EVALUATION OF HYPOGLYCAEMIC EFFECTS OF LEAF EXTRACTS OF MISTLETOES (*Globimetula oreophila* OLIV. AND *Globimetula braunii* ENGL. Van Tiegh) IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Folklore medicine has it that some mistletoes are used in the management of diabetes in Nupeland. The aqueous and ethylacetate extracts of two mistletoe plants, *Globimetula braunii* and *Globimetula oreophila* were investigated for hypoglycaemic effects in diabetic rats. Diabetes was induced intraperitoneally in rat with 100mg/kg body weight (b.w) of alloxan and rats with blood glucose >135 mg/kg b.w were considered diabetic. The design consisted of twenty one (21) rats with each group having three rats. The diabetic rats were given 500mg/kg b.w of respective extracts once daily for fourteen days. Blood glucose was monitored every five days and it reduced during the period of treatment when compared with diabetic untreated group (DNT). The experiment lasted for twenty one (21) days, the blood serum and fresh organs were collected after euthanizing the animals. The extracts showed significant ($P<0.05$) hypoglycaemic effects with 58.37%, 50.46%, 65.50% and 50.65% for Aqueous *Globimetula braunii* (AGB), Aqueous *Globimetula oreophila* (AGO), Ethylacetate *Globimetula Braunii* (EGB) and Ethylacetate *Globimetula oreophila* (EGO) respectively. There was no significant ($P>0.05$) gain in body weight for all the groups treated with the extracts but normoglyceamic group (NNT) showed significant ($P<0.05$) increase (8.04%) in total body weight. The mean percentage ratio of fresh organs to whole body weight for liver and heart reveals no significant ($P>0.05$) difference between the groups. While lungs and pancreas show significant ($P<0.05$) reduction for all the groups treated with the extracts. The kidney of the AGB treated group showed a significant ($P<0.05$) increase when compared with NNT groups. There was no significant difference ($P<0.05$) in serum total protein and cholesterol levels between the groups. Triglyceride level significantly ($P<0.05$) increased (173.50 ± 16.57) in the Diabetes untreated group (DNT) alone when compared with the NNT group. Only EGO group showed a significant ($P< 0.05$) increase (1.57 ± 0.2) in the level of direct bilirubin, total bilirubin was not significantly ($P>0.05$) increase in all the groups when compared with the NNT group. AGB group showed significant increase in the activities of Alkaline phosphatase. EGB, EGO and DNT, showed significant ($P<0.05$) increase (78.90 ± 8.90, 63.30 ± 6.10 and 73.07 ± 2.40 respectively) in the activities of Alanine aminotransferase when compared with the NNT group. All groups showed significant ($P<0.05$) increase in the activities of Aspartate aminotransferase when compared with the NNT groups. Thus, the research work has shown that all the extracts of *G.braunii* and *G.oreophila* at 500mg/kg b.w have significant anti-diabetic effects on the diabetic animal model (rats) in addition to ameliorating the biochemical abnormalities arising from diabetes.

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ABBREVIATIONS, GLOSSORY AND SYMBOLS

ADA:	American Diabetes Association
AGE:	Advanced Glycation End Protein
AGI:	Alpha Glucosidase Inhibitor
AIDS:	Aquired Immunodeficiency syndrome
AKT:	Protein Kinase B
ALP:	Alkaline Phosphate
ALT:	Alanine Aminotransferase
AST:	Aspartate Aminotransferase
ATP:	Adenosine Triphosphate
CCAC:	Canadian Council for Animal Care
CDC:	Disease Control and Prevention
CEBPB:	Ubiquitosly Expressed Transcription Factors
CVD:	Cardiovascular Diseases
DAG:	Diacylglycerol
DKA:	Diabetic Ketoacidosis
DM:	Diabetes Mellitus
DNA:	Deoxyribonucleic acid
DPP-4:	Dipeptidyl Peptidase-4
DP-W:	Dipeptidyl Peptidase-w
FCPD:	Fibrocalculus Pancreatic Diabetes
FDA:	Food and Drug Administration
FFA:	Free Fatty Acids
FOXO:	Forkhead Box Protein O
G.braunii:	*Globimetula braunii*
G.oreophila:	*Globimetula oreophila*

G6pase:	Glucose-6-Phosphatase
GDM:	Gestational Diabetes Mellitus
GFAT:	Glucosamine-Glutamine, Fructose-6-Phosphate Amido Transferase
GIGT:	Gestational Impaired Glucose Tolerance
GIP:	Gastric Inhibitory peptide
GLP-1:	Glucagon Like-Peptide-1
Glut 4:	Glucose Transporter 4
GSK-3:	Glycogen Synthase Kinase-3
HAAF:	Hypoglycaemia Associated Autonomic Failure
HDL:	High Density Lipoprotein
HIV:	Human Immune Virus
HMGA1:	Human Transcription Factor
HNS:	Hyperosmolar Non-ketotic State
ICD:	International Classification of Diabetes
ICS-UNIDO:	International Centre for Science- United Nations Industrial Development Organization
IDA:	International Diabetes Association
IDDM:	Insulin Dependent Diabetes Mellitus
IDN:	International Nomenclature of Diabetes
IFG:	Impaired Fasting Glycaemia
IGT:	Impaired Glucose Tolerance
IR:	Insulin Receptor
IRS:	Insulin Receptor Substrate
IRS-1 -/-:	IRS-1 Homozygous Null Mice
IRS-1:	Insulin Receptor Substrate 1
IRS-2 -/-:	IRS-1 Homozygous Null Mice
IRS-2:	Insulin Receptor Substrate 2
LADA:	Latent Autoimmune Diabetes of Adulthood

LDL:	Low Density Lipoproteins
MCR-4:	Melanocortin-4 Receptor
MODY:	Maturity –onset Diabtes of the Young
MRDM:	Malnutrition Related Diabetes Mellitus
mTOR:	Molecular Target of Rapamycin
NAD^+:	Nicotinamide Adenine Dinucleotide (Oxidized form)
NADH:	Nicotinamide Adenine Dinucleotide (reduced form)
$NADP^+$:	Nicotinamide Adenine Dinucleotide Phosphate (oxidized form)
NADPH:	Nicotinamide Adenine Dinucleotide Phosphate (reduced form)
NIDDM:	Non- Insulin Dependent Diabetes Mellitus
NIH:	National Institutes of Health
OECD:	Organisation for Economic Corporation and Development
OGTT:	Oral Glucose Tolerance Test
PDK:	Phosphoinositide Dependent Kinase
PDPD:	Protein Deficient Pancreatic Diabetes mellitus
PEPCK:	Phosphoenolpyruvate Carboxykinase
PGC-1:	PPAR-γ co-activator-1
PI-3-K:	Phosphatidyl Inositol -3-OH Kinase
PIP2:	Phosphatidyl Inositol-3,4-bisphophate
PIP3:	Phosphatidyl Inositol-3,4,5-bisphophate
PKB:	Protein Kinase B
PKC:	Protein Kinase C
TCA:	Tricarboxylic Acid Cycle
TNF:	Adipokine Tumour Necrosis Factor
TZD:	Thiazolidinedione
VEGF:	Vascular Endothelial Growth Factor
WHO:	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The number of people with diabetes mellitus was estimated in 2011 to be 336 million adults aged 20-79 years of the world's 7 billion population. This gives a comparative prevalence of 8.5% (International Diabetes Federation (IDF), 2011). Accord to the International Diabetes Federation (IDF) data of 2003, shows that West Africa, Nigeria has the highest number of people living with diabetes with approximate estimate of 1,707,000 people affected (IDF, 2003).

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both (American Diabetic Association (ADA), 2000). The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, Liver, heart, and blood vessels (Zeller, Whittaker, Sullian, Raskin, and Jacob, 1991; Pedrini, Levey, Lau, Chalmers, and Wang, 1996).

The basis of the abnormalities in carbohydrate, fat, and protein metabolism noted in diabetes is as a result of the deficient action of insulin on target tissues which then lead to abnormalities of lipoprotein metabolism, hypertriglyceridemia and hypercholesterolemia (Granner, 2000). There is also marked elevation in the activities of some enzymes in a diabetic condition (Eskander, Won Jun, Ibrahim, and Abdelal, 1995; Hassan, 2007). Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action (Lernmark, Haggloff, Freedman, Irvine, Ludvigsson and Holmgren, 1981).

Today, medicinal plants are increasingly being used in most parts of the world as antidiabetic regimen because of their hypoglycaemic effects (Saidu, Mann and Onuegbu 2012). Mistletoe plants are well known for their medicinal importance from time immemorial which has been highly revered in different cultures (Marzell, 1923). They have been reported for their anticancer, antihypertensive, and fertility functions (Obatomi, Bikomo and Temple, 1994). A tea prepared from leaves of the *Viscum album* mistletoe is used traditionally to treat diabetes in the West Indies (Peters 1957). This treatment has been shown also to relieve the diabetic symptoms of severely hyperglycaemic streptozotocin-diabetic mice, including polydipsia, polyphagia and body weight loss (Swanston-Flatt, Day, Bailey and Flatt, 1989).

Mistletoe, which is called 'Afomo' in Yoruba, 'Kauchi' in Hausa, 'Apari' in Igbo, 'Osuochi' in Ebira, and 'Etu' in Nupe, is ethnomedicinally used by various ethnicities in Nigeria as a remedy for several human and animal ailments such as diarrhea, dysentery, wound, cancer, hemorrhage, convulsion (Adesina, 1979; Adesina and Ojewole, 1983), gynaecology problems and cardiovascular diseases (Adodo, 2004). The genus *Globimetula* is in the major group Angiosperms (Flowering plants) and it has about twelve (12) known species which are *anguliflora, assiana, braunii, cornutibracteata, Cupulata, dinklagei, elegantiflora, mayombensis, mweroensis, oreophila, pachyclada and rubripes. G.braunii* and *G.oreophila* medicinal importance is well documented (Le and Zam, 2008; Ochuko, Joseph, Adeniyi, Rabiat and Michael, 2011; Huaxing and Micheal, 2012). *G.braunii* and *G.oreophila* are also used as folkloric treatment for diabetes by the Nupe speaking people of Niger state. The medicinal efficacy of mistletoe has been proven to be dependent on several factors (Bako, 2001; Wahab, Ayodele and Moody, 2010). Thus it is of the interest of this research to investigate and compare the varying degree of the antidiabetic properties of *G.braunii* and *G.oreophila* which are different species from different host plant.

1.2 Justification for this Research

Folkloric usage of mistletoe plants for the treatment of diabetes is well known and highly revered by many cultures all over the world. The Nupe speaking people of Niger state of Nigeria use the plant locally for the treatment of many illnesses including diabetes. The varying degree of the medicinal properties of mistletoe and its efficacy depend on the extract, the species and the host plant it parasitized. Hence the need to scientifically appraise the antidiabetic property of selected species from two different host plants (Locust beans and shea butter plant).

1.3 Aim and Objectives

1.3.1 Aim

To investigate the hypoglycemic properties of aqueous and ethylacetate leaves extracts of *Globimetula braunii* (grown on Locust beans) and *Globimetula oreophila* (grown on shea butter) in alloxan induced diabetic wistar rats.

1.3.2 Objectives

I. To determine the phytochemical constituents of the plants extracts

II. To investigate the LD_{50} of aqueous and ethylacetate extracts of *G.braunii* and *G.oreophila* in Wistar rats

III. To investigate the effect of the crude aqueous and ethylacetate extracts on the blood glucose level of diabetic rats.

IV. To determine the effects of the extracts on the relative organs weights of diabetic rats.

V. To check the effects of extract on selected marker enzymes and on some biochemical parameters of diabetic rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diabetes Mellitus

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs (ADA, 2000).

Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. The long–term effects of diabetes mellitus include progressive development of the specific complications of vascular damages (Mayfield, Reiber, Sanders, Janisse and Pogach, 1998). People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease.

Several pathogenetic processes are involved in the development of diabetes. These include processes which destroy the beta cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin (Granner, 2000).

2.2 Pathophysiology of Diabetes Mellitus

The pancreas plays a primary role in the metabolism of glucose by secreting the hormones insulin and glucagon (Figure 2.1). The islets of Langerhans secrete insulin and glucagon directly into the blood. Insulin is a protein that is essential for proper regulation of glucose and for maintenance of proper blood glucose levels (Worthley, 2003).

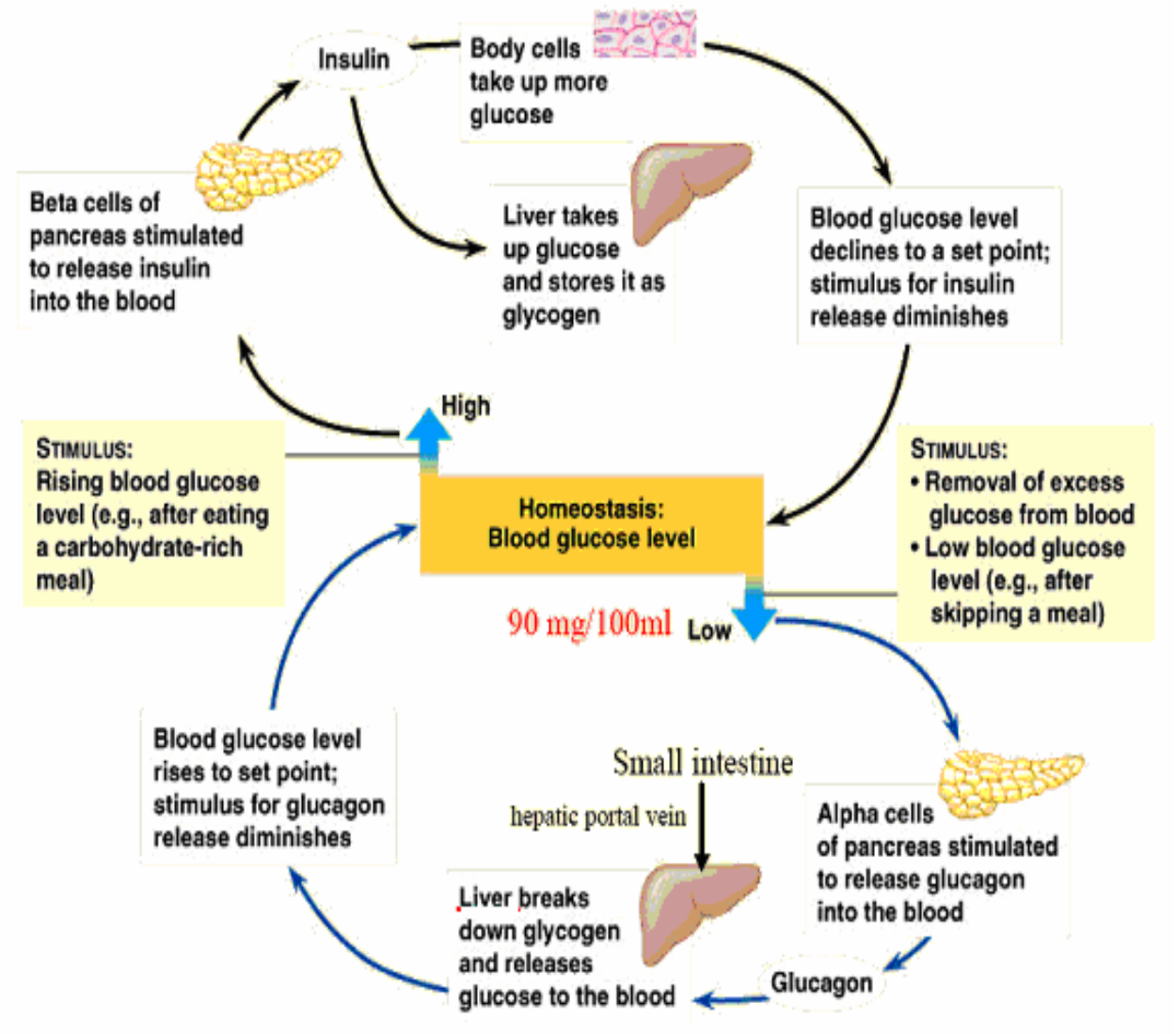

Figure 2.1: The Endocrine Role of Pancreas in the Body
Source**: (**Tedesse**,** 2008**).**

Glucagon is a hormone that opposes the action of insulin. It is secreted when blood glucose level falls. It increases blood glucose concentration partly by breaking down stored glycogen in the liver by a pathway known as glycogenolysis. Gluconeogenesis is the production of glucose in the liver from non-carbohydrate precursors such as glycogenic amino acids (Sowka, Gurwood and Kabat, 2001).

Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells (primarily muscle and fat cells, but not central nervous system cells). Therefore deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus (Najjar, 2001).

2.3 Classification of Diabetes Mellitus

2.3.1 Earlier Classifications

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 (WHO, 1980) and, in modified form, in 1985 (WHO, 1985). The 1980 and 1985 classifications of diabetes mellitus and allied categories of glucose intolerance included clinical classes and two statistical risk classes. The 1980 Expert Committee proposed two major classes of diabetes mellitus and named them, IDDM or Type 1, and NIDDM or Type 2. In both the 1980 and 1985 reports other classes of diabetes included Other Types and Impaired Glucose Tolerance (IGT) as well as Gestational Diabetes Mellitus (GDM). The recommended classification includes both staging of diabetes mellitus based on clinical descriptive criteria and a complementary aetiological classification (See appendix I).

2.3.2 Revised Classification

The classification encompasses both clinical stages and aetiological types of diabetes mellitus and other categories of hyperglycaemia, as suggested by Kuzuya and Matsuda (1997). The clinical staging reflects that diabetes, regardless of its aetiology, progresses through several clinical stages during its natural history. Moreover, individual subjects may move from stage to stage in either direction. Persons who have, or who are developing, diabetes mellitus can be categorized by stage according to the clinical characteristics, even in the absence of information concerning the underlying aetiology. The classification by aetiological type results from improved understanding of the causes of diabetes mellitus.

2.3.3 Aetiological Classification of Diabetes Mellitus

The aetiological types designate defects, disorders or processes which often result in Diabetes Mellitus. They are Type 1 Diabetes Mellitus, Type 2 Diabetes Mellitus, Gestational Diabetes Mellitus and other specific types. They are discussed in the following paragraphs.

2.3.3.1 Type 1 Diabetes Mellitus

Type 1 indicates the processes of beta–cell destruction that may ultimately lead to diabetes mellitus in which “insulin is required for survival” to prevent the development of ketoacidosis, coma and death. An individual with a Type 1 process may be metabolically normal before the disease is clinically manifest, but the process of beta–cell destruction can be detected. Type 1 is usually characterized by the presence of anti–GAD, islet cell or insulin antibodies which identify the autoimmune processes that lead to beta–cell destruction (Yki-Jarvinen, 1984).

2.3.3.2 Type 2 Diabetes Mellitus

Type 2 is the most common form of diabetes and is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature. Both are usually present at the time that this form of diabetes is clinically manifest. By definition, the specific reasons for the development of these abnormalities are not yet well elucidated (David and McCulloch, 2013).

2.3.3.3 Gestational Diabetes Mellitus

Gestational diabetes is carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy. It does not exclude the possibility that the glucose intolerance may antedate pregnancy but has been previously unrecognized. Individuals at high risk for gestational diabetes include older women, those with previous history of glucose intolerance, those with a history of large for gestational age babies, women from certain high–risk ethnic groups, and any pregnant woman who has elevated fasting, or casual, blood glucose levels (Di-Cianni, Miccoli, Volpe, Lencioni and Del-Prato, 2003)

2.3.3.4 Other Specific Types

Other specific types are currently less common causes of diabetes mellitus, but are those in which the underlying defect or disease process can be identified in a relatively specific manner (see appendix I). They include, for example, fibrocalculous pancreatopathy, a form of diabetes which was formerly classified as one type of malnutrition–related diabetes mellitus.

2.4 Complications Associated with Diabetes Mellitus

The complications of diabetes mellitus are far less common and less severe in people who have well-controlled blood sugar levels (DCCTRG, 1995; Nathan, Cleary, and 2005). Wider health problems accelerate the deleterious effects of diabetes. Complications can be divided into acute (short term) complications and chronic (long term) complications.

2.4.1 Acute Complications of Diabetes Mellitus

2.4.1.1 Diabetic Ketoacidosis

Diabetic ketoacidosis (DKA) is an acute and dangerous complication that is always a medical emergency. Low insulin levels cause the liver to turn fatty acid to ketone for fuel, ketone bodies are intermediate substrates in that metabolic sequence. This is normal when periodic, but can become a serious problem if sustained. Elevated levels of ketone bodies in the blood decrease the blood's pH, leading to DKA. Patient with diabetic ketoacidosis is characterised with dehydration, breathing rapidly and deeply, abdominal pain, hypotension, lost of consciousness, shock and then death. Urine analysis will reveal significant levels of ketone bodies (which have exceeded their renal threshold blood levels to appear in the urine, often before other overt symptoms). Ketoacidosis is much more common in type 1 diabetes than type 2 (Keller, 1986).

2.4.1.2 Hyperglycemia Hyperosmolar State

Hyperosmolar nonketotic state (HNS) is an acute complication sharing many symptoms with DKA, but an entirely different origin and different treatment. The diabetic hypersomolar state is defined by serum glucose greater than 600 mg/dl and a serum osmolarity greater than 320 m Osm/L (Wachtel, 1990). A person with very high

(usually considered to be above 300 mg/dl (16 mmol/L)) blood glucose levels, water is osmotically drawn out of cells into the blood and the kidneys eventually begin to dump glucose into the urine. This results in loss of water and an increase in blood osmolarity. If fluid is not replaced (by mouth or intravenously), the osmotic effect of high glucose levels, combined with the loss of water, will eventually lead to dehydration. The body's cells become progressively dehydrated as water is taken from them and excreted. Electrolyte imbalances are also common and are always dangerous. As with DKA, urgent medical treatment is necessary, commonly beginning with fluid volume replacement. Lethargy may ultimately progress to a coma, though this is more common in type 2 diabetes than type 1 (Stoner, 2005).

2.4.1.3 Hypoglycaemia

Hypoglycemia, or abnormally low blood glucose, is an acute complication of several diabetes treatments. It is rare otherwise, either in diabetic or non-diabetic patients. The patient may become agitated, sweaty, weak, and have many symptoms of sympathetic activation of the autonomic nervous system resulting in feelings akin to dread and immobilized panic (Philip *et al.*, 2003). Consciousness can be altered or even lost in extreme cases, leading to coma, seizures, or even brain damage and death. In patients with diabetes, this may be caused by several factors, such as too much or incorrectly timed insulin, too much or incorrectly timed exercise (exercise decreases insulin requirements) or not enough food (specifically glucose containing carbohydrates). The variety of interactions may cause identification difficult in many instances.

2.4.1.4 Diabetic Coma

Diabetic coma is a medical emergency in which a person with diabetes mellitus is comatose (unconscious) because of one of the acute complications of diabetes:

1. Severe diabetic hypoglycemia

2. Diabetic ketoacidosis advanced enough to result in unconsciousness from a combination of severe hyperglycemia, dehydration and shock, and exhaustion

3. Hyperosmolar nonketotic coma in which extreme hyperglycemia and dehydration alone are sufficient to cause unconsciousness.

In most medical contexts, the term diabetic coma refers to the diagnostical dilemma posed when a physician is confronted with an unconscious patient about whom nothing is known except that he has diabetes (http://en.wikipedia.org/wiki/Diabetic_coma).

2.4.2 Chronic Complications

The chronic complications of diabetes mellitus affect many organ systems and are responsible for the majority of morbidity and mortality (Alberti, 1998). Chronic complications can be divided into vascular and nonvascular complications. The vascular complications are further subdivided into microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular complications (coronary artery disease, peripheral vascular disease, and cerebrovascular disease) (Michael, 2008). Nonvascular complications include problems such as gastroporesis, sexual dysfunction, and skin changes. As a consequence of its chronic complications, DM is the most common cause of adult blindness, a variety of debilitating neuropathies, and cardiac and cerebral disorders. Treating the complications of diabetes costs more than controlling the disease.

Early in the course of diabetes, intracellular hyperglycaemia causes abnormalities in blood flow and increased vascular permeability (Gera *et al.*, 1999). This reflects decreased activity of vasodilators such as nitric oxide, increased activity of

vasoconstrictors such as angiotensin II and endothelin-1, and elaboration of permeability factors such as vascular endothelial growth factor (VEGF) (John, 1991). In diabetic arteries, endothelial dysfunction seems to involve both insulin resistance specific to the phosphotidylinositol–3-OH kinase pathway and hyperglycaemia (More detail in the section of Biochemistry of Diabetes mellitus and its complications).

2.4.2.1 Diabetic Retinopathy

Diabetic retinopathy occurs in three-quarter (3/4) of all persons having diabetes for more than fifteen years and is the most common cause of blindness. There is appearance of retinal vascular lesions of increasing severity, culminating in the growth of new vessels (Barbara *et al.,* 1984). Diabetic retinopathy is classified into two stages: nonproliferative and proliferative. The non-proliferative stage appears late in the first decade or early in the second decade of disease and is marked by retinal vascular microneurisms, blot hemorrhages, and cotton-wool spots and includes loss of retinal pericytes, increased retinal vascular permeability, alterations in regional blood flow, and abnormal retinal microvasculature, all of which lead to retinal ischemia. In proliferative retinopathy there is the appearance of neovascularisation in response to retinal hypoxia. The newly formed vessels may appear at the optic nerve and/or macula and rupture easily, leading to vitreous haemorrhage, fibrosis, and ultimately retinal detachment (Aiello *et al*., 1998).

2.4.2.2 Neuropathy

About half of all people with diabetes have some degree of neuropathy, which can be polyneuropathy, mono-neuropathy, and/or autonomic neuropathy. In polyneuropathy there is loss of peripheral sensation which, when coupled with impaired microvascular and macrovascular junction in the periphery, can contribute to non-healing ulcers, the

leading cause of non-traumatic amputation. There is thickening of axons, decrease in microfilaments, and capillary narrowing involving small myelinated or non-myelinated C-fibres (http://diabetes.niddk.nih.gov/dm/pubs/neuropathies/). It can occur both from direct hyperglycaemia-induced damage to the nerve parenchyma and from neuronal ischemia leading to abnormalities of microvessels, such as endothelial cell activation, pericyte degeneration, basement membrane thickening, and monocyte adhesion. Mono-neuropathy is less common than polyneuropathy and includes dysfunction of isolated cranial or peripheral nerves. Autonomic neuropathy can involve multiple systems, including cardiovascular, gastrointestinal, genitourinary, sudomotor, and metabolic systems (Chen and Reaven, 1997).

2.4.2.3 Nephropathy

This is a major cause of end-stage renal disease. There are glomerular hemodynamic abnormalities resulting in glomerular hyper-filtration, leading to glomerular damage as evidenced by microalbuminurea. There is overt proteinuria, decreased glomerular filtration rate, and end-stage renal failure. Dysfunction of the glomerular filtration apparatus is manifested by microalbuminurea and is attributed to changes in synthesis and catabolism of various glomerular basement membrane macromolecules such as collagen and proteoglycans, leading to an increase in glomerular basement thickening.

Another possible mechanism to explain the increase in permeability of the glomerulus is the increase in renal VEGF levels observed in preclinical models of diabetes, since VEGF is both an angiogenic and a permeability factor (Ritz and Orth, 1999).

2.4.2.4 Cardiovascular Morbidity and Mortality

In diabetes mellitus there is marked increase in several cardiovascular diseases, including peripheral vascular disease, congestive heart failure, coronary artery disease, and myocardial infarction, and a one to fivefold increase in sudden death. The absence of chest pain (silent ischemia) is common in individuals with diabetes, and a thorough cardiac evaluation is indicated in individuals undergoing major surgical procedures (Isomaa *et al.*, 2001)

Individuals with DM have increased incidence of congestive heart failure (diabetic cardiomyopathy). The aetiology of this abnormality is probably multifactorial and includes factors such as myocardiac ischemia from atherosclerosis, hypertension, and myocardial cell dysfunction secondary to chronic hyperglycaemia (Fuller *et al*., 1980).

Though DM itself does not increase levels of Low Density Lipoproteins (LDL), LDL particles found in type 2 DM are more atherogenic and are more easily glycated and susceptible to oxidation (Grundy *et al*., 1999).

2.4.2.5 Hypertension

Hypertension can accelerate other complications of diabetes mellitus, particularly cardiovascular disease and nephropathy. Antihypertensive agents should be selected based on the advantages and disadvantages of the therapeutic agent in the context of the individual patient's risk-factor profile (Grossman and Messerli, 2008).

2.4.2.6 Infections

Individuals with diabetes mellitus exhibit a greater frequency and severity of infection. The reasons for this include incompletely defined abnormalities in cell-mediated

immunity and phagocyte function associated with hyperglycaemia as well as diminished vascularisation secondary to long-standing diabetes (Lipsky *et al.,* 2004). Many common infections are more frequent and severe in the diabetic population, whereas several rare infections are seen almost exclusively in the diabetic population (such as rhinocerebral mucormycosis and malignant otitis externa, which is usually secondary to *P. aeruginosa* infection in the soft tissue surrounding the external auditory canal). Pneumonia, urinary tract infection, and skin and soft tissue infections are all more common in the DM population (Lipsky *et al.,* 1987). Gram-negative organisms, for example, *S. aureus* and *Mycobacterium tuberculosis*, are more frequent pathogens in patients of DM. Diabetic patients have an increased rate of colonization of *S. aureus* in skin folds and also have a greater risk of postoperative wound infections (ADA, 1999).

Respiratory Infections

The immune response is impaired in individuals with diabetes mellitus. Cellular studies have shown that hyperglycaemia both reduces the function of immune cells and increases inflammation. The vascular effects of diabetes also tend to alter lung function, all of which leads to an increase in susceptibility to respiratory infections such as pneumonia and influenza among individuals with diabetes. Several studies also show diabetes associated with a worse disease course and slower recovery from respiratory infections (Ahmed *et al*., 2008).

Periodontal Disease

Diabetes is associated with periodontal disease (gum disease) (Mealey, 2006) and may make diabetes more difficult to treat (Lakschevitz *et al*., 2011) Gum disease is frequently related to bacterial infection by organisms such as *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* (Mombelli, 2012). A number of trials have

found improved blood sugar levels in type 2 diabetics who have undergone peridontal treatment (Lakschevitz *et al*., 2011).

