IN VITRO AND *IN VIVO* STUDIES ON THE ALPHA GLUCOSIDASE INHIBITORY EFFECTS OF THE LEAF EXTRACT OF *Carica papaya* LINN

BY

ABUBAKAR MUJAHEED
(P13SCBC8011)

DEPARTMENT OF BIOCHEMISTRY
FACULTY OF LIFE SCIENCE
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

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By

Mujaheed,ABUBAKAR
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DEPARTMENT OF BIOCHEMISTRY
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MARCH, 2017

DECLARATION

I declare that the work in this dissertation entitled *IN VITRO* AND *IN VIVO* STUDIES ON THE ALPHA GLUCOSIDASE INHIBITORY EFFECTS OF THE LEAF EXTRACT OF *Carica papaya* LINN has been carried out by me in the Department of Biochemistry, Faculty of Life Science, Ahmadu Bello University, Zaria under the supervision of Prof. E. Onyike and Dr. M. A. Ibrahim. The information derived from the literature has been duly acknowledged in the text and the list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other university.

AbubakarMujaheed		
Name of student	Signature	Date

CERTIFICATION

This dissertation entitled *IN VITRO* AND *IN VIVO* STUDIES ON THE ALPHA GLUCOSIDASE INHIBITORY EFFECTS OF THE LEAF EXTRACT OF *Carica papaya*LINNby Mujaheed ABUBAKAR meets the regulations governing the award of the degree of Master of Science of theAhmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. E. Onyike _____ _____ _____
(Chairman, Supervisory Committee) Signature Date

Dr. M. A. Ibrahim _____ _____ _____
(Member, Supervisory Committee) Signature Date

Prof. M.N. Shuaibu _____ _____ _____
(Head of Department) Signature Date

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

DEDICATION

This dissertation is dedicated to my late parents AlhajiAbubakarAbdullahi andHajiyaZubaidah Ali Abdallah. Your love has being my driving force up till now. May your gentle soul rest in perfect peace, amin.

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ABSTRACT

Alpha glucosidase inhibitors regulate postprandial hyperglycemia (PPHG) by impeding the rate of carbohydrate digestion in the small intestine and thereby hampering the diet associated acute glucose excursion. In this study, *in vitro* and *in vivo* alpha glucosidase inhibitory effects of the leaf extract of *Carica papaya*linnwere evaluated. The dried powdered leaf of *C. papaya* was extracted for 48 hours using four different solvents (water, methanol, ethyl acetate and n-hexane), after which the extracts were subjected to *in vitro* alpha glucosidase inhibitory studies.Methanolic extract being the most activewith an IC_{50} value of 171.83 ± 26.89 µg/ml was subjected to fractionation via column chromatography separation and six fractions were obtained which were subjected to further *in vitro* alpha glucosidase inhibitory studies.Fraction A (FA) had the lowest IC_{50}(78.62 ± 25.23 µg/ml) than the other fractions and even lower than the standard drug acarbose (161.08 ± 41.01). Therefore, FA was selected for the *in vivo* maltose and sucrose tolerance tests as well asgas chromatography - mass spectrometry(GC -MS) analysis. The *in vivo* tests showed that FA had active inhibitory effects on maltose tolerance within the first 30 mins and on sucrose tolerance within the first 60 mins. The computed area under the curves ($AUC_{(0-120)}$) further indicate that FA (at 200 mg/kg bw) have better inhibitory activity on maltase than sucrase. The GC - MSanalysis of FA indicates the presence of phenolics, various fatty acids and their derivatives. It was concluded that the leaf extract of *C. papaya* possess alpha glucosidase inhibitory effects and may provide a natural and safe remedy for the reduction of postprandial hyperglycemia which will therefore help to reduce the risk of developing metabolic disorders.

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LIST OF ABBREVIATIONS

ANOVA	Analysis Of Variance
BMI	Basal Metabolic Index
CFRD	Cystic Fibrosis–Related Diabetes
CVD	Cardiovascular Disease
GCMS	Gas Chromatography-Mass Spectrometry
GDM	Gestational Diabetes Mellitus
GLUTs	Glucose Transporters
IC_{50}	Median Inhibitory Concentration
IDF	International Diabetes Federation
IPF	Insulin Promoter Factor
MDGs	Millennium Development Goals
MODY	Maturity-Onset Diabetes of the Young
NICE	National Institute for Health andClinical Excellence
NIST	National Institute of Standard and Technology
RT	Retention Time
SGLTs	Sodium-GlucoseCo-Transporters
TLC	Thin Layer Chromatography
TZD	Thiazolidinediones
UKPDS	United Kingdom Prospective Diabetes Study

CHAPTER ONE

1.0 INTRODUCTION

Plants have in one way or the other provided the basic need of mankind – food, shelter, clothing and protection from disease causing agents. The use of plants and its extracts to treat various diseases can be traced to the early days of human history. Medicinal plants contain substances that modulate the physiology of sick mammals and the knowledge of these healing properties has been passed down through generations. About 70 to 80 per cent of the world population, especially inhabitants of rural areas, use medicinal plants for primary health care, also known as herbalism (Cunningham, 1993; Dossou-Yovo et al., 2014). Pharmaceutical companies also use medicinal plants as a basis for production of therapeutic drugs. Medicinal plants have a promising future because there are about half million plants around the world, and most of them have their medicinal potentials not investigated yet. Therefore, pharmacological potential of plants could be decisive in present or future studies (Bassam, 2012) needed for the production of more effective drugs. In recent times, the term "alternative medicine" has begun to crop up in western culture, focusing on the idea of using the plants for medicinal purpose. It is therefore important to identify the inhibitors of enzymes in medicinal plants to elucidate their specific role in disease treatment and drug production in order to support primary health care.

Diabetes mellitus (DM) is a common metabolic disease characterized by high blood glucose levels due to the lack of pancreatic insulin secretion, with or without an interruption in the action of insulin (Porter and Barrett, 2005). The prevalence and incidence of type 2 diabetes, representing 90% of all cases of diabetes, are increasing rapidly throughout the world. It has been estimated that the number of people with diabetes is expected to rise from 415 million in 2015 to 642 million by 2040 if no urgent action is taken (IDF, 2015). Epidemiological studies and clinical trials have demonstrated that hyperglycemia is the main cause of complications associated with coronary artery disease, cerebrovascular disease, kidney failure, blindness, limb amputation, neurological complications and premature death (Lopez-Candales, 2001). Studies reported that postprandial hyperglycemia may induce non-enzymatic glycosylation of proteins, resulting in the development of chronic complications (Gholamhoseinian et al., 2008). Hence, control of postprandial plasma glucose levels is critical in the initial treatment of DM as well as reducing chronic vascular complications (Shim et al., 2003).

One therapeutic approach used to reduce postprandial hyperglycemia in patients with DM is preventing the absorption of carbohydrates following the consumption of food (Gholamhoseinian et al., 2008). Only monosaccharides, such as glucose and fructose can be transported from the intestinal lumen into the blood stream. Complex starches, oligosaccharides and disaccharides must be broken down into individual monosaccharides before being absorbed in the duodenum and upper jejunum. Enteric enzymes including pancreatic alpha-amylase and alpha-glucosidase which are attached to the brush border of the intestinal cells facilitate this digestion. An increasing number of studies are available screening alpha-glucosidase inhibitors from plants and synthetic sources (Christudas et al., 2009). Inhibitors of these enzymes such as acarbose and miglitol have been developed from natural sources (Shim et al., 2003).

Alpha glucosidase inhibitors (acarbose and miglitol) are used as oral anti diabetic drugs for treating type 2 diabetes mellitus. They act by preventing the digestion of carbohydrates such as starch (Bischcoff, 1994; Sindhu et al., 2013). Interestingly, alpha glucosidase inhibitors fall under the third category of oral hypoglycemic agents (Du et al.,2005). Some alpha glucosidase inhibitors, such as acarbose and voglibose obtained from natural sources, can effectively control blood glucose levels after food intake and have been used clinically in the treatment

of DM(Playford et al., 2013). However,only few alpha glucosidase inhibitors are commercially available and usually contained sugar moieties and their synthesis involves tedious multistep procedures (Yin et al., 2014). Moreover, they have been associated with serious gastrointestinal side effects. Therefore, it is necessary to search for alternatives that can display alpha glucosidase inhibitory activity but without side effects especially from medicinal plants. This is due to the fewer side effects of plant derived products.
Carica papaya Linn (family: Caricaceae) known as pawpaw tree is widely distributed throughout the world, mostly grow in tropics and it grows up to 5 to 10 m tall. Its leaves are large, 50-70 cm in diameter, deeply palmately lobed with 7 lobes (Duke, 1984; Khalid et al., 2013). The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present (Nisar et al., 2011). In some parts of Asia, the young leaves of the papaya are steamed and eaten like spinach. Papaya leaf juice was found to increase white blood cells and platelets, normalizes clotting, and repairs the liver(Aravind et al., 2013). Papaya leaves are also made into tea as a treatment for malaria (Udohet al., 2005). The leaves of the papaya plants facilitate digestion because they contains chemical compounds (karpain) which kills microorganisms that often interfere with the digestive function (Aravind et al., 2013). Also the aqueous leaf extract of C. papaya exerts a hypoglycemic and antioxidant effect, it also improves the lipid profile in diabetic rats(Juarez-Rojop et al., 2012). In addition, the leaf extract positively affects the integrity and function of both liver and pancrease (Juarez-Rojop et al., 2012). However, detailed investigation of the alpha glucosidase inhibitory activity of the leaf extract from this plant has not been conducted to date.

1.1 STATEMENT OF RESEARCH PROBLEM

The frequently used alpha glucosidase inhibitors, acarbose and miglitol, are often reported to produce diarrhea and other intestinal disturbances, with the corresponding bloating, flatulence, cramping and abdominal pain (Fujisawa et al., 2005). Controlled trials with alpha glucosidase inhibitors report these gastrointestinal side effects as the most common reasons for non-compliance and early subject withdrawal (Neuser et al., 2005). In Africa, apart from South Africa and Democratic Republic of Congo, Nigeria has thehighest number of people with diabeteswith approximately 3.9 million people (IDF, 2015). Also, the cost and availability of such alpha glucosidase inhibitors is troubling most of the people with diabetes in Africa.The foregoing therefore makes the search for alternative agents with potent anti-hyperglycemic properties that could also decrease postprandial hyperglycemiaand other diabetic complications is of utmost importance.

1.2 JUSTIFICATION

Traditional medicinal plants with their various biological constituents have been used effectively by the communities for a long time to treat diseases. Naturally available alpha-glucosidase inhibitors from medicinally important plants are shown to be effective in managing postprandial hyperglycemia (PPHG) which is a major concern in type2 diabetes and can also provide benefits by controlling PPHG without side effect.Interestingly, plant based agents were reported to be more acceptable source of alpha glucosidase inhibitors due to their low cost and relatively better safety levels, including lower incidence of serious gastrointestinal side effects (Benalla et al., 2010).Despite the knowledge of the pathological processes involved in causation/progression of the disease and the wide range of therapeutic agents designed to fight hyperglycemia, the statistical projections are still alarming and the stability of communities is being threatened.Alternative strategies to the current

pharmacological options of DM management are therefore urgently needed (WHO, 2002), to manage this crippling global health problem.Ethnobotanical and ethnopharmacological surveys report that more than 1200 plants are being used in many ethnic societies around the world in traditional medicine for their alleged hypoglycemic activity (Akah et al., 2002; Mohammed et al., 2014 and Osigwe et al., 2015) and C.papaya is one of such plants. In view of this, the current study was designed to evaluate the anti-hyperglycemic activity of extracts of leaf of C. papaya.

1.3 AIM AND OBJECTIVES

1.3.1 AIM

The study is aimed at determining the alpha glucosidase inhibitory effects of the leaf extract of C. papaya.

1.3.2 OBJECTIVES

The specific objectives are:

To determine the inhibitory activity of the different solvent extracts of the leaf of C. papaya on alpha glucosidase activity.

To partially purify the best inhibitory extract of the leaf of C. papaya.

To determine the effect of C. papaya leaf fraction on maltose and sucrose tolerance in rats.

To identify the possible compounds of the best inhibitory fraction by GC-MS analysis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycemia from defects in insulin secretion, insulin action, or both. The abnormalities in carbohydrate, fat and protein metabolism that are found in diabetes are due to deficient action of insulin on target tissues (Craig et al., 2009). The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of different organs especially the eyes, kidneys, nerves, heart and blood vessels.

Insulin is a hormone synthesized by the β-cells at the pancreatic islets of Langerhans and its primary role is to tightly control blood glucose levels which usually rise after dietary intake. Thus, insulin is released from the pancreatic β-cells to normalize the glucose level. Therefore, in diabetic patients, anomalies in the production of insulin and/or its utilization cause hyperglycemia. Symptoms of marked hyperglycemia include polyuria, polydypsia, weight loss, sometimes with polyphagia and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia (ADA, 2009). Acute, life threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis. Long term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations and charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular symptoms and sexual dysfunction (Craig et al.,

2009).Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral, arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes (ADA, 2009).

2.1.1. Classification of diabetes mellitus

Assigning a type of diabetes to an individual often depends on the circumstances presentatthetimeofdiagnosis,withindividualsnotnecessarily fittingclearlyintoa single category. For example, some patients cannot be clearly classified as having type 1 or type 2 diabetes. Clinical presentation and disease progression may vary considerably in both types of diabetes. The traditional paradigms of type 2 diabetes occurring only in adults and type 1 diabetesonlyinchildrenarenolongeraccurate,asbothdiseasesoccurinbothcohorts. Occasionally, patients with type 2 diabetes may present with diabetic ketoacidosis (DKA). Children with type 1 diabetes typically present with the hallmark symptoms ofpolyuria/polydipsia andoccasionallywithDKA.Theonsetoftype1 diabetes maybe variable in adults and may not present with the classic symptoms seen in children. However, difficulties in diagnosis may occur in children, adolescents, and adults, with the true diagnosis becoming more obvious over time (ADA, 2015). However there are different types of DM and they include;

Type1 diabetes mellitus:This form, previously called “insulindependent diabetes” or “juvenile-onset diabetes,” accounts for 5 - 10% of diabetes andisduetocellular-mediatedautoimmune destruction of the pancreatic β-cells (ADA, 2015). Autoimmune markers include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to Glutaic Acid Decarboxylase (GAD65), autoantibodies to the tyrosine phosphatasesIA-2andIA-2b,andautoantibodies to zinc transporter 8 (ZnT8). Type 1 diabetes is defined by the presence of one or more of these autoimmune markers. The disease has strong human leukocyte antigen(HLA) associations, with linkage to the alpha chain (DQA) andbeta chain (DQB) genes. These HLA-DR/DQ alleles can be either predisposing or protective (Ziegler and Nepom, 2010).

