ANTI-DIABETIC EFFECT OF LEMONGRASS (*CYMBOPOGON CITRATUS STAPF*) TEA IN TYPE 2 DIABETIC RATS

BY

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DECLARATION

I declare that the work in this dissertation entitled 'Anti-Diabetic Effect of Lemongrass (*Cymbopogon Citratus Stapf*) Tea in Type 2 Diabetic Rats' was carried out by me in the Department of Biochemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertationwas previously presented for another degree or diploma at this or any other institution.

Husaina Garba Anchau ---------- ----------

Signature Date

CERTIFICATION

This dissertation titled "Anti-Diabetic Effect of Lemongrass (*Cymbopogon citratus Stapf*) Tea in Type 2 Diabetic Rats' by **Husaina Garba ANCHAU** meets the regulations governing the award of the Degree of Master of Science in Biochemistry of Ahmadu Bello University Zariaand is approved for its contribution to knowledge.

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DEDICATION

This research work is dedicated to my children, may Almighty Allah continue to blessand guide them.

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ABSTRACT

Lemongrass (Cymbopogon citratus Stapf) tea is commonly consumed as food and for the traditional treatment of diabetes mellitus locally. This study was conducted to investigate the effect of lemongrass tea (LGT) in type 2 diabetes mellitus (T2DM) rats model. Forty-two (42) animals were randomly divided into 6 groups of seven animals each: Normal Control (NC), Diabetic Control (DBC), Diabetic Lemongrass Tea Low (DLTL, was administered 0.25g/100ml/kg BW of the lemongrass tea), Diabetic treated with metformin (DMF 150mg/kg BW), Diabetic Lemongrass Tea High (DLTH) and Normal Lemongrass Tea High (NLTH) were administered 0.5g/100ml/kg BW of the lemongrass tea. T2DM was induced in rats by feeding 10% fructose solution ad libitum for two weeks followed by intra-peritoneal (i.p) injection of streptozotocin (STZ, 40mg/kg BW) in all animals except NC and NLTH groups. The phytochemical analysis of lemongrass tea and cold water extract was compared using standard methods. From the results, the hot LGT showed higher phytochemical constituents compared to cold water extract, except for saponins. The LGT treatment at both dosages significantly ($p < 0.05$) reduced blood glucose level and dyslipidemia compared to DBC while NLTH was within the normal range. There were significant ($p < 0.05$) improvements in oral glucose tolerance ability, weight gain, decreased food and fluid intakes. Similarly, serum insulin concentration, pancreatic β-cell function and liver glycogen content were significantly ($p < 0.05$) increased in DLTL, DLTH groups when compared to DBC group. The results obtained in this study validate the traditional usage of LGT for management of diabetes treatment and suggest that both lower and higher (0.25/100ml/kg and 0.5/100ml/kg) doses of LGT are effective to reduce most of the diabetes associated complications in a T2D model of rats

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LIST OF ABBREVIATIONS

ALP…… Alkaline Phosphatase

ALT…… Alanine Aminotransferase

AST…… Aspartate Amino Transferase

DBC……. Diabetic Control

DLTH……....Diabetic Lemongrass Tea High

DLTL…….Diabetic Lemongrass Tea Low

DM…….Diabetes Mellitus

DMF……...Diabetic with Metformin

GDM……..Gestational Diabetes Mellitus

HDL……High Density Lipoprotein

HOMA-β……. Homeostatic Model Assessment of Beta Cell Function

HOMA-IR ……...Homeostatic Model Assessment of Insulin Resistance

IDF……...International Diabetes Federation

LDL……. Low Density Lipoprotein

LGT…….. Lemongrass Tea

NC……. Normal Control

NLTH……... Normal Lemongrass Tea High

OGTT…… Oral Glucose Tolerance Test

T1DM……. Type1 Diabetes Mellitus

T2DM……. Type 2 Diabetic Mellitus

TC ……Total Cholesterol

TG…… Triglycerides

CHAPTER ONE

1.0 Introduction

Diabetes mellitus (DM)is a condition that causes hyperglycaemia due to either decreased insulin secretion or insulin sensitivity of target tissue (Panini,2013).It is a progressive metabolic disorder that eventually leads to micro and macro vascular changes such as nephropathy, neuropathy, retinopathy and cardiovascular diseases(Osadebe*et al*., 2010). It is broadly classified into three major classes, type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM) and gestational diabetes mellitus. T1DM results from inadequate production of insulin by β-cells of the pancreas, while T2DM occurs when the pancreas does not produce enough insulin or when the body does not effectively use the insulin that is produced (Goldenberg and Panthakee, 2013). Furthermore, it has been reported that T2DM is most prevalent, accounting for more than 90% of diabetes cases (International Diabetes Federation, 2015). As a multifactorial disorder, management of diabetes requires comprehensive and holistic approaches. The currently available synthetic oral hypoglycaemic drugs such as biguanides (example include metformin), insulin secretagogues (example include glipizide) and α-glucosidase inhibitors (acarbose, miglitol, voglibose) are widely used in the treatment of T2DM. However, in view of the expensive, non-availability coupled with adverse effects associated with the use of these drugs, an alternative source with less toxic effects is of enormous interest. The use of plant-derived products is receiving much attention attributed to their less adverse consequences and functional foods are receiving much attention (Gomathi *et al*., 2013).

Considerable amount of data hasshown that functional foods play crucial role in the prevention and control of chronic diseases such as diabetes (Jacobson, 2004). The concept of functional foods entail utilizing food for the prevention and reduction of risk factors for

several diseases or enhancing certain physiological function beyond adequate nutritional effects(Tahrani*et al*., 2010). Among the prominent functional foods is the *Cymbopogon citratusStapf,* commonly known as lemongrass.It is an aromatic perennial plant belonging tothe *Poacae* family, having a long slender green leaf. It is widelydistributed and extensively used worldwide (Ekpenyong*et al*. 2015). It is frequently consumed for recreational as well as therapeutic purposes attributed to the ingredients, taste, distinctive lemony smell and colour. In some African countries, such as Nigeria and Egypt, lemongrass leaf tea is consumed in management of fever, jaundice, throat and chest infections,moderate-to-severe pain, hypertension, obesity and diabetes (Mansour*et al*. 2002). Interestingly, Boaduo*et al*. (2014) reported that various extracts from lemongrassinhibited α-glucosidaseactivity *in vitro*and improved insulin release. However, detailed anti-diabetic action of lemongrasstea in T2DM rats' model is not widely available.

1.1 Statement of Research Problem

The prevalence of diabetes is increasing annually estimated to be 463 million people globally with 19.4 million people in Africa and the global projection is well above 700 million by 2045 (International Diabetes Federation, 2019). However, the current estimation also suggests that in Nigeria there were more than 2.7 million cases of diabetes in 2019. With the current statistics, DM not only takes a heavy toll on lives around the world but imposes a serious financial burden on the sufferers and their family members (Nasli-Esfahani *et al*., 2017). These geometric increases in the number of diabetics in recent times cannot be dissociated from unhealthy life style, urbanization, aging and ravaging influence of free radicals (American Diabetes Association, 2015).

Diabetes burden and allied complications like amputation, stroke or end stage renal diseases are soaring.T2DM is managed by a combination of diet, exercise and conventional therapy. Some of these conventional or synthetic drugs can cause side-effects including

haematological, gastrointestinal reactions, hypoglycaemic coma, and disturbances in the liver and kidney metabolism. In addition, these drugs are not ideal for use during pregnancy (Gomathi*et al.,* 2013).

1.2 Justification

Like other parts of the world, diabetes has also become a serious issue in Africa.It is quite obvious that the prevalence of DM and its complications are increasing at an alarming rate, especially among the black race (Ahren, 2013). The International Diabetes Federation's (IDF) Diabetes Atlas estimates that, 60% of people with DM in Africa are undiagnosed (IDF, 2019). Among the types of DM, T2DM is known to have the highest prevalence, which is between 90% of all diabetes cases (IDF, 2019).

There has recently been a rapid increase in consumer interest in the health enhancing roles of specific foods or physiologically active food components, so called functional food (Hasler, 1998). The crucial role of functional food in the treatment and reduction of complications associated with T2DM cannot be denied. Extracts and essential oils from Lemongrass have been reported to reduce blood glucoseandlipid profileslevels in normal(Adeneye and Agbaje, 2007; Ademuyiwa*et al*. 2015; Ekpenyong*et al*. 2015) and hyperlipidemic rats(Bharti*et al*. 2013; Ekpenyong*et al*. 2015).Lemongrass as a functional food is consumed in form of tea for nourishment and in the traditional treatment of diabetes and related disorders (Lunyera *et al*. 2016)**.**Also**,** several studies have already investigated various alkaloids, flavanoids and phytochemicals in *Cymbopogoncitratus*, to exhibit anti-diabetic effects (Ekpenyong*et al*. 2015; Boaduo *et al*., 2014). These effects include inhibition of α-glycosidase, amylase enzymes activity (Boaduo *et al*.2014), reducing carbohydrate metabolism and therefore lowering blood glucose levels (Boaduo *et al.* 2014).

1.3 Aim and Objectives

1.3.1 Aim

The aim of this study is to investigate the anti-diabetic potential of lemongrasstea in a T2DM model of rats.

1.3.2 The Specific Objectives are:

1. To compare the phytochemical composition of lemongrass tea and cold water extract.
2. The determine the effect of lemongrasstea on body weight, feed and fluid intakes, serum glucose levels, insulin, insulin resistance indices in T2DM induced rats.
3. To determinethe effect of lemongrass tea on lipid profiles (total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol) and some serum biochemical parameters (creatinine, total proteins, albumin, ALT, ALP, AST, and liver glycogen) in T2DM induced rats.
4. To determine the pancreatic integrity of T2DM induced rats administered with the LGT.

1.4 Research Hypothesis:

Null hypothesis: Consumption of lemongrass tea has no anti-diabetic effects.

Alternative hypothesis: Lemongrass tea consumption possess anti-diabetic potential in the management of diabetes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus can be described as a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2013). The hormone is responsible for the uptake of glucose from the blood mainly into muscle and fat cells of the body and it also inhibit hepatic glucose production, and therefore regulate the concentration of blood glucose(Darkwa, 2011).The basis of abnormalities in carbohydrate, fat and protein metabolism in diabetes is due to impaired action of insulin on target tissues (Golden and Sapir, 2012). The chronic hyperglycaemia is associated with long-term damage, dysfunction, and failure of different organs especially the eyes, kidneys, nerves, heart and blood vessels (American Diabetes Association, 2019). It is also associated with an enhanced risk for developingpremature atherosclerosis as evident by an increase in the concentration of serum triglycerides (TG), increase in low density lipoprotein (LDL), and decrease in high density lipoprotein (HDL) (Dixit and Kar, 2010).

Several pathogenic processes are involved in the development of DM. Theserange from autoimmune destruction of the β cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Insulin resistance is characterised by decreased tissue sensitivity to insulin and marked compensatory hyperinsulinemia. Initially, plasma glucose levels are maintained in the normal range. The first glucose abnormality is detected by a rise in the postprandial glucose levels because of reduced first-phase of insulin secretion. With time, further decline in β cells function lead to elevation of the fasting blood glucose levels. Eventually, DM occurs with more insulin secretory loss (Yadav*et al.*, 2008).

Symptoms of marked hyperglycaemia include polyuria, polydipsia, weight loss, sometimes polyphagia and blurred vision. Impairment of growth and susceptibilityto certain infections may also accompany chronic hyperglycaemia (American Diabetes Association, 2014).

2.1.1 Prevalence of Diabetes

The prevalence and incidence of T2DM, representing 90% of all cases of DM, are increasing rapidly throughout the world. It has been estimated that the number of people with DM is expected to rise from 415 million in 2015 to 700 million by 2045 if no urgent action is taken (International Diabetes Federation, 2019).Moreover, the prevalence of DM is higher in men than women, but there are more women with DM than men (Ogurtsova *et al*., 2015). The most important demographic change to DM prevalence across the world appears to be the increase in the proportion of people greater than 65 years of age (Ogurtsova*et al.*, 2015).All nations, rich and poor are suffering the impact of the DM 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 (International Diabetes Federation, 2019).

Globally in 2019, it is estimated that almost 463 million people suffer from DM with a prevalence of 9.3%. The prevalence is higher in urban (10.8%) than in rural (7.2%) areas, and in high income (10.4%) than low income countries (4.0%). An estimated 19.4 million adults aged 20-79 have DM in the African region, representing a regional prevalence of 2.1-6.7%. The Africa region has the highest proportion of undiagnosed DM; over two thirds (66.7%) of people with DM are unaware they have the disease(International Diabetes Federation, 2019).

Some of Africa's most populous countries have the highest numbers of people with DM, including South Africa (4.6 million), Democratic Republic of Congo (1.8 million), Nigeria

(2.7 million) and Ethiopia (1.7 million). Nearly half of all adults with DM in the region live in these four countries (International Diabetes Federation, 2019).