2.5 Biochemistry of Diabetes and its Complications

The understanding of biochemistry of diabetes stems from the basic understanding on how glucose is metabolized starting from its absorption into the blood stream. Therefore the starting event in the biochemistry of diabetes is when glucose is not utilized. The search for what happen in diabetes cause many authors and researchers to actually come up with amazing biochemical events that leads to diabetes and its associated complication. Events like; Insulin secretion and action, some hormone actions (such as Glucagon), carbohydrate metabolism, protein metabolism, lipid metabolism, hexozamine pathways and sorbitol pathway. All have been indicated in the biochemistry of diabetes mellitus. While some literature took their explanation base on pathogenesis, others just looked at it generally on events that happen in all type of diabetes. Thus the hallmark of diabetes mellitus is inability to control blood glucose giving two major clinical features: Type 1 and Type 2 (Taylor and Agius, 1988)

2.5.1 Insulin Secretion and Diabetes Mellitus

Physiologically, plasma glucose concentration is a major regulator of insulin secretion. This effect may be mimicked and potentiated by amino acids (Floyd *et al*.,1966), potentiated by glucose insulinotropic peptide (Dupre *et al*., 1973; Jones *et al.*, 1987) and inhibited by catecholamines which act predominantly via the α-adrenergic receptors (Robertson *et al*., 1976). It has been argued that the capacity of molecules to stimulate insulin secretion is directly proportional to their suitability as, cell fuels for glycolysis (Ashcroft, 1981; Sener and Malaisse, 1984). Glyceraldehyde and pyruvate are

respectively good and ineffective insulin secretagogues (Sener *et al*., 1978; Zawalich *et al*., 1978). Whatever the precise nature of the initiating signal, it is clear that insulin release is closely associated with a rise in intracellular free calcium (Rorsman *et al*., 1984). However, under a number of experimental conditions, glucose brings about a paradoxical decrease in intracellular free calcium.

Glucose affects cellular uptake and efflux of Ca^{2+} and movement of free Ca^{2+} into organelle-bound calcium, these processes exhibiting different latencies. Assessment of pancreatic, cell capacity/responsiveness in NIDDM is complicated by prevailing blood glucose concentrations. Although NIDDM subjects have no absolute deficiency of insulin as assessed by diurnal plasma insulin profiles (Liu *et al*., 1983) and normal or raised fasting plasma insulin (DeFronzo and Ferrannini, 1982) this cannot be interpreted as indicating normality of cell function. When normal subjects are examined during hyperglycaemia, resulting rates of insulin secretion are far higher than in equivalently hyperglycaemic NIDDM subjects (Halter *et al*., 1979; Ferner *et al*., 1986). Conversely, when NIDDM subjects are rendered normoglycaemic by prior insulin infusion, steady state insulin levels after a wash-out period are lower than in weight-matched normal subjects (Turner *et al*., 1976).

Initial assessment of the effect of such non-glucose insulin secretagogues as isoproterenol or arginine suggested that, cells of NIDDM subjects responded normally (Pfeifer *et al*., 1981). Examination of the slope of glucose potentiation demonstrated that, although the insulin response to arginine in NIDDM subjects at blood glucose around 22 mmol/l was equivalent to that of normal subjects, the latter exhibited a 5-fold enhancement of response when blood glucose was raised to 22 mmol/l (Robertson and Chen, 1977; Ward *et al*., 1984).

The glucose level required for half-maximal responsiveness to arginine was normal in NIDDM. These findings imply a generalized loss of, cell capacity rather than a problem of glucose sensing as the basis of the insulin secretory abnormality in NIDDM. In support of this, post mortem studies have suggested a 40-60 % decrease in mean, cell mass in NIDDM (Saito *et al*., 1979; Gepts and Lecompte, 1981). A 70-90% pancreatectomy does not usually bring about overt diabetes (Brooks, 1979), emphasizing the importance of concurrence of a moderate decrease in both, cell function and tissue sensitivity in the pathogenesis of NIDDM. Dysfunction of a normal number of, cells induced by somatostatin in man and partial pancreatectomy in the rat both give rise to impaired first and second phase insulin responses to glucose and normal responses to non-glucose stimuli (Ward *et al*., 1983; Leahy *et al*., 1984).

It is now recognized that IDDM is a disease of slow onset, even though clinical presentation may be sudden and dramatic (Tarn *et al*., 1987). The preclinical phase may last for five years or more, may be characterized by a fluctuating rate of, cell attrition (Spencer *et al*., 1984) and first phase insulin response may decrease slowly before disappearing (Srikanta *et al*., 1983). It is highly likely that an immunological attack causes the progressive, cell damage (Bottazzo, 1986). However, immunological cell attack may not always progress to IDDM, and mild abnormalities in insulin secretion may result (Millward *et al*., 1986; Heaton *et al*., 1987).

2.5.2 Hormones Actions and Diabetes Mellitus

2.5.2.1 Insulin Action and Diabetes Mellitus

The action of insulin on cells requiring its effect to utilized glucose has created the point of analysis in terms of the understanding of biochemistry of diabetes especially type 2 which occurs as a result of the condition known as insulin resistance. The mechanism of

insulin action has been explained which gives a glimps to the understanding of the genesis of insulin resistance that is known to be the root cause of type 2 diabetes.

In the event of insulin action, insulin binds to insulin receptor to effects the influx of glucose into the cells. The insulin receptor (IR) is a heterotetramer consisting of two α subunits and two β subunits that are linked by disulphide bonds. Insulin binds to the α-subunit of the insulin receptor and activates the tyrosine kinase in the β-subunit. Once the tyrosine kinase of insulin receptor is activated, it promotes autophosphorylation of the β subunit, where phosphorylation of three tyrosine residues (Tyr-1158, Tyr-1162, and Tyr-1163) is required for amplification of the kinase activity (White *et al*., 1988). Most of the metabolic and antiapoptotic effects of insulin are mediated by the signalling pathway involving the phosphorylation of the insulin receptor substrate (IRS) proteins, and the activation of the phosphatidylinositol (PI) 3-kinase, Akt (also known as protein kinase B), the molecular target of rapamycin (mTOR), and p70 S6 kinase (Cheatham *et al*., 1994; Shepherd *et al*., 1995). The insulin receptor tyrosine kinase phosphorylates the IRS proteins, and phosphotyrosine residues on IRS proteins become good targets for the p85 regulatory subunit of PI3-kinase.

The activated PI3-kinase generates 3′-phosphoinositides (phosphatidyl-inositol-3,4-bisphosphate (PIP2) and phosphatidyl-inositol-3,4,5-trisphosphate (PIP3)) (Alessi and Cohen 1998) which bind to the phosphoinositidedependent kinase 1 (PDK1). Known substrates of the PDKs are the protein kinase B (PKB) and also atypical forms of the protein kinase C (PKC) (Kotani *et al.*, 1998).

Downstream from PI3-kinase, the serine/threonine kinase Akt (also called PKB), triggers insulin effects on the liver, such as glycogen synthesis and the suppression of hepatic glucose production. Akt plays an important role by linking glucose transporter

(GLUT4), the insulin dependent glucose transporter protein, to the insulin signalling pathway. It activates GLUT4 which moves to the cell surface to transport glucose into the cell (Kupriyanova and Kandror 1999; Pessin *et al*., 1999). Recent data from PKB knockout animal models offer a clearer answer to the question of whether PKB is required for normal glucose homeostasis. While disruption of PKB/Akt1 isoform in mice did not cause any significant perturbations in metabolism, mice with a knock-out of the PKB (Akt2) isoform show insulin resistance, ending up with a phenotype closely resembling type 2 diabetes in humans (Cho *et al*., 2001). Consistently, recent studies on inherited insulin post-receptor mutations in humans detected a missense mutation in the kinase domain of PKB (Akt2) in a family of severely insulin resistant patients. The mutant kinase was unable to phosphorylate downstream targets and to mediate inhibition of phosphoenolpyruvate carboxykinase (PEPCK), a gluconeogenic key enzyme (George *et al*., 2004). This suggests that the impairment of insulin activity leading to insulin resistance is linked to insulin signalling defects. These insulin signalling pathways are shown in Figure 2.2.

Free fatty acids are known to play a key role in promoting loss of insulin sensitivity in type 2 diabetes mellitus (Sarina, 2010). It has been postulated that an increase in the intracellular concentration of fatty acid metabolites activates a serine kinase cascade, which leads to defects in insulin signaling downstream to the insulin receptor (Qiao *et al*., 1999). In addition, the complex network of adipokines released from adipose tissue modulates the response of tissues to insulin. Among the many molecules involved in the intracellular processing of the signal provided by insulin, the insulin receptor substrate-2, the protein kinase B and the forkhead transcription factor Foxo 1a are of particular interest, as recent data has provided strong evidence that dysfunction of these proteins results in insulin resistance in vivo (Shepherd *et al*., 1998; Nakae *et al.*, 2002; Ueki *et*

al., 2002). Recently, studies have revealed that phosphoinositidedependent kinase-1-independent phosphorylation of protein kinase Cε causes a reduction in insulin receptor gene expression. Additionally, it has been suggested that mitochondrial dysfunction triggers activation of several serine kinases, and weakens insulin signal transduction (Moller, 2001; DiMauro and Schon, 2003; Petersen *et al*., 2003).

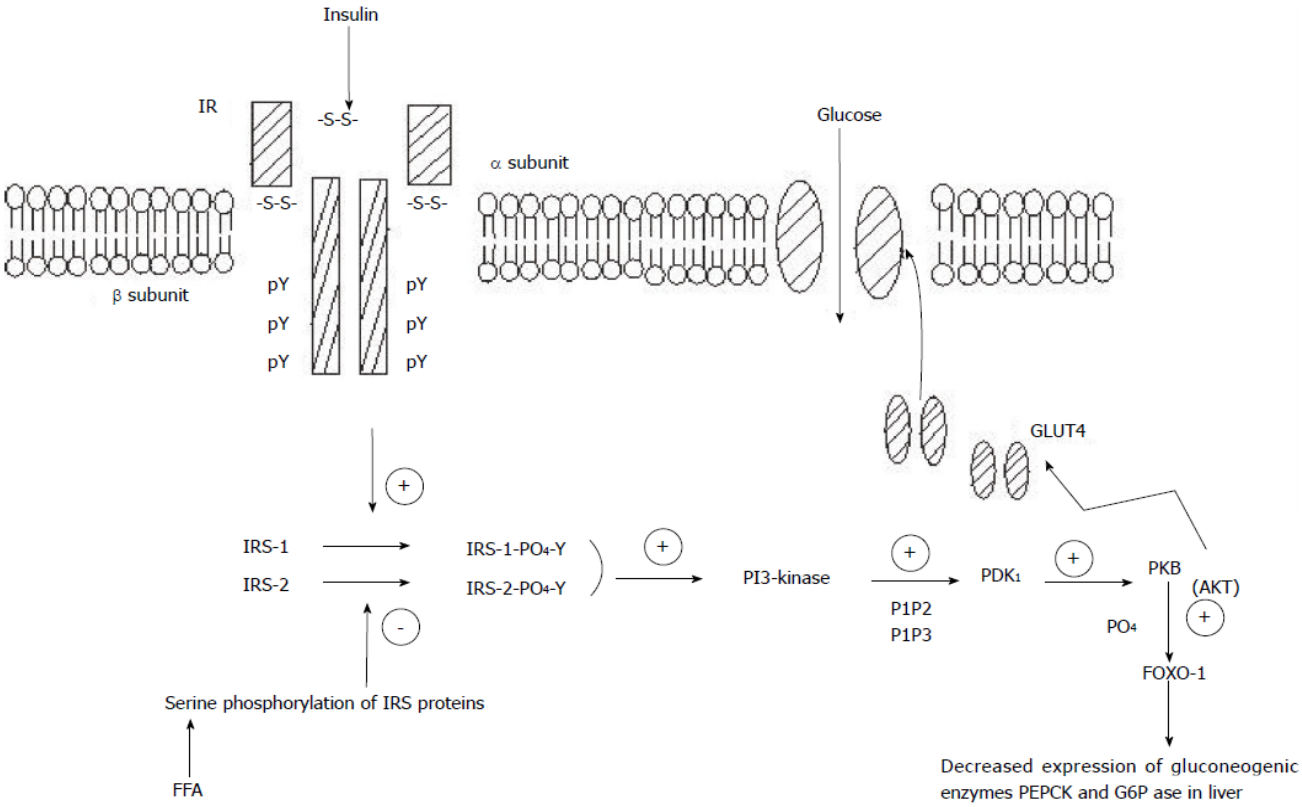

Figure 2.2: Insulin signalling pathway showing binding of insulin with the insulin receptor leading to the activation of glucose transporter 4 which imports glucose into the cell.

Source: Sarika, 2010

Key: Binding of insulin to the IR activates PI3-k which produces PI4, 5P2 and PI3, 4, 5P3. These serve as docking sites for PDK1 which then mediates activation of PKB. Activated PKB can regulate transcription of target genes-PEPCK and G6Pase via Foxo-1. Increased free fatty acids may cause serine phosphorylation of IRS proteins, which in turn decreases IRS-tyrosine phosphorylation, impairing downstream effectors. pY: phosphorylated tyrosine; IR: insulin receptor; IRS: insulin receptor protein; PI3-k: phosphatidylinositol 3-kinase; PDK1: phosphoinositidedependent kinase 1; PKB: protein kinase B; Foxo-1: forkhead box protein O; PEPCK: phosphoenolpyruvate carboxykinase; G6Pase: glucose-6-phosphatase; FFA: free fatty acids; PIP2: phosphatidyl-inositol-3,4-bisphosphate; PIP3: phosphatidyl-inositol- 3,4,5-tris-phosp-hate; GLUT4: glucose transporter 4.

2.5.2.2 Glucagon

Plasma glucagon levels are high in untreated diabetes (Gerich *et al*., 1976) and particularly during episodes of diabetic ketoacidosis (Muller *et al*., 1973), but are rapidly suppressed by insulin treatment (Gerich *et al.*, 1976; Stark *et al.*, 1987). In IDDM, glucagon secretion in response to hypoglycaemia is frequently blunted or absent. This subnormal response develops within 1-5 years after diagnosis and becomes more marked in later years (Gerich *et al.*, 1973; Benson *et al*., 1977; Bolli *et al.*, 1983). Impaired glucagon secretion is also observed in NIDDM (Bolli *et al.*, 1984).

Glucagon has a primary role in increasing hepatic glucose output by stimulating glycogenolysis and gluconeogenesis. In diabetic subjects, inhibition of glucagon secretion markedly suppresses the development of hyperglycaemia after insulin withdrawal. The stimulatory effect of a glucagon infusion on glucose output appears transient, because it returns to normal within about 90 min despite persistently high glucagon concentrations, probably due to feedback inhibition via insulin secretion (Bratusch-Marrain *et al*., 1979). Though the stimulation of glycogenolysis may be transient (Komjati *et al.,* 1985), that of gluconeogenesis is probably sustained because high glucagon levels unaccompanied by appropriate increases in insulin secretion result in a sustained increase in hepatic glucose output (Rizza *et al.*, 1979b). At physiological levels, glucagon infusion has been shown to have either no effect on glucose uptake or to cause mild glucose intolerance (Bajorunas *et al*., 1986), whether the latter is due to a direct effect of glucagon on extrahepatic tissues or to stimulation of catecholamine secretion is not clear.

2.5.2.3 Catecholamines

Catecholamine levels (adrenaline and noradrenaline) are elevated in insulin-dependent diabetics during ketoacidosis or poor metabolic control (Christensen, 1974). In man, adrenaline causes a transient increase in hepatic glucose production (Rizza *et al*., 1980) mediated through a β-receptor mechanism, while noradrenaline is weakly hyperglycaemic in normal and insulin-deficient man (Schade and Eaton, 1978). When glucagon secretion is normal, blockade of the adrenergic receptor system does not modify glucose counter-regulation following insulin induced hypoglycaemia (Rizza *et al.*, 1979a), suggesting that catecholamines may not be essential for glucose homoeostasis in hypoglycaemia. However, during infusion of insulin and somatostatin, to inhibit glucagon secretion, adrenergic blockade prevents the increase in hepatic glucose production in response to hypoglycaemia (Hansen *et al*., 1986), suggesting that catecholamines might be important for glucose counter-regulation when glucagon secretion is impaired. Adrenaline, noradrenaline and dopamine have been shown to be ketogenic in man; their effect is largely due to stimulation of lipolysis in adipose tissue, but they may also have a direct ketogenic effect on the liver (Keller *et al*., 1984; Keller, 1986). Stimulation of the sympathetic nerves in the perfused rat liver increases glucose production and ketogenesis (Beuers *et al*., 1986), suggesting that noradrenaline may have a role in regulation through sympathetic nerve stimulation.

2.5.2.4 Growth Hormone

Plasma growth hormone (GH) profiles are elevated in diabetics with long term complications and poor metabolic control (Fineberg and Merimee, 1974; Gerich, 1984). It is uncertain whether the high GH levels are the cause or the consequence of the metabolic imbalance. Infusion of physiological levels of GH in normal and

hypophysectomized subjects is associated with an early insulin-like effect on blood glucose followed by insulin resistance. The former is due to increased glucose clearance and suppressed glucose output, the latter is due to impaired suppression of glucose output and decreased glucose clearance (MacGorman *et al.*, 1981; Bratusch-Marrain *et al*., 1982). Physiological doses of GH increase ketone body levels in man (Schade *et al.*, 1978; Metcalfe *et al*., 1981; Keller *et al.*, 1984). This is due to increased lipolysis and possibly also increased ketogenesis at the hepatic level. Although some direct effects of GH on the liver have been demonstrated, including stimulation of glucose transport and oxidation (Fix and Moore, 1981), increased activity of phosphatidate phosphohydrolase (Pittner *et al*., 1986) and acute inactivation of acetyl-CoA carboxylase at micromolar GH concentrations (Bornstein *et al.*, 1983), there is as yet no evidence in vitro for a direct ketogenic effect of GH on liver cells at physiological hormone levels. In adipose tissue in vitro, acute insulin-like effects of GH on glucose transport and oxidation, leucine oxidation and glycogen synthesis and stimulation of lipolysis after prolonged exposure are well documented, although the effects are more prominent in adipose tissue from hypophysectomized than from normal animals (Davidson, 1987).

The high GH levels in IDDM have been implicated to be involved in the long-term complications associated with neovascularization (Gerich, 1984). GH regulates the production of insulin-like growth factors (IGF) by the liver, although IGF levels do not always correlate with GH (Herington *et al*., 1983). IGF I levels are raised in plasma and ocular vitreous in diabetics with proliferative retinopathy (Grant *et al*., 1986) and evidence in vitro has shown that IGF I increases the proliferation of bovine retinal endothelial cells in culture (King *et al*., 1985).

2.5.2.5 Glucocorticoids

Cortisol excess increases hepatic glucose output and ketogenesis in insulin-deprived diabetics (Barnes *et al.*, 1978) and in normal subjects treated with somatostatin to inhibit insulin secretion, but not when insulin secretion is unimpaired (Johnston *et al*., 1982). High cortisol levels have been observed in diabetic ketosis and have been implicated to aggravate increased lipolysis in adipose tissue and increased fractional diversion of fatty acids towards mitochondrial ketogenesis at the hepatic level (Johnston and Alberti, 1982). Glucocorticoid increased ketogenesis and gluconeogenesis in rat hepatocytes in the absence of insulin, but not in its presence (Agius *et al*., 1986a). The stimulatory effect is much smaller than that of glucagon, supporting the clinical evidence that cortisol has a relatively minor role as regulator of ketogenesis.

2.5.3 Metabolic Disturbance and Diabetes Mellitus

2.5.3.1 Carbohydrate Metabolism

Fasting State: In normal subjects, fasting blood glucose is maintained constant by control of hepatic glucose output. After an overnight fast, approx. 75 % of hepatic glucose output is accounted for by glycogenolysis and the rest by gluconeogenesis from lactate, alanine, glycerol, and pyruvate in decreasing order of importance (Hers and Hue, 1983). Hepatic glucose output is controlled by basal levels of insulin and glucagon. Basal hepatic extraction of insulin and glucagon is approximately 500 (Waldhausl *et al*., 1982) and hence insulin and glucagon concentrations in the peripheral circulation are lower than in the portal vein. At least 70 % of extrahepatic glucose utilization occurs in insulin-insensitive tissues (brain, red blood cells and renal medulla) (Vranic and Wrenshall, 1968). In NIDDM, fasting blood glucose is raised in direct proportion to hepatic glucose output (Bogardus *et al*., 1984; Revers *et al*., 1984;

DeFronzo *et al*., 1985), and appears unlikely to be a result of decreased insulin action at the periphery as it has not been shown to correlate closely with insulin-stimulated glucose disposal (DeFronzo *et al*., 1982; Bogardus *et al*., 1984). As fasting plasma insulin and C-peptide concentrations are normal in NIDDM, the elevated hepatic glucose output is likely to reflect a degree of hepatic insulin insensitivity. Study of the insulin dose-response of suppression of hepatic glucose output supports this concept (Revers *et al*., 1984).

Postprandial State: In normal subjects, first-pass hepatic glucose extraction is low and over 90 % of an oral glucose load reaches the peripheral circulation (Radziuk *et al.*, 1978; Pehling *et al.,* 1984). Over the subsequent few hours approximately one-third of glucose is cleared by the liver (Katz *et al*., 1983). Uncertainty persists about the proportion of glucose stored by the liver which is derived from 3-carbon precursors formed in the periphery, but this is currently thought to be considerable (Katz and McGarry, 1984). The post-meal hyperinsulinaemia inhibits lipolysis and stimulates storage of glucose and fatty acids as glycogen in muscle and liver and as triacylglycerol in adipose tissue.

In NIDDM subjects, study of plasma insulin levels throughout the day has demonstrated that, although the incremental responses to meals are decreased and delayed, the prolonged postprandial peaks ensure that mean diurnal plasma insulin levels are elevated (Liu *et al.,* 1983). Postprandial rates of gluconeogenesis are not suppressed (Wahren *et al*., 1972; Hall *et al*., 1979) and total hepatic glucose release as assessed isotopically is excessive (Firth *et al*., 1986). Gluconeogenesis from meal-derived 3-carbon precursors is somewhat greater in NIDDM than in normal subjects.

Although the insulin-mediated increment in muscle glucose uptake is subnormal in NIDDM, absolute postprandial rates of glucose uptake are normal when measured directly by the forearm technique at prevailing blood glucose concentrations despite the marked insulin insensitivity (Jackson *et al*., 1973; Firth *et al*., 1986). This reflects the mass action effect of glucose on tissue uptake (Revers *et al*., 1984).

Relatively few studies on human muscle enzymes have been carried out. Falholt *et al.* (1987) found decreased fasting activity of hexokinase and phosphofructokinase, suggesting a potential decrease in glycolysis in NIDDM subjects, but glucose-6-phosphate dehydrogenase and malic enzyme activities were elevated and so was muscle triacylglycerol. However, the patients studied were remarkably hyperinsulinaemic, fasting plasma insulin levels being 6-fold elevated, and thus not representative of the majority of NIDDM subjects.

In subjects with IDDM, postprandial hyperglycaemia is the consequence of the lack of an appropriate sharp increase in circulating insulin levels, and reflects inadequacies in the dynamics of insulin delivery. Hepatic release of glucose is relatively uninhibited and this, together with the demonstrated resistance to insulin in peripheral tissues (Proietto *et al*., 1983; Yki-Jarvinen *et al*., 1984), results in postprandial hyperglycaemia. A striking feature is the high lactate in IDDM and poorly controlled NIDDM. Strong correlations have been reported between mean 24 h plasma insulin and lactate concentrations (Alberti *et al*., 1975; Capaldo *et al*., 1984). The high lactate persists during establishment of normoglycaemia over a 24 h period, although in this situation measured Cori cycle activity is suppressed (Nosadini *et al*., 1982). It is possible that the hyperlactataemia may represent an imbalance of insulin action on peripheral tissues compared with liver, as subcutaneous or intravenous insulin administration leads to

equal levels of insulin in the peripheral circulation and portal vein, unlike the normal situation. Restoration of the portal-peripheral insulin gradient returns lactate concentrations towards normal (Stevenson *et al*., 1983; Jimenez *et al*., 1985). In poorly controlled NIDDM subjects, the portal-peripheral insulin gradient is preserved, but it is possible that peripheral tissues become relatively more resistant to insulin action.

2.5.3.2 Disturbances in Lipid Metabolism

Fatty acid mobilization, production and utilization of ketone bodies. One of the most prominent features of insulin deficiency is rapid mobilization of fatty acids from adipose tissue. In IDDM, excessive lipolysis during insulin deficiency is the combined result of insulin lack and insulin resistance (Singh *et al*., 1987). One of the consequences of excessive mobilization of fatty acids in IDDM is the production of ketone bodies (acetoacetate, 3-hydroxybutyrate and acetone) in liver. Fatty acids taken up by the liver, after conversion to their CoA esters, are either esterified to glycerolipid or oxidized to acetyl-CoA in mitochondria. A high proportion of the acetyl-CoA formed is converted to acetoacetate and 3-hydroxybutyrate. The rate of transfer of fatty acyl units to the mitochondria is regulated by the activity of carnitine palmitoyltransferase I (EC 2.3.1.21), which faces the intermitochondrial membrane space and catalyses the first step specific to mitochondrial fatty acid oxidation. Carnitine palmitoyltransferase I is regulated by malonyl-CoA, an intermediate in fatty acid synthesis, and it is also regulated by a phosphorylation mechanism. Malonyl-CoA decreases the affinity of the enzyme for its fatty acyl-CoA substrate (McGarry *et al.*, 1977), while phosphorylation increases the affinity for substrate (Harano *et al.*, 1985). In insulin deficiency, the rate of fatty acid synthesis in liver declines and consequently the concentration of malonyl-CoA also decreases, and the affinity of carnitine palmitoyltransferase for malonyl-CoA

also decreases (Gamble and Cook, 1985), thus relieving the inhibition of carnitine palmitoyltransferase by malonyl-CoA. Changes in the kinetic properties of detergent-solubilized carnitine palmitoyltransferase following exposure of liver cells to glucagon (activation) or insulin (inactivation) have also been observed (Harano *et al*., 1985; Agius *et al*., 1986b), indicating that additional mechanisms contribute to activation (or deinhibition) of carnitine palmitoyltransferase in insulin deficiency, thereby favouring increased transfer of long-chain fatty acids into mitochondria.

The utilization of acetoacetate and 3-hydroxybutyrate as oxidative fuels (or lipogenic substrates) by a variety of tissues is well established (Robinson and Williamson, 1980) and increases with blood ketone body concentration in the fed-to-fasted transition (Miles *et al*., 1980). In muscle, at ketone body concentrations attained in prolonged starvation or diabetic ketosis, the rate of uptake reaches saturation (Owen and Reichard, 1971). Consequently, with increasing ketone body production and thereby plasma concentration, there is a progressive decrease in total fractional clearance (Fery and Balasse, 1985).

Although the biochemical routes of metabolism of acetoacetate and 3-hydroxybutyrate are well established, the conversion of acetoacetate to acetone and its subsequent excretion or metabolism has only recently received attention. In man, plasma acetone correlates with, and is generally higher than, acetoacetate in fasting and diabetic ketosis (Owen *et al*., 1982) and the production rate is estimated to be about half the rate of ketogenesis (Reichard *et al*., 1986). Conversion of acetoacetate to acetone can occur either non-enzymically or catalysed by acetoacetate decarboxylase (EC 4.1.1.4) (Argiles, 1986). The occurrence of this enzyme has been demonstrated in various rat tissues, but only in human plasma (Koorevaar and van Stekelenburg, 1976). The high

acetone production rate in man suggests the occurrence of the enzyme in other tissues. At low plasma acetone concentrations (1-2 mM), as occur in fasting ketosis, about 20 % is excreted (in breath and urine) and the rest metabolized, whereas at higher concentrations, as in diabetic ketosis (7-9 mM), about 80 % is excreted (Owen *et al*., 1982). In the rat, acetone is hydroxylated to acetol which is either converted to methylglyoxal or to propane-1,2-diol (Figure 2.3). The former is converted to pyruvate either directly or indirectly (Cassava *et al*., 1984), whereas propane- 1 ,2-diol is either oxidized to L-lactate or converted to acetate and formate (Kosugi *et al*., 1986a) (Figure 2.3). Studies on the incorporation of 2-^{14}C acetone into glucose in the rat have shown that at low plasma, acetone is metabolized primarily to lactate and pyruvate, but at high plasma acetone, acetate formation predominates (Kosugi *et al*., 1986b). There is evidence that suggested that in human diabetic ketosis acetone metabolism may be very similar (Reichard *et al*., 1986). 2-^{14}C Acetone was incorporated into glucose via pyruvate and lactate in the majority of patients, but, in one subject with high plasma acetone, label incorporation was predominantly via acetate formation (Reichard *et al*., 1986). These findings suggest that, in moderate ketosis in man, acetone is a potential gluconeogenic substrate.

Triacylglycerol secretion and clearance: The plasma triacylglycerol concentration is elevated in both IDDM and NIDDM (Nikkila, 1984). In IDDM, decreased clearance seems to be the main cause of the high triacylglycerol level (Bagdade *et al*., 1968; Nikkila *et al*., 1977; Taskinen and Nikkila, 1979), whereas in NIDDM the triacylglycerolaemia may be due to increased production by the liver (Greenfield *et al*., 1980; Kissebah *et al*., 1982; Dunn *et al*., 1984) and decreased clearance (Nikkila *et al*., 1977; Taskinen *et al*., 1982; Pfeifer *et al*., 1983).

Production of triacylglycerol by the liver involves the esterification of fatty acid, either synthesized de novo in the liver from dietary carbohydrate and amino acid, or derived from adipose tissue reserves. The triacylglycerol is either stored intracellularly or it is packaged with apoproteins and secreted in the form of very low density lipoprotein (VLDL). Under certain conditions fatty acid esterification increases with increasing fatty acid concentration and appears to be non-saturable (Ontko, 1972). VLDL secretion, in contrast, may be limited by the availability of apoproteins or other components of the lipoprotein particle. When VLDL secretion reaches saturation, triacylglycerol accumulates inside the liver. The elevated plasma triacylglycerol in diabetes is generally associated with VLDL, although increases in other lipoproteins (LDL and HDL) also occur (Gibbons, 1986).