The rate of β-cell destruction is quite variable, being rapid in some individuals (mainlyinfantsandchildren)andslowin others (mainly adults). Children and adolescents may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severehyperglycemiaand/orketoacidosis with infection or other stress. Adults may retain sufficient β-cell function to prevent ketoacidosis for many years; such individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis. At this latter stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide. Immune-mediated diabetescommonly occurs in childhood and adolescence, but it can occur at any age, even in the 8th and 9th decades of life (ADA, 2009).

Autoimmunedestructionof β-cellshas multiple genetic predispositions and is also related to environmental factors that are still poorlydefined. Although patients are not typically obese when they present with type 1 diabetes, obesity should not preclude the diagnosis. These patients are also prone to other autoimmune disorders such as Graves’ disease, Hashimoto’s thyroiditis, Addison’s disease, vitiligo, celiac disease, autoimmune hepatitis, myasthenia gravis, and pernicious anemia.

Type 2 diabetes mellitus: This form, previously referred to as “non-insulin-dependent diabetes” or “adult onset diabetes,” accounts for;90 - 95% of all diabetes (ADA, 2015). Type 2 diabetes encompasses individuals who have insulin resistance and usually relative (rather than absolute) insulin deficiency (ADA, 2015). At least initially, and often throughout their lifetime, these individuals may not need insulin treatment to survive.

There are various causes of type 2 diabetes. Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur, and patients do not have any of the other

known causes of diabetes. Most, but not all, patients with type 2 diabetes are obese. Obesity itself causes some degree of insulin resistance. Patients whoarenotobesebytraditionalweight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region (ADA, 2009).

Ketoacidosis seldom occurs spontaneously in type 2 diabetes; when seen, it usually arises in association with the stress of another illness such as infection. Type 2 diabetes frequently goes undiagnosedformanyyearsbecausehyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice the classic diabetes symptoms. Nevertheless, such patients are at an increased risk of developing macrovascular and microvascular complications.

Whereas patients with type 2 diabetes may have insulin levels that appear normal orelevated, thehigherblood glucose levelsinthesepatientswouldbeexpected to result in even higher insulin value shad their β-cell function been normal. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemiabutisseldomrestoredtonormal.

The risk of developing type 2 diabetes increases with age, obesity, and lack of physicalactivity.Itoccursmorefrequentlyin women with prior GDM, in those with hypertension or dyslipidemia, and in certain racial/ethnic subgroups (African American, American Indian, Hispanic/ Latino, and Asian American). It is often associated with a strong genetic predisposition, more so than type 1 diabetes. However,thegeneticsoftype2diabetes is poorly understood(ADA, 2015).

Gestational Diabetes Mellitus (GDM):For many years, GDM was defined as any degree of glucose intolerance that was first recognized during pregnancy, regardless of whether the condition may have predated the pregnancy or persisted after the pregnancy. This definition facilitated a uniform strategy for detection and classification of GDM, but it was limited by imprecision (ADA, 2015).

The ongoing epidemic of obesity and diabetes has led to more type 2 diabetes inwomenofchildbearingage,resultingin an increase in the number of pregnant womenwithundiagnosedtype2 diabetes. Because of the number of pregnant women with undiagnosed type 2 diabetes, it is reasonable to test women with riskfactorsfortype2diabetes at their initial prenatal visit, using standard diagnostic criteria. Women with diabetes in the first trimester would be classified as having type 2 diabetes. GDM is diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes (ADA, 2015).

Other specific types of diabetes:

Maturity-Onset Diabetes of the Young (MODY):MODY is characterized by impaired insulin secretion with minimal or no defects in insulin action. It is inherited in an autosomal dominant pattern. Abnormalities at six genetic loci on different chromosomes have beenidentifiedtodate.Themostcommon formis associatedwithmutationsonchromosome12inahepatictranscriptionfactor referred to as hepatocyte nuclear factor (HNF)-1a. A second form is associated with mutations in the glucokinase gene on chromosome 7p and results in a defective glucokinase molecule. Glucokinase converts glucose to glucose-6-phosphate, the metabolism of which, in turn, stimulates insulin secretion by the b-cell. The less common forms of MODY result from mutationsinothertranscriptionfactors,including HNF-4a, HNF-1b, insulin promoter factor (IPF)-1, and NeuroD1 (ADA, 2009).

Neonatal Diabetes:Diabetesdiagnosedinthe first6monthsof life has been shown not to be typical autoimmune type 1 diabetes. This so-called neonatal diabetes can either be transient

or permanent. The most common genetic defectcausingtransientdiseaseisadefect on ZAC/HYAMI imprinting, whereas permanent neonatal diabetes is most commonly a defect in the gene encoding the Kir6.2 subunit of the b-cell KATP channel.Diagnosingthelatterhasimplications, since such children can be well managed with sulfonylureas (ADA, 2015).

Cystic Fibrosis - Related Diabetes (CFRD):CFRDisthemostcommoncomorbidity in people with cystic fibrosis, occurringin about20% of adolescents and40 - 50% of adults. Diabetes in this population is associated with worse nutritional status, more severe inflammatory lung disease, and greater mortality from respiratory failure. Insulin insufficiency related to partial fibrotic destruction of the islet mass is the primary defect in CFRD. Genetically determined function of the remaining β-cells and insulin resistance associated with infection and inflammationmayalsoplayarole.Whilescreening for diabetes before the age of 10 years can identify risk for progression to CFRD inthosewithabnormalglucosetolerance, there appears to be no benefit with respecttoweight,height,BMI,orlungfunction compared with those with normal glucose tolerance,10 years of age. The use of continuous glucose monitoring may be more sensitive than OGTT to detect risk for progression to CFRD, but this likely needs more evidence (ADA, 2015).

Genetic defects in insulin action: There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes. Some individuals with these mutations may have acanthosis nigricans. Women may be virilized and have enlarged, cystic ovaries. In the past, this syndrome was termed type A insulin resistance. Leprechaunism and the Rabson Mendenhall syndrome are two pediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance. The former has characteristic facial features and is usually fatal in infancy, while the latter is associated with abnormalities of teeth and nails and pineal gland hyperplasia. Alterations in the structure and function of the insulin receptor cannot be demonstrated in patients with insulin resistant lipoatrophic diabetes.Therefore, it is assumed that the lesion(s) must reside in the postreceptor signal transduction pathways (ADA, 2009).

Diseases of the exocrine pancreas: Any process that diffusely injures the pancreas can cause diabetes. Acquiredprocesses include pancreatitis, trauma, infection, pancreatectomy and pancreatic carcinoma. With the exception of that caused by cancer, damage to the pancreas must be extensive for diabetes to occur; adrenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. This implies a mechanism other than simple reduction in β-cell mass. If extensive enough, cysticfibrosis and hemochromatosis will also damage β-cells and impair insulin secretion. Fibrocalculous pancreatopathy may be accompanied by abdominal pain radiating to the back and pancreatic calcifications identified on x-ray examination. Pancreatic fibrosis and calcium stones in the exocrine ducts have been found at autopsy (ADA, 2015).

Endocrinopathies: Several hormones (e.g., growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g., acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, respectively) can cause diabetes. This generally occurs in individuals with pre-existing defects in insulin secretion and hyperglycemia typically resolves when the hormone excess is resolved. Somatostatinoma and aldosteronoma-induced hypokalemia can cause diabetes, at least in part, by inhibiting insulin secretion. Hyperglycemia generally resolves after successful removal of the tumor.

Drug or chemical-induced diabetes: Many drugs can impair insulin secretion. These drugs may

not cause diabetes by themselves, but they may promote diabetes development in individuals with insulin resistance. In such cases, the classification is unclear because the sequence or relative importance of β-cell dysfunction and insulin resistance is unknown. Certain toxins such as vacor (a rat poison) and intravenous pentamidine can permanently destroy pancreatic β-cells. Such drug reactions fortunately are rare. There are also many drugs and hormones that can impair insulin action. Examples include nicotinic acid and glucocorticoids. Patients receiving α-interferon have been reported to develop diabetes associated with islet cell antibodies and in certain instances, severe insulin deficiency (ADA, 2015).

Viruses and infections: A virus cannot cause diabetes on its own, but people are sometimes diagnosed with type 1 diabetes during or after a viral infection, suggesting a link between the two. Also, the onset of type 1 diabetes occurs more frequently during the winter when viral infections are more common. Viruses possibly associated with type 1 diabetes include coxsackievirus B, cytomegalovirus, adenovirus, rubella, and mumps. Scientists have described several ways these viruses may damage or destroy beta cells or possibly trigger an autoimmune response in susceptible people. For example, anti-islet antibodies have been found in patients with congenital rubella syndrome, and cytomegalovirus has been associated with significant beta cell damage and acute pancreatitis—inflammation of the pancreas. Scientists are trying to identify a virus that can cause type 1 diabetes so that a vaccine might be developed to prevent the disease.

Other genetic syndromes associated with diabetes: Many genetic syndromes are accompanied by an increased incidence of diabetes. These include the chromosomal abnormalities of Down syndrome, Klinefelter syndrome and Turner syndrome. Wolfram's syndrome is an autosomal recessive disorder characterized by insulin deficient diabetes and the absence of β-cells at autopsy. Additional manifestations include diabetes insipidus, hypogonadism, optic artrophy and neural deafness (ADA, 2015).

2.1.2 Prevalence of diabetes

The prevalence and incidence of type 2 diabetes, representing 90% of all cases of diabetes, are increasing rapidly throughout the world. It has been estimated that the number of people with diabetes is expected to rise from 415 million in 2015 to 642 million by 2040 if no urgent action is taken (IDF, 2015).

Moreover, the prevalence of diabetes is higher in men than women, but there are more women with diabetes than men.The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people greater than 65 years of age (Wild et al.,2004).

All nations, rich and poor are suffering the impact of the diabetes epidemic. The impact is worse in those countries that are socially and economically disadvantaged. Diabetes threatens the achievement of the Millennium Development Goals (MDGs), increases the risk of developing tuberculosis and is closely linked with other infections (IDF, 2011).

Globally in 2013, it is estimated that almost 382 million people suffer from diabetes for a prevalence of 8.3%. North America and the Caribbean is the region with the highest prevalence of 11% having 37 million people with diabetes followed by the Middle East and North Africa with a prevalence of 9.2% having 35 million people with diabetes. Western Pacific is the region with higher number of people living with diabetes (138 million).However its prevalence is 8.6%, close to the prevalence of the World (IDF, 2013).

An estimated 14.2 million adults aged 20-79 have diabetes in the Africanregion, representing a regional prevalence of 2.1-6.7%. The Africa region has the highest proportion of undiagnosed diabetes; over two thirds (66.7%) of people with diabetes are unaware they

have the disease. The majority (58.8%) of people with diabetes live in cities, even though the population in the region is predominantly (61.3%) rural. Diabetes in adults is in general much higher on islands in the Africa Region, compared to the mainland. The highest prevalence is found in the Seychelles (17.4% age-adjusted comparative prevalence, 17.4% raw prevalence), followed by the island of Reunion (15.8% age-adjusted, 18.2% raw) and Comoros (9.9% age-adjusted, 7.5% raw). Some of Africa's most populous countries have the highest numbers of people with diabetes, including South Africa (2.3 million), Democratic Republic of Congo (1.8 million), Nigeria (1.6 million) and Ethiopia (1.3 million). Nearly half of all adults with diabetes in the region live in these four countries (IDF, 2015).

Nigeria with over 250 tribes and different culture and food values, the prevalence values has not been uniform though the International Diabetes Federation recorded that there were more than 1.56 million cases of diabetes in Nigeria (IDF, 2015).

2.2 Postprandial Hyperglycemia

Hyperglycemia, a condition characterized by an abnormal excess of sugar in the blood, has been linked to the onset of type 2 diabetes mellitus and associated cardiovascular complications including hypertension (Hayden and Tyagi, 2003) and poor control of hyperglycemia appears to play a significant role in the development of CVD in diabetes (Laakso, 1999). Recently, there has been increasing evidence that the postprandial state is an important contributing factor to the development of atherosclerosis (Bonora and Muggeo, 2001). In diabetes, the postprandial phase is characterized by a rapid and large increase in blood glucose levels and the possibility that these postprandial "hyperglycemic spikes" may be relevant to the pathophysiology of latediabetes complications is recently receiving much attention.

A large number of epidemiologic studies have documented the strong link between chronic hyperglycemia typically reflected by glycosylated hemoglobin (A1c) and long term morbidity and mortality in patients with diabetes. Results from a cohort of 879 individuals with type 1 diabetes who were followed for 20 years indicated that A1c was significantly associated with all cause and cardiovascular mortality. Subjects were divided into quartiles based on A1c. The risk of death for subjects in the highest quartile (average A1c 11.4%)was 2.42 times that for those in the lowest quartile (average A1c 8.7%). A similar pattern was seen in the risk of cardiovascular mortality for subjects in the highest quartile which was 3.28 times that for subjects in the lowest A1c quartile (Bash et al., 2008).

Epidemiologic studies also have demonstrated that hyperglycemia increases the risk of microvascular complications. Investigators for the Pittsburgh Epidemiology of Diabetes Complications Study calculated hyperglycemia exposure in A1c months (A1c units above normal × months) in 353 patients with insulin dependent diabetes mellitus. They found that the risk of developing proliferative retinopathy, microalbuminuria, overt nephropathy and distal symmetrical polyneuropathy all rose with increasing A1c months. Subjects with 1000 A1c months and above appeared to be at risk for developing most microvascular complications; however, the majority of complications arose in individuals with less exposure (Orchard et al., 1997). Results from ARIC study showed that the risk of chronic kidney disease increases progressively with A1c. in this study, A1C concentrations of 6% to 7%, 7% to 8% and > 8% were associated with hazard ratios for chronic kidney disease of 1.4×, 2.5× and 3.7×, respectively versus an A1c< 6% (Bash et al., 2008).