2.1.2Overviewon Glucose Metabolism and Insulin Signalling

Diabetes mellitus is a group of diseases characterised by high fasting blood glucose levels. Glucose is a simple sugar that provides energy to all of the cells (Neeland *et al*., 2012). The cells take glucose from the blood and break it down for energy. Glucose gets absorbed from the intestines and distributed by thebloodstream to keep a constant supply of sugar, by maintaining a constant glucose concentration in the blood (Neeland*et al*., 2012). To maintain a constant blood glucose level, the two antagonist hormones (insulin and glucagon) are produced in the pancreas.

Insulin is an important signalling molecule required by almost all of the cells, but its major targets are liver, fat and muscle cells. When glucose goes too high, the pancreas releases insulin into the blood stream and insulin stimulates the liver to convert the blood glucose into glycogen for storage. If the blood glucose goes too low, the pancreas release glucagon which causes the liver to turn stored glycogen back into glucose and release it into the blood (Evan and Bruce, 2006). Metabolic action of insulin result from its interaction with the insulin receptor (IR) found in all insulin responsive target cells like liver, muscle and adipose tissue (Hu *et al*., 2013).

2.1.3 Classification of Diabetes Mellitus

Assigning a type of DM to an individual often depends on the circumstances present at the time of diagnosis, with individual not necessarily fitting clearly into a single category. For example, some patients cannot be clearly classified as having T1DM or T2DM. Clinical presentation and disease progression may vary considerably in both types of DM. The traditional paradigm of T2DM occurring only in adults and T1DMonly in children are no longer accurate, as both diseases occur in both groups. Occasionally, patients with T2DM

may present with diabetic ketoacidosis (DKA). Children with T1DM typically present with the hallmark symptoms of polyuria/polydipsia and occasionally with DKA.The onset of T1DM 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 (Genuth*et al.,* 2003).

Type 1 diabetes mellitus:This form, previously called "insulindependent diabetes mellitus" or "juvenile-onset diabetes" accounts to 5-10%of diabetes isduetocellular-mediatedautoimmune destruction of the pancreatic β-cells (American Diabetes Association, 2020). Autoimmune markers include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to GAD (GAD65), autoantibodies to the tyrosine phosphatasesIA-2andIA-2b,andautoantibodies to zinc transporter 8 (ZnT8). T1DM is defined by the presence of one or more of these autoimmune markers. The disease has strong HLA associations, with linkage to the DQA and 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 (American Diabetes Association, 2020).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 T1DM, obesity should not preclude the diagnosis. These patients are also prone to other autoimmune disorders such as Graves' disease, Addison's disease, celiac disease, autoimmune hepatitis, and pernicious anemia.

Type 2 diabetes mellitus: This form was previously referred to as "non-insulin-dependent diabetes mellitus" or "adult onset diabetes. T2DM encompasses individuals who have insulin resistance and usually relative (rather than absolute) insulin deficiency (Genuth*et al.,* 2003). Initially, and often throughout their lifetime, these individuals may not need insulin treatment to survive.There are various causes of T2DM. 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 DM. Most, but not all, patients with T2DM 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 (American Diabetes Association, 2009).Ketoacidosis seldom occurs spontaneously in T2DM; when seen, it usually arises in association with the stress of another illness such as infection. T2DM frequently goes undiagnosedformanyyearsbecausehyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice the classic DM symptoms. Nevertheless, such patients are at an increased risk of developing macro vascular and micro vascular complications.Whereas patients with T2DM may have insulin levels that appear normal orelevated, thehigherblood glucose levelsinthesepatientswouldbeexpected to result in even higher insulin value and their β-cell function been normal (Panini, 2013). 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 hyperglycemiabutis rarely restoredtonormal (Droumaguet*et al.*, 2006). The risk of developing T2DM 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 T1DM. However,thegeneticsof T2DM is poorly understood (Droumaguet*et al.,* 2006).

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 (Metzger *et al.,* 2008).The ongoing epidemic of obesity and diabetes has led to more T2DM inwomenofchildbearingage,resultingin an increase in the number of pregnant womenwithundiagnosed T2DM. Because of the number of pregnant women with undiagnosed T2DM, it is reasonable to test women with riskfactorsfor T2DM at their initial prenatal visit, using standard diagnostic criteria. Women with diabetes in the first trimester would be classified as having T2DM. GDM is diabetes diagnosed in the second or third trimester of pregnancy that is not clearly diabetes.

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 (American Diabetes Association, 2020). 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 β-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 TIDM. 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 β-cell KATP channel.Diagnosingthelatterhasimplications, since such children can be well managed with sulfonylurea (International Expert Commission, 2009).

Cystic Fibrosis–Related Diabetes(CFRD):CFRDisthemostcommonin people with cystic fibrosis, occurringin about20% of adolescents' and40–50% of adults. DM 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 DM 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 to 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(Droumaguet*et al.*, 2006).

Genetic defects in insulin action: There are unusual causes of DM 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 DM. Some individuals with these mutations may have acanthosisnigricans. Women may be virilized and have enlarged, cystic ovaries. In the past, this syndrome was termed typeA 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 post receptor signal transduction pathways (Droumaguet*et al.*, 2006).

Diseases of the exocrine pancreas: Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy and pancreatic carcinoma. With the exception of that caused by cancer, damage to the pancreas must be extensive for DM to occur; adrenocarcinomas that involve only a small portion of the pancreas have been associated with DM. This implies a mechanism other than simple reduction in β-cell mass. If extensive enough, cystic fibrosis and hemochromatosis will also damage β-cells and impair insulin secretion. Fibrocalculouspancreatopathy 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 and epinephrine) antagonize insulin action. Excess amounts of these hormones 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 (Droumaguet*et al.,* 2006).

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 DM associated with islet cell antibodies and in certain instances, severe insulin deficiency (Droumaguet*et al.,* 2006).

Viruses and infections: A virus cannot cause diabetes on its own, but people are sometimes diagnosed with T1DM during or after a viral infection, suggesting a link between the two. Also, the onset of TIDM occurs more frequently during the winter when viral infections are more common (Principi *et al*., 2017). Viruses possibly associated with T1DM include coxsackievirusB, 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 T1DM so that a vaccine might be developed to prevent the disease (American Diabetes Association, 2015).

Streptozotocin: it is a nitrosourea analogue in which the N-Methyl-N-nitrosourea (MNU) moiety is linked to the carbon-2 of a hexose. The toxic action of streptozotocin and

chemically related alkylating compounds requires their uptake in to the cells. nitrosoureas are usually lipophilic and tissue uptake trough the plasma membrane is rapid; however as a result of the hexose substitution, streptozotocin is less lipophilic. Streptozotocin is selectively accumulated in the pancreatic beta cells via the low affinity GLUT2 glucose transporter in the plasma membrane (Lenzen, 2008). It is generally assumed that the toxicity of streptozotocin is dependent upon the DNA alkylating activity of its methylnitrosourea moiety (Wilson et al., 1988), especially at the O6 position of the guanine (Lenzen, 2008). The transfer of the methyl group from streptozotocin to the DNA molecule causes damage, result in the fragmentation of the DNA (Lenzen, 2008). Protein glycosylation may be an additional damaging factor (Konrad and Kudlow, 2002). In the attempt to repair DNA, poly (ADP-ribose) polymerase (PARP) is over stimulated. This diminishes cellular NAD+, and subsequently ATP, stores (Sandler and Swenne, 1983). The depletion of the cellular energy stores ultimately results in beta cell necrosis(Lenzen,2007).

Fructose: it is a 6-carbon sugar that is monosaccharide found in fruits and honey. Fructose can be toxic to humans when ingested in large amount. After absorption of fructose, it is transported to the liver where it is effectively absorbed by liver cells. In the liver, fructose can enter metabolic pathways; it can be oxidized, converted to glucose (and glycogen) or converted lactic acid, or enter *de novo lipogenesis* (DNL). Conversion of fructose to glucose thus increase the risk of type 2 diabetes. Numerous studies (Bantle, 2009) have shown increase in insulin resistance upon ingestion of fructose.

Other genetic syndromes associated with diabetes: Many genetic syndromes are accompanied by an increased incidence of DM. 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 DM and the

absence of β-cells at autopsy. Additional manifestations include diabetes insipidus, hypogonadism, optic atrophy and neural deafness (Droumaguet*et al.,* 2006).

2.1.4 Diagnostic Tests for Type 2 Diabetes Mellitus

Diabetes can be diagnosed by the presence of four classic signs that include polyuria, polyphagia, polydipsia and foremost hyperglycemia (Vasudex and Jann, 2011). T2DM is characterized by recurrent or persistent hyperglycemia, and it is diagnosed by demonstrating one of the following tests:

1. Fasting blood glucose test (most common): fasting blood glucose levels are checked after fasting for 12 to 14 hours.
2. Random blood glucose test: blood glucose levels are checked at various times during the day. Blood glucose levels tend to stay constant in a person who does not have diabetes.
3. Oral glucose tolerance test: a high glucose drink is given. Blood samples are checked at regular intervals for two hours.
4. Glycohemoglobin HbA1c: measures how much glucose is stuck to red blood cells. It also shows how well diabetes has been controlled in the last 2 to 3 months and whether diabetes medicine needs to be changed. HbA1c of 6.5% is recommended as the cut point for diagnosing diabetes. A value of less than 6.5% does not exclude diabetes mellitus, diagnosed using glucose tests (World Health Organisation, 2011).

Table 2.1 The standard of values of diagnostic tests in type 2 diabetes mellitus.

Test to diagnosis	Normal (mg/dL)	Pre-Diabetes(mg/dL)	Diabetes(mg/dL)
Fasting blood sugar	70-99	100-125	≥126
Random blood sugar	70-139	140-199	≥200
2 hours glucose tolerance test	70-139	140-199	≥200

2.1.5 Postprandial Hyperglycemia

Diabetes mellitus is characterized by a high incidence of cardiovascular disease (CVD) (Kannel and McGee, 1979) 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 postprandial state is an important contributing factor to the development of atherosclerosis (Bonora and Muggeo, 2001). In diabetes, 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 late diabetes complications are 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 T1DM who were followed for 20 years indicated that A1C was significantly associated with all cause and cardiovascular mortality (Bash *et al.,* 2008).Several studies have demonstrated the

effectiveness of targeting postprandial glucose to decrease the risk of diabetes complications (Lunyera *et al*., 2016: Misra, 2012). The Campanian postprandial hyperglycemia study compared theeffects of repaglinide and glyburide on PPG, carotid intimamedia thickness and markers of systematic vascular inflammation in 175 patients with T2DM. After 12 months, peak PPG was 148 mg/dL in the repaglinide group versus 180 mg/dL in the glyburide group. Regression of carotidintimamedia thickness (a decrease greater than 0.020 nm) was observed in 52% of patients in the repaglinide group versus 18% of those in the glyburide group. Reductions in C-reactiveprotein and IL6 were significantly greater with repaglinide than with glyburide. These results show that targeting postprandial glucose can promote atheroma regression in patients with T2DM (Esposito *et al.*,2004).

2.1.6 Complications of Diabetes Mellitus

Diabetic retinopathy

During hyperglycemia, reduction of glucose to sorbitol by aldose reductase constitutes the first and the rate-limiting step of the polyol pathway that converts glucose to fructose via sorbitol dehydrogenase (SDH). Inthis pathway both NADPH and NAD are consumed as cofactors for the enzymes aldose reductase and sorbitol dehydrogenase (Srivastava *et al.*, 2005). Osmotic stress due to accumulation of sorbitol and oxidative stress due to changes in the ratio of NADPH/NADP and reduced NAD (NADH)/NAD are the major cause of retinopathy complications of secondary diabetes. (Srivastava*et al*., 2005)

Diabetic neuropathy

Diabetic neuropathy, a lifethreatening complication involves both peripheral and autonomic nerves; affecting almost half of the diabetic population (Chawla*et al.,* 2016). The risk of developing of diabetic neuropathy is directly proportional to both the duration and magnitude of hyperglycemia. In addition, some individuals may also possess genetic facets that influence their predisposition in developing such complications (Fowler, 2008). Peripheral

neuropathy in diabetes appears in several forms depending on the site, manifesting as sensory, focal/multifocal, and autonomic neuropathies. Diabetic neuropathy has resulted in more than 80% amputations after foot ulceration or injury (Chawla*et al.,* 2016).

Diabetic nephropathy

The pathogenic mechanisms underlying diabetic nephropathy involve generation of reactive oxygen species (ROS), accumulation of advanced glycation end products (AGE), and activation of intracellular signaling molecules such as protein kinase C (PKC) (Cade, 2008). PKC activation, increased polyol flux, and hexosamine formation are also linked to oxidative stress in promoting macro vascular complications through multiple mechanisms (Chawla*et al.,* 2015).

Vascular Complication

Oxidative stress, caused by the overproduction of ROS plays an important role in the activation of other pathogenic pathways involved in diabetic complications which in turn lead to the development of micro and macro vascular complications (Chawla*et al.,* 2016). It also inactivates two critical anti-atherosclerotic enzymes, endothelial nitric oxide synthase, and prostacyclin synthase (Folli*et al.,* 2011). ROS-mediated cellular damage may be a form of pathologic “memory” in the microvasculature that persists even after glucose normalization.