In absolute insulin deficiency, the plasma concentration and turnover of fatty acids increases, whereas fatty acid synthesis de novo decreases and the proportion of fatty acyl-CoA that is esterified as opposed to oxidized also decreases. Any absolute increase in hepatic fatty acid esterification depends on whether the increase in fatty acid availability compensates for the increased fractional diversion towards mitochondrial oxidation. In patients with IDDM, intensive insulin therapy, which is associated with higher mean plasma insulin compared with conventional insulin therapy, results in a decrease in triacylglycerol secretion rate (Pietri *et al.*, 1983; Dunn *et al*., 1987). Whether the effect of the increased insulinization was primarily due to a decrease in fatty acid availability or to inhibition of VLDL secretion is not clear.

Triacylglycerol secretion rates are generally higher in NIDDM than in IDDM (Greenfield *et al*., 1980). In normolipaemic NIDDM, triacylglycerol secretion and clearance are both increased, whereas in hyperlipaemic NIDDM, fractional turnover rate

is reduced such that increased triacylglycerol removal does not compensate for the increase in secretion rate (Kissebah *et al*., 1982).

In NIDDM with decreased VLDL clearance, the composition of VLDL is abnormal, with a high triacylglycerol/ apoprotein B ratio (Taskinen *et al*., 1986), indicating multiple abnormalities in VLDL metabolism. A key issue is whether changes in adipose tissue lipolysis, or intrahepatic mechanisms involving either changes in fractional esterification of fatty acid or in the assembly and secretion of VLDL, are responsible for the increase in triacylglycerol secretion rate in NIDDM.

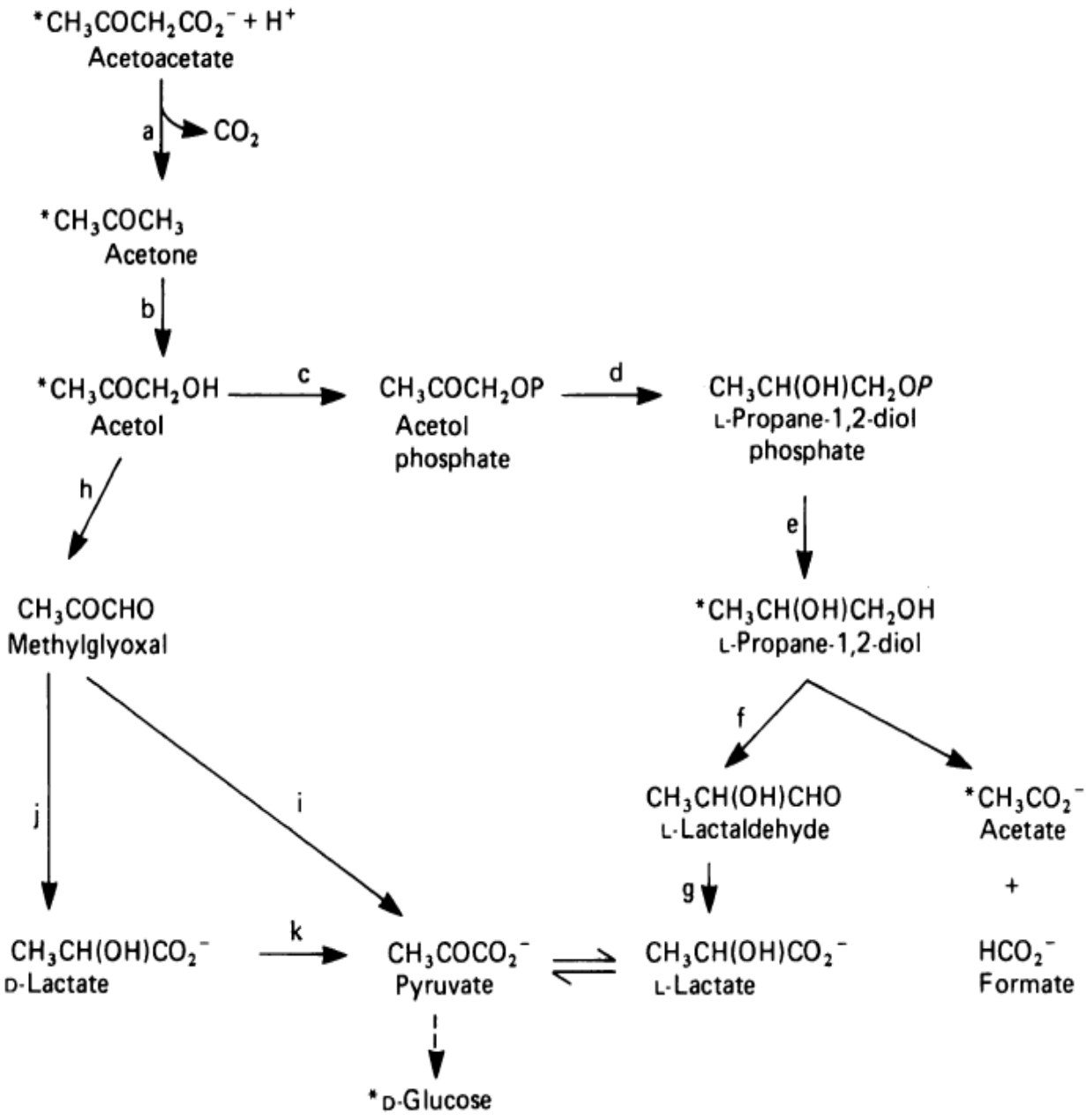

Figure 2.3: Postulated pathways for the metabolism of acetone to glucose and Acetate

Source: Taylor and Agius, 1988

Key: Intermediates marked by * increase in human plasma during ketosis (Reichard et al., 1986). Enzymes: a, acetoacetate decarboxylase; b, acetone mono-oxygenase (NADP+); c, acetol kinase; d, propanediol phosphate dehydrogenase (NADP+); e, phosphatase; f, lactaldehyde reductase [NAD(P)+]; g, lactaldehyde dehydrogenase (NAD+); h, acetol mono-oxygenase (NADP+); i, 2-oxoaldehyde dehydrogenase [NAD(P)+]; j, glyoxylase I and II; k, D-2-hydroxyacid dehydrogenase (FAD); 1, lactate dehydrogenase (NAD+).

2.5.4 Biochemistry of Diabetes-Induced Damage

Many hypotheses about how hyperglycemia causes diabetic complications have generated a large amount of data as well as several clinical trials based on specific inhibitors of these mechanisms. The main hypotheses are: the Aldose Reductase theory (Sorbitol (polyol) pathway), Advanced Glycation End Product (AGE) theory, Activation of Protein Kinase C (PKC) isoform theory, Increased Hexosamine Pathway Flux theory, and the Reactive Oxygen Intermediate theory.

2.5.4.1 The Sorbitol (polyol) Pathway

One of the consequences of hyperglycaemia in human diabetes mellitus is increased metabolism of glucose by the sorbitol pathway. This involves the reduction of glucose to sorbitol catalysed by aldose reductase (EC 1.1. 1.21) and the oxidation of sorbitol to fructose by sorbitol dehydrogenase (EC 1.1.1.14). Aldose reductase is present in human brain, nerves, aorta, muscle, erythrocytes and ocular lens (Srivastava *et al*., 1984; Das and Srivastava, 1985). Although the purified enzyme has a low affinity for glucose (Km approximately 100 mM) (Moonsammy and Stewart, 1967), it can be activated by glucose 6-phosphate, NADPH and glucose (Das and Srivastava, 1985).

Sorbitol is not permeable to cell membranes and tends to accumulate in the cell. At high glucose the flux through the sorbitol pathway in rabbit lens may account for one-third of glucose metabolism (Gonzalez *et al.*, 1984). This has important implications in terms of redox changes of NADP and NAD couples and metabolism of glucose by alternative pathways (Jeffrey and Jornvall, 1983). Conversion of glucose to sorbitol by aldose reductase requires NADPH and forms $NADP^+$ (Figure 2.4) and thereby competes with other NADPH-requiring reactions.

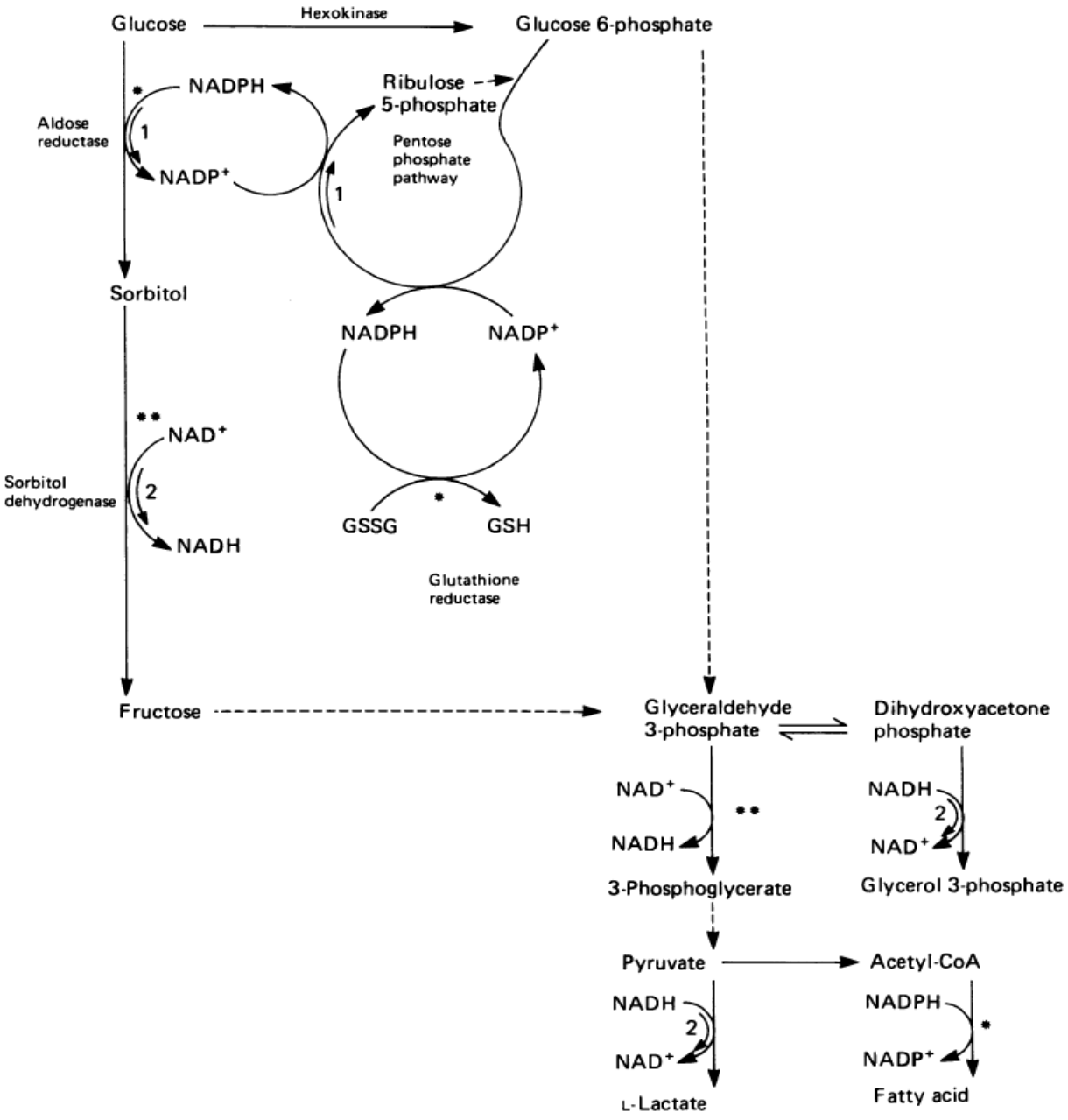

Figure 2.4: The sorbitol pathway and its links with the pentose phosphate pathway and glycolysis through the $NADP^+$ and NAD^+ redox couples.

Source: Taylor and Agius, 1988

Key: The sorbitol pathway involves: conversion of glucose to sorbitol by aldose reductase and sorbitol to fructose by sorbitol dehydrogenase. Reactions coupled to oxidation of NADPH are indicated by * and reactions coupled to reduction of NAD^+ are indicated by **. Aldose reductase competes with glutathione reductase for NADPH and sorbitol dehydrogenase competes with glyceraldehyde-3-phosphate dehydrogenase for NAD^+. (1) An increased flux through aldose reductase favours an increased activity of the pentose phosphate pathway (Gonzales *et al.*, 1986) and increased flux through sorbitol dehydrogenase favours increased conversion of dihydroxyacetone phosphate to glycerol 3-phosphate (Gonzalez *et al.*, 1983) and decreased conversion of glyceraldehyde 3-phosphate to 3-phosphoglycerate.

NADPH is required for the conversion of oxidized to reduced glutathione, a powerful antioxidant which protects cellular components from oxidative damage, and for fatty acid and cholesterol biosynthesis. The pentose phosphate pathway is the major source of NADPH in most tissues and its flux is generally determined by the $NADP^+$/NADPH ratio. Conversion of sorbitol to fructose is coupled to reduction of NAD+ to NADH and this competes with glycolysis at the glyceraldehyde dehydrogenase step for NAD^+ (Gonzalez *et al*., 1986). An increase in the NADH/NAD^+ ratio favours increased conversion of dihydroxyacetone phosphate to glycerol-3-phosphate. In rat lens, increased flux through aldose reductase is associated with increased pentose phosphate pathway activity, decreased glycolysis (Gonzalez *et al.*, 1986), accumulation of glycerol-3-phosphate and depletion of reduced glutathione (Whikehart and Soppet, 1981; Gonzalez *et al*., 1984).

2.5.4.2 Advanced Glycation End Products (AGEs)

AGEs are found in increased amounts in diabetic retinal vessels (Stitt *et al.*, 1997) and renal glomeruli (Horit *et al*., 1997). AGE inhibitors partially prevented various functional and structural manifestations of diabetic microvascular diseases in retina, kidney, and nerve. The AGE inhibitor amino guanidine lowered total urinary protein and slowed progression of neuropathy (Nakamura *et al*., 1997). Production of intracellular AGE precursors damages target cells by three general mechanisms: intracellular proteins modified by AGEs have altered function; extracellular matrix components modified by AGE precursors interact abnormally with other matrix components and with the receptors for matrix proteins (integrins) on cells; and plasma proteins modified by AGE precursors bind to receptors of AGE (RAGE) on endothelial

cells, mesangial cells, and macrophages, inducing receptor-mediated production of reactive oxygen species (Degendardt, 1998).

The AGE receptor ligation activates the pleiotropic transcription factors, causing pathological changes in gene expression along with other cellular signalling events, such as activation of mitogen-activated protein (MAP) kinase or PKC, which can lead to cellular dysfunction (Ishii *et al*., 1996).

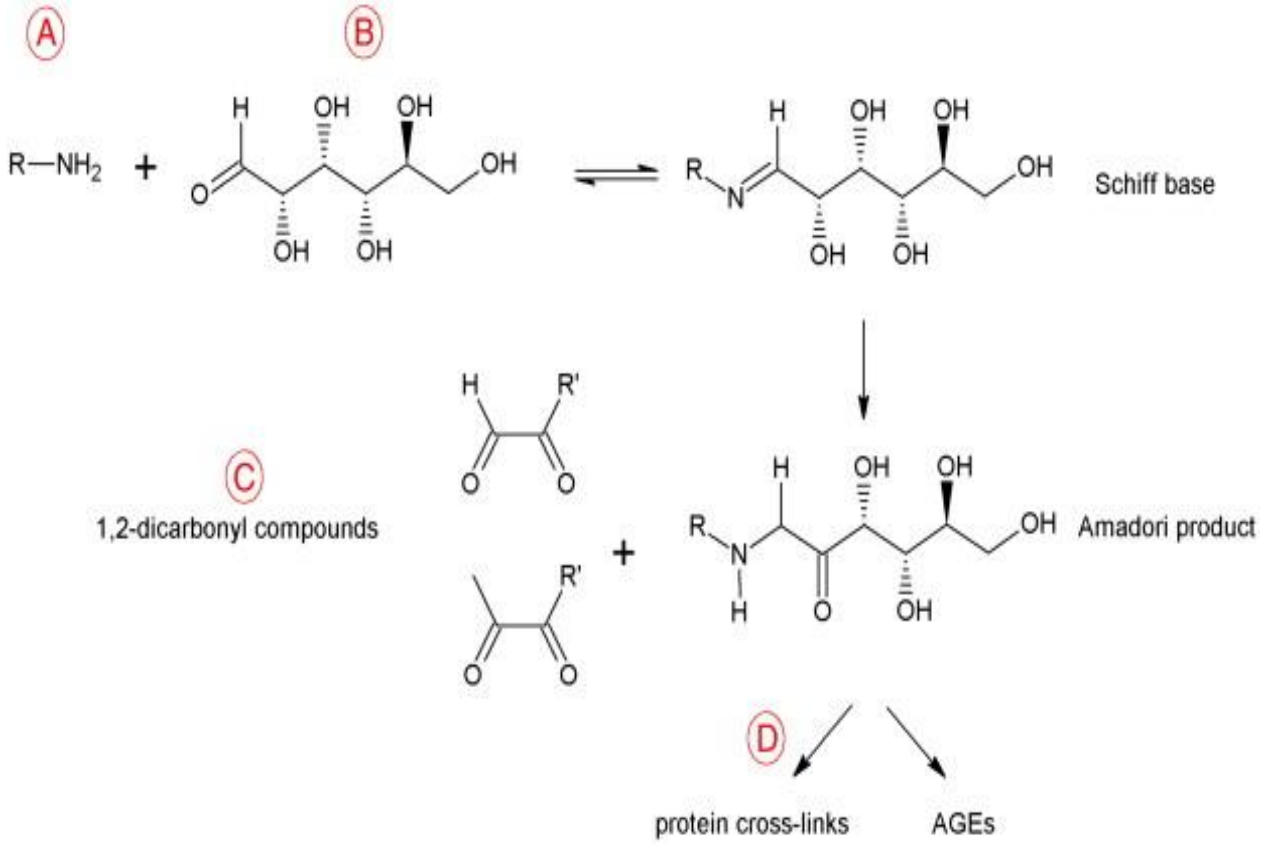

Figure 2.5: Formation of Advanced glycosylation end product
Source: Martina and Gerald, (2010)

Key: (A) proteins , (B) Glucose, (C) Carbonyl compound, (D) Products

2.5.4.3 Diacylglycerol (DAG) and Protein Kinase C (PKC)

These are critical intracellular signaling molecules that can regulate many vascular functions, including permeability, vasodilator release, endothelial activation, and growth factor signalling. The PKC family comprises at least eleven isoform, nine of which are activated by the lipid second messenger DAG. Intracellular hyperglycemia increases the amount of DAG in cultured microvascular cells and in the retina and renal glomeruli of diabetic animals (Koya *et al*., 1997). Increased *de novo* synthesis of DAG leads to the activation of PKC b isoforms which have been shown to mediate retinal and renal blood flow abnormalities. Activation of PKC by raised glucose also induces expression of the permeability-enhancing factor VEGF in smooth muscle cells. Treatment with an inhibitor specific for PKC b significantly reduced PKC activity in the retina and renal glomeruli of diabetic animals (Koya *et al*., 2002). Concomitantly, treatment significantly reduced diabetes-induced increases in retinal mean circulation time, normalized increases in glomerular filtration rate, and partially corrected urinary albumin excretion.

2.5.4.4 Hexosamine Pathway

Shunting of excess intracellular glucose into the hexosamine pathway might also cause several manifestations of diabetic complications (Kolm *et al*., 1998). In this pathway, fructose 6-phosphate is diverted from glycolysis to provide substrates for reactions that require UDP-N-acetylglucosamine, such as proteoglycans synthesis and the formation of O-linked glycoproteins (see Figure 2.6).

Inhibition of the rate-limiting enzyme in the conversion of glucose to glucosamine-glutamine, fructose-6-phosphate amidotransferase (GFAT), blocks hyperglycemia-

induced increases in the transcription of TGF α, TGF β, and PAI-1. This pathway has also an important role in hyperglycemia induced and fat-induced insulin resistance.

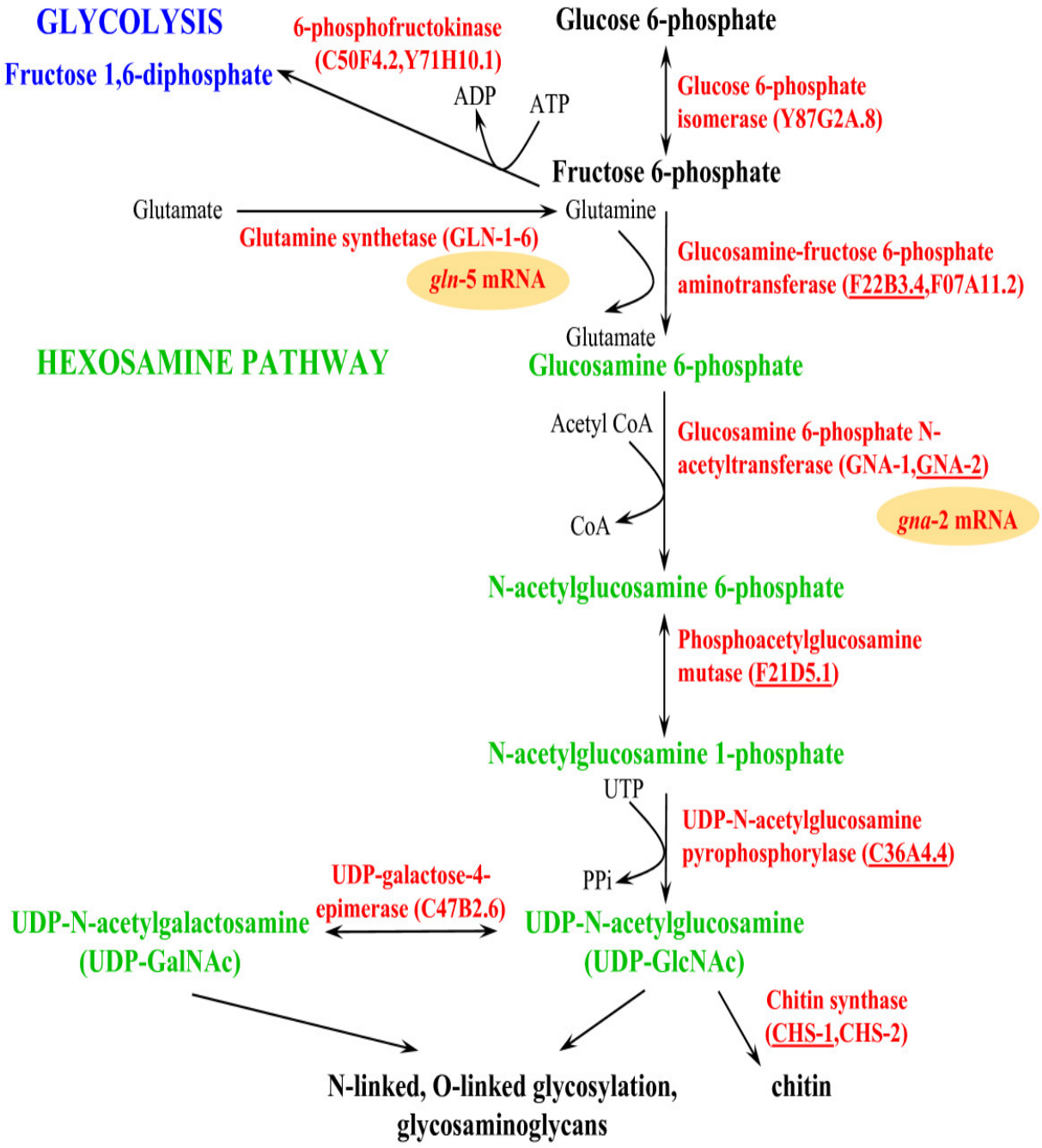

Figure 2.6: Hexosamine pathway

Source: http://www.biomedcentral.com/content/download/figures/1741-7007-4-35-1-pdf.

2.5.4.5 Reactive Oxygen Intermediate Theory

Hyperglycemia can increase oxidative stress through both enzymatic and non-enzymatic processes. Glucose metabolism through the glycolytic pathway and TCA cycle produces reducing equivalents used to drive the synthesis of ATP via oxidative phosphorylation in mitochondria. By products of mitochondrial oxidation include free radicals such as superoxide anion, whose generation increases with increased glucose levels. Glucose oxidation also produces free radicals which damage cellular proteins as well as mitochondrial DNA (Santos, *et al.*, 2013).

Increased oxidative stress reduces nitric oxide levels, damages cellular proteins, and promotes leukocyte adhesion to the endothelium while inhibiting its barrier function. Levels of antioxidants such as GSH, vitamin C, and vitamin E have been reported to be decreased in patients with diabetes, while the levels of some markers of oxidative stress, oxidized low-density lipoprotein cholesterol are increased (John, 1991).

2.6 Epidemiology of Diabetes Mellitus

2.6.1 Prevalence and Incidence

A global epidemic of diabetes mellitus was predicted for the first quarter of the twenty first century (Greenberg and Sacks, 2002). Diabetes is amongst the five leading causes of deaths these days (WHO, 1994). Each year, over three million deaths worldwide are attributed to diabetes-related causes (International Diabetes Federation (IDF), 2003)

Diabetes prevalence increases with increasing age, affecting 18.4% of those 65 years of age or older (Centre for Disease Control and Prevention, 2012). Diabetes is particularly prevalent in minorities. Indeed, the prevalence of type 2 diabetes is highest in ethnic minorities in the United States, such as African Americans, Hispanic populations

(Latinos and Mexican Americans), Native Americans and Alaska Natives, Asian Americans, and Pacific Islanders.

Diabetes mellitus contributes to a considerable increase in morbidity and mortality rates, which can be reduced by early diagnosis and treatment. In 2002 diabetes costs in the United States were $132 billion. Direct medical expenditures, such as inpatient care, outpatient services, and nursing home care, totalled $91.8 billion. Indirect costs, totalling $39.8 billion, were associated with lost productivity, including pre-mature death and disability. Total medical expenditures incurred by people with diabetes totalled $91.8 billion, or an average annual total direct cost of medical care of $13,243 per person compared with $2560 per person without diabetes (ADA, 2003).

In 2000, about 15 million U.S. adults 18 years of age or older had diagnosed diabetes (6.3 million men and 8.7 million women), representing an increase from 4.9% of the adult population in 1990 to 7.3% in 2000. If undiagnosed diabetes is considered as well, it is likely that almost 10% of U.S. adults have diabetes (Mokdad *et al.,* 2001). Much of the increase is because type 2 diabetes is no longer a disease that affects mainly older adults. Between 1990 and 1998, the prevalence of diabetes increased by 76% among people in their thirties (Mokdad *et al.,* 2001). Among children with newly diagnosed diabetes, the prevalence of type 2 diabetes also increased dramatically in the past decade, growing from less than 4% in the years preceding 1990 to as high as 45% in certain racial/ethnic groups in recent years (ADA, 2000).

2.6.1.1 Global Epidemic

Diabetes is reaching epidemic proportions. In 2003, the International Diabetes Federation (IDF) estimated that approximately 194 million people around the world had

diabetes. By 2025 this figure is expected to rise to 333 million, amounting to 6.3% of the world's population living with diabetes.

As of 2010 an estimated 285 million people had diabetes, with type 2 making up about 90% of the cases (Mbanya *et al.*, 2010). The prevalence of type 2 diabetes is rising at alarming rate throughout the world, due to increases in life expectancy, obesity and sedentary lifestyles. Of particular cause for concern is the dramatic rise of type 2 diabetes in children and adolescents.

2.6.1.2 Diabetes in Africa

The Africa continent counts approximately 13.6 million people with diabetes. The Africa Region of IDF, which mainly includes sub-Sahara Africa, counts approximately 7 million people with diabetes. Estimate for the region for 2025 are likely to double and reach 15 million.

Whereas Nigeria has the highest number with diabetes (with approximately 1,218,000 people affected). Reunion has the highest prevalence in the African Region (13.1%). Nigeria also has the highest number of people with impaired glucose tolerance with an estimated 3.85 million people.

The studies from Tanzania (urban/rural ratio of 5:1) and Cameroon (ratio of 2:1) both confirm the marked urban/rural discrepancy in diabetes prevalence. It is estimated that undiagnosed diabetes accounts for 60% of those with the disease in Cameroon, 70% in Ghana and over 80% in Tanzania.

Table 2.1 Prevalence of diabetes and its future projection worldwide

year	2000	2003	2025	2030
World	171,000,000	194,000,000	333,000,000	366,000,000

Source: International Diabetes Federation (IDA) estimate, 2003

Table 2.2: Prevalence of Diabetes in West Africa

S/N	**COUNTRY**	**2000**	**2030**
1.	Nigeria	1,707,000	4,835,000
2.	Benin	87,000	266,000
3.	Niger	108,000	382,000
4.	Zambia	70,000	186,000
5.	Senegal	143,000	421,000
6.	Mali	140,000	405,000
7.	Liberia	40,000	154,000
8.	Ghana	302,000	851,000
9.	Gambia	22,000	61,000
10.	Gabon	8,000	14,000
11.	Côte d'Ivoire	264,000	636,000
12.	Chad	97,000	269,000
13.	Central African Republic	18,000	38,000
14.	Cape Verde	7,000	24,000
15.	Burkina Faso	124,000	388,000
16.	Equatorial Guinea	8,000	21,000
	TOTAL	**2,902,400**	**8,951,000**

Source: International Diabetes Federation (IDF) estimate, 2003

2.6.2 Risk Factors

The causes of diabetes are complex and only partly understood. This disease is generally considered multifactorial, involving several predisposing conditions and risk factors. In many cases genetics, habits and environment may all contribute to a person's diabetes.