Several studies have demonstrated the effectiveness of targeting postprandial glucose to decrease the risk of diabetes complications (Ceriello, 2010). The campanian postprandial hyperglycemia (PPHG)study compared the effects of repaglinide and glyburide on PPHG, carotid intimamedia thickness and markers of systematic vascular inflammation in 175

patients with type 2 diabetes. These results show that targeting postprandial glucose can promote atheroma regression in patients with type 2 diabetes (Esposito et al.,2004).

2.3 Alpha Glucosidase

The digestion of carbohydrates begins in the mouth by the action of salivary α-amylase which hydrolyzes the α-1,4 bonds in starch, the products of this process are maltose, maltriose and small dextrins (Robyt, 2008). The starch digestion process continues in the small intestines by the action of pancreatic α-amylase. The digestion process is completed by enzymes in the brush border of the small intestine (maltase, sucrase and lactase, also known as disaccharidases or alpha glucosidase) which yields the absorbable monosaccharides glucose, fructose and galactose. A small proportion of monosaccharides can be absorbed passively; however, a carrier protein is required to absorb the amount ingested in a normal diet (Grabitske and Slavin, 2009).

Alpha glucosidase (EC 3.2.1.20) (alpha-D-glucoside glucohydrolase) is an exo-type enzyme distributed widely in microorganisms, plants and animal tissues, (Kimura et al. 2004) which catalyzes the liberation of α-glucose from the non-reducing end of the substrate. Inhibiting this enzyme slows the elevation of blood sugar following a carbohydrate meal (Lebovitz, 1997). It is a membrane bound enzyme present in the epithelium of the small intestine, which works to facilitate the absorption of glucose by the small intestine by catalyzing the hydrolytic cleavage of oligosaccharides into absorbable monosaccharides (Irfan, 2002). By the inhibition of α-glucosidase in the intestine, the rate of hydrolytic cleavage of oligosaccharides decreases and the process of carbohydrate digestion spreads to the lower part of small intestine. This spreading of digestion process delays the overall absorption rate of glucose into the blood. This has proved to be one of the best strategies to decrease the postprandial rise in blood glucose and in turn help avoid the onset of late diabetic complications (Irfan, 2002).

2.4 Current treatment of type 2 diabetes

Several clinical and experimental studies have provided strong evidences that T2D and its related complications can be controlled, prevented or delayed by achieving tight glycemic control (Nyenweaet al., 2011).Therefore much effort has been vowed to the search and development of optimal therapeutic agents for the management of type 2 diabetes.

Most existing antidiabetic drugs were developed without a prior definition of molecular targets. The advancement being made in understanding the pathogenesis of type 2 diabetes and the molecules involved provide the opportunity to develop new treatment interventions. However, the most commonly used approach is via oral therapies and at present, several classifications exist for the currently available oral anti-type 2 diabetes drugs. The drugs that are available and are in use currently are summarized below;

2.4.1 Sulfonylureas

Sulfonylurea (SU) drugs have been available in the United States since 1954. Second-generation SUs (glyburide, glipizide, and glimepiride) are more potent and probably safer than first-generation SUs (chlorpropamide, tolbutamide, acetohexamide, and tolazamide) but essentially of equal efficacy (Cohen and Harris, 1987). The SUs bind to the SU receptor, found on the surface of pancreatic beta cells. This interaction leads to a closure of voltage-dependent potassium adenosinetriphosphate (KATP) channels, facilitating cell membrane depolarization, calcium entry into the cell, and insulin secretion (Zimmerman, 1997). Thus, SUs allow for insulin release at lower glucose thresholds than normal. They partially reverse the attenuated insulin secretion that characterizes type 2 diabetes mellitus. Understandably, in the face of SU therapy, circulating insulin concentrations are increased (Doaret al., 1976). As a result, and despite the presence of insulin resistance, glucose concentrations fall. The possibility that such agents may also directly enhance peripheral glucose disposal (ie,

decrease insulin resistance) has also been raised (Simonson et al., 1984). However, the peripheral effects of SUs are most likely secondary to a reduction in glucotoxicity.
Most sulfonylureas are metabolized by the liver and cleared by the kidney and are therefore not recommended in patients with advanced liver or kidney disease. Given the epidemiological association between hyperinsulinemia and cardiovascular disease, there have been concerns that sulfonylureas might increase cardiovascular morbidity (Henry, 1998). Therefore, they must be used cautiously in those with advanced forms of either hepatic or renal impairment. Sulfonylureas are approved for use as monotherapy and in combination with all other oral agent classes (except the non-SU secretagogues) and insulin.
With all theanti-type 2 diabetes activity, the sulfonylureas have their own side effects. Hypoglycemia and weight gain are the major adverse effects of these drugs. The sulfonylureas-associated hypoglycemia results from the direct stimulation of insulin secretion from the pancreatic β-cells regardless of blood glucose concentrations (Cheng and Fantus, 2005). On the other hand, the weight gain could also be detrimental, especially to obesity prone patients. Other side effects of the sulfonylureas are skin rash, hyponatreamia and alcoholinduced flushing.

2.4.2 Biguanides

Although available internationally for decades, metformin, a biguanide, was not released in the United States until 1995 (Bailey and Turner, 1996). The earlier biguanides,phenformin and buformin, were introduced into the markets since 1970s but they were withdrawn from circulation because of their association withlactic acidosis (Kolata, 1979). In contrast to the SUs, metformin does not stimulate insulin secretion (Johansen, 1999). The precise mode of action of metformin remains somewhat controversial, but its predominant effect is to reduce hepatic glucose production in the presence of insulin (Hundal et al., 2000). Increased peripheral glucose disposal has also been measured, although this is most likely a secondary phenomenon caused by lowering of glucotoxicity and not a direct effect of the drug itself (Yu et al., 1999).
As with the sulfonylureas, biguanides reduce HbA1c by approximately 1-2% (Inzucchi, 2002). In contrast to sulfonylurea therapy, metformin monotherapy is associated with weight loss (or little to no weight gain) and a lower incidence of hypoglycemia. In the UKPDS, obese patients randomized to metformin gained only 1-2kg compared with gains of 5-7kg in patients receiving sulfonylurea or insulin treatment (UK Prospective Diabetes Study, 1998). The drug also has non-glycemic benefits, including reducing low-density lipoprotein cholesterol and triglycerides and reducing the antifibrinolytic factor lasminogen activator inhibitor. In addition, its lack of β-cell stimulation may have consequent positive effects on other cardiovascular risk factors.
The side effects of metformin therapy includes; gastrointestinal discomfort, such as nausea, abdominal pain, bloating, anorexia and diarrhea in approximately 50% of patients (Bailey and Turner, 1996) the reasons for these effects is not completely known. Also, metformin causes weight loss in some patients (DeFronzoet al., 1995) and the risk of lactic acidosis observed with earlier biguanides is also reported, though rarely in some patients. Approximately, the risk of the lactic acidosis is 100 times less than that with phenformin therapy (Misbinet al., 1998).

2.4.3 Thiazolidinediones.

In 1997, troglitazone, a thiazolidinedione(TZD), was introduced in the United States. It was later withrawn from the market because of rare idiosyncratic hepatocellular injury (Murphy et al.,2000).This novel class of drugs, currently represented by rosiglitazone and pioglitazone, has a unique mechanism of action that remains incompletely understood. Thiazolidinediones

are pharmacological ligands for a nuclear receptor known as peroxisome-proliferator-activited receptor gamma. When activated, the receptor binds with response elements on DNA, altering transcription of a variety of genes that regulate carbohydrate and lipid metabolism (Mudaliar and Henry, 2001). The most prominent effect of TZDs is increased insulin-stimulated glucose uptake by skeletal muscle cells (Frias et al., 2000). Thus, these agents decrease insulin resistance in peripheral tissues. Hepatic glucose production is decreased, although perhaps only at the highest doses (Maggs et al., 1998). Peroxisome-proliferator-activited receptor gamma activation also reduces lipolysis and enhances adipocyte differentiation. It is interesting to consider that the receptor is most highly expressed in adipocytes, while expression in myocytes is comparatively minor. Therefore, the increase in glucose uptake by muscle may largely be an indirect effect mediated through TZD interaction with adipocytes (Chaoet al., 2000). Candidates for the intermediary signal between fat and muscle include leptin, free fatty acids, tumor necrosis factor α, adiponectin, and the more recently isolated resistin (Steppan et al., 2001).

As with metformin, the TZDs do not stimulate pancreatic islet cells to secrete more insulin. Indeed, insulin concentrations are usually reduced, perhaps to an even greater extent than with metformin (Hoffmann and Spengler, 1997). Thiazolidinediones enhance the responsiveness and efficiency of beta cells, presumably by decreasing glucose and free fatty acid levels, both of which have deleterious effects on insulin secretion (Cavaghan et al., 1997). Preliminary data also suggest that this drug class may actually prolong beta cell survival (Finegood et al., 2001).

In placebo-controlled trials, TZDs generally lower HbA1cas much as SUs and metformin do (Aronoff et al., 2000) and more than AGIs do. Head-to-head studies have been performed on TZDs vs metformin (Kirk et al., 1999) and SUs, with equivalent reductions in HbA1c. No long-term outcome studies on microvascular end points are available. In relatively short-term studies, TZDs appear to lower microalbumin excretion, perhaps a manifestation of their beneficial effect on endothelial function (Lebovitz et al., 2001). Since TZDs decrease insulin resistance and because the latter is associated with macrovascular disease, some have wondered whether these drugs might provide cardiovascular protection. Preliminary evidence suggests that this notion may be justified (Parulkaret al., 2001).

In addition to their ability to lower insulin levels, the TZDs also have certain lipid benefits. High-density lipoprotein cholesterol concentrations, for instance, increase with TZD therapy and triglyceride concentrations frequently fall. The effect on low-density lipoprotein cholesterol concentrations is more variable, with increases reported with some, but not all, agents. Any rise in low-density lipoprotein cholesterol concentrations may be due to a shift from small and dense to large and buoyant low-density lipoprotein particles, which are less atherogenic (Fonseca et al., 2000).

Adverse effects of TZDs include weight gain, which can be as great or greater than that with the SUs. Weight gain appears to involve mostly peripheral subcutaneous sites, with a reduction in visceral fat depots (Kelly et al., 1999), the latter being better correlated with insulin resistance. Edema can also occur. Both weight gain and edema are more common in patients who receive TZDs with insulin. Anemia may also occur infrequently. Although the Food and Drug Administration still recommends periodic measurement of hepatic function, the available TZDs, unlike troglitazone, have not been convincingly associated with liver injury. Patients with advanced forms of congestive heart failure and those with hepatic impairment should not receive TZDs. Thiazolidinediones are the most expensive class of antidiabetic medication and are indicated as monotherapy and in combination with metformin, SUs, and insulin (pioglitazone only).

2.4.4 α-Glucosidase Inhibitors

The α-glucosidase inhibitors (AGIs; eg, acarbose and miglitol) were introduced in 1996. Their mechanism of action is unique, and this is the sole drug class not targeted at a specific pathophysiological defect of type 2 diabetes mellitus. The α-glucosidase inhibitors inhibits the activity of α-glucosidases located at the brush border of the small intestine as well as α-amylase found in the pancreas in a competitive manner. The α-amylase breakdown starch and other polysaccharides to oligosaccharides whereas α-glucosidases hydrolyze these oligosaccharides into absorbable glucose. Therefore, these agents interact with the carbohydrate-binding region of these enzymes and inhibit the digestion of the ingested carbohydrates (Koski, 2004). Consequently, the absorption of monosaccharides (mainly glucose) derived from dietary carbohydrates is delayed. This impedes the entry of glucose into the bloodstream and reduces postprandial blood glucose levels (Cheng and Fantus, 2005). Maltase and sucrase are amongst the most important isoenzymes of α-glucosidase. Adverse effects of AGIs include flatulence, abdominal discomfort, and diarrhea, frequently leading to discontinuation of the drug. The AGIs are rarely used as monotherapy because of their comparatively mild efficacy. They are approved for use as monotherapy and in combination with SUs. One caveat regarding AGI therapy (specifically, when combined with secretagogues or insulin) is the requirement that hypoglycemia be reversed by consuming glucose itself, as opposed to more complex carbohydrates (Inzuuchi, 2002).

2.4.5 Dipeptidyl peptidase (DPP) IV inhibitors

DPP-4 inhibitors are orally available agents that prevent the degradation of GLP-1 and GIP, thus increasing endogenous incretin levels. DPP-4 is rapidly and sustainably inhibited with these agents: it is seen within 30 minutes of administration and lasts for 24 hours. Sitagliptin was the first oral DPP-4 inhibitor to be approved in the US (October 2006) for use either as monotherapy or in combination with metformin or TZDs; in Europe it is currently indicated for use only in combination with metformin or TZDs. In a trial of 552 patients, sitagliptin monotherapy was shown to reduce HbA1c over a 12-week period by 0.4% (at 25mg once daily [QD]) and 0.6% (100mg QD) from a baseline of 7.7% (Hanefeldet al., 2005). Similar effects were reported in a study involving 743 patients over 12 weeks (Scott et al., 2005). Sitagliptin does not appear to have an effect on bodyweight and it has been shown to be generally well tolerated, with no increased risk for hypoglycemia compared with placebo. Adverse events reported in at least 5% of patients more frequently in the study group include upper respiratory tract infections and nasopharyngitis. Vildagliptin over 12 weeks at 25mg BID was shown to reduce both fasting and prandial glucose, while HbA1c was reduced by 0.6% (Pratleyet al., 2006). In a 24-week parallel-group study in drug-naïve type 2 diabetes patients, vildaglipin (50mg or 100mg BID) significantly reduced HbA1c compared with placebo (Pi-sunyer et al., 2007).