2.1.7Therapeutic Agents for Treatment Options

There are several anti-hyperglycemicand glucose lowering agents that are used in the treatment of DM. They act differentlyto maintain lowblood glucose. Most of them are orally administered, except for insulin and insulin preparations which are administered parenterally (Warjeet, 2011). Most of these anti-diabetic drugs have been tried on experimental models

like rodents, in which diabetes has been induced by pharmacological, surgical or genetic manipulations to examine anti-diabetic effects (Fröde and Medeiros, 2008). Depending on their mechanism of action, anti-diabetic drugs or agents used in the treatment of DM are classified into several groups such as: insulin and insulin secretagogues, insulin sensitizers, suppressors of hepatic glucose production, alpha glucosidase, aldose reductase inhibitors (Miller *et al.,* 2013)

Insulin and Insulin Secretagogues

Most diabetic patients may eventually develop progressive pancreatic beta-cell failure and impaired insulin secretion, and as such may need insulin to control hyperglycemia (Hamaty, 2011). Insulin as a drug is an injectableanalogue of the normal physiological insulin with similar function of promoting cell uptake of glucose, glucose disposal and storage of glucose as glycogen, thus lowering blood glucose level. Therapeutic insulin is divided into two types depending on the mode of action; long-acting (basal) insulin, which is usually taken at bed time to maintain normal blood glucose and rapid acting (prandial or bolus) insulin which is usually injected after a meal to control postprandial blood glucose rise (Hamaty, 2011). Insulin secretagogues (ISs) are diabetic medications that are used to remedy impaired insulin secretion in T2DM patients. They help the pancreas to produce and secrete insulin for blood glucose homeostasis (Patel *et al*., 2012). Some existing commonly used insulin secretagogues are the sulphonylureas like glyburide, gliclazide and glipizide, which are sometimes used in combination with other hypoglycemic agents. However, it has been reported that this class of ISs are associated with hyperinsulinemia; risk of hypoglycemia; inadequate glycaemic control (in T2DM patients); gradual failure in beta-cell function; and weight gain, while meglitinides like repaglinde, which is another class of ISs and a prandial glucose regulator is believed to have better glycaemic control and lower risk of hypoglycemia than sulphonylureas (Davies, 2002).

Insulin Sensitizers

Insulin resistance and insensitivity in the body, especially in the muscles are major metabolic defects associated withT2DM.Insulin produced cannot adequately induce blood glucose uptake or disposal in these tissues, which contributesto hyperglycemia (Hauner, 2002). Insulin sensitivity improvement drugs, such as thiazolidines (TZDs)and biguanides like metforminwork by improving the insulin insensitivity and insulin dependent glucose uptake in muscles and other tissues, hence improving metabolic control in T2DM patients (Klip and Leiter, 1990; Sirtori and Pasik, 1994;Hauner, 2002). It has been reported that TDZs can reduce serum glucose, insulin and triglyceride level and also increase blood glucose uptake when used to treat T2DM patients (Kahn *et al*.,2000). Hauner (2002) reported that the mechanism of action of TDZs involves a Peroxisome proliferator-activated receptor *gamma* (PPAR-γ)-dependent transcription control of genes involved in glucose and fat metabolism in adipose and muscle tissues, which are promoted via an endocrine signal from adipocytes. Other anti-diabetic drugs belonging to this class include biguanides (e.g. metformin); troglitazone, rosiglitazone and pioglitazone have been hypothesized to have potential therapy against T2DM related complications such ascardiovascular diseases (Hauner, 2002; Charbonnel*et al*., 2004). Despite this, some TDZs like rosiglitazone are believed to be associated with some adverse effects like risk of weight gain, coronary heart disease, heart attack and some other vascular disease (Hussein *et al*., 2004). Metformin treatment has been reported to be associated with vitamin B12deficiency and increased risk of lactic acidosis especially in individuals with renal or CVD (Bailey and Turner, 1996; Liu*et al*., 2006). Studies conducted by Ekström et al., (2012) showed lower risk of CVD and acidosis or serious infection in metformin treated T2D patients than some oral glycaemic drugs and insulin, concluding that the beneficial effects of metformin outweigh the risk of adverse effects.

Suppressors of Hepatic Glucose Production

Hepatic glycogenolysis and gluconeogenesis are two glucagon-dependent glucose production processes that serves as major sources of glucose for body metabolic functions, during fasting and when blood glucose drops. The inability of insulin to suppress these processes and the hormonal and enzymatic factors that promote them is a major etiological factor in the development of hyperglycemia in diabetic patients (Postic*et al*., 2004). These processes, which are cyclic AMP (cAMP) dependent, are believed to be major targets for some classes of anti-diabetic drugs (Miller*et al*., 2013).Glucagon binding to cell membrane receptors causes the activation of adenylatecyclase, and subsequent cAMP production, which activates protein kinase A (PKA). Activated PKA phosphorylates and activates target proteins that signals or up regulates hepatic glucose output (Jiang and Zhang, 2005).Anti-diabetic drugs belonging to this class act by down regulating glycogenolysis and gluconeogenesis, which are major processes that increase hepatic glucose output. Although the molecular mechanism behind the anti-diabetic action of biguanides have been previously suggested to be via the enhancement of glucose disposal and activation of the enzyme AMP-activated protein kinase (AMPK), a cellular glucose uptake inducer; (Goodarzi and Bryer- Ash, 2005) recent studies have reported biguanides to be a suppressor of hepatic glucose production (Viollet*et al*., 2012), and metformin an antagonist of glucagon action (Miller *et al*., 2013).According to Miller *et al*. (2013) metformin treated mice exhibited accumulation of AMP (an adenylatecyclase inhibitor) in the liver cells and increased PKA activity and target protein phosphorylation, indicatingsuppression or blocking of glucagon-dependent hepatic glucose output and hypoglycemic function.

Enzyme (α-glucosidase and α-amylase)Inhibitors

This class of oral glycaemic drugs works by preventing postprandial hyperglycemia (Reuser and Wisselaar, 1994). They delay the digestion of carbohydrates-like starch and sucrose, and

also delay the subsequent absorption of absorbable monosaccharides like glucose resulting from carbohydrate digestion (Bischoff, 1994; Patel *et al*., 2012). It is believed that α-glucosidase inhibitors are saccharides that competitively inhibit carbohydrate hydrolyzing enzyme like α-glucosidase and α-amylase (Horii *et al*., 1986; Bischoff, 1995; Kim*et al*., 1999). Studies have shown that *Acarbose:* is an oral α-glucosidase and α-amylase inhibitor for use in the management of T2DM (Wang *et al.,* 2014). It is chemically known as O-4, 6-dideoxy-4-[[(1S, 4R, 5S, 6S)-4, 5, 6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]-α-D glucopyranosyl - (1→4) – O – α - D-glucopyranosyl - (1→4) - D-glucose. The anti-hyperglycemic action of acarbose results from a competitive, reversible inhibition of pancreatic α-amylase and a membrane bound intestinal α-glucosidase hydrolase enzyme. Acarbose is shown to reduce and slow down the intestinal absorption of glucose, which subsequently minimize the postprandial rise of blood glucose and insulin concentration (Wang *et al.,* 2014).These effects and further beneficial effects of acarbose against nephropathy, neuropathy, retinopathy, endothelial dysfunctions and CVD problems make it a potential therapy for prevention of T2DM complications (Bischoff, 1995; Standl and Schnell, 2012). It was first extracted from the culture broths of actinomycetes and was applied in clinical studies for more than 10 years (Coniff and Krol, 1997; Scheen, 1998). It reversibly inhibits α-glucosidase that exists in the brush-border of the small intestinal mucosa (Clissold and Edwards, 1988).

Sodium Glucose co-transporters 2 inhibitors

Sodium glucose co-transporters 2 (SGLT2) inhibitors represent a new strategy in the treatment of DM. These drugs inhibit glucose re-absorption 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, re-absorption 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 families, which have 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,whichhasninememberswithknown functions, of these, SGLT1 and SGLT2 are primarily responsible for renal glucose re-absorption (Nair and Wilding, 2010).SGLT2playsamajorroleinrenalglucosere-absorptionand accounts for approximately 90% of renal glucose re-absorption.TheevidenceforSGLT2beingamajorpathwayforrenal glucose re-absorption 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 normaloralglucosetolerancetest results (Nair and Wilding, 2010).

AnumberofSGLT2inhibitorsarein various stagesofclinical 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 anti-diabetic 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 anti-diabetic drugs. The novel mechanism of action suggests their potential use in combination with other anti-diabetic agents to exert additive or synergistic effects in lowering glucoselevelsin T2D mellitus. Moreover, these drugs present so many advantages among which is the prevention of blood pressure elevation in hypertension prone patients because of reduced sodium re-absorption thereby causing a decrease in the activity of the renin-angiotensin-aldosterone system.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).

2.2 Medicinal Plants

The use of medicinal plants is as old as human civilization; plants have been used for the treatment or prevention of various human diseases. Throughout history, humans have found that some plants and herbs cannot only enhance the flavor of foods but also to restore health

(Zahmatkesh and Khodashenas, 2013).Medicinal plants and traditional medicines play an important role in the health care system of most developing countries. Many of these plants found in our tropical rain forest areas in Nigeria are associated with some potential medicinal properties and are being exploited for their medicinal values by traditional herbal practitioners in the preparation of herbal medicines in the treatment of various ailments and diseases (Alqasim, 2013).

Medicinal plants play an important role in the management of type 2 diabetes mellitus, especially in developing countries where resources are meager. The treatment of diabetes relies heavily on dietary measures, which includes the use of traditional plants therapies (Dusane and Joshi, 2013). Some of the reports in ethno-botany suggested that about 800 medicinal plants possess anti-diabetic potential and the bioactive compounds such as glycosides, alkaloids, flavonoids (phenols), terpenoids are effective medications both in preclinical and clinical studies (Auddy*et al*., 2003).

Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as anti-diabetic, anti-hyperlipidemia, and renal dysfunction remedies. Despite the presence of known anti-diabetic medicine in the pharmaceutical market, diabetes and related complications continued to be a major medical problem (Campbell *et al*., 2012). Anti-hyperglycemic, anti-dyslipidemia and anti-renal failure effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or the facilitation of metabolites in insulin dependent processes (Amreen*et al*., 2012). There is strong desire to use herbs or plants for treatment, due to less side effects, easier consumption or availability (Joan *et al*., 2012).Today, some pharmaceutical drugs are derived from excellent ingredients in medicinal plants (e.g.galegine and diosgenin). In folk medicine, many natural formulations have the potential to treat many diseases and disorders.

Traditional medicinal plant practitioners of the study area reported that leaves were the dominant plant part used to prepare medications (31.9%), seeds (19%), roots (15.3%), bulb (5.52%), shoot tip (4.29%), stem and stem bark (3.68%), fruits (1.84%), latex of stem, rhizome, flowers, gum of stem and whole plant (1.23%) and others (8.6%). The administration of remedy preparations was mainly through oral (74.8%), dermal (20.3%), nasal (3.7%) and optical (1.2%) (Regassa, 2013).

2.2.1 Lemongrass

Lemongrass*Cymbopogoncitratus* is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root. It has short underground stems with ringed segments, coarse, green slightly leathery leaves in dense clusters (Carlin *et al.,* 1986).

Classification

Kingdom -*plantae*- plants

Subkingdom-*tracheobionta*-vascular plants

Super division-*spermatophyta*-seed plants

Division-*magnoliophyta*-flowering plants

Class – *liliopsida*-monocotyledons

Subclass - *commlinidae*

Order – *cyperales*

Family – *Poacae*- Grass family

Genus – *Cymbopogonspreng.*-Lemon grass

Species – *Cymbopogoncitratus*(DC.)Stapf- lemongrass.

plate 1: Lemongrass Image (Godwin Ihesie, 2017)

Ethnopharmacology of Cymbopogon species

Lemongrass is native to tropical regions such as Africa, Asia, southeast Asia, Australiaand oceanic where it has been used traditionally for medicinal, cosmetic and culinary purposes. Traditional application of *Cymbopogon* genus in different countries shows a high applicability as a common tea, medicinal supplement, insect repellant, insecticide, have been used locally in cosmetics, in flu control, and as anti-inflammatory and analgesic.*Cymbopogon citratus* is ranked as one of the most widely distributed of the genus which is used in every part of the world. In Nigeria, its applications include cures for upset stomach, malaria therapy, insect repellent and as antioxidant (tea)(Fagbemi*et al*.,2007)

2.2.2 Phytochemicals Compounds

Lemongrass contains several important bioactive compounds which are useful in several health issues. Essential oil is one of the important components of lemongrass extracts and its

application includes co-ingredients for perfumes and cosmetics. Its high citral composition has made it important for several chemical syntheses (Negrelle and Gomes, 2007).

Shah *et al* 2011, identified terpenes, alcohols ketones, aldehyde and esters as main compounds in *Cymbopogoncitratus*. The major phytoconstituents are essential oils (that contain citral α, citral β, NerolGeraniol, Citronellal, Terpinolene, Geranyl acetate, Mycerene and TerpinolMethylheptenone.) flavonoids and phenolics compounds, which consist of luteolin, isoorientin 2 -0-rhamnoside, quercetin, kaempferol and apiginin.

