloxan-induced diabetic rats. At the end of the 21days treatment, methanol crude extracts and fractions of *L.recemosa* significantly reduced the blood glucose level in a dose dependant manner, it was observed with the highest activity of the crude extract adminsterd at 300mg/kg body weight,were as methanol fractionaat 150mg/kg body weight showed the least activity.Metformin(standard drug)suppressed the rise of blood glucose level with the highest activity at 300mg/kg body weight.A consistent rise in blood glucose level was observed in the negative control(untreated)group,while the normal control group had no influences on blood glucose level.

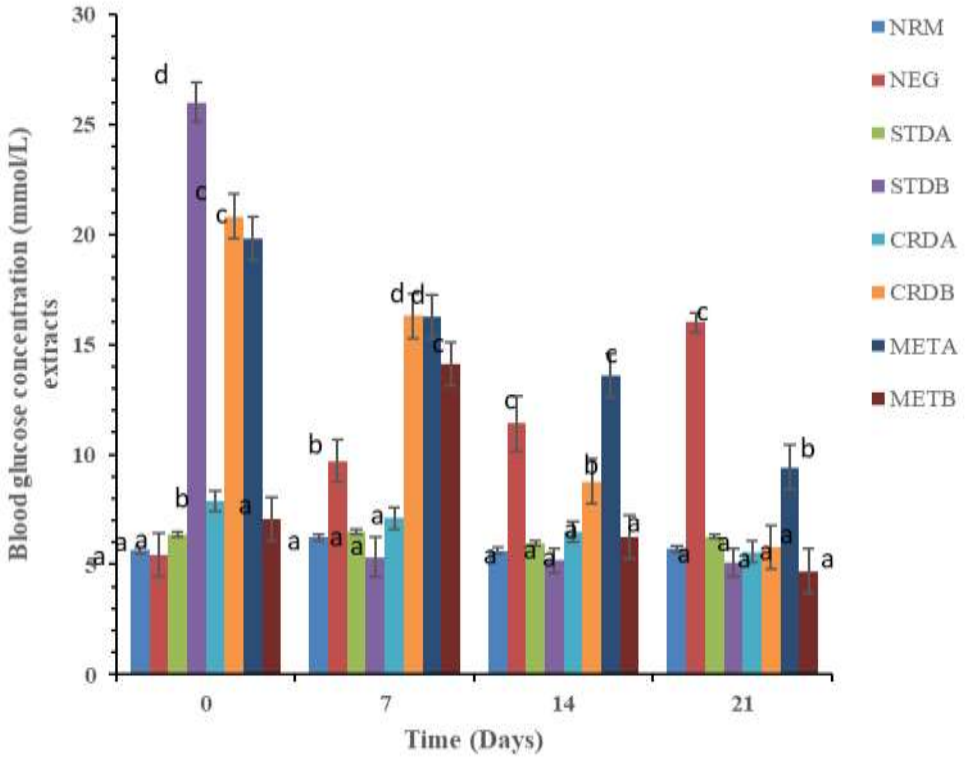


Figure 4.2: Blood glucose concentration of alloxan induced diabetic rats treated with methanol and fractions extract of *L. racemosa*

Key; NEG= negative control (untreated), NORM= normal control, STD A= standard drug (metformin) 150 mg/kgbw, STD B = standard drug (metformin) 300 mg/kgbw CRD A =crude (70 % methanol) 150 mg/kgbw, CRD B= crude (70 % methanol) 300 mg/kgbw, MET A= (methanol fractions) 150 mg/kgbw, MET B= (methanol fractions) 300 mg/kgbw.

4.1.7 Effect of Hot Crude Methanolic (70%) Extract and Methanol Fractions on the Blood Level of DPP-4 in Alloxan-Induced Diabetic Rats

The effect of methanolic (70%) extract and fractions on the blood level of DPP4 in alloxan-induced diabetic rats was presented in table 4.8.*L.recemosa* shows a non-dose dependant effects on blood levels of DDP-4.normal control group (1095 pg/ml ± 0.00375), methanol fraction (150mg/kg bodyweight) (1247.26 pg/ml ± 0.02325), standard metformin (150mg/kg bodyweight) (1404 pg/ml ± 0.00575), standard metformin (300mg/kg bodyweight) (1465.38 pg/ml ± 0.0081), crude (300mg/kg bodyweight) (1641.08 pg/ml ± 0.06995), methanol fraction (300mg/kg bodyweight) (1610.79 pg/ml ± 0.1385) as compared to the least effective; negative (untreated) group (1828.91 pg/ml ± 0.2215).