Compared with other medications, DPP-4 inhibitors generally show non-inferiority, although there as have yet been no comparisons with insulin. In a 24-week study on vildagliptin as monotherapy (50mg BID) in 459 patients, DDP-4 inhibition showed a reduction in HbA1c by 1.1% from a baseline of 8.7% and was non-inferior compared with rosiglitazone. Patients on vildagliptin did not gain weight, while those on rosiglitazone did (Rosenstocket al., 2007). A 52-week study using vildagliptin as monotherapy at 50mg BID in 526 subjects with type 2 diabetes showed a reduction in HbA1c of 1% from a baseline of 8.7%, although non-inferiority to metformin was not reached. Overall, vildagliptin is generally well tolerated, with no increased risk for hypoglycemia compared with placebo. Adverse events reported in at least 5% of vildagliptin patients include upper respiratory tract infections, nasopharyngitis, dizziness, influenza and headache. A number of DPP-4 inhibitors are currently in late stage

clinical development,including alogliptin and sexagliptin. The efficacy and safety of both agents have been evaluated as monotherapy and as combination therapy. Early data suggest that both alogliptin and saxagliptin also effectively lower glucose and HbA1c as monotherapy in treatment naïve patients (DeFronzoet al., 2008; Rosenstocket al., 2008). These agents also appear to be effective as combination therapy in type 2 diabetes patients inadequately controlled on monotherapy with available antidiabetic agents (Allenet al., 2008).
DPP-4 inhibitors have been recently approved as forms oftherapeutic intervention for the management of type 2 diabetes mellitus.They can be used in combination with other existing oral anti-diabetic agents or independently. The National Institute for Health andClinical Excellence (NICE) and German Diabetes Association,among other entities, advocates the use of DPP-4 inhibitors asan additional hypoglycaemic agent (second and third line therapy) in type 2 diabetes mellitus, alongside biguanides, thiazolidinediones or sulfonylureas (Gallwitz, 2013).

2.4.6 SGLT 2 inhibitors

Sodium glucose co-transporters 2 (SGLT2) inhibitors represent a new strategy in the treatment of diabetes. These drugs inhibit glucose reabsorption from renal tubules, thereby promoting urinary glucose excretion and decreasing plasmaglucoselevels.Thesedrugshaveauniquemechanismof action that is independent of pancreatic β-cell function or modulation of tissue insulin sensitivity. Thus, they have the potential to be used as combination therapy with other oral anti-diabetic drugs as well as insulin. A number of SGLT2 inhibitors are in various phases of preclinical and clinical development, and dapagliflozin is the furthest advanced compound (Misra, 2012).
Glucose absorption at the enterocytes, reabsorption at the renal tubules, transport across the blood-brain barrier and uptake and release by all cells in the body is mediated by two groups of transporters. These include glucose transporters (GLUTs)andsodium-glucoseco-transporters(SGLTs) (Wood and Trayhurn, 2003).GLUTs are facilitative or passive transporters that transport glucose along the concentration gradient. They belong to the solute carrier family 2 (SLC2) gene family, which has 13 members: GLUT1-12 and the H+-myoinositolco-transporters. GLUTs are expressed in every cell of the body. SGLTs transport sodium and glucose into cells using the sodium gradient produced by sodium/potassium ATPase pump at the basolateral cell membrane. These transportersbelongtothesolutecarrierfamily5(SLC5)gene family,whichhasninememberswithknownfunctions.Of these, SGLT1 and SGLT2 are primarily responsible for renal glucose reabsorption (Nair and Wilding, 2010).
SGLT2playsamajorroleinrenalglucosereabsorptionand accounts for approximately 90% of renal glucose reabsorption.TheevidenceforSGLT2beingamajorpathwayforrenal glucose reabsorption has been derived from the genetic studies of individuals with familial renal glycosuria. Mutations in the SLC5A2 gene encoding SGLT2 lead to familial renal glycosuria. This benign disorder is inherited as an autosomal recessive trait and is characterized by isolatedpersistent glycosuria with normal blood glucose levels and normaloralglucosetolerancetestresults (Nair and Wilding, 2010).AnumberofSGLT2inhibitorsareinvariousstagesofclinical development for the treatment of diabetes.SGLT2 inhibitors interfere with the function of SGLT2 in proximal convoluted tubules of kidney and induce glycosuria.Animal studies and clinicaltrialshaverevealedthatSGLT2inhibitionbenefitsthe diabetic state by lowering plasma glucose levels, decreasing glucotoxicity and reducing plasma insulin and glycosylated hemoglobinlevels (Boldys and Okopien, 2009).Reductionintheplasmaglucoselevel improves liver sensitivity to insulin. This suppresses hepatic glucose production, leading to an

improvement in the diabetic state.By causing glycosuria, SGLT2 inhibitors not only reduce plasma glucose levels,but also cause a net loss of calories from the body and maintain overall negative energy balance.

Inaddition,theseinhibitorsalsohaveabloodpressure lowering effect, which might be related to their mild diuretic and weight-reducing action (Kipnes, 2010). As compared to currently used antidiabetic drugs, SGLT2 inhibitors do not stimulate insulin secretion or pose the risk of hypoglycemia or cause gastrointestinal side effects (Boldys and Okopien, 2009). The convenience of oral administration is another advantage of this new class of antidiabetic drugs. The novel mechanism of action suggests their potential use in combination with other antidiabetic agents to exert additive or synergistic effects in lowering glucoselevelsintype 2 diabetes mellitus.

Despite interesting reports on several potential beneficial effects of these drugs, they are still not without adverse effects.Common side effects include symptoms of thirst, urinary tract infections and mycotic genital infections. Less common side effects are hypoglycemia, dehydration, hypovolemia and serum cholesterol and creatinine elevations (Tahrani et al., 2013).

2.5 Herbal Therapy for Type 2 Diabetes

Long before the birth of orthodox Western medicine, medicinal herbs were applied to treat a wide range of disease categories (Basch et al., 2003). Due to emphasis on scientism and other complicated reasons, Western medicine now prevails over “traditional” forms of medicine including herbal medicine systems. Although herbal medicine systems are sometimes misinterpreted as being unscientific and anachronistic, their long-term existence proves they are able to compete with Western drugs at some level. The use of a medicinal herb, alone or in combination with other herbs, can be thought of as a type of combination therapy because of the complexity of the phytochemicals and bioactivities in the plant. Thus, a single antidiabetic herb with thousands of phytochemicals may have multiple benefits by targeting several metabolic pathways and essentially “killing several birds with one stone.” One study supported this principle by demonstrating that a combination therapy of orthodox medicine and herbal medicine exhibited a better (synergistic) effect than either medicine alone (Kaur et al., 2013). Therefore, herbal medicine can complement orthodox therapy in type 2 diabetes and provides hope for a cure.

Medicinal herbs have never become obsolete and still play a prominent role in human health care. Among them, over 1200 plants have been claimed to be remedies for diabetes (Habeck, 2003). Over 400 plants as well as 700 recipes and compounds have been scientifically evaluated for type 2 diabetestreatments (Singhet al., 2011). Metformin was developed based on a biguanide compound from the antidiabetic herb, French lilac, and is now a first-line drug for type 2 diabetes (Oubre et al., 1997). Medicinal herbs contain diverse bioactive compounds and can have multiple actions on insulin action, insulin production, or both.

2.6 Carica papaya Linn.

2.6.1 Classification of Carica papaya.

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Sub-class: Dilleniidae
Order: Violales
Family: Caricaceae
Genus: Carica
Specie:papaya

Name: Carica papaya
Other names: Gwanda (Hausa),Ibepe (Yoruba),Okwere(Igbo), Pawpaw, Papaw, Papaya, (English)

2.6.2 Description, distribution and habitat of Carica papaya

Carica, the genus to which the papaya belongs, is taxa of flowering plants in the family Caricaceae.Formerly treated as including about 20-25 species of short-lived evergreenshrubs or small treesgrowing up to 10 meters tall and native to tropical Centraland South America-recent genetic evidence has resulted in the genus being restricted to the single species C. papaya (papaya; syn. C. peltata, C. posoposa). Most of the other species have been transferred to the genus Vasconcellea, with a few to the genera Jacaratia and Jarilla. Moreover, it is now cultivated in tropical and subtropical regions worldwide (Oderinde et al., 2002).

The papaya, is a small tree, with the single stem growing from 5 to 10 meters in height. It is considered a horticultural wonder in that it grows from a seed to a seven meter (20 foot), fruit-bearing tree in less than 18 months (Herbst, 2001). The tree is usually unbranched if unlopped.

The papaya has spirally arranged leaves confined to the top of the trunk. The leaves are large, 50-70 centimeters (cm) diameter, deeply palmately lobed with 7 lobes. The lower trunk is conspicuously scarred where leaves and fruit were borne. The flowers are similar in shape to the flowers of the Plumeria but are much smaller and wax-like. They appear on the axils of the leaves, maturing into the fruit.

The papaya fruit tends to be large, being about 15-45 cm long (6 to 17 inches) and 10-30 cm in diameter (4 to 12 inches). The Solo variety grown in Hawaii and Florida and particularly popular in the United States is a pear-shaped, golden-yellow variety that reaches about 16 centimeters (6 inches) and 1 to 2 pounds in weight (Herbst, 2001). The fruit has a large center cavity that is packed with shiny, grayish-black seeds, which are also edible but generally not eaten (Herbst, 2001). The fruit of the papaya is ripe when it feels soft (like a ripe avocado or a bit softer) and its skin has attained amber to orange hue. The fruit's taste is vaguely similar to pineapple and peach, although much milder without the tartness, as well as creamier and more fragrant, with a texture of slightly over-ripened cantaloupe.



Figure 2.1: Carica papaya (Caricaceae).

2.6.3 Biological activities of Carica papaya

Many biologically active phytochemicals have been isolated from papaya and studied for their action. Recently an antifungal chitinase has been gene cloned and characterized from papaya fruit. The chitinase is classified as chitinase IV chitinase based on its amino acid sequence homologywith other plant chitinases. The recombinant papaya chitinase also has antibacterial activity(Chen et al., 2007). The purified chemopapain from commercially available spray dried

latex of the fruits has shown immunological properties(Buttle and Barrett, 1984). The anthelmintic activity of papaya seed has been variously ascribed to carpaine (an alkaloid) and carpasemine (later identified as benzylthiourea) and benzylisothiocyanate(Kermanshai et al., 2001), cysteine proteinases from papaya fruit have also been reported(Stepek et al., 2005). Carpaine, an alkaloid with an intensively bitter taste and a strong depressant action on the heart, has been obtained from the fruit and seed, but especially from the leaves(Kirtikar and Basu, 1998).

The leaf extracts of C. papayawas reported to have an antimicrobial activity against various species of bacteria and fungi. It has been proposed that phytochemical compounds such as tannin, coagulate the wall proteins, saponins, facilitates the entry of toxic material or leakage of vital constituents from the cell. Thus blocks the growth of microorganisms (Baskaran et al., 2012).

Effective antioxidant supplementation Bionormalizer, a natural Japanese health food prepared by the fermentation of papaya, is able to improve the haemorrheoligy in alcoholics either by directly affecting the ethanol related lipoperoxidation and xanthine oxidase system activation and/or by modifying red blood cell membrane characteristics (Marotta et al., 2001). It also exhibits therapeutic properties against various pathological disorder including tumours and immunodeficiency. Its protective action is based on the free radical scavenging activity as well as normalization of an organism's superoxide level. It is proposed that, normalization of an organism's superoxide level, which is due to inactivation of the ferrous ions, the catalyst of the superoxide driven Fenton reaction, is one of the molecular mechanism of Bionormalizer activity (Osanta et al., 1995). Papaya markedly increases iron (Fe) absorption from rice meal, which was measured in parous Indian women, using the erythrocyte utilization of radioactive Fe method. The fruit is rich in vitamins, minerals, proteins, polysaccharides, lectins, saponins and flavonoids and can be used in the prevention of complications of diabetes mellitus (Savickiene et al., 2002).

The latex of papaya and Fluconazole has synergestic action on the inhibition of Candida albicans growth. This synergestic effect results in partial cell wall degradation (as indicated by transmission electron microscopy observations) (Giordani et al.,1997). Latex alone is statically effective on C. albicanswhen added to a culture during the exponential growth phase and approximately 60% was achieved. This fungistatic effect is the result of cell wall degradation due to a lack of polysaccharides constituents in the outermost layers of the fungal cell wall and release of cell debris into the culture medium. Latex proteins appear to be responsible for antifungal action and minimum protein concentration for producing a complete inhibition was reported as about 138mg/ml (Giordani et al.,1996).

Seed extract showed pronounced hypertrophy and hyperplasia of pituitary gonadotrophs. Whereas the male rats treated with seed extract revealed gradual degeneration of Germ, Sertoli and Leydig cells as well as germinal epithelium, which confirmed its antifertility activity (Udoh et al.,2005)., Three weeks of administration aqueous extract of papaya seeds showed that the lumina of the seminiferous tubles were more prominent and empty in the experimental animals with no evidence of spermatozoa (Uche-Nwachi et al.,2001). Verma and Chinoy, (2001)have reported its effect on cauda epididymal microenvironment.The benzene chromatographic fraction of the chloroform extract of the seeds possesses reversible male contraceptive potential and the effect appears to be mediated through the testis and may be directly rendered on the spermatozoa (Pathak et al., 2002) without adverse toxicity. Another study revealed inhibition of sperm motility due to other epididymis (Maniyannan et al., 2004). A possible mechanism of action and preliminary studies on the antifertility effect of crude seeds on the gonads of male albino rats has been done by Udoh

and Kehinde (Udoh and Kehinde, 1999). A recent report revealed that ithas good contraceptive efficacy in langur monkey and the action is mediated through inhibition of sperm motility (Lohiya et al., 2008).

Sharma and Mahanta, (2000) have reported that the composite root extract as one of the constituent, induces morphological changes in the endometrial surface epithelium in albino rat uterus. The characteristic smooth regular pattern of normal epithelium appears to have changed at places haphazardly oriented groups of cells and loss of microvilli indicating a disorganized picture. Whereas seeds aqueous extract has shown abortifacient properties on female Sprague Dawley ratsand the petroleum ether, alcoholic and aqueous extracts inhibits ovulation in rabbits (Oderinde et al., 2002).