To complicate matters, there can be contrary risk factors for the various forms of the disease. For example, autoimmune diabetes (type 1 and latent autoimmune diabetes of adulthood, LADA) is more common in white people, but metabolic diabetes (type 2 and gestational diabetes) is more common in people of other races and ethnicities. Type 1 is usually diagnosed in children, but advancing age is a risk factor for type 2 and gestational diabetes. Insulin resistance, prediabetes and metabolic syndrome are strong risk factors for type 2 diabetes. Other diabetic risk factors and causes include: (http://www.mayoclinic.com/health/diabetes/DS01121/DSECTION=risk-factors)

Genetics and family history: Certain genes are known to cause maturity-onset diabetes of the young (MODY) and Wolfram syndrome. Genes also contribute to other forms of diabetes, including types 1 and 2.

Family medical history is also influential to varying degrees: For example, a person whose parents both have type 1 diabetes has a 10 to 25% chance of developing that disease, according to the American Diabetes Association, and someone whose parents both have type 2 diabetes has a 50% chance of developing that disease.

Weight and body type: Overweight and obesity are leading factors in type 2 diabetes and gestational diabetes. Excess fat, especially around the abdomen (central obesity), promotes insulin resistance and metabolic syndrome.

Most people with autoimmune diabetes (type 1 and LADA) are of normal weight, and excess weight has not traditionally been considered to be related to these conditions. However, recent research indicates that obesity may hasten the development of type 1 diabetes and that the increasing rate of type 1 diabetes may be at least partly due to the rise of childhood obesity. Furthermore, patients with autoimmune diabetes who gain weight are susceptible to insulin resistance and double diabetes.

Sex: Though men make up less than 49% of the U.S. adult population, they account for 53% of the adult cases of diabetes, according to the National Institutes of Health (NIH). The prevalence of diabetes in American men and women was similar until 1999, when a growing disparity began, according to an analysis of statistics published by the U.S. Centers for Disease Control and Prevention (CDC). Little or no research has been conducted to explain this trend. One factor may be the documented increase in recent years of low testosterone levels (male hypogonadism), which scientists have linked to insulin resistance.

Level of physical activity: Lack of regular exercise is blamed for much of the twin global epidemics of obesity and diabetes.

Diet: The effect of diet in the development of diabetes is controversial. Some studies have linked heavy consumption of soft drinks and other simple carbohydrates to risk of metabolic diabetes, and foods low in the glycemic index, such as whole grains, to reduced risk. Yet the ADA states that eating foods containing sugar does not cause the disease. The culprit, rather, is the weight gain due to sedentary habits and excess intake of calories, according to the ADA.

Another dispute centers around whether being fed cow's milk early in life might be linked to type 1 diabetes. Some researchers have noted a connection, but others have not. Further scientific research is needed on this topic.

Other diseases: Medical conditions including high blood pressure, hyperlipidemia (unhealthy levels of cholesterol), polycystic ovarian syndrome, asthma and sleep apnea have been linked to type 2 diabetes. Celiac disease (gluten intolerance) and other autoimmune diseases have been linked to type 1. The many conditions that may cause secondary diabetes include pancreatitis, hemochromatosis, endocrine disorders including hyperthyroiddism, Cushing's disease and acromegaly, and genetic conditions including cystic fibrosis, Down syndrome and some forms of muscular dystrophy, Diabetic foot and urinary tract infection (Lipsky *et al*., 2004; Mokabberi and Ravakhah, 2007).

Hormones: These chemical messengers can contribute to diabetes in various ways. For example, stress hormones such as cortisol have been linked to fluctuating glucose levels in type 2 diabetes, and stress hormones in women during pregnancy have been linked to risk of type 1 diabetes in the child. The release of growth and sex hormones during adolescence may make some teens more susceptible to diabetes. A wide range of hormonal treatments including anabolic steroids, growth hormone, estrogens, injected contraceptives, androgen deprivation therapy for prostate cancer and corticosteroids have been linked to secondary diabetes.

Medical treatments: In addition to hormonal therapies, medications including diuretics, beta blockers (another class of antihypertensives), immunosuppressives, antiretrovirals (AIDS/HIV drugs) antipsychotics, lithium, and some antidepressants, anticonvulsants and chemotherapy drugs have been linked to an increased risk of

secondary diabetes. Pancreatectomy and radiation therapy may also result in secondary diabetes. Drugs including pentamidine (used to treat pneumonia) and L–asparaginase (used to treat leukemia) have been linked to type 1 diabetes.

Other chemicals: In addition to these pharmaceuticals, some studies have linked PCBs, other pollutants and certain pesticides including the defoliant Agent Orange and dioxin (its active ingredient) to insulin resistance and type 2 diabetes. Common consumer plastics and plastics ingredients including phthalates and bisphenol A have also been linked to insulin resistance in some cases.

Exposure to agricultural pesticides during pregnancy has been tentatively linked to gestational diabetes. A rat poison called pyriminal has been linked to type 1 diabetes.

Other environmental factors: Some researchers theorize that free radicals may contribute to the development of type 1 and possibly other forms of diabetes. Free radicals are formed as a result of chemical reactions in the body. Smoke, air pollution and even genetics contribute to the formation of free radicals. When these radicals build up, they can destroy cells, including those involved in the production of insulin.

Cold weather is another possible environmental factor in type 1 diabetes. This disease occurs more commonly in cold climates and develops more frequently in the winter than the summer.

Viruses: Some people are diagnosed with type 1 diabetes after a viral infection. Viruses thought to be related to type 1 diabetes include mumps, rubella and coxsackie virus (related to the virus family that causes polio and hepatitis).

Smoking: Cigarette smoking is a risk factor for type 2 diabetes and possibly other forms of diabetes.

Alcohol: Excessive use of alcohol is a risk factor for diabetes. For example, it can cause pancreatitis. However, some research has found that light drinking may decrease the risk of becoming diabetic.

Most of these risk factors can be described as either uncontrollable, such as genetics and age, or controllable, such as exercise and diet. Some, such as obesity, may involve genetics and lifestyle choices. People cannot alter their uncontrollable risk factors, but they can lower their risk of developing diabetes by reducing controllable risk factors through improved health habits.

2.7 Diagnosis of Diabetes Mellitus

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following (WHO, 1999):

a) Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl)

b) Plasma glucose ≥ 11.1 mmol/l (200 mg/dL) two hours after a 75 g oral glucose load as in a glucose tolerance test.

c) Symptoms of hyperglycemia and casual plasma glucose ≥ 11.1 mmol/l (200 mg/dl)

d) Glycated hemoglobin (Hb A1C) ≥ 6.5%. (American Diabetes Association, 2010)

A positive result, in the absence of unequivocal hyperglycemia, should be confirmed by a repeat of any of the above methods on a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test (Saydah *et al*., 2001). According to

the current definition, two fasting glucose measurements above 126 mg/dl (7.0 mmol/l) are considered diagnostic for diabetes mellitus.

People with fasting glucose levels from 110 to 125 mg/dl (6.1-6.9 mmol/l) are considered to have impaired fasting glucose (WHO, 2006). Patients with plasma glucose at or above 140 mg/dL (7.8 mmol/L), but not over 200 mg/dL (11.1 mmol/L), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two prediabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus, as well as cardiovascular disease (Santaguida *et al*., 2013). Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause (Selvin *et al*., 2010).

2.8 Prevention, Management and Treatment

2.8.1 Prevention Methods for Diabetes

There is no known way of preventing autoimmune forms of diabetes (type 1 and latent autoimmune diabetes of adulthood) or genetic conditions such as maturity-onset diabetes of the young and Wolfram syndrome. Genetic tests can reveal who is at risk, and clinical trials are investigating potential methods of preventing type 1 including vaccines and pre-emptive use of insulin. People can take many steps to reduce their risk of developing metabolic forms of diabetes (type 2 and gestational diabetes). The primary focus is on managing weight through regular exercise and a sensible diet. Such practices also help people with other forms of diabetes avoid insulin resistance and double diabetes. It is also beneficial to control blood pressure and cholesterol, avoid smoking and restrict alcohol.

People with risk factors for diabetes should be screened regularly with glucose tests. Early detection and treatment can avert many diabetic complications. Those who already have diabetes can help prevent complications such as diabetic retinopathy and diabetic neuropathy by maintaining control over their glucose levels and following other aspects of their care plan, as the Diabetes Control and Complications Trial and many other research projects have affirmed.

Scientists are conducting a great deal of research into the diagnosis, monitoring, treatment and prevention of diabetes. Recent advances include inhaled insulin, new antidiabetic agents, and continuous glucose monitoring and implantable insulin pumps. Projects in development range from stem cell research to refinement of an artificial pancreas. Though there is still no cure for diabetes, a disease that was once a death sentence has become a chronic condition that can be managed (Naila *et al*., 2009).

2.8.2 Management and Treatment of Diabetes Mellitus

According to the WHO (1994) guidline for the management of diabetes mellitus, management is based on some certain principles and also stipulated objectives. These are highlighted below:

1. Correct diagnosis is essential. Thus emphasis should be placed on using appropriate diagnostic criteria.
2. Treatment should not only consider lowering the blood glucose level but also should focus on the correction of any associated risk factors and complications.
3. The doctor should work closely with the nurse and other members of the diabetes health care team, whenever available, and with the person with diabetes.

4. Self-care is an essential strategy. Education of the person with diabetes and his/her family is the cornerstone of management. Without appropriate education, the desired therapy targets are difficult, or even impossible to achieve. People with diabetes should be encouraged and enabled to participate actively in managing and monitoring their condition.
5. Good control is important. Self-monitoring improves the quality and safety of therapy.
6. The health care system should ensure that people with diabetes have access to the basic requirements essential to practise self-care.
7. Record-keeping is critically needed and should be considered a basic requirement for the management and follow-up of all cases.
8. Objectives and priorities of treatment must be tailored to individual needs; therapy targets should be individually determined for each case.
9. These objectives include; To relieve symptoms, To correct associated health problems and to reduce morbidity, mortality and economic costs of diabetes, To prevent as much as possible acute and long-term complications; to monitor the development of such complications and to provide timely intervention, To improve the quality of life and productivity of the individual with diabetes

Mangament and treatment of diabetes mellitus depends basically on the type of diabetes. Generally, there are Pharmacological, Novel ways and non-pharmacological therapy of managing and treating diabetes mellitus (Brajendra and Arvind, 2006). Thus, the hallmark in the management and treatment of diabetes mellitus is to make sure that glucose is controlled and to avoid complications usually caused by diabetes.

2.8.2.1 Pharmacological Therapy

Pharmacological Therapeutic Strategies and Targets

The current therapeutic approaches were largely developed in the absence of the fine molecular targets or understanding of the pathogenesis of the diseases. In last few years a large number of molecular drug targets involving various biochemical pathways have been worked out. These are based on the predicted roles in modulating one or more key aspects of the pathogenesis of the diabetes and metabolic syndrome. These are: Reducing excessive glucose production by liver, targeting β-cells, targeting insulin-signaling pathways, and targeting lipid metabolism (Figure 2.7).

Reducing Excessive Hepatic Glucose Production

The liver, by way of gluconeogenesis and glycogenolysis, plays a very important role in regulating endogenous glucose production by the synthesis or breakdown of glycogen. Increased rates of hepatic glucose production are largely responsible for the development of overt hyperglycemia. Glucagon contributes to hyperglycemia through induction of the gluconeogenesis and glycogenolytic pathways (Unger, 1971; Shah *et al*., 2000). Its receptor, a seven-transmembrane-domain G protein receptor, could be a target for the development of small-molecule antagonists (Connell, 1999). Besides, several enzymes that regulate rate-controlling steps in the gluconeogenesis or glycogenolytic pathways can also be used as molecular targets for therapeutic intervention. One such enzyme is inhibition of hepatic glycogen phosphorylase (Treadway *et al*., 2001), an enzyme that catalyses the release of glucose from glycogen. Others are fructose-1, 6-bisphosphatase and glucose-6-biphosphatase (Zhang and Moller, 2000). Whereas inhibition of fructose-1, 6-bisphosphatase would selectively block gluconeogenesis by disrupting the conversion of fructose-1,6-biphosphate to

fructose-6-phosphate, inhibition of glucose-6-phosphatase would attenuate the final step in hepatic glucose production common to the gluconeogenic and glycogenolytic pathways.

Treating β-cells

Two distinct gut-derived peptide hormones, glucagon-likepeptide-1 (GLP-1) and gastric inhibitory peptide (GIP), act through their respective G protein-coupled receptors on β-cells to potentiate glucose-stimulated insulin secretion (Drucker, 2001). Administration of any of these two hormones to humans can potentiate insulin secretion. Since both hormones are subject to rapid amino terminal degradation by dipeptidylpeptidase- IV (DP-IV), use of modified GLP-1 peptide agonists resistant to this enzyme has been recommended. It is observed that DP-IV null mice have increased circulating active GLP-1 along with enhanced insulin secretion, and an otherwise healthy phenotype (Marguet, 2000). Thus, development of GLP-1 analogues and DP-IV inhibitors is likely to yield important new therapeutic approaches that might circumvent the liabilities of hypoglycemia, weight gain, and secondary failures associated with sulphonylureas use.

Targeting the Insulin Signaling Pathways

Insulin resistance can be due to multiple defects in signal transduction, such as impaired activation of insulin receptor- tyrosine kinase and reduced activation of insulin-stimulated phosphatidylinositol-3-OH kinase (PI-3-K). A number of molecular targets are now being investigated as ways of enhancing insulin-mediated signal transduction. Elevated expression of PTP 1B has been reported in insulin-resistant patients (Drake and Posner, 1998). Overexpression of this enzyme prevents insulin receptor kinase activation. A PTP 1B knockout mouse was more insulin sensitive than control littermates. Thus inhibition of PTP 1B represents a good target for drug discovery

(Goldstein *et al*., 1998). Serine kinases may phosphorylate and thus inhibit the tyrosine phosphorylation of IRS 1 in experimental paradigms of insulin resistance. Identification of these kinases and specific inhibitors represents another rich area for antidiabetic therapy. Similarly, products of PI-3-kinase play a critical role in insulin action and might be reduced during insulin resistance. Other putative negative regulators of insulin signalling have recently been implicated as independent drug targets.

Glycogen synthase kinase-3 (GSK-3) has a clear role in opposing the effect of insulin by inhibiting the activation of glycogen synthase and the subsequent accumulation of glycogen in muscle (Weston and Davis, 2001). Recent results with selected inhibitors of GSK-3 activity *in vivo* could indeed augment insulin action (Henriken *et al*., 2001). The SH2 domain containing inositol 5-phophatase type 2 (SHIP 2) may function to dephosphorylate key phospholipids, such as phosphatidyl inositol phosphate generated by insulin-mediated PI-3-K activation.

Recently, heterozygous null mice have been shown to display enhanced sensitivity to insulin, implicating this enzyme as a diabetic target (Clement *et al*., 2001). Protein kinase C θ could be an additional drug target, as increased muscle PKC θ activity has been observed in the context of fatty acid-induced insulin resistance (Shulman, 2000).

Targeting Lipid Metabolism

Since obesity plays an important role in the development of insulin resistance, attenuating the appetite and/or enhancing energy expenditure will be of great use in treating type 2 diabetes. Melanocortin-4 receptor (MCR-4) offers the prospects of ameliorating obesity and type 2 diabetes.

Thus, either an increase in the expression of a natural MCR- 4 antagonist or knockout of the receptor itself produces a strong phenotype with multiple features of metabolic syndrome (Klebig *et al.,* 1995; Huszar *et al*., 1997). Appetite reduction through central inhibition of fatty acid synthase also offers a new target (Loftus *et al*., 2000). cAMP-activated protein kinase, acetyl CoA carboxylase, adipocyte- related complement protein 30, PPAR-γ, and PPAR-α represent some of the mechanisms that could be exploited to reverse or prevent obesity-related lipotoxicity (Winder, 1999).

PPARs are ligand-activated transcription factors which are members of a nuclear receptor family offering a promising therapeutic target for metabolic syndrome. PPAR-γ is a predominant molecular target for insulin-sensitizing thiazolidinedione (TZD) drugs (Willson *et al*., 2000; Moller and Greene, 2001). PPAR- γ affects the gene transcription in adipose tissues leading to induction of adipocyte genes, such as those for lipoprotein lipase and fatty acid transporter-1, which results in improvement in insulin action along with lowering of triglyceride and FFA levels (Moller and Greene, 2001). A closely related nuclear receptor, PPAR-α, is the molecular target for the fibrate class of lipid-modulating drugs. (Willson *et al*., 2000). PPAR-α agonists have an independent insulin-sensitizing effect arising from reduction in muscle lipid content (Ye *et al*., 2001).

Antidiabetic Drugs

Hyperglycemia in patients with diabetes mellitus is always the result of a mismatch between the quantity of insulin necessary to regulate the person's metabolic processes and the amount of insulin being secreted by the person's β-cells. Patients with insulin resistance start with a relative insulin deficiency and with passing years frequently progress to an absolute insulin deficiency. Antihyperglycemic agents such as Insulin

(Extended Insulin, zinc insulin (ultralente), Insulin gargine (Lantus) and (Insulin determir) Levemir) reduces the level of glucose in the blood plasma, thiazolidinediones (Rosiglitazone (arandia) , Pioglitazone (Actos), Troglitazone (Rezuline), Hinterthuer, 2008) or Biguanides (metformin (glucophage), Phenformin (DBI), and Buformin) decrease insulin resistance (Bennett *et al*., 2011), α-glucosidase inhibitors (Miglitol (Glyser), Acarbose (Precose/Glucobay) and Voglibose) decrease postprandial insulin needs (Elizabeth and Steven, 2008), insulin secretagogues (Sulfonylureas;Tolbutamide (Orinase branded name), Acetohexamide (Dymelor), Tolazamide (Tolinase), Chlorpropanide (Diabenese) are known as the first generation agents and Glipizide (Glucotrol), Glyburide or Glibenclamide (Diabeta, micronase, Glynase), Glimepiride (Amanyl), Gliclazide (Diamicron), Glycopyramide and Gliquidone, are the second generation agents) improve and increase endogenous insulin secretion (Cambon-Thompson *et al*., 2007) and insulin and its analogues replace endogenous insulin secretion by exogenous insulin administration.

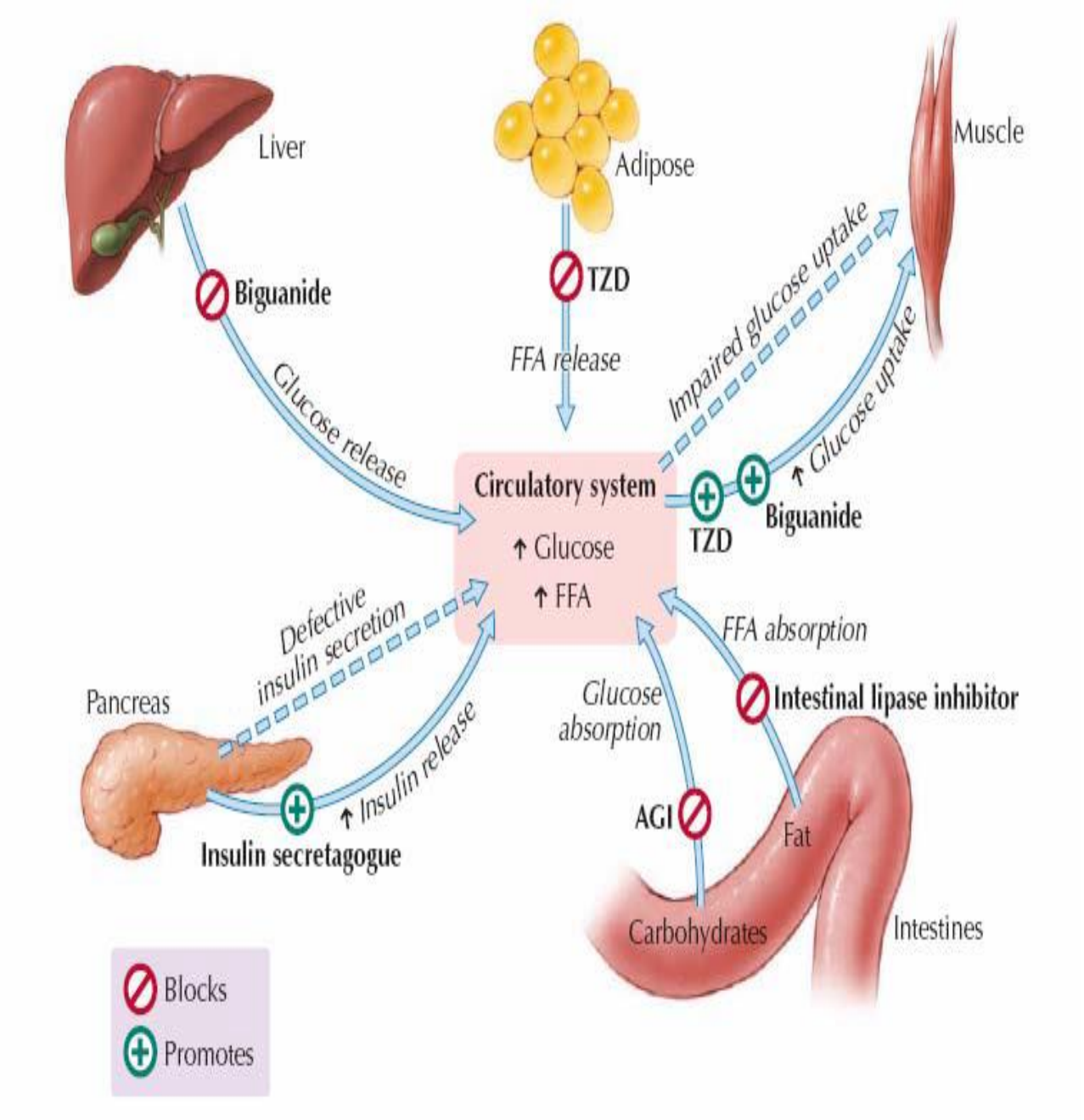

Figure 2.7: Major target organs and mechanism of actions of orally administered antihyperglycemic agents in type II diabetes mellitus

Source: Cheng and Fantus, 2005

Key: TZD = thiazolidinedione; FFA = free fatty acid; AGI = α-glucosidase inhibitor

2.8.2.2 Novel Ways of Managing and Treating Diabetes Mellitus

The Role of Nanotechnology in Diabetes Management.

It is imperative that effective monitoring and treatment options be available to patients. Nanotechnology is a focal point in diabetes research, where nanoparticles in particular are showing great promise in improving the treatment and management of the disease (Dennis *et al*., 2011). Due to their ability to potentially enhance drug delivery to areas where there are barriers or unfavourable environments for macromolecules, nanoparticles are being explored as vehicles for improved oral insulin formulations. This also goes inline with the use of nanomaterials to deliver certain antidiabetic drugs (Yamaguchi, 2006; Karthikeyan *et al.*, 2012). The use of nanotechnology in the development of glucose sensors is also a prominent focus in non-invasive glucose monitoring systems. Finally, compelling but relatively unexplored applications of nanotechnology, such as the development of an artificial pancreas is one of the prospecting area for the feature (Renzo, *et al.*, 2006). Overall, nanotechnology has the potential to significantly impact several areas of current and future diabetes research.

Regenerative Medicine and Organ Transplanting

Regenerative medicine will be crucial in achieving the ultimate goal in diabetes treatment in other to free diabetic patients from the need to inject insulin. While conventional medication serves purely a disease management, regenerative medicine may actually allow restoring endogenous insulin production by providing stem-cell based therapeutic modalities capable of conserving and rebuilding pancreatic islets. A breakthrough in this technology may finally provide curative therapies for both type 1 and type 2 diabetes. In addition, regenerative medicine therapeutic strategies could also play a significant role in the management of the most serious and complex complication

of diabetes by providing therapies with intelligent nanostructured biomaterials releasing bioactive molecules with the purpose of efficient healing of diabetic complication (diabetic foot ulcers) (Renzo, *et al.*, 2006).

The current alternative to insulin injections is a pancreas transplant but there are many side effects and complications associated with this type of treatment. As this is organ donation the patient is put on a waiting list which usually entails a very long waiting period and could take years, sometimes too late for the patient. There are other factors adding to the problem of the long waiting lists such as cross matching tissue, this is so that the donor organ and recipient are a match so that the body does not reject the organ. Following the operation the patient would also be required to use immune suppressant drugs, leaving them susceptible to hospital viruses such as the H1N1 super virus. With all these factors the success rate of the transplant are around 90% giving the patients freedom from insulin dependency. The organs can approximately function up to 10 years after surgery, it is then estimated that only half the transplants are expected to continue working successfully.

2.8.2.3 Non-Pharmacological Therapy of Managing Diabetes Mellitus

Nutrition therapy

Caloric content

Most patients with NIDDM are overweight or obese, and it is now well recognized that this is a major factor in insulin resistance. Consequently, reduction of excess weight is a primary component in the management of NIDDM. When extreme caloric restriction and/or rapid weight loss seem desirable, a very low caloric diet or protein-sparing modified fast may be considered (ADA, 2004).

Macronutrients

The ideal balance of carbohydrate, protein, or fat intake in patients with NIDDM is still a matter of discussion. It has recently been recognized that a diet containing 60% carbohydrates, even if not including sugar, may predispose to the development of dyslipidemia (Garg *et al*., 1988). Carbohydrates should be predominantly complex and high in soluble fiber; foods with an aglycemic index (Jenkins *et al*., 1989) are preferred, although moderate intake of simple sugar such as sucrose does not seem to be detrimental (Brenner *et al*., 1982). Protein intake should not exceed the daily requirement, since high protein intake appears to have a detrimental effect on renal function (Brenner *et al*., 1982).

Dietary fibers

Numerous studies reviewed by Hoewitz (1990) have shown that addition of certain types of soluble fiber, particularly guar gum and pectin, may result in significant reduction of postprandial glucose and insulin levels in patients with NIDDM.

Fish oils

There is some evidence that fish oils or fish-derived omega- 3 fatty acids may play some role in preventing atherosclerotic vascular disease by reducing plasma triglyceride and lipoprotein levels (Axelrod, 1989). However, there is also evidence that in NIDDM the decrease in plasma triglyceride levels is counterbalanced by adverse effects on blood glucose or low density lipoprotein (LDL)-cholesterol (Hendra *et al*., 1990).

Physical Activity (Exercise)

Recent clinical investigations have shed light on the mechanism by which exercise may help in controlling excessive blood glucose levels (Berger *et al.*, 1982). Furthermore, there is good evidence that regular exercise has a positive influence via various cardiovascular risk factors that worsen diagnostics in patients with type 2 diabetes (ADA, 1990). However, in a small proportion of patients exercise may be harmful and, therefore, should not be prescribed. Regular exercise improves insulin sensitivity and, as a consequence, may improve glucose tolerance (Horton, 1986). Such effects result partly from enzymatic adaptation in skeletal muscles, considered to be responsible for improvement in maximal oxygen uptake, and partly from a decrease in body weight, body fat and, possibly, also cell size. Such effects are beneficial in patients with type 2 diabetes since they enhance work capacity and quality of life and may also help to reduce the requirement for insulin or oral hypoglycemic agents.

Medicinal Plants Therapy

Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine. It has been estimated that about 80-85% of population both in developed and developing countries rely on traditional medicine for their primary health care needs and it is known that a major part of traditional therapy involves the use of plant extracts or their active principles (Tomlinson and Akerele, 1998; Elujoba *et al*., 2005; Ignacimuthu *et al*., 2006). Due to lack of organized health care systems in developing countries like Nigeria, people with chronic diseases like diabetes are among the worst suffers in their communities today. Hence, the majority of the populations instead of orthodox medicine prefers traditional medicines for a range of diseases

including diabetes and its complications (Kochhar and Nagi, 2005; Zibula and Ojewole, 2000).

The active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents. Different species of medicinal plants are used in the treatment of diabetes mellitus. For diabetes treatment, before the discovery of insulin by Banting and Best in 1922, the only options were those based on traditional practices (Ribnicky *et al*., 2006). Metformin is an example of antidiabetic drug derived from medicinal plant *Galega officinalis* for the treatment of non insulin dependent diabetes mellitus patients (Oubre *et al*., 1997; Eshrat, 2002). Some examples of medicinal plant used in the treatment of diabetes mellitus are listed (Appendix I).

2.9 Mistletoe Plants and the Medicinal Value

Mistletoe is the common name for obligate hemi-parasitic plants in several families in the order Santalales. These plants grow attached to and penetrating within the branches of a tree or shrub by a structure called the haustorium, through which they absorb nutrients from the host plant. Among the families of mistletoe include *Viscaceae* and *Loranthaceae*, both of these plant families are taxonomically related and are part of the order *Santalales*.

The *Viscaceae* family is composed of seven genera namely *Arceuthobium, Ginalloa, Dendrophthora, Phoradendron, Korthalsella, Notothixos, Viscum.* The *Loranthaceae* is a large family of about 75 genera and over 900 species (Judd *et al*., 2002). It is speculated to have originated in Eastern Asia (Barlow, 1983) or on the Gondwanan landmass with a further intrusive element spreading south into the Malesian and Australian regions. The family has three terrestrial, root parasitic genera and 72 genera of aerial branch parasites (Wilson *et al*., 2006).

The Loranthacean mistletoes are tropical and occur as parasites on both angiosperms and gymnosperms (Dembele *et al*., 1994). Six major genera are found in Nigeria namely: *Tapinanthus* (Blume) Reichb, *Agelanthus* Tieghem, *Loranthus* L., *Globimetula* Tieghem, *Phragmanthera* Tieghem *and Englerina* Tieghem (Johri and Bhatnagar, 1972; Omolaja and Gamaye, 1998).

Mistletoe plants are well known for their medicinal importance from time immemorial which has been highly revered in different cultures (Marzell, 1923). In folklore medicine, majority of the species of mistletoe have been reported for their anticancer, antihypertensive, antidiabetic, antiepileptic and fertility functions (Obatomi *et al.,* 1994). A tea prepared from leaves of mistletoe is used traditionally to treat diabetes in the West Indies (Peters 1957). This treatment has been shown also to relieve the diabetic symptoms of severely hyperglycaemic streptozotocin-diabetic mice, including polydipsia, hyperphagia and body weight loss (Swanston-Flatt *et al.,* 1989). Mistletoe which are also called 'Afomo' in Yoruba, 'Kauchi' in Hausa, 'Apari' in Igbo, 'Osuochi' in Ebira, and 'Esu' in Nupe, is ethnomedicinally used by various ethnicities in Nigeria as a remedy for several human and animal ailments such as diarrhoea, dysentery, wound, cancer, haemorrhage, convulsion (Adesina, 1979; Adesina and Ojewole, 1983), gynaecology problems and cardiovascular diseases (Adodo, 2004). It has been found to have anti-microbial properties against certain multiple-drug-resistant bacterial and fungal isolates of farm animals (Deeni and Sadiq, 2002).