Asaola*et al* 2009analyzed the phytochemical constituents of the leaves of *Cymbopogon citratus* using the methods of (Sofowora*et al.,* 1982). It shows that lemongrass contains alkaloids, saponins, tannins, anthraquinones, steroids, phenolics and flavonoids. Each of these phytochemicals is known for various protective and therapeutic effects. For instance, phenols are known to be an erythrocyte membrane modifier, (Adesanya and sofowora, 1983).

2.2.3 Biological Activities of Lemongrass

2.2.3.1 Antioxidant properties of lemongrass

Oxidation isa fundamental process in human cells, tissues and system leading to formation of reactive oxygen species (ROSs) which include hydrogen peroxide (H_2O_2), superoxide anions (O_2) and free radicals (Heo*et al*., 2003). Due to its reactivity, ROSs damage biochemical components like cell membrane, cellular lipids, proteins and DNA (Devasagayam*et al.*,2004). Additionally, ROSs functions as major inducer of several health issues like atherosclerosis, rheumatoid arthritis and muscle destruction. Others are cataracts, certain neurological disorders, cancer and ageing. Antioxidants have to be present in the body to offer protective mechanism against damaging effects of oxidation process caused by these radicals (Finkel, 1998; Thannickal and Fanburg, 2000).

Research have identified antioxidant potentials of lemon grass extracts and documented their abilities to reduce ROSs. Such mechanism include inhibition of lipoperoxidation and decolorizationof 2,2-diphenyl-1-pierylhydrazyl (DPPH) (Sharma and Bhat, 2009; Mirghani*et al*., 2012). Infusions and decoctions prepared from lemon grass showed antioxidant properties by scavenging superoxide anion, inhibiting lipoperoxidation anddecolorizing DPPH. These effects are higher in infusion than decoction (Cheel*et al*.,2005). Similarly, lemon grass infusion exhibited stronger antioxidant activities in relation to other extracts (methanolic, 80% aqueous ethanol and decoction. Further studies revealed that tannins and flavonoids fractionsof oil free infusion extract were most active anti-oxidative agents compared to phenolic acids fraction (Figueirinha*et al*., 2008). Aqueous ethanol extract was reported to exhibit antioxidant properties by decreasing reactive oxygen species production and lipid peroxidation, as well as, increasing superoxide dismutase activity and glutathione formation (Tiwari *et al*., 2010). Recently, essential oil of lemon grass was also reported to show antioxidant property by DPPH scavenging test. The results showed that both leaves and stalk extracts posse's radicals scavenging ability in a dose dependent manner (Mirghani*et al*., 2012).

2.2.3.2 Anti-bacteria potential of lemongrass.

Anti-bacterial activity in extracts of plant materials has been elucidated from various sources in recent times with promising results. This characteristic has also been investigated in the volatile oil portion of the aqueous extract of lemon grass (Grace *et al.*, 1984). Among the major bioactive compounds identified in the oil were α-citral (geranial) and β-citral (neral) components. These components demonstrate their antibacterial activity by inhibiting the growth of both Gram positive and Gram-negative bacteria. However, the third component mycerenepossess no anti-bacterial activity individually but do enhance activity when combined with others (Grace *et al*., 1984)

2.2.3.3 Anti-obesity and anti-hypertensive activity of lemongrass

Several investigations have been carried out on the potentials of lemon grass extract asa source of hypolipidemic and hypoglycemic substances which may lower the risks of hypertension and obesity. Available reports showed that citratus aqueous extracts when fed to rats at 500mg/kg/day led to significant reduction in hypoglycemic index inspite of counter-regulatory factors such as catecholamine, cortisol and glucagon. Hypolipidemic effect was recorded with noticeable reduction in low density lipids levels in the blood stream. The mechanism by which the tea effectively performs these effects remained elusive but several researchers have associated it with increased insulin synthesis and secretion (hyperinsulinemia) or increased peripheral glucose utilization(Adejuwonand Esther, 2007; Olorunnisola et al., 2014)

The presence of anti-hypertensive compounds such as flavanoids and alkaloids has been reported to assist in the hypoglycemic properties exhibited by lemongrass aqueous extract since it contains essential oil and other extractants (Onabanjo*et al*., 1993; Oladele*et al*., 1995). Similarly, lemon grass extracts were efficacious in reducing cholesterol levels in the blood stream. Investigators opined that this could be due to the presence of endogenousligand of central-type benzodiazepine receptors known as endozepineoctadecaneuropeptide (ODP), which are inhibitors of food intake in small animals (Do Rego *et al*., 2007).

2.2.3.4 Cytotoxicity and anti-mutagenicity of lemongrass

Several studies (both in-vivo and in-vitro) have been conducted to investigate cytotoxicity and mutagenicity effects of lemon grass extracts in order to confirm the safety of lemon grass tea. All phenolic compounds isolated from methanolic extract of lemon grass were non toxic to human lung fibroblasts even at high concentration (1 mM) (Cheel*et al*., 2005). In another

study, adult rats subjected to oral consumption of lemon grass tea for two months did not cause any toxicity effect on both the rats andtheir offspring (Lucia *et al*., 1986).

2.2.3.5 Anti-diabetic potentials of lemongrass

Research has identified the anti-diabetic potentials of lemon grass essential oils and lemon grass extracts in both normal and diabetic rats.Bharti *et al*., (2013), reported the blood glucose lowering activities of the essential oil of the lemon grass leaf sheath in poloxamer-407 induced hyperlipidemic model in wistar rats. When compared to the diabetic control rats, the diabetic rats treated with the lemon grass essential oils show a significant amelioration of glycaemia, insulinemia, and lipid metabolism dysfunction. Histopathological analysis of the pancreas has shown an increase in β-cell mass, islet number, and severity insulitus. According to Abbas*et al*., (2018), they stated that administration of extract of root and flower of lemon grass reduced the fasting and postprandial blood sugar levels, bringing them down towards normal in dexamethasone induced hyperglycemia in mice.

In a study performed byModak and Mukhopadhaya (2011),citral-treated rats show a dose-dependent reduction in body weight gain. Treated animals exhibit lower fasting glucose levels. Improved glucose tolerance, lower fasting plasma glucose, higher metabolic rate, and smaller adipocytes after drug administration. These findings suggest that citral increases the energy dissipation and reduces lipid accumulation, consequently preventing and ameliorating diet-induced obesity. Otherbenefitsoflemongrassincludes; relieves menstrual pains,burnsfats, servesasasourceofvitaminsAandC, regulatehighbloodpressure, helpsin constipation andindigestionetc.Researcheshavenotbeenconductedtoprovemostoftheseclaimedbenefits.

CHAPTER THREE

3.0MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals, Reagents and Equipment

Glucometer(on Call Plus^{R}, San Diego, USA), strip, pH meter, desiccator, refrigerator, spectrophotometer.

Chemicals and reagents used were: Streptozotocin (STZ) which was purchased from Sigma Aldrich Company, India.Fructose, glucose, metformin(Ranbaxy Ltd, Gurgaon, India).All other chemicals(formalin, citric acid, sodium hydroxide, sodium citrate) used were of analytical grade.

3.1.2Plant material, Collection and Identification

Fresh sample of lemongrass wascollected at Anguwan Liman Zaria city, Zaria, Kaduna State in the month of April. The plant was identified at the Herbarium,Department of Botany, Ahmadu Bello University and a voucher sample (1882) for future references was deposited at herbarium unit.

3.1.3Experimental Animals

A total of forty-two (42) apparently healthy albino rats of both sex was obtained from Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were kept in a well aerated laboratory cages in the animal house and allowed for one-week acclimatization before the commencement of the experiment. The animals were fed with commercially available diet (Vital Feed, Jos, Nigeria) and water was provided *ad libitum* during the stabilization period.

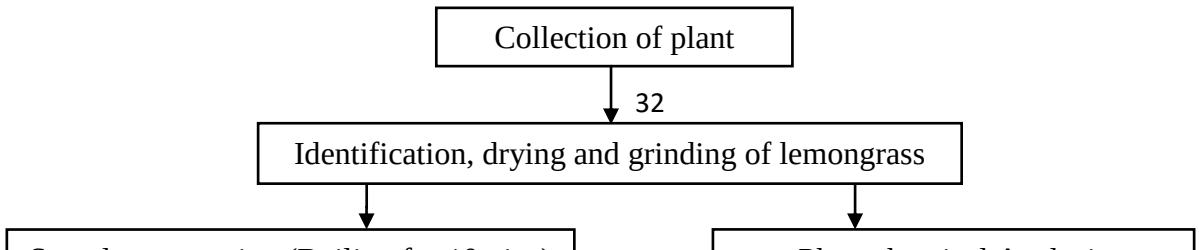

Figure 3.1:Experimental Design for the Study

3.2METHODOLOGY

3.2.1 InductionofT2DM

The T2DM was induced in the animals in DBC, DLTL, DLTH and DMFgroups by feeding 10% fructose solution in the first two (2) weeks for induction of insulin resistance followed

by intraperitoneal injection of STZ (40mg/kg b.w) in citrate buffer (pH 4.5) in order to induce partial pancreatic β-cell dysfunction while the animals in NC and NLTH group were fed with normal drinking water and injected with citrate buffer, respectively (Wilson and Islam, 2012). Fasting Blood glucose (FBG) level was measured seven (7) days after STZ injection using a portable glucometer (On Call Plus^{R}) and animals with blood glucose level ≥ 200 mg/dl were considered diabetic.

3.2.2 Tea Preparation and Intervention (Islam, 2011)

The fresh lemongrass leaveswere rinsed in a clean water to remove all contaminants, shade dried at the Department of Biochemistry Ahmad Bello University Zaria, grinded to powder and was constituted in the following concentrations: 0.25 g/100ml/kg and 0.5 g/100ml/kg by boiling for 10 minutes in water, cooled to room temperature at 25^{0}C and administered*ad libitum*to the respective groups during the four (4) weeks intervention period, starting from one week after STZ injection.

3.2.3. Quantitative phytochemical analysis

Possible phytochemicals present *in Cymbopogon citratus*tea was determined using standard methods with slight modifications in all the methods.

3.2. 3.1 Alkaloids determination using Harborne (1998) method

Harbone method of Alkaloids determination was carried out. Five (5) g of *Cymbopogon citratus powdered leaves* were weighed each for both cold water extraction and for the hot tea and 500ml of distilled water was added in each of the containers, for the hot tea the mixture was boiled for 10minutes while for the cold water extraction the mixture was soaked for 24 hours, cooled and then filtered. The filtrate of each of the mixture was put in a water bath to evaporate to dryness and the residue were collected. 200ml of 10% acetic acid in ethanol was added to each of the residue obtained, covered and was allowed to stand for four (4) hours

each. The extracts were concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to each of the extract until the precipitation was complete. The whole solutions were allowed to settle and the precipitate were collected and washed with dilute Ammonium hydroxide and then filtered. The residue was the alkaloids, which was dried and weighed.

3.2.3.2 Flavonoids determination by the method of Bohm and Koupai-Abyazani (1994)

The method of Bohm and Koupai was adopted in the determination of Flavonoids. Ten (10) g of *Cymbopogon citratus*powdered leaves were weighed each for both cold water extraction and for the hot tea. 500ml of distilled water was added in each of the containers, for the hot tea the mixture was boiled for 10minutes while for the cold water extraction the mixture was soaked for 24 hours, cooled and then filtered. The filtrate of each of the mixture was put in a water bath to evaporate to dryness and the residue were collected. The residue of each of the mixture was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125mm). The filtrate was transferred into crucible and evaporated into dryness over a water bath and weighed to a constant weight.

3.2.3.3Saponins determination:

The method of Obadoni and Ochuko (2001) was used. 10g of the *Cymbopogon citratus*powdered leaves was weighed each for both cold water extraction and for the hot tea. 500ml of distilled water was added in each of the containers, for the hot tea the mixture was boiled for 10minutes while for the cold water extraction the mixture was soaked for 24 hours, cooled and then filtered. The filtrate of each of the mixture was put in a water bath to evaporate to dryness and the residue were collected. The residue of each of the mixture obtained was put into a conical flask and 100ml of 20% aqueous ethanol was added. The sample was heated over a hot water bath for four (4) hours with continuous stirring at about

55^0C. The mixture was filtered and the residue re-extracted withanother 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^0C, the concentrate was transferred into 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was covered while the ether layer was discarded. The purification process was repeated and 60ml of n-butanol was added. The combined n-butanol extract was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples was dried in the oven to a constant weight; the saponin content was calculated as percentage.

3.2.3.4 Tannin determination by Van-Buren and Robinson (1981) method

The method of Van-Buren and Robinson was adopted in determining tannin concentration. Five hundred (500) mg of *Cymbopogon citratus*powdered leaves were weighed each for both cold water extraction and for the hot tea and 100 ml of distilled water was added in each of the containers, for the hot tea the mixture was boiled for 10minutes while for the cold water extraction the mixture was soaked for 24 hours, cooled and then filtered. The filtrate of each of the mixture was put in a water bath to evaporate to dryness and the residue were collected. 50ml of distilled water was added to each of the residue obtained and shaken for one (1) hour on a mechanical shaker. It was then filtered into a 50ml volumetric flask and made up to a mark. Then 5ml of the filtered was pipetted out into a test tube and mixed with 2ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120nm within 10 minutes.