The antibacterial and antifungal ability of both fresh and dried leaves of C. papaya against bacteria and fungi of medical importance was carried out. The aqueous, ethanol and acetone extract of both the dried and fresh leaves were tested at 25, 50 and 100 mg/ml concentrations on both the bacteria and fungi isolates using the disc diffusion method. Results showed very significant broad spectrum antimicrobial activity against Gram-negative and Gram-positive bacteria and fungi. The organic extracts were more effective than aqueous extracts. The result further showed that the dry sample was effective against both Gram-positive and Gram-negative bacteria while the fresh sample was more effective against Gram-negative bacteria. The dried leaf extract was potent against some of the bacteria which standard antibiotics were not able to inhibit. C. papaya leaves showed a better antibacterial activity than antifungal activity. Demonstration of antimicrobial activity against the test isolates is an indication that there is possibility of sourcing alternative antibiotic substances in this plant for the development of newer antibacterial agents (Okunola et al., 2012).Aqueous extract of C. papaya leaves at concentration of 400 mg/kg and800 mg/kg were given to cyclophosphamide induced thrombocytopenic rats for a period of fifteen days. Blood was withdrawn at various time intervals to determine the platelet count. Also, the clotting time was determined on the 15th day of the study by capillary method. C. papaya leaf extract was found toincrease the platelet count and also to decrease the clotting time in rats (Patil et al., 2013).

Sri Lankan traditional medicine use mature leaf concentrate of C. papaya to treat thrombocytopenia. This claim was scientifically validated. Adult wistar rats (N=6 per group) with Hydroxyurea - induced thrombocytopenia (model established for the first time), were orally administered, once daily on 3 consecutive days with three doses of fresh mature leaf concentrate of C. papaya (0.18, 0.36 and 0.72 ml/100g), while controls received water. Standard protocols were used to establish their platelet, WBC and RBC counts. Effects of mature leaf concentrate of C. papaya on carrageenan induced edema in rats, on rat erythrocyte membrane stabilization, and on acetic acidinduced vascular permeability in mice, as well as acute toxicity studies were conducted using standard methodology. High dose of mature leaf concentrate of C. papaya in thrombocytopenic rats significantly ($P<0.05$) increased platelets by 76.5%, WBC by 30.51% and RBCs by 9.08%, when compared with controls. High dose of mature leaf concentrate of C. papaya also significantly ($P<0.5$) inhibited carrageenan induced rat paw edema and impaired in vivo vascular permeability in mice (by 82%), while inducing maximum (10.11%) membrane stabilizing activity of rat RBCs at 8mg/ml of mature leaf concentrate of C. papaya, suggestive of effective anti-inflammatory activity. Administration of high dose of mature leaf concentrate of C. papaya on 3 consecutive days neither provoked overt signs of toxicity nor stress, where hepatotoxicity, renotoxicity, hematotoxicity and neurotoxicity were also ruled out. Thus freshly prepared mature leaf concentrate of C. papaya is orally active, effectively increases rat platelet, WBC and RBC

counts with no acute toxicity, and possesses potent anti-inflammatory activity, that overly justify claims of traditional medicine (Gammulle et al., 2012).

CHAPTER THREE

3.0MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

Alpha glucosidase from yeast of Saccharomyces cerevisiae, p-nitrophenyl-α-D-glucopyranoside (pNPG), acarbose, dimethyl sulfoxide (DMSO), were purchased from Sigma-Aldrich, Germany. All solvents and all other chemicals were purchased from Haddis International, Samaru Zaria-Nigeria.

3.1.2 Plant materials

The fresh plant sample of C. papaya wascollected from a local farm at Samaru village, Sabongari Local Government area, Kaduna State, Nigeria and authenticated at the herbarium unit of Biological Sciences Department, Ahmadu Bello University, Zaria, Nigeria where a voucher number V/No: 1708was obtained.

3.1.3 Preparation of plant extract

The collected plant leaf were rinsed in a clean water and air dried at room temperature. The dried leaf were pulverized into powder using laboratory mill, the powder obtained was used to prepare the extracts. The dried powdered leaf of C. papaya was extracted for 48 hours using four different solvents (n-hexane, ethyl-acetate, methanol and water) respectively. Fifty (50)grams of the powdered plant was soaked in 500ml of hexane for 48 hours at room temperature, after which it was filtered using muslin cloth and Whatmann filter paper No 1 to obtain the hexane extract. The same procedure was repeated to the rest of the three different solvents. The hexane, ethyl-acetate and methanolic extracts were concentrated using a rotary evaporator while the aqueous extract was concentrated to dryness at 40oC using water bath. Figure 3.1 shows the experimental design of this study.

3.1.4 Animals

A total of twenty (20) apparently healthy wistar rats weighing between 150-200 g were obtained and kept in a well aerated laboratory cages in the animal house, Department of

Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria. They were allowed to adjust to the laboratory environment for a period of two weeks before the commencement of experiment. The animals were fed with Growers mash from the Vital Feeds Company and water was provided ad libitum during. The animals were then divided into treatment groups and control groups.Furthermore, the rules and regulations of experimental animal ethics committee of Ahmadu Bello University, Zaria were duly followed in maintaining the animals.

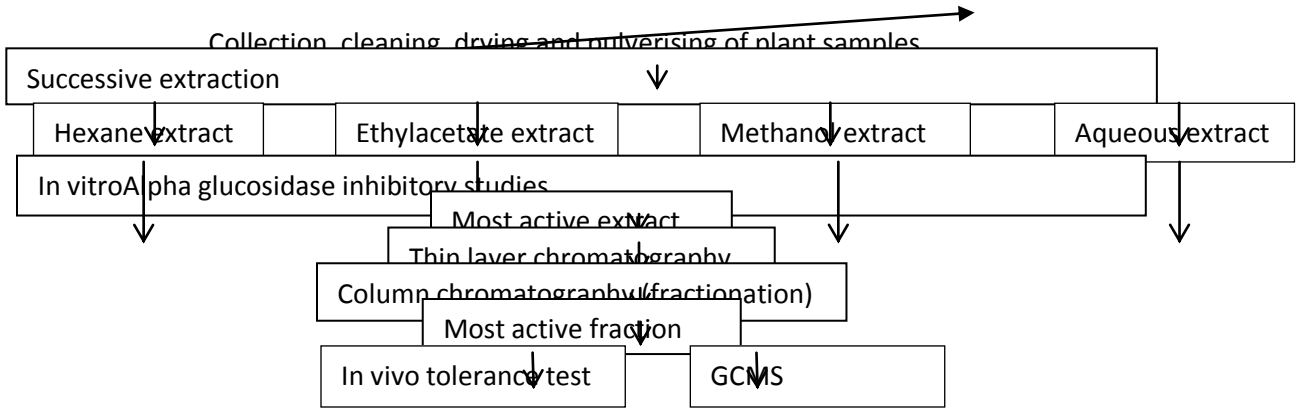


Figure 3.1: Experimental design of the invitro and invivo studies on the alpha glucosidase inhibitory effects of the leaf extract of C. papaya.

3.2 Methodology

3.2.1 Determination of alpha glucosidase inhibition

Method: The effect of the plant extracts on α-glucosidase activity was determined according to the method described by Kim et al. (2005) with some modifications.

Principle: p-Nitrophenyl-α-D-Glucopyranosideα-D-

α-glucosidase

Procedure: Alpha-glucosidase from Saccharomyces for the assay.

One hundred microliters of α-glucosidase (1U/ml) was added to tubes containing 40µL of

different concentrations of the extract. After which 60µL of 5.0mMpara-nitropheynyl-α-D-glucopyranoside (pNPG) in phosphate buffer pH 6.9 was then added to start the reaction. The reaction mixture was incubated at 37ºC for 1hour and stopped by adding 2ml of 0.1 M Na2CO3. The α-glucosidase activity was determined by measuring the yellow colored para-nitrophenol released from pNPG at 400nm. The blank solution was prepared by adding the Na2CO3 to the reaction mixture before adding the enzyme. The results were expressed as percentage of the negative control in which the extract was replaced with DMSO and distilled water mixture in equal volume. Acarbose was used as the positive control. All the reactions were conducted in triplicates.

Percentage inhibition was calculated as:

% Inhibition = [(Abscontrol-Absextract)/Abscontrol] x 100

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC50) were determined graphically using the concentration/inhibition plots.

3.2.2 Bioassay guided fractionation of the leaf extract

The extract that showed the lowest IC50(methanolic extract) was subjected to column chromatographic separation.

3.2.2.1Thin Layer Chromatography (TLC)

Thin Layer Chromatography was carried out to determine the best solvent system for the column chromatography. A thin layer chromatographic plate pre-coated with silica gel was used. The crude extract was dissolved solvent and applied to the plate. The plates were placed in chromatographic tanks with a mixture of different solvent systems. The different solvent systems used include n-hexane 100%, n-hexane and ethylacetate 9:1, 8:2, 7:3, ethylacetate 100%, ethylacetate and methanol 9:1, chloroform and methanol 9:1 and chloroform 100%. Thereafter, the plates were removed, sprayed with p-anisaldehyde and followed by heating at 110 °C for 5 minutes.

3.2.2.2 Column Chromatography

The column was packed with 100g of the silica gel (60-120 mesh) and 250ml of n-hexane. After the packing, 5g of the crude methanolic extract was loaded and eluted with 500ml of n-hexane 100%, 500 ml of 100% chloroform, chloroform and methanol (9:1, 8:2, 7:3; each 500ml) and 500 ml of 100% methanol. After collecting 154 fractions (20ml each), TLC was carried out and those fractions with similar Rf TLC profilewere pooled together which gave six pooled fractions (A – F). The fractions were tested against alpha glucosidase in order to ascertain their inhibitory potentials on the alpha glucosidase. The fraction with the highest inhibitory activity was fraction A.

3.2.3 Animal grouping

After two weeks adaptation period, the rats were randomly divided in to 4 groups of five animals each namely; NC: treated with distil water, CPF100: treated with 100 mg/kg bw of C. papaya fraction, CPF200: treated with 200 mg/kg bw of C. papaya fraction, Acarbose: treated with 100 mg/kg bw of a standard drug, acarbose.

3.2.4 The effects of the fraction on thein vivo maltose and sucrose tolerance in rats.

The rats in all the experimental groups were fasted overnight for 12 h and after which, the animals were orally co-administered with maltose or sucrose (2g/kg bw) and the respective dose of the fraction in the treatment groups whereas distilled water and acarbose where administered instead of the fraction in the NC and acarbose groups respectively The blood glucose concentrations of all animals were measured in the blood collected from the tail vein by using a portable glucometer (Glucoplus Inc., Saint-Laurent, Quebec, Canada) at 0 (just before maltose or sucrose ingestion), 30, 60, 90 and 120 min after the dose of maltose or

sucrose. A one day wash out period was given between the two experiments for maltose and sucrose tolerance in rats. The area under the curve (AUC) for all the experimental groups was calculated according to the following formula:

$$AUC_{tk} = \sum_{i=1}^{k} \frac{(C_{i-1} + C_i)}{2}(t_i - t_{i-1})$$

Where C = blood glucose concentration in mmol/L andt = time in minutes.

3.3 Gas chromatography-Mass spectrometry analysis

The Gas chromatography-Mass spectrometry (GCMS) analysis of the best alpha glucosidase inhibitory fraction was performed using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30m length, 0.25mm diameter, and 0.25mm film thickness.The carrier gas was ultra-pure helium at a flow rate of 0.7 mL min-1 and a linear velocity of 37 cm s-1. The injector temperature was set at 250 °C. The initial oven temperature was 60 °C, which was programmed to 280 °C at the rate of 10 °C min-1 with a hold time of 3 min. Injections of 2 µL were made in the splitless mode with a manual split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230 °C, quadrupole temperature 150 °C, solvent delay 4 min and scan range 50-700 amu.Compounds were identified by direct comparison of the retention times andinterpretation of mass spectrum of fraction was conducted using the database of National Institute of Standard and Technology (NIST) library having more than 62,000 spectral patterns.

3.4 Statistical Analysis

The results are presented as mean ± standard deviation (SD). Comparisons between the groups were performed by the analysis of variance (ANOVA) (using SPSS 20.0 for Windows Computer Software Package). Significant differences were compared by Duncan's Multiple Range test; a probability level of less than 5% ($P< 0.05$) was considered significant (Duncan, 1955).

CHAPTER FOUR

4.0 RESULTS

4.1 Extraction and fractionation yield.

Table 4.1 shows the percentage yield of the extracts recovered from various solvents extract of the plant sample.

Table 4.1: Percentage yield of extracts recovered.

Solvent	Yield (%)	Colour
Aqueous	14.30	Brownish
Methanol	10.24	Greenish
Ethylacetate	6.08	Greenish

Hexane	2.80	Oily green

Table 4.2 shows the percentage yield of various fractions recovered from the methanolic extract.

Table 4.2: Percentage yield of fractions recovered.

Fraction	Yield (%)	Colour
A	12.11	Oily greenish
B	7.42	Oily dark green
C	4.10	Oily brownish
D	2.15	Greenish
E	3.52	Brownish
F	25.39	Light brownish

4.2 The in vitroinhibition of alpha glucosidase by the aqueous, methanol, ethylacetate and

hexane extracts of C. papaya leaf.

Figure 4.1 shows the variation of the percentage inhibition of alpha glucosidase as the concentration of the extracts increases. It was observed from the figure that methanolic and ethylacetate extractsas well as the standard drug (acarbose) had a dose dependent inhibitory effect with the methanolic extract showing the highest inhibitory effect against alpha glucosidase when compared to the other extracts.

The activity of the extracts was evaluated by determining the inhibitory concentrations (IC50) of all the extracts on the enzyme. From the table 4.3, all the extracts had inhibitory activity against the enzyme. However, the methanolic extract was the most active against the enzyme with the lowest IC50 (171.83 ± 26.89µg/ml) and it was selected for the column chromatography in order to partially purify the extract.