Table 4.7: Effect of Hot Crude Methanolic (70%) Extract and Methanol Fractions on the Blood Level of DPP4 in Alloxan-Induced Diabetic Rats

Extract	Concentration (pg/ml)
Normal	1095.79 ± 0.00375[a]
Negative control	1828.91 ± 0.2215[b]
Standard (metformin 150mg/kg)	1404.79 ± 0.00575[a]
Standard (metformin 300mg/kg)	1465.38 ± 0.0081[a]
Crude extract (150mg/kg)	1122.15 ± 0.0069[b]
Crude extract (300mg/kg)	1133.66 ± 0.06995[b]
Methanol fraction (150mg/kg)	1168.19 ± 0.02325[b]
Methanol fraction (300mg/kg)	1179.10± 0.1385[c]
Crude extract (single dose 300mg/kg)	1008.24± 0.0009[a]

4.2 Discussion

Currently, diabetes is controlled by a handful of available drugs such as oral hypoglycaemic agents and insulin, but their use is limited due to their own drawbacks like secondary failure of hypoglycaemic drugs.to overcome the side effects or unwanted effects of synthetic drugs and hormones, there is a need to find a safer and more effectic antidiabetic drugs that can also take the care of the associated discorders and can be used as maintenance therapy.

The significant differences in percentage yield of *L. racemosa* for both hot and cold 70% methanolic extracts of *L. racemosa* shows that differences in temperature of the solvent used can affects the yield of the extracts.

Acute toxicity measures an experimental animals toxicological reaction to single or instant exposure to a sample substance measuring an unidentified substances acute toxicity is the first guide to pharmacological research, *L. racemosa* extracts revealed non-mortality for a single dose of the extracts at 5000 mg/kg bodyweight this suggest that the LD_{50} of the extracts could be greater than 5000 mg/kg. Therefore the 70% methanolic extracts of *L. racemosa* for both hot and cold extraction may be considered relatively safe on acute toxicity testing (Bruce, 1985).

The oral glucose tolerance test (OGTT) is the most sensitive test for detecting borderline diabetes melittus, ability of the body to utilize glucose in the circulation. It widely used to evaluate apparent insulin release and insulin resistance in various clinical settings (Chaimum-aom *et al.,* 2017). The extract shows a dose dependent in blood glucose reduction with time and the highest activity was observed in the cold extracts, indicating the presence of bioactive compounds that have glucose supressing potentials.

The anti-hyperglycaemic effects that results from treatments with plants are often due to their ability to improve the performances of pancreatic tissues which is done by increasing insulin secraetion or reducing the intestinal absorption of glucose this is due to the presences of secondary metabolites present in some plants. Most plants operate through different meachanism that affect blood sugar some of them may incresase insulin kinase, some of them may inhibit insulinase activity others may increase reconstruction of pancreatic β cells. Moreover, fibers of plants may also interfere in the absorption of carbohydrates and thus have an effect on blood glucose (Bahmani *et al*., 2014).

Due to this, knowledge about medicinal plants could encourage the production of phytotherapeutics from different plants or the isolation of the bioactive molecules with known action meachanism. Phytochemical screening showed that some plants extracts contain tannins, alkaloids, flavonoids, amino acids and protein, triterpenes and phenolic compound (Gireesh *et al.,* 2009). Most plants containing these secondary metabolites are frequently implicated as having antidiabetic effects through the insulinomimetic activity of the plant extracts (Malviyan *et al.,* 2010). showed the phytochemical constituents of methanol extracts of *L. racemosa* contains important phytoconstituents like tannis, saponins, alkaloids phenols and flavonoids this collaborated the report of some studies of *L. racemosa* accounted for by (Yahaya *et al.*, 2018) who carried out quantitative phytochemical screening on the leaf of *L. racemosa*. The presence of these phytochemicals in the extract assumes significant part in the counteraction and treatment of diseases and infections (Kurmukov, 2013; & Miaffo *et al.,* 2019). Phytochemicals such as phenols and flavonoids have been reported as a good source of anti-inflammatory, antiseptic, antioxidant and hemostatic agents (Miaffo *et al.,* 2019). Report has also shown that saponins possess hypocholesterolemic and anticarcinogenic properties (Kurmukov, 2013). Extract of plant rich in tannins are good source of astringents against diarrhea, as diuretics against stomach and duodenal tumors and as anti-inflammatory,