3.2.3.5 Determination of total phenolics by spectrophotometric method (Chang *et al.*, 2002)

The concentration of total phenolics content of the lemongrass tea was determined according to the method described by Chang *et al* (2002). 5g *Cymbopogon citratus*powdered leaves were weighed each for both cold water extraction and for the hot tea and 100 ml of distilled

water was added in each of the containers, for the hot tea the mixture was boiled for 10minutes while for the cold water extraction the mixture was soaked for 24 hours, cooled and then filtered. The filtrate of each of the mixture was put in a water bath to evaporate to dryness and the residue were collected. The residue of each sample were boiled in 50ml of ether for 15mins and then 5ml of the extracts were pipetted into 50ml flask. 2ml of ammonium hydroxide solution and 5ml of amyl alcohol were also added to the samples and make up to the mark. It was left to react for 30mins for colour development. The absorbance was measured at 550nm. Gallic acid was used for calibration of a standard curve. The results are expressed as mg gallic acid equivalent (mgGAE)/g dry weight of the plant tissue.

3.2.4. Body Weight, Fluid and Feed Intake.

The body weight of all the animals in each group was measured weekly using weighing balance. A respective concentration of lemongrass tea was administered using feeder bottles*ad libitum*to the respective groupsof animals (DLTL, DLTH and NLTH) which serves as their drinking waterand the fluid intake was calculated by subtracting the remaining ml in the bottle from the original volume, this was done daily during the four (4) weeks intervention period, started after the confirmation of diabetes. At the same time the animals in the NC, DMF and DBC groups were given normal drinking water instead of the lemongrass tea using the same method. Daily feed intake was also calculated by subtracting the remaining feed from the original quantity given to the rats for each group during the experimental period.

3.2.5. Blood Glucose Level

The blood concentration of each animal was measured after an overnight fasting. A small drop of blood from each rat was obtained by pricking the rat tails with a lancet and placed on a disposable test strip which the glucometer(ON CALL Plus $^{R)}$ reads and calculated the blood

glucose concentration in mg/dL. The blood glucose level of each animal was recorded weekly.

3.2.6 Oral glucose tolerance test (OGTT)

In the last week of intervention period, an OGTT was performed in order to measure the glucose tolerance ability of each animal. The animals were fasted overnight and a single dose of glucose solution (2.0 g/kg BW) was orally administered to each animal and the subsequent blood glucose concentration was measured in the blood collected from the tail veins at 0 min (just before glucose injection), 30, 60, 90 and 120 minutes after the glucose ingestion.

3.2.7. Determination of Serum Insulin

Principle of the assay

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

3.2.8 Insulin Resistance and Pancreatic β-cell Function Indices

Homeostatic Model Assessment (**HOMA**) was used. It is a method used to quantify β-cell function and insulin resistance (**IR**) from basal (fasting) glucose and insulin or C-peptide concentrations.

$$\text{HOMA-IR} = \frac{\text{Glucose x Insulin}}{405}$$

$$\text{HOMA-}\beta = \frac{360 \text{ x Insulin}}{}$$

Glucose – 63

Glucose in mass unit mg/dL.

The formula used was first described under the name HOMA by Matthews et al. (1985).

3.2.9. Biochemical Test

At the end of the experimental period, animals were fasted for 14hours and euthanized by anaesthesia, blood, pancreas and liver samples were thereafter collected. The blood of each animal was collected via cardiac puncture and it was preserved immediately in a refrigerator until required for use. The blood sample was centrifuged at 3000 rpm for 15 minutes and serum from each blood sample was separated and preserved infreezer at 4^0C for further analysis of various biochemical parameters. TC, TG, HDL, total protein, albumin, creatininewas estimated using chemistry analyser, while LDL level was calculated using Friedewald*et al*.(1972). The liverwas collected from each animal, washed with normal saline, wipedwith filter paper, weighed and preserved until subsequent analysis for the determination of liver glycogen content.

3.2.9.1.Serum lipid profile (Total Cholesterol, Triglycerides, High Density Lipoprotein and Low-Density Lipoprotein).

3.2.9.1.1Triglyceride Level

Triglyceride (TG) level was estimated by the enzymatic colorimetric method (Werner *et al*., 1981).TG was measured using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol and free fatty acids by the enzyme lipase. The glycerol formed isphosphorylated to glycerol-3-phosphate by glycerol kinase. The glycerol-3-phophate was oxidized by glycerol phosphate oxidase producing dihydroxyacetone phosphate and hydrogen peroxide. Then, Peroxidase catalyzes the redox-coupled reactions of $H_2O_2$with 4-aminoantipyrine (4-AAP), producing a bright purple color. The absorbance is measured at 540 nm. The reaction sequence is as follows:

Triglycerides + $3H_2O$ $\xrightarrow{\text{Lipase}}$ glycerol + fatty acid

Glycerol + ATP $\xrightarrow{\text{Glycerol kinase}}$ glycerol-3-phosphate + ADP

Glycerol-3-phosphate+O_2 $\xrightarrow{\text{Glycerol phosphate oxidase}}$dihydroxyacetonephosphate+H_2O_2

H_2O_2 + 4-aminoantipyrine $\xrightarrow{\text{Peroxidase}}$ 4-(p-benzoquinone-monoimino)-phenazone+ $2H_2O$ + HCl.

Enzymatic assay was done at 540nm wavelength, 1cm optical path, 37^0C temperature and measurement was done against reagent blank. The sample used for this study was the serum of the experimentalrat while the reagents are standard and ready for use on automated analyzer. Triglycerides Reagents: the components of the Triglycerides (GPO) system pack include (from package insert): 50mmol/L PIPES buffer, pH 6.8, 40mmol/L Mg^{++}, 0.20 mmol/L Sodium cholate, ≥1.4mmol/L ATP, ≥0.13 mmol/L 4-Aminophenazone, 4.7mmol/L 4-Chlorophenol, 1µmol/L Potassium hexacyanoferrate (ıı), 0.65% Fatty alcohol polyglycolether, ≥ 5.0 U/mL lipoprotein lipase (EC 3.1.1.13; Pseudomonas species, 25^oC), ≥0.19 U/mL glycerolkinase (EC2.7.1.30; Bacillus stearotheraphilus; 25^oC), ≥2.5 U/mL glycerophosphate oxidase (EC 1.1.3.21; E. coli; 25^oC), ≥0.10 U/mL peroxidase (EC1.11.17; horse radish; 25^oC), the reagent was supplied as a solution and ready for use.

Procedure: Samples, standard and blank were preincubated for 5 minutes at 37 °C. Reagent blank (1000 µL reagent + 10µL distilled water) and samples (10 µL sample + 1000µL reagent) or standard (10 µL standard + 1000µL reagent) were pipetted into cuvette and mixed gently by inversion. The cuvettes were inserted into the cell holder and stopwatch was started to count. The absorbance of sample, standard and blank was measure at 540 nm. Finally, the absorbance of the sample (ΔA sample) and the standard (ΔA standard) against the reagent blank were calculated.

$$\text{TG level concentration (mg/dL)} = \frac{\Delta A_{sample} \times C_{standard}}{\Delta A_{standard}}$$

3.2.9.1.2 Total Cholesterol

Cholesterol was determined by enzymatic colorimetric method (Allain*et al.*,1974). Cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase. The free cholesterol produced was oxidized by cholesterol oxidase to cholest -4-ene-3-one with the simultaneousproduction of hydrogen peroxide, which oxidatively couples with 4-aminophenazone and phenol in the presence of peroxidase to yield a chromogen. The absorbance was measured at 400 nm. The reaction sequence is as follow:

Cholesteryl ester + H2O$\xrightarrow{\text{Cholesteryl Ester Hydrolase}}$Cholesterol + Fatty acid

Cholesterol + O2$\xrightarrow{\text{Cholesterol oxidase}}$Cholest-4-en-3-one + H_2O_2

$2H_2O_2$+ 4-aminophenazone + Phenol$\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $4H_2O$

Enzymatic assay was adjusted at 400 nm wavelength, 1 cm optical path, 37^{o}C temperature and measurement was done against reagent blank. Cholesterol reagents: The components of Cholesterol High Performance System Pack Reagents included (taken from package insert): Cholesterol Reagent (16 X 50 mL), 75mmol/L PIPES buffer, pH 6.8, 10mmol/L Mg^{2+}, 0.20 mmol/L Sodium cholate, 0.15mmol/L 4-Aminophenazone, ≥4.2mmol/L phenol, 1% Fatty alcohol-polyglycol ether, ≥ 0.5 U/mL Cholesterol esterase (EC 3.1.1.13; Pseudomonas species, 25^{o}C), ≥0.15 U/mL Cholesterol oxidase (EC 1.1.3 6; E. coli; 25^{o}C), ≥0.25 U/mL peroxidase (EC1.11.17; horse radish; 25^{o}C), the reagent was supplied as a solution and ready for use.

Procedure: Samples, standard and reagent blank were pre-incubated for 5 minutes at 37 °C. Samples (10 µL sample + 1000µL reagent) or standard (10 µL + 1000µL reagent) and reagent blank (1000 µL + 10µL distilled water) were pipetted into cuvette and mixed thoroughly by inversion. The cuvettes were inserted into the cell holder and stopwatch was started to count. The absorbance of sample, standard and the reagent blank were measured at

400 nm within 60 minutes. Finally the absorbance of thesample (ΔA sample) and the standard (ΔA standard) against the reagent blank were calculated.

$$\text{TC level concentration (mg/dL)} = \frac{\Delta A_{sample} \times C_{standard}}{\Delta A_{standard}}$$

3.2.9.1.3 High Density Lipoprotein

The VLDL and LDL from serum are precipitated by phosphotungstate in the presence of magnesium ions. After removal by centrifugation, the clear supernatant containing high density lipoproteins (HDL)-fraction and their cholesterol content was determined enzymatically.

The reactions are as follows:

Cholesteryl ester + H_2O $\xrightarrow{\text{Cholesteryl Ester Hydrolase}}$ Cholesterol + Fatty acid

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol oxidase}}$ Cholest-4-en-3-one + H_2O_2

$2H_2O_2$+ 4-aminophenazone + Phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $4H_2O$

Enzymatic assay was done at 593 nm wavelength, 1 cm optical path, 37 °C temperature and measurement was done against reagent blank. The HDL-Cholesterol reagent components are: ≥ 1 kU/l PEG cholesterol esterase (EC 3.1.1.13; Pseudomonas species, 25°C), ≥5.6 kU/l PEG cholesterol oxidase (EC 1.1.3.6; Pseudomonas species, 25°C), ≥30 kU/l peroxidase (EC1.11.17; horse radish; 25°C), 0.5g/l 4-aminophenazone, 10mmol/l MOPS (3-morpholino-propane sulfonic acid) buffer, pH 7.0.

Procedure for precipitation: 100 µL of reagent and 10µL of sampleswere pipetted into centrifuge tube, mixed well, allowed to stand for 5 minutes at 37 °C and centrifuged at 3000 rpm for 20 minutes. The supernatant (sample) was collected for HDL test.

Procedure for determination of HDL: Reagent blank, samples and calibratorwere preincubated for 5 minutes at 37 °C. Reagent blank (10 µL distilled water + 750 µLreagent andsamples (10 µL samples + 750 µL reagent) or calibrator (10 µL calibrator + 750 µL

reagent) were pipetted into cuvette and mixed gently by inversion. The cuvettes were inserted into the cell holder and stopwatch was started to count. The absorbance of sample, standard and the reagent blank were measured at 593 nm after 5 minutes. Finally, the absorbance of the samples (ΔA sample) and the calibrator (ΔA standard) against the reagent blank were calculated.

HDL concentration (mg/dL) $= \frac{\Delta A_{sample} \times C_{calibrator}}{\Delta A_{calibrator}}$

3.2.9.1.4 Low Density Lipoprotein

LDL cholesterol was calculated frommeasuredvaluesoftotalcholesterol, triglyceridesand HDL by use of the empirical Freidewald Formula equation (Tremblay, *et al.*, 2004):

Concentration of LDL –cholesterol (ml/dl)= [Total cholesterol]-$\frac{[\text{Triglyceride}]}{5}$ - [HDL]

3.2.9.2. Serum Creatinine

Colorimetric estimation of serum creatinine was done by using the alkaline picrate method via Jaffe's Method (Peake and Whiting, 2006). Creatinine in an alkaline medium forms a colored complex with picric acid. The formation rate of thecomplex measured calorimetricallythrough the increase of the absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample.

Creatinine+ Picric acid $\xrightleftharpoons{NaOH}$ Creatinine picrate

Yellow (alkaline medium) (Orange)

This enzymatic assay was done at 400nm wavelength, 1cm optical path, 37^{o}C temperature and measurement done against air (increasing absorbance). The creatinine working reagent was formed by combining equal volume of creatinine buffer reagent and creatinine picrate reagent in the creatinine reagent kit.