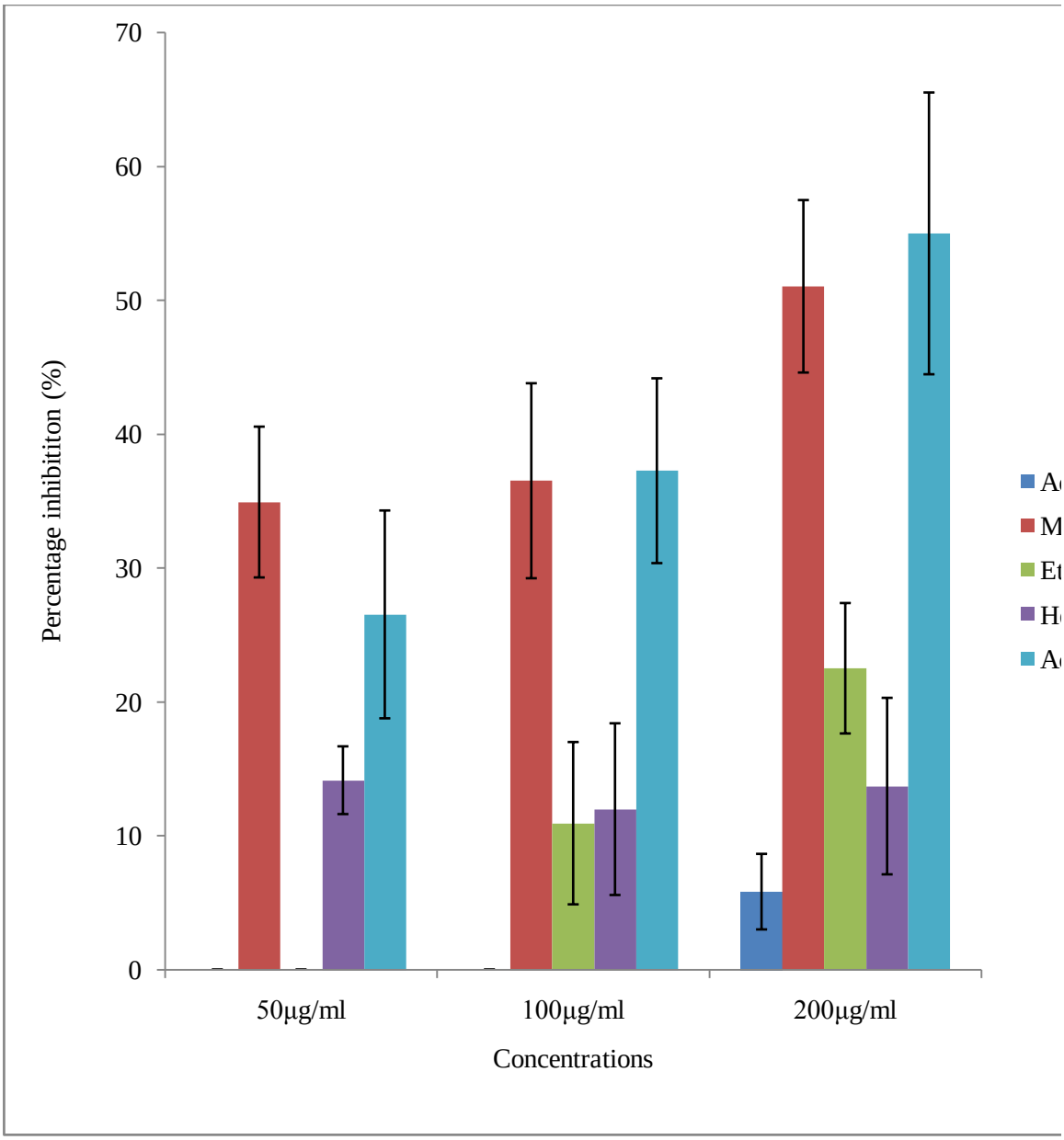


Figure 4.1: Variation in percentage inhibition of α glucosidase activity of aqueous, methanol, ethylacetate and hexane extract of C. papaya leaf. Data are presented as mean ± SD of triplicate determination.

Table 4.3: IC50 values for the inhibition of alpha glucosidase activity by the extracts of the leafof C. papaya.

Extracts	IC50 of extract on alpha glucosidase (µg/ml)
Aqueous	572.08 ± 71.06b
Methanol	171.83 ± 26.89a

Ethylacetate	292.15 ± 199.29a
Hexane	518.80 ± 67.07b
Acarbose (standard drug)	161.08 ± 41.01a

Results are presented as mean ± SD. Values with different superscripts down the column are significantly different from each other at $P< 0.05$.

4.3 Results of thin layer chromatography.

The chromatographic profile of methanolic extract (ME) is shown in plates I – III.

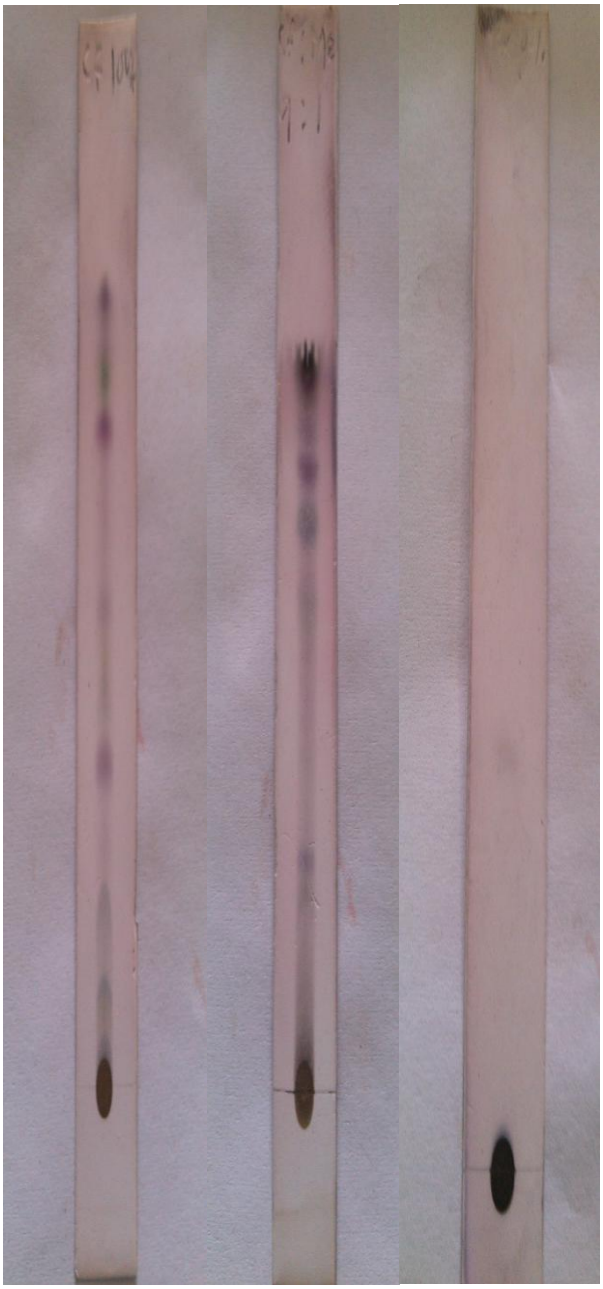


Plate I: ME TLC CHCl3 MeOH:CHCl3 100%9:1100%

Plate II: ME TLC HEX

Plate III: ME TLC

4.4 Result of column chromatography.

A total of one hundred and fifty four (154) fractions, 20ml each were collected. The fractions were pooled together based on their TLC profile to give five (5) major fractions and the column was finally washed with methanol to give the sixth fraction (Table 4.4).

Table 4.4: Column chromatography of methanolic extract of C. papaya.

Fraction	Collection	Number of Spots
A	1 - 41	0
B	42 - 87	4
C	88 - 98	1
D	99 - 120	9
E	121 - 126	8
F	127 - 154	5

4.4.1 TLC profile of methanolic fractions from column chromatography.
Plate IV - VII shows the TLC profile of column chromatography ofmethanolic fractions.

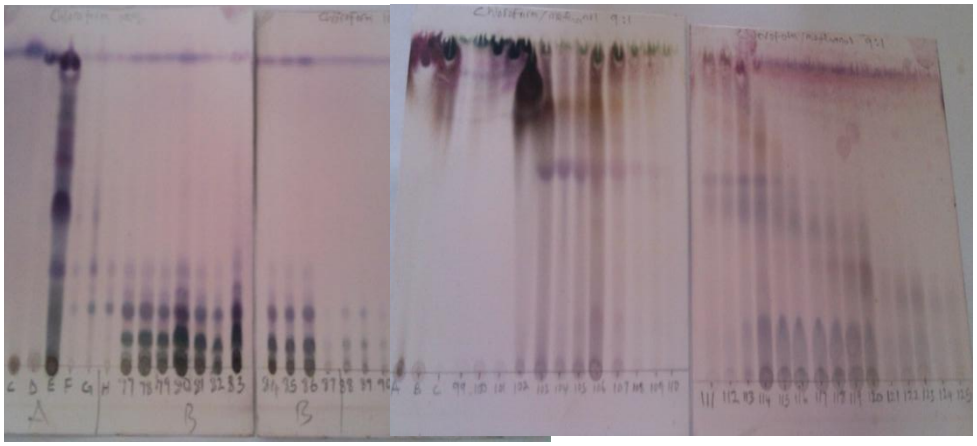


Plate IV: TLC of 1 – 92Plate V: TLC of 1 – 125
CHCl3 100% CHCl3 :MeOH (9 : 1)

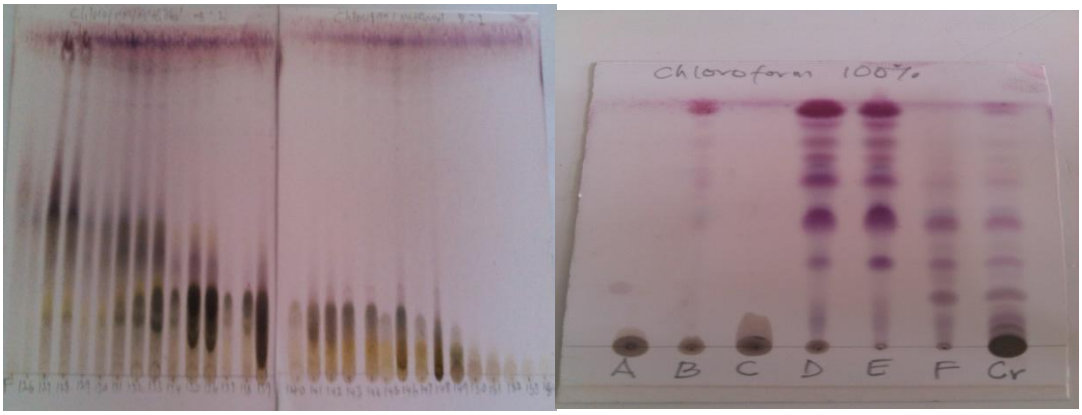


Plate VI: TLC of 126 - 154 Plate VII: TLC of A - Cr (Crude extract)
CHCl3 : MeOH (9 : 1) CHCl3 100%

4.5 The in vitro inhibition of alpha glucosidase by the methanolic extract fractions of C. papaya leaf.
Figure 4.2 shows the variation of the percentage inhibition of alpha glucosidase as the concentration of the fractions increases. It was observed from the figure that only fraction A had ahigher dose dependent percentage inhibition when compared to the other fractions. Table 4.5shows the result for the effect of the different solvents fractions on alpha glucosidase. The activity of the fractions was evaluated by determining the inhibitory concentrations (IC50) of all the fractions on the enzyme. From the table, all the fractions had

inhibitory activity against the enzyme and they are significantly different atP< 0.05. However, fraction A was the most active against the enzyme having the lowest IC50 value (76.62 ± 25.23µg/ml) and it was selected for the in vivo tolerance test and GCMS analysis.

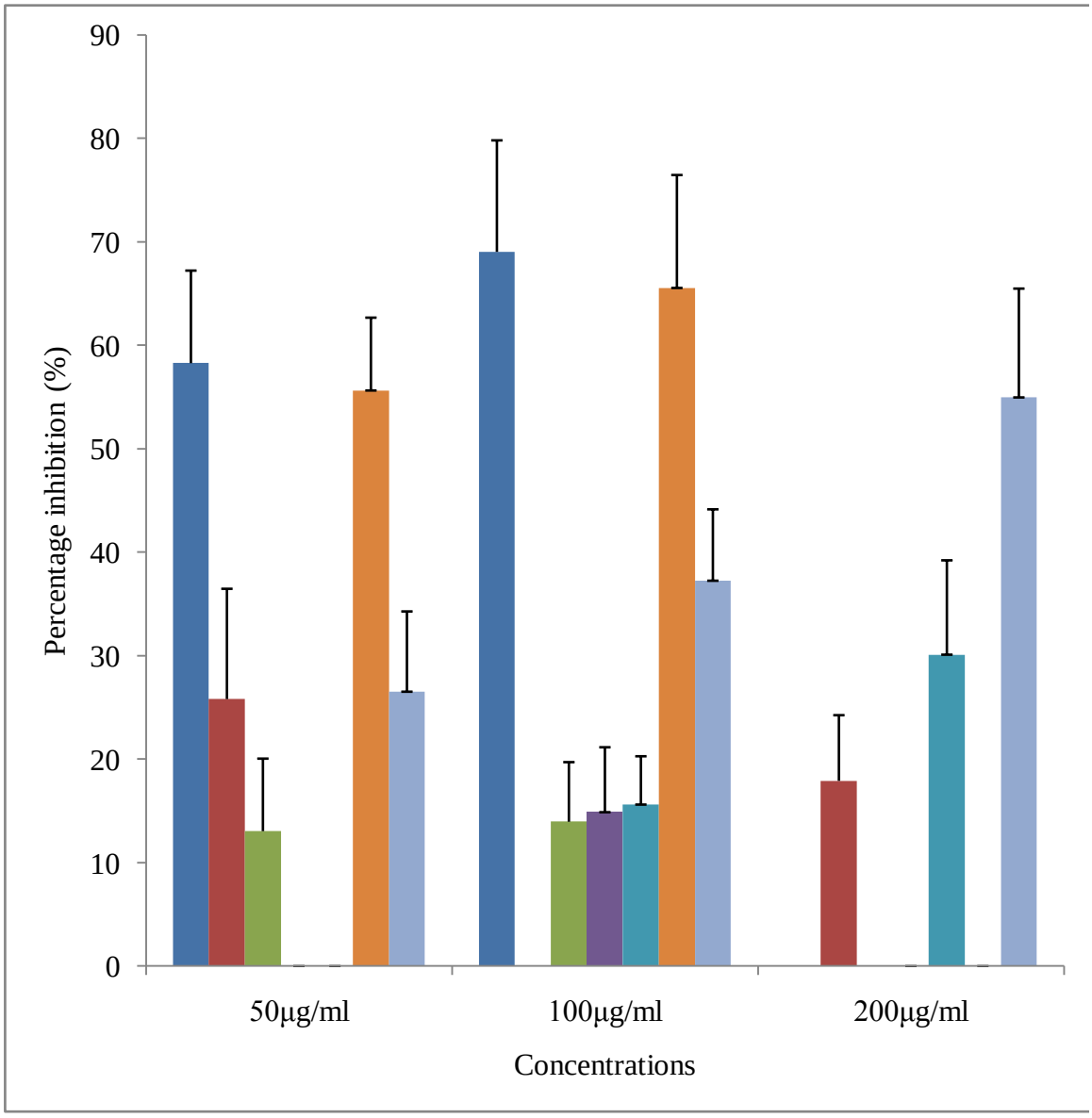


Figure 4.2: Variation in percentage inhibition of α-glucosidase activity of different solvent fractions of the methanolic extract of C. papaya leaf. Data are presented as mean ± SD of triplicate determination.