Mistletoe plant has provided a number of products including those derived for the treatment of cancer. This product called Iscador has other variant products known as Eurixor, Helixor, Lektinol, Isorel and Abnoba-viscum. It is well known that the medicinal functions of mistletoe are dependent on the specie type, the host plant it

parasitized and the extract type (Bako, 2001; Wahab *et al.,* 2010). *Globimetula braunii* and *Globimetula oreophila* grows on locust beans plant and shea butter respectively which have been used locally for treating many ailments including diabetes mellitus. Thus, these two species were actually selected in other to appraise their purported local usage for treating diabetes mellitus.

2.9.1 General Profile of *Globimetula braunii*

2.9.1.1 Taxonomy

Kingdom : Plantae

Phylum : Tracheophyta

Class : Magnoliopsida

Order : Santalales

Family : Loranthaceae

Genus : Globimetula

Species : Globimetula braunii

Name: *Globimetula braunii* (ENGL.) Van tiegh.
Source: *arctos.database.museum/TaxonomyResults.cfm?full...Globimetula*

2.9.1.2 Ecology

Globimetula braunii is a hemi-parasitic plant that grows on deciduous trees preferring those with soft bark like old Apple trees, Guava, Coffea, Hevea, cocoa, citrus and other trees.

2.9.1.3 Botanical Description of Plant

Twigs terete or elliptic in section, glabrous. Leaves opposite, subopposite or sometimes (in Zaire basin) ternate; petiole 5–15 mm. long; lamina fleshy, reddish when young,

turning light to dark green, elliptic, elliptic-oblong or elliptic-ovate, 4–10 cm. long, 1.5–7 cm. wide, blunt or rounded at the apex, broadly cuneate to shortly cordate at the base; second and third main lateral nerves curved-ascending to upper half, the distal ones more spreading. Umbels 3–6, mostly at older leafless nodes; peduncle 3–20 mm. long, glabrous or sometimes scurfy; pedicels 4–9(–15), 2.5–5 mm. long; bract-cup 0.5 mm. long, limb triangular or bifid, 0.5–1.2 mm. long. Receptacle 1–1.5 mm. long; calyx 0.5–0.7 mm. long. Corolla red or pink, the apical swelling darkening in ripe buds, 2.5–3.5 cm. long; apical swelling of mature buds subhemispherical, slightly pointed, pentagonal, slightly reduplicate proximally, 2.5–3.5 mm. across, 2–2.5 mm. tall; basal swelling usually subglobose, 2–4 mm. long, 2–3 mm. in diameter, rarely ellipsoid. Inner anthers 1.5–2 mm. long; outer ones 1–1.5 mm. Style-constriction 1.5–2 mm. long; stigma peltate to depressed-turbinate, 1.5–2 mm. across. Berry orange or red, 5–6 mm. long, 5 mm. in diameter; seed orange or red, 3 mm. long, 1.5 mm. in diameter (Appendix II).

2.9.1.4 Medicinal and Functional Uses

The leaves of *Globimetula braunii* is usually use to hasten delivery in traditional medicine practice. According to local herb sellers of the Yoruba tribe residing in South western Nigeria, *Globimetula braunii* is locally called Afomo onishano is reputed to be effective for treating many diseases ranging from headache, leg pain, rheumatism to pulmonary troubles (Okpuzor *et al*., 2009a). There are claims that the leaves, fruits and flowers of the subject plant have been implicated in the management of high blood pressure, while the roots attaching it to the host plant are used for other therapeutic applications like ulcer and cancer treatment (Burkill, 1985). Folklore usage of *G.braunii* is also well known for the treatment of diabetes and sickle cell anaemia by the Nupes of North-west part of Nigeria. The aqueous extract of *G.braunii* have been shown to

augment labour when compared with convectional drugs atropine and salbutamol in rat, these gives a further appraisal for its local use as a regimen to hasten quick child birth during labour (Le and Zam, 2008). Antilipemic/hypocholesteremic activities of the methanolic extracts of *Globimetula braunii* were also studied in the tissues of normo and hypercholesteremic rats and it was found out that there was significant increase in the level of High Density Lipoproteins cholesterol as compared to hypercholesteremic control (Ochuko *et al.,* 2011). The laxative property of *G.braunii* has also been appraised scientifically (Fred-Jaiyesimi *et al.,* 2008). Also a number of researches has shows it antimicrobial properties against various strains of microorganism (Inuwa *et al.,* 2012).

2.9.2 General Profile of *Globimetula oreophila*

2.9.2.1 Taxonomy

Kingdom : Plantae
Phylum : Magnoliophyta
Class : Magnoliopsida
Order : Santalales
Family : Loranthaceae
Genus : Globimetula
Species : Globimetula oreophila

Name: *Globimetula oreophila* (Oliv.) Van tiegh.
Source: http://www.*arctos.database.museum/TaxonomyResults.cfm?full...Globimetula*

2.9.2.2 Ecology

A parasite on trees and shrubs such as shea butter tree, guava forming a bush to about 1 m diameter; in North-Eastern Nigeria and Western Cameroons, and in Eastern Cameroun (Burkhil, 1985).

2.9.2.3 Botanical Description of Plant

Young branchlets brown, slightly glossy, compressed above, subterete and lenticellate below, glabrous, older ones yellowish-brown, longitudinally lenticellate; internodes 1–1 3/4 in. long. Leaves opposite, ovate-oblong or lanceolate, obtuse or shortly acuminate to an obtuse apex, rounded or subcordate at the base, 2 1/2–4 in. long, 1–1 1/2 in. broad, coriaceous, glabrous, slightly glossy; lateral nerves 4–5 on each side, patulous, arcuate-ascending, anastomosing well within the margin; petiole 4–7 lin. long. Umbels axillary, fascicled, about 20-flowered; peduncle 1/2– 3/4 in. long; pedicels 2–2 1/2 lin. long; bract obliquely cupular, 1–1 1/8 lin. long, ciliate, dorsal margin 7/8 lin. long, umbonate on the lower half, ventral margin 3/8 lin. long, more or less toothed. Receptacle and calyx together campanulate, 1–1 1/8 lin. long. Calyx irregularly lobed, ciliate, 1/2 lin. long. Corolla 1 1/2 in. long, split unilaterally to within 6 1/2 lin. above the base, apical swelling of the bud not winged, basal swelling obovoid, pentagonal, 2 lin. long; tube glandular within; lobes elongate-spathulate, 8 1/2 lin. long from the point of insertion of the filaments, remaining united nearly to the apex. Filaments 7 lin. long, broadened towards the apex; anthers oblong, emarginate, 7/8 lin. long. Disc 1/4 lin. high, fleshy. Style glabrous, neck 1 lin. long; stigma cushion-shaped, 1/2– 5/8 lin. in diam., grooved on the upper surface (Appendix II).

2.9.2.4 Medicinal and Functional Uses

Globimetula oreophila has been notably used locally against hypertension, skin diseases and also headache in Chinese folklore medicine (Huaxing and Michael, 2012). The Products derived from *Globimetula oreophila* have been used in farming, forestry, hunting and the production of fishing apparatus (Burkhill, 1985).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Material

The two mistletoe species (*Globimetula braunii* and *Globimetula oreophila*) leaf parts were collected from the host plants (*Parkia bigblibosa* and *Vitellera paradoxa* respectively) in College of Education, Minna between April and May 2012 and were identified and authenticated at the Herbarium of the Department of Biological Science, Ahmadu Bello University, Zaria. Voucher specimens (V/no. 2829 and V/no. 2998 respectively) were deposited at the Herbarium of the same Department for future reference.

3.1.2 Animals

A total of one hundred and fifty healthy male and female wistar albino rats of 6-8 weeks old were obtained from the Animal House Centre, College of Health Sciences, Benue State University Makurdi, Nigeria. They were conveniently housed in the Department of Biochemistry Laboratory II, Federal University of Technology, Minna, under standard environmental conditions (temperature 27$\pm$ 2^{o}C, 70% relative humidity, 12hrs day light/night cycle) and had free access to commercial feed pellets (by growers) and water. They were then allowed to acclimatize to the laboratory conditions for two weeks before the commencement of the experiments (Nagappa *et al*., 2003; Miura *et al*., 2005; Kumar *et al*., 2006). Prior to each study, the animals were made to fast for 12 hours but had free access to water (Ragavan and Krishnakumari, 2006). The cages were cleaned regularly throughout the experimental periods. Animals were catered for in compliance with internationally acceptable principles for humane handling and use of laboratory

animals in the Canadian Council on Animal Care Guidelines and Protocol Review (CCAC, 1997) and National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, 1985).

3.1.3 Reagents and Apparatus

IVD Dialab Kits (Protein, cholesterol and Triglyceride analysis), Randox kit (Alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST)), Teco Diagnostics Kit (Alkaline phosphatase (ALP) and Bilirubin), Alloxan monohydrate (Qualikem, Fine Chem. Pvt. Ltd, Vadodara, India), Finetest Glucometer (Infopia Co., Ltd, Korea) were used for the current work. All other chemicals were of analytical grades.

3.2 Methods

3.2.1 Plant Sample Preparation and Extraction

3.2.1.1 Plant Sample Preparation

The leaves samples of both *G. braunii* and *G.oreophila* were collected and rinsed with clean water, and air dried (Rocha *et al*., 2011). The dried leaves of each were separately grinded into powder with mortar and pestle and electric blender, and kept at room temperature until required. The standard conduct for the collection and preparation of herbal medicine was adopted (WHO, 2007).

3.2.1.2 Extraction of Prepared Plant Samples

Aqueous Extracts

The powdered plant samples (50g) were weighed and dissolved in 300ml of distilled water and then refluxed for 3hrs at 80^{o}C (Edwin *et al*., 2009). They were then filtered using Muslin cloth. The filtrates were then transferred to water bath at 70^{o}C in other to concentrate the extract by evaporating the water present. The extracts were transferred

into sterile bottle and kept in the refrigerator. This was conducted four times making a total of 200gms of the powdered samples that were extracted (ICS-UNIDO, 2008).

Ethylacetate Extracts

The powdered plants samples (200g) were weighed separately into 1000ml conical flask, 800ml of ethylacetate was added into it, shook very well and then covered tightly for 72hrs and shake intermittently. The macerates were then filtered and the filtrates were transferred to rotary evaporator at 62^{o}C in other to concentrate the extracts (ICS-UNIDO, 2008).

3.2.2 Phytochemical Screening

Standard screening test of the extract was carried out for various plant constituents. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins and anthraquinones using standard procedures of Hymete, (1986), Trease, and Evans (1985), Sofowora (1993) and Edeoga *et al*., (2005).

3.2.2.1 Test for Alkaloid

The crude extract (0.2g) was dissolved in 5ml of 2% hydrochloric acid and then heated on water bath for 10 minutes. Solution was clarified by filtration. To 1ml of the filtrate, Wagner reagent was added and observed for reddish brown precipitate. To another 1.0 ml portion of the Filtrate was tested with Dragendroff's and Mayer's reagents. The treated solutions were observed for any cream coloured precipitation.

3.2.2.2 Test for Anthranoid

The crude extract (0.2g) was boiled with 3ml Sodium hydroxide. Three drops of acetic acid and 3ml of toluene were added. The solution was mixed and allowed to settle, the upper layer was transferred to fresh test tube using a pipette, and 0.2ml of sodium

hydroxide was added. A red coloration of the solution indicates the presence of anthronoids.

3.2.2.3 Test for Anthraquinone

The crude extracts (0.2g) were dissolved in 2ml of water and heated in a water bath for 10 minutes. They were then allowed to and shaken vigorously with 10 ml of benzene, filtered and 5 ml of 10% ammonia solution added to the filtrate. Shake the mixture and the presence of a pink, red or violet colour in the ammonia (lower) phase indicated the presence of free anthraquinones.

3.2.2.4 Test for Cardiac Glycoside

The crude extracts (0.2g) were dissolved in 1ml glacial acetic acid containing 1 drop of ferric chloride solution. It was then under layered with 0.5 ml of concentrated sulphuric acid. A brown ring formation at the inter phase will indicates the presence of deoxy sugar characteristics of cardiac glycosides.

3.2.2.5 Test for Flavonoid

Two methods were used to determine the presence of flavonoid in the plant sample (Sofowora, 1993). 3ml of dilute ammonia solution were added to a 0.2g of the extracts followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. Sodium hydroxide solution was added to a solution of 0.2g of the extract dissolved in water. The mixture was inspected for the production of yellow colour which was considered as positive test for flavonoids.

3.2.2.6 Test for Phlobatannin

To 0.2g of extracts, 1% aqueous hydrochloric acid was added and then boiled for 15 minutes. Deposition of red precipitate was taken as evidence for the presence of phlobatannins.

3.2.2.7 Test for Saponins

The froth test method was used to test for the presence of saponin, in which 0.2g of the extracts was dissolved in 5ml of distilled water in a test tube. The test tube was covered and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over a 30 minute period of time. If a “honey comb” froth above the surface of liquid persists after 30 min. the sample is suspected to contain saponins.

3.2.2.8 Test for Steroid

The Salkowski’s test was used to test for the presence of steroid in which 0.2g of the extracts was dissolved in 1ml chloroform in a test tube. Concentrated sulfuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish brown colour at the interface indicated the presence of a steroid ring (for example the aglycone portion of the glycoside).

3.2.2.9 Test for Tannins

About 0.2g of the crude extracts was boiled in 5ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

3.2.2.10 Test for Terpenoid

Exactly 0.2g of each extract was mixed in 2ml of chloroform and concentrated 3ml H_2SO_4 was carefully added to form a layer. A reddish brown coloration formation of the interface shows positive results for the presence of terpenoids.

3.2.2.11 Test for Total Phenol

The 0.2g extracts was dissolved in water. Few crystals of ferric sulfate were added to the mixture. Formation of dark-violet colour indicated the presence of phenolic compounds.

3.2.3 Preliminary Toxicological Studies

3.2.3.1 Acute Oral Toxicity Test.

Acute toxicity study of the crude aqueous and ethylacetate plant extracts was carried out using female Wister albino rats by adopting the up and down guideline of Organisation for Economic Corporation and Development standards (Rispin *et al.,* 2000). The Rats weighing (121.47g – 221.54g) were fasted overnight and the weight of each rat was recorded just before use. Animals were divided randomly into groups based on their relative body weight with each group consisting of three rats each; a control group and three stepwise treatment dose groups of 300mg/kg body weight (b.w), 1200mg/kg b.w, 4800mg/kg b.w for every extracts of *G.braunii* and *G.oreophila* used. The vehicle for aqueous extracts was distilled water while that of ethylacetate was Dimethylsulphuroxide (DMSO). First level of dosing (300mg/kg) was observed for any mortality within four hours and further observed for 24hrs. Since there was no mortality, different groups of animals were gavage again with the next dose (1200mg/kgb.w) and this continues with the third dose (4800mg/kg b.w) for another different groups. The guideline stipulated that the study should be terminated if there is no death in the group at dose levels of 5000mg/kg b.w of the extract. All animals were left for seven days and observed daily for any changes in general behaviour and/or other physical activities. They were then finally reweighed before they were euthanized.

3.2.3.2 Estimation of Median Lethal Dose (LD_{50})

Several methods were described according to the OECD standard in regards to the estimation of LD50 (Rispin *et al.,* 2000). These are described below.

The methods for estimating the LD50 (median lethal dose) are the same for staged tests as for other types of dose response tests. Certain features of the staged designs are based on the assumptions that the underlying form of the dose response curve approximates

the probit model, so the use of a probit regression model (with the logarithm of dose as the independent variable) to estimate the LD50 was used. For the probit model, the maximum likelihood estimate of the Log (LD50) was –a/b, where a is the intercept and b is the slope.

3.2.4 Induction of Diabetes

Twenty four (24) healthy male rats were randomly selected and weighed. They were then assigned into six groups based on their body weight, fasted overnight (12hrs) and subjected to single intraperitoneal injection of alloxan monohydrate at dose of 100mg/kg b.w using distilled water as vehicle and allow access to food after 5 minutes. The dose used was determined after series of pilot studies. Diabetes was confirmed after 72hrs of induction with fasting blood glucose level $\geq$ 135mg/dl-350mg/dl (Etuk, 2010) which was measured using autocoding glucometer (Finetest). This was done by collecting the blood through the orbital puncture of the tail vein. The diabetic condition was then allowed to stabilize by completing six days starting from the first day of induction (Joussen *et al*. 2001).

3.2.5 Animal Grouping and Treatments

3.2.5.1 Animal Grouping

Eighteen rats drawn from the diabetic pool which were able to meet the standard of diabetic conditions together were assigned into six groups with normal rat groups making a total of seven groups, each group comprise of three rats each.

1. Diabetic animals treated with standard drug (DST): Glibenclamide were given at the dose level of 5mg/kg body weight, daily by gavage. However, the administered dose was calculated equivalent to the human therapeutic dose according to the United state of America Food and Drug Administration (FDA) Guidance for Industry and Reviewers (2000) (Edwin *et al.,* 2009).

2. Normoglycemic control (NNT): This group were not induced with diabetes and was gavaged with 20ml/kg bw of distilled water for fourteen days.
3. Aqueous *Globimetula braunii* (AGB): Rats in this group were diabetic and gavaged with 500mg/kg b.w aqueous *G.braunii* from the 7th day after diabetic stabilization for fourteen days.
4. Aqueous *Globimetula oreophila* (AGO): Rats in this group were diabetic and gavaged with 500mg/kg b.w aqueous *G.oreophila* from the 7th day after diabetic stabilization for fourteen days.
5. Ethylacetate *Globimetula braunii* (EGB): Rats in this group were diabetic and gavaged with 500mg/kg b.w ethylacetate *G.braunii* from the 7th day after diabetic stabilization for fourteen days.
6. Ethylacetate *Globimetula oreophila* (EGO): Rats in this group were diabetic and gavaged with 500mg/kg b.w ethylacetate *G.braunii* from the 7th day after diabetic stabilization for fourteen days.
7. Hyperglycemic control (DNT): This group were made diabetic without treatment.

3.2.5.2 Treatment of Animals

The rats were allowed to fast overnight but with access to water prior to every treatment. Treatment starts on the seventh day of diabetes induction with standard drug and 500mg/kg body weight (b.w) of the extracts which was derived from the pilot studies conducted prior to the experiment. The choice of the doses used for the pilot study was based on the different doses reported by other literatures on the other mistletoe extracts used in their research (Gray and Flatt, 1999; Adediwura *et al*., 2008; Okpuzor, 2009b Ekhaise *et al.*, 2010; Inuwa *et al*., 2012). The diabetic animals were treated with the extracts and standard drugs once daily for fourteen days, making the

duration of the experiment to last for twenty one days. They were allowed access to food fifteen minutes after treatment.

3.2.6 Determination of Fasting Blood Glucose

The animals were fasted over night prior to the day of taking the fasting blood glucose with only access to water (Tap water). The blood glucose level in mg/dl was checked five days intervals during the period of treatment using Finetest autocoding glucometer by collecting the blood through orbital puncture of the tail vein of rats. The glucometer principle is based on electrochemical method. Test strips contain a capillary that sucks up a reproducible amount of blood. The glucose in the blood reacts with an enzyme electrode containing glucose oxidase (or dehydrogenase). The enzyme is reoxidized with an excess of a mediator reagant, such as a ferricyanide ion, a ferrocene derivative or osmium bipyridyl complex. The mediator in turn is reoxidised by reaction at the electrode, which generates an electrical current. The total charge passing through the electrode is proportional to the amount of glucose in the blood that has reacted with the enzyme which is then inprinted on the glucometers screen as the glucose concentration present in the blood (http://en.wikipedia.org/wiki/Glucose_meter).

3.2.7 Total Body Weight

The weight of all the rats including diabetic rats were determined before and during the period of treatment with the extracts at five days intervals. The animals were always allowed to fast over night but allowed access to water before taking the reading of their weight using weighing balance. The values obtained were then used to calculate the percentage change in body weight.

3.2.8 Organ-Body Weight Ratio

At the end of the experiment, the animals were euthanized and the organs, liver, kidneys, lungs, liver and pancreas were excised from individual animals and weighed.

The ratio of organ to body weights was then calculated and the values obtained were used to calculate percentage ratios of organ – body weight.

3.2.9 Blood Collection and Serum Preparation

Collection of sample for biochemical analyses was done as described before by Yakubu *et al.,* (2005). Twelve hour after the final treatment, the rats were anaesthetized using chloroform and blood sample collected through jugular puncture. The blood samples were allowed to stand at room temperature and then centrifuge at 1000rpm for 10minutes.

3.2.10 Biochemical Analysis

Biochemical analysis was carriedout in other to determine the effect of the plant extracts in the biochemical state of the diabetic rats which is characterised with biochemical anormalities. Specific kits were used for the analysis of serum biochemical parameters. The serum total protein was analysed using IVD Dialab kits as described by Thomas, (1998); Johnson *et al*., 1999; Guder *et al*., (2001). Methods of Artiss and Zak, (1997); Deeg and Ziegenhorn, (1983) were used for serum total cholesterol. Triglyceride was estimated by the method of Cole *et al*., (1997). Randox kit was used to estimate Alanine aminotransferase and Aspartate aminotransferase as described by Reitman and Frankel (1957). Teco Diagnostics Kit was used for Alkaline phosphatase as described by Tietz (1976) and Demeterious *et al.,* (1974) and Bilirubin was estimated as described by Tietz, (1976).

3.2.10.1 Estimation of Total Protein

The serum protein was estimated using Dialab kit based on photometric test according to Biuret method. Together with copper ions, protein forms a violet blue colour complex

in alkaline solution. The absorbance at 540nm of this coloured complex was then recorded for the calculation of concentration of total proteins.

3.2.10.2 Estimation of Total Cholesterol

Cholesterol was determined after enzymatic hydrolysis and oxidation (Artiss and Zak, 1997; Deeg and Ziegenhorn, 1983). The colorimetric indicator is quinoneimine which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidise (Trinder's reaction).

Cholesterol Ester + $H_2O \xrightarrow{CHE}$ Cholesterol + Fatty Acid

Cholesterol + $O_2 \xrightarrow{CHO}$ Cholesterol-3-one + H_2O_2

H_2O_2 + Phenol +4 Aminoantipyrine $\xrightarrow{POD}$ Quinonimine + 4 H_2O

The intensity of the pink/red colour is proportional to the cholesterol concentration in the sample and the absorbance (500nm) was taken and concentration obtained using formula.

3.2.10.3 Estimation of Triglyceride

Diablab kit was used in the determination of triglyceride. Triglyceride was determined after enzymatic splitting with lipoprotein lipase. Indicator is quinoneimine which is generated from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase (Cole *et al*., 1997). The manual procedures was followed. After preparing the samples in test tubes at 10µl with the blank and standard at 1000µl, they were then mixed incubated for 10minute at 37^{o}C. The absorbance (500nm) of the coloured content was then determined using spectrophotometer.

Triglycerides $\xrightarrow{LPL}$ Glycerol + Fatty Acid

Glycerol + ATP $\xrightarrow{GK}$ Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + $O_2 \xrightarrow{GPO}$ Dihydroxyaceton phosphate + H_2O_2

H_2O_2 + Aminoantipyrine + 4-Chlorophenol $\xrightarrow{POD}$ Quinoneimine + HCl + 4H_20

3.2.10.4 Estimation of Bilirubin

Teco diagnostic kit was used in the determination of Direct and Total bilirubin. Bilirubin reacts with diazotized sulfanilic acid to produce azobilirubin, which has an absorbance maximum at 560nm in the aqueous solution. The intensity of the colour produced is directly proportional to the amount of direct bilirubin concentration present in the sample. The subsequent addition of methanol accelerates the reaction of unconjugated bilirubin in the serum, and a value for total bilirubin was obtained after five (5) minutes. The total bilirubin value represents the sum of the bilirubin glucuronide (direct) and the unconjugated (indirect) bilirubin. The colour produced measured at 560nm is proportional to the amount of the total bilirubin concentration present in the sample (Tietz, 1976).

3.2.10.5 Estimation of Serum Enzymes

Assay for Alkaline Phosphatase

Principally, Alkaline phosphatase acts upon the AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically with the Absorbance of 590nm. The procedures described by Teco diagnostics manual were followed as described below:

For each sample, 0.5 mL of Alkaline Phosphatase Subtrate was dispensed into labelled test tubes and equilibrates to 37^{o}C for three (3) minutes. At timed intervals, 0.05ml of each standard control and sample was added to its respective test tubes which were then mixed gently. They were then incubated for ten (10) minutes at 37^{o}C. Following the same sequence described above, 2.5mL of Alkaline Phosphatase Color Developer was added at timed intervals and then mixed well. The wavelength of the spectrophotometer

was set at 590nm which was zero with reagent blank (wavelength range: 580-630). The absorbance of samples were then read and recorded.

Assay for Alanine Aminotransferase

The procedures described by Randox Diagnostic Kit (Cat No. AL 100) manual was followed and absorbance at 546nm was read for further calculation of concentration.

Principally, Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine. The concentration of ALT was determine by calculating it from the plot of standard values of Absorbance against Concentration

$$\alpha\text{-oxoglutarate} + \text{L-Alanine} \xrightarrow{\text{GPT}} \text{L-glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^+$$

Assay for Aspartate Aminotransferase

The procedures described by Randox Diagnostic Kit (Cat No. AS 101) manual was followed and absorbance at 546nm was read for further calculation of concentration. The concentration of AST was then determined by calculating it from the plot of standard values of Absorbance against Concentration

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

$$\alpha\text{-oxoglutarate} + \text{L-aspartate} \xrightarrow{\text{GOT}} \text{L-glutamate} + \text{Oxaloacetate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} \text{L-Lactate} + NAD^+$$

3.2.11 Statistical Analysis

Data obtained were statistically evaluated using one-way ANOVA, followed by post hoc Duncan test of comparison using statistical package for social sciences (SPSS) version 16 software. The results were expressed as mean ± standard error of the mean. Values were considered to be significant if $p<0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Yields of Extracts

The weight obtained, percentage yield, colour, and appearance of the extract are presented in Table 4.1. 48.45, 65.31, 18.73 and 14.19 grams were obtained for Aqueous *G.braunii*, Aqueous *G.oreophila*, Ethylacetate *G.braunii*, and Ethylacetate *G.oreophila* respectively from 200g of powered plant material. 24.22%, 32.66%, 9.37% and 7.10% were calculated for AGB, AGO, EGB, and EGO respectively. Aqueous *G.braunii* and *G.oreophila* extracts were brown and deep brown colour respectively. Both ethylacetate *G.braunii* and *G.oreophila* extracts were dark in colour. Only ethylacetate extract of *G.braunii* and *G.oreophila* shows a greasy appearance which reflects a significant difference from the appearance of aqueous, *G.braunii* and *G.oreophila* extracts of the plants.

Table 4.1: Yields of plant materials after extraction with aqueous and ethylacetate Solvents

	Plant Extracts			
Description	**A*GB***	**A*GO***	**E*GB***	**E*GO***
Weight (g)	48.45	65.31	18.73	14.19
Percentage (%)	24.22	32.66	9.37	7.10
Color of Extracts	Brown	Deep brown	Dark green	Dark green
Appearance & touch	Muggy	Sticky	Greasy	Greasy

Key:

AGB*: Aqueous Globimetula braunii* AGO*:Aqueous Globimetula oerophila* EGB*: Ethylacetate Globimetula braunii* EGO*:Ethylacetate Globimetula oeorphila*

4.2 Phytochemical Screening

The preliminary phytochemical screening of aqueous and ethylacetate extracts of *G.braunii* and *G.oreophila* (Table 4.2), reveals the presence of Flavonoid, Phlobatannin, Saponin, Tannins, Terpenoid, and Total phenols for all the extracts. Only ethylacetate extracts revealed the presence of Anthraquinone, Cardiac glycoside and Steroid for *G.braunii* and *G.oreophila* but alkaloid was present in ethylacetate extracts of both *G.braunii* and *G.oreophila* however, there were traces of anthraquinone, cardiac glycoside and steroids in the aqueous extracts of both *G. braunii* and *G.oreophila.* Aqueous and ethylacetate extracts of *G.braunii* and *G. oreophila* exhibited trace presence of alkaloid, likewise the same trace presence was noticed for ethylacetate extracts of *G.braunii* and *G.oreophila* for Anthranoid while aqueous extracts of *G.braunii* and *G.oreophila* showed absence of anthranoid.

Table 4.2: Preliminary phytochemical constituents of the extracts of *G.braunii* and *G.oreophila*

	Plant extracts			
Phytochemicals tested	**A*GB***	**A*GO***	**E*GB***	**E*GO***
Alkaloid	±	±	+++	++
Anthranoid	-	-	±	±
Anthraquinone	±	±	++	+
Cardiac glycoside	±	±	++	++
Flavonoid	++	++	+++	+++
Phlobatannin	++	++	+++	+++
Saponin	+++	++	++	+
Steroids	±	±	++	++
Tannins	+	+	+++	+
Terpenoid	+	+	+++	++
Total Phenols	+	+	+++	++

Key:
+++ Highly present, ++moderately present, + Present ± Trace, - Absent.
AGB: Aqueous *Globimetula braunii,* AGO: Aqueous *Globimetula oerophila*
EGB: Ethylacetate *Globimetula braunii,* EGO: Ethylacetate *Globimetula oeorphila*

4.3 Preliminary Toxicological Studies

4.3.1 Acute Oral Toxicity Test

There was significant difference (soft, very soft with mucus, linked mucus, solid and thick feaces) in the appearance of the animal's faeces between the different dose levels. No significant changes in behaviours such as alertness, motor activity, breathing, restlessness, diarrhoea, tremor, convulsions, coma and appearance of the animals dosed with 300mg/kg, 1200mg/kg, and 4800mg/kg body weight of the crude aqueous and ethylacetate extracts of *G.braunii* and *G.oreophila* (see Tables in the appendices).

No death was observed up to the dose of 4.8g/kg body weight. There were no significant changes in the body weight also. The rats were physically active. These effects were observed during the experimental period (72 hrs) and the animals were left for seven days before sacrifice.