Procedure: creatinine reagent, sample and standard were pre-incubated at 37°C. The spectrophotometer was adjusted to zero absorbance with air. Working reagent (10µl reagent

+10µl distilled water) and sample (10µl sample + 10µl reagent) or standard (10µl standard + 10µl reagent) were pipetted into cuvette and mixed gently. The cuvettes were put into the cell holder and stopwatch started to count. The absorbance was recorded at 400 nm after 30 seconds (A1) and after 90 seconds (A2) of the sample or standard addition.

Concentration of Creatinine (mg/dL) =(A2- A1)Sample / (A2- A1)Standardx Con. of Standard(mg/dL)

3.2.9.3 Serum Total Protein (Bradford, 1976).

In alkaline solution, peptide bonds bind with Cu^{2+}ions to form a blue violet colored complex. This complex is formed between the Cu^{2+}ion, the carbonyl oxygen and amide hydrogen atoms. Each Cu^{2+}ion is combined to six peptide bonds. The intensity of the color is proportional to the reacting number of peptide bonds, and therefore to the amount of protein present in the medium, in which the absorbance is measured at 546nm. The reaction is described as follows:

Cu^{2+} + Protein $\rightleftharpoons$ Cu-Protein Complex
37^0C (Blue violet color)

The assay was done at 546nm wavelength, 1cm optical path, 37^oC temperature and measurement was done against the blank. The biuret reagents components are Sodium Hydroxide, Copper(ıı) Sulfate and Potassium Sodium tartrate.

Procedure: Samples (1000µL biuret reagent + 10µL sample), calibrator (1000 µL biuret reagent + 10µL calibrator) and blank reagent (1000 µL biuret reagent + 10 µL distilled water) were put into cuvettes and mixed at 37 °C by inversion. Then, the cuvettes were inserted into the cell holder and stopwatch was started to count. The absorbance of both the sample and the reagent blank were measured at 546 nm after 10 minutes. Finally, the absorbance of the samples (ΔA sample) and the calibrator (ΔA calibrator) were calculated against the blank reagent.

Concentration of total protein(mg/dL) =$((A_{sample}- A_{blank})/(A_{calibrator}- A_{blank}))$x Concentration of Calibrator(mg/dL)

3.2.9.4. Assay for Serum Albumin Concentration

This assay was carried out using commercial test kits according to the method described by Doumas*et al.* (1997).

Principle: Sample albumin binds to an indicator, 3,3',5,5'-tetrabrom-cresol sulphonapthalein (bromcresol green, BCG). The intensity of the formed albumin-BCG complex is directly proportional to the concentration of albumin.

Albumin + BCG → albumin-BCG complex.

Procedure: This assay was carried out using commercial test kits and the procedure was as follows: Using a micropipette, 10 µL of appropriate sample (serum), standard and blank was pipetted into clean test tubes labeled sample, standard and blank respectively. 1000 µL of working reagent comprising of succinate buffer (pH 4.20, 75 mmol/L) and bromcresol green (0.14 g/L) was pipetted into all the test tubes, including another test tube labelled as blank. The reaction mixture was mixed and incubated for 1 minute. The absorbance of the standard and sample (serum) was measured against the reagent blank at wavelength of 630 nm. The concentration of albumin was calculated by using the formula below:

$$\text{Albumin Concentration (g/dL)} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times 3$$

3.2.9.5. Assay of Aspartate and Alanine Aminotransferases Activity

This assay was carried out using the method described by Reitman and Frankel(1957).

Principle:

1. α-oxoglutarate + L-aspartate $\xrightarrow{\text{AST}}$ L-glutamate + Oxoloacetate

   AST is measured by monitoring the concentration of oxaloacetate hydrazoneformed with 2,4-dinitrophenylhyrazine and measured at 546 nm.

2. α-oxoglutarate + L-alanine $\xrightarrow{\text{ALT}}$ L-glutamate + pyruvate

ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhyrazine and measured at 546 nm.

Procedure: Exactly 0.5 ml of test reagent 1 was pipetted into test tubes labeled as sample test and blank test. Then, 0.1 ml of sample (serum) was pipetted and added to the content in the tube labeled as sample test whereas 0.1 ml of distilled water was added to the blank test. The mixture was mixed by inversion and then incubated at 37^{o}C for 30 minutes. Then,0.5 ml of reagent II was pipetted and added to the content in both tubes followed by the addition of 0.1 ml of distilled water to the content in the tube labeled as sample test whereas 0.1 ml of sample (serum) was added to the blank test. The mixture was mixed by inversion and then incubated at 25^{o}C for 20 minutes. Then,5.0 ml of NaOH solution was added to the content in both tubes mixed and allow to stand for 5 minutes then the absorbance of test was read against the blank at 546 nm.

Calculation: AST and ALT activity are extrapolated from the standard curve.

NB: AST Reagent 1 contained phosphate, L-aspartate and alphaoxoglutarate (100mmol/L). ALT Reagent 1 contained phosphate, L-alanine and α-oxoglutarate (100 mmol/L), Reagent 2 contained 2,4-dinitrophenylhyrazine (2 mmol/L).

3.2.9.6. Assay for Alkaline Phosphatase (ALP, EC 3.1.3.1) Activity

This assay was carried out using commercial kit according to the method of Schlebusch*et al.* (1974).

Principle: In the presence of magnesium and waterion in form of magnesium ion, alkaline phosphate convert ρ-nitrophenylphosphate to inorganic phosphate and ρ-nitrophenol, which are yellow in colour. The activity of the enzyme corresponds to the intensity of yellow colourformed which is then measured spectrophotometrically.

$$\rho\text{-nitrophenylphosphate} + H_2O \xrightarrow{\text{Alkaline phosphatase}} \text{phosphate} + \rho\text{-nitrophenol (yellow)}$$

Procedure: Alkaline phosphatase assay was carried out according to the following procedures: Exactly 1000 µL of working reagent 1 (125 mmol/L of diethanolamine buffer pH 10.2, 0.625 mmol/L of $MgCl_2$, p-nitro phenyl phosphate) was added to 20 µL of sample (serum). The reaction mixtures were incubated for 1 minute at 37^{o}C. Change in absorbance per minutes was measured for 3 minutes. The absorbance was obtained at 405 nm spectrophotometrically. ALP activity was estimated by using the equation below:

$$\text{Alkaline phosphatase activity (U/L)} = \frac{\text{abs2} - \text{abs1}}{\text{minutes}} \times 2750$$

Where abs = absorbance of the reaction mixture.

3.2.9.7. Determination of Hepatic Glycogen Content

The isolation and hydrolysis of glycogen in the liver tissues was done by the method described by Lo *et al*., (1970).

Principle: When tissue is heated with strong alkali, glycogen is released which precipitates upon addition of ethanol. Glycogen is then hydrolyzed in acid to release glucose.

Isolation of glycogen: 1.0g of Liver tissue was placed in 4.0 ml of KOH (30 %)then heated in boiling water for 10 min. After cooling, 0.2 ml of Na_2SO_4(20 %) and 5.0 ml of ethanol (95%) were added and kept at 20^{o}C for 5 min. After precipitation was completed, the mixture was centrifuged at 3000 x g for 10 min and decanted. The packed glycogen in the tube was dissolved by addition of 5.0 ml distilled water with gentle warming.

Hydrolysis and estimation of glycogen: Exactly 10 ml of HCl (1.2 mol/L) was added to 10 ml of the sample in test tubes then neutralized by the addition of 2 drops of 0.5 mol/L NaOH and a drop of phenol red as indicator and then cooled.Glucose in the sample was measured by the method described by kasetti *et al*., 2010. A reagent blank was prepared by pipetting 1.0 ml of distilled water into a clean test tube. Exact 1.0 ml of sample and

standard glucose solution (0.5 mg/ml of glucose), was pipetted into a similar tube. Then, 5.0 ml of anthrone reagent was delivered into each tube and tightly capped. The test tubes were placed in a cold-water bath. After all tubes have reached the temperature of the cold water, they were immersed in a boiling water bath to a depth a little above the level of the liquid in the tubes for 15 minutes and were then removed and placed in a cold-water bath and cooled to room temperature. Absorbance of test and standard were read against the reagent blank at 620 nm.

Calculation:

$$\text{Glycogen (mg/g liver tissue)} = \text{ODu} \times \frac{0.5}{\text{ODs}} \times \frac{\text{Vol. of sample}}{\text{g of the tissue}} \times 100 \times 0.9,$$

where,

OD_U = optical density of the unknown, OD_S = optical density of the standard, 0.5 = g of glucose in 0.5 ml of standard solution, 0.9 = factor for converting glucose value to glycogen value. Anthronereagent (contained 0.05 % anthrone, 1 %thiourea, and 72 % H_2S0_4acid).

3.2.10 Histopathological study

A small piece of the pancreatic tissue from each animal was cut and placed in a 10% formalin solution and preserved at room temperature for histopathological study. The formalin solutionof each pancreatic tissue wasreplaced weekly during the entire preservation period. After the preservation period, sections were cut laterally at sizes of 3µm. The slides were deparaffinised in p-xylene and rehydrated in varyingpercentages of ethanol and subsequentlyrinsed in water. The sections were stained with hematoxylin and eosin (H&E). All slides were examined using light microscopy.

3.2.11.Data Analysis

Data obtained in this study were analysed using statistical software package (SPSS version 22) using one-way ANOVA followed by *post–hoc* test. The values were considered significant at $p \leq 0.05$. Data are presented mean ±SD

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Composition of Lemongrass Tea and Lemongrass Cold-Water Extract

Thequantitative phytochemical analysis of the lemongrass tea and cold-water extract ispresented in Table 4.1. The result revealed that the lemongrass tea had higher amount of alkaloids, flavonoids, tannins and saponins though insignificant ($p \geq 0.05$) compared to the cold water-extract. However, the amount of total phenolics in lemongrass tea was significantly ($p \leq 0.05$) higher than cold water-extract (Table 4.1).

Table 4.1 Phytochemical Compositionof Lemongrass Tea and Lemongrass Cold-Water Extract

Phytochemicals	**Lemongrass tea**	**Lemongrass cold-water extract**
Alkaloids (mg/g)	38.00±2.65[a]	32.00±1.73[a]
Flavonoids (mg/g)	68.00±7.55[a]	58.05±1.64[a]
Saponins (mg/g)	22.00±2.65[a]	24.00±5.29[a]
Tannins (mg/g)	69.00±4.58[a]	51.00±2.00[a]
Totalphenolics (mg/g)	111.00±5.57[a]	88.00±3.61[b]

Data are presented as mean±SD of triplicate determinations. Values with different superscript across the rows for a given parameter are significantly different from each other.

4.2. Body Weight of Animal Groups DuringFour Weeks Experimental Period

The body weightsof all the animals throughout the study period are presented in Figure 4.1. The result indicated that there was significant ($p \leq 0.05$) decrease in the body weight of the fructose treated animals compared to NC although not significantly ($p \geq 0.05$) different with the NLTH group (Table 4.2). Similarly, STZ injection further decreased the body weights of diabetic groups compared to the non-diabetic groups. Despite 4-weeks treatment with lemongrass tea to diabetic animals, the increase in the body weight observed was not significant ($p \geq 0.05$) compared to diabetic untreated group (Table 4.2).

Table 4.2 Body Weight (g/wk) of Animal Groups duringFour Weeks Experimental Period.

	NC	DBC	DLTL	DLTH	DMF	NLTH
WK-2	225±18.5[a]	217±66.4[a]	205±62.6[a]	194±27.1[a]	215±23.8[a]	211±17[a]
WK-1	246±27[b]	198±55.6[a]	214±56.2[a]	190±25.1[a]	200±32.3[a]	219±23.9[a,b]
WK 0	250±24.3[b]	189±46.1[a]	200±59.8[a]	185±23.6[a]	194±28.9[a]	225±31.5[a,b]
WK 1	254±22.3[b]	183±48.7[a]	189±21.9[a]	182±20.5[a]	196±18.1[a]	229±28[a,b]
WK 2	258±23.7[b]	179±41.4[a]	179±23[a]	197±26.8[a]	192±34.5[a]	257±41[b]
WK 3	261±21.6[b]	176±40.7[a]	171±34.5[a]	196±25.6[a]	193±32.7[a]	258±38.8[b]
WK 4	255±25.4[b]	170±35.2[aa]	187±46.2[a]	196±29.4[a]	190±37.8[a]	246±33.3[b]

Data are shown as mean ± SD. Values with different superscript letters for a given week are significantly different from each other. NC- Normal control; DBC- Diabetic control; DLTL- Diabetic lemongrass tea low; DLTH- Diabetic lemongrass tea high; DMF- Diabetic metformin; NLTH- Normal lemongrass tea high

4.3 Fluid Intake in Animal Groups During Four Weeks Experimental Period

The results of the daily fluid intake of different animal groups are presented in table 4.3. The fructose administered group had significant($p \leq 0.05$) increase in fluid intake when compared to the normal groups. Also, after STZ administration the DB groups had significantly ($p \leq 0.05$) further higher mean fluid intake when compared to the NC group (Table 4.3). Treatment with lemongrass tea and drug insignificant ($p \geq 0.05$) decreased the fluid intake of the diabetic treated groups in the last week of experiment. Also significant ($p \leq 0.05$) increase in the fluid intake in the NLTH group was observed when compared to the NC group.