Table 4.5: IC50 values for the inhibition of alpha glucosidase activity by the fractions of the methanolic extract of the leaf of C. papaya.

Fractions	IC50 of fractions on alpha glucosidase (μg/ml)
A	78.62 ± 25.23a
B	182.63 ± 57.29bcd
C	287.70 ± 8.87e
D	261.07 ± 73.05de
E	229.92 ± 31.07cde
F	121.25 ± 43.61ab
Acarbose (standard drug)	161.08 ± 41.01bc

Results are presented as mean ± SD. Values with different superscripts down the column are significantly different from each other at $P< 0.05$.

4.6 The invivo inhibition of alpha glucosidase by the fraction (A) of the methanolic extract of

C. papaya leaf on maltose and sucrose tolerance in rats.
In the maltose tolerance test, the fraction A treated groups had reduced blood glucose levels in a dose dependent manner compared to the NC group, though the difference was insignificant ($P< 0.05$) in some cases (Figure 4.3). However, in the sucrose tolerance test, the fraction A treated groups had a non-dose dependent reduced blood glucose levels for most times during the 120 min experimental period (Figure 4.4). It was noteworthy that the fraction A treated groups had a profound decreased blood glucose levels from the 60 min.

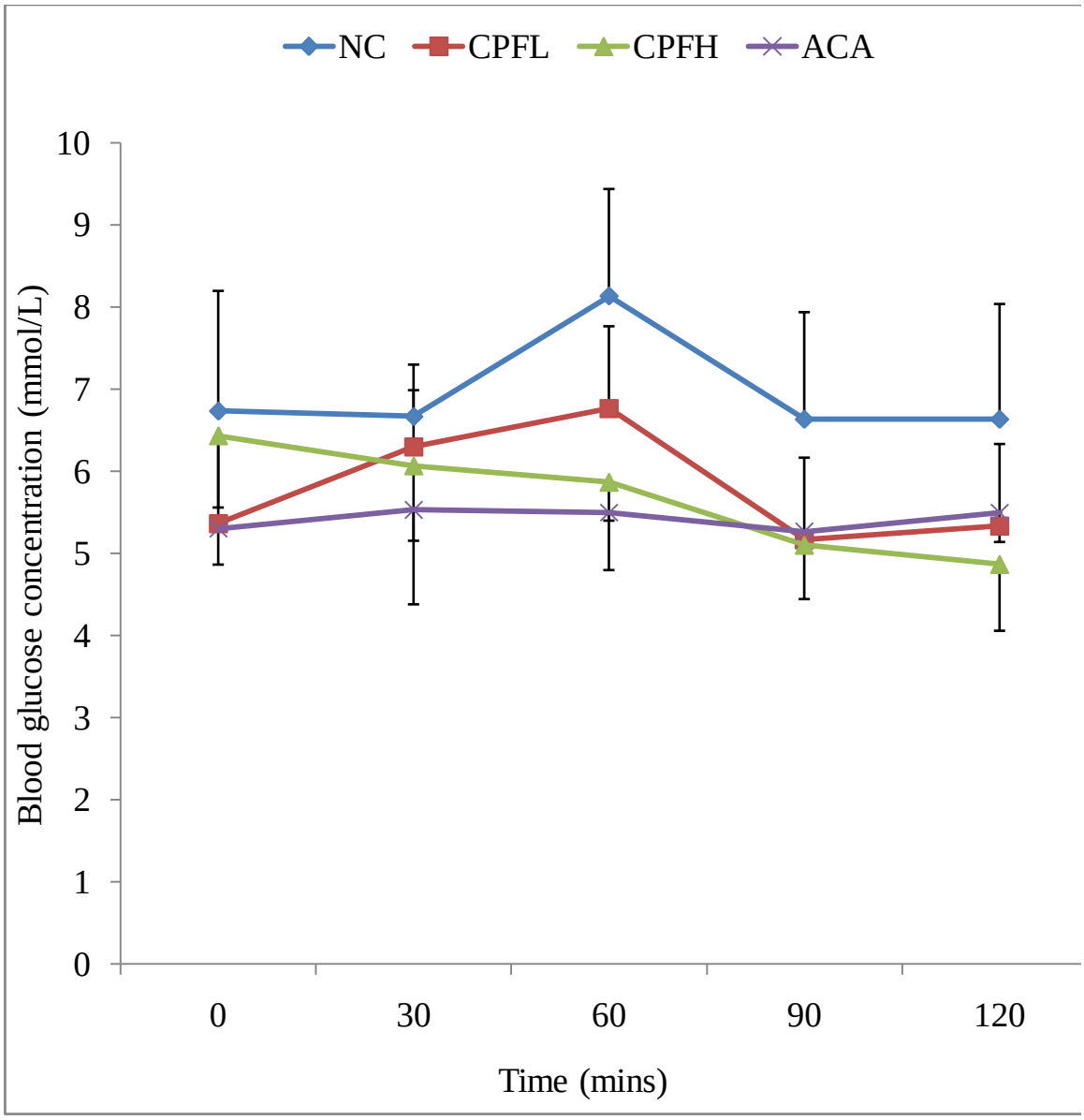


Figure 4.3:Blood glucose response during oral maltose tolerance test in normal rats treated with different concentrations of fraction (A) of C. papaya methanolic extract and Acarbose. NC, CPFL, CPFH and ACA refers to Normal control, Carica papaya fraction low, Carica papaya fraction high and Acarbose respectively.

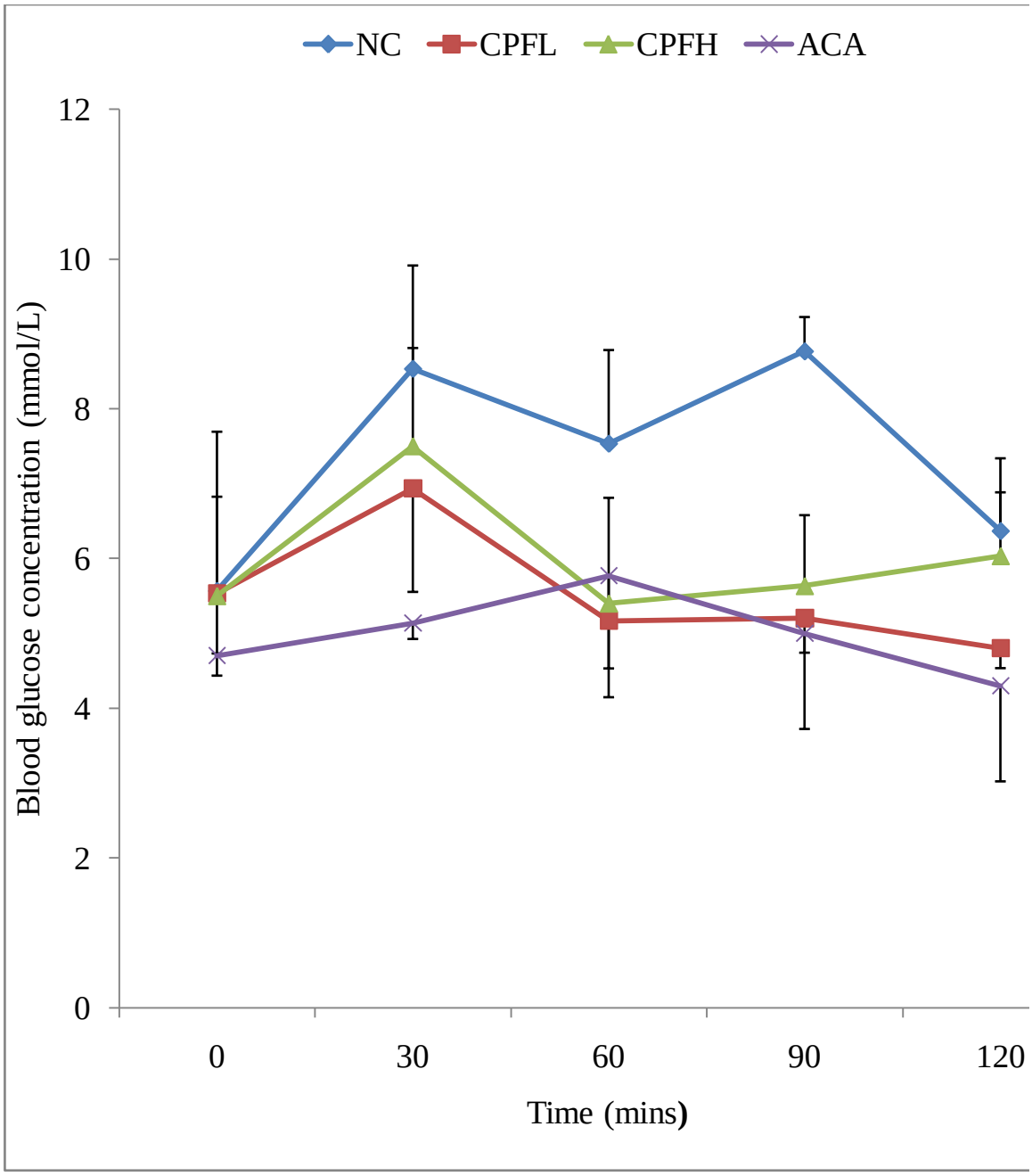


Figure 4.4: Blood glucose response during oral sucrose tolerance test in normal rats treated with different concentrations of fraction (A) of C. papaya methanolic extract and Acarbose. NC, CPFL, CPFH and ACA refers to Normal control, Carica papaya fraction low, Carica papaya fraction high and Acarbose respectively.

4.7 Area under the curve of blood glucose of rats subjected to maltose and sucrose tolerance test.

Table 4.6 shows the results for the computed area under the curve (AUC) values for the maltose and sucrose tolerance tests over the period of 2 hours.The data for the computed area under the maltose tolerance curves revealed that the fraction A treated groups had lower, but insignificant (P> 0.05), AUC values compared to the NC group. On the other hand,

the AUC values for sucrose tolerance test revealed that the fraction A treated groups also had lower but insignificant (P> 0.05) AUC values compared to the NC. However, the CPFL had lower AUC value compared to CPFH further supporting the non-dose dependent effect of the fraction A in the sucrose tolerance test.

Table 4.6:Computed area under the curve (AUC) values for the maltose and sucrose tolerance tests over the 2 hour period.

Groups	AUC0-120mins (Minutes.mmol/L)	
	Maltose	S
NC	843.5 ± 127.73b	9
CPFL	707.5 ± 62.17ab	6
CPFH	680.5 ± 125.20ab	7
ACA	648.5 ± 25.15a	6

All values are presented as mean ± SD. Values with different superscripts are significantly different from each other at P< 0.05.
NC, CPFL, CPFH and ACA refers to Normal control, Carica papaya fraction low, Carica papayafraction high and Acarbose respectively.

4.8Gas chromatography-mass spectrometry (GC-MS) analysis of fraction A of the methanolic extract of the leaf of C. papaya.

The GC-MS chromatogram of fraction A of the methanolic extract of the leaf of C. papaya showed the presence of 13compounds (appendix I).The interpretation of mass spectrum was done by National Institute standard and Technology (NIST) database that contained more than 62,000 spectral patterns. Table 4.7 shows the peak number, retention time (RT), compound name, molecular weightand similarity index of the compounds of fraction A of the methanolic extract of the leaves of C. papaya.

These compounds identified from fraction A were; 2,4-di-tert-butylphenol, cetene, cetyl alcohol,hexahydrofarnesyl acetone, palmitic acid,z-(13,14-epoxy)tetradec-11-en-1-ol acetate, linolelaidic acid, phytol,1-eicosene, 5-methyl-5-(4,8,12-trimethyltridecyl) dihydrofuran-2(3H)-one, 1-heptatriacotanol, 17-pentatriacontene and squalene.