antiseptic, antioxidant and hemostatic pharmaceuticals (Innalegwu *et al.,* 2017; & Miaffo *et al.,* 2019). presents the quantity of phytochemical in *L. racemosa* extracts tannis been the most abundants (8.88 ± 0.63 mg/g) followed by flavonoids, saponins phenols and and alkaloids been the least, according to (Afolabi *et al.,* 2007). antioxidants (tannis, flavonoids, vitamin C and E) have been show to prevent the distruction of β cells by inhibiting the peroxidation chain reaction and thus they may provide protection against the development of diabetes.

The 1,1-diphenyl-2-picryhydrazyl (DPPH) antioxidant assay is based on the ability of DPPH a stable free radical to decolorize in the presences of antioxidants.the DPPH radical contains an odd electron which is responsible for the absorbance at 517 nm and also for visible deep purple colour, concentration of sample at which the inhibition percentage reaches 50 % is its IC_{50} value. IC_{50} value is negatively related to the antioxidant activity,as it expresses the amount of antioxidant needed to decrease its radical concentration by 50 %.the lower the IC_{50} value, the higher is the antioxidants activity of the test sample in the present study ,the methanolic extract of *L. racemosa* shows the inhibition of DPPH activity by the extract was in a dose dependent manner with the highest inhibition (76.86 ± 1.51 µg/mL) obtained at 100 with an IC_{50} of 48.14 ± 2.05 µg/mL which is significantly different with the standard (Gallic acid) with an inhibition of 86.89±0.76 µg/mL and an IC_{50} of 19.48 ± 3.45 µg/mL. The IC_{50} is the concentration of antioxidant substance that inhibits 50 % of DPPH radicals. According to Marjoni and Zulfisa, (2017). antioxidants substances are classified based on their IC_{50} as either highly active (<50 µg/mL), active (50-100 µg/mL), moderate (101-250 µg/mL), weak (250-500 µg/mL) and inactive (>500 µg/mL).

For the measurement of the reducing ability, the Fe^{3+}- Fe^{2+} was investigated in the presence of *L. racemosa* extract.the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidant has been assigned to

various meachanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. thus, antioxidants in *L. racemosa* extract was proved to have electron donating capacity that can reduce or scavenge the free radicals.

However, this trend of concentration of *L. racemosa* extracts in a dose-dependent manner is the same for percentage inhibition of lipid peroxidation.

Diabetes and other degenerative diseases have been reported to be responsible for changes in the oxidative status of an individual (Awad *et al.,* 2016). Oxidative stress causes variable consequences on the activity of antioxidant enzymes (Awad *et al.,* 2012). The restoration of antioxidant status is a key and significant step to assess the impact of antidiabetic compounds or extracts (Awad *et al.,* 2012). Oxidative stress occurs as a result of either elevated level of reactive oxygen species (ROS) or reduced scavenging ability of the organ or both. Shows the effect of methanol extracts on *L. racemosa* on blood glucose level, the significant ($p \leq 0.05$) blood glucose lowering effect of *L. racemosa* methanol extracts may be attributed to the presences of flavonoids, saponins, tannis, phenols and alkaloids.that have been associated with hypoglycaemic activity (Chan-Yong *et al*., 2010) various reserachers have demonstrated the antidiabetic activity of flavonoids, flavonoids glycosides such as strictinin,isostrictinin and pedunculagin are the effective constituents of *psidium guajava*,which have been used in clinical treatment of diabetes due to improved sensitivity of insulin (Chauchan *et al*., 2010). Presence of saponins in this extract could be responsible for the hypoglycaemic activity for instance ginseng and its saponins have been shown to lower blood glucose in alloxan treated, genetically diabetic and normal mice (Chan-Yong, 2010). Clinically, all the forms of tannins may participate in managing glucose level in blood tannis stimulates the receptor cells to utilize carbohydates (Kumari *et al.,* 2014).