Table 4.3 Fluid Intake (ml/kg/day) in Animal Groups During the Four Weeks of Experimental Period

	NC	DBC	DLTL	DLTH	DMF	NLTH
WK-2	227±93[a]	310±140[b]	234±77[a]	234±134.1[a]	245±79.5[a]	243±94.2[a]
WK-1	326±120.5[a]	457±61.7[c]	333±110.2[a]	410±71.4[b]	386±102.7[b]	324±44.8[a]
WK0	206±54.8[a]	433±106.9[c]	443±40.7[c]	447±53.5[c]	468±16.1[c]	278±36.9[b]
WK1	180±66.7[a]	435±49[c]	404±85[c]	421±79.1[c]	410±49.7[c]	322±113.2[b]
WK2	271±81.9[a]	422±16.5[c]	444±53.6[c]	419±38.3[c]	423±60.8[c]	359±40.3[b]
WK3	342±70.1[a]	431±25.3[b]	422±63.4[b]	434±43.2[b]	415±63[b]	420±56.5[b]
WK4	320±22.4[a]	455±38.8[b]	430±39.8[b]	436±15.9[b]	425±41.1[b]	340±25.5[a]

Data are shown as mean ± SD. Values with different superscripts for a given week are significantly different from each other. NC- Normal control; DBC- Diabetic control; DLTL- Diabetic lemongrass tea low; DLTH- Diabetic lemongrass tea high; DMF- Diabetic metformin; NLTH- Normal lemongrass tea high

4.4Feed Intake in Animal Groups during Four Weeks Experimental Period

The results of the daily feed intake in different animal groups are presented in table 4.4. There was significantly higher feed intake in the fructose administered groups in the first week of fructose administration when compared to the normal groups. However, a decrease of feed intake was observed in the second week of fructose administration to the administered groups when compared to NC group except for DLTL group.Similarly, after STZ injection, a significant ($p \leq 0.05$) decrease in the feed intake of the diabetic groups was observed except for DLTL as shown in table 4.4. However, treatment with lemongrass tea significantly ($P \leq 0.05$) increased the feed intake of the diabetic treated groups when compared to the DBC group. Lemongrass treated groups had better increase in feed intake when compared to metformin treated group.

Table 4.4 Feed Intake (g/kg/day) in Animal Groups during Four Weeks Experimental Period

	NC	DBC	DLTL	DLTH	DMF	NLTH
WK-2	109±31.9[a]	132±54[a]	124±46[a]	123±43[a]	128±66[a]	106±29[a]
WK-1	138±26.6[a]	187±60.2[b]	179±72[b]	185±52.8[b]	184±53.4[b]	148±66[a]
WK0	152±12.2[b]	114±22.6[a]	150±42[b]	92±8.5[a]	94±35.9[a]	95±26[a]
WK1	134±24.3[b]	109±22.2[a]	120±13.4[a]	132±13.3[b]	122±24.5[a]	119±27.7[a]
WK2	142±51.4[b]	101±29.9[a]	111±20.5[a,b]	115±26.1[a,b]	116±47.8[b]	129±19.6[b]
WK3	147±27.6[b]	95±22.7a	117±18.3[a,b]	126±24.7[b]	117±29.3[a,b]	132±42[b]
WK4	163±19[c]	81±28.9[a]	127±22[b,c]	130±14.5[b,c]	108±19.9[a,b]	159±19.3[c]

Data are shown as mean ± SD. Values with different superscript letters for a given week are significantly different from each other. NC- Normal control; DBC- Diabetic control; DLTL- Diabetic lemongrass tea low; DLTH- Diabetic lemongrass tea high; DMF- Diabetic metformin; NLTH- Normal lemongrass tea high

4.5Weekly Fasting Blood Glucose levels in Animal Groups During Four Weeks Experimental Period

Theanti-hyperglycemic effects of lemongrass tea extract on the fasting blood glucose levels of STZ induced diabetic rats is presented in table 4.5. After STZ injection, the FBG levels were significantly ($p \leq 0.05$) higher in the diabetic groups when compared to the Normal groups throughout the study. However, treatment with lemongrass tea and drug significantly ($p \leq 0.05$) decreased blood glucose level of the diabetic treated groups when compared to the DBC group. Administration of lemongrass tea to the NLTH group did not significantly ($p \geq 0.05$) affect the blood glucose level when compared to the NC group.

Table 4.5 Fasting Blood Glucose Levels (mg/dl) in Animal Groups during the Four Weeks Experimental Period

	NC	DBC	DLTL	DLTH	DMF	NLTH
WK-1	75±10.9[a]	76±9.4[a]	72±11.4[a]	91±11.7[a]	72±12[a]	78±12.1$_a$
WK0	75±10.9[a]	173±85[b]	292±128[c]	200±49.4[b]	304±175.9[c]	74±13.3[a]
WK1	84±19.4[a]	189±68.1[d]	305±153[d]	188±48[b]	248±148.9[c]	83±6.7[a]
WK2	89±9[a]	198±70.6[d]	274±130.7[d]	173±47[b]	228±139[c]	94±7.9[a]
WK3	89±12[a]	206±64.6[b,c]	164±48.1[b]	138±26.4[b]	184±93.7[b]	91±5.8[a]
WK4	90±13.8[a]	237±72.2[c]	154±23.6[b]	116±15.2[a]	133±14.2[b]	97±6.9[a]

Data are shown as mean ± SD. Values with different superscripts for a given week are significantly different from each other. NC- Normal control; DBC- Diabetic control; DLTL- Diabetic lemongrass tea low; DLTH- Diabetic lemongrass tea high; DMF- Diabetic metformin; NLTH- Normal lemongrass tea high

4.6. Oral Glucose Tolerance Test (OGTT) in Animal Groups during the Last Week of Four Weeks Experimental Period

The result for the OGTT shows that, induction of diabetes significantly ($p \leq 0.05$) affected the glucose tolerance ability of the Diabetic groups when compared to the normal groups. However, better glucose tolerance ability was observed in the diabetic treated groups when compared to DBC group.

Table 4.6 Oral Glucose Tolerance Test (mg/dl) in Animal Groups during the Last Week of Four Weeks Experimental Period.

	NC	DBC	DLTL	DLTH	DMF	NLTH
0 MINS	83 ± 8.1^{a}	215 ± 62.2^{c}	132 ± 20.6^{b}	122 ± 21.3^{b}	129 ± 21.1^{b}	100 ± 11.5^{a}
30 MINS	113 ± 14.3^{a}	274 ± 60.2^{c}	249 ± 29.7^{c}	200 ± 15^{b}	256 ± 63^{c}	134 ± 10.3^{a}
60 MINS	91 ± 9.8^{a}	270 ± 72.7^{c}	254 ± 37^{b}	230 ± 13^{b}	242 ± 34^{b}	96 ± 11.8^{a}
90 MINS	82 ± 10^{a}	286 ± 75.3^{c}	224 ± 30.5^{b}	173 ± 8.8^{b}	214 ± 33^{b}	89 ± 8.8^{a}
120 MINS	80 ± 7.6^{a}	301 ± 79.9^{c}	198 ± 44.8^{b}	148 ± 25.2^{b}	210 ± 10^{b}	87 ± 9.9^{a}

Data are shown as mean ± SD. Values with different superscript for a given parameter are significantly different from each other. NC- Normal control; DBC- Diabetic control; DLTL- Diabetic lemongrass tea low; DLTH- Diabetic lemongrass tea high; DMF- Diabetic metformin; NLTH- Normal lemongrass tea high.

4.7The Influence of Lemon Grass Tea on Serum Insulin, HOMA IR and HOMA β Scores on Diabetic Rats.

The serum insulin level and HOMA β scores were significantly ($p \leq 0.05$) lower in the DB groups when compared to the normal groups (Table 4.7). However, treatment with lemon grass tea and drug significantly ($p \leq 0.05$) increased both the serum insulin and HOMA β scores of the DB treated groups. Similarly, there was significantly ($p \leq 0.05$) higher HOMA IR in the diabetic groups when compared to the normal groups. Also, treatment significantly ($p \leq 0.05$) reduced the IR of the treated diabetic groups when compared to the DBC group.

Table 4.7 SerumInsulin Level, HOMA-IR & HOMA-β in Animal Groups

Groups	**NC**	**DBC**	**DLTL**	**DLTH**	**DMF**	**NLTH**
Insulin (pmol/l)	251.60±50.10[d]	147.40±80.90[a]	208.10±80.80[b]	214.70±21.00[b,c]	225.30±24.40[c]	268.90±57.90[d]
HOMA-IR	55.90±11.10[a]	86.30±47.30[c]	76.40±28.60[b]	61.50±6.00[a]	74.00±8.00[b]	64.40±13.90[a]
HOMA-β	15.90±3.18[d]	3.55±1.95[a]	7.72±3.00[b]	10.60±1.03[c]	9.68±1.04[c]	15.40±3.24[d]

Data are shown as mean ± SD. Values with different superscripts across the rows for a given parameter are significantly different from each other. NC- Normal control; DBC- Diabetic control; DLTL- Diabetic lemongrass tea low; DLTH- Diabetic lemongrass tea high; DMF- Diabetic metformin; NLTH- Normal lemongrass tea high

4.8Serum Lipid Profile in Animal Groups at the End of the Four Weeks Experimental Period

The results of serum lipid profile in animal groups are presented in figure 4.6 below. Significant ($p \leq 0.05$) increases in total cholesterol, triglyceride, high density lipoproteins and lowdensity lipoproteins were observed within the diabetic groups. However, treatment significantly ($p \leq 0.05$) reduced the total cholesterol, triglyceride, high density lipoproteins and low density lipoproteins of the diabetic treated groups when compared to the untreated group.

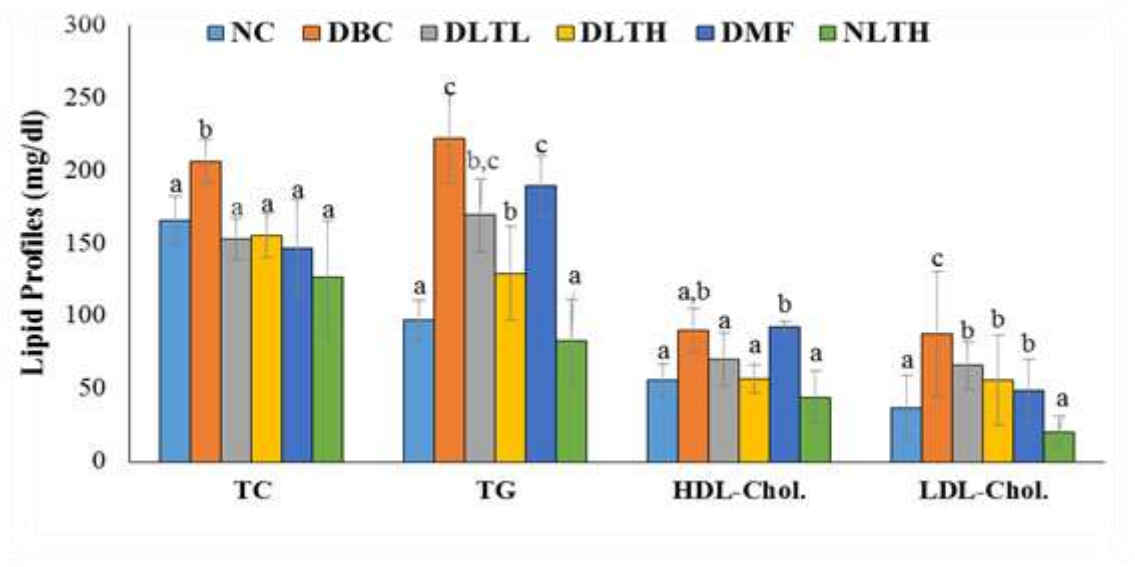

Figure 4.1:Serum lipid profile in animal groups at the end of the 4 weeks experimental period.

Data are shown as mean ± SD. Values with different superscript over the bars for a given parameter are significantly different from each other. NC- Normal control; DBC- Diabetic control; DLTL- Diabetic lemongrass tea low; DLTH- Diabetic lemongrass tea high; DMF- Diabetic metformin; NLTH- Normal lemongrass tea high.

4.9 Serum Biochemical Parameters and Liver Glycogen Content in Different Animal Groups

The result of the serum biochemical parameters and liver glycogen content are presented below in Table 4.9. Significant ($P \leq 0.05$) increase was observed in the ALT level of the DB groups when compared to the normal groups. However, treatment significantly reduced the ALT level of the treated DB groups when compared to the DBC group. Better reduction of the ALT level was observed in the LG treated groups when compared to the DMF treated group. Also, significant decrease was observed in the AST and ALP level of the DB groups when compared to the normal groups. However, after treatment a significant increase in both levels were observed in the treated groups when compared to the DBC. As for the albumin, a significant ($p \leq 0.05$) elevation was observed in the DB groups when compared to the normal groups. Treatment significantly decreased the albumin concentration of the treated groups when compared to the DBC group, with better reduction of albumin concentration observed in DLTL group when compared to DLTH and DMF group. A significant elevation ($p \leq 0.05$) in total protein level was observed in the DB groups when compared to the normal groups. However, treatment significantly ($p \leq 0.05$) decreases the total protein levels of the treated groups when compared to DBC group except for DLTH group which was found to be higher to that of DBC group. Better reduction of total protein level was observed in DLTL group when compared to DLTH and DMF groups. The glycogen content was significantly reduced in the diabetic groups when compared to normal groups. However, treatment significantly ($p \leq 0.05$) elevated the glycogencontent of the treated groups when compared to DBC group. The creatinine level was neither affected by the induced T2DM nor by the LG treatment. There was no significant ($p \leq 0.05$) difference between NC and NLTH groups in all the parameters.