Table 4.7:Identified components of the fraction A of the methanolic extract of the leaves of C. papaya by GC-MS

S/No	Retention time	Compound	Molecular weight	Simil
1	28.731	2,4-di-tert-butylphenol	206.32	95
2	30.994	Cetene	226.45	94
3	36.634	Cetyl alcohol	242.45	91
4	37.427	Hexahydrofarnesyl acetone	268.48	90
5	38.323	Palmitic acid	256.43	96
6	39.501	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	268.39	90

7	38.556	Linolelaidic acid methyl ester	294.47	99
8	39.682	Phytol	296.54	91
9	40.129	1-Eicosene	280.54	91
10	40.923	5-methyl-5-(4,8,12-Trimethyltridecyl) dihydrofuran-2(3H)-one	324.54	91
11	41.072	1-heptatriacotanol	537.01	90
12	41.111	17-pentatriacontene	490.95	96
13	44.607	Squalene	410.73	93

CHAPTER FIVE

5.0 DISCUSSION

The use of alpha glucosidase inhibitors in the control of rise in the postprandial glucose level is desirable as itconstitutes a noninvasive mechanism for controlling hyperglycemia. The STOP NIDDM study published in 2002 has revealed the advantage of alpha glucosidase inhibitors in prevention of progress of potential diabetic patients into Type II diabetic patients in addition to controlling the risk of cardiovascular damage (Abesundaraet al., 2004). Diabetic patients generally undergoa hyperglycemic shoot up after meals which take almost 4-5hours to reduce back to the original glucose level. The shoot up is attributed to increased disaccharidases activity by 1.5 fold (Tormo et al., 2002) and glucose transporters GLUT2 and SGLT1 activity by almost 3-4 fold in diabetic animals as compared to normal (Kwon et al., 2007). In this study, we therefore validated the use of the leaf of C. papayaby folk medicine in the treatment of type 2 diabetes and the results suggest that the plant might be good candidate for the control of postprandial hyperglycemia.

Report from previous research has shown that the aqueous leaf extract of C. papaya exerts a hypoglycemic, hypolipidemic and antioxidant effects in diabetic rats (Juarez-Rojop et al., 2012). These report and others on the anti-diabetic effect of the extracts of the leaf of C. papaya did not however investigate the inhibitory potentials of the extract on alpha glucosidase. The result of this study shows that the aqueous, methanol, ethylacetate and hexane extracts of the leafcould inhibit alpha glucosidase and consequently might reduce postprandial hyperglycemic shoot up.Among all the extracts, the methanolic extract had the best alpha glucosidase inhibitory activity which could be as a result of the presence of flavonoids, phenolics, coumarins, tannins, alkaloids and organic acids that were reported in the methanolic extract of the leaf of C. papaya(Khuzhaev and Aripova, 2000). According to

Caniniet al., (2007), the main compounds present in C. papaya leaf are phenolic acids, as well as trace amounts of chlorogenic acid, compared to flavonoids and coumarin compounds. Also, the authors suggested that the presence of such phenolic and coumarin compoundsin C. papaya leaf could partially explain the pharmacological properties of this plant. Moreover,administration of the extract of C. papaya leaf induced a significant reduction in glucose and triacylglycerol plasma concentrations (0.75 and 1.5 g/100 mL)(Juarez-Rojop et al., 2012). In addition,diabetic rats when treated with the C. papaya extract in the dose 400 mg/kg b.w. showed 4.15%, 6.52%, and 8.56% decline in the blood glucose level in the initial 2, 4, and 6 h, respectively. Then they showed 12.63%, 22.63%, 30.14%, and 38.19% decline in the blood glucose level on day 1, 7, 14, and 21, respectively. There were statistically significant differences in the serum cholesterol, triglycerides, and total protein levels when diabetic rats received the C. papaya extract at a dose of 400 mg/kg b.w. (Maniyar and Bhixavatimath, 2012).Another study also showed that both 31 and 62 mg/kg chloroform extract of C. papaya doses improved AST and ALT levels in diabetic rats, but the 125 mg/kg dose did not change aminotransferase concentrations(Akharaiyi, 2011).

Since the methanolic extract of the leaf of C. papaya had the least IC50 against the enzyme, it was selected for the column chromatography in order to have a better insight into the part of the extract that is responsible for the inhibition of the enzyme. Hence, the methanolic extract was subjected to column chromatography using different solvents of varying polarity to separate the compounds in the extract based on their polarity in the solvents. The solvents used as the mobile phase were hexane, chloroform and methanol. This now resulted in 154 fractions and those fractions with similar TLC profile were pooled together leading to six fractions (A, B, C, D, E and F). The fractions were subjected to inhibition studies on alpha glucosidase as it was done for the extract, the IC50 of the fractions were determined so as to ascertain their relative inhibitory activity against the enzyme. All the fractions were significantly different from each other ($P< 0.05$) andFA had the lowest IC50(78.62 ± 25.23) than the other fractions and even lower than the standard drug acarbose (161.08 ± 41.01), which makes it the most active amongst others. The result for alpha glucosidase inhibitory activity of the fractions was consistent with the previous findings on alpha glucosidase inhibitor from Musa spp (Zhanwuet al., 2014) in which the inhibitory activityof the most active fraction with IC50 (788.36 ± 19.32 mg/L) was significantly ($P< 0.05$) higher than the standard drug acarbose.

The identified phenolic compounds may be partly responsible for the alpha glucosidase inhibitory activity. Phenolic compounds have an electron donor capability and are readily oxidized to form phenolate ion or quinone, which is an electron acceptor (Michalak, 2006) thus, they have the ability to block or enhance specific enzymes responsible for digestion of carbohydrates. Comparing the IC50 of FA and that of the methanolic extract, you can observe that the possible compounds such as the phenolics might be present more in the fraction due to the partial purification of the extract. However, since the FAof the methanolic extract of the leaf of C. papaya had the least IC50 against the enzyme, it was selected for the in vivomaltose and sucrose tolerance tests and GCMS analysis.

The rise in plasma glucose in normoglycemic individuals after consumption of carbohydrate begins at about 10min and reaches maximum in~60min (ADA, 2001). In this study,the peak in plasma glucose level was observed within the first 60mins of maltose and30mins of sucrose dose, after which a decrease was observed until the level returns to normal. Moreover, in the maltose tolerance test all the FA treated groups showed reduced blood glucose levels in a dose dependent manner as the group treated with the high concentration of the FA showed the most reduction in blood glucose level, while in the sucrose tolerance test all the FA

treated groups showed reduced blood glucose levels but not in a dose dependent manner as the group treated with the low concentration of the FA showed the most reduction in blood glucose level.The observed effects of the FA within the initial 30mins on maltase and 60mins on sucrase suggest a fast inhibitory activity against intestinal maltase. Interestingly,it was also evident from the computed AUC data that FA (at 200mg/kg bw) have better inhibitory activity on maltase than sucrase which is similar to that reported by Ibrahim et al., (2016).This could possibly be due to the presence of additional phytochemicalsintheextractthatspecificallytargetmaltaseinadditionto non-specific α-glucosidaseinhibitors.Thepresentin vivotestfindings suggest that FA probably exerted its anti-diabetic effect via suppressing postprandial hyperglycemia as similarinhibitoryeffectswereseenforFA andacarbosein vitroandin vivo.Based on the results, it can be hypothesized that FA, in part, diminishes glucose absorption by inhibition of the activity of intestinal glucose transporters (the same mechanism acarbose exhibits).
Phenolic compounds are also known to interact with proteins and can inhibit enzymatic activity including alpha glucosidase and alpha amylase. In fact, previous studies indicated that the alpha amylase and alpha glucosidase inhibitory effects of Ocimum basilicum, Citrusmaxima, cinnamon and tea extracts are mediated by phenolics (El-Beshbishy and Bahashwan, 2011; Oboh and Ademosun, 2011). Indeed, alpha amylase inhibitory activities of some phenolics have been proposed to be through the formation of hydrogen bonds between the OH groups of the phenolics and the carboxylate groups of Asp197 and Glu233 in the active site of the enzyme (Piparo et al., 2008).In fraction A, prominent compounds were identified by GC-MS and out of the 13 compounds identified, 2,4-di-tert-butylphenol, 5-methyl-5-(4,8,12-trimethyltridecyl) dihydrofuran-2(3H)-one, phytol, squalene and palmitic acid were the most prominent amongst others and the observed activity could be as a result of their presence. Interestingly, palmitic acid, squalene (Widyawati et al., 2015) and phytol (Jananie et al., 2011) havealready been proven to have an anti-diabetic activity by causing insulin secretion, insulin stimulation, α-glucosidasethe common characteristics of alloxan-induced diabetes mellitus in experimental rats (Luoet al., 2004).
Phytol has a potential role in the management of insulin resistance and metabolic disorders that accompany diabetes and/or obesity, through activating RXR via its metabolite, and modulating other factors that imply in metabolic disorders. Moreover, molecular docking studies of phytanic acid on the two crystal structures of PPARc binding protein, and RXRa/PPARc heterodimer showed good alignment with the experimental findings and verified/confirmed the antidiabetic biological activity of phytol and its active metabolite, phytanic acid. Phytol can be administered with lower doses of TZDs to maintain the full therapeutic action, but with lesser side effects (Elmazar et al., 2013). Squalene has been presented in several studies as a compound that contributes to the anti-hyperglycemic activity of plants. Palmitic acid, octadecanoic acid and squalene were present among the phytochemicals in Mucunapruriens and Cynodondactylon and showed an anti-hyperglycemic activity on STZ-induced diabetic rats (Tiong et al., 2013). Squalene obtained from plants (Castellano et al., 2013) acts as an alpha glucosidase inhibitor (Hou et al., 2009) and may increase insulin secretion. Therefore, the presence of this prominent phytochemicals in our FA could explore the anti-hyperglycemic effect of the leaf of C. papaya. And also plant extracts have been valuable anti-diabetic agents and may involve one or more active components responsible for blood glucose reduction (Groveret al., 2002).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The result of this study shows that the aqueous, methanol, ethylacetate andhexane extracts of C. papaya leaf possess possible inhibitory potential against alpha glucosidase with the methanolic extract being the most potent inhibitor in comparison with the other extracts. The fraction A of the methanolic extract also showed strong inhibitory potentials against alpha glucosidase.Moreover, the fraction A of the methanolic extract possesses intestinal maltase and sucrase inhibitory effects in rats. The GC-MS results of the fraction A of the methanolic extract shows the presence of palmitic acid, squalene and phytolwhich has already been proved to possess an anti-diabetic property. These findings therefore validate the use of the leaf of C. papayaby folk medicine in the treatment of type 2 diabetes as the results suggest that the plant is a good candidate for the control of postprandial hyperglycemia.

6.2 Recommendations

The fraction A of the methanolic extract of the leaf of C. papaya should be subjected to a bioassay guided fractionation in order to isolate the specific active compound(s) that is responsible for the inhibition of alpha glucosidase and the mode of interaction of the compound and the enzyme should also be studied.

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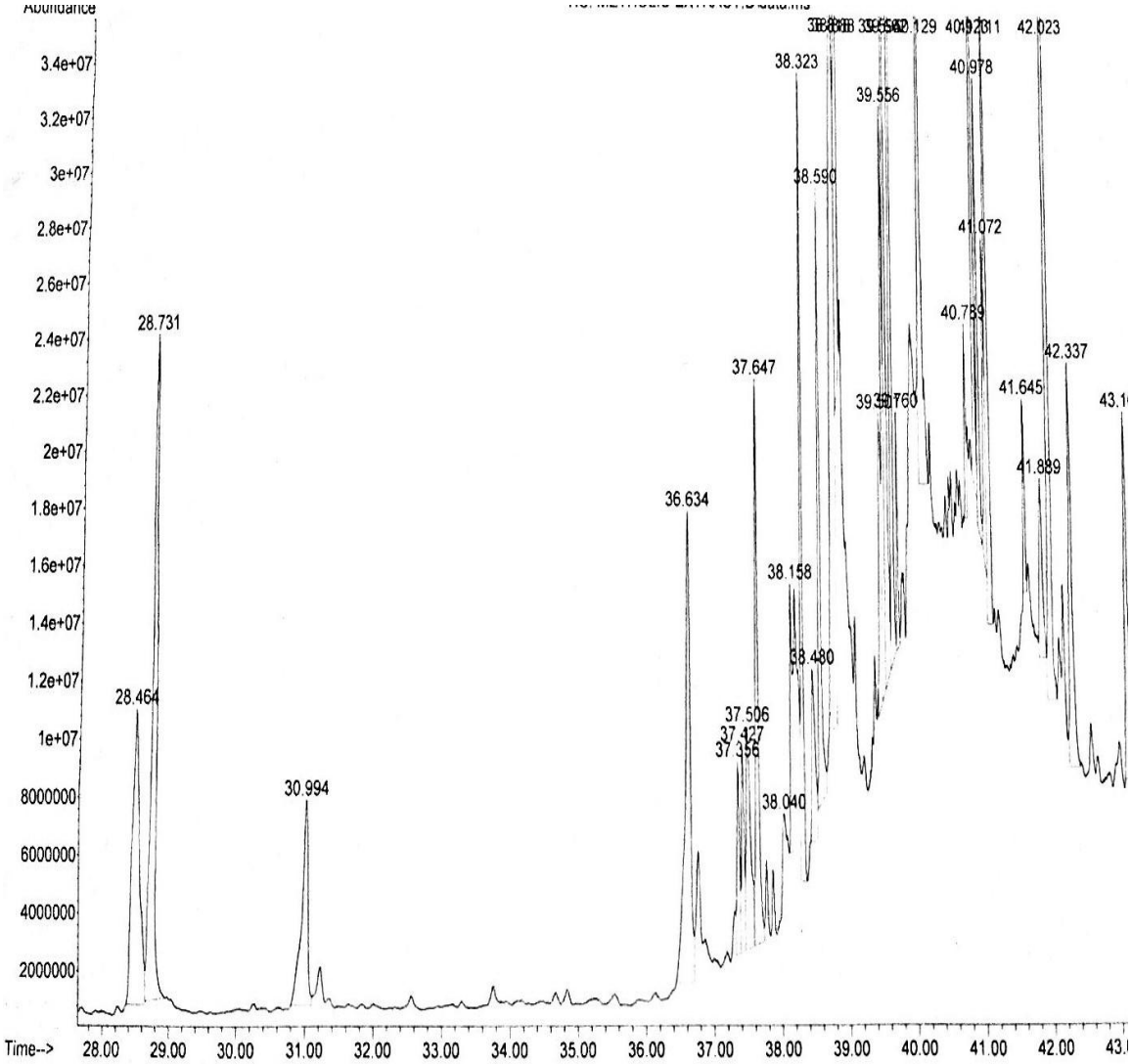
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APPENDIX I

GC-MS chromatogram of the fraction A of the C. papaya leaf methanolic extract.



APPENDIX II

Some collected fractions at the column chromatographic stage.