The LD50 of the crude aqueous and ethylacetate extracts of *G.braunii* and *G.oreophila* (Table 4.3) appears to be >4800mg/kg since there was zero mortality recorded for all the different doses used.

Table 4.3 Acute toxicity studies of *G.braunii* and *G.oreophila* extracts

Groups	Dose (mg/kg)	Number of Rats (n)	Mortality
B	300	3	0
C	1200	3	0
D	4800	3	0
E	300	3	0
F	1200	3	0
G	4800	3	0
H	300	3	0
I	1200	3	0
J	4800	3	0
K	300	3	0
L	1200	3	0
M	4800	3	0

Key:

B=Aqeous G.braunii (300mg/kg) b.w C=Aqeous G.braunii (1200mg/kg) b.w
D=Aqeous G.braunii (4800mg/kg) b.w E=Aqeous G.oreophila (300mg/kg) b.w
F=Aqeous G.oreophila (1200mg/kg) b.w G=Aqeous G.oreophila (4800mg/kg) b.w
H=Ethylacetate G.braunii (300mg/kg) b.w I=Ethylacetate G.braunii (1200mg/kg) b.w
J=Ethylacetate G.braunii (4800mg/kg) b.w

4.4 Hypoglycaemic Effects of Extracts in Alloxan Induced Diabetic Rats

Figure 4.1 shows the effects of both aqueous and ethylacetate extracts of *G.braunii* and *G.oreophila* on the fasting blood glucose concentration (mg/dl) of rats that were induced with diabetes which was measured every five days intervals starting from the first day treatment commence. Only EGB shows a significant ($p<0.05$) hypoglycaemic effects (78.67 ± 7.26) compared with the day treatment started. AGB, AGO, EGO only shows a significant reduction in fasting blood glucose (83.67 ± 9.35, 89.67 ± 6.01 and 100.67 ± 2.35 respectively) between the fifth day of treatment and the fourteenth day when treatment ends. However, there appeared a significant increase in the blood glucose of untreated diabetic group (DNT) compare with the non-diabetic groups (NNT).

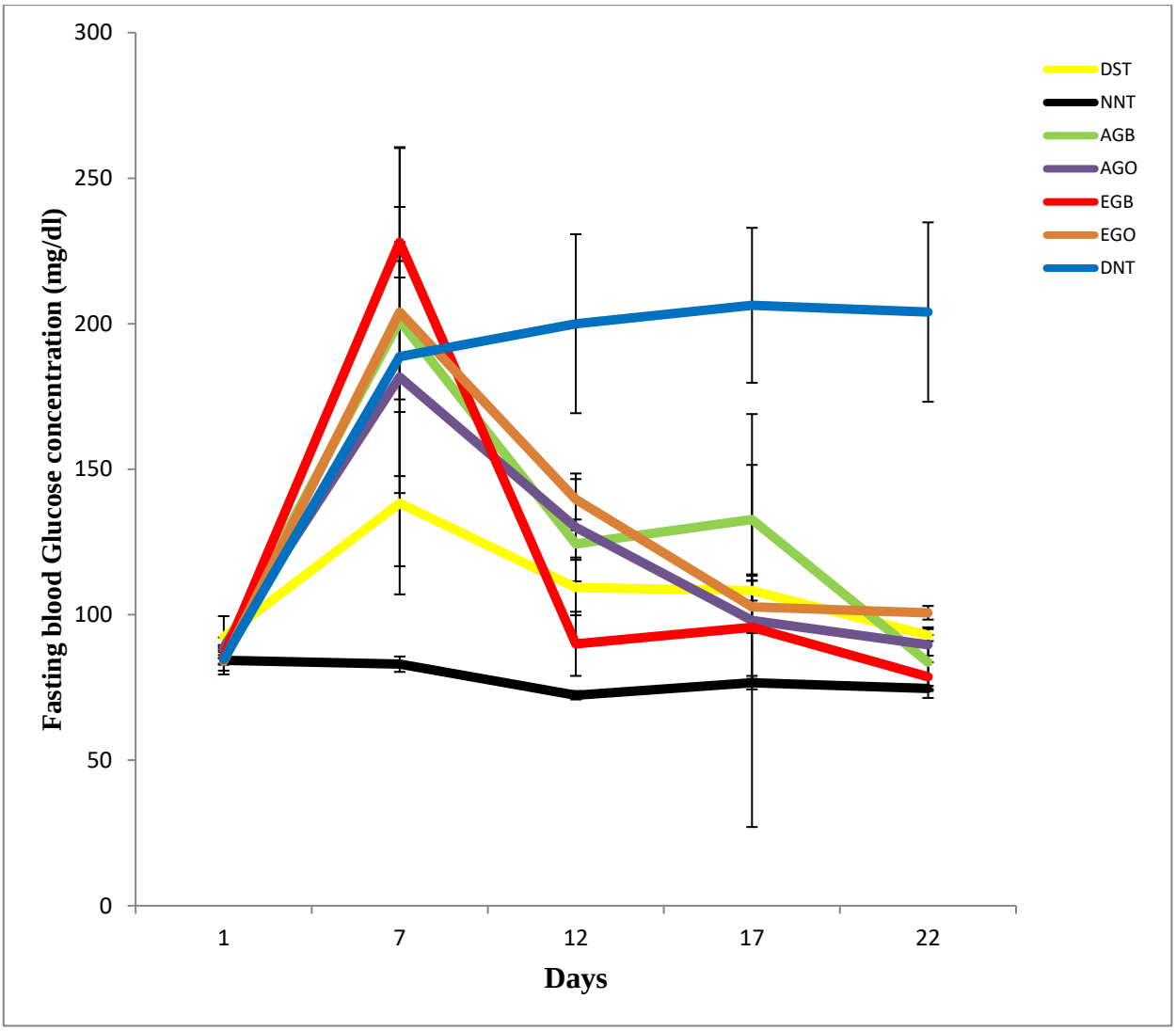

Figure 4.1: Effects of aqueous and ethylacetate extracts of *G.braunii* and *G.oreophila* on blood glucose levels of alloxan induced with diabetic rats

Key

DST: (Glibenclamide), 5mg/kg body weight (b.w),
NNT: Normoglycemic (2ml/kg b.w of distilled water),
AGB: Aqueous G.braunii extracts (500mg/kg b.w),
AGO: Aqueous G.oreophila extract, (500mg/kg b.w),
EGB: Ethyl acetate G.braunii extract, (500mg/kg b.w.),
EGO: Ethyl acetate G.oreophila extracts, (500mg/kg b.w),
DNT=Diabetic without treatment.

4.5 Effects of Extracts in Diabetes Induced Rats

4.5.1 Average Body Weight of Rats

Figure 4.2 represents variation in whole body weight of rats in the various experimental groups. The NNT, which is the control group shows significant increase ($p<0.05$) in the body weight (174.68 ± 5.72) while DST, AGB, AGO, EGB, EGO, (which are groups treated with standard drug and extracts) shows no significant increase ($P>0.05$) in the body weight throughout the experimental periods. Likewise DNT shows no significant reduction in their body weight.

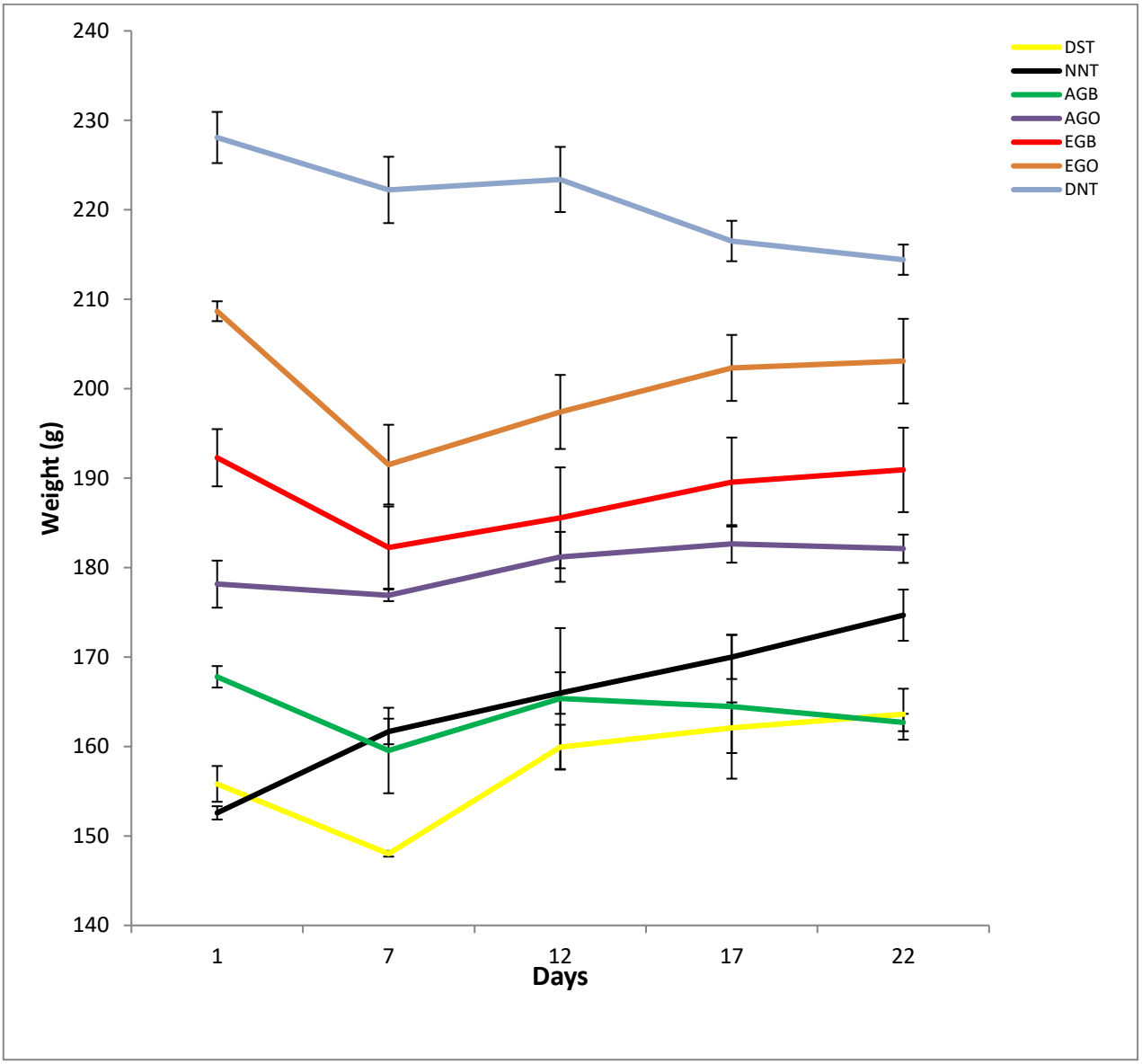

Figure 4.2: Body weight changes of rats treated with extracts of *G.braunii* and *G.oreophila*.

Key:

DST: (Glibenclamide), 5mg/kg body weight (b.w),
NNT: Normoglycemic (2ml/kg b.w of distilled water),
AGB: Aqueous G.braunii extracts (500mg/kg b.w),
AGO: Aqueous G.oreophila extract, (500mg/kg b.w),
EGB: Ethyl acetate G.braunii extract, (500mg/kg b.w.),
EGO: Ethyl acetate G.oreophila extracts, (500mg/kg b.w),
DNT=Diabetic without treatment.

4.5.2 Organ-Body Weight Ratios

Table 4.4 reveal the mean percentage ratios of fresh organ (Liver, Heart, Lungs Kidneys, and pancreas) to the final (day 22 of the experiment before sacrifice) whole body weight of animals of the experimental groups. There was no significant effect ($P>0.05$) of extracts (AGB, AGO, EGB & EGO) on the relative liver and heart weight, the Lungs to whole body weight percentage of AGB, AGO, EGB, EGO significantly ($P<0.05$) (0.67 ± 0.01, 0.55 ± 0.03, 0.56 ± 0.02, and 0.60 ± 0.03 respectively) reduced when compared to the control (NNT). The lowest percentage of kidney-whole body weight was noticed in EGO which shows its significance ($p<0.05$, 0.28 ± 0.003) when compared to the control groups (NNT). As for the pancreas, the control groups (NNT) shows the highest percentage and which is significant ($P<0.05$) when compared with the other groups that receive induction of diabetes.

Table 4.4: Mean percentage ratios of fresh organs to whole body weight of rats treated with *G.braunii* and *G.oreophila* extracts

	Fresh organs (%)				
Experimental Groups	**Liver**	**Heart**	**Lungs**	**Kidneys**	**Pancreas**
DST	2.83 ± 0.14^{a}	0.34 ± 0.03^{a}	0.72 ± 0.16^{ab}	0.32 ± 0.01^{abc}	0.23 ± 0.03^{a}
NNT	3.09 ± 0.10^{a}	0.36 ± 0.02^{a}	1.03 ± 0.24^{b}	0.29 ± 0.01^{ab}	0.39 ± 0.04^{b}
AGB	2.96 ± 0.19^{a}	0.38 ± 0.02^{a}	0.67 ± 0.01^{a}	0.36 ± 0.03^{c}	0.21 ± 0.04^{a}
AGO	2.85 ± 0.17^{a}	0.40 ± 0.04^{a}	0.55 ± 0.03^{a}	0.33 ± 0.003^{abc}	0.17 ± 0.007^{a}
EGB	2.75 ± 0.05^{a}	0.36 ± 0.01^{a}	0.56 ± 0.02^{a}	0.30 ± 0.003^{ab}	0.19 ± 0.01^{a}
EGO	2.70 ± 0.02^{a}	0.34 ± 0.03^{a}	0.60 ± 0.03^{a}	0.28 ± 0.003^{a}	0.22 ± 0.02^{a}
DNT	2.98 ± 0.06^{a}	0.35 ± 0.3^{a}	0.61 ± 0.03^{a}	0.33 ± 0.003^{bc}	0.22 ± 0.02^{a}

Values are expressed as Means$\pm$Standard Error of Means ($\bar{X}\pm SEM$), (n=3 per group),

Values in the same vertical column with superscripts that are not related represent means that are significantly different ($p < 0.05$).

Key:

DST: (Glibenclamide), 5mg/kg body weight (b.w),
NNT: Normoglycemic (2ml/kg b.w of distilled water),
AGB: Aqueous G.braunii extracts (500mg/kg b.w),
AGO: Aqueous G.oreophila extract, (500mg/kg b.w),
EGB: Ethyl acetate G.braunii extract, (500mg/kg b.w.),
EGO: Ethyl acetate G.oreophila extracts, (500mg/kg b.w),
DNT=Diabetic without treatment.

4.5.3 Biochemical Analysis

4.5.3.1 Estimates of Total protein

Figure 4.3 shows the different serum concentration of total protein between the various groups. There was no significant difference (P<0.05) between the different experimental groups.

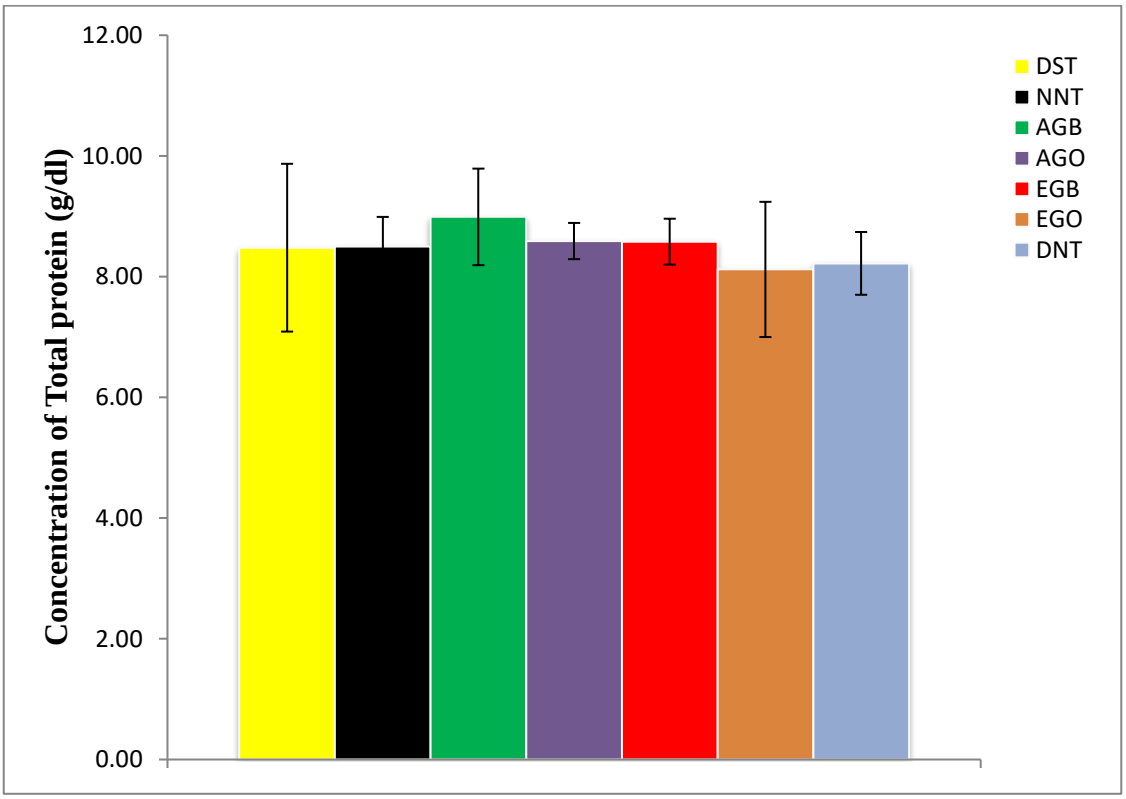

Figure 4.3: Serum Total proteins of rats treated with *G.braunii* and *G. oreophila* Extracts

Values are expressed as Means±Standard Error of Means ($\bar{X}$±SEM), (n=3 per group), Significant level (P <0.05).

Key:

DST: (Glibenclamide), 5mg/kg body weight (b.w), NNT: Normoglycemic (2ml/kg b.w of distilled water), AGB: Aqueous G.braunii extracts (500mg/kg b.w), AGO: Aqueous G.oreophila extract, (500mg/kg b.w), EGB: Ethyl acetate G.braunii extract, (500mg/kg b.w.), EGO: Ethyl acetate G.oreophila extracts, (500mg/kg b.w), DNT=Diabetic without treatment.

4.5.3.2 Estimates of Triglyceride

The serum triglyceride level of the different experimental groups is represented in figure 4.4. Only DNT shows significant (P<0.05) higher concentration (173.50±16.87) of triglyceride when compared with other groups whereas none of the other groups show significant difference (P>0.05) in concentration of triglyceride.

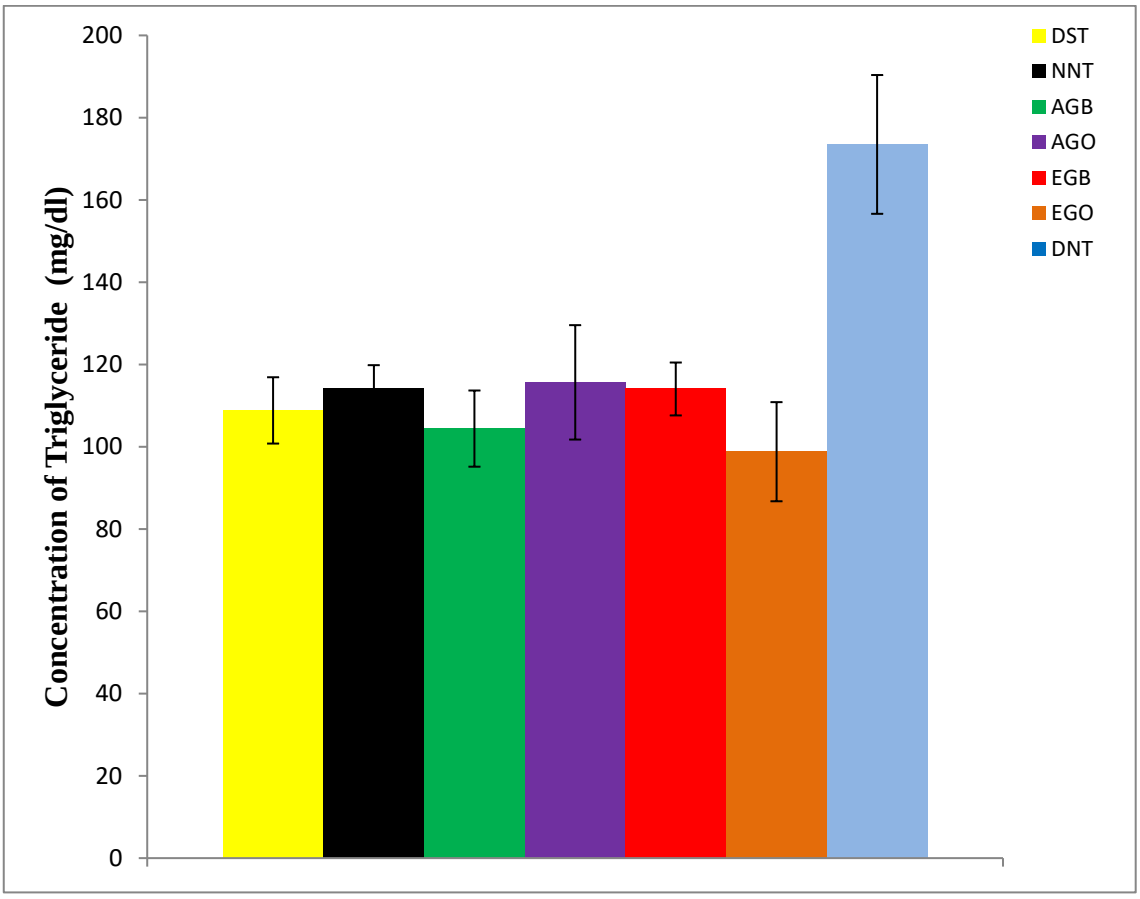

Figure 4.4: Serum Triglyceride of rats treated with *G.braunii* and *G.oreophila* extracts.

Values are expressed as Means±Standard Error of Means ($\bar{X}$±SEM), (n=3 per group), Significant level (P <0.05).

Key:

DST: (Glibenclamide), 5mg/kg body weight (b.w), NNT: Normoglycemic (2ml/kg b.w of distilled water), AGB: Aqueous G.braunii extracts (500mg/kg b.w), AGO: Aqueous G.oreophila extract, (500mg/kg b.w), EGB: Ethyl acetate G.braunii extract, (500mg/kg b.w.), EGO: Ethyl acetate G.oreophila extracts, (500mg/kg b.w), DNT=Diabetic without treatment.

4.5.3.3 Estimates of Total Cholesterol

Figure 4.5 represent the level of total cholesterol. The result shows that there was no significant difference (P>0.05) in the level of total cholesterol between all the experimental groups.

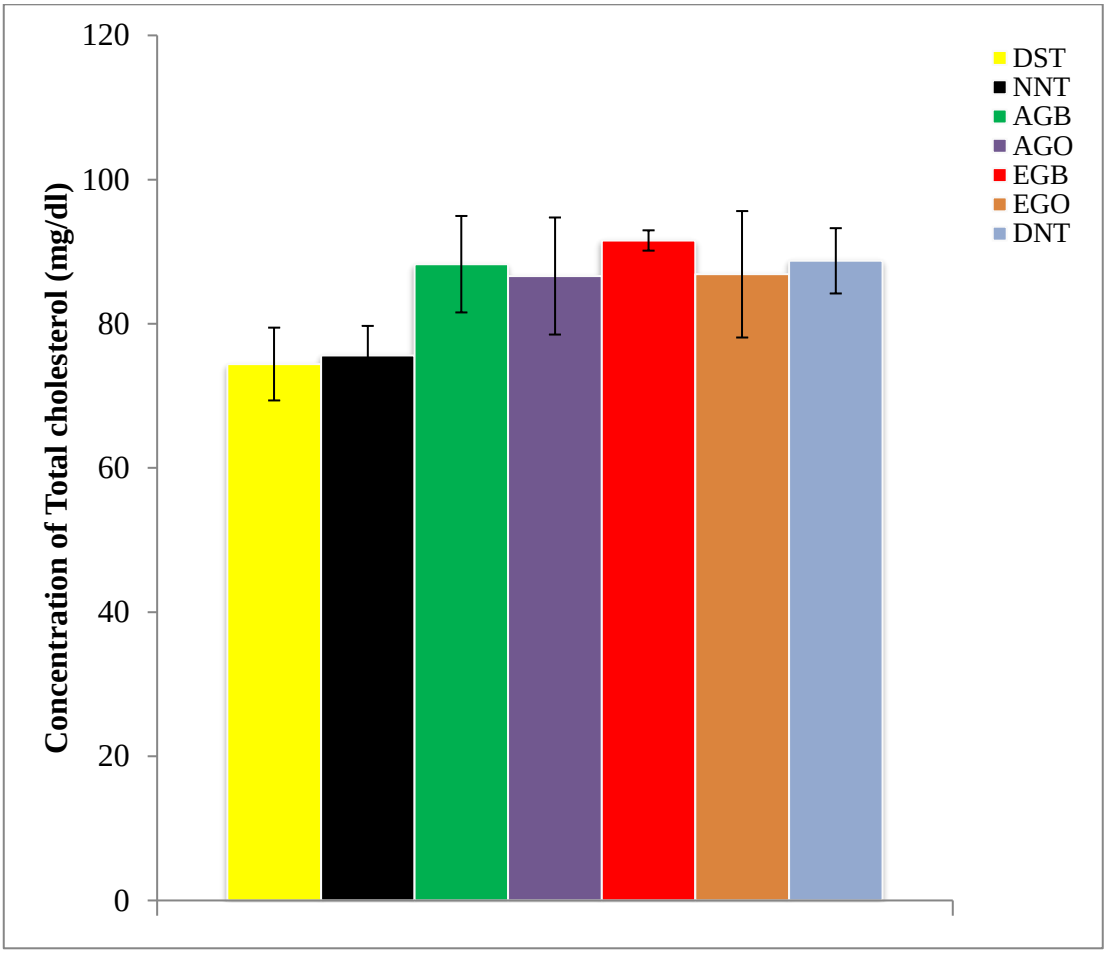

Figure 4.5: Serum Total cholesterols of rats treated with *G. braunii* and *G.oreophila* extracts.

Values are expressed as Means±Standard Error of Means ($\bar{X}$±SEM), (n=3 per group), Significant level (P <0.05).

Key:

DST: (Glibenclamide), 5mg/kg body weight (b.w), NNT: Normoglycemic (2ml/kg b.w of distilled water), AGB: Aqueous G.braunii extracts (500mg/kg b.w), AGO: Aqueous G.oreophila extract, (500mg/kg b.w), EGB: Ethyl acetate G.braunii extract, (500mg/kg b.w.), EGO: Ethyl acetate G.oreophila extracts, (500mg/kg b.w), DNT=Diabetic without treatment.

4.5.3.4 Estimates of Bilirubin

The level of total bilirubin in the serum is represented in figure 4.6. When the level of the serum total bilirubin of the other experimental groups was compared with NNT group, they show no significant (P>0.05) higher level of total bilirubin.

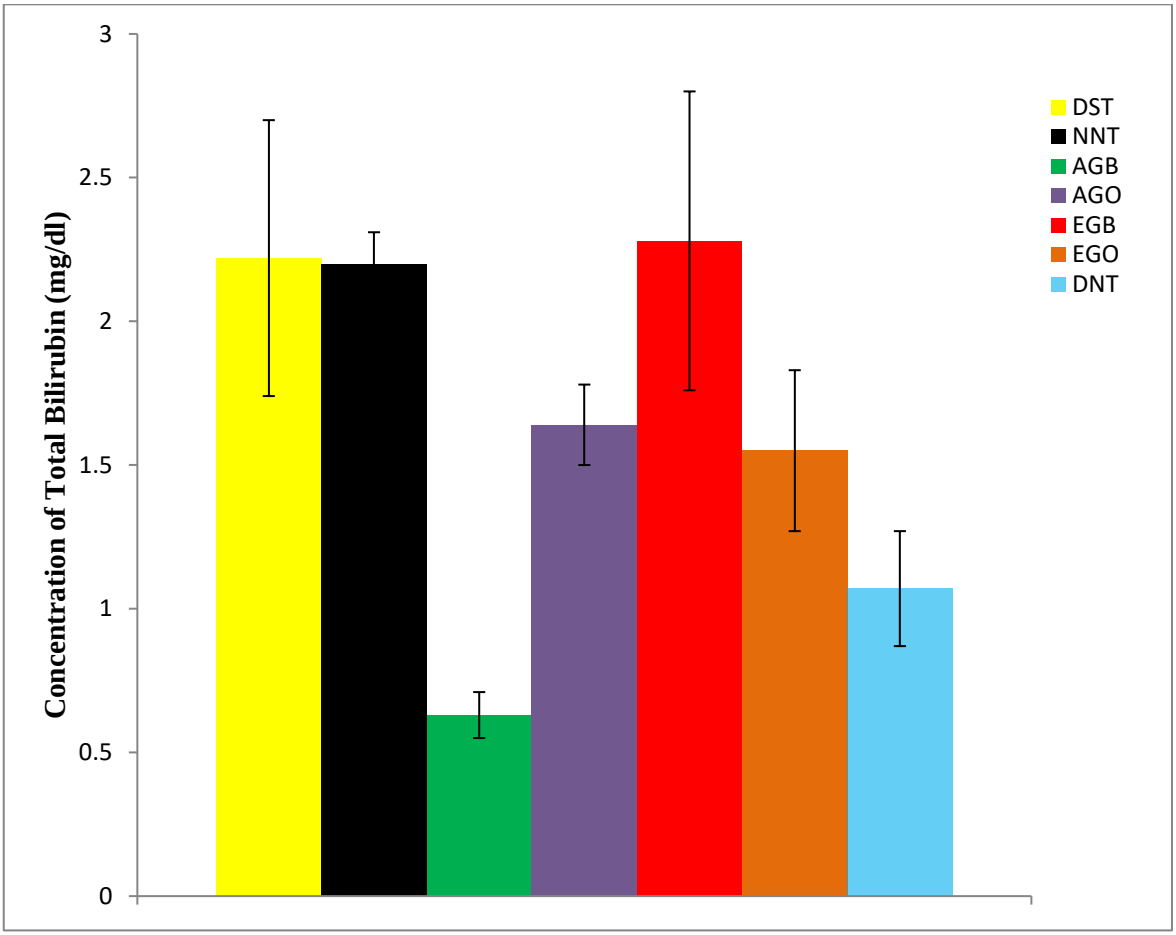

Figure 4.6: Serum Total Bilirubin of rats treated with *G.braunii* and *G.oreophila* extracts.