Table 4.8 Serum Biochemical Parameters and Liver Glycogen Content in Different Animal Groups

	NC	DBC	DLTL	DLTH	DMF	NLTH
ALT (U/l)	11.60±1.52[a]	19.40±2.30[c]	15.40±2.30[b]	14.80±2.59[b]	18.40±1.67[c]	11.00±2.71a
AST (U/l)	183.00±22.90[e]	87.60±14.35[a]	138.40±21.56[b,c]	145.4±23.14[c]	136.40±21.02[b]	173.75±23.00[d]
ALP (U/l)	10.72±2.88[b]	6.70±0.76[a]	8.50±1.46[a,b]	7.99±0.92[a]	9.00±0.59[b]	10.80±3.00[b]
Albumin (g/dl)	2.49±0.58[a]	3.24±0.20[b]	2.90±0.51[a,b]	3.14±0.31[b]	3.48±0.20[b]	2.30±0.34[a]
Total Protein (g/dl)	4.00±1.34[a]	4.87±0.91[b]	4.15±0.95[a]	5.78±0.97[c]	4.59±0.89[b]	4.06±0.40[a]
Glycogen (mg/g tissue)	10.72±1.07[c]	3.68±0.29[a]	6.32±1.29[b]	6.66±0.64[b]	5.78±1.28[b]	8.82±2.75[c]
Creatinine (mmol/l)	0.86±0.18[a]	1.16±0.38[a]	0.88±0.25[a]	1.14±0.39[a]	1.22±0.36[a]	0.90±0.14[a]

Data are shown as mean ± SD. Values with different superscript across the rows for a given parameter are significantly different from each other. NC- Normal control; DBC- Diabetic control; DLTL- Diabetic lemongrass tea low; DLTH- Diabetic lemongrass tea high; DMF- Diabetic metformin; NLTH- Normal lemongrass tea high.

4.10 Histology of the Pancreatic Tissues of Different Animal Groups at the End of the Intervention Period.

The histopathological studies of the pancreases have shown reduction in the concentration of the islets within the diabetic groupsindicating diabetes. The induction of diabetes causes distortion of the pancreatic tissues of the diabetic groups when compared to the normal groups. However, treatment with lemongrass tea and drug for four weeksrelatively improved islets with better organized β-cells of the treatment groups when compared to the DBC group. The NC and NLTH had a larger islet with high number of β-cells while the DBC had a smaller islet and morphologically deformed β-cells

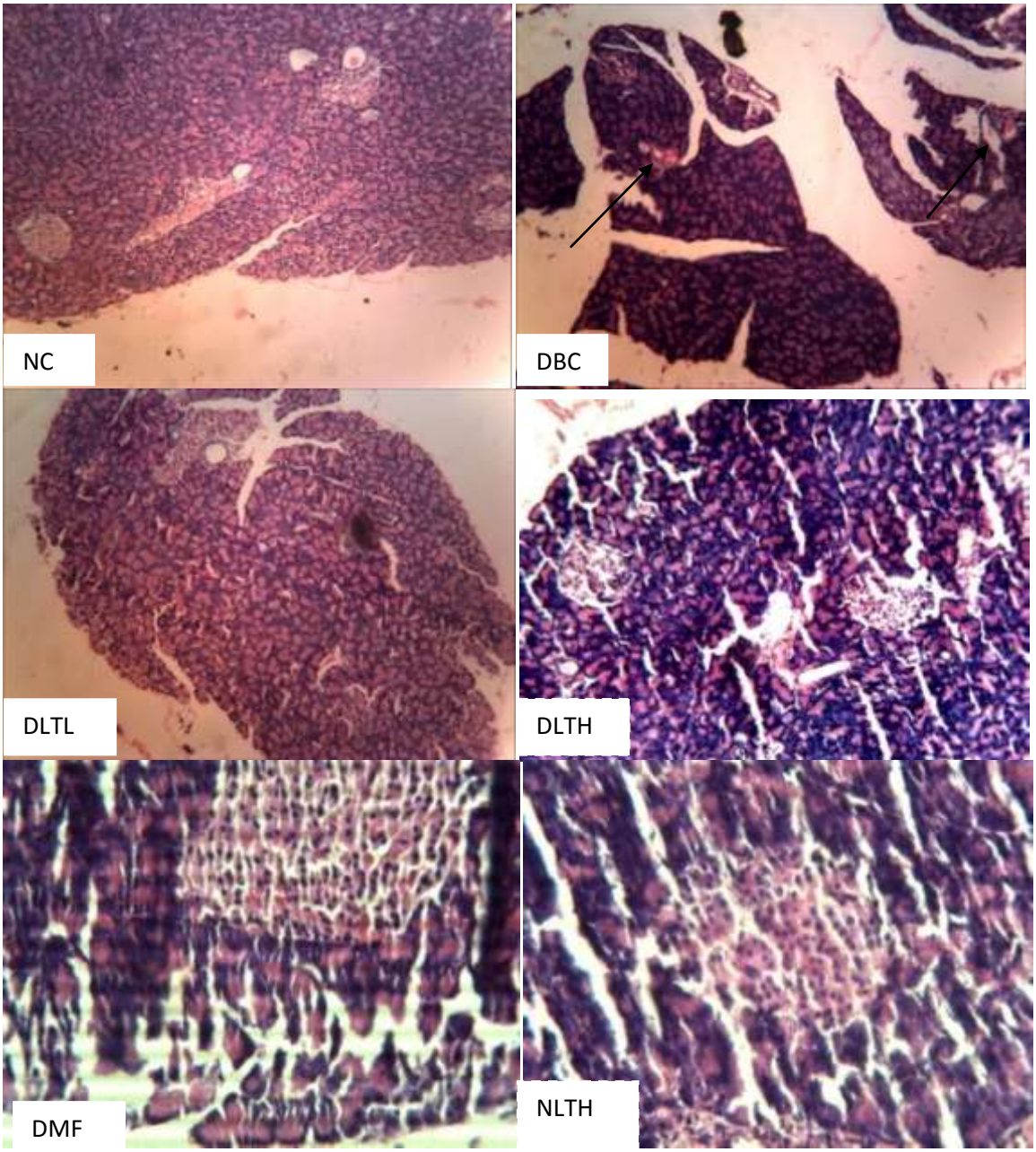

Plate 2: Photomicrograph of the Pancreatic Tissues of Different Animal Groups at the End of the Intervention Period.

CHAPTER FIVE

5.0 DISCUSSION

Diabetes mellitus is a heterogeneous group of metabolic disorder characterised by hyperglycaemia and glucose intolerance, due to inadequate insulin secretion, decreased insulin action or both (Sicree*et al*., 2016).As a multifactorial disorder, management of diabetes requires comprehensive and holistic approaches. The present study demonstrates a significant role of lemongrass tea in normalizing body weight, reducing blood glucose levels, decreasing cholesterol levels in type 2 diabetic rats. According to ADA (2007), the major symptoms of DM are polyphagia, polydipsia and body weight loss which were also observed in the diabetic groups of our experiment. However, treatment with LG tea significantly reduced these alterations close to normal in the diabetic treated groups.

The study showed that the LGT consumption for four weeks at both doses reduced blood glucose level, improved postprandial glucose utilization, ameliorated insulin resistance, hyperlipemia and alterations in some biochemical parameters in T2D rat model. The LGT had higher phytochemical content compared to cold water extract, with phenolics having the highest content (table 4.1). This finding is in agreement to several previous studies (Shaima *etal*., 2016; Ornelas-Paz *et al*., 2010 and Turkmen and Velioglu, 2005) that show dramatic increase in total phenolics, tannins and alkaloid contents of fruits and vegetables when boiled for less than 30minutes than the cold-water extracts, though reason remain speculative. It has been proposed that boiling improves the contact of the phytochemicals with water molecules which subsequently enhances extraction efficiency and in turn greater content of the ingredients in the boiled extract (Vilkhu *et al*., 2008). Interestingly, Oboh *et al.*, have shown an increased antioxidant potential of LGT compared to cold water extractdue to higher content of ingredients in LGT. This again supports the selection of LGT for the present study

in addition to the widely acclaimed health benefits in the treatment of several diseases including T2D.

The increase in fasting blood glucose concentration is a primary characteristic feature of type 2 diabetes mellituswhich occurs because of low insulin level and/or by resistance to insulin at the cellular level (Sommerfield*et al*., 2004) and causes life threatening complications linked to the disease.Therefore, maintaining glucose homeostasis is vital in preventing the effects of hyperglycaemia and its associated complications (Gin and Rigalleau, 2000). In this study, there were elevations in the fasting blood concentrations of the diabetic groups and was sustained throughout the study period, indicating the success of the induction. However, the lemongrass tea significantly ($p \leq 0.05$) reduced the fasting blood glucose level in the diabetic treated groups. The observation of reduction in fasting blood glucose in this study is in agreement with previous work on effects of lemongrass essential oil (Bharti*et al*. 2013).This study was further supported with oral glucose tolerance ability of the diabetic treated groups in a dose dependent manner as the group with the higher concentration of lemongrass tea showed the most reduction in the blood glucose level. This may be due to the regeneration of pancreatic β-cells of the treated groups.

Hypoglycaemic and hypolipidemic effects of fresh leaf aqueous extract of lemongrass was previously reported (Adeneye and Agbaje, 2007). Despite amelioration in glucose metabolism by lemongrass tea treatment, it was unable to induce beneficial effects in the lipid profile (HDL). It has also been demonstrated that lemongrass essential oil improved the lipid profile of the diabetic rats by reducing the total cholesterol and triglycerides contents. Hypolipidemic effect was recorded with noticeable reduction in low density lipoproteins levels in the blood stream. The mechanism by which the lemongrass extracts perform these effects remains elusive but several researchers have associated it withincreased insulin synthesis and secretion or increased peripheral glucose utilization (Adejuwon and Esther,

2007; Celso*et al*., 2011). Similarly, lemongrass extracts were effective in reducing cholesterol levels in the blood stream. Investigators have opined that this could be due to the presence of an endogenous ligand of central-type benzodiazepine (ODN) which is an inhibitors of food intake in small animals (Do Rego*et al*., 2007). Studies have shown that chronic uncontrolled T2D may led to reduction of circulating insulin levels and alter the pancreatic integrity and function (Murdolo *et al*., 2013; Bonner-Weir and O'Brien, 2008) which were observed in our present study. Consumption of LGT in both doses significantly increased serum insulin level accompanied by a decline in insulin resistance, calculated by HOMA- IR. These favourable effects of LG tea may be attributed to the synergistic action of their phytoconstituents. Another important feature of experimentally induced diabetes is a reduction in liver glycogen level (Ibrahim and Islam, 2014) which is caused by reduced activity of glycogen synthase and increased glycogen phosphorylase activity during the disease. Previous studies (Habibuddini*et al.*, 2008: Jain *et al.*, 2010) have demonstrated that a number of plant materials elicited anti diabetic potential partly through stimulation of hepatic glycogenesis. Therefore, the increase inglycogen level observed in the LG treated groupswhen compared to DBC indicated the anti-diabetic activity of LG tea through stimulating hepatic glycogenesis and/or inhibiting glycogenolysis which is in agreement with the result reported by Ibrahim and Islam (2014). Prospective studies have shown that increasing liver enzyme levels, particularly Alanine aminotransferase (ALT), predicts incident diabetes (Hanley *et al*., 2004: Vozarova*et al.*, 2002). According to this study a significant increase in ALT level not ALP and AST was observed. The increase in ALT and albumin levels indicates an impaired liver function. There was also an increase in the total protein in the diabetic group while the creatinine level was not affected in this study. The photomicrograph of this study has shown distorted islets and morphologically deformed beta-cells in the DBC. Kaku (2010) reported that reduction in the number of islets greatly affect

the long term control of blood glucose leading to diabetes complications. However, treatment with LG tea led to regeneration of beta-cells, which justifies the reduced blood glucose levelandimproved beta-cell function.Therefore, despite the limitations of our present study to specifically isolate the active compound(s) responsible for the observed inhibitory effect,it can be concluded that, the activities exhibited by LG tea is due to the presence of the phytoconstituents identified, either individually or in synergy.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The results of this study have shown that lemongrass tea reduced hyperglycaemia, improved blood glucose tolerance, ameliorated insulin resistance and hyperlipidemia in T2D rats model. The anti-diabetic potential of lemongrass tea could be due to the phytochemicals present in the extract.

6.2 RECOMMENDATION

Although this study shows the anti-diabetic effect of lemongrass tea, there was no detailed phytochemical analysis, hence more research should be done on the isolation of bioactive compounds of this promising herb and their mechanism of actions.

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