The results showed that the blood glucose level of the group of rats fed rat chow and standard drug (group 2) had a significant decrease in blood glucose (17.23 %) on day 21. This outcome might be ascribed to the presence of phytochemicals like phenols, flavonoids, tannins and saponins that can mimic insulin or invigorate the discharge of β-cells by the islets of Langerhans (Chikezie *et al.,* 2018; & Miaffo *et al.,* 2019). Another conceivable mechanism of the extract potentials might be the recovery of β-cells which may have been compromised by alloxan, mobilization of blood glucose in peripheral tissue, the stimulation of glucose uptake by peripheral tissues, the inhibition of endogenous glucose production or the activation of gluconeogenesis in the liver and muscles (Ikewuchi *et al.,* 2011; & Maiffo *et al.,* 2019). In diabetic condition, hyperglycemia may occur as result of uninhibited activities of lipolytic hormones on the fat depot and an increase in activation of unsaturated fats (Ikewuchi *et al.,* 2011).

Although, the methanolic crude extracts of *L. racemosa* showed a better suppression of blood glucose level at a dose level of 300 mg/kg bodyweight significantly reduced blood glucose level from (20.82 to 5.78 mmol/l)while the dose of 150 mg/kg reduced the blood glucose level from (7.88 to 5.78 mmol/l) standard anti –diabetic drugs metformin administered orally also significantly lower blood glucose concentration at 300 mg/kg from (26.0 to 5.08 mmol/l)when compared to the dose administered at 150 mg/kg which lowered the blood glucose level from (6.38 to 6.28 mmol/l). These study shows that the crude extracts of *L. recemosa* contains important phytochemicals that have beneficial effects in reducing blood glucose level.

Dpp-4 (dipeptidyl-peptidase-4) inhibitors or gliptins repesent a class of oral anti-hyperglycemic agents that inhibits the enzyme Dpp-4, thus augmenting the biological activity of the incertin, hormones (glucagon-like peptide-1GLP-1)and glucose dependant insulinotropic polypeptide (GIP) and restoring many of the pathophysiological problems of diabetes these enzymes are

expressed on the surface of most cell types and deactivates a variety of other bioactive peptides such as (GLP) and (GLP-1) (Holst *et al.,* 2009).

Methanol extracts and fractions of *L racemosa* showed a non-dependant activity in the inhibition of DPP-4, the highest activity was observed in methanol fraction (300 mg/kg) which is consistent with the treatments trend metformin (standard) shows some level of activity against DPP-4 theses may be as a result of DPP-4 not being a target molecule for metformin however metformin is often used as a combination therapy with DPP4 inhibitors.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this study, several models of *in vitro* and *in vivo* assays were applied to evauate the hypoglycaemic effect of methanol extracts of *L. racemosa*. it shows that *L. racemosa* to be safe at 5000 mg/kg body weight, in the in vivo studies, the extracts exhibited hypoglycaemic activity, therefore, it could be speculated that the observed hypoglycaemic activity of methanol extract of *L. racemosa* might be related to the presences of phytoconstituents such as flanonoids, saponins, and tannins, having the potential to impart beneficial therapeutic effect in diabetes *L. racemosa* shows a high level of antioxidants , Involvement of free radicals appears to be the feature of most human diseases. Therefore, the inhibition of lipid peroxidation and radical scavenging power of plants might be important in fighting diabetic diseases.

5.2 Recommendations

Based on the outcome of this study, the followings are recommended:

i. estimation of insulin level should be carried out in the experimental animals, as this may give more insight into the mechanism of antidiabetic activity exhibited by the plant extract.
ii. chronic toxicity studies of this plant should be carried out so as to ascertain the safety of usage of this plant extract on various body orssgans.
iii. Further investigations are needed in other higher primate so as to validate the traditional used of the plant in the management of diabetes**.**

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