Values are expressed as Means±Standard Error of Means ($\bar{X}$±SEM), (n=3 per group), Significant level (P <0.05).

Key:

DST: (Glibenclamide), 5mg/kg body weight (b.w), NNT: Normoglycemic (2ml/kg b.w of distilled water), AGB: Aqueous G.braunii extracts (500mg/kg b.w), AGO: Aqueous G.oreophila extract, (500mg/kg b.w), EGB: Ethyl acetate G.braunii extract, (500mg/kg b.w.), EGO: Ethyl acetate G.oreophila extracts, (500mg/kg b.w), DNT=Diabetic without treatment.

Figure 4.7 shows the level of direct bilirubin. Only EGO groups shows significant (P<0.05) increase (1.57±0.2) in the level of direct bilirubin when compared with NNT group.

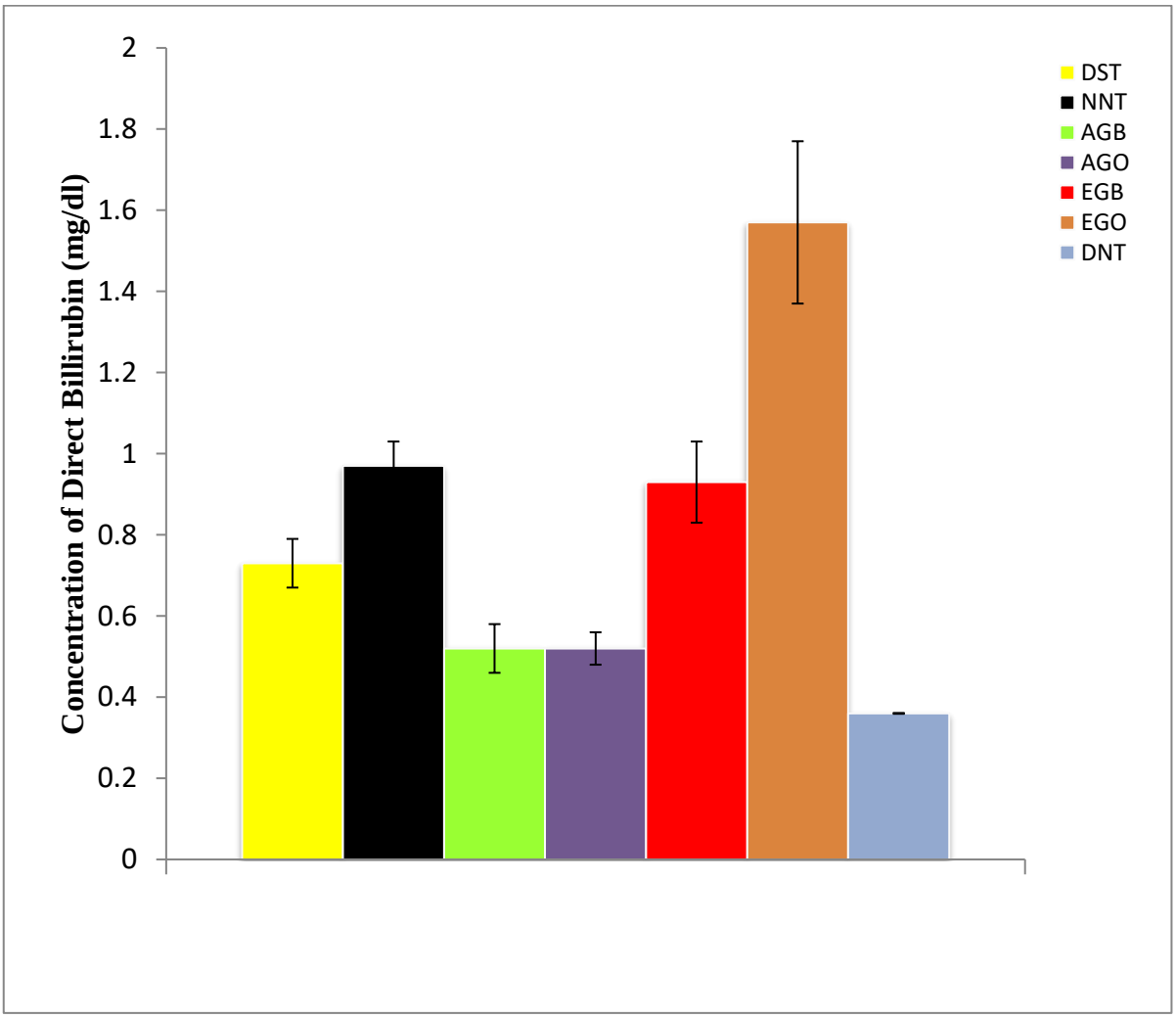

Figure 4.7: Serum direct bilirubin of rats treated with *G.braunii* and *G.oreophila* extracts.

Values are expressed as Means±Standard Error of Means ($\bar{X}$±SEM), (n=3 per group), Significant level (P <0.05).

Key:

DST: (Glibenclamide), 5mg/kg body weight (b.w), NNT: Normoglycemic (2ml/kg b.w of distilled water), AGB: Aqueous G.braunii extracts (500mg/kg b.w), AGO: Aqueous G.oreophila extract, (500mg/kg b.w), EGB: Ethyl acetate G.braunii extract, (500mg/kg b.w.), EGO: Ethyl acetate G.oreophila extracts, (500mg/kg b.w), DNT=Diabetic without treatment.

4.5.3.5 Activities of some Serum Enzymes

Assay of Alkaline Phosphatase

The concentratioin of serum alkaline phosphatase is represented in figure 4.8. Only AGB was significantly higher (125.30±5.60) in the level of serum alkaline phosphatase when compared with NNT groups.

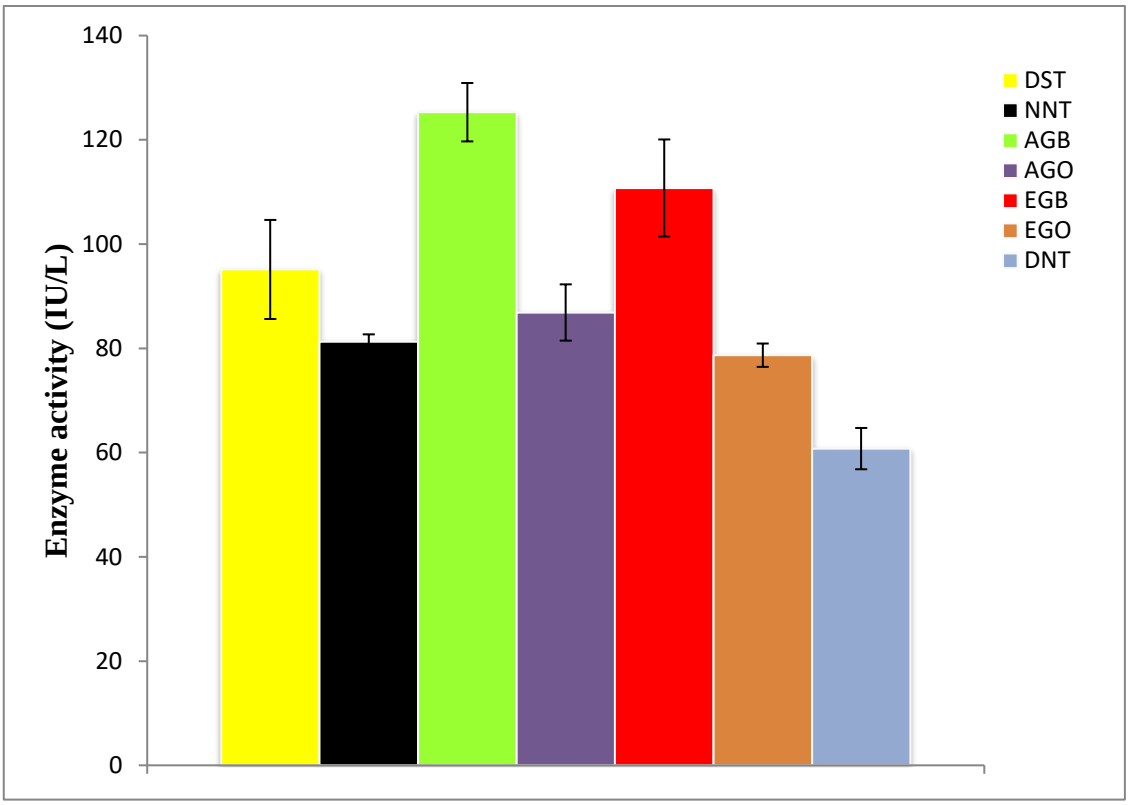

Figure 4.8: Serum Alkaline Phosphatase (ALP) of rats treated with *G.braunii* and *G.oreophila* extracts.

Values are expressed as Means±Standard Error of Means ($\bar{X}$±SEM), (n=3 per group), Significant level (P <0.05).

Key:

DST: (Glibenclamide), 5mg/kg body weight (b.w), NNT: Normoglycemic (2ml/kg b.w of distilled water), AGB: Aqueous G.braunii extracts (500mg/kg b.w), AGO: Aqueous G.oreophila extract, (500mg/kg b.w), EGB: Ethyl acetate G.braunii extract, (500mg/kg b.w.), EGO: Ethyl acetate G.oreophila extracts, (500mg/kg b.w), DNT=Diabetic without treatment.

Assay of Alanine Aminotransferase

The serum level of alanine aminotransferase is represented in figure 4.9. There was significant difference in the level of serum alanine aminotransferase (78.90 ±8.90, 63.30±6.10 and 73.07±2.40 respectively) for EGB, EGO, and DNT groups when compared with NNT.

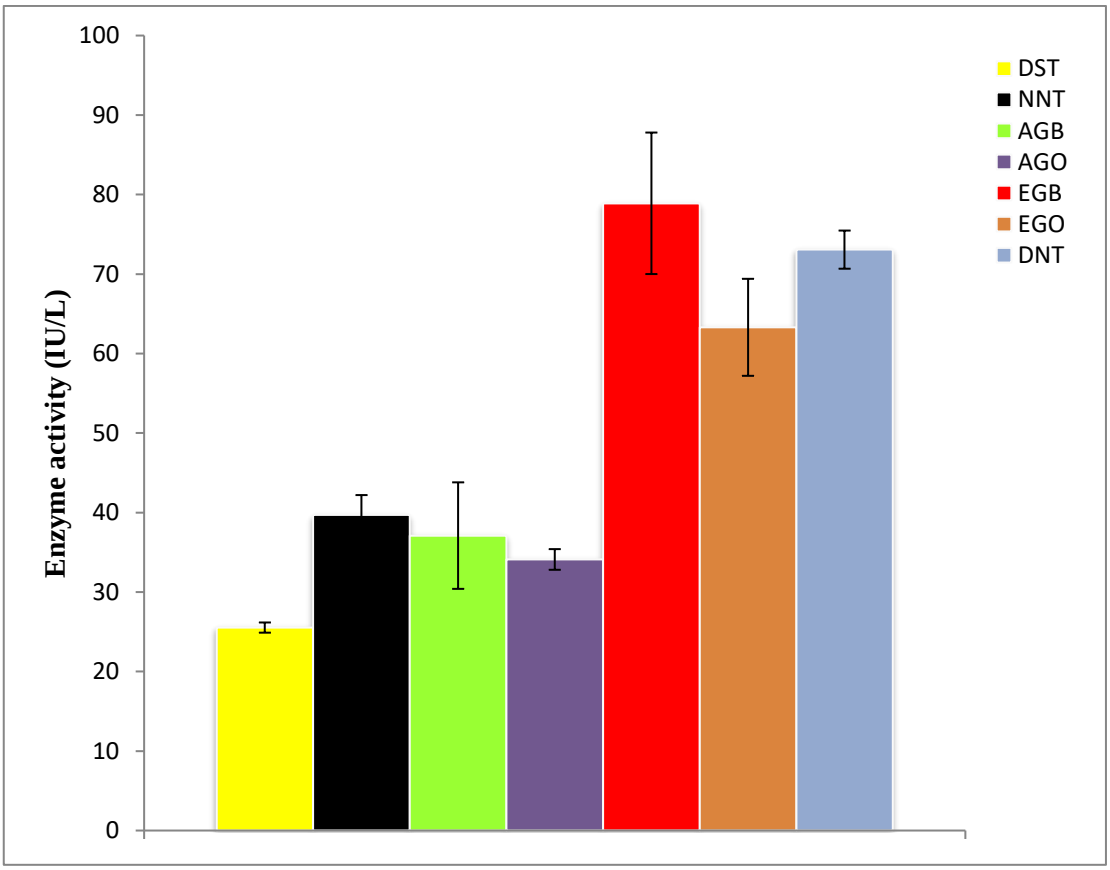

Figure 4.9: Serum Alanine aminotransferase (ALT) of rats treated with G.braunii and G.oreophila extracts

Values are expressed as Means±Standard Error of Means ($\bar{X}$±SEM), (n=3 per group), Significant level (P <0.05).

Key:

DST: (Glibenclamide), 5mg/kg body weight (b.w), NNT: Normoglycemic (2ml/kg b.w of distilled water), AGB: Aqueous G.braunii extracts (500mg/kg b.w), AGO: Aqueous G.oreophila extract, (500mg/kg b.w), EGB: Ethyl acetate G.braunii extract, (500mg/kg b.w.), EGO: Ethyl acetate G.oreophila extracts, (500mg/kg b.w), DNT=Diabetic without treatment.

Assay of Aspartate Aminotransferase

Figure 4.10 represent the serum concentration of aspartate aminotransferase. All the groups (DST, AGB, AGO, EGB, EGO and DNT) shows a significant ($P<0.05$) increase in the level of serum aspartate aminotransferase activities (60.00±5.50, 43.25±2.25, 49.75±8.75, 39.25±7.25, 57.00±5.50 and 53.75±3.98 respectively) when compared with the NNT group.

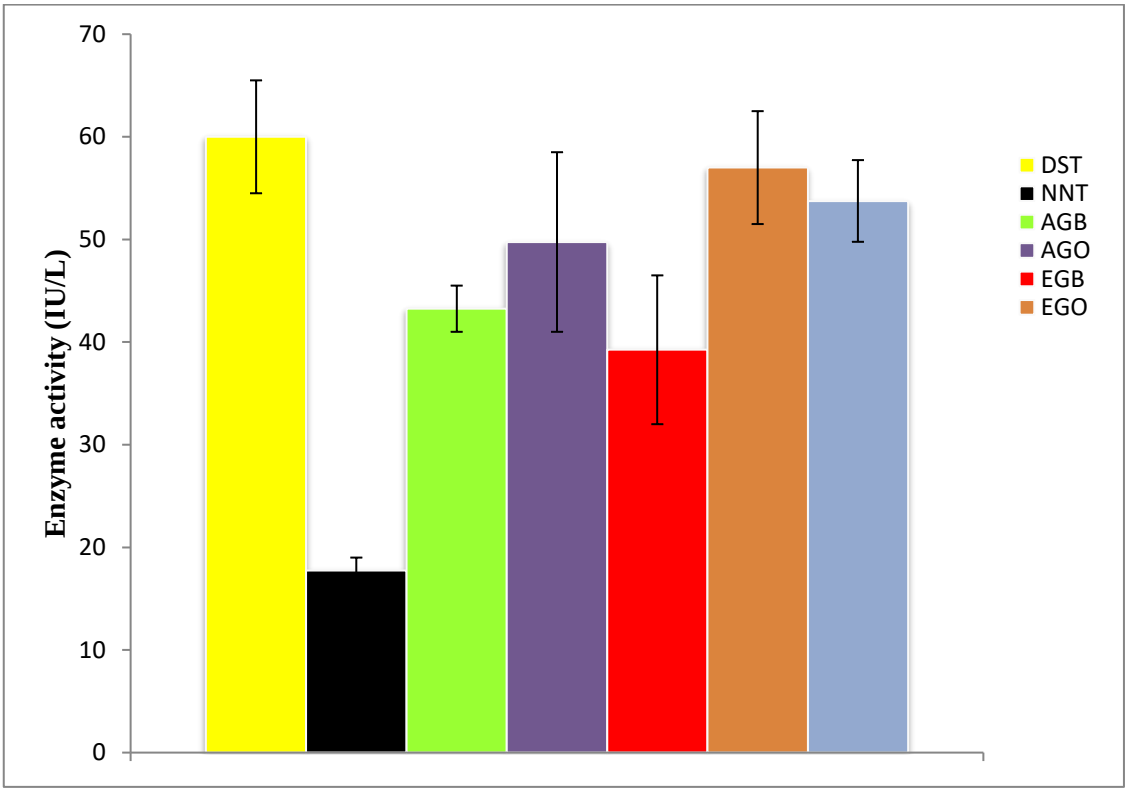

Figure 4.10: Serum Aspartate Aminotransferase (AST) of rats treated with *G.braunii* and *G.oreophila* extracts

Values are expressed as Means±Standard Error of Means ($\bar{X}$±SEM), (n=3 per group), Significant level ($P<0.05$).

Key:

DST: (Glibenclamide), 5mg/kg body weight (b.w), NNT: Normoglycemic (2ml/kg b.w of distilled water), AGB: Aqueous G.braunii extracts (500mg/kg b.w), AGO: Aqueous G.oreophila extract, (500mg/kg b.w), EGB: Ethyl acetate G.braunii extract, (500mg/kg b.w.), EGO: Ethyl acetate G.oreophila extracts, (500mg/kg b.w), DNT=Diabetic without treatment.

4.6 Discussion

Diabetes is associated with lots of biochemical abnormalities which in turn affects the physiological conditions of the system (Francesco *et al*., 1973; AbouSeif and Youssef, 2004; Muhammad *et al*., 2009). The most prominent biochemical abnormality in diabetes is hyperglycemia, that is, uncontrolled elevation of blood glucose (David, 2013). In these study, Type I diabetes which is characterized with hyperglycemia was modeled by the destruction of Beta cells of the islet of Langerhans located in the pancreas of the rat with alloxan based on the method review by Etuk (2010a). However, the result of the study of aqueous and ethylacetate extracts of both *Globimetula braunii* and *Globimetula oreophila* significantly ($p<0.05$) alleviated the hyperglycemic condition (Figure 4.1). But ethylacetate *Globimetula braunii* crude extracts has a swift and highest hypoglycemic effects with a reduction of 60.53% between the first and the fifth day of treatment. This is similar to other reports that use ethylacetate and methanol *Globimetula braunii* extracts from different host plant (Ekhaise *et al*., 2010). The hypoglycemic effect may be due to any of following mechanisms; the ability of the plant extracts to inhibit endogenous glucose production (Eddouks *et al.,* 2003) or interfere with gastrointestinal glucose absorption (Musabayane *et al.,* 2006), have insulin -like substances (insulin mimetic) (Gray and Flatt, 1999), or may inhibit insulinase activity and may increase secretion of insulin from the β cells of the pancreas, that is, pancreatotrophic action (Gray and Flatt 1999; Khan *et al*,1990; Trivedi *et al.,* 2004; Yadav *et al.,* 2008), it may also be possible to increase beta cells in pancreas by activating the regeneration of these cells (Jelodar *et al.,* 2007). In the current work the mechanism by which the extracts exert hypoglycaemic effect was not studies.

The bodyweight of the groups treated with the aqueous and ethylacetate extracts increases as compared with the diabetic untreated group (Figure 4.2). But the weight gain is not significant enough in their differences within the days of treatment. The induction of hyperglycemia has been found to greatly affect weight (Nelson and Cox, 2000). Weight variation is also part of the clinical features of diabetes (AbouSeif and Youssef, 2004). Thus the transient gain in the body weight of the groups treated with the extracts indicates the healing effects of the extracts on the diabetic rats. This transient weight gain may also be attributed to glucose utilization, which further explains the hypoglycemic properties of the plant extracts. The marked variation in the percentage weight of organs to body ratio especially for the pancreas, translate to the destructive effects of alloxan on the beta cells of the islet of Langerhans in the pancreas of rats (Etuk, 2010) and the effects of extracts. In a diabetic condition, organs are characterized with damages in tissues which in turn affect their weight (Gavin *et al*., 1999; Muhammad *et al*., 2009). Thus, regeneration of the organs may be achieved through ameliorating the diabetic conditions with extracts of strong tissue repair ability which may be the behavior of the extract. However, a significant gain in weight of the kidney was observed in the groups treated with ethylacetate *G.braunii* extracts. These extrapolations can be well explained by histopathological findings of the effects of the extracts on the organs in relation to a diabetic state.

Diabetes mellitus has been associated with altered protein metabolism as much of the structural proteins are used in gluconeogenesis. This is because insulin is an important physiological factor that plays a key role in the maintenance of protein balance, since it not only stimulates the uptake of amino acids and protein synthesis, but also inhibits protein degradation (Pathak and Dhawan, 1988). It has however been observed that no

significant difference ($p<0.05$) in the levels of protein (Figure 4.3) in all the experimental groups.

The significant increase in triglyceride seen in diabetic group (Figure 4.4) reflects the abnormalities in lipid metabolism associated with diabetes (Santini *et al.*, 1997) which was not observed in the groups treated with the extracts. Insulin can affect the adipocytes by inhibiting lipolysis and promoting storage of triglycerides in adipocytes (Zaahkouk, 2001). Thus, lack of insulin or inadequate utilization in diabetes enhances hydrolysis of triglycerides into diglycerides, unesterified fatty acids and free glycerol (Ebara, 1994). These fatty acids may diffuse out of the cells or may be re-esterified into triglycerides for storage or secretion of Very Low Density Lipoprotein (VLDL). Because insulin is known to suppress VLDL secretion, the lack of this suppression by deficient insulin secretion in diabetes may lead to hypertriglyceridemia, which possibly can mediate the mechanism by which diabetes causes hyperlipidemia (Saudek and Eder, 1979). As the concentration of triglycerides increases in the circulation, this increases the hydrolysis of triglycerides from VLDL, yielding Low Density Lipoprotein. These particles carry most of the cholesterol in the blood and are cleared mainly by the hepatic LDL receptor, which are increased by insulin (Saudek and Eder, 1979). Consequently, the clearance of LDL can be delayed resulting in hypercholesterolemia (Tsutsumi *et al.*, 1995). In other words, the reduction in the rate of cholesterol removal from circulation appears responsible for hypercholesterolemia recognized in diabetic states. It can then be deduced that the crude extracts have a gentle and yet inhibiting powers against hypertriglyceridemia and hypercholesterolemia noted in diabetic complication, especially for ethyl acetate extracts of *G.braunii* whose report has shown the antihyperlipidemic, hypercholesterolemic and antioxidant properties from the host

plants *cola acuminate,* orange plants and coffee plants (Adediwura *et al.*, 2008; Okpuzor *et al.*, 2009b; Ochuko *et al*., 2011).

Significant increase in direct bilirubin can only be seen in the group treated with ethylacetate *G. oreophila* when compared with the normoglycemic groups (Figure 4.7) whereas increased level of total bilirubin was only noticed in the group treated with ethylacetate *G. braunii* which is not significantly different when compared with normoglyceamic group (Figure 4.6). Bilirubin is a metabolite of the heme portion of heme proteins, mainly hemoglobin. Normally it is excreted into the intestine and the bile from the liver. The site of the catabolism of hemoglobin is the reticuloendothelial system (RES). Bilirubin is then released into the bloodstream where it binds tightly to albumin and is transported to the liver. Upon uptake by the liver, bilirubin is conjugated with glucuronic acid to form bilirubin mono and diglucuronide which are water-soluble metabolites. The metabolites will react with aqueous diazo reagent and are commonly referred as "direct bilirubin" (Goran, 2009). Elevation of total serum may occur due to excessive hemolysis or destruction of the red blood cells. For example, hemolytic disease of the newborn, liver diseases; hepatitis and cirrhosis, obstruction of the biliary tract disease; gallstones. There is information in the literature indicating elevated levels of direct bilirubin in patients with liver or biliary tract disease, even though, total bilirubin levels are normal. However the elevated increase seen in the group treated with ethylacetate *G. oreophila* may indicate an obstruction in the biliary tract of the rats.

The measurement of the activities of various enzymes in tissues and body fluids play a significant and well known aid in disease investigation, diagnosis and tissues cellular damage (Malomo, 2000). It is also a means of assessing drug or herbal safety as well as toxicity risk (Jigam *et al.,* 2011; Jigam *et al.,* 2012). Alkaline phosphate (ALP) is a

marker enzyme for the plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974). It is often employed to assess the integrity of the plasma membrane (Akanji *et al*., 1993). An elevated increase in ALP was only observed in the groups of rats treated with aqueous *G.braunii* crude extract (Figure 4.8). ALP is distributed almost in every tissue of the body; the levels are of interest in the diagnosis of hepatobiliary disorder and noted to be increased in diabetic state (Kochmar and Moss, 1976; Eskander *et al.*, 1995). Therefore Aqueous *G.braunii* seems to aggravate the elevated activities of ALP in diabetic condition. The increase in the activities of Alanine aminotransferases (ALT) was noted in the groups treated with Ethylacetate *G.braunii* extracts and Ethylacetate *G.oreophila* extracts (Figure 4.9). The increase was not significantly ($p<0.05$) different from the diabetic groups. As for aspartate aminotransferase (AST) activities, all the groups treated with the extracts except for the group treated with ethylacetate *G.braunii* extracts shows a significant increase which was significantly different from diabetic groups that was not treated (Figure 4.10). Increase in the activities of serum AST, ALT and ALP in diabetic animals have been reported (Eskander *et al.*, 1995; Hassan, 2007). These findings were further confirmed by Rawi *et al.* (1998). This elevation in enzymatic activities was attributed to their greater need for gluconeogenic substrates. Eskander *et al.* (1995) found that liver was necrotized in diabetic rats. Moreover, striking elevations of serum AST and ALT activities were observed in patients with acute hepatic necrosis. In addition, the elevation of the serum ALP activity in diabetic rat; may be attributed to the elevation of a small intestinal alkaline phosphatase activity in these animals (Unakami *et al.,* 1990; Mansour *et al.,* 2002). ALT is considered a more specific and sensitive indicator of hepatocellular injury than AST in rats. The magnitude of ALT increase is usually greater than that of AST when both are increased due to hepatic injury, in part because of the longer half-

life of ALT and the greater proportion of AST that is bound to mitochondria (Carakosta *et al*., 1986; Boyd 1988; Blair *et al*., 1991; Travlos *et al.,* 1996). Increased serum ALT activity, with or without increased AST activity, include hepatocellular necrosis, or regenerative/reparative activity (Zimmerman, 1999; Hall 2001; Mayer and Harvey, 2004). Decreases in ALT activity have been observed with concurrent hepatic microsomal enzyme induction in the rat (Boone *et al*., 1998). Increases in ALT activity also have been reported with concurrent hepatic microsomal induction in rat but were not considered indicative of hepatic injury because no substantive concurrent changes in liver histology or liver weight were observed (Amacher *et al*., 1998; Valentine *et al*., 1990). Increased serum ALT activity can also be affected by extrahepatic factors. Muscle injury can cause increases in serum transaminase activity, but AST is generally higher than ALT when both are concurrently increased (Boone *et al*., 2005). Therefore, there is no conclusive remark regarding the effects of the crude extracts on organ function and injuries for the diabetic groups until a substantial histopathological studies are performed, hence the need for further histopathological studies on the organs.

The acute toxicity effect of the extracts reveals no lethal mortality at 4800mg/kg of the crude extracts (Table 4.3) and it was mainly conducted to determine the LD50 of the crude extracts of plants. This shows that the safe dose could be above 4800mg/kg. LD50 is just an index in acute toxicity investigation of substance, therefore other observations can also tell the nature of crude extract but there was no significant difference in the clinical signs observed. Thus, acute toxicity effects can also be substantiated if histopathological studies are conducted.

The exhibition of medicinal properties by plants is known to be due to the presence of some secondary metabolite and they act as the lead compound in the development of

drugs (Alan, 2000). These chemical constituents usually found in plants have various forms and different mechanism of actions for the properties they exhibited. Some of this constituents are refers to as anutrients (xenobiotics) of which their presence in the crude extract when taken may initiate a synergistic, antagonistic, potentiating and agonistic forms of effects in the body (Ernest and Patricia, 1994) and this reflect their medicinal properties as the case may be. Thus, the varying degree of the hypoglycaemic effects of the *G.braunii* and *G.oreophila* extracts and the effects on the biochemical parameters of the diabetic rats can be attributed to their phytochemical constituents present in the extracts. Flavonoid, alkaloid, saponin, tannins, phlobatannin, terpenoid, total phenols, anthraquinone, and cardiac glycoside were detected in all the extracts of *G.braunii* and *G.oreophila*. Whereas anthranoid was detected in trace amount (Table 4.2). Flavonoid has been reported to posses antidiabetic properties. Flavonoid has many other bioforms and their mechanisms of action as antidiabetic agents have been elucidated (Brahmanchari, 2011). Among the mechanism are; ability to initiate hypoglycaemia through insulin mimetic ability, ability to stimulate the β-cells of islets of langerhans of the pancreas to secret insulin, stimulation of glucose uptake in peripheral tissue and ability to express the rate-limiting enzymes involved in carbohydrate metabolism pathway (Brahmanchari, 2011).

Some of the hypoglycaemic mechanisms of these phytochemicals have been elucidated by many researchers (Evans 1989; Wahab *et al*., 2010; Brahmanchari 2011). Therefore the hypoglycaemic activities of the extracts observed may be due to the presence of these phytochemical constituents.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The research work has shown that all the extracts (Aqueous *G.braunii*, Aqueous *G.oreophila*, Ethylacetate *G.braunii*, and Ethylacetate *G.oreophila* extract*)* at 500mg/kg body weight have significant hypoglycaemic effects on the diabetic animal model (rats). However, ethylacetate extracts of *G.braunii* had higher activity (65.50%) and may alleviate some biochemical abnormalities in alloxan induced diabetic rat. Thus, this shows an appraisal for the folkloric consumption of mistletoe as antidiabetic medicament.

5.2 Recommendations

The continue struggle to find a therapeutic regimen to cure diabetes is still ongoing, therefore further prudent experimental design is required to investigate the histopathological effects of the extracts on diabetic rat especially for ethylacetate *G.braunii*. The quantitative phytochemical analysis of the plant extracts and the mechanism of action of the exact lead compound present in the extracts responsible for hypoglycaemic effects should be investigated.

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APPENDICES

APPENDIX I

(a) Values for diagnosis of diabetes mellitus and other categories of hyperglycaemia

	Glucose concentration, $mmoll^{-1}$ ($mgdl^{-1}$)		
	Whole Blood		Plasm
	Venous	Capillary	Venous
Diabetis Mellitus:	≥ 6.1 (≥ 110)	≥ 6.1 (≥ 110)	≥ 7.0 (≥ 126)
Fasting			
or			
2-h post glucose load	≥ 10.0 (≥ 180)	≥11.1 (≥ 200)	≥ 11.1 (≥ 200)
or both			
Impaired Glucose Tolerance (IGT):			
Fasting (if Measured)	< 6.1 (<110)	< 6.1 (<110)	< 6.1 (<110)
and			
2-h post glucose load	≥ 6.7 (≥ 120) and < 10.0 (< 180)	≥ 7.8 (≥ 140) and < 11.1 (< 120)	≥ 7.8 (≥ 140) and < 11.1 (< 120)
Impaired fasting glycaemia (IFG): Fasting	≥ 5.6 (≥ 100) and < 6.1 (< 110)	≥ 5.6 (≥ 100) and < 6.1 (< 110)	≥ 6.1 (≥ 110) and < 7.0 (< 126)
***and* (**if measured**)** 2-h post glucose load	< 6.7 (<120)	< 7.8 (<140)	< 7.8 (<140)

(b) Aetiological classification of Disorders of glycaemia

Type 1 (beta cell destruction, usually leading to absolute insulin deficiency
Autoimmune
Idiopathic

Type 2 (May from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with or without insulin resistance).

Other specific Types

Genetic defects of beta-cell function
Genetic defects in insulin action
Diseases of the exocrine pancreas
Endocrinopathies
Drug or chemical-induced
Infections
Uncommon forms of immune-mediated diabetes
Other genetic syndromes sometimes associated with diabetes

Gestational diabetes

(c) Other specific types of diabetes

Genetic defects of beta-cell function

- Chromosome 20, HNF4α (MODY 1)
- Chromosome 7, glucokinase (MODY 2)
- Chromosome 12, HNF1α(MODY3)
- Chromosome 13, IPF-1 (MODY 4)
- Mitochondrial DNA 3243 mutation
- Others

Genetic defects in insulin action

- Type A insulin resistance
- Leprechaunism
- Rabson-Mendenhall syndrome
- Lipoatrophic diabetes
- Others

Diseases of the exocrine pancreas

- Fibrocalculous pancreatopathy
- Pancreatitis
- Trauma/pancreatectomy
- Cystic fibrosis
- Haemochromatosis
- Others

Endocrinopathies

- Cushing's syndrome
- Acromegaly
- Phaeochromocytoma
- Glucagonoma
- Hyperthyroidism
- Somatostatinoma
- Others

Drugs or chemical induced

Infection

- Congenital rubella
- Cytomegalovirus
- Others

Uncommon forms of immune-mediated diabetes

- Insulin autoimmune syndrome (antibodies to insulin)
- Anti-insulin receptor antibodies
- "Stirr Man" Syndrome
- Others

Other genetic syndromes

- Down's syndrome
- Friedreich's ataxia
- Huntington's chorea
- Klinefelter's syndrome
- Lawrence-Moon-Biedel syndrome
- Myotonic dystrophy
- Porphyria
- Prader-Willi syndrome
- Turner's syndrome
- Wolfram's syndrome
- Others

Drug or chemical induced

- Nicotinic acid
- Glucocorticoids
- Thyroid hormone
- Alpha-adrenergic agonists

Beta-adrenergic agonists
Thiazides
Dilantin
Pentamidine
Vacor
Interferon-alpha therapy
Others

(g) Some ethnobotanically surveyed medicinal plants used in the treatment of diabetes mellitus in northern west part of Nigeria.

S/N	Botanical Name	Family	Common name	Local name (Hausa)
1	*Mangifera indica*	Anacarbiaceae	Mango	Mangoro
2	*Angeissus leiocarpus*	Combretaceae	Giant fern	Marke
3	*Fiscus thonnigii*	Moraceae	Loin cloth fig tree	Cediya
4	*Khaya senegalensis*	Meliaceae	Mahogany	Maddacii
5	*Euphorbia convuludiodes*	Euphobiaceae	Corn cob cactus	Noonan kurciya
6	*Acacia nilotica*	Mimosaceae	Egyptian mimosa	Bagaruwa
7	*Vernona amydalina*	Asreraceae	Biter leaves	Shuwaka
8	*Cassia arereh*	Caesalpiniaceae	Indian senna	Marga
9	*Cassia goratensis*	Caesalpiniaceae	Golden shower	Runhu
10	*Calotropis procera*	Asclepiadaceae	Sodom apple	Tumfafiya
11	*Senna occidentalis*	Fabaceae	Coffee senna	Sangasanga
12	*Alluvium cepa*	Liliaceae	Onion	Albasa
13	*Ipomoea batatas*	Convolulaceae	Sweet	Dankali
14	*Vitex gekowskii*	Verbenaceae	Black plum	Dinya
15	*Citrus medica*	Rutaceae	Sour orange	Lenum tsami
16	*Parkta filicoidae*	Mimosaceae	African locust bean tree	Dorawa
17	*Allium sativum*	Liliaceae	Garlic	Tafarnuwa
18	*Anacardium occidentalis*	Anacardiaceae	Cashew	Kanju
19	*Azadirachta indica*	Meliaceae	Neem	Dongo yaro

20	*Vitillarta paradoxa*	Sapotaceae	Shea butter tree	Kade
21	*Gossypium hirsutum*	Malvaceae	Cotton	Auduga
22	*Lawsonia inermis*	Lythraceae	Egyptians priest	Lelle
23	*Moringa oleifera*	Moringaceae	Horseradish tree	Zogale
24	*Psidium guajava*	Myrtaceae	Gwava	Goba
25	*Bauchinia reticulate*	Casalpiniaceae	Camel foot tree	Kalgo
26	*Balanites aegyptiaca*	Zygophyliaceae	Desert date tree	Aduwa
27	*Lannea kerstingii*	Anacardiaceae	-	Faarun
28	*Zizyphus mucronata*	Rhamnaceae	Buffalo	Magarya
29	*Daucus carota*	Apiaceae	Carrot	Carrot
30	*Zizyphus spina*	Rhamnaceae	Christ's thorn	Kurna
31	*Anana senegalensis*	Anonaceae	Wild custard apple	Gwardar daji
32	*Eugenia caryophyllata*	Myrtaceae	Clove	Kanumfari
33	*Blighia sapida*	Sapindaceae	Akee apple	Gwaja kusa
34	*Ficus sycomorus*	Moraceae	Sycamore tree/tree of life	Baure

Source: Etuk *et al.,* 2010

(h) List of some plants that have been scientifically appraised as anti-diabetic plants

S/N	Botanical	Family	Parts used	Main active components
1	*Allium sativum*	Alliaceae	Bulbs	Allyl propyl disulphide, allicin
2	*Annona squamosal*	Annonaceae	Fruits	Liriodenine, moupinamide
3	*Areca catechu*	Arecaceae	Seed	Arecaine and arecoline
4	*Artemisia pallens*	Asteraceae	Leaves and flowers	Germacranolide
5	*Azadirachta indica*	Meliaceace	Leaves, flowers & seed	Azadirachtin and nimbin
6	*Bauchinia forficate*	Leguminosae	Leaf	Astragalin kaempferitin
7	*Beta vulgaris*	Amaranthaceae	Root	Phenolics, betacyanins
8	*Boerhavia diffusa*	Nyctaginaceae	Whole plant	punarnavine and ursolic acid
9	*Camellia sinensis*	Theaceae	Leaves	Caffeine and catechins
10	*Capparis decidua*	Cappardaceae	Fruit	Spermidine Isocodonocarpine
11	*Cinnamomum zeylanicum*	Lauraceae	Bark	Cinnamaldehyde, eugenol
12	*Combretum micranthum*	Combretaceae	Leaves	Polyphenols
13	*Elephantopus scaber*	Asteraceae	Whole plant	Terpenoid and 2,6,23-trienolide
14	*Ficus bengalensis linn*	Moraceae	Bark	Leucodelphinidin and leucopelargonin
15	*Gymnema sylvestre*	Asclepiadeceae	Leaf	Dihydroxy gymnemic triacetate
16	*Gynandropsis gynandra*	Capparidaceae	Root	N,N-diethyltoluamide
17	*Lantana camara*	Verbenaceae	Leaves	Lantanoside, lantanone

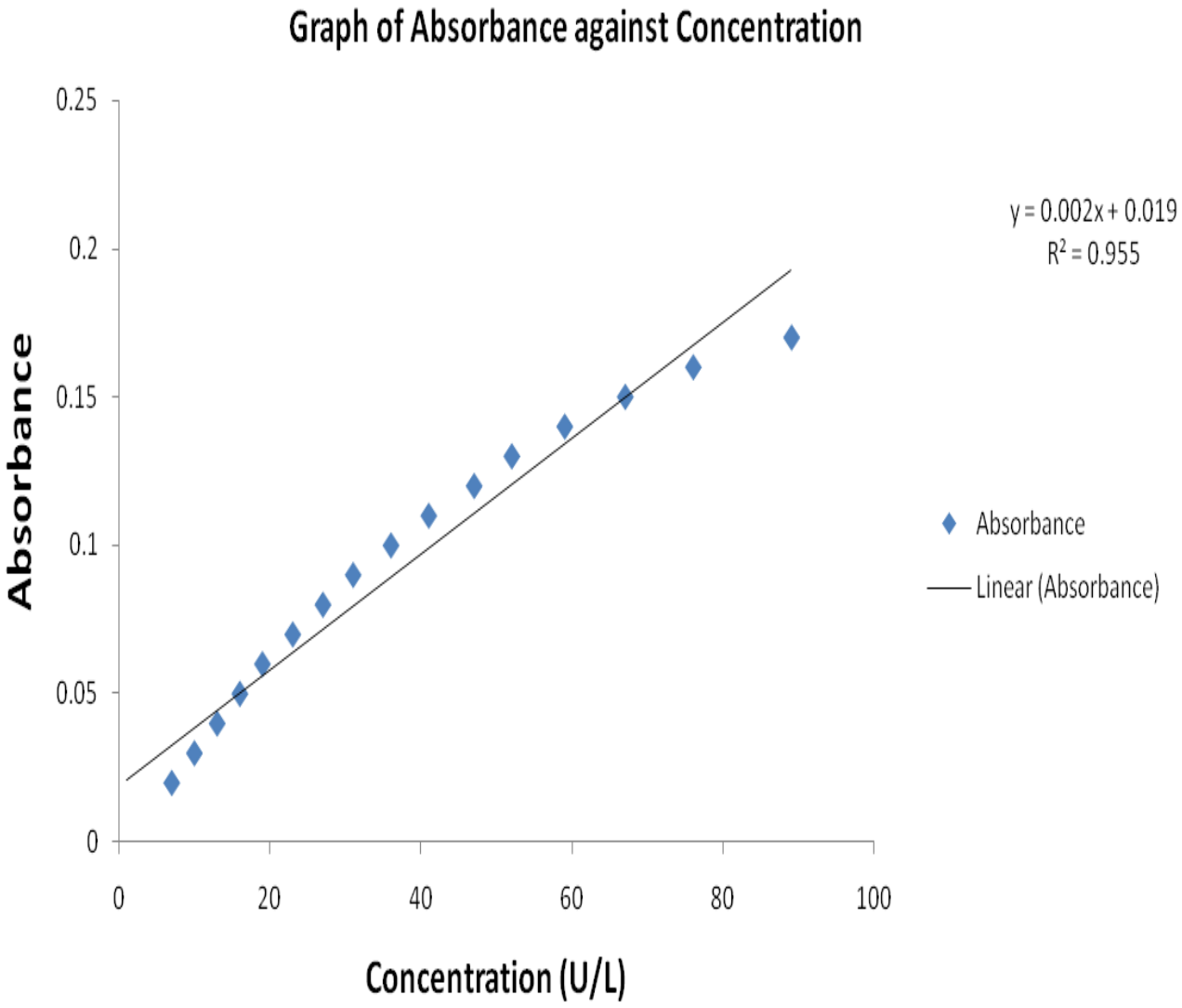
18	*Liriope spicata*	Liliaceae	Root	Beta-sitosterol, stigmasterol
19	*Momordica charantia*	Cucurbiataceae	Leaves	Charantin, sterol
20	*Ocimum sanctum*	Labiatae	Root	Eugenol
21	*Panax quinquefolius*	Araliaceae	Root	Ginsenosides, protopanaxadiol
22	*Phyllanthus amarus*	Phyllanthaceae	Whole plant	Phyllanthin
23	*Prunus amygdalus*	Rosaceae	Seeds	Amygdalin
24	*Parinari excels*	Chryssobalanaceae	Bark	Myricetin, quercertin
25	*Pterocarpus marsupium*	Leguminosae	Whole plant	Kenotannic acid, pyrocatechin
26	*Punica granatum*	Lythraceae	Fruit	Punicalagin, punicalin
27	*Ricinus communis*	Euphorbiaceae	Root	Ricinolic acid
28	*Salacia oblonga wall*	Celastraceae	Root bark	Salacinol
29	*Sarcopoterium spinosum*	Rosaceae	Root	Catechin and epicatechin
30	*Smallanthus sonchifolius*	Asteraceae	Leaves	Sonchifolin, uvendalin enhydrin, fluctuanin
31	*Swertia puniceae*	Gentianaceae	Whole plant	Methyl swertianin and bellidifolin
32	*Tinospora cordifolia*	Menispermaceae	Root	Tinosporone, tinosporic acid
33	*Trigonella foenum gracecum*	Fabaceae	Leaves and seeds	4-hydroxy isoleucine
34	*Vernonia anthelmintica*	Asteraceae	Seed	Epoxy acid or vernolic acid
35	*Withania somnifera*	Solanaceae	leaves	Somniferine, withananine and cuscohygrine
36	*Rosmarinus officinalis*	Labiatae	Leaves	Caffeic acid, Carnaso, Rosmaridephenol, Rosmerinic acid.
37	*Ximenia americana*	Olacacecae	Stem bark	Alkaloids, saponin, flavonoid

Source: M.Upendra *et al*., 2010

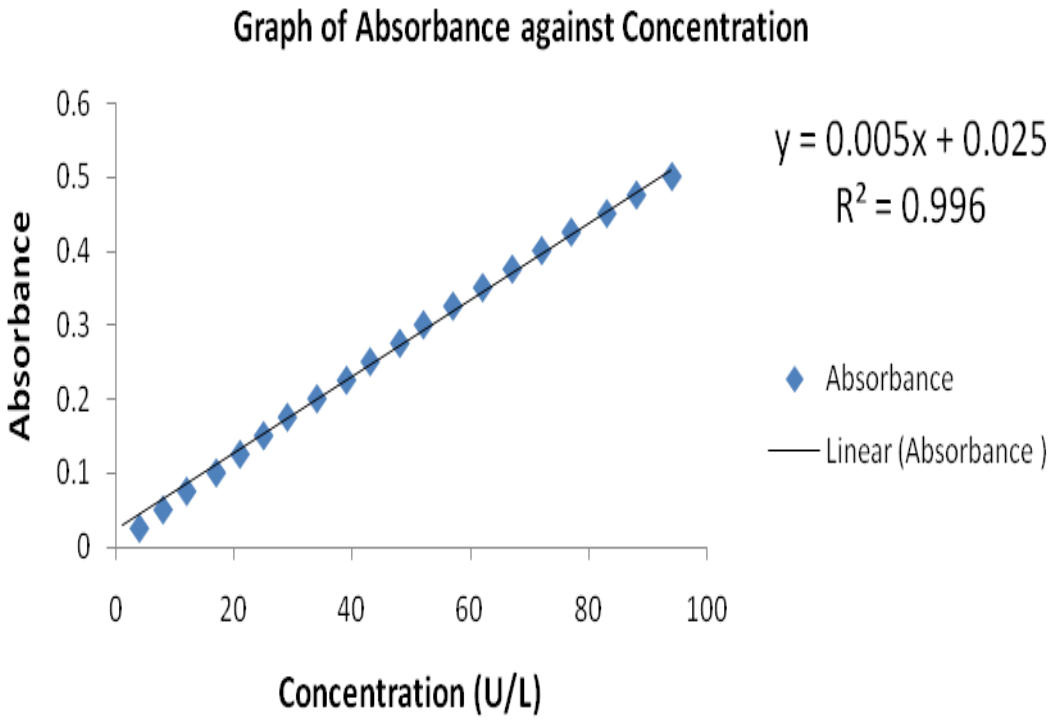
(a) ***G.braunii***

(b) ***G.oreophila***

Appendix II



B1: Standard plot for the determination of serum concentration of aspartate aminotransferase



B2: Standard plot for the determination of serum concentration of Alanine aminotransferase

B3: Formulas for the calculation of Biochemical parameters

Cholesterol (mg/dl) = $\frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard (std)}}$ X Conc. of Std (g/dl)

Triglycerides (mg/dl) = $\frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard (std)}}$ X Conc. of Std (g/dl)

Concentration of ALP = $\frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard (std)}}$ X Conc. of Std (IU/L)

Appendix III

a) : Results of acute toxicity test of Aqueous *G.braunii* extracts

Groups/Dosage (mg/kg)Body weight (b.w)	Initial Body Weight	Final Body Weight	Differences in Body weight	Percentage differences (%)	Observations	Mortality
A	121.47±0.96	125.30±1.18	3.83	3.15**	Normal	No death
B	155.56±0.55	161.08±2.85	5.52	3.55**	Normal	No death
C	146.00±0.36	147.02±1.2	1.02	0.70**	Thick and solid Feces with small mucus at the tip, Behavior normal	No death
D	150.86±1.77	151.23±2.54	0.37	0.25**	Thick and solid feces with enough mucus at the tip, behavior and other signs normal.	No death

Key:
*=Percentage Reduction
**= Percentage Increase
A= Control (20ml/kgb.w Distilled water)
C=Aqeous G.braunii (1200mg/kg) b.w
B=Aqeous G.braunii (300mg/kg) b.w
D=Aqeous G.braunii (4800mg/kg) b.w

b): Results of acute toxicity test of Aqueous *G.oreophila* extracts

Groups/Dosage (mg/kg)Body weight (b.w)	Initial Body Weight	Final Body Weight	Differences in Body weight	Percentage differences (%)	Observations	Mortality
A	121.47±0.96	125.30±1.18	3.83	3.15**	Normal	No death
E	140.19±0.25	145.02±2.46	4.83	3.45**	Solid feces, behaviors and other signs normal.	No death
F	148.86±0.94	151.44±2.35	2.58	1.73**	Solid feces with little mucus all over, behaviors and other signs normal.	No death
G	129.63±0.79	132.99±1.62	3.36	2.59**	Solid feces with enough mucus allover and much at the tip. Behavior and other signs normal.	No death

Key:

*=Percentage Reduction **= Percentage Increase

A= Control (20ml/kgb.w Distilled water) F=Aqeous G.oreophila (1200mg/kg) b.w

E=Aqeous G.oreophia (300mg/kg) b.w G=Aqeous G.oreophila (4800mg/kg) b.w

c): Results of acute toxicity test of Ethylacetate *G.braunii* extracts

Groups/Dosage (mg/kg)Body weight (b.w)	Initial Body Weight	Final Body Weight	Differences in Body weight	Percentage differences (%)	Observations	Mortality
A	121.47±0.96	125.30±1.18	3.83	3.15**	Normal	No death
H	191.84±0.57	195.99±0.90	4.15	2.16**	Normal.	No death
I	219.83±7.13	221.54±5.22	1.71	0.78**	Soft feaces with no mucus, behavior and other signs normal.	No death
J	199.38±0.66	203.03±4.37	3.65	1.83**	Very soft feaces with no mucus, behavior and other signs normal.	No death

Key:
*=Percentage Reduction
**=Percentage Increase
A= Control (20ml/kgb.w Distilled water)
I=Ethylacetate G.braunii (1200mg/kg) b.w
H=Ethylacetate G.braunii (300mg/kg) b.w
J=Ethylacetate G.braunii (4800mg/kg) b.w

d): Results of acute toxicity test of Ethylacetate *G.oreophila* extracts

Groups	Initial Body Weight	Final Body Weight	Differences in Body weight	Percentage differences (%)	Observations	Mortality
A	121.47±0.96	125.30±1.18	3.83	3.15**	Normal	No death
K	175.19±1.93	178.10±1.93	2.91	1.66**	Normal feces with linked together with mucus, behaviors and other signs normal.	No death
L	178.10±1.56	169.56±2.02	-8.54	4.8*	Solid feces with linked together with mucus, behavior and other signs normal.	No death
M	154.31±3.15	157.29±5.63	2.98	1.93**	Solid and thick feces linked together with mucus, behavior and other signs normal.	No death

Key:
*=Percentage Reduction **= Percentage Increase
A= Control (20ml/kgb.w Distilled water) L=Ethylacetate G.oreophila (1200mg/kg) b.w
K= Ethylacetate G.oreophila (300mg/kg) b.w M=Ethylacetate G.oreophila (4800mg/kg) b.w

e): Values obtained from acute toxicity test for LD_{50} determination

Groups	Dose (mg/kg)	Number of Rats (n)	Mortality
B	300	3	0
C	1200	3	0
D	4800	3	0
E	300	3	0
F	1200	3	0
G	4800	3	0
H	300	3	0
I	1200	3	0
J	4800	3	0
K	300	3	0
L	1200	3	0
M	4800	3	0

Key:

B=Aqeous G.braunii (300mg/kg) b.w C=Aqeous G.braunii (1200mg/kg) b.w
D=Aqeous G.braunii (4800mg/kg) b.w E=Aqeous G.oreophia (300mg/kg) b.w
F=Aqeous G.oreophila (1200mg/kg) b.w G=Aqeous G.oreophila (4800mg/kg) b.w
H=Ethylacetate G.braunii (300mg/kg) b.w I=Ethylacetate G.braunii (1200mg/kg) b.w
J=Ethylacetate G.braunii (4800mg/kg) b.w

f): Hypoglycemic effects of Aqueous and Ethylacetate extracts of *Globimetula braunii* and *Globimetula oreophila* in rats induced with diabetes.

	Days of Glucose Measurement				
Experimental Groups	**Day 1**	**Day 7**	**Day 12**	**Day 17**	**Day22**
DST	92.33±7.17^{a}	138.33±31.33^{a}	109.33±9.53^{a}	108.33±3.48^{a}	93.00±2.08^{a}
NNT	84.33±4.84^{c}	83.00±2.65^{bc}	72.33±1.45^{a}	76.00±2.31^{abc}	74.67± 0.88^{ab}
AGB	88.33±3.84^{a}	201.00±27.02^{b}	124.33±4.67^{a}	132.67±18.81^{a}	83.67±9.35^{b}
AGO	88.67±0.88^{a}	181.00±39.87^{b}	130.00±8.52^{ab}	98.00±70.95^{a}	89.67±6.01^{a}
EGB	85.67±2.85^{a}	228.00±12.12^{b}	90.00±11.02^{a}	95.67±17.70^{a}	78.67±7.26^{a}
EGO	84.00±3.21^{a}	204.00±56.37^{b}	139.67±6.94^{ab}	102.67±8.95^{a}	100.67±2.35^{a}
DNT	84.67±1.49^{a}	188.00±72.00^{a}	200.00±30.75^{a}	206.33±26.62^{a}	204.00±30.83^{a}

Values are expressed as Means±Standard Error of Means ($\bar{X}$±SEM), (n=3),

Values in the same row with superscripts that are not related represent means that are significantly different ($p < 0.05$).

g): The mean Percentage changes in Fasting blood glucose concentration (mg/dl) between the days of treatment with the extracts.

	Treatment days			
Experimental Groups	**Day 7-day12**	**Day 12-day17**	**Day 17-day22**	**Day1-Day22**
DST	20.96*	0.91*	14.15*	32.77*
NNT	12.89*	5.07**	1.75*	10.04*
AGB	38.14*	6.71**	36.93*	58.37*
AGO	28.18*	24.61*	8.5*	50.46*
EGB	60.53*	6.3**	17.77*	65.50*
EGO	31.53*	26.49*	1.95*	50.65*
DNT	6.38**	3.17**	1.13*	8.51**

Key: * Percentage Reduction ** Percentage Increase

h): Changes in whole body weight of rats between days of treatment

Experimental Groups	Days of Weight Measurement				
	Day 1	**Day 7**	**Day 12**	**Day 17**	**Day22**
DST	155.82± 4.0[a]	148.01± 0.64[a]	159.92± 5.02[a]	162.10± 5.67[a]	163.61± 5.69[a]
NNT	152.58±1.47[a]	161.68±2.85[ab]	165.97±4.63[ab]	170.00±4.91[b]	174.68±5.72[b]
AGB	167.79±2.39[a]	159.55± 9.56[a]	165.36±15.75[a]	164.45±16.10[a]	162.68±1.93[a]
AGO	178.15±5.25[a]	176.90±1.29[a]	181.20±5.58[a]	182.65±4.19[a]	182.11±3.15[a]
EGB	192.28±6.39[a]	182.24±9.17[a]	185.56±1.27[a]	189.56±9.95[a]	190.92±9.44[a]
EGO	208.66±2.22[a]	191.51±8.91[a]	197.40±8.28[a]	202.32±7.37[a]	203.08±9.46[a]
DNT	228.08±5.71[a]	222.22±7.41[a]	223.39±7.27[a]	216.51±4.51[a]	214.20±3.38[a]

Values are expressed as Means±Standard Error of Means ($\bar{X}\pm SEM$), (n=3 per group),

Values in the same row with superscripts that are not related represent means that are significantly different ($p < 0.05$).

I): Mean Percentage (%) changes in whole body weight of rats between days of treatment.

	Days of Experiment				
Experimental Groups	**Day 1-day7**	**Day 7-day12**	**Day 12-day17**	**Day 17-day22**	**Day1-Day22**
DST	5.02*	8.05**	1.36**	0.93**	10.54**
NNT	5.96**	2.65**	2.43**	2.75**	8.04**
AGB	4.91*	3.64**	0.55*	1.08*	1.96**
AGO	0.70*	2.43**	0.80**	0.30*	3.51**
EGB	5.22*	1.82**	2.16**	0.72**	4.21**
EGO	8.22*	3.06**	2.49**	0.38**	6.04**
DNT	2.57*	0.53**	3.08*	1.07*	3.61*

Key: * = Percentage Reduction ** = Percentage Increase

J): Levels of serum Transaminases (ALT/AST) and Alkaline Phosphatase (ALP)

	Enzymes (U/L)		
Experimental Groups	**ALP (U/L)**	**ALT (SGPT) (U/L)**	**AST (SGOT) (U/L)**
DST	95.13 ± 9.50^{bc}	25.53 ± 0.64^{a}	60.00 ± 5.50^{c}
NNT	81.28 ± 1.40^{ab}	39.70 ± 2.5^{a}	17.75 ± 1.25^{a}
AGB	125.30 ± 5.60^{c}	37.10 ± 6.70^{a}	43.25 ± 2.25^{bc}
AGO	86.87 ± 5.40^{ab}	34.10 ± 1.30^{a}	49.75 ± 8.75^{bc}
EGB	110.76 ± 9.31^{bc}	78.90 ± 8.90^{c}	39.25 ± 7.25^{b}
EGO	78.68 ± 2.24^{ab}	63.30 ± 6.10^{b}	57.00 ± 5.50^{bc}
DNT	60.75 ± 3.96^{a}	73.07 ± 2.40^{bc}	53.75 ± 3.98^{bc}

Values are expressed as Means±Standard Error of Means ($\bar{X} \pm SEM$), (n=3 per group),

Values in the same column with superscripts that are not related represent means that are significantly different ($p < 0.05$).

h): Serum concentration of Total Protein, Total Cholesterol, Triglyceride, Direct Bilirubin and Total Bilirubin.

	Biochemical parameters				
Experimental Groups	**Total Protein(g/dl)**	**Total cholesterol (mg/dl)**	**Triglyceride (mg/dl)**	**Direct Bilirubin (mg/dl)**	**Total Bilirubin (mg/dl)**
DST	8.48 ± 1.39^{a}	74.41 ± 5.05^{a}	108.84 ± 8.06^{a}	0.73 ± 0.06^{bc}	2.22 ± 0.48^{cd}
NNT	8.50 ± 0.49^{a}	75.59 ± 4.11^{a}	114.05 ± 5.79^{a}	0.97 ± 0.06^{c}	2.20 ± 0.11^{cd}
AGB	8.99 ± 0.80^{a}	86.26 ± 6.69^{a}	104.42 ± 9.26^{a}	0.52 ± 0.06^{ab}	0.64 ± 0.08^{a}
AGO	8.59 ± 0.30^{a}	86.62 ± 8.12^{a}	115.66 ± 13.91^{a}	0.52 ± 0.04^{ab}	1.64 ± 0.14^{bc}
EGB	8.58 ± 0.38^{a}	91.55 ± 1.41^{a}	114.05 ± 6.43^{a}	0.93 ± 0.1^{c}	2.86 ± 0.52^{d}
EGO	8.12 ± 1.12^{a}	86.86 ± 8.77^{a}	98.80 ± 12.05^{a}	1.57 ± 0.2^{d}	1.55 ± 0.28^{abc}
DNT	8.22 ± 0.52^{a}	88.73 ± 4.53^{a}	173.50 ± 16.87^{b}	0.36 ± 0.00^{a}	1.07 ± 0.2^{ab}

Values are expressed as Means±Standard Error of Means ($\bar{X} \pm SEM$), (n=3),

Values in the same column with superscripts that are not related represent means that are significantly different ($p < 0.05